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Thomson et al.

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(54) **GENERATING ARTERIAL ENDOTHELIAL CELL POPULATIONS**

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Related U.S. Application Data

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(51) **Int. Cl.**

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G01N 33/50	(2006.01)
A61K 35/44	(2015.01)
C12N 5/00	(2006.01)

(52) **U.S. Cl.**

CPC ***C12N 5/069*** (2013.01); ***A61K 35/44*** (2013.01); ***C12N 5/0031*** (2013.01); ***C12N 5/0056*** (2013.01); ***G01N 33/5064*** (2013.01); *C12N 2500/35* (2013.01); *C12N 2500/90* (2013.01); *C12N 2500/98* (2013.01); *C12N 2501/10* (2013.01); *C12N 2501/113* (2013.01); *C12N 2501/15* (2013.01); *C12N 2501/155* (2013.01); *C12N 2501/16* (2013.01); *C12N 2501/165* (2013.01); *C12N 2501/415* (2013.01); *C12N 2501/42* (2013.01); *C12N 2506/02* (2013.01); *C12N 2506/45* (2013.01)

(58) **Field of Classification Search**

None

See application file for complete search history.

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2018/0023051	A1 *	1/2018	Esmeraldo De Campos Vaz O ...	C12N 5/069 506/39

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WO	2014040020 A2	3/2014	
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(74) *Attorney, Agent, or Firm* — McDonnell Boehnen Hulbert & Berghoff LLP

(57) **ABSTRACT**

Methods for generating human arterial endothelial cells under defined conditions in the absence of insulin are described. In particular, provided herein are efficient, defined, and scalable methods for generating human arterial endothelial cells from human pluripotent stem cells. Also provided herein are uses of human arterial endothelial cells obtained according to these methods. For example, methods of treating peripheral arterial disease and methods of screening agents for that effect adhesion of leukocytes to arterial endothelial cells are also provided.

12 Claims, 17 Drawing Sheets
(17 of 17 Drawing Sheet(s) Filed in Color)

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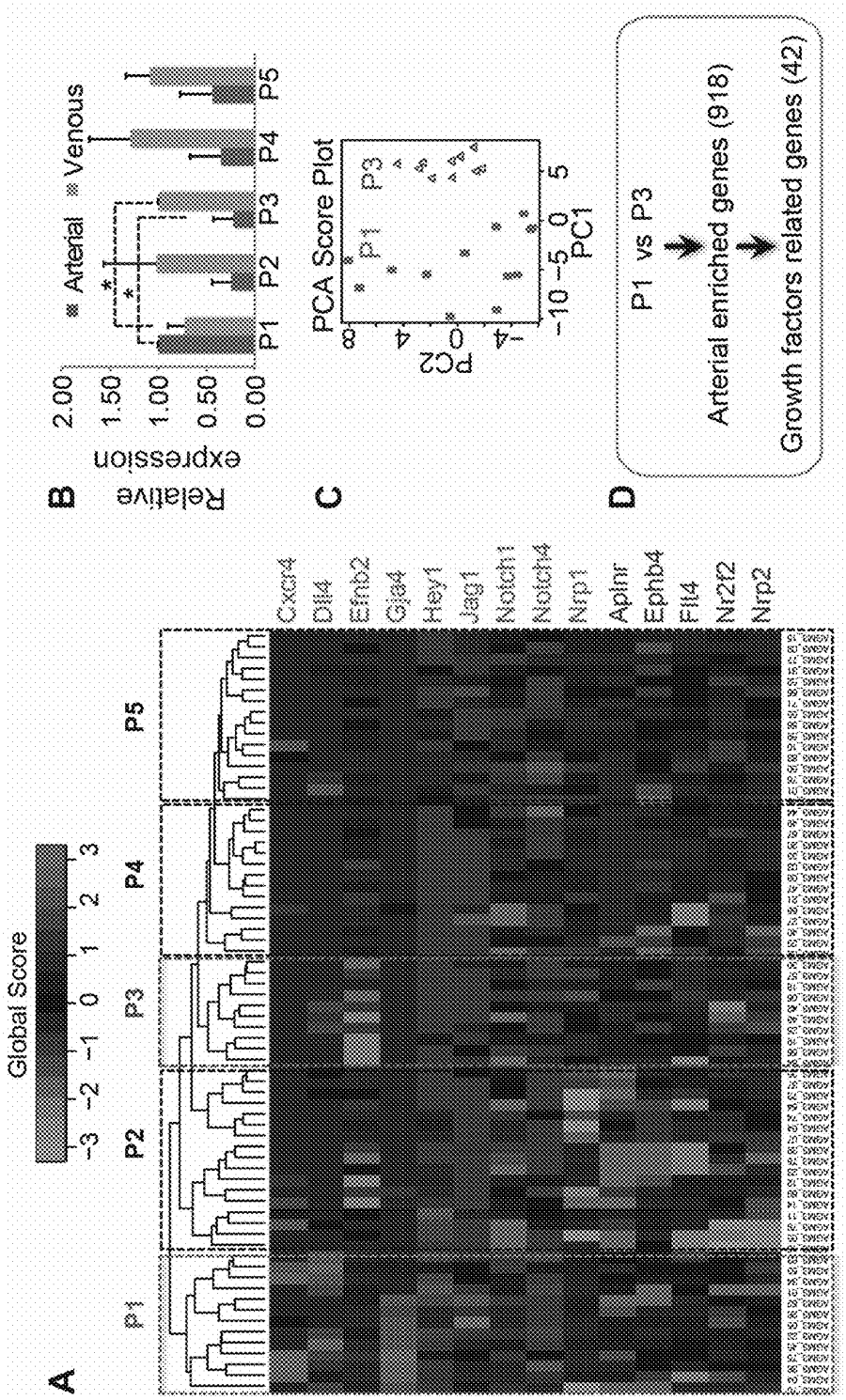
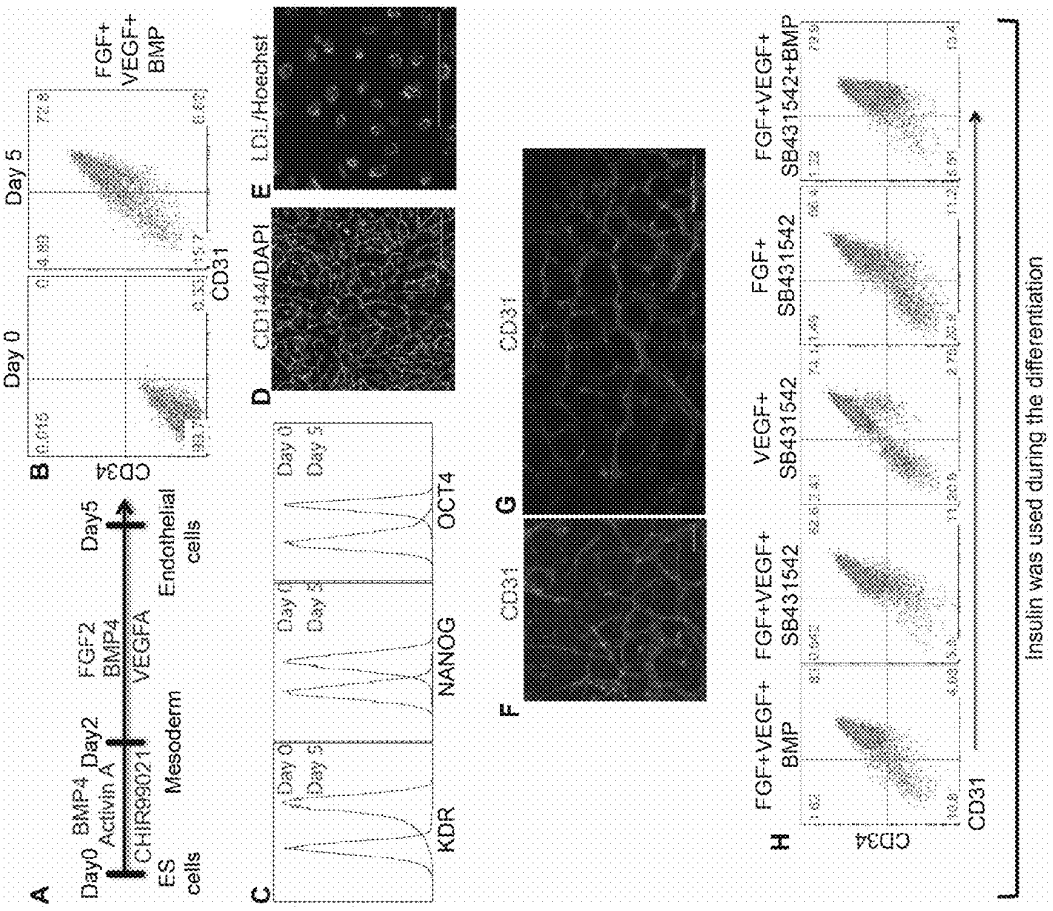
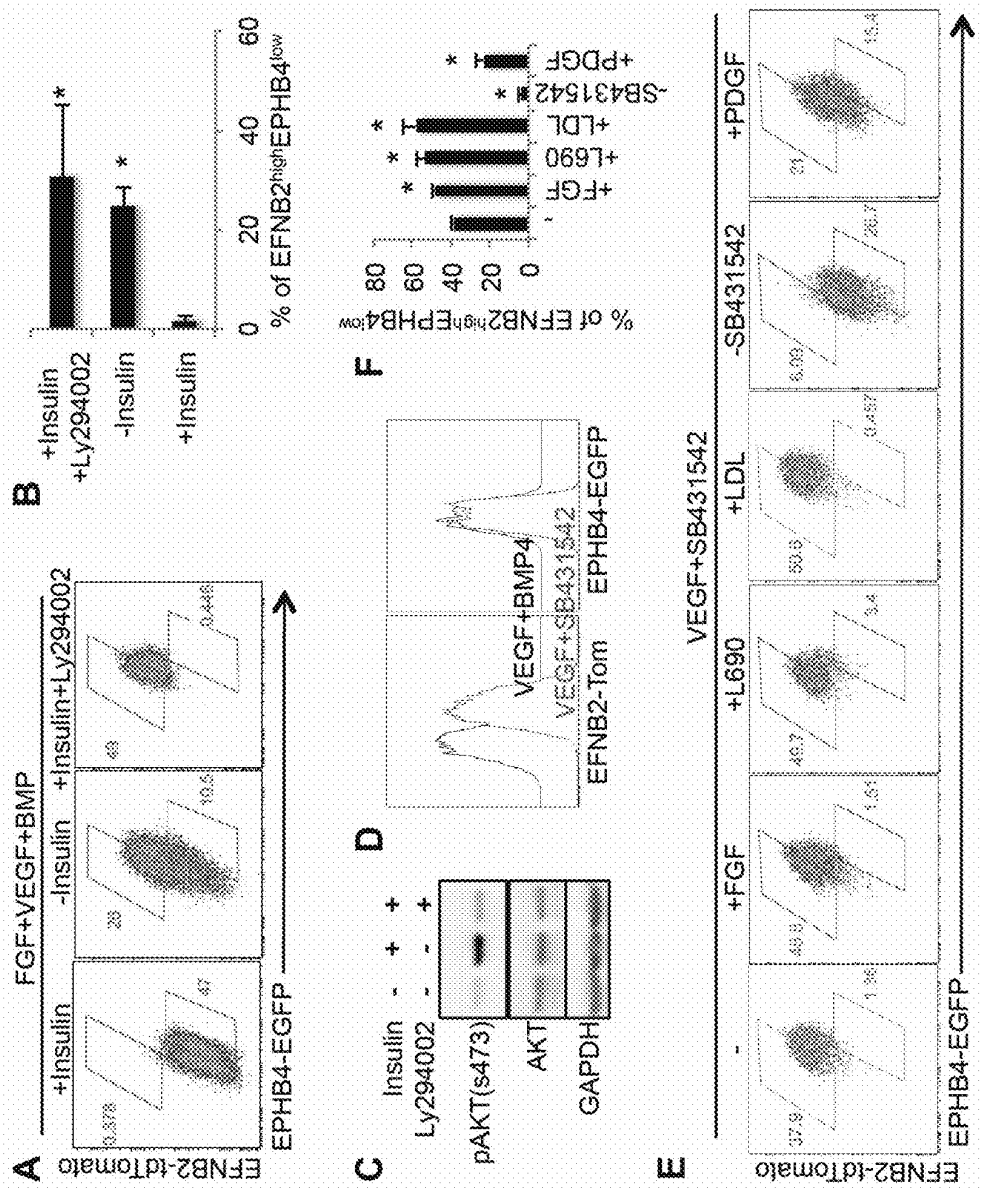
FIGS. 1A-1D


A
P1
P2
P3
P4
P5
Global Score
−3 −2 −1 0 1 2 3
Cxcr4
Dll4
Efnb2
Gja4
Hey1
Jag1
Notch1
Notch4
Nrp1
Apln
Ephb4
Flt4
Nr2f2
Nrp2
B
Relative expression
2.00
1.50
1.00
0.50
0.00
Arterial
Venous
P1 P2 P3 P4 P5
C
PCA Score Plot
PC2
PC1
P1
P3
D
P1 vs P3
Arterial enriched genes (918)
Growth factors related genes (42)

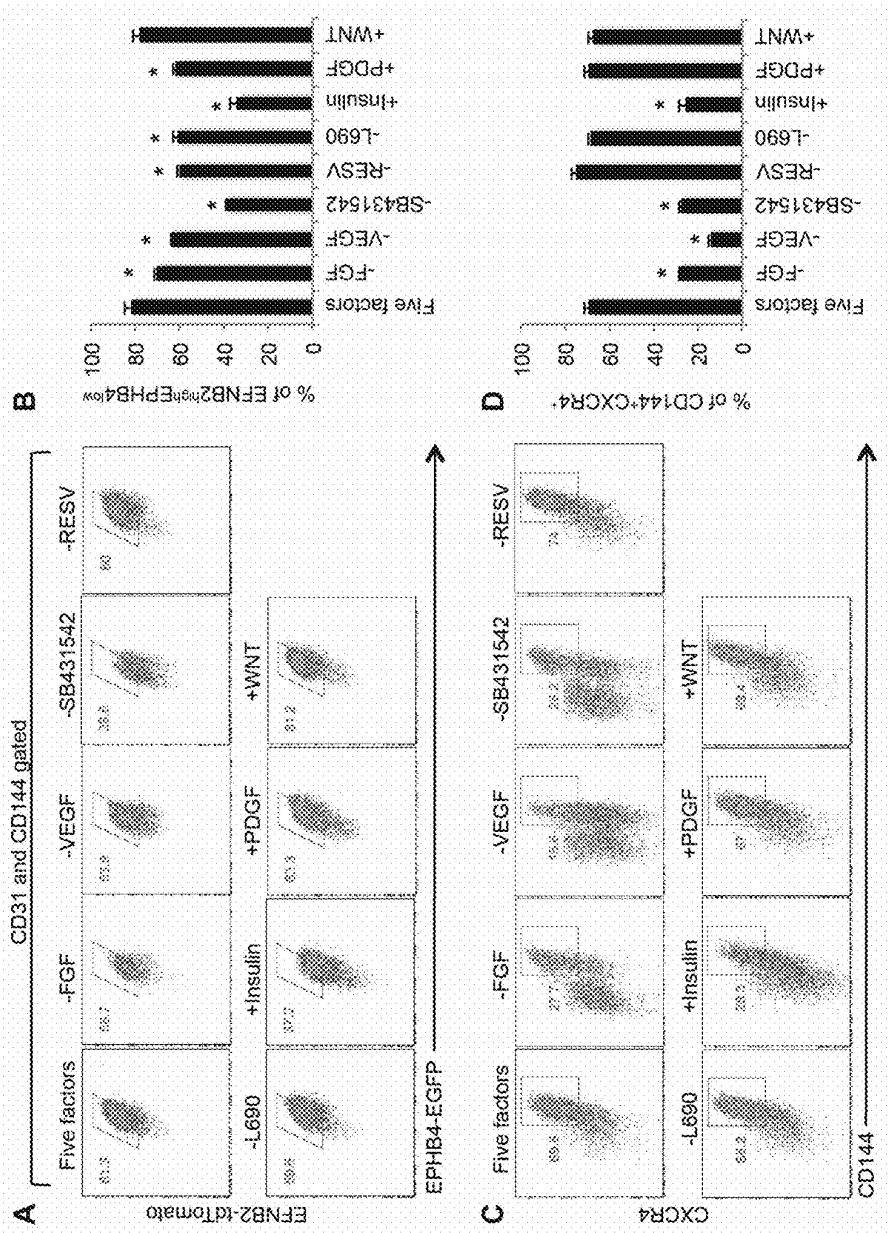
FIGS. 2A-2H


A
Day0
BMP4
Activin A
CHIR99021
Day2
FGF2
BMP4
VEGFA
Day5
ES cells
Mesoderm
Endothelial cells
B
Day 0
Day 5
FGF+ VEGF+ BMP
CD34
CD31
C
Day 0
Day 3
KDR
NANOG
OCT4
D
CD144/DAPI
E
I.Di./Hoechst
F
CD31
G
CD31
H
FGF+VEGF+ BMP
FGF+VEGF+ SB431542
VEGF+ SB431542
FGF+ SB431542
FGF+VEGF+ SB431542+BMP
CD34
CD31
Insulin was used during the differentiation

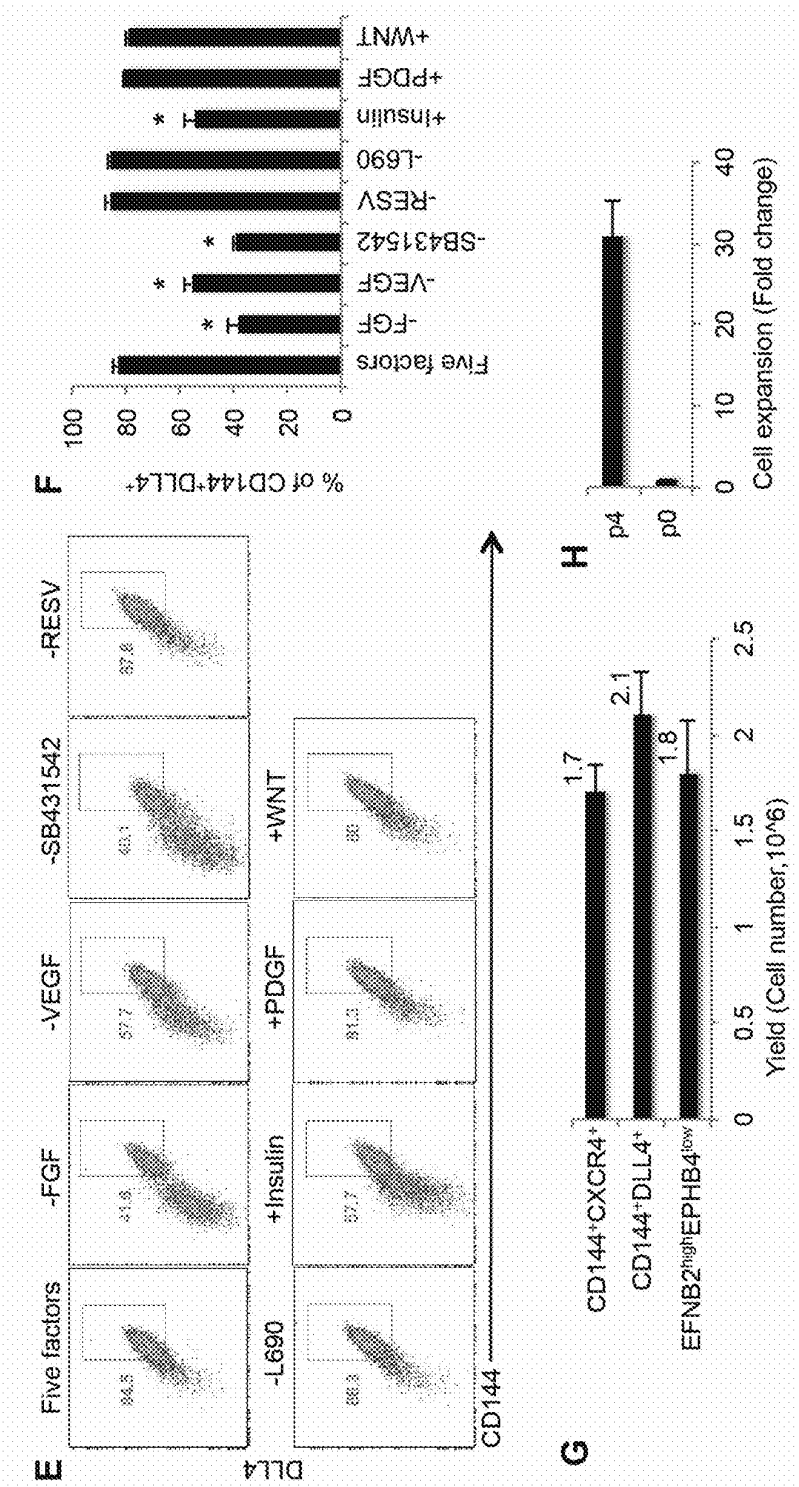
FIGS. 3A-3F


A
FGF+VEGF+BMP
+Insulin
-Insulin
+Insulin+Ly294002
EFNB2-tdTomato
EPHB4-EGFP
B
+Insulin
+Ly294002
-Insulin
+Insulin
% of EFNB2highEPHB4low
C
Insulin
Ly294002
pAKT(s473)
AKT
GAPDH
D
VEGF+BMP4
VEGF+SB431542
EFNB2-Tom
EPHB4-EGFP
E
VEGF+SB431542
+FGF
+L690
+LDL
-SB431542
+PDGF
EFNB2-tdTomato
EPHB4-EGFP
F
% of EFNB2high EPHB4low
+FGF
+L690
+LDL
-SB431542
+PDGF

FIGS. 4A-4H


A
CD31 and CD144 gated
Five factors
-FGF
-VEGF
-SB431542
-RESV
-L690
+Insulin
+PDGF
+WNT
EFNB2-tdTomato
EPHB4-EGFP
B
% of EFNB2highEPHB4low
Five factors
-FGF
-VEGF
-SB431542
-RESV
-L690
+Insulin
+PDGF
+WNT
C
CXCR4
CD144
D
% of CD144+CXCR4+

FIGS. 4A-4H, CONTINUED



FIGS. 5A-5G

A

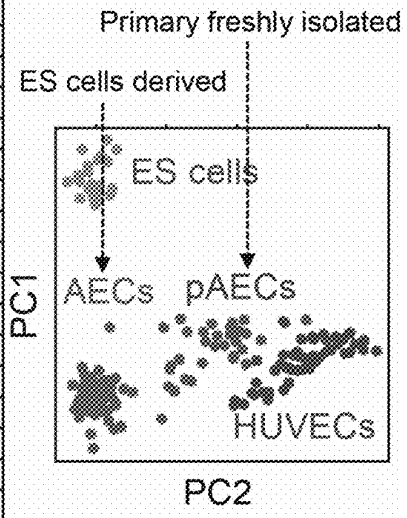
Bulk RNA-seq (TPM)

ES cells derived Primary cultured

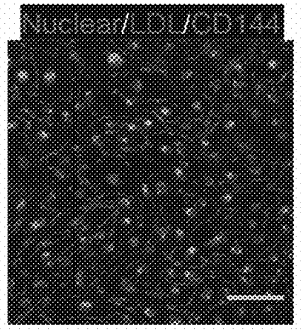
Gene ID	H1_1	H1_2	HUVEC	AEC1	AEC2	AoEC	AECs/HUVEC
CXCR4	0.70	0.97	18.75	1146.78	830.89	69.21	*52.73*
DLL4	0.11	0.24	8.20	485.38	335.06	11.01	*50.04*
EFNB2	11.40	11.35	26.50	542.73	415.18	66.18	*18.07*
GJA4	8.00	7.03	3.30	1921.76	1482.60	18.89	*515.53*
GJA5	1.96	1.77	3.48	44.76	34.19	0.44	*11.33*
HEY1	0.68	1.59	3.86	477.77	430.53	7.11	*117.54*
HEY2	13.57	11.48	7.94	70.68	59.79	1.69	8.21
JAG1	11.81	13.73	56.03	19.29	15.53	44.00	0.31
NOTCH1	18.53	19.53	68.29	82.24	62.68	41.02	1.06
NOTCH4	1.04	1.19	18.92	48.45	42.75	32.85	2.41
NRP1	0.68	0.07	233.85	312.82	221.54	435.78	1.14
APLNR	0.40	0.41	0.57	106.95	125.38	0.00	203.40
EPHB4	104.38	108.19	258.35	118.74	102.17	130.46	0.43
FLT4	5.98	7.41	7.48	62.43	50.00	4.41	7.52
NR2F2	0.00	0.00	209.06	2.31	1.94	153.76	*0.01*
NRP2	10.74	9.55	126.31	93.90	82.43	173.74	0.70

B

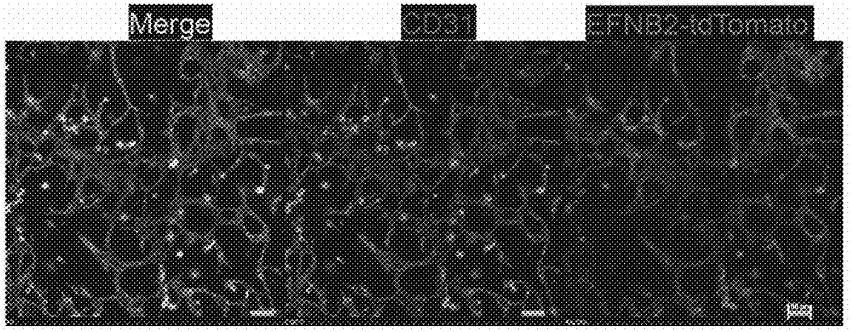
Single-cell RNA-seq



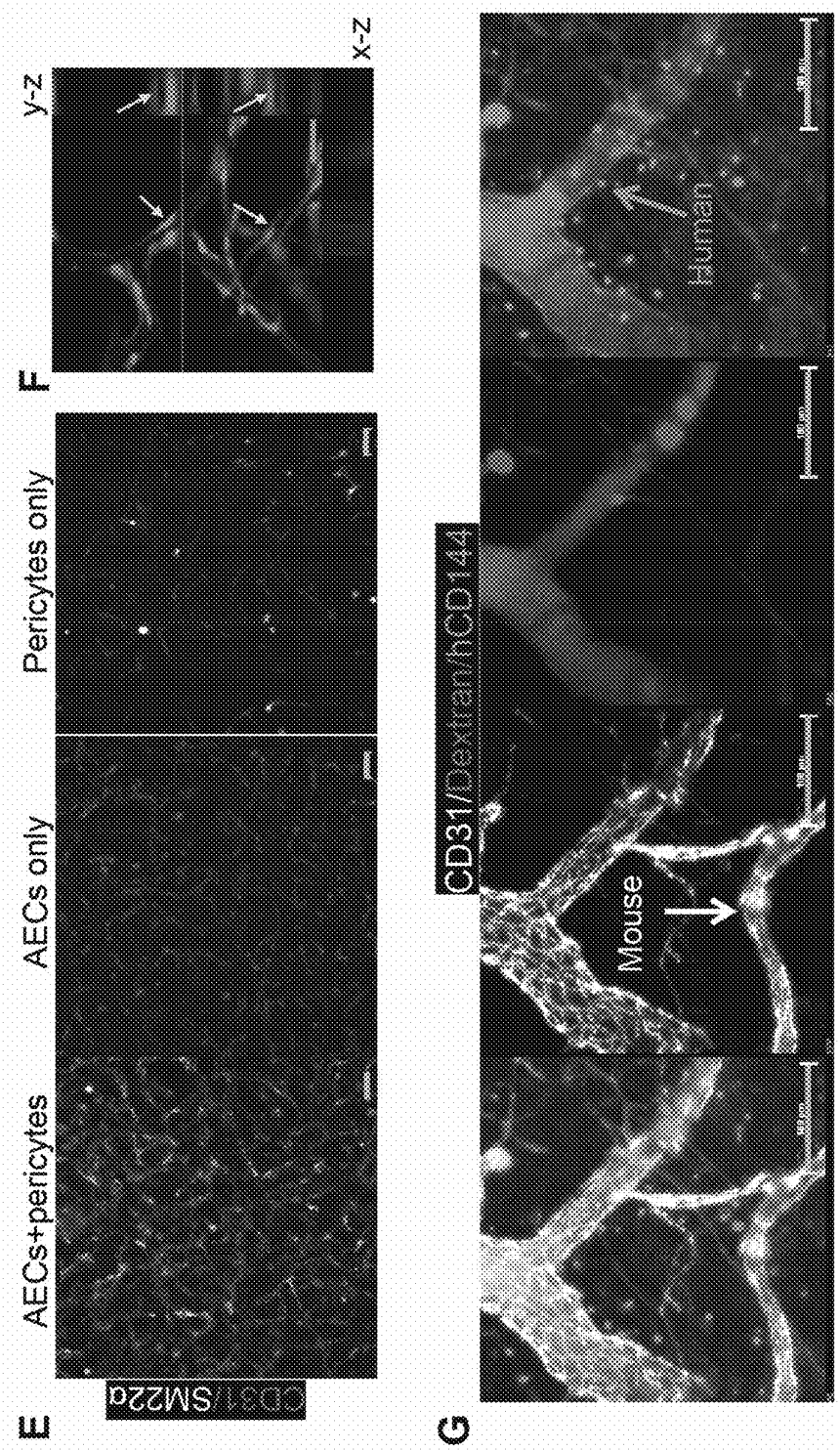
C



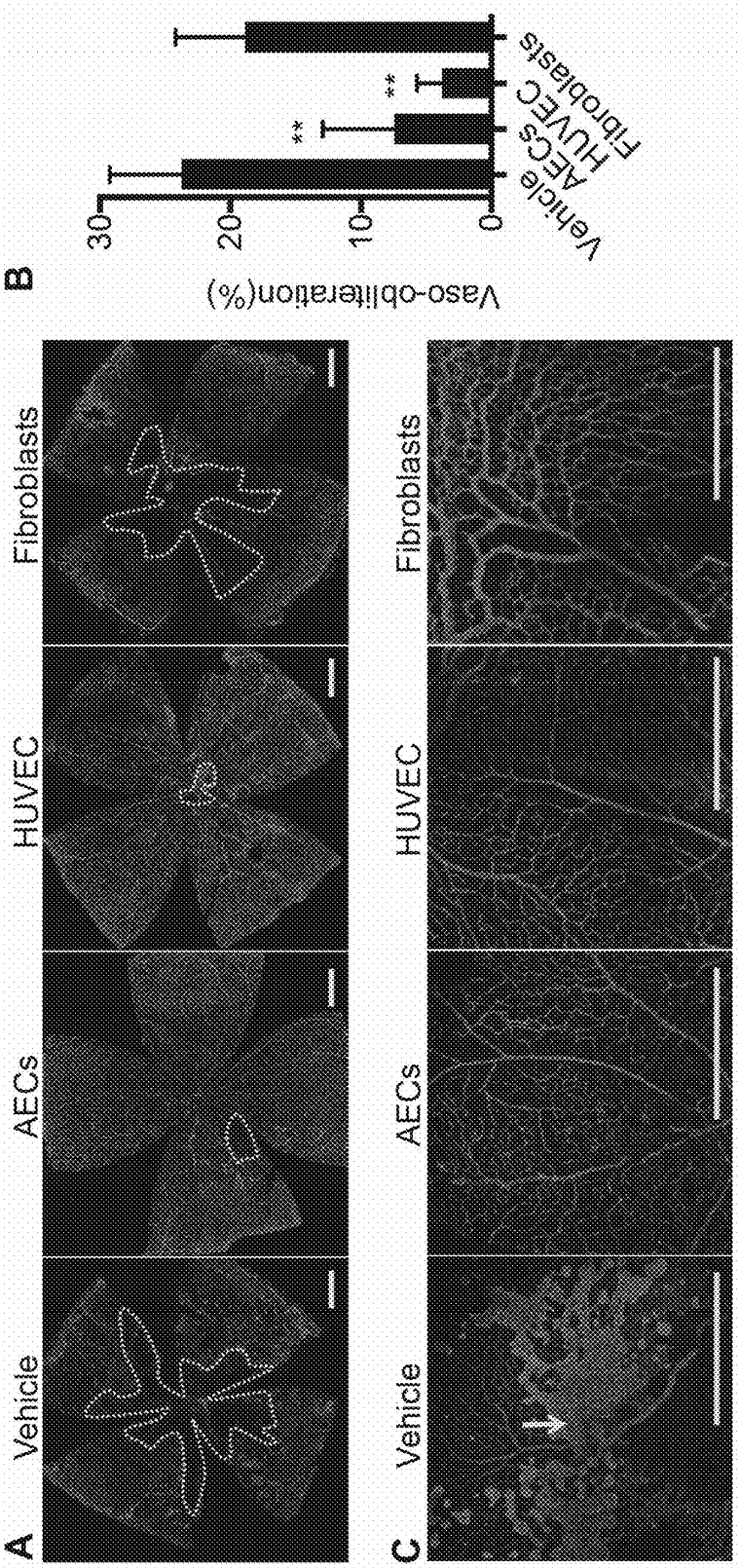
D



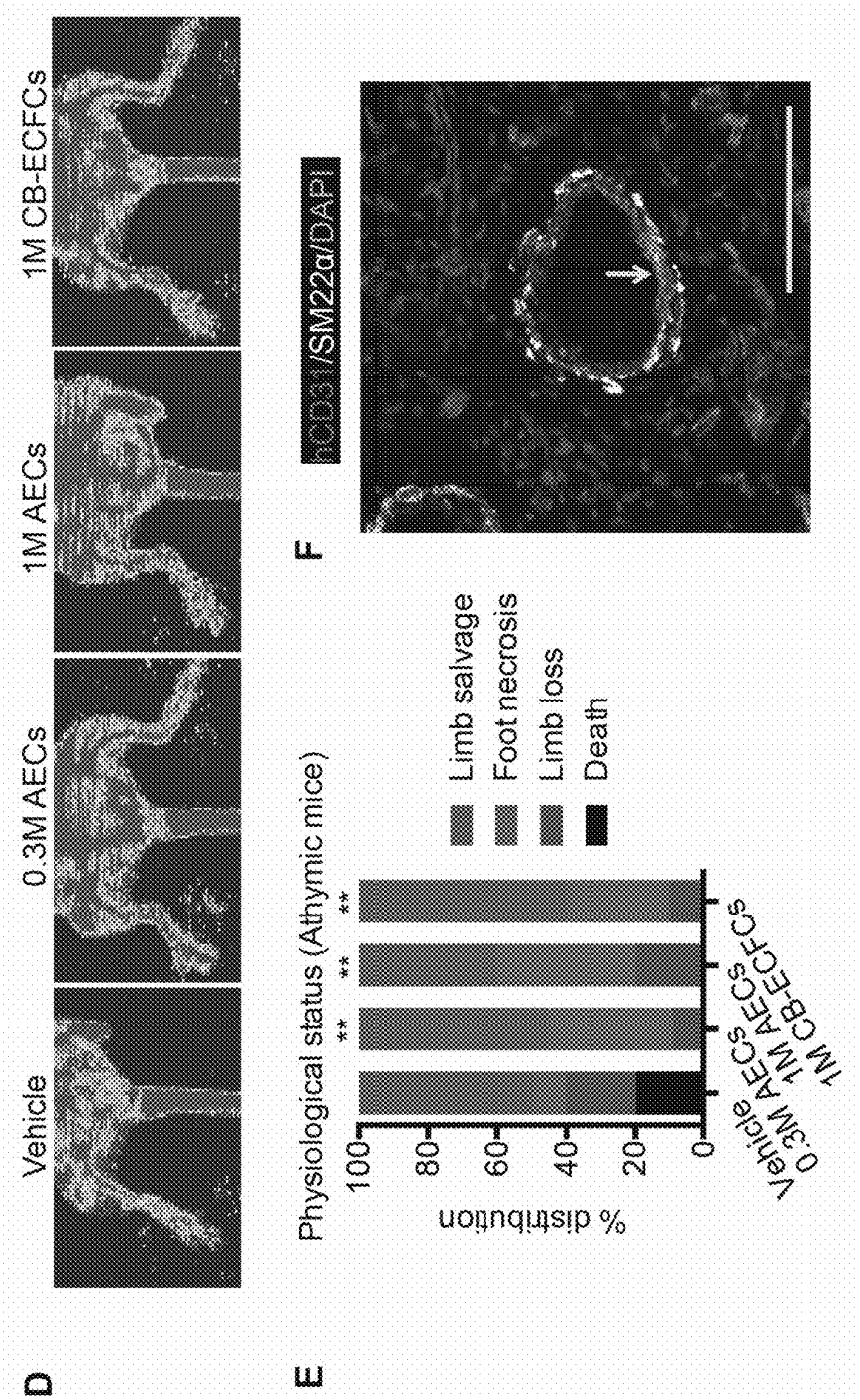
FIGS. 5A-5G, CONTINUED



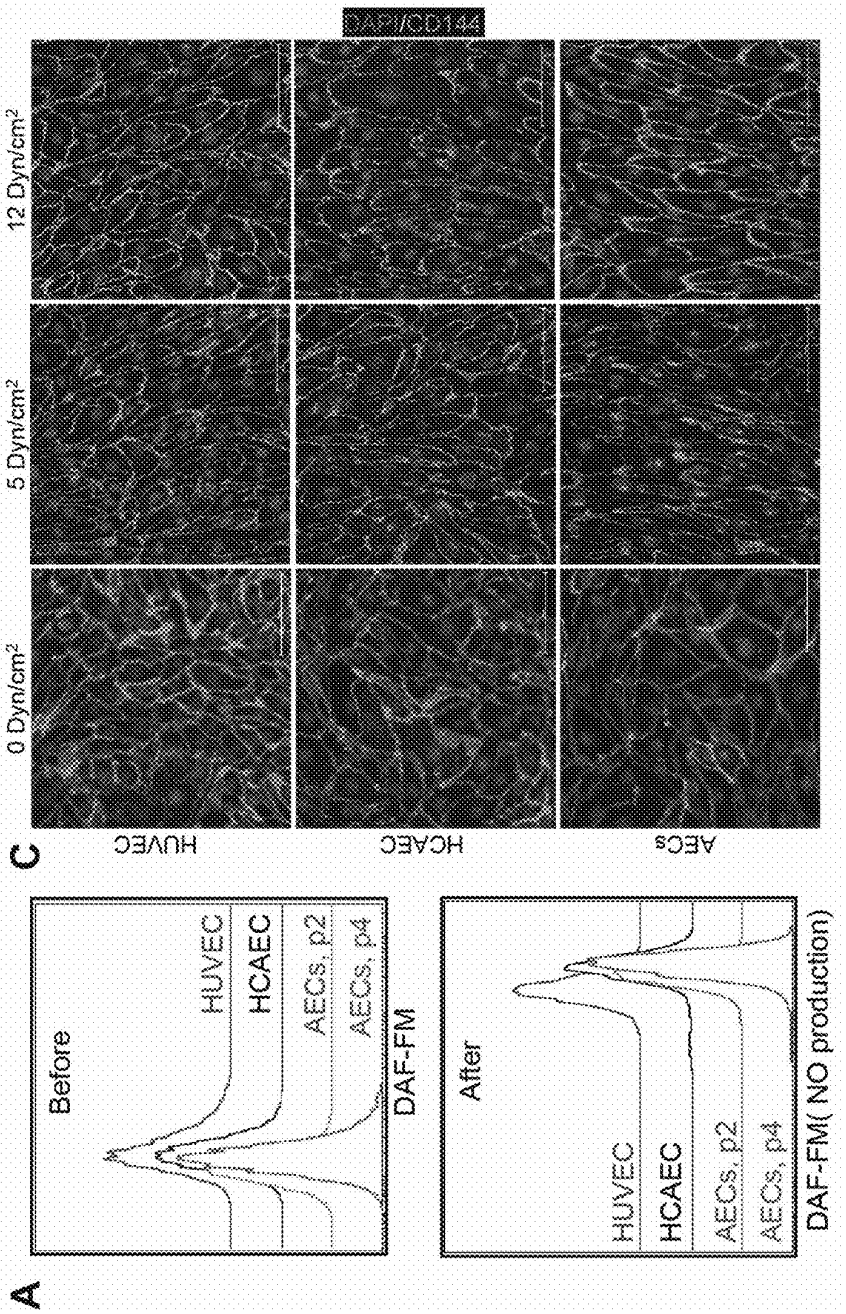
FIGS. 6A-6F


A
Vehicle
AECs
HUVEC
Fibroblasts
B
Vaso-obliteration(%)
0
10
20
30
**
**
Vehicle
AECs
HUVEC
Fibroblasts
C
Vehicle
AECs
HUVEC
Fibroblasts

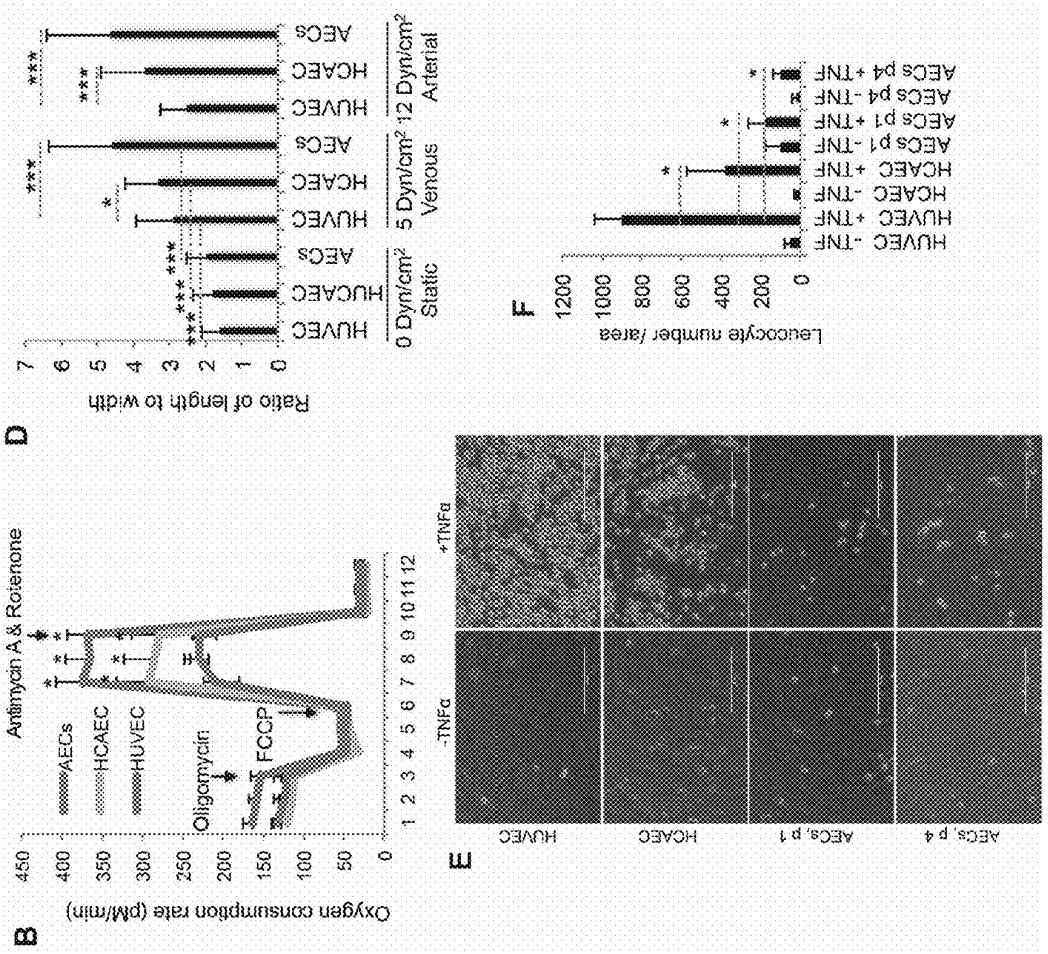
FIGS. 6A-6F, CONTINUED



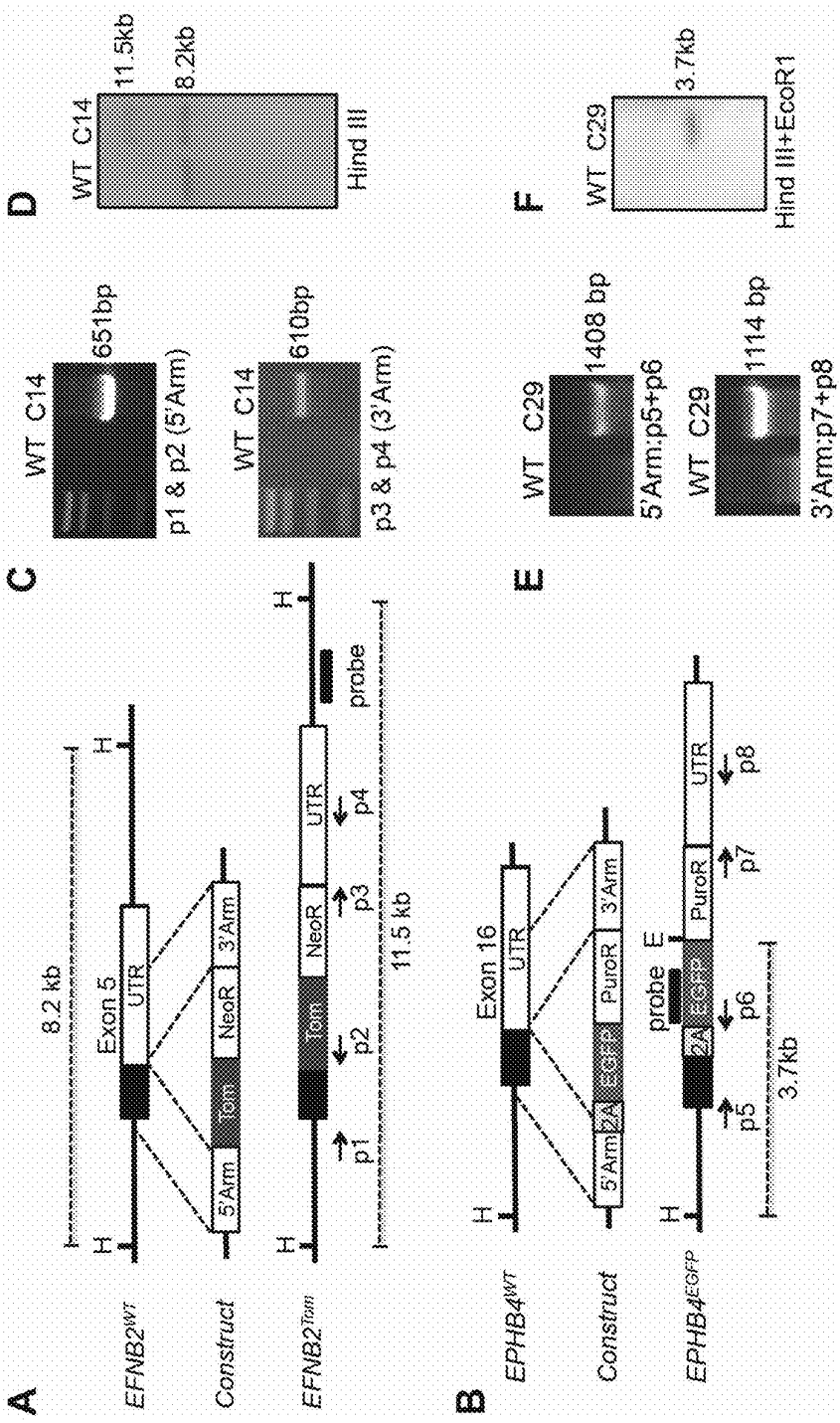
FIGS. 7A-7F


A
Before
HUVEC
HCAEC
AECs, p2
AECs, p4
DAF-FM
After
DAF-FM(NO production)
C
DAF/CD144
HUVEC
HCAEC
AECs
0 Dyn/cm²
5 Dyn/cm²
12 Dyn/cm²

FIGS. 7A-7F, CONTINUED



FIGS. 8A-8J


A
8.2 kb
Exon 5
UTR
H
H
EFNB2WT
Construct
5'Arm
Tom
NeoR
3'Arm
EFNB2Tom
NeoR
UTR
p1
p2
p3
p4
probe
11.5 kb
B
Exon 16
UTR
EPHB4WT
Construct
5'Arm
2A
EGFP
PuroR
3'Arm
probe E
EPHB4EGFP
PuroR
UTR
p5
p6
p7
p8
3.7kb
C
WT C14
651bp
p1 & p2 (5'Arm)
610bp
p3 & p4 (3'Arm)
D
WT C14
11.5kb
8.2kb
Hind III
E
WT C29
1408 bp
5'Arm:p5+p6
1114 bp
3'Arm:p7+p8
F
WT C29
3.7kb
Hind III+EcoR1

FIGS. 8A-8J, CONTINUED

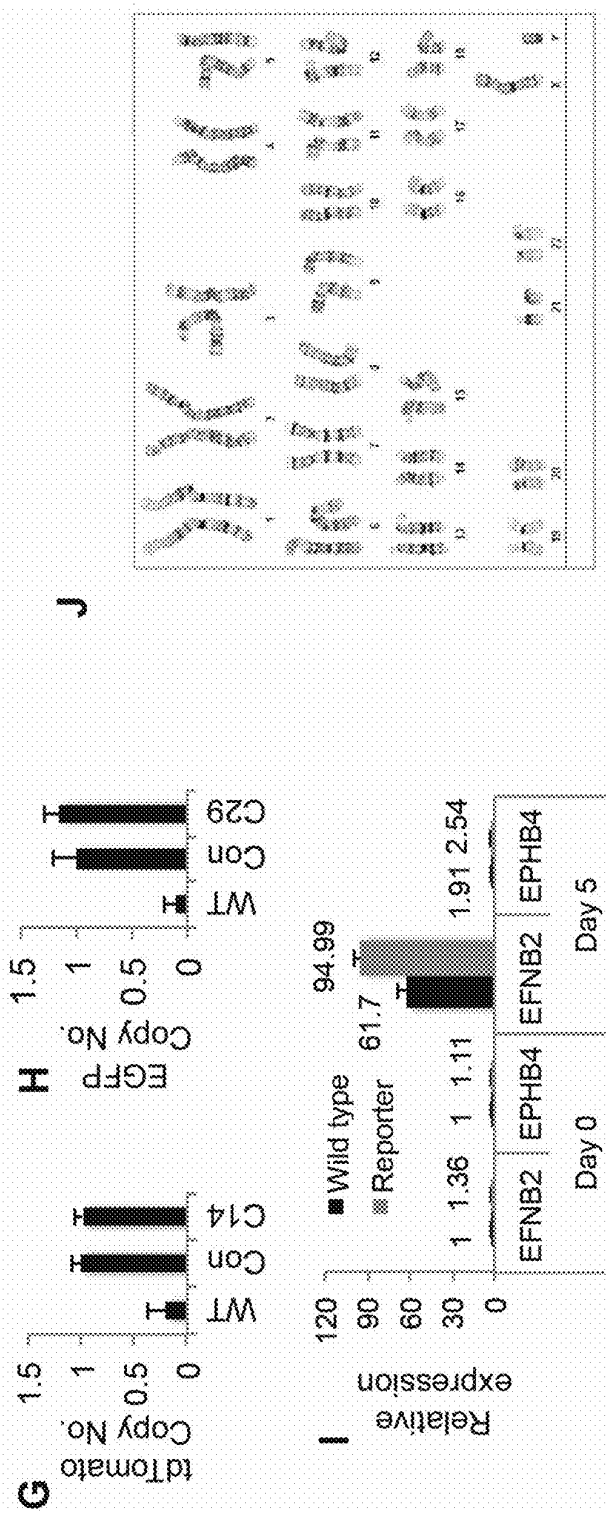
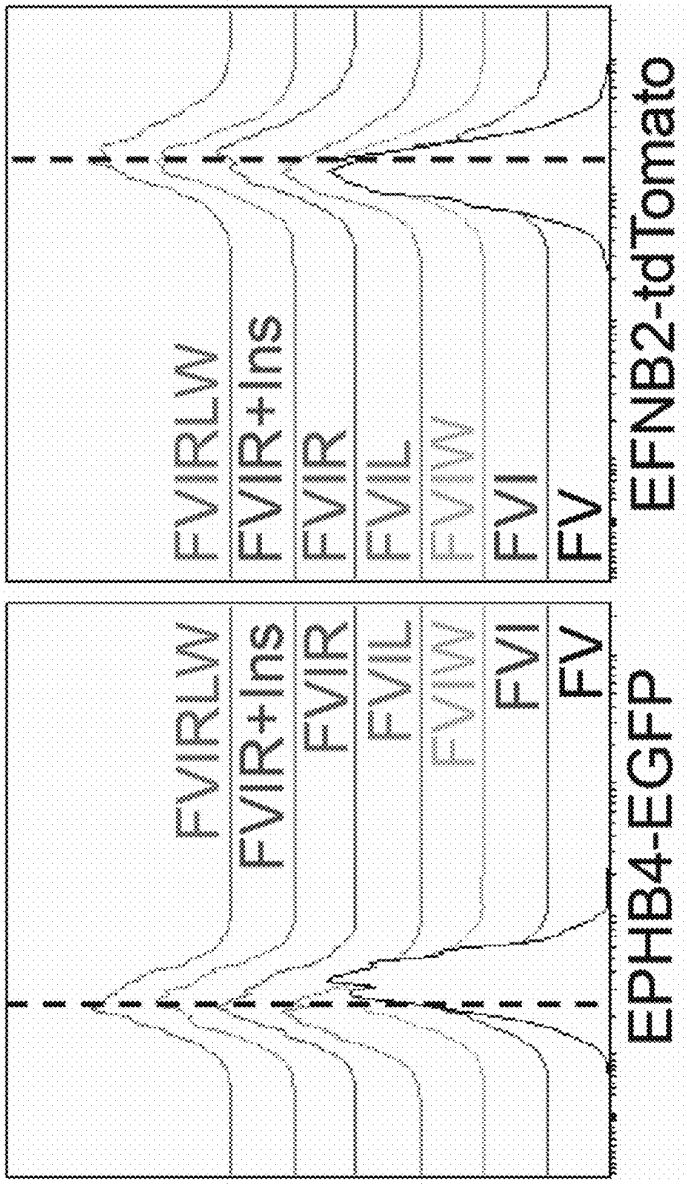


FIG. 9


FVIRLW
FVIR+Ins
FVIR
FVIL
FVIW
FVI
FV
EFNB2-tdTomato
FVIRLW
FVIR+Ins
FVIR
FVIL
FVIW
FVI
FV
EPHB4-EGFP

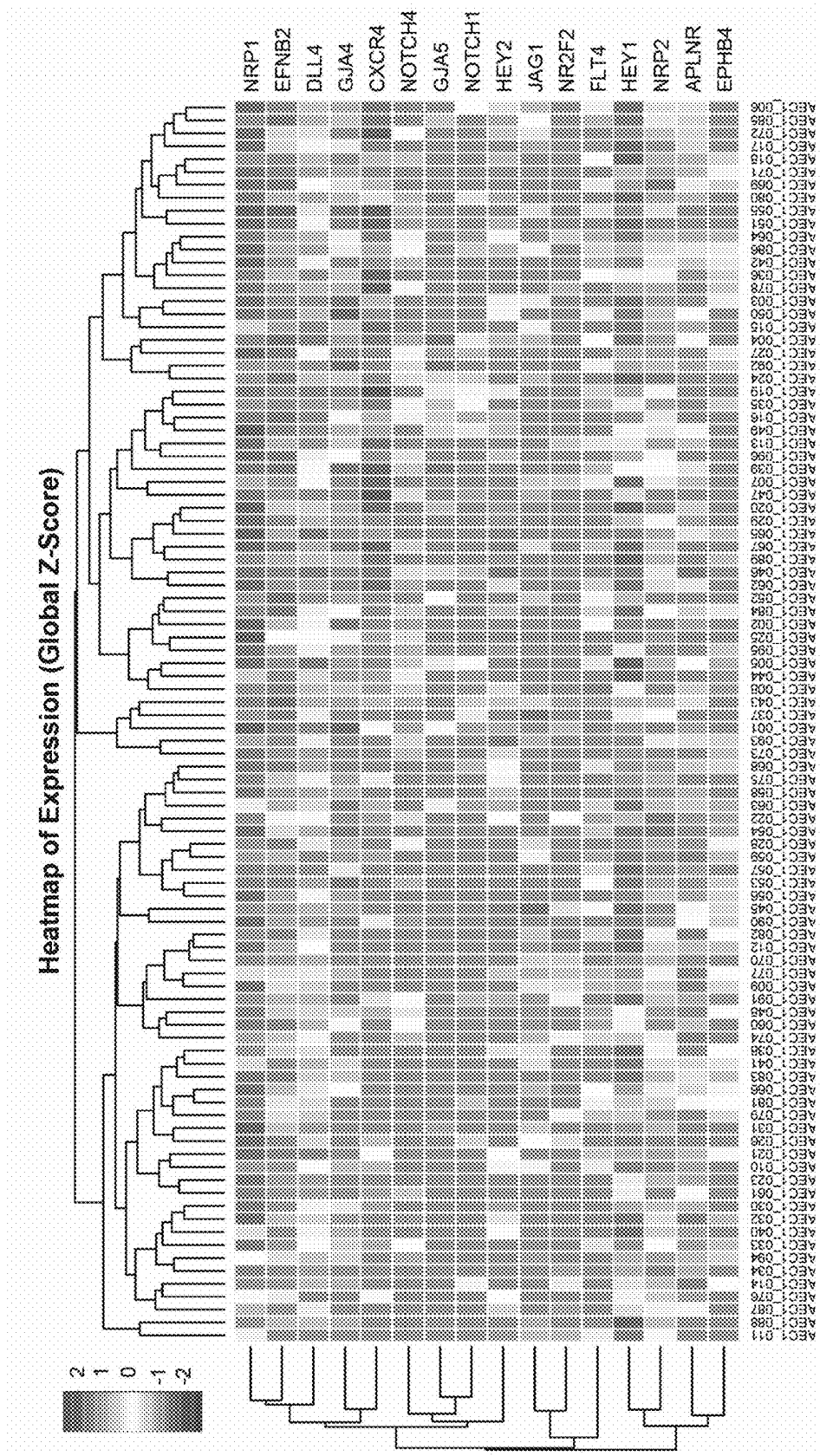
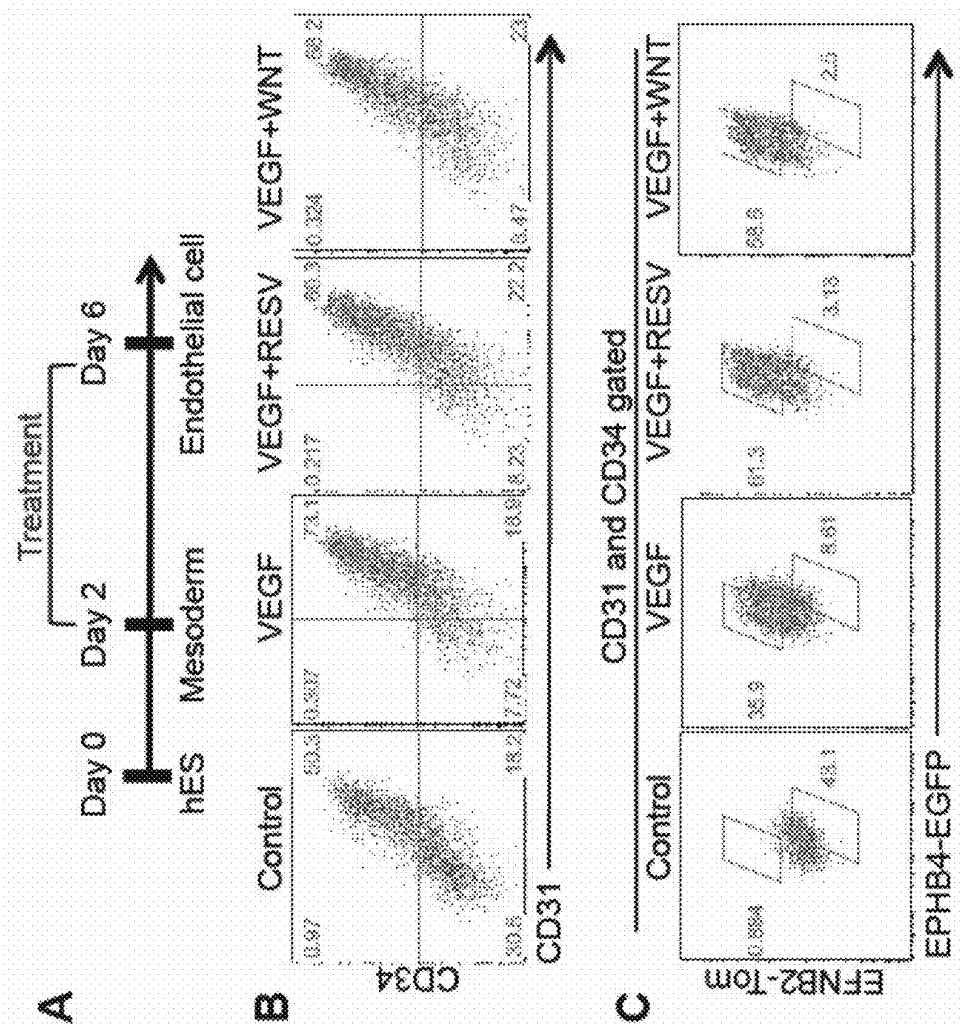

Heatmap of Expression (Global Z-Score)
NRP1
EFNB2
DLL4
GJA4
CXCR4
NOTCH4
GJA5
NOTCH1
HEY2
JAG1
NR2F2
FLT4
HEY1
NRP2
APLNR
EPHB4
2
1
0
-1
-2

FIG. 10

FIGS. 11A-11C


A
Treatment
Day 0
Day 2
Day 6
hES
Mesoderm
Endothelial cell
B
Control
VEGF
VEGF+RESV
VEGF+WNT
CD34
CD31
C
CD31 and CD34 gated
Control
VEGF
VEGF+RESV
VEGF+WNT
EFNB2-Tom
EPHB4-EGFP

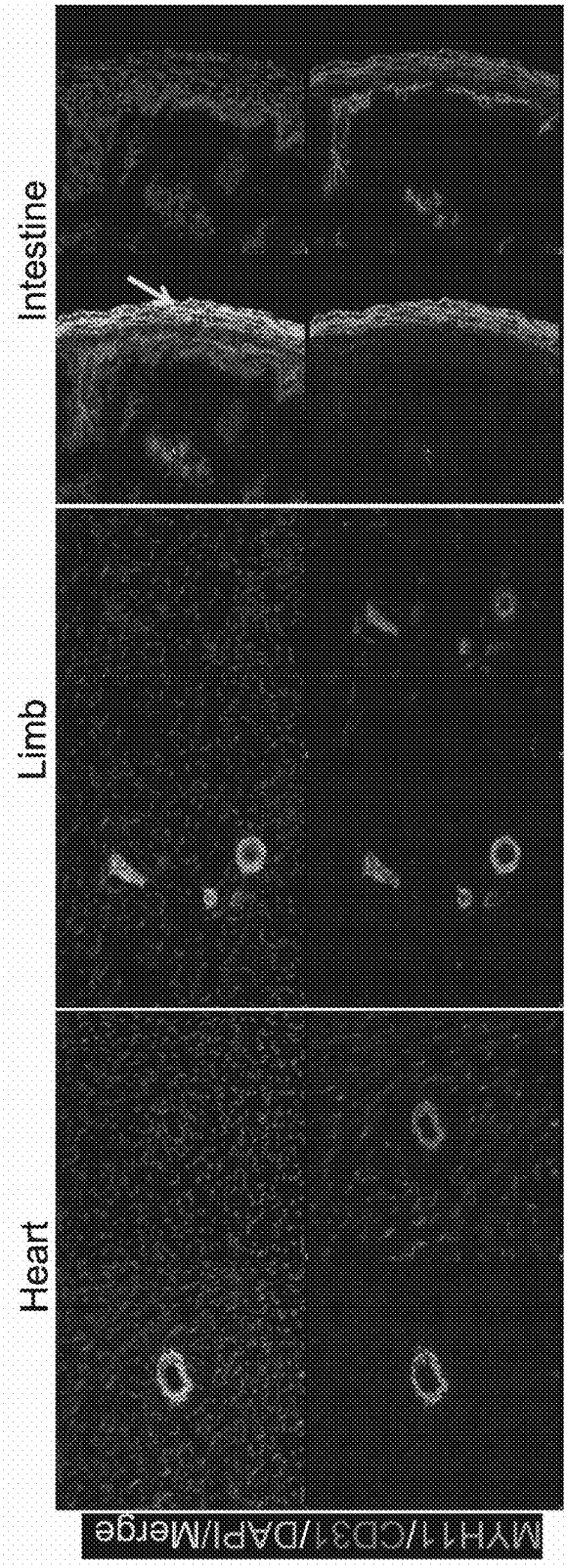


FIG. 12

GENERATING ARTERIAL ENDOTHELIAL CELL POPULATIONS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application No. 62/118,553, filed Feb. 20, 2015, which is incorporated herein by reference in its entirety.

LENGTHY TABLES

The patent contains a lengthy table section. A copy of the table is available in electronic form from the USPTO web site (https://seqdata.uspto.gov/?pageRequest=docDetail&DocID=US11674123B2). An electronic copy of the table will also be available from the USPTO upon request and payment of the fee set forth in 37 CFR 1.19(b)(3).

STATEMENT REGARDING FEDERALLY FUNDED RESEARCH OR DEVELOPMENT

This invention was made with government support under UH2-TR000506 awarded by the National Institutes of Health (National Center for Advancing Translational Sciences). The government has certain rights in the invention.

BACKGROUND

Cardiovascular disease is the leading cause of death in U.S., and most vascular diseases, such as atherosclerosis, occur in the arteries. Atherosclerosis is a chronic inflammatory disease that is initiated by activation, dysfunction, and structural changes of the endothelial cells, leading to increased leukocyte-endothelial adhesion.

Generating arterial endothelial cells from pluripotent stem cells holds great promise for the development of therapies for diseases or conditions that would treat cardiovascular disease. However, arterial endothelial development is challenging, as primary arterial endothelial cells undergo dedifferentiation in culture. For instance, U.S. Published Patent Application No. 2009/0104159 to Prosper et al. describes methods of culturing and using vascular endothelial cells that demonstrate the "potential" for arterial differentiation (paragraph [0136]). Further, U.S. Published Patent Application No. 2012/0064040 to McCloskey et al. describes chemically defined culture conditions to derive endothelial cells from embryonic stem cells. However, here again, this disclosure appears to merely demonstrate the potential for differentiating arterial endothelial cells from embryonic stem cells, and achieves very low results.

Existing protocols for deriving arterial endothelial cells from human embryonic stem cells have been largely unsuccessful. Accordingly, there remains a need in the art for efficient, defined, and scalable methods for generating human arterial endothelial cells from human pluripotent stem cells.

SUMMARY OF THE INVENTION

In a first aspect, provided herein is a method of obtaining arterial endothelial cells. The method can comprise or consist essentially of culturing mesodermal cells in a serum-free, albumin-free, chemically defined culture medium that is substantially free of insulin and comprises a fibroblast growth factor (FGF), a vascular endothelial growth factor (VEGF), and at least one of a Notch agonist, a TGF-beta inhibitor, and an inhibitor of inositol monophosphatase, whereby a cell population comprising arterial endothelial cells is obtained. The arterial endothelial cells of the population can express one or more markers selected from the group consisting of neuropilin1 (NRP-1), Delta-like 4 (DLL4), ephrin-B2 (EFNB2), CD44, CXCR4/CD184, Gap Junction Protein Alpha-4 (GJA4), Hey1, Jagged-1 (JAG1), Notch1, and Notch4. The cell population can comprise at least 80% arterial endothelial cells.

The serum-free, albumin-free, chemically defined culture medium can comprise a FGF, a VEGF, a Notch agonist, a TGF-beta inhibitor, and an inhibitor of inositol monophosphatase. The mesodermal cells can express one or more mesodermal markers selected from the group consisting of Brachyury (T), EMOS, FOXA2, MIXL1, MSX1, and MSX2.

In some cases, the mesodermal cells are obtained by culturing human pluripotent stem cells for a period of about two days in a serum-free, albumin-free, chemically defined cell culture medium comprising a Bone Morphogenetic Protein (BMP), Activin A, and an activator of Wnt/β-catenin signaling to obtain a cell population comprising mesodermal cells. The mesodermal cells can express one or more mesodermal markers selected from the group consisting of Brachyury (T), EMOS, FOXA2, MIXL1, MSX1, and MSX2. The pluripotent stem cells can be human embryonic stem cells or human induced pluripotent stem cells. The activator of Wnt/β-catenin signaling can be a Gsk3 inhibitor. The Gsk3 inhibitor can be selected from the group consisting of CHIR 99021, CHIR 98014, BIO-acetoxime, BIO, LiCl, SB 216763, SB 415286, AR A014418, 1-Azakenpaullone, and Bis-7-indolylmaleimide. The Notch agonist can be selected from the group consisting of Resveratrol (3,4',5-trihydroxystilbene), valproic acid, and suberoyl bishydroxamic acid. The TGF-beta inhibitor can be SB431542. The inhibitor of inositol monophosphatase can be L-690, 330.

In another aspect, provided herein is a substantially pure, isolated population of arterial endothelial cells obtained according to a method provided herein. The isolated population can comprise at least 90% arterial endothelial cells or at least 99% arterial endothelial cells.

In another aspect, provided herein is a substantially pure, isolated population of pluripotent stem cell-derived arterial endothelial cells obtained according to a method provided herein. The isolated population can comprise at least 90% arterial endothelial cells or at least 99% arterial endothelial cells.

In a further aspect, provided herein is a method of in vitro screening test agents. The method can comprise contacting a test agent to arterial endothelial cells obtained according to a method provided herein; and detecting an effect of the agent on the contacted arterial endothelial cells. Detecting

can comprise performing a method selected from the group consisting of leukocyte adhesion assay, RNA sequencing, gene expression profiling, transcriptome analysis, metabolome analysis, detecting reporter or sensor, protein expression profiling, Förster resonance energy transfer (FRET), metabolic profiling, and microdialysis.

In yet another aspect, provided herein is a kit for obtaining arterial endothelial cells, the kit comprising: (i) a serum-free, albumin-free, chemically defined culture medium suitable for differentiation of mesodermal cells into arterial endothelial cells, wherein the culture medium is substantially free of insulin and comprises a fibroblast growth factor (FGF), a vascular endothelial growth factor (VEGF), and at least one of a Notch agonist, a TGF-beta inhibitor, and an inhibitor of inositol monophosphatase; and (ii) instructions describing a method for differentiating mesodermal cells into arterial endothelial cells, the method employing the culture medium. The kit can further comprise (a) a serum-free, albumin-free, chemically defined culture medium suitable for differentiation of human pluripotent stem cells into mesodermal cells, where the culture medium comprises a BMP, Activin A, and an activator of Wnt/β-catenin signaling; and (b) instructions describing a method for differentiating human pluripotent stem cells into arterial endothelial cells, the method employing the culture medium of (a).

These and other features, aspects, and advantages of the present invention will become better understood from the description that follows. In the description, reference is made to the accompanying drawings, which form a part hereof and in which there is shown by way of illustration, not limitation, embodiments of the invention. The description of preferred embodiments is not intended to limit the invention to cover all modifications, equivalents and alternatives. Reference should therefore be made to the claims recited herein for interpreting the scope of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the compositions and methods provided herein. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

FIGS. **1**A-**1**D demonstrate single-cell RNA-seq. (A) Hierarchical clustering analysis of arterial and venous genes of single cells. (B) Average arterial and venous gene expression of five cell subpopulations. In each subpopulation, the average TPM (transcript per million) of each gene was calculated and normalized to population P1 (arterial genes) or P3 (venous genes). The normalized expression of all arterial genes or venous genes was further averaged and shown in a bar graph. Data were represented as mean±SD. *: $P<0.05$, n=13 cells in P1, n=10 cells in P3. (C) Principal component analysis of P1 and P3. The plot was generated by Singular™ Analysis Toolset 2.1. (D) Arterial enriched genes. The mean TPM of each gene for P1 was compared to P3 to calculate fold change. P-value was also calculated by comparing P1 to P3. Arterial enriched genes were identified with fold change>2, P value<0.1, and the mean TPM of P1>10. $P<0.1$ was used as the cut off since the P value of the previously reported arterial genes, VEGFa, Fzd4, Fzd7, Fzd10, D114, and Notch4, was between 0.01 to 0.1.

FIG. **2**A provides a schematic representation of a protocol for generating arterial endothelial cells from human pluripotent stem cells using a chemically-defined medium listed in Table 1. Human embryonic stem (ES) cells were first differentiated into mesoderm cells using E8BAC medium. E6 medium supplemented with growth factors or small molecules (E6FVB) was then used to induce mesoderm cells to differentiate into endothelial cells. FIG. **2**B is flow cytometric data for of CD31 and CD34 expression at day 0 (undifferentiated pluripotent state) and at day 5 (differentiated state). FIG. **2**C is flow cytometric data for KDR, NANOG, and OCT4 expression at day 0 and at day 5. FIG. **2**D indicates CD144 expression on purified endothelial cells by immunostaining. FIG. **2**E shows a LDL (low density lipoprotein) uptake assay. FIG. **2**F shows an in vitro MATRIGEL® (BD Biosciences, Falcon®) encapsulation assay. FIG. **2**G shows the results of an in vivo MATRIGEL® gel plug assay for analysis of migration and angiogenesis. Anti-human CD31-specific antibody was used for immunostaining to detect vascular formation in recovered gel plugs. FIG. **2**H shows flow cytometric analysis of CD31 and CD34 expression after five days of differentiation in the indicated combinations of growth factors and small molecules. Insulin was included in each combination. When the differentiation medium included a TGF inhibitor, SB431542 was included at 10 μM.

FIGS. **3**A-**3**F present candidate pathways critical for arteriovenous specification. (A) Flow cytometric analysis of EFNB2-tdTomato and EPHB4-EGFP expression on CD31 and CD144 gated endothelial cells. EFNB2-tdTomato/EPHB4-EGFP dual reporter cells (hetero knock-in in H1 cells for each gene) were first differentiated into mesoderm cells by E8BAC medium (E8 medium supplemented with BMP4, Activin-A, and CHIR99021). E5 (E8 medium minus FGF2, TGFβ1, and insulin) medium supplemented with 100 ng/ml FGF2, 50 ng/ml VEGFA, and 50 ng/ml BMP4 was used to induce mesoderm cells to differentiate into endothelial cells from day 2 to day 6. Either Insulin (20 μg/ml) or Ly294002 (16 μM, a PI3K inhibitor) was added to the medium as indicated from day 2 to day 6. (B) Statistics of EFNB2-tdTommato^{high}/EPHB4-EGFP^{low} cells. Data are represented as mean±SD. *: $P<0.05$, n=5. (C) Western blots showing AKT activity. Protein was harvested at day 3. (D) Flow cytometric analysis of EFNB2-tdTomato and EPHB4-EGFP expression on CD31 and CD144 gated endothelial cells. E5 medium supplemented with 50 ng/ml VEGFA, 50 ng/ml BMP4 or 10 μM SB431542 was used to induce mesoderm cells to differentiate into endothelial cells from day 2 to day 6. (E) Flow cytometric analysis of EFNB2-tdTomato and EPHB4-EGFP expression on CD31 and CD144 gated endothelial cells. ES cells were first differentiated into mesoderm cells as mentioned above. E5 medium supplemented with 50 ng/ml VEGFA and 10 μM SB431542 was used as the base medium induce mesoderm cells to differentiate into endothelial cells from day 2 to day 6. Other factors were added to the base medium as indicated. (F) Statistics of EFNB2-tdTomato^{high}/EPHB4-EGFP^{low} cells. 5 μM L690, 5 μg/ml LDL, and 100 ng/ml PDGF-BB were used. *: $P<0.05$, n=3.

FIGS. **4**A-**4**H present flow cytometric analysis of expression of EphrinB2 and Ephrin Type B Receptor 4 reporter constructs (EFNB2-tdTomato and EPHB4-EGFP) on CD31− and CD144-gated endothelial cells obtained after differentiation in various medium, as described. FIG. **4**B illustrates the percentage of arterial endothelial cells (AEC) (EFNB2-tdTomato^{high}/EPHB4-EGFP^{low}) obtained after differentiation in the various medium conditions of FIG. **4**A.

Data are represented as mean±SD. FIG. **4**C shows flow cytometric analysis of expression of CXCR4 and CD144 on endothelial cells obtained after differentiation in various medium. FIG. **4**D illustrates the percentage of $CXCR4^+$ $CD144^+$ arterial endothelial cells obtained after differentiation in the various medium conditions of FIG. **4**C. FIG. **4**E shows flow cytometric analysis of expression of DLL4 and CD144 on endothelial cells obtained after differentiation in various medium. FIG. **4**F illustrates the percentage of $DLL4^+CD144^+$ arterial endothelial cells obtained after differentiation in the various medium conditions of FIG. **4**E. 5 μM L-690,330 (a bisphosphonate inhibitor of inositol monophosphatase), and 100 ng/ml PDGF-BB (platelet derived growth factor BB) were used. FIG. **4**G shows the statistics of arterial endothelial cell number generated from 1.0×10^6 starting ES cells after six days of differentiation ("five factors" medium). FIG. **4**H shows the expansion of EFNB2-$tdTomato^{high}$/EPHB4-$EGFP^{low}$ cells from passage 0 to passage 4.

FIGS. **5**A-**5**G present characterization of arterial endothelial cells. All arterial endothelial cells were derived by "five factors" medium. (A) TPM of bulk RNA-seq was shown. EFNB2-$tdTomato^{high}$/EPHB4-$EGFP^{low}$ (AECs) were sorted for RNA-seq. The ratio of AECs (average TPM from AEC 1 and AEC2) to HUVEC was calculated. AoEC, cultured aortic endothelial cells. (B) PCA of single-cell RNA-seq. AECs: sorted EFNB2-$tdTomato^{high}$/EPHB4-$EGFP^{low}$ cells, pAECs: primary arterial endothelial cells from freshly isolated human fetus dorsal aorta, ES: H1 ES cells. (C) LDL uptake. Scale bar=100 Arterial endothelial cells (passage 2) derived from wild type H1 cells were used. The purity was about 93% after being passaged, so cells used in panels C—H were not purified. (D) Matrigel encapsulation assay. Arterial endothelial cells (passage 3) derived from reporter cell line were used. Scale bar=100 (E) Vascular formation in fibrin gel. Arterial endothelial cells (passage 2) derived from wild type H1 cells were used for panels E-F. Scale bar=100 (F) Lumen formation of endothelial cells and pericytes co-cultured in fibrin gel. To visualize the lumen, cells were stained with CMFDA (green). Y-z and x-z projection was shown. Scale bar=100 μm. (G) Endothelial cells formed functional vessels in vivo. Wild type H1 derived-arterial endothelial cells (passage 2, purified by CD144 microbeads) were mixed with Matrigel™ and injected into SCID mice. After four weeks, rhodamine-dextran was retro-orbital injected to highlight perfused vessels. Scale bar=100 μm. CD31: anti human and mouse CD31 antibody, Santa Cruz, cat #SC-1506. hCD144: anti-human CD144-647 antibody, BD biosciences, cat #561567.

FIGS. **6**A-**6**F demonstrate that arterial endothelial cells improve vascular function. (A) Flat-mounted retinas of oxygen-induced retinopathy. Endothelial cells were stained by CD31 antibody. Vaso-obliteration area was outlined. Scale bar=0.5 mm. (B) Statistics of vaso-obliteration. **: P<0.01. The P value was calculated by comparing to vehicle group. Vehicle group: n=12, from three independent experiments, PBS was used. AECs group: n=16, from two independent experiments. HUVEC group: n=5. Fibroblast group: n=5. (C) Neovascular tuft was indicated by the arrow. Endothelial cells were stained by CD31 antibody. Scale bar=0.5 mm. (D) Representative laser Doppler perfusion imaging showing the blood flow in ischemia athymic mice. (E) A stacked bar graph showing the physiological status at post-operative day 40. Vehicle group: n=10, DF12 medium was used. 0.3M AECs group: n=11, 3×10^5 AECs were injected per mouse. 1M AECs group: n=10, 1×10^6 AECs were injected per mouse. 1M cord blood-derived endothelial colony forming cells (CB-ECFCs) group: n=10, 1×10^6 CB-ECFCs were injected per mouse. The animal death was caused by ischemia related infection. **, P<0.001 (Chi-squared test, compared to vehicle group). (F) AECs formed vessels and recruited smooth muscle cells in mouse limb. AECs were stained with human specific CD31 antibody. Scale bar=100 μm. hCD31: anti human CD31 antibody, BD biosciences, cat #550274.

FIGS. **7**A-**7**F present arterial-specific functional characterization of endothelial cells. (A) Nitric oxide (NO) production was revealed by the intensity of 4-amino-5-methylamino-2',7'-difluororescein diacetate (DAF-FM), which is a NO-sensitive reagent that is useful for qualitative assessment of cellular NO production. Arterial endothelial cells (AECs) were derived from wild type H1 cells by "five factors" medium and used for experiments at passage 2 or 4. DAF-FM is nonfluorescent until it reacts with NO to form a fluorescent benzotriazole. The fluorescent intensity was measured by flow cytometry. Experiment was performed three times and typical data from one assay was shown. (B) Oxygen consumption rate was measured on XF24 analyzers (Seahorse Bioscience). Oligomycin was used to abolish the oxygen consumption. Carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone (FCCP), which is a potent uncoupler of oxidative phosphorylation in mitochondria that disrupts ATP synthesis by transporting protons across cell membranes, was used to measure maximal respiration capacity. Antimycin A and Rotenone were applied simultaneously to completely block the electron transport chain. *: P<0.05, n=3. The P value was calculated by comparing to HUVEC. HCAEC, human coronary arterial endothelial cells. (C) Shear Stress response performed on ibidi Pump System (Red perfusion set, μ-Slide VI 0.4. (D) The statistics data of shear stress response. Ratio of cell length to width was used to demonstrate the elongation of cells in response to shear stress. For each cell type, 100 cells were measured to do the statistics. Data are represented as mean±SD. *: P<0.05; ***:P<0.001, n=100 cells from three independent experiments. (E) Leukocyte (round cells) adhesion assay. Scale bar=200 μm. AECs were used at passage 1 or 4. (F) Statistics of leukocyte adhesion assay. Leukocyte number was counted for each image. Data are represented as mean±SD. *: P<0.05, n=3 images from three independent experiments. The P value was calculated by comparing to HUVEC with TNFα treatment.

FIGS. **8**A-**8**J present data for the generation and characterization of the reporter cell line. (A) Schematic of wild type and targeted EFNB2-tdTomato allele. H1 ES cells were used for gene targeting. Tom: tdTomato. (B) Schematic of wild type and targeted EPHB4-EGFP allele. (C) Junction PCR of 5' arm and 3' arm of EFNB2-tdTomato alleles. WT: wild type, C14: clone 14 of targeted cells. (D) Southern blot of EFNB2 wild type and knock-in (EFNB2-tdTomato) alleles. (E) Junction PCR of 5' arm and 3' arm of EPHB4-EGFP allele. C29: clone 29 of targeted cells. (F) Southern blot of EPHB4-EGFP allele. (G) qPCR analysis of tdTomato copy number of EFNB2-tdTomato cell line (clone 14). Data are represented as mean±SD. n=3. Con: control samples with one copy of tdTomato. (H) qPCR analysis of EGFP copy number of EFNB2-tdTomato/EPHB4-EGFP cell line (clone 29). Data are represented as mean±SD. n=3. Con: control samples with one copy of EGFP. (I) Comparing of endogenous EFNB2 and EPHB4 gene expression of wild type and reporter cell lines by RT-qPCR. Day 5, differentiation for five days. Data are represented as mean±SD. n=3. (J) Karyotyping of EFNB2-tdTomato/EPHB4-EGFP cell line (clone 29).

FIG. **9** presents flow cytometric analysis of EFNB2-tdTomato and EPHB4-EGFP expression. Purified AECs were cultured in E5 medium supplemented with growth factors or small molecules for three days. F: 100 ng/ml, V: 50 ng/ml VEGFA, I: 10 μM SB431542, W: 100 ng/ml WNT3A, L: 10 μM L-690,330, R: 5 μM RESV. Ins: 10 μg/ml insulin. Lower EPHB4-EGFP and higher EFNB2-tdTomato expression were observed in cultures with FVIR, FVIR+Ins, and FVIRLW medium.

FIG. **10** presents a heat-map of expression obtained using single-cell RNA-seq. Hierarchical clustering analysis was performed for arterial and venous genes of EFNB2-tdTomato^{high}/EPHB4-EGFP^{low} AECs derived by the "five factors" protocol as described herein.

FIGS. **11**A-**11**C present arterial endothelial cell differentiation data. (A) Schematic of arterial endothelial cell differentiation protocol. ES cells were first differentiated into mesoderm cells by E8BAC medium (E8 medium supplemented with 5 ng/ml BMP4, 25 ng/ml Activin A, and 1 μM CHIR99021). E5 (E8 medium minus FGF2, TGFβ1, and insulin) medium supplemented with 100 ng/ml FGF2 and 10 μM SB431542 was then used to induce mesoderm cells to differentiate into endothelial cells. (B) Flow cytometric analysis of CD31 and CD34 expression. E5+100 ng/ml FGF2+10 μM SB431542 medium ("Control") supplemented with 50 ng/ml VEGF, 5 μM RESV (resveratrol, a Notch activator), or 50 ng/ml WNT3A was used to induce mesoderm cells to differentiate into arterial endothelial cells. (C) Flow cytometric analysis of EFNB2-tdTomato and EPHB4-EGFP expression.

FIG. **12** demonstrates MYH11-positive vascular smooth muscle in mouse heart, limb, and intestine. In mouse heart and limb, MYH11-positive vascular smooth muscle is recruited to the blood vessels. In the intestine, smooth muscle cells express both MYH11 and CD31 (arrow indicated), demonstrating that MYH11^{+}CD31^{-} cells are vascular smooth muscle cells while MYH11^{+}CD31^{+} cells are intestinal smooth muscle cells.

DETAILED DESCRIPTION OF THE INVENTION

All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as though set forth in their entirety in the present application.

The present invention is based, at least in part, on the Inventors' discovery that arterial endothelial cell differentiation was greatly improved by combining several specific factors as compared to single factors. As described herein, the inventors further discovered that certain factors (insulin, TGFβ, and PDGF) inhibit arterial endothelial differentiation.

Methods

In exemplary embodiments, the methods provided herein comprise differentiating mesodermal stem cells into arterial endothelial cells. As used herein, the term "arterial endothelial cell" (AEC) refers to cells of the arterial vascular endothelial lineage obtained according to a method provided herein. AECs of the present invention are characterized by high levels of expression of arterial endothelium markers such as EphrinB2, DLL4, Hey-2, jagged-1, and jagged-2. AECs are also characterized by low leukocyte adhesion, higher NO production and oxygen consumption, response to shear stress, and capacity to form vascular networks in vitro and in vivo while maintaining expression of arterial markers in such networks. AECs are distinguishable from other cell types, including endothelial cells (ECs), venous endothelial cells, and endothelial progenitor cells, on the basis of characteristic expression profiles and functional attributes of the cells in vitro as described herein.

In a first aspect, provided herein is a method of obtaining arterial endothelial cells. In exemplary embodiments, the method comprises directing differentiation of mesodermal cells into cells of the arterial endothelial cell lineage. As used herein, the terms "mesodermal cell" and "mesoderm cell" are used interchangeably and refer to a cell having mesoderm-specific gene expression and being capable of differentiating into a mesodermal lineage such as bone, muscle such as cardiac muscle, skeletal muscle and smooth muscle (e.g., of the gut), connective tissue such as the dermis and cartilage, kidneys, the urogenital system, blood or hematopoietic cells, heart and vasculature. Mesoderm-specific biomarkers include Brachyury (T).

Throughout the AEC differentiation steps provided herein, mesodermal cells are typically cultured in a culture medium that is free, substantially free, or essentially free of insulin, albumin, or any component derived from a non-human animal (i.e., free of xenogeneic material). As used herein, the term "substantially free" refers to cell culture conditions substantially devoid of a certain component or reagent. Accordingly, substantially free of insulin means that the culture medium comprises less than 2% of insulin by weight, and preferably contains less than 1%, less than 0.5%, less that 0.2% or less that 0.1% of insulin.

In addition, the culture medium comprises, or consists essentially of, one or more of a Fibroblast Growth Factor (FGF), a vascular endothelial growth factor (VEGF), an inhibitor of TGF-beta signaling (e.g., SB431542), Resveratrol (RESV), and an inhibitor of inositol monophosphatase, where culturing occurs for a length of time sufficient for the cultured mesoderm cells to differentiate into arterial endothelial cells. In some embodiments, the cell culture medium used for AEC differentiation methods described herein comprises each of these components. In other cases, the culture medium is substantially free of one or more of these ingredients. Culturing can take place on any appropriate surface (e.g., in two-dimensional or three-dimensional culture).

In some cases, a mesodermal cell (including, in some cases, a pluripotent stem-cell derived mesodermal cell) is cultured in medium that comprises an FGF, VEGF, a Notch agonist, a TGFβ receptor inhibitor, and an inhibitor of inositol monophosphatase in amounts effective to direct differentiation of a mesodermal cell to the arterial endothelial lineage. In some cases, the FGF is FGF2. VEGF is a heparin-binding glycoprotein that acts as a specific endothelial cell mitogen. In some cases, the VEGF is VEGF-A (vascular endothelial growth factor A) or an isoform thereof (e.g., VEGF-165). Exemplary human VEGF-A protein sequences comprise Genbank: AAH65522.2 and GenBank: AAH1 1177.2, and the nucleic acids encoding all of or encoding the non-precursor part of such are encompassed.

TGFβ receptor inhibitors appropriate for use in a method of the present invention include, without limitation, SB-431542, SB-525334, A83-01, LY2157299, LY210976, GW788388, RepSox, SB-505124, D4476, GW788388, SD208, and EW-7197. Preferably, the inhibitor of TGF-beta signaling is SB431542, a small molecule inhibitor of endogenous activin and the type I receptor (TGFβ Receptor I) (Inman et al., *Mol Pharmacol.* 62(1):65-74 (2002).

Notch is a single-pass cell-surface receptor that binds to a family of cell-surface ligands including the Delta-like and Jagged families. As used herein, the terms "Notch agonist"

and "Notch activator" refer to molecules (e.g., biomolecules, small molecules, chemicals) that bind to Notch receptor and initiate or mediumte signaling events associated with Notch activation. Resveratrol (3,4',5-trihydroxystilbene) belongs to a class of polyphenolic compounds called stilbenes and is an activator (agonist) of Notch signaling. Other Notch agonists appropriate for use according to methods for promoting arterial differentiation provided herein include valproic acid and suberoyl bishydroxamic acid. In addition, immobilized or multimerized soluble Notch ligands such as immobilized DLL4 and immobilized Jagged-1 peptide also can be used as Notch activators.

Inositol monophosphatase (IMPase) catalyses the hydrolysis of myo-inositol monophosphates to myo-inositol, which is required in the phosphoinositide cell signaling pathway. In some cases, an inhibitor of IMPase is the biphosphonate L-690,330 ([144-Hydroxyphenoxy)ethylidene]bisphosphonic acid). Lithium also inhibits IMPase to attenuate phosphoinositide signaling (Berridge et al., *Cell* 59:411-419 (1989)). Other inhibitors of the phosphoinositide signaling pathway include, without limitation, phosphoinositide 3-kinase (PI3K) inhibitor Ly294002, Pictilisib, HS-173, GSK2636771, Duvelisib, TG100-115, GSK1059615, PF-04691502, PIK-93, BGT226, AZD6482, SAR245409, BYL719, CUDC-907, IC-87114, TG100713, Gedatolisib, CH5132799, PKI-402, BAY 80-6946, XL147, PIK-90, PIK-293, PIK-294, Quercetin, Wortmannin, ZSTK474, AS-252424, AS-604850, and Apitolisib.

A suitable working concentration range for chemical inhibitors such as those described herein is from about 0.1 μM to about 100 μM e.g., about 2 μM, 5 μM, 7 μM, 10 μM, 12 μM, 15 μM, 18 μM, or another working concentration of one or more the foregoing chemical inhibitors between about 0.1 μM to about 100 μM.

Preferably, mesodermal cells are cultured in the AEC differentiation medium until at least about 80% (e.g., at least 80%, 85%, 90%, 95%, 99%) of the resulting cell population are arterial endothelial cells. Arterial endothelial cells characteristically have the following expression profile: $CD31^+/CD144^+/CD41^-/CD45^-$.

For several of the biological markers described herein, expression will be low or intermediumte in level. While it is commonplace to refer to cells as "positive" or "negative" for a particular marker, actual expression levels are a quantitative trait. The number of molecules on the cell surface can vary by several logs, yet still be characterized as "positive." Accordingly, characterization of the level of staining permits subtle distinctions between cell populations. Expression levels can be detected or monitored by flow cytometry, where lasers detect the quantitative levels of fluorochrome (which is proportional to the amount of cell surface antigen bound by the antibodies). Flow cytometry or fluorescence-activated cell sorting (FACS) can be used to separate cell populations based on the intensity of antibody staining, as well as other parameters such as cell size and light scatter. Although the absolute level of staining may differ with a particular fluorochrome and antibody preparation, the data can be normalized to a control.

Any appropriate method can be used to detect expression of biological markers characteristic of cell types described herein. For example, the presence or absence of one or more biological markers can be detected using, for example, RNA sequencing (e.g., RNA-seq), immunohistochemistry, polymerase chain reaction, quantitative real time PCR (qRT-PCR), or other technique that detects or measures gene expression. RNA-seq is a high-throughput sequencing technology that provides a genome-wide assessment of the RNA content of an organism, tissue, or cell. Alternatively, or additionally, one may detect the presence or absence or measure the level of one or more biological markers of AECs using, for example, via fluorescent in situ hybridization; (FISH; see WO98/45479 published October 1998), Southern blotting, Northern blotting, or polymerase chain reaction (PCR) techniques, such as qRT-PCR. In exemplary embodiments, a cell population obtained according to a method provided herein is evaluated for expression (or the absence thereof) of biological markers of arterial endothelial cells such as EFNB2, Cxcr4, Delta-like 4 (DLL4), Gja4, Hey1, Jag1, Notch1, Notch4, and Nrp1. Preferably, AECs express one or more of the following arterial endothelial cell markers: Ephrin B2 (EFNB2), Neuropilin-1 (NRP-1)/CD304, Delta-like 4 (DLL4), and CD184 (cluster of differentiation 184). The Ephrin B2 (EFNB2) gene encodes an EFNB class ephrin that binds to the EPHB4 and EPHA3 receptors. Neuropilin-1 (NRP1), which is also known as vascular endothelial cell growth factor 165 receptor (VEGF165R), is primarily expressed in arterial endothelial cells. DLL4 is a Notch ligand expressed in arterial endothelial cells (Shutter et al., *Genes & Dev.* 14:1313-18 (2000)). CD184 is also known as CXCR4 (C—X—C chemokine receptor type 4) or fusin. Quantitative methods for evaluating expression of markers at the protein level in cell populations are also known in the art. For example, flow cytometry is used to determine the fraction of cells in a given cell population that express or do not express biological markers of interest.

The terms "defined culture medium," "defined medium," and the like, as used herein, indicate that the identity and quantity of each medium ingredient is known. As used herein, the terms "chemically-defined culture conditions," "fully defined, growth factor free culture conditions," and "fully-defined conditions" indicate that the identity and quantity of each medium ingredient is known and the identity and quantity of supportive surface is known. As used herein, the term "albumin-free conditions" indicates that the culture medium used contains no added albumin in any form, including without limitation Bovine Serum Albumin (BSA), any form of recombinant albumin, or any other animal components.

Human pluripotent stem cells (hPSCs), either embryonic or induced, provide access to the earliest stages of human development and offer a platform on which to derive a large number of vasculogenic cells for cellular therapy and tissue engineering. Accordingly, in exemplary embodiments, the methods provided herein further comprise differentiating human pluripotent stem cells under conditions that promote differentiation of mesodermal stem cells into arterial endothelial cells. In such, a method of producing an arterial endothelial cell comprises culturing human pluripotent stem cells in a serum-free, albumin-free, chemically defined culture medium that promotes mesoderm differentiation. In this manner, pluripotent stem cell-derived mesodermal cells are differentiated according to the AEC differentiation methods provided herein, thus producing pluripotent stem cell-derived AECs. In exemplary embodiments, the serum-free, albumin-free, chemically defined culture medium that promotes mesoderm differentiation comprises Activin A, Bone Morphogenetic Protein 4 (BMP4), FGF2, and an activator of Wnt/β-catenin signaling.

Defined medium and substrate conditions for culturing pluripotent stem cells, as used in the methods described herein, are well known in the art. The medium used herein are limited only in that they are albumin-free. In some cases,

pluripotent stem cells to be differentiated according to the methods disclosed herein are cultured in a serum-free, albumin-free medium.

As will be appreciated by those of ordinary skill in the art, Wnt/β-catenin signaling can be activated by modulating the function of one or more proteins that participate in the Wnt/β-catenin signaling pathway to increase β-catenin expression levels or activity, TCF and LEF expression levels, or β-catenin/TCF/LEF induced transcriptional activity.

In some embodiments, activation of Wnt/β-catenin signaling is achieved by inhibition of Gsk3 phosphotransferase activity or Gsk3 binding interactions. While not wishing to be bound by theory, it is believed that inhibition of Gsk3 phosphorylation of β-catenin will inhibit tonic degradation of β-catenin and thereby increase β-catenin's level and activity to drive differentiation of pluripotent stem cells to an endodermal/mesodermal lineage. Gsk3 inhibition can be achieved in a variety of ways including, but not limited to, providing small molecules that inhibit Gsk3 phosphotransferase activity, RNA interference knockdown of Gsk3, and overexpression of dominant negative form of Gsk3. Dominant negative forms of Gsk3 are known in the art as described, e.g., in Hagen, T. et al. *J Biol Chem,* 277:23330-5 (2002), which describes a Gsk3 comprising a R96A mutation.

In some embodiments, the Wnt/β-catenin signaling pathway is activated by inhibiting Gsk3 in pluripotent stem cells by contacting the pluripotent stem cells with a small molecule that inhibits Gsk3 phosophotransferase activity or Gsk3 binding interactions. Suitable small molecule Gsk3 inhibitors include, but are not limited to, CHIR 99021, CHIR 98014, BIO-acetoxime, BIO, LiCl, SB 216763, SB 415286, AR A014418, 1-Azakenpaullone, Bis-7-indolymaleimide, and any combinations thereof. In some embodiments, any of CHIR 99021, CHIR 98014, and BIO-acetoxime are used to inhibit Gsk3 in pluripotent stem cells in the differentiation methods described herein. In one embodiment, the small molecule Gsk3 inhibitor to be used is CHIR99021 at a concentration ranging from about 1 μM to about 9 μM, e.g., about 1 μM, 2 μM, 3 μM, 4 μM, 5 μM, 6 μM, 7 μM, 8 μM, 9 μM or another concentration of CHIR99021 from about 1 μM to about 9 μM. In another embodiment, the small molecule Gsk3 inhibitor to be used is CHIR 98014 at a concentration ranging from about 0.1 μM to about 1 μM, e.g., about 0.1 μM, 0.2 μM, 0.3 μM, 0.4 μM, 0.5 μM, 0.6 μM, 0.7 μM, 0.8 μM, 0.9 μM, 1.0 μM or another concentration of CHIR-98014 from about 0.1 μM to about 1 μM. In another embodiment, the small molecule Gsk3 inhibitor to be used is BIO-acetoxime at a concentration ranging from about 0.1 μM to about 1 μM, e.g., about 0.1 μM, 0.2 μM, 0.3 μM, 0.4 μM, 0.5 μM, 0.6 μM, 0.7 μM, 0.8 μM, 0.9 μM, 1.0 μM or another concentration of BIO-acetoxime from about 0.1 μM to about 1 μM.

In other embodiments, Gsk3 activity is inhibited by RNA interference knockdown of Gsk3. For example, Gsk3 expression levels can be knocked-down using commercially available siRNAs against Gsk3, e.g., SignalSilence® GSK-3α/β siRNA (catalog #6301 from Cell Signaling Techology®, Danvers, Mass.), or a retroviral vector with an inducible expression cassette for Gsk3, e.g., a commercially available Tet-inducible retroviral RNAi system from Clontech (Mountainview, Calif.) Catalog No. 630926, or a cumate-inducible system from Systems Biosciences, Inc. (Mountainview, Calif.), e.g., the SparQ® system, catalog no. QM200PA-2. In other embodiments, the Wnt/β-catenin signaling pathway is activated by overexpressing β-catenin itself, e.g., human β-catenin (GenBank Accession Nos: X87838 and CAA61107.1 for nucleotide and protein sequences, respectively). In one embodiment, β-catenin overexpression is inducible β-catenin overexpression achieved using, e.g., any of the just-mentioned inducible expression systems. Alternatively, a constitutively active, stabilized isoform of β-catenin is used, which contains point mutations S33A, S37A, T41A, and S45A as described, e.g., in Baba, Y. et al. Constitutively active β-catenin confers multi-lineage differentiation potential on lymphoid and myeloid progenitors. *Immunity* 23:599-609 (2005).

In yet other embodiments, Wnt/β-catenin signaling pathway activation in pluripotent stem cells is achieved by contacting the cells with an agent that disrupts the interaction of β-catenin with Axin, a member of the β-catenin destruction complex. Disruption of the Axin-β-catenin interaction allows β-catenin to escape degradation though the destruction complex thereby increasing the net level of β-catenin to drive β-catenin signaling. For example, the Axin-β-catenin interaction can be disrupted in pluripotent cells by contacting them with the compound 5-(Furan-2-yl)-N-(3-(1H-imidazol-1-yl)propyl)-1,2-oxazole-3-carboxamide ("SKL2001"), which is commercially available, e.g., as catalog no. 681667 from EMD4 Biosciences. An effective concentration of SKL2001 to activate Wnt/β-catenin signaling ranges from about 10 μM to about 100 μM, about 20 μM, 30 μM, 40 μM, 50 μM, 60 μM, 70 μM, 80 μM, 90 μM or another concentration of SKL2001 from about 10 μM to about 100 μM. In some embodiments the activator of Wnt/β-catenin signaling is a Gsk3 inhibitor. In some embodiments the Gsk3 inhibitor is selected from the group consisting of CHIR99021, CHIR98014, BIO-acetoxime, BIO, LiCl, SB216763, SB415286, AR A014418, 1-Azakenpaullone, and Bis-7-indolylmaleimide. In some embodiments the Gsk3 inhibitor is CHIR99021 or CHIR98014 at a concentration between about 0.1 μM to about 10 μM in the medium. In one embodiment, the small molecule Gsk3 inhibitor to be used is CHIR99021 at a concentration ranging from about 1 μM to about 9 μM, e.g., about 1 μM, 2 μM, 3 μM, 4 μM, 5 μM, 6 μM, 7 μM, 8 μM, 9 μM or another concentration of CHIR99021 from about 1 μM to about 9 μM. In another embodiment, the small molecule Gsk3 inhibitor to be used is CHIR98014 at a concentration ranging from about 0.1 μM to about 1 μM, e.g., about 0.1 μM, 0.2 μM, 0.3 μM, 0.4 μM, 0.5 μM, 0.6 μM, 0.7 μM, 0.8 μM, 0.9 μM, 1.0 μM or another concentration of CHIR98014 from about 0.1 μM to about 1 μM.

In exemplary embodiments, pluripotent stem cells are cultured in a chemically defined culture medium comprising or consisting essentially of DMEM/F12 culture medium, L-ascorbic acid-2-phosphate magnesium, sodium selenium, human FGF2, insulin, $NaHCO_3$, transferrin, TGFβ1, BMP4, Activin-A, and CHIR99021 ("E8BAC medium") for two days. Preferably, the culture medium comprises or consists essentially of DMEM/F12 medium; L-ascorbic acid-2-phosphate magnesium (64 mg/l); sodium selenium (14 μg/l); human FGF2(100 μg/l); insulin (20 mg/l); NaHCO3 (543 mg/l); transferrin (10.7 mg/l); TGFβ1 (2 μg/l); BMP4 (5 μg/l); Activin A (25 μg/l); and CHIR99021 (1 μM). Human pluripotent stem cells are cultured in the culture medium for about two days. After about two days, at least about 80% (e.g., at least about 80%, 85%, 90%, 95%, 99%) of the resulting cell population are mesoderm cells. As used herein, the term "mesoderm cell" refers to a cell having mesoderm-specific gene expression, capable of differentiating into a mesodermal lineage such as bone, muscle such as cardiac muscle, skeletal muscle and smooth muscle (e.g., of the gut),

connective tissue such as the dermis and cartilage, kidneys, the urogenital system, blood or hematopoietic cells, heart and vasculature. Mesoderm-specific biomarkers include Brachyury (T). Culturing can take place on any appropriate surface (e.g., in two-dimensional or three-dimensional culture).

As used herein, "pluripotent stem cells" appropriate for use according to a method of the invention are cells having the capacity to differentiate into cells of all three germ layers. Suitable pluripotent cells for use herein include human embryonic stem cells (hESCs) and human induced pluripotent stem (iPS) cells. As used herein, "embryonic stem cells" or "ESCs" mean a pluripotent cell or population of pluripotent cells derived from an inner cell mass of a blastocyst. See Thomson et al., *Science* 282:1145-1147 (1998). These cells express Oct-4, SSEA-3, SSEA-4, TRA-1-60 and TRA-1-81. Pluripotent stem cells appear as compact colonies comprising cells having a high nucleus to cytoplasm ratio and prominent nucleolus. ESCs are commercially available from sources such as WiCell Research Institute (Madison, Wis.). As used herein, "induced pluripotent stem cells" or "iPS cells" mean a pluripotent cell or population of pluripotent cells that may vary with respect to their differentiated somatic cell of origin, that may vary with respect to a specific set of potency-determining factors and that may vary with respect to culture conditions used to isolate them, but nonetheless are substantially genetically identical to their respective differentiated somatic cell of origin and display characteristics similar to higher potency cells, such as ESCs, as described herein. See, e.g., Yu et al., *Science* 318:1917-1920 (2007).

Induced pluripotent stem cells exhibit morphological properties (e.g., round shape, large nucleoli and scant cytoplasm) and growth properties (e.g., doubling time of about seventeen to eighteen hours) akin to ESCs. In addition, iPS cells express pluripotent cell-specific markers (e.g., Oct-4, SSEA-3, SSEA-4, Tra-1-60 or Tra-1-81, but not SSEA-1). Induced pluripotent stem cells, however, are not immediumtely derived from embryos. As used herein, "not immediumtely derived from embryos" means that the starting cell type for producing iPS cells is a non-pluripotent cell, such as a multipotent cell or terminally differentiated cell, such as somatic cells obtained from a post-natal individual.

Human iPS cells can be used according to a method described herein to obtain AECs having the genetic complement of a particular human subject. For example, it may be advantageous to obtain AECs that exhibit one or more specific phenotypes associated with or resulting from a particular disease or disorder of the particular mammalian subject. In such cases, iPS cells are obtained by reprogramming a somatic cell of a particular human subject according to methods known in the art. See, for example, Yu et al., *Science* 324(5928):797-801 (2009); Chen et al., *Nat. Methods* 8(5):424-9 (2011); Ebert et al., *Nature* 457(7227):277-80 (2009); Howden et al., *Proc. Natl. Acad. Sci. U.S.A.* 108(16):6537-42 (2011). Induced pluripotent stem cell-derived AECs allow modeling of drug responses in tissue constructs that recapitulate vascular tissues in an individual having, for example, a particular disease. Even the safest drugs may cause adverse reactions in certain individuals with a specific genetic background or environmental history. Accordingly, human subject specific iPS cell-derived AECs are useful to identify genetic factors and epigenetic influences that contribute to variable drug responses.

Subject-specific somatic cells for reprogramming into iPS cells can be obtained or isolated from a target tissue of interest by biopsy or other tissue sampling methods. In some cases, subject-specific cells are manipulated in vitro prior to use in a three-dimensional hydrogel-based tissue construct of the invention. For example, subject-specific cells can be expanded, differentiated, genetically modified, contacted to polypeptides, nucleic acids, or other factors, cryo-preserved, or otherwise modified prior to introduction to a three-dimensional tissue construct.

Defined medium and substrate conditions for culturing pluripotent stem cells, as used in the methods described herein, are well known in the art. In some cases, pluripotent stem cells to be differentiated according to the methods disclosed herein are cultured in mTESR-1® medium (StemCell Technologies, Inc., Vancouver, British Columbia.), or Essential 8® medium (Life Technologies, Inc.) on a MATRIGEL™ substrate (BD Biosciences, N.J.) according to the manufacturer's protocol or on a Corning® Synthemax surface.

Preferably, human pluripotent stem cells (e.g., human ESCs or iPS cells) are cultured in the absence of a feeder layer (e.g., a fibroblast feeder layer), a conditioned medium, or a culture medium comprising poorly defined or undefined components. As used herein, the terms "chemically defined medium" and "chemically defined culture medium" also refer to a culture medium containing formulations of fully disclosed or identifiable ingredients, the precise quantities of which are known or identifiable and can be controlled individually. As such, a culture medium is not chemically defined if (1) the chemical and structural identity of all medium ingredients is not known, (2) the medium contains unknown quantities of any ingredients, or (3) both. Standardizing culture conditions by using a chemically defined culture medium minimizes the potential for lot-to-lot or batch-to-batch variations in materials to which the cells are exposed during cell culture. Accordingly, the effects of various differentiation factors are more predictable when added to cells and tissues cultured under chemically defined conditions. As used herein, the term "serum-free" refers to cell culture materials that do not contain serum or serum replacement, or that contains essentially no serum or serum replacement. For example, an essentially serum-free medium can contain less than about 1%, 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.3%, 0.2%, or 0.1% serum. "Serum free" also refers to culture components free of serum obtained from animal (e.g., fetal bovine) blood or animal-derived materials, which is important to reduce or eliminate the potential for cross-species viral or prion transmission. For avoidance of doubt, serum-containing medium is not chemically defined.

The methods provided herein produce isolated populations of pluripotent stem cell-derived AECs, where the isolated population is a substantially pure population of AECs. As used herein, "isolating" and "isolated" refer to separating, selecting, or enriching for a cell type of interest or subpopulation of cells from surrounding, neighboring, or contaminating cells or from cells of another type. As used herein, the term "substantially pure" refers to a population of cells that is at least about 80% (e.g., at least about 80%, 82%, 83%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more) pure, with respect to AECs making up a total cell population. In other words, the term "substantially pure" refers to a population of AECs of the present invention that contains at least about 80% (e.g., at least about 80%, 82%, 83%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more) of AECs when directing differentiation to obtain cells of the arterial endothelial cell lineage. The term "substantially pure" also refers to a population of AECs of the present invention that contains fewer than about 20%, about 10%, or about 5% of

non-AECs in an isolated population prior to any enrichment, expansion step, or differentiation step. In some cases, a substantially pure isolated population of AECs generated according to a method provided herein is at least about 95% (e.g., at least about 95%, 96%, 97%, 98%, 99%) pure with respect to AECs making up a total cell population.

An important difference between arterial endothelial cells produced from iPS cells from a specific individual and primary arterial endothelial cells isolated from that same individual is that the iPS cell-derived cells are infinitely scalable and are capable of exceeding the Hayflick limit (a certain number of cell divisions). As used herein, the term "Hayflick limit" refers to a finite number of population doublings in vitro before a cell can no longer proliferate and enters senescence (Hayflick L. *Exp Cell Res* 37:614-36, 1965). While the inherent self-renewal capacity of primary cultured arterial endothelial cells is limited, an almost inexhaustible supply of arterial endothelial cells can be obtained according to the methods provided herein from a single source (e.g., a somatic cell of an individual). Accordingly, in an embodiment of the invention, the AECs are capable of expansion within the tissue culture laboratory such that the numbers of cells obtained is sufficient to treat more than one patient and, in the preferred embodiment, are capable of cell banking.

In some embodiments, the proportion of arterial endothelial cells in a population of cells obtained in the described methods is enriched using a cell separation, cell sorting, or enrichment method, e.g., fluorescence activated cell sorting (FACS), enzyme-linked immunosorbent assay (ELISA), magnetic beads, magnetic activated cell sorting (MACS), laser-targeted ablation of non-endothelial cells, and combinations thereof. Preferably, FACS is used to identify and separate cells based on cell-surface antigen expression.

The methods of the present invention provide scalable, inexpensive, and reproducible generation of human AECs. For instance, after obtaining a cell population comprising human AECs according to a method described herein, the human AEC population can be expanded in a culture medium appropriate for proliferating human AECs including, without limitation, Human Endothelial Serum-Free Medium (Life Technologies, Cat. No. 11111-044), EGM-2 (Lonza, Cat. No. CC-3162), and Endothelial Cell Culture Medium (BD Biosciences, Cat. No. 355054).

TABLE 1

Chemically Defined Culture Medium Components

Medium Name	Protocol Step	Chemically Defined Components
E8		DMEM/F12 medium + L-ascorbic acid-2-phosphate magnesium (64 mg/l); sodium selenium (14 μg/l); human FGF2 (100 μg/l); insulin (20 mg/l); $NaHCO_3$ (543 mg/l); Transferrin (10.7 mg/l); and TGFβ1 (2 μg/l)
E8BAC	Human pluripotent stem cells to mesodermal cells	E8 medium + BMP4 (5 μg/l); Activin A (25 μg/l); and CHIR99021 (1 μM)
E7		DMEM/F12 medium + L-ascorbic acid-2-phosphate magnesium (64 mg/l); sodium selenium (14 μg/l); human FGF2 (100 μg/l); insulin (20 mg/l); $NaHCO_3$ (543 mg/l); and Transferrin (10.7 mg/l)
E7BVi		E7 medium + VEGFA (50 μg/l); BMP4 (50 μg/l); and SB431542 (5 μM)
E7Bi		E7 medium + BMP4 (50 μg/l); and SB431542 (5 μM)
E7Vi		E7 medium + VEGFA (50 μg/l); and SB431542 (5 μM)
E7V		E6 medium + FGF2 (100 ng/ml); and 50 ng/ml VEGFA
E6		DMEM/F12 medium + L-ascorbic acid-2-phosphate magnesium (64 mg/l); sodium selenium (14 μg/l); insulin (20 mg/l); $NaHCO_3$ (543 mg/l); and transferrin (10.7 mg/l)
E6FVB		E6 medium + human FGF2 (100 μg/l); VEGFA (50 μg/l); and BMP4 (50 μg/l)
E6V		E6 medium + VEGFA (50 μg/l)
E5		DMEM/F12 medium + L-ascorbic acid-2-phosphate magnesium (64 mg/l); sodium selenium (14 μg/l); $NaHCO_3$ (543 mg/l); and transferrin (10.7 mg/l)
FVIRL	Differentiating pluripotent stem cell-derived mesodermal cells into arterial endothelial cells	E5 medium + Human FGF2 (100 μg/l) VEGF-165 (50 μg/l) SB431542 (10 μM) RESV (5 μM) L-690,330 (10 μM)
FVIRLW		FVIRL + WNT3A (100 ng/ml)
FVIRL-5		E5 medium + Human FGF2 (100 μg/l); VEGF-165 (50 μg/l); SB431542 (10 μM); RESV (5 μM); and L-690,330 (5 μM)
FVIRL-5-I		FVIRL-5 + insulin (20 mg/l)
FVIRL-5-W		FVIRL-5 + WNT3A (50 ng/ml)
FVIRL-5-BB		FVIRL-5 + PDGF-BB (100 ng/ml)
FVIR	Maintaining and expanding arterial endothelial cells	E5 medium + Human FGF2 (100 μg/l); VEGF-165 (50 μg/l); SB431542 (10 μM); and RESV (5 μM)
FVIL		E5 medium + Human FGF2 (100 μg/l); VEGF-165 (50 μg/l); SB431542 (10 μM); and L-690,330 (10 μM)
FVIW		E5 medium + Human FGF2 (100 μg/l); VEGF-165 (50 μg/l); SB431542 (10 μM); and WNT3A (100 ng/ml)
FVB		E5 medium + Human FGF2 (100 μg/l); VEGF-165 (50 μg/l); and BMP4 (50 μg/l)

TABLE 1-continued

Chemically Defined Culture Medium Components

Medium Name	Protocol Step	Chemically Defined Components
FVI		E5 medium + Human FGF2 (100 μg/l); VEGF-165 (50 μg/l); and SB431542 (10 μM)
FV		E5 medium + Human FGF2 (100 μg/l) VEGF-165 (50 μg/l)
BVIn	Differentiating pluripotent stem cell-derived mesodermal cells into endothelial cells	E5 medium + BMP4 (50 μg/l); VEGF-165 (50 μg/l) Insulin (20 mg/l)
VI		E5 medium + VEGF-165 (50 μg/l) SB431542 (5 μM)
Control		E5 medium Human FGF2 (100 μg/l) SB431542 (10 μM)
Control + VEGF		Control medium + VEGF-165 (50 ng/ml)
Control + RESV		Control medium + RESV (5 μM)
Control + WNT3A		Control medium + WNT3A (50 ng/ml)

In another aspect, provided herein are therapeutic compositions including arterial endothelial cells obtained according to methods provided herein and methods of using them for the treatment of subjects.

In a further aspect, therefore, the present invention provides methods and compositions for cell transplantation, cell replenishment, and cell or tissue replacement and enhancing vasculogenesis. The method can comprise providing to a subject in need thereof a therapeutically effective amount of arterial endothelial cells derived according to a methods provided herein, whereby providing arterial endothelial cells treats the subject. Disorders requiring cell or tissue replacement and improving vasculogenesis include, without limitation, myocardial and peripheral vascular ischemia, other peripheral artery diseases, myocardial infarction (MI), stroke, and diabetic neuropathy, and any other disorder or disease for which the stricken individual would benefit from angiogenic regenerative medicine. Preferred individual subjects according to the present invention are mammals including, without limitation, humans and non-human primates, as well as canines, felines, ovines, porcines, equines, and bovines. In some cases, a substantially pure population of arterial endothelial cells is obtained using a pluripotent cell (e.g., induced pluripotent stem cell) of the subject in need of treatment. However, a substantially pure population of arterial endothelial cells also can be obtained using pluripotent stem cells of, preferably, a syngeneic or allogeneic donor. Less preferably, a xenogeneic donor is used.

In another aspect, this document provides methods for improving vascular perfusion. In particular, provided herein is a method for treating peripheral arterial disease in a patient, where the method comprises administering to the patient a therapeutic dose of arterial endothelial cells obtained as described herein. As used herein, the term "peripheral arterial disease" refers to acute and chronic critical limb ischemia and ischemia associated with a disorder affecting blood supply to tissues such as diabetes or arteriosclerosis. In some cases, arterial endothelial cells obtained according to the methods provided herein are directly injected into the patient subject to treat the peripheral artery disease. Without being bound to any particular theory, it is expected that such arterial endothelial cells would be therapeutic for limb ischemia (e.g., ischemia associated with diabetes or cardiac infarcts) and more beneficial than treatment with a non-arterial endothelial cell. In exemplary embodiments, in vitro-derived AECs are patient specific or HLA-matched cells for transplantation to a patient to treat ischemia. For example, AECs can be derived from iPS cells obtained by reprogramming a somatic cell of the patient to pluripotency and then using the iPS cells according to a method provided herein to obtain a population comprising patient specific AECs. AECs obtained from patient-derived iPS cells can be administered to the patient in any pharmaceutically acceptable carrier, buffer, or excipient. The route of administration of the cells to the patient may be via intravenous or intramuscular injection. In some cases, for example, AECs derived from human pluripotent stem cells are resuspended in a saline solution and injected intramuscularly at one or more sites of limb ischemia.

Any appropriate dosage can be used for a therapeutic method provided herein. The cell dose will depend on the extent and severity of the ischemia but a preferred range is from about 1×10^8 cells/patient to about 1×10^{10} cells/patient per dose. In some cases, AECs obtained as described herein are co-administered to a subject with other cell types including, for example, smooth muscle cells (e.g., vascular smooth muscle cells).

After administering the cells into the subject, the effect of the treatment method may be evaluated, if desired, using any appropriate method known to practitioners in the art. The treatment may be repeated as needed or required. Following treatment according to the methods provided herein, the treated subject can be monitored for any positive or negative changes in limb ischemia. In a preferred embodiment, a therapeutic increase in blood supply to an ischemic tissue is a result of an increase in blood vessel formation (angiogenesis) following implantation of the said cells. The methods provided herein provide cells that are pro-angiogenic following transplantation. In some cases, positive changes include, without limitation, increased blood supply to ischemic tissue, increased amputation-free survival, decreased need for limb amputation, decreased limb pain when the subject is a rest, and improvements in pain-free walking (e.g., pain-free walking over greater distances).

In another aspect, AECs obtained according to the methods provided herein are useful for methods in which the production of nitric oxide (NO) has a therapeutic or preventative benefit for a subject. For example, provided herein is a method for administering AECs to a subject as a method for providing NO to the subject, whereby administering the AECs treats or prevents atherosclerosis, reduces DNA damage, and/or relaxes smooth muscle cells to improve blood vessel function.

Administration of a therapeutically effective amount of AECs into the recipient subject is generally effected using methods well known in the art, and usually involves directly injecting or otherwise introducing a therapeutically effective AECs into the subject using clinical tools known to those skilled in the art (e.g., U.S. Pat. Nos. 6,447,765; 6,383,481; 6,143,292; and 6,326,198). For example, introduction of AECs of the present invention can be effected locally or systemically via intravascular administration, such as intravenous, intramuscular, or intra-arterial administration, intraperitoneal administration, and the like. Cells can be injected into an infusion bag (e.g., Fenwal infusion bag (Fenwal, Inc.)) using sterile syringes or other sterile transfer mechanisms. The cells can then be immediately infused via IV

administration over a period of time, such as 15 minutes, into a free flow IV line into the patient. In some embodiments, additional reagents such as buffers or salts are provided to the recipient subject concurrently with the cells.

In exemplary embodiments, AECs of the present invention are provided to the subject as a pharmaceutical composition comprising the cells and one or more pharmaceutically acceptable carriers, buffers, or excipients. The pharmaceutical composition for administration must be formulated, produced, and stored according to standard methods that provide proper sterility and stability. A pharmaceutical composition of the present invention may also comprise one or more growth factors or cytokines (e.g., angiogenic cytokines) that promote the survival or engraftment of transplanted cells, promote angiogenesis, modulate the composition of extracellular or interstitial matrix, and/or recruit other cell types to the site of transplantation.

In another aspect, provided herein is a method for producing an engineered blood vessel using arterial endothelial cells obtained according to a method provided herein. AECs also can be used as raw materials, optionally in combination with additional cell populations, for creating blood vessels in vitro or in vivo. Such vessels will be useful, for example, in revascularizing damaged tissues and in treating peripheral artery disease. Engraftment of and vasculogenesis by externally injected cells has been shown by in vivo animal studies. See, for example, Kim et al., *J. Am. Coll. Cardiol.* 56:593-607 (2010).

Also provided herein are methods of using in vitro-derived AECs for in vitro blood vessel formation and for vascularization of engineered tissues that lack a vascular network such as engineered cardiac muscle tissue or heart. For example, AECs are useful in methods for producing tissue-engineered vascular grafts for clinical applications such as replacing diseased vessels. In some cases it will be advantageous to use patient-specific or HLA matched AECs for methods of treating a patient with a tissue-engineered vascular graft, an in vitro-produced blood vessels, or other vascularized engineered tissue. As described above, AECs can be derived from iPS cells obtained by reprogramming a somatic cell of the patient to pluripotency and then using the iPS cells according to a method provided herein to obtain a population comprising patient-specific AECs. In some cases, it will be advantageous to co-culture the AECs with other cell types such as vascular smooth muscle cells (VSMC) to obtain a vascularized engineered tissue construct such as an engineered blood vessel for clinical application such as bypass surgery. Preferably, AECs are combined with patient-specific in vitro-derived vascular smooth muscle cells for these methods. Vascular smooth muscle cells are positive for expression of ACTA2, TAGLN, MYH11, and ELN, but CD31 negative. In other cases, AECs can be co-cultured with cardiomyocytes to form a vascularized cardiac tissue patch useful for improving cardiac function.

In a further aspect, provided herein is a method of in vitro screening of an agent. For example, provided herein are methods of using in vitro-derived arterial endothelial cells for high throughput screening of candidate. For example, AECs obtained as described herein can be screened to identify agents that decrease leukocyte adhesion as a potential therapeutic or preventative for atherosclerosis. Screening methods can comprise or consist essentially of (a) contacting a test agent to an arterial endothelial cell or population of arterial endothelial cells obtained as described herein; and (b) detecting an effect of the agent on the cell or cells (e.g., decreased leukocyte adhesion to AECs). In some cases, screening methods include screening candidate compounds to identify test agents that promote the development of vascular tissue. In other cases, candidate compounds can be screened for toxicity to human arterial endothelial cells or vascular tissue. In some cases, detecting comprises detecting at least one positive or negative effect of the agent on morphology or life span of cells, whereby an agent that increases or reduces the life span of the cells or has a positive or negative impact on the morphology of the cells is identified as having an effect on human arterial endothelial cells or vascular tissues. In some cases, detecting comprises performing a method selected from the group consisting of adhesion assays, RNA sequencing, gene expression profiling, transcriptome analysis, metabolome analysis, detecting reporter or sensor, protein expression profiling, Förster resonance energy transfer (FRET), metabolic profiling, and microdialysis. The agent can be screened for an effect on gene expression, and detecting can comprise assaying for differential gene expression relative to an uncontacted cell or cell population.

In exemplary embodiments, detecting and/or measuring a positive or negative change in a level of expression of at least one gene following exposure (e.g., contacting) of a test compound to arterial endothelial cells comprises whole transcriptome analysis using, for example, RNA sequencing. In such cases, gene expression is calculated using, for example, data processing software programs such as Light Cycle, RSEM (RNA-seq by Expectation-Maximization), Excel, and Prism. See Stewart et al., *PLoS Comput. Biol.* 9:e1002936 (2013). Where appropriate, statistical comparisons can be made using ANOVA analyses, analysis of variance with Bonferroni correction, or two-tailed Student's t-test, where values are determined to be significant at $P<0.05$. Any appropriate method can be used to isolate RNA or protein from neural constructs. For example, total RNA can be isolated and reverse transcribed to obtain cDNA for sequencing.

Test compounds can be dissolved in a solvent such as, for example, dimethyl sulfoxide (DMSO) prior to contacting to AECs provided herein. In some cases, identifying agents comprises analyzing the contacted AECs for positive or negative changes in biological activities including, without limitation, gene expression, protein expression, cell viability, and cell proliferation. For example, microarray methods can be used to analyze gene expression profiles prior to, during, or following contacting the plurality of test compounds to the AECs. In some cases, a method of the present invention further comprises additional analyses such as metabolic assays and protein expression profiling.

Compositions

In another aspect, provided herein are preparations of AECs. For example, provided herein are AECs, substantially purified populations of AECs, pharmaceutical preparations comprising AECs, and cryopreserved preparations of the AECs. The AECs described herein may be substantially free of at least one protein, molecule, or other impurity that is found in its natural environment (e.g., "isolated"). The AECs may be mammalian, including, human AECs. The invention also provides human AECs, a substantially purified population of human AECs, pharmaceutical preparations comprising human AECs, and cryopreserved preparations of the human AECs. The preparation may be a preparation comprising human embryonic stem cell-derived AECs, human iPS cell-derived AECs, and substantially purified (with respect to non-AECs) preparations comprising differentiated pluripotent stem cell-derived AECs.

Cell preparations provided herein are useful for various in vitro and in vivo applications such as engineering new blood

vessels, endothelial cell transplantation into the heart for myocardial regeneration, induction of angiogenesis for treatment of regional ischemia, and screening for drugs affecting vasculature such as angiogenesis inhibition to slow cancer progression. Since most vascular disease occurs in arteries (Go et al., 2014), arterial cells are extremely valuable for disease modeling, as they can be used for investigating how arterial endothelial cells are activated, and for screening drugs to prevent the activation, which will facilitate understanding and curing atherosclerosis. Because it has been very difficult to obtain AECs, these cells have been largely omitted from tissue-engineered vascular grafts and prevascularization of tissue transplants (Bae et al., 2012; Campbell and Campbell, 2007), which could contribute to poor clinical outcome. The disclosed methods facilitate production and use of AEC populations.

Preparations comprising AEC cells useful for clinical applications must be obtained in accordance with regulations imposed by governmental agencies such as the U.S. Food and Drug Administration. Accordingly, in exemplary embodiments, the methods provided herein are conducted in accordance with Good Manufacturing Practices (GMPs), Good Tissue Practices (GTPs), and Good Laboratory Practices (GLPs). Reagents comprising animal derived components are not used, and all reagents are purchased from sources that are GMP-compliant. In the context of clinical manufacturing of a cell therapy product, such as in vitro populations of human arterial endothelial cells, GTPs govern donor consent, traceability, and infectious disease screening, whereas the GMP is relevant to the facility, processes, testing, and practices to produce a consistently safe and effective product for human use. See Lu et al. *Stem Cells* 27: 2126-2135 (2009). Where appropriate, oversight of patient protocols by agencies and institutional panels is envisioned to ensure that informed consent is obtained; safety, bioactivity, appropriate dosage, and efficacy of products are studied in phases; results are statistically significant; and ethical guidelines are followed.

In another aspect, provided herein is a culture medium or a culture system comprising a culture medium for differentiating human pluripotent stem cell-derived mesodermal cells into AECs, where the culture medium comprises or consists essentially of a Fibroblast Growth Factor (FGF), a vascular endothelial growth factor (VEGF), an inhibitor of TGF-beta signaling (e.g., SB431542), a Notch agonist (e.g., Resveratrol (RESV)), and an inhibitor of inositol monophosphatase. In exemplary embodiments, the culture medium comprises or consists essentially of E5 medium supplemented with human FGF2 (100 μg/l), VEGF-165 (50 μg/l), SB431542 (10 μM), RESV (5 μM), and L-690,330 (10 μM). Such a culture medium does not comprise insulin.

Articles of Manufacture

The invention also provides a kit for differentiating human pluripotent stem cells into AECs, comprising (i) a first culture medium suitable for differentiation of human pluripotent stem cells into mesodermal cells; (ii) a second culture medium suitable for differentiation of pluripotent stem cell-derived mesodermal cells into arterial endothelial cells; and (iii) instructions describing a method for differentiating human pluripotent stem cells into $CD31^+/CD144^+/CD41^-/CD45^-$ arterial endothelial cells, the method employing the first culture medium and the second culture medium.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention belongs. Although any methods and materials similar to or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described herein.

In the specification and in the claims, the terms "including" and "comprising" are open-ended terms and should be interpreted to mean "including, but not limited to . . . ." These terms encompass the more restrictive terms "consisting essentially of" and "consisting of." As used herein and in the appended claims, the singular forms "a", "an", and "the" include plural reference unless the context clearly dictates otherwise. As well, the terms "a" (or "an"), "one or more" and "at least one" can be used interchangeably herein. It is also to be noted that the terms "comprising," "including," "characterized by," and "having" can be used interchangeably.

As used herein, "a medium consisting essentially of" means a medium that contains the specified ingredients and those that do not materially affect its basic characteristics.

As used herein, "effective amount" means an amount of an agent sufficient to evoke a specified cellular effect according to the present invention.

As used herein, "about" means within 5% of a stated concentration range, density, temperature, or time frame.

The invention will be more fully understood upon consideration of the following non-limiting Examples. It is specifically contemplated that the methods disclosed are suited for pluripotent stem cells generally. All papers and patents disclosed herein are hereby incorporated by reference as if set forth in their entirety.

EXAMPLES

Example 1—Protocol for Directed Differentiation of Pluripotent Stem Cells into AECs

To investigate arterial differentiation, an endothelial cell differentiation protocol was developed using a defined culture medium lacking both serum and bovine serum albumin. Xeno-free pluripotent stem cells were first differentiated into mesoderm cells in a culture medium supplemented with BMP4, Activin-A, and CHIR99021 (E8BAC medium) for two days. Mesoderm cells were then treated with FGF2, VEGFA, and BMP4 for another three days, yielding a 70% $CD31^+/CD34^+$ endothelial cell population (FIGS. **2**A-B). Insulin was included in this mesoderm-to-endothelium differentiation medium. Endothelial cell fate was further confirmed by the down-regulation of NANOG and OCT4 (FIG. **2**C), the up-regulation of KDR/VEGFR2, the expression of CD144 (CDH5/VE-cadherin) (FIG. **2**D), the internalization of LDL (FIG. **2**E), and the formation of capillary networks in vitro and in vivo (FIGS. **2**F-G). With this protocol, we were able to investigate the effect of individual medium components under completely defined culture conditions (FIG. **2**H).

Since cells of the $CD31^+/CD34^+$ endothelial cell population largely failed to express markers of AECs (data not shown), we isolated a population of $CD31^+/CD144^+/CD41^-/CD45^-$ endothelial cells from the aorta-gonad-mesonephros (AGM) region of embryonic mesoderm of a E11.5 day mouse embryo. These cells were isolated from the AGM to identify new factors having capacity to induce arterial differentiation.

Single-cell RNA-Seq was performed for the $CD31^+/CD144^+/CD41^-/CD45^-$ endothelial cells to characterize global gene expression profiles of individual endothelial cells. To distinguish arterial and venous endothelial cell populations, a set of arterial markers (Efnb2, Cxcr4, D114,

Gja4, Hey1, Jag1, Notch1, Notch4, and Nrp1) and venous markers (Aplnr, Ephb4, Flt4, Nr2f2, and Nrp2) were analyzed using SINGuLAR™ Analysis Toolset. Many of the markers clustered into either the arterial group or venous group, but Aplnr and Notch1 did not cluster with either group (FIG. **1**A). This result is consistent with a previous study suggesting that some arteriovenous markers are transiently non-specific (Chong et al., 2011). Based on marker expression, the $CD31^{+}/CD144^{+}/CD41^{-}/CD45^{-}$ endothelial cells were clustered into five subpopulations (FIG. **1**A). The average normalized expression of arterial and venous gene sets within each subpopulation was calculated to distinguish arterial and venous cells (FIG. **1**B). Population 1 (P1) was identified as arterial endothelial cells, as it had the highest arterial and the lowest venous marker expression (FIG. **1**B). By contrast, Population 3 (P3) had the lowest arterial gene expression (FIG. **1**B). Principal component analysis revealed a clear separation between the P1 and P3 cells (FIG. **1**C), and 918 genes were determined to be enriched in P1 cells (arterial endothelial cells) ($p<0.1$, FC>2, TMP>1) compared to P3 cells (see Table 4).

To identify growth factor related genes within the 918 arterial enriched genes, five AmiGo gene ontology data "terms" were combined: growth factor binding (GO: 0019838), growth factor activity (GO:0008083), growth factor receptor binding (GO:0070851), receptor activity (GO:0004872), and receptor binding (GO:0005102). The combined list was then intersected with plasma membrane genes (GO:0005886) to remove non-cell surface genes (FIG. **1**D and Tables 2 and 4). Some of the resulting 42 genes were not growth factors or their receptors, but were either upstream or downstream of a growth factor signaling pathway. Some well-known arteriovenous regulators, including VEGFA, Wnt signaling (FZD4, FZD7, FZD10), and Notch signaling (DLL4 and Notch4) were present in these 42 genes (Table 2).

In order to test candidate factors in human arterial differentiation, we made a dual human ES cell reporter line using clustered regularly interspaced short palindromic repeats (CRISPR (clustered regularly interspaced short palindromic repeats)-Cas9 technology to target EFNB2 (ephrin B2) with tdTomao and EPHB4 (ephrin type B receptor 4) with EGFP (FIGS. **8**A-**8**B). See, e.g., Hou et al., 2013. EFNB2 and EPHB4 are the most characterized embryonic arterial and venous endothelial cell markers, respectively (Wang et al., 1998). Specific targeting of the EFNB2 and EPHB4 loci was confirmed by junction PCR and southern blot (FIGS. **8**C-**8**F). Only single copies of each reporter were integrated into genome (FIGS. **8**G-**8**H), and the endogenous expression of EFNB2 and EPHB4 in the reporter cell line was similar to that in wild type cells (FIG. **8**I). Karyotypes were normal after dual targeting (FIG. **8**J), and DNA sequencing revealed no CRISPR induced insertions or deletions in the wild type alleles.

We used the EFNB2-tdTomato/EPHB4-EGFP dual reporter cell line to test the function of individual growth factor related genes identified by single-cell RNA-Seq analysis. Consistent with their previously described roles, VEGFA, WNT3A, and RESV (a Notch agonist) all promoted increased arterial specification (FIG. **11**).

We then investigated the other growth factors/signaling pathways during endothelial cell differentiation by adding or removing recombinant proteins/small molecules, such as insulin, as it is widely used in endothelial cell differentiation protocols. Surprisingly, removing insulin after mesoderm formation triggered AEC differentiation, as evidenced by the increased number of EFNB2-tdTomato^{high}/EPHB4-EGFP^{low} cells (FIGS. **3**A-B). Since insulin is able to activate AKT (Mackenzie and Elliott, 2014), a negative regulator of arteriovenous specification (Hong et al., 2006), we examined AKT activity. Phosphorylated AKT (pAKT) was increased by the presence of insulin, and inhibiting PI3K activity using Ly294002 (a reversible inhibitor of phosphoinositide 3-kinases (PI3Ks)) decreased pAKT (FIG. **3**C) and reversed the inhibitory effect of insulin during arterial differentiation (FIGS. **3**A-**3**B). These results demonstrate that the insulin-AKT pathway played a key role in suppressing arterial differentiation.

In addition, we found that the following factors increased arterial endothelial cell differentiation: FGF2, L-690,330 (a inositol monophosphatase inhibitor), and LDL (low-density lipoprotein), as evidenced by the increase of EFNB2-tdTomato^{high}/EPHB4-EGFP^{low} cells (FIG. **3**F). In contrast, removing SB431542 (a TGF-β receptor inhibitor) or adding PDGF-BB inhibited arterial differentiation (FIGS. **3**E-**3**F).

To further confirm these results, EFNB2-tdTomato^{high}/EPHB4-EGFP^{low} putative arterial endothelial cells and EFNB2-tdTomato^{low}/EPHB4-EGFP^{high} putative venous endothelial cells were sorted by FACS and analyzed by RT-qPCR. Arterial genes were significantly up-regulated in EFNB2-tdTomato^{high}/EPHB4-EGFP^{low} cells. These data demonstrate that FGF, L-690,330, and LDL promote arterial endothelial differentiation of human pluripotent stem cells while insulin, TGF-β, and PDGF inhibit arterial endothelial differentiation.

To further improve arterial differentiation, we examined combinations of individual factors. Arterial endothelial cell differentiation was greatly improved by combining FGF, VEGFA, SB431542, RESV, and L-690,330 ("five factors") in a chemically defined medium ('FVIRL medium" in Table 1; see also FIGS. **4**A-**4**B) when compared to the differentiation observed when single factors were employed. Removing FGF, VEGF, SB431542, or RESV individually led to a decrease of EFNB2-tdTomato^{high}/EPHB4-EGFP^{low} cells (FIGS. **4**A-**4**B). Two other arterial markers, CXCR4 and DLL4, were similarly decreased upon removal of FGF, VEGF, SB431542, or RESV (FIGS. **4**C-**4**F). However, when RESV or L-690,330 was removed, or when PDGF as added, fewer EFNB2-tdTomato^{high}/EPHB4-EGFP^{low} putative arterial endothelial cells were obtained, but no reduction of $CD144^{+}CXCR4^{+}$ and $CD144^{+}DLL4^{+}$ cells was observed (FIGS. **4**C-**4**F). Although as a single factor WNT3A promoted arterial differentiation, exogenous WNT3A did not further increase arterial differentiation in the context of the other five factors (FIGS. **4**A-**4**F, FIG. **9**).

Endothelial cells generated with the five-factor protocol took up LDL, formed vascular networks, and maintained EFNB2 (Ephrin B2) expression in those networks (FIGS. **5**C-**5**D). Another characteristic feature of functional AECs is decreased leukocyte adhesion relative to that of venous endothelial cells (Hauser et al., *J Immunol* 151, 5172-5185 (1993); Kalogeris et al., *Am J Physiol* 276, L9-L19 (1999)). Thus, we analyzed the ability of TNFα, a proinflammatory cytokine to induce leukocyte adhesion in different types of endothelial cells (De Caterina et al., 1995).

Finally, we examined whether the human ES cell-derived AECs exhibit arterial-specific functional characteristics. First, the "five factors" AECs produced NO at levels comparable to primary human coronary arterial endothelial cells (HCAECs), and at higher levels than HUVEC cells (FIG. **7**A). Second, AECs consumed oxygen at rates similar to primary arterial endothelial cells, and at higher rates than HUVEC cells (FIG. **7**B). Third, the AECs elongated in response to shear stress to a similar degree as primary arterial endothelial cells, and to a greater degree than HUVEC cells (FIGS. **7**C-**7**D). The AECs exhibited low levels of TNFα-induced leukocyte adhesion (Hauser et al., *J Immunol* 151, 5172-5185 (1993); Kalogeris et al., *Am J Physiol* 276, L9-L19 (1999)) that was comparable to primary arterial endothelial cells and was much lower than that of HUVEC cells (FIGS. **7**E-**7**F). In summary, our combined results demonstrate that the AECs are characterized by gene expression and functional properties, which are distinct from venous endothelial cells but consistent with arterial endothelial cells.

Previous studies revealed that the vascular smooth muscle expressed ACTA2 (smooth muscle actin or "SMA"), TAGLN (Smooth muscle protein 22-alpha or "SM22A"), MYH11 (myosin, heavy chain 11, smooth muscle), and elastin (ELN). Owens et al., *Physiol Rev* 84, 767-801 (2004). We further demonstrated that CD31 could be used to distinguish intestinal and vascular smooth muscle cells. As shown in FIG. **12**, MYH11-positive vascular smooth muscle is recruited to the blood vessels. In the intestine, smooth cells express both MYH11 and CD31 (arrow indicated), demonstrating that $MYH11^{+}CD31^{-}$ cells are vascular smooth muscle cells while $MYH11^{+}CD31^{+}$ cells are intestinal smooth muscle cells.

TABLE 2

Arterial Enriched Growth Factor Related Genes

Related Pathway	Gene	Description	Treatment
Adiponectin	ADIPOR2	Adiponectin receptor protein 2	Add adiponectin (ADIPO)
Angiopoietin	ANGPT2	Angiopoietin-2	Add ANGPT2
BMP	CRIM1	Cysteine-rich motor neuron 1 protein. Antagonist of BMP4/7	Remove BMP4
Chemerin	CMKLR1	Chemokine-like receptor 1	Add Chemerin
EFNA1	EFNA1	Ephrin-A1. Positively regulated by TNF and VEGF	Add TNF or VEGF
EFNB2	EFNB2	Ephrin-B2. Arterial endothelial cell marker	N/A
EGF and VEGF related	LDLR	Low-density lipoprotein receptor. Positive regulation of KDR and EGF	Add EGF or VEGFA
EGF and VEGF related	SSFA2	Sperm-specific antigen 2. Positively regulated by KRAS, which can be activated by EGF and VEGF	Add EGF or VEGFA
EGF related	CELSR1	Cadherin EGF LAG seven-pass G-type receptor 1. Interacts with EGFR	Add EGF
EGF related	CD2AP	CD2-associated protein. Interacts with EGFR	Add EGF
EGF related	CD9	CD9 antigen Binds to EGF	Add EGF
EGF related	THBD	Thrombomodulin. Positive regulation of EGFR	Thrombomodulin (THBD)
FGF	FGFR2	Fibroblast growth factor receptor 2	Add FGF2
FGF related	ARRB2	Beta-arrestin-2. FGFR1 recruits ARRB2 upon agonist treatments	Add FGF2
IFN	IFNGR2	Interferon gamma receptor 2	Add IFNg
Insulin/IGF	IGFBP3	Insulin-like growth factor 2 mRNA-binding protein 3 Inhibits IGF pathway	Add insulin
Interleukin	IL13RA1	Interleukin-13 receptor subunit alpha-1	Add IL13
LIF	STAT3	Signal transducer and activator of transcription	Add LIF
LIF or Wnt related	YES1	Tyrosine-protein kinase Yes. Regulated by LIF and Wnt	Add LIF or Wnt
NGF	PTPRG	Receptor-type tyrosine-protein phosphatase gamma. Inhibits NGF function	Add B-NGF to reverse its effects
Notch	DLL4	Delta-like protein 4	NOTCH signaling, previously reported
Notch	NOTCH1	Neurogenic locus notch homolog protein 1	NOTCH signaling, previously reported
Notch related	APLP1	Amyloid-like protein 1. Processed by γ-secretase, which is also part of Notch signaling	Add APLP1
Notch related	PALM	Paralemmin-1. Overexpressed in ESR1+ breast cancer cells. Notch actives ESR dependent transcription	Add PALM
Notch related	DAG1	Dystroglycan: acts downstream of Notch signaling pathway	Add DAG1
NPR	NPR2	Atrial natriuretic peptide receptor 2	Add C-type natriuretic peptide
PDGF related	SLC9A3R2	Na(+)/H(+) exchange regulatory cofactor NHE-RF2	Increase PDG-BB activity, so add PDGF-BB
PDGF related	ABCA1	ATP-binding cassette sub-family A member 1. Suppressed by PDGF	Add PDGF-BB to suppress it

TABLE 2-continued

Arterial Enriched Growth Factor Related Genes

Related Pathway	Gene	Description	Treatment
SDF	CXCR4	C—X—C chemokine receptor type 4	Add CXCL12/SDF
Semaphorin	PLXNA2	Plexin-A2	Add Semaphorin (SEMA)
Slit	ROBO2	Roundabout homolog 2; response to slit ligand	Add Slit2-N
TGF	BRCA1	Breast cancer type 1 susceptibility protein; expression is inhibited by TGFβ1.	Add or remove SB431542 (TGF receptor inhibitor)
TNF	SLC20A1	Sodium-dependent phosphate transporter 1. Upregulated by TNF	Add TNFα
VEGF	VEGFA		VEGF signaling, previously reported
VEGF related	GRIA2	Glutamate receptor 2, GluR2, upregulated by VEGF	Add GlutaMAX ™ medium (Glu)
VEGF related	LEPR	Leptin receptor. Leptin upregulates VEGF signaling	Add LEPTIN
VEGF related	XPR1	Xenotropic and polytropic retrovirus receptor 1. Binds to ACTR6, which can be activated by VEGF	Add VEGF
VEGF related	INPP5K	Inositol polyphosphate 5-phosphatase K. Inositol pathway suppresses VEGF	Add L-690,330 (inositol monophosphatase inhibitor) (L690)
Wnt	FZD4	Frizzled-4	WNT signaling, previously reported
Wnt	FZD7	Frizzled-7	WNT signaling, previously reported
Wnt	FZD10	Frizzled-10	WNT signaling, previously reported
N/A	SLC20A2	Sodium-dependent phosphate transporter 2	N/A

Materials and Methods

Isolation of mouse endothelial cells for single-cell RNA-seq: Twenty-four E11.5 mouse (CD-1 background) embryos were harvested. The head, tail, limb, internal organ, and somite were removed. The aorta-gonad-mesonephros (AGM) tissue was incubated in 2 mg/ml collagenase type IV (Life Technologies, cat #17104-019) and 0.25 mg/ml dispase (Life Technologies, cat #17105-041) solution for 15 minutes on ice to let enzyme penetrate into the tissue. The tissue with enzyme was then incubated at 37° C. for 10 minutes. The enzyme was neutralized by 2% FBS-HBSS and pipetted up and down to further dissociate the cells. The cells were immunostained and $CD31^{+}CD144^{+}CD41^{-}CD45^{-}$ endothelial cells were sorted out by flow cytometry. CD41 and CD45 were used to deplete hematopoietic stem cells.

Isolation of human fetus arterial endothelial cells for single-cell RNA-seq: Human fetal aorta tissue (14 weeks gestation) was dissected from the aortic arch to the abdominal bifurcation. Tissue was obtained from the Human Fetal Tissue Repository at Albert Einstein College of Medicine of Yeshiva University (Bronx, N.Y.). This work was done under approval from the UW-Madison Health Sciences IRB and the IRB of Albert Einstein College of Medicine. The adventitia layer of human fetus dorsal aorta was completely removed and the rest of the tissue was cut into small pieces. The tissue was then digested by 300 U/ml collagenase/elastase (Worthington Biochem, cat #LK002067) for one hour at 37° C., and the tissue was pipetted up and down every 20 minutes. The endothelial cells were sorted by flow cytometry using anti-CD31 antibody.

Single cell RNA-sequencing. For mouse AGM cells, 15 μl cell suspension (containing 5×10^4 cells) was loaded into a Fluidigm C_1™ chip. RNA isolation, cDNA library preparation were performed on Fluidigm C_1™ Single-Cell Auto Prep System as manufacturer suggested (Smarter-seq1 protocol). cDNA concentration was measured by Quant-iT™ PicoGreen® dsDNA assay kit (Life Technology, cat #P7589) and diluted to 0.1-0.3 ng/μl. cDNA was tagmented (by a modified transposition reaction) and barcoded by using Nextera XT DNA Sample Prep Kit (Illumina, cat #FC-131-1024). For sequencing (Illumina, HiSeq2500), 18-24 samples were pooled. In total, 84 cells were sequenced. After doublet exclusion and outlier removing, 70 cells were used for further analysis.

For AECs derived by the "five factors" protocol, $CD144^{+}$/EFNB2-$tdTomato^{high}$/EPHB4-$EGFP^{low}$ cells were sorted and loaded into a Fluidigm C_1™ chip. cDNA were prepared and sequenced as mentioned above. In total, 96 cells were sequenced and used for further analysis.

For primary AECs ("pAECs;" freshly isolated from 14 weeks old human fetus dorsal aorta), smarter-seq2 protocol was applied to Fluidigm C_1™ single-cell auto prep system for cDNA preparation. Smarter-seq2 is has been shown to improve cDNA yield and sequencing sensitivity[41], thus it's suitable for samples with relative low RNA quality. In total, 48 cells were sequenced and used for further analysis.

H1 ES and HUVEC cells were prepared by Fluidigm C_1™ single-cell auto prep system using smarter-seq2 protocol. In total, twenty-four H1 and 48 HUVEC cells were sequenced and used for further analysis.

Hierarchical clustering: Single-cell RNA-seq data (TPM) were generated from RSEM. For each gene, the log 2 TPM was scaled to z scores with mean 0 and variance 1. Prior to taking the logarithm, TPM below 1 was imputed as 1. Hierarchical clustering was performed using the Euclidean distances between cells (FIG. **1**).

Data analysis by SINGuLAR Analysis Toolset 2.1: Single-cell RNA-seq data (TPM) was loaded to SINGuLAR Analysis Toolset 2.1. The outliers were removed by the "identifyOutliers( )" command. Arterial and venous markers of the samples were them analyzed by the "autoAnalysis( )" command. As a result, PCA plot of FIG. **1**C was automatically generated. Heat map of AECs data (FIG. **10**) was also generated by "autoAnalysis( )" of SINGuLAR.

Principle component analysis by R program: Principle component analysis (PCA) was performed on single-cell

RNA-seq data (FIG. 5B). To adjust for sequencing depth variation across different cells, expected counts were normalized by mediumn-ratio normalization. To reduce the effect of potential outliers, for each gene, values that were greater than the 95th quantile of the gene-specific expression had been imputed using the 95th quantile. Prior to PCA, the gene-specific normalized expression was rescaled to values with mean 0 and standard deviation 1 for all genes. The PCA analysis was performed using prcomp( ) function in R.

Generation of the growth factor related gene list: Five Amigo Go Terms (version 1.8) growth factor binding (GO:0019838), growth factor activity (GO:0008083), growth factor receptor binding (GO:0070851), receptor activity (GO:0004872), and receptor binding (GO:0005102) were combined. The combined list was then joined with plasma membrane (GO:0005886) to generate the growth factor related gene list. The list was further joined with "arterial-enriched genes" from Table 5 to generate an arterial enriched growth factor related gene list of Table 6.

Gene targeting on the H1 ES cells: The 5' and 3' homology arms of EFNB2 targeting vector were synthesized by IDT (gBlock) with introducing Sal I and BamH I (5' arm), Bmt I and Mlu I (3' arm) restriction sites to facilitate subcloning into the targeting vector. The 5' and 3' homology arms of EPHB4 targeting vector were PCR amplified from BAC (bacterial artificial chromosome).

To achieve the best electroporation efficiency, human ES cells (H1) were EDTA passaged (1:4 split) and cultured to reach 80-90% confluence two days before the experiment. At the day of the experiment, ES cells were dissociated by Accutase, washed once with E8 medium, and resuspended at densities of 5×10^6 cells/mL in E8 medium with 10 mM Hepes buffer (pH 7.2-7.5) (Life Technologies). For electroporation, 400 μL of cell suspension, 7.5 μg gRNA plasmid, 7.5 μg spCas9 plasmid, and 10 μg linearized DNA template plasmid were mixed in a 4-mm cuvette (Bio-Rad) and immediumtely electroporated with a Bio-Rad Gene Pulser. Electroporation parameters were 250 V, 500 μF, and infinite resistance. Cells were then plated on Matrigel™-coated plate in E8 medium (10 μM Y27632 was added for the first day). For the EFNB2-tdTomatom cell line, 100 μg/ml Geneticin was added to the medium when cells reached to 20% confluence (usually 3-4 days after electroporation) and drug selection was used for the last five days. For the EPHB4-EGFP cell line, 0.5 μg/ml puromycin was added to the medium when cells reached to 20% confluence. Due to the drug sensitivity of cells in E8 medium, eight hours/day puromycin treatment was performed for five days. Surviving colonies were picked four to six days after drug selection and expanded in E8 medium.

Karyotyping: Karyotyping was performed by WiCell Research Institute.

Southern blot: The probe was synthesized by using PCR DIG Probe Synthesis Kit (Roche, Cat #11 636 090 910). The southern blot was performed following DIG Application Manual for Filter Hybridization from Roche.

TABLE 3

Culture Medium Components						
Medium components	E8	E8BAC	E6	E5	E7V	Five factors
DMEM/F12	+	+	+	+	+	+
L-ascorbic acid-2-phosphate magnesium (64 ng/ml)	+	+	+	+	+	+
Sodium selenium (14 ng/ml)	+	+	+	+	+	+
$NaHCO_3$ (543 μg/ml)	+	+	+	+	+	+
Transferrin (10.7 μg/ml)	+	+	+	+	+	+
Insulin (20 μg/ml)	+	+	+		+	
FGF2 (100 ng/ml)	+	+			+	+
TGFβ1 (2 ng/ml)	+	+				
BMP4 (5 ng/ml)		+				
Activin A (25 ng/ml)		+				
CHIR99021 (1 μM)		+				
$VEGFA_{165}$ (50 ng/ml)					+	+
SB431542 (10 μM)						+
RESV (5 μM)						+
L690 (10 μM)						+

Human pluripotent stem cell culture and differentiation: iPS cell line 005B23.1 was derived from skin punch fibroblast and maintained on recombinant vitronectin-coated plates. DF19.11 was derived from foreskin fibroblast. CD-3-1 was derived from cord blood cells. PBMC was derived from peripheral blood mononuclear cells. H1 and H9 ES cells were derived from male and female embryos, respectively.

Human pluripotent stem cells were cultured in E8 medium on a Matrigel™-coated plate (excepted 005B23.1). To achieve the best differentiation results, ES cells were split by EDTA at 1:4 ratios two days before the differentiation. The cells reached 80-90% confluency two days later. At the day of the differentiation, ES cells were dissociated by Accutase (Invitrogen) for 3 minutes at 37° C. The cells were plated on vitronectin-coated plate (recombined vitronectin, 50 μg/10-cm dish) at 1:3 ratios ($1.1\text{-}1.5\times10^5$ cells/cm^2). The cells reached 100% confluence after 36 hours. To improve cell survival, 10 μM Y27632 was used for the first day. The cells were cultured in E8BAC medium (see Table 3: E8 medium supplemented with 5 ng/ml BMP4, 25 ng/ml Activin A, and 1 μM CHIR99021) for two days. E6 (E8 medium minus FGF2, and TGFβ1) medium supplemented with growth factors or small molecules was then used to induce endothelial cell differentiation for another three days. Medium was changed every day. The cells were harvested at day 5. To isolate $CD31^+CD34^+$ cells, the cells were labeled with CD34 magnetic beads and processed through autoMACS (Miltenyi Biotec). The purified cells were cultured on fibronectin-coated (Life Technologies, Cat #33016-015) (100 μg/10-cm dish) or vitronectin-coated (50 μg/10-cm dish) dishes with E7V (E6+100 ng/ml FGF2+50 ng/ml VEGFA) medium.

Arterial endothelial cell differentiation and expansion: It took six days for AECs differentiation. From day 0 to day 2, human ES/iPS cells were first differentiated into mesoderm cells as mentioned above. From day 2 to day 6, E5 medium was used and growth factors or small molecules were added as indicated. With the combination of "five factors," AECs were induced by E5 medium supplemented with 100 ng/ml FGF, 50 ng/ml VEGF, 10 μM SB431542, 5 μM RESV, and 10 μM L690 from day 2 to day 6.

AECs were purified by CD144 microbeads (Miltenyi Biotec, cat #130-097-857) for some of the functional assays. After optimization (FIG. 9), AECs were maintained in FVIR (E5+100 ng/ml FGF, 50 ng/ml VEGF, 10 μM SB431542, 5 μM RESV) or FVIR+Ins (FVIR medium+10 μg/ml insulin) medium on fibronectin- or vitronectin-coated dishes.

LDL-uptake assay: To perform the LDL-uptake assay, 2 μg/ml acetylated-LDL-FITC was added to the medium and

cultured for 4 hours. Ten minutes before imaging, 2 μg/ml Hoechst was added to medium. To co-stain with CD144, anti-CD144-647 antibody was added to medium two hours before imaging. The medium was removed and HBSS was added for live cell imaging. It is important to image the cell in live because fixing the cell will diminish LDL-FITC signal.

MATRIGEL™ encapsulation assay: 1.5×10^3 endothelial cells/pi and 0.75×10^3 pericytes/μl (ScienCell, cat #1200) were encapsulated in 6.5 mg/ml Matrigel™. A 10 μL Matrigel™/cell solution was spotted in the middle of 24-well plate and incubated for 5 mins at 37° C. for solidification. E7V mediumwas then applied. Immunostaining was performed on day 4 and the structures were imaged using Nikon confocal microscopy.

In vivo MATRIGEL™ plug angiogenesis assay: 5×10^5 endothelial cells were resuspended in 100 μl E7V medium and 200 μL Matrigel and then the 300 μL cell/Matrigel™ mixture was subcutaneously injected into the neck of nude mice. After two weeks of inoculation, the Matrigel™ was harvested, fixed, and immunostained. For dextran injection, 100 μg rhodamine-conjugated dextran was retro-orbital injected into mice after four weeks of inoculation. Ten minutes after dextran injection, the Matrigel™ plug was harvested, fixed, and immunostained.

Fibrin Gel Encapsulation Assay: 1.5×10^3 endothelial cells/pi and/or 0.75×10^3 pericytes/μl were encapsulated in fibrin gel. Fibrin gel was prepared by 2.5 mg/ml fibrinogen (EMD, cat #341578) and 0.5 U/ml thrombin (Sigma, cat #T-9326). A 10 μL fibrin gel/cell solution was spotted in the middle of 24-well plate and incubated for 10 mins at 37° C. for solidification. E7V medium was then applied. Immunostaining was performed on day 4 and the structures were imaged using confocal microscopy.

Oxygen-Induced Retinopathy Model: The experiments were performed under approval from UW-Madison Ophthalmology and Visual Science IRB. Oxygen-induced retinopathy was induced in C57/BL6 wild-type mice as previously described[21]. Briefly, postnatal day seven mice were exposed in 75% oxygen for five days. At day postnatal day 12, they were transfer back to room air and received 1 μl intravitreal injection containing 5×10^4 cells. Phosphate buffered saline (PBS) was used as vehicle and injected as the control. Five days later, retinas were harvested and immunostaining was performed.

Hind Limb Ischemia Model: The experiments were performed under approval from UW-Madison Cardiovascular Physiology Core Facility IRB. The Hind limb ischemia model was generated as previously described[22]. Briefly, 10-12 weeks old female athymic nude mice (Crl:NU(NCr)-Foxn1^{nu}, Charles River Laboratories, Chicago, Ill.) were used. Ten to twenty weeks old instead of four to six weeks old mice were used as the recovery of the older mice was slower and more similar to human limb ischemia. The common iliac artery was ligated in the abdominal cavity and just caudal to the inguinal ligament, the femoral artery was ligated in two locations and removed. The mice were randomly assigned into four groups right after surgery and injected cells or DF12 medium. The cells (0.3×10^6, 1×10^6, or 3×10^6 cells per mouse) were suspended in 300 μl DF12 medium and injected intramuscularly into six site of the gracilis muscle in the ischemic leg. The surgery was performed on seven to eight mice per day.

Nitric Oxide Production Assay: The endothelial cells were seeded on vitronectin coated 24-well plate (1×10^5 cells/well). AECs were cultured in FVIR+Ins medium. HUVEC (Lonza, cat #CC-2519) was cultured in EGM2 (Lonza, cat #CC-3202)) medium. HCAEC (Lonza, cat #CC-2585) was cultured in EGM2 medium for one day and then in FVIR+Ins medium for another day. Two days later, all the medium were changed to fresh FVIR+Ins medium containing 1 μM DAF-FM (Life technologies, cat #D-23844). Cells were cultured for 30 mins and then harvested for flow cytometric analysis. DAF-FM is nonfluorescent until it reacts with NO to form a fluorescent benzotriazole. To achieve the consistent results, the same cell density and same medium is used after adding DAF-FM.

Oxygen Consumption Assay: 4×10^4 cells/well were seeded on the XF24-well plate (Seahorse Bioscience) for overnight. AECs were cultured in FVIR medium, HCAEC and HUVEC were culture in EGM2 medium. One day later, medium was changed to Mito Assay medium (Seahorse Bioscience) and oxygen consumption rate was measured by XF24 analyzers according to manufacture's instruction (Seahorse Bioscience). Oligomycin (0.5 μM) was injected at time point 3 to abolish the oxygen consumption by inhibiting ATP-synthase. FCCP (2 μm, Mitochondrial uncoupler) was injected at time point 6 to uncouple the electron transport chain from the oxidative phosphorylation thus measuring the maximal respiration capacity. To measure non-mitochondrial respiration, 1 μm Antimycin A and 1 μm Rotenone were applied simultaneously at time point 9 to completely block the electron transport chain at cytochrome bc1 (complex III) and NADH dehydrogenase (complex I), respectively.

Shear Stress Response: Shear stress response was assayed using an ibidi Pump System (Red perfusion set, μ-Slide VI 0.4). For each channel of μ-Slides, 30 μl cell suspension (5×10^5 cells/ml, with 10 μM Y27632) was loaded. After cell attached, 130 μl fresh medium was added to each channel. Two days later, μ-Slide was perfused by ibidi Pump System. After perfusion for 24 hours, cells were harvested and immunostained.

Since FVIR+Ins medium promoted the elongation of endothelial cells, E7V medium was used to culture "five factors" AECs before and during the 24-hour shear stress response experiment.

Leukocyte Adhesion Assay: All endothelial cells were cultured on a fibronectin coated 24-well plate. AECs were cultured in FVIR medium; HUVEC and HCAEC were cultured in EGM-2 medium (Lonza). When the cells reached to 100% confluence, they were treated with or without 10 ng/ml TNFα for four hours. Then 1×10^6 U937 cells were suspended in 0.5 ml fresh RMPI1640+10% FBS and added to each well. Twenty to 60 minutes later, cold medium (RMPI1640+10% FBS) was used to gently wash away the non-attached cells. Washing was repeated two more times. Cells were imaged immediumtely.

Antibody Reagents: Anti-mouse CD41-FITC (Biolegend, Catalog #133904), Anti-mouse CD45-FITC (STEMCELL technologies, Catalog #10710), Anti-mouse CD144-PE (BD, Catalog #562243), Anti-mouse CD31-APC (BD, Catalog #551262), Anti-human CD31-FITC (BD, Catalog #555445), Anti-human CD31-V421 (BD, Catalog #564089), Anti-human CD31-PE (BD, Catalog #555446), Anti-human CD34-647 (BD, Catalog #555824), Anti-human CD144-647 (BD, Catalog #561567), Anti-human DLL4-APC (Miltenyi, Catalog #130-096-560), Anti-human CXCR4-APC (BD, Catalog #560936), Anti-CD34 Micro bead (Miltenyi, 130-046-703), Anti-CD144 Micro bead (Miltenyi, 130-097-857), Anti-pAKT (ser473) (Cell signaling, Catalog #4060), Anti-

AKT (Cell signaling, Catalog #4691), Anti-GAPDH (EMD Millipore, Catalog #MAB374).

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The invention has been described in connection with what are presently considered to be the most practical and preferred embodiments. However, the present invention has been presented by way of illustration and is not intended to be limited to the disclosed embodiments. Accordingly, those skilled in the art will realize that the invention is intended to encompass all modifications and alternative arrangements within the spirit and scope of the invention as set forth in the appended claims.

TABLE 4

Genes Enriched in P1 (arterial endothelial cells) vs. P3 (venous cells)

SYMBOL	Average Venous	Average arterial	Arterial/Venous	p
EFNB2	3.385	132.8169231	39.2369049	9.19431E-05
ZFP91	3.728333333	40.26538462	10.79983494	0.000240266
ABI1	10.46833333	103.5061538	9.887548529	0.000275893
RB1CC1	1.223333333	26.68538462	21.8136659	0.000475352
PAM16	1.233333333	109.8853846	89.0962578	0.000478996
NDUFB2	68.985	204.7292308	2.967735461	0.000997087
ECE2	32.285	241.0869231	7.467459287	0.001219051
GLOD4	29.72	115.0207692	3.870147013	0.001231445
ATP6V1H	9.261666667	96.89230769	10.4616492	0.001476268
UQCR11	400.9133333	867.3530769	2.163442831	0.001500257
BCR	0.273333333	17.03230769	62.31332083	0.001570238
KLF7	6.853333333	59.80615385	8.726578869	0.001641459
YES1	5.766666667	44.61384615	7.736505113	0.001805012
GPX4	195.8316667	519.4053846	2.652305388	0.001808462
CRAMP1L	2.33	12.18153846	5.228128095	0.001919003
PDLIM7	22.71166667	104.6307692	4.606917263	0.002007834
SUV420H2	0.21	42.26923077	201.2820513	0.002058135
CLTA	9.336666667	93.24230769	9.986680581	0.0021114
SIK3	0.288333333	9.756923077	33.83903957	0.002112886
APOOL	28.395	227.7223077	8.019803053	0.002179598
2410004B18RIK	0.103333333	16.97615385	164.2853598	0.002200093
PMPCA	5.285	89.88615385	17.00778692	0.002332837
SLC20A1	13.90833333	141.1692308	10.14997465	0.002452212
CRYZ	3.031666667	81.01	26.72127543	0.002540742
STIP1	93.86166667	275.8192308	2.93857163	0.002676347
2310033P09RIK	1.333333333	40.97461538	30.73096154	0.002701952
SNRPN	1.75	34.89384615	19.93934066	0.002947216
SNURF	1.75	34.89384615	19.93934066	0.002947216
PKIG	27.89166667	135.9292308	4.873471076	0.002989368
ZFP69	1.728333333	74.69538462	43.21815889	0.003117547
ZEB1	19.37	42.88769231	2.214129701	0.003365306
NFKB1	4.178333333	28.84	6.902273634	0.003383717
UBQLN1	8.751666667	82.31230769	9.405329388	0.003450139
SCP2	40.35	131.2307692	3.252311505	0.003458867
NFAT5	1.718333333	12.03384615	7.003208237	0.00356317
GALNT7	2.171666667	24.92846154	11.47895389	0.003740709
SLC11A1	6.113333333	15.63692308	2.557839107	0.003873468
MAPK7	4.216666667	32.58384615	7.727394345	0.00388953
DNAJA2	43.11333333	155.7569231	3.612732095	0.004169191
3200002M19RIK	31.69166667	128.7215385	4.061684096	0.004182439
MYCT1	19.67166667	109.9984615	5.591720488	0.004228163
UNC5B	0.413333333	53.98923077	130.6191067	0.004302974
NOTCH1	6.468333333	25.01153846	3.86676709	0.004390574
VCP	87.10333333	236.8469231	2.719148786	0.004391778
MTX2	3.993333333	42.71384615	10.69628869	0.004393955
MYO6	4.123333333	22.79769231	5.528947205	0.004450296
EIF1A	51.38666667	182.0284615	3.54232865	0.004523565
SFT2D1	29.595	145.1507692	4.904570679	0.004672191
HNRPDL	89.60333333	201.6476923	2.250448558	0.004872772
OS9	0.843333333	18.93846154	22.45667376	0.005041053
OSBPL11	1.883333333	67.16923077	35.66507828	0.005042374
TMEM2	46.21	126.8084615	2.744177917	0.005122916
NCLN	5.511666667	53.11923077	9.637598567	0.00512415
CCNH	26.70166667	91.59307692	3.430238197	0.005293571
PODXL	2.241666667	22.92230769	10.22556477	0.005298181

TABLE 4-continued

Genes Enriched in P1 (arterial endothelial cells) vs. P3 (venous cells)

SYMBOL	Average Venous	Average arterial	Arterial/Venous	p
CPSF2	11.525	69.92	6.06681128	0.005372826
RERE	2.495	10.02230769	4.016956991	0.005444336
EIF4E2	63.23833333	151.6823077	2.398581678	0.005508351
CSGALNACT1	1.436666667	27.72384615	19.29734071	0.005582996
STAG2	16.92666667	99.05769231	5.852167722	0.005604699
CATSPER2	0	4.33	#DIV/0!	0.005764138
ORC4	29.08833333	125.2469231	4.305744219	0.005852601
FNTA	11.80333333	66.92307692	5.669845546	0.006003204
MDM2	2.74	52.58384615	19.19118473	0.006038551
AAAS	4.686666667	83.18307692	17.74887843	0.006204517
CCNG2	6.808333333	77.16	11.33317013	0.006289792
XPNPEP1	42.52166667	156.0361538	3.669568154	0.006315041
ABCA1	1.843333333	31.11153846	16.87786897	0.006324339
CENPB	3.355	24.35076923	7.258053422	0.006437385
ATP6V1B2	15.295	154.7784615	10.11954636	0.006463226
1810011O10RIK	13.18333333	116.0692308	8.804240008	0.00664935
PALM	9.896666667	56.17153846	5.675803819	0.006659886
1110057K04RIK	15.285	106.5630769	6.97174203	0.006710143
TMED10	86.775	189.76	2.186804955	0.007271129
RELL1	6.05	54.70769231	9.04259377	0.007359743
MICALL1	0.476666667	10.91461538	22.89779451	0.007489279
CYFIP1	37.99833333	86.40923077	2.274026864	0.007616271
GMFG	1.781666667	45.36538462	25.46233	0.007680945
MMGT1	11.83833333	57.67461538	4.87185263	0.007898165
MRPL19	3.635	29.56461538	8.133319225	0.007954454
ZFP770	0.115	12.44153846	108.187291	0.008008639
HEY1	1.525	49.74769231	32.62143758	0.008135942
AI462493	19.63166667	68.19	3.473469734	0.008317939
MAP3K3	4.02	48.54923077	12.07692308	0.008461151
ARL8A	4.375	16.96153846	3.876923077	0.009161649
PTPRG	2.28	19.35153846	8.487516869	0.009360361
KIF1C	0.446666667	3.813076923	8.53673938	0.00945174
SLC16A13	0.035	32.42461538	926.4175824	0.009459486
TANC1	1.568333333	17.25153846	10.99991825	0.009468885
CARKD	0.571666667	65.80307692	115.1074232	0.009662523
IRF2	5.738333333	28.24538462	4.922227932	0.009848846
TMEM126A	51.09333333	159.66	3.12486952	0.010061198
SLC9A3R2	2.42	60.53769231	25.01557533	0.010070149
RBM17	11.22833333	109.3530769	9.739030155	0.010121604
NDFIP2	5.515	61.11384615	11.08138643	0.01026201
WDR37	6.125	60.00307692	9.796420722	0.010282102
ANKRD13A	0.711666667	22.15615385	31.13276887	0.010510297
CAMK2N1	0.038333333	10.04	261.9130435	0.010661901
CYBA	61.34333333	211.6038462	3.449500291	0.010705172
9430020K01RIK	6.446666667	33.73153846	5.232399968	0.010782094
PON3	47.42166667	235.8323077	4.973091928	0.010947494
ZIK1	1.445	32.63846154	22.58717061	0.011011179
TAF6	6.866666667	143.5169231	20.90052278	0.011094949
ARPC1B	15.71333333	118.2546154	7.525749812	0.011196008
HERPUD1	0.075	80.46692308	1072.892308	0.011239847
ZC3H7A	19.84833333	94.36769231	4.754439112	0.011339573
USP34	16.32166667	40.75307692	2.496869821	0.01139032
VPS41	51.05166667	151.2569231	2.962820471	0.011548761
PTRF	100.8383333	262.8869231	2.607013765	0.011974959
SPARCL1	26.95166667	141.1661538	5.237752292	0.012197776
ATPAF1	0	8.841538462	#DIV/0!	0.012432885
RTF1	7.266666667	19.70769231	2.712067749	0.012510393
TRAF3IP2	0.021666667	10.25307692	473.2189349	0.012650349
MPV17L	62.90166667	133.8876923	2.128523764	0.012821527
METTL1	0.608333333	21.49538462	35.33487882	0.012864088
AP2A2	30.55333333	66.98615385	2.192433576	0.012937774
BC052040	0.421666667	35.65923077	84.5673457	0.012992012
OTUD6B	1.256666667	134.4076923	106.9557233	0.013133609
RBM27	6.238333333	20.85692308	3.34334861	0.013572071
STOX2	4.311666667	16.90692308	3.921203651	0.013704227
DUSP1	22.30833333	115.6538462	5.184333784	0.013959937
APRT	143.845	392.3446154	2.727551291	0.013992332
KLF11	1.945	13.22615385	6.800079098	0.014138226
MBNL1	54.94166667	162.4823077	2.957360371	0.014235892
NDUFB6	176.9133333	435.2423077	2.460200707	0.01426922
HS6ST1	0.331666667	18.20692308	54.89524546	0.014290794
RNF103	0.058333333	3.42	58.62857143	0.014436475
GPR108	0	34.85230769	#DIV/0!	0.014485379
CABLES2	0.03	40.96384615	1365.461538	0.014513813
1110012L19RIK	0.075	51.40076923	685.3435897	0.014638158
FAM102B	6.878333333	48.90153846	7.109504017	0.015105008

TABLE 4-continued

Genes Enriched in P1 (arterial endothelial cells) vs. P3 (venous cells)

SYMBOL	Average Venous	Average arterial	Arterial/Venous	p
GIT2	31.37	66.93461538	2.13371423	0.015337561
SH2D3C	11.65166667	63.51	5.450722357	0.015462751
NDUFAF4	6.903333333	27.92923077	4.045760131	0.015588477
TACC1	23.90333333	66.06692308	2.763920921	0.015677661
TMEM107	0	54.48615385	#DIV/0!	0.016146777
MAP3K11	0.808333333	21.93384615	27.13465504	0.016217106
HDHD2	12.73833333	69.06307692	5.421672923	0.016455021
ST3GAL5	2.035	27.55615385	13.54110754	0.016545693
TFDP2	1.631666667	8.296923077	5.084937534	0.017053524
FAM70A	72.96666667	231.5038462	3.172734301	0.017064899
ATP6V1C1	21.12333333	70.67461538	3.345807893	0.017762152
ARL4C	0	10.77384615	#DIV/0!	0.017765223
WDR1	118.985	361.9530769	3.042005941	0.017812468
SPPL3	24.87666667	90.63538462	3.643389439	0.018003641
GM10406	29.53833333	83.45769231	2.825402888	0.018369717
RNASEK	36.47	153.1953846	4.200586362	0.01843833
SAT1	207.5716667	736.1584615	3.546526717	0.018518169
NGFRAP1	163.3833333	366.7930769	2.244984659	0.018834065
SCNM1	5.593333333	59.61384615	10.65801779	0.01890972
TMEM135	0.041666667	40.40076923	969.6184615	0.018917381
THBD	4.881666667	38.83615385	7.955511201	0.018990321
PIGC	4.391666667	45.81769231	10.43287111	0.01906959
FAM108B	2.291666667	30.18769231	13.17281119	0.019131469
GTF2A1	152.4783333	318.5053846	2.088856677	0.019181905
KLF3	16.465	83.83461538	5.091686328	0.019245785
CELSR1	0.176666667	9.797692308	55.4586357	0.019332519
BAG1	6.505	38.77692308	5.961095016	0.019352643
OMA1	0.8	71.75	89.6875	0.019386958
WTAP	53.74333333	133.7023077	2.487793358	0.019456377
SRPRB	13.555	97.11846154	7.164770309	0.019611167
GOT1	3.796666667	55.56615385	14.63551023	0.019997176
UBE2J1	8.225	31.62076923	3.844470423	0.020059233
LNPEP	0.87	4.605384615	5.293545535	0.020273547
CLK2	7.611666667	49.52307692	6.506206734	0.020375008
PSG25	0.573333333	1.700769231	2.966457961	0.020531763
PLK2	269.0066667	586.1761538	2.179039505	0.02053319
GNGT1	0.228333333	2.098461538	9.190342504	0.020576308
BC004004	1.505	63.48615385	42.18349093	0.020608049
PRSS23	0.18	38.21153846	212.2863248	0.020670808
TMEM179B	0.498333333	48.33923077	97.00180087	0.020676696
POFUT2	8.811666667	76.23692308	8.651816502	0.020698889
PDCD7	0.131666667	34.84153846	264.6192795	0.020842697
MYO10	24.63833333	75.11153846	3.048564099	0.021072143
ZFP830	3.733333333	13.74615385	3.682005495	0.02125756
CUL1	53.66166667	151.5253846	2.823717451	0.021439414
PHLPP2	0.356666667	2.52	7.065420561	0.021609083
TCF4	65.40666667	136.8769231	2.092705989	0.021644076
FAM82B	0.046666667	6.852307692	146.8351648	0.021781048
MTHFS	1.378333333	40.47230769	29.36322203	0.021829631
SEC11C	18.31	100.5761538	5.492963072	0.021830525
CYP20A1	2.475	63.35307692	25.5972028	0.021853266
GJA4	0	341.8130769	#DIV/0!	0.021930681
MRPS17	53.04333333	152.1369231	2.868162944	0.021976091
TCN2	0.865	63.31461538	73.19608715	0.022099139
ERICH1	7.178333333	26.62923077	3.709667625	0.022136522
CUL4A	8.706666667	89.80307692	10.31428908	0.022308948
GALT	9.176666667	85.35846154	9.301684875	0.022314096
SLC41A1	0.126666667	11.60615385	91.62753036	0.022449628
PPP2R3C	1.751666667	35.29384615	20.14872283	0.022578109
RFTN2	2.033333333	37.20307692	18.29659521	0.022607793
TNS1	0.323333333	19.85461538	61.40602696	0.022707466
N4BP1	3.005	22.25307692	7.405350058	0.022840249
AP1B1	7.47	45.62461538	6.107712903	0.022906176
PHB	107.2916667	246.0061538	2.29287289	0.022956614
UBAC1	2.656666667	13.19846154	4.968053277	0.023037392
RAB5C	5.825	17.75153846	3.047474414	0.023133369
DARS2	1.083333333	40.68538462	37.55573964	0.023179456
FZD4	3.078333333	42.66692308	13.86039732	0.023271942
REEP5	15.91833333	56.58	3.55439221	0.023371665
ARF6	14.16666667	35.28230769	2.490515837	0.023533819
NMD3	61.78833333	170.4692308	2.758922625	0.023562786
MKNK2	16.75666667	66.63230769	3.976465548	0.023626939
0910001L09RIK	0.408333333	26.54769231	65.01475667	0.023758909
TPP1	12.98	59.35846154	4.573070997	0.02376108
3110056O03RIK	0.93	18.67692308	20.08271299	0.023780867
ADAM15	3.48	19.44692308	5.588196286	0.023832747

TABLE 4-continued

Genes Enriched in P1 (arterial endothelial cells) vs. P3 (venous cells)

SYMBOL	Average Venous	Average arterial	Arterial/Venous	p
ANGPT2	0	3.886923077	#DIV/0!	0.023913439
TRMT2B	0.348333333	18.88384615	54.21199853	0.024410915
XPR1	2.316666667	18.66692308	8.057664638	0.0245685
LAMA4	38.52	123.1223077	3.196321591	0.024670168
RBM15B	1.35	10.13230769	7.505413105	0.024726345
PLAG1	2.741666667	14.81923077	5.405190554	0.024945921
1110001J03RIK	42.435	114.24	2.692117356	0.025236719
DNAJC21	1.855	32.87076923	17.72009123	0.025455253
ANKRD37	0	19.33384615	#DIV/0!	0.025456764
HEBP1	0.083333333	2.872307692	34.46769231	0.025492603
EBF1	9.5	74.50538462	7.842672065	0.02550248
TOMM70A	25.685	96.77769231	3.767868106	0.025592427
NFRKB	0.12	8.373846154	69.78205128	0.025651677
RNF34	6.471666667	75.87769231	11.72459835	0.025710175
TMEM161A	0	6.103076923	#DIV/0!	0.025750694
FDXR	0	5.233076923	#DIV/0!	0.025890656
HEXA	25.26166667	115.6738462	4.5790267	0.025964577
RHOG	8.52	69.45615385	8.152130733	0.02611705
MAPKAPK2	0.276666667	19.44153846	70.27062095	0.026290641
A430005L14RIK	14.65666667	116.7207692	7.963664037	0.026423167
ECHDC1	3.351666667	50.15307692	14.96362315	0.026501523
TRAF7	27.59833333	92.11923077	3.33785485	0.026596211
CXCR4	0	22.95230769	#DIV/0!	0.026654915
ZFP169	0.053333333	1.375384615	25.78846154	0.026750673
FERMT2	35.28	100.5438462	2.849882261	0.026789286
MYLK4	0.515	1.243846154	2.41523525	0.026929464
FAM172A	5.861666667	44.64769231	7.616893769	0.027185609
NIT2	22.61	127.0746154	5.620283741	0.027205719
GOLGA2	0.39	8.923846154	22.8816568	0.027392671
ATP6V1E1	46.32166667	168.2253846	3.631678148	0.027790291
2310016C08RIK	6.558333333	79.30769231	12.09265956	0.028063943
LRRC47	0.076666667	18.86461538	246.0602007	0.028071959
VPS13B	0.915	11.73615385	12.82639765	0.028077765
RFESD	1.87	34.06076923	18.2143151	0.028109316
STRBP	9.725	27.59923077	2.837967174	0.028230906
NDRG3	0.303333333	29.64692308	97.73710904	0.028272119
SFT2D2	0.026666667	15.18615385	569.4807692	0.028377348
WSB1	248.3766667	560.1653846	2.255305992	0.028735922
MRE11A	0.725	49.67230769	68.51352785	0.028793407
IFNGR2	15.70166667	59.68307692	3.801066357	0.028817418
1700123O20RIK	39.35666667	94.47	2.400355721	0.02904505
MKI67IP	17.88	83.63692308	4.677680262	0.029243856
SIRT2	10.35166667	67.20692308	6.492377048	0.029404512
2510012J08RIK	0.23	7.200769231	31.30769231	0.029411528
TMEM188	2.975	37.80923077	12.70898513	0.02958981
USP28	0.366666667	27.24538462	74.30559441	0.029612383
HRSP12	7.301666667	74.51769231	10.20557302	0.029670977
FCER2A	0.435	1.655384615	3.805481874	0.029723822
INPP5K	0.178333333	49.50230769	277.5830338	0.029804456
TMEM134	5.673333333	23.44769231	4.132965742	0.029850332
HSDL1	2.213333333	117.4130769	53.04807692	0.029853034
TRIM24	14.90666667	51.56692308	3.459319527	0.029874672
1600012H06RIK	1.013333333	30.41153846	30.01138664	0.02993989
MIER2	0.113333333	25.69153846	226.6900452	0.029975659
EPN2	0.393333333	67.31153846	171.13103	0.030066295
CKB	6.455	74.98307692	11.61627838	0.030084135
ATG5	36.51666667	108.35	2.967138293	0.030330758
BIN3	1.095	72.92230769	66.59571479	0.030339038
SIRT6	11.55166667	30.65	2.653296783	0.030360402
PFKL	6.498333333	72.58615385	11.16996469	0.030418381
CXXC5	11.08666667	51.99923077	4.690249318	0.030572682
2700078K21RIK	0.695	95.28307692	137.0979524	0.030787086
SNIP1	0.418333333	7.924615385	18.94330371	0.030894971
EXOC6	3.431666667	56.72615385	16.5302051	0.030922331
ANGEL2	16.89833333	54.69846154	3.23691458	0.03096661
PPP5C	5.806666667	44.66153846	7.691424534	0.031218815
ECE1	111.9	274.79	2.45567471	0.031225536
STARD3NL	19.37333333	86.21307692	4.450089999	0.031238384
HPCAL1	10.92833333	67.22692308	6.15161718	0.031429075
NDUFS2	73.09333333	241.6146154	3.305562961	0.031732727
BCL6B	43.01166667	120.15	2.79342814	0.031754776
AP3S1	12.56166667	55.00769231	4.379012257	0.031755004
PPPDE2	0.086666667	23.73307692	273.8431953	0.031827411
TRIB1	3.995	18.30384615	4.581688649	0.031860429
AP3S2	6.46	20.70307692	3.204810669	0.031865004
TRIM35	14.28	62.37307692	4.36786253	0.031931969

TABLE 4-continued

Genes Enriched in P1 (arterial endothelial cells) vs. P3 (venous cells)

SYMBOL	Average Venous	Average arterial	Arterial/Venous	p
UBE2M	0.461666667	8.538461538	18.49486254	0.03200187
GADD45GIP1	1.156666667	7.919230769	6.846597207	0.032007181
GM7244	0.346666667	2.086153846	6.017751479	0.032191261
DTL	14.22166667	76.84615385	5.403456265	0.032255499
GSTM1	0	90.04923077	#DIV/0!	0.032400938
SSH1	0.678333333	4.932307692	7.271215271	0.032554474
CARS	3.118333333	40.93	13.12560128	0.032685951
GM6654	20.50833333	75.04	3.659000406	0.032959343
RBM41	5.021666667	20.57846154	4.097934591	0.033106439
HSPA4L	0.036666667	5.396153846	147.1678322	0.033300757
VPS28	103.3033333	249.6053846	2.416237468	0.033470154
RFK	8.44	54.41615385	6.447411593	0.03351173
MCCC2	0.193333333	48.14153846	249.0079576	0.033669052
TADA2B	0.238333333	5.889230769	24.71005917	0.033685451
NDOR1	1.638333333	16.99769231	10.37499022	0.033873322
USP3	26.95333333	75.01538462	2.783157975	0.033890802
MAN1C1	3.095	29.26	9.453957997	0.033934677
ANKRD54	0.041666667	30.51307692	732.3138462	0.034039372
TTLL12	1.216666667	25.01769231	20.56248683	0.034336372
VAC14	0.415	5.976153846	14.40037071	0.034356148
CLPTM1	2.968333333	16.86	5.679955081	0.03444528
1110020G09RIK	4.076666667	54.71692308	13.42197622	0.034502276
MLH3	0.658333333	8.206153846	12.46504382	0.034602385
SCYL2	0.018333333	1.806923077	98.55944056	0.034995114
MECOM	6.53	97.88538462	14.99010484	0.035000679
LPCAT3	5.005	48.18384615	9.627142089	0.035029409
ZFP457	0.336666667	2.023846154	6.011424219	0.035086209
ZFP637	44.54	132.8969231	2.983765673	0.035138186
CEP250	0.098333333	5.122307692	52.09126467	0.035266592
CAMK2G	2.091666667	36.67538462	17.53404842	0.035309188
GRPEL2	1.341666667	15.22538462	11.34811276	0.035436744
PKMYT1	1.156666667	35.49615385	30.68831745	0.035543986
IFT46	38.585	94.58153846	2.451251483	0.035658758
DPM3	45.605	99.79230769	2.188187867	0.035678768
RASSF2	2.685	16.88923077	6.290216301	0.035699489
PXK	1.29	19.44692308	15.07513417	0.035717616
FOXP2	1.498333333	25.56769231	17.0640883	0.035990592
SLC25A1	3.498333333	48.71846154	13.92619196	0.03616713
2610002117RIK	9.57	57.04923077	5.961257134	0.036249471
SLC38A7	0	2.803076923	#DIV/0!	0.03650076
NCSTN	19.76333333	70.50384615	3.567406619	0.036654248
1810048J11RIK	0	25.67769231	#DIV/0!	0.036656075
RPP40	7.81	40.37846154	5.170097508	0.036738428
SMTN	10.585	37.49692308	3.542458486	0.036844152
MTHFD2	2.961666667	67.39230769	22.7548591	0.036927106
CSAD	2.518333333	22.9	9.093315685	0.03721687
PLCB4	0.061666667	20.81769231	337.5841996	0.037255767
MAP3K7	8.493333333	41.92692308	4.936450912	0.037295572
DNAJC17	0.176666667	31.84461538	180.2525399	0.037395996
5430437P03RIK	16.90333333	45.21384615	2.674847929	0.037578453
CCND2	71.25	177.8453846	2.496075574	0.037648986
DPM2	37.36666667	129.3623077	3.461970768	0.037677582
2310036O22RIK	19.51333333	51.35307692	2.631691677	0.037728855
KLHL21	0.893333333	20.34384615	22.77296211	0.038085178
FAM165B	61.485	142.6907692	2.320741144	0.038115442
FKBP14	0.071666667	54.78	764.372093	0.038340315
ARHGAP5	17.34333333	71.04461538	4.096364523	0.038370987
2310008H04RIK	2.466666667	20.33923077	8.245634096	0.038383648
ZFP592	0.285	20.45615385	71.77597841	0.038495223
TMTC4	5.511666667	31.10307692	5.64313461	0.038498387
HIGD1A	3.58	19.04153846	5.318865492	0.038573935
SMPDL3B	0	8.326923077	#DIV/0!	0.038797907
RCC2	22.76666667	50.06769231	2.199166573	0.039044589
KBTBD2	8.396666667	77.70538462	9.254313372	0.039141354
TERF1	2.76	27.59230769	9.997212932	0.039151289
ACER2	0.85	10.87153846	12.79004525	0.039158693
MGLL	0.093333333	25.18307692	269.8186813	0.03921294
SRGAP1	3.741666667	13.83384615	3.697241734	0.039355605
APPL2	1.191666667	31.36461538	26.31995697	0.039489916
LNX1	0	1.966153846	#DIV/0!	0.039521684
MIPOL1	0.363333333	17.92	49.32110092	0.039567716
SLC23A2	7.303333333	29.13538462	3.989326967	0.039581995
PAIP2	219.0333333	454.2284615	2.073786919	0.039729082
CERK	3.871666667	21.01307692	5.427398258	0.039748408
LAGE3	30.33833333	133.3246154	4.394592607	0.039911992
NOP14	5.015	56.62	11.29012961	0.040136837

TABLE 4-continued

Genes Enriched in P1 (arterial endothelial cells) vs. P3 (venous cells)

SYMBOL	Average Venous	Average arterial	Arterial/Venous	p
GMPPB	11.83333333	66.38692308	5.610162514	0.040183033
PRR5L	22.68833333	63.24384615	2.787505156	0.040241287
ZCWPW1	0.04	27.26846154	681.7115385	0.040286692
2510006D16RIK	123.25	263.7661538	2.140090498	0.04028986
PPM1E	0	5.566153846	#DIV/0!	0.040391847
PCYT1A	2.993333333	15.98076923	5.338787048	0.04052893
1110034B05RIK	12	84.33230769	7.027692308	0.04072132
PGAP2	0	50.55923077	#DIV/0!	0.040730276
SRGN	0.238333333	33.19846154	139.2942442	0.040969616
LPCAT1	1.751666667	12.03461538	6.870379858	0.041139103
LYL1	0.573333333	11.66307692	20.34257603	0.041144687
GM14430	0.275	8.703846154	31.65034965	0.041267689
GM14434	0.275	8.703846154	31.65034965	0.041267689
AGGF1	4.063333333	55.85384615	13.7458194	0.041344615
GTL3	77.075	248.4761538	3.22382295	0.041361201
KCNQ1	0.031666667	1.834615385	57.93522267	0.04147258
ANXA11	0.123333333	1.683846154	13.65280665	0.041566921
GIMAP1	15.75666667	50.43076923	3.200598851	0.041692451
DNAJB9	0	28.25307692	#DIV/0!	0.041693969
COX18	2.021666667	16.60461538	8.213329951	0.04188168
PRODH	0	31.34384615	#DIV/0!	0.041905819
GM5113	9.601666667	22.49923077	2.343263055	0.041978051
HINT2	11.93166667	55.91615385	4.686365737	0.042079062
LYPLA2	2.881666667	35.32923077	12.25999911	0.042084923
MAP3K2	0.046666667	2.787692308	59.73626374	0.042146552
GALE	23.73666667	96.33307692	4.058407959	0.042260317
PLAT	0.16	19.67230769	122.9519231	0.042532177
ZFP358	1.115	21.94	19.67713004	0.042682574
ZCCHC3	16.50166667	44.49923077	2.696650688	0.042818734
PPP2R4	6.496666667	21.63307692	3.329873308	0.043018399
AKR1B3	103.7033333	277.6669231	2.677512035	0.043535478
GM10345	0	32.37153846	#DIV/0!	0.043551944
ZHX1	0.88	22.74615385	25.8479021	0.043595271
RAPGEF5	3.076666667	17.74384615	5.767230603	0.043739677
2310003L22RIK	0	20.38615385	#DIV/0!	0.043829833
FBRSL1	0.531666667	5.803076923	10.91487823	0.044093031
TMOD1	12.35166667	38.17461538	3.090644883	0.044263895
TBC1D23	2.17	21.87384615	10.08011343	0.044386935
ELAC2	0.556666667	57.29923077	102.9327499	0.044398266
SPINT2	0	25.24769231	#DIV/0!	0.04442694
API5	67.80333333	162.0369231	2.389807626	0.044541725
SKIL	62.55166667	144.3038462	2.306954456	0.044578008
ASS1	2.425	50.87076923	20.9776368	0.044658449
ZFP809	0.548333333	6.85	12.49240122	0.044775293
PECAM1	87.48666667	216.1353846	2.470495138	0.044778294
LCLAT1	0.03	12.22153846	407.3846154	0.044791712
LIN7C	34.86666667	92.33692308	2.648286513	0.044837935
DAZAP1	3.816666667	16.06538462	4.209271078	0.044855774
BMP1	12.96666667	59.58846154	4.595511173	0.044873594
ALAS2	0.033333333	12.3	369	0.045035951
ARFGEF2	0.013333333	12.11538462	908.6538462	0.045037558
KHNYN	0	2.626923077	#DIV/0!	0.045084098
ECM1	0	27.87153846	#DIV/0!	0.045122243
MTSS1L	0.92	8.325384615	9.049331104	0.045141405
RIC8B	1.546666667	17.55923077	11.35295093	0.045459795
FAM100A	0.071666667	7.908461538	110.3506261	0.045489009
POP7	27.48833333	108.7146154	3.954936593	0.045527616
TMEM8B	0.453333333	14.72538462	32.48246606	0.04554072
HECTD1	18.975	49.41769231	2.604357961	0.04578972
PIH1D1	7.455	49.70076923	6.66676985	0.045985496
GNE	0.055	2.502307692	45.4965035	0.046063511
LYSMD3	0.82	42.79846154	52.19324578	0.046070404
1110003E01RIK	0.468333333	34.33153846	73.30577607	0.046077666
FUT10	0.02	5.203846154	260.1923077	0.046298882
PEX11B	4.003333333	39.44923077	9.854095946	0.046489188
RNF31	0.471666667	24.92153846	52.83718402	0.046492936
OGFR	0.033333333	14.2	426	0.046557415
GCFC1	30.21166667	75.21	2.489435648	0.04657836
WDR55	7.323333333	60.16923077	8.216098876	0.046768358
FAM117A	0	3.892307692	#DIV/0!	0.046981029
EIF2B5	14.40833333	66.86153846	4.640476932	0.047035175
PPFIBP2	0.528333333	13.06461538	24.72797865	0.047178928
TMEM109	9.633333333	56.26846154	5.841016769	0.047417695
MMP15	8.233333333	24.76538462	3.007941451	0.047597294
TRMT11	7.843333333	31.60692308	4.029781948	0.048005041
ALAS1	14.72	49.46384615	3.360315635	0.048187395

TABLE 4-continued

Genes Enriched in P1 (arterial endothelial cells) vs. P3 (venous cells)

SYMBOL	Average Venous	Average arterial	Arterial/Venous	p
CCPG1	0.54	22.89076923	42.39031339	0.048240471
GAK	5.171666667	28.74461538	5.558095144	0.048289197
RANBP6	1.16	8.937692308	7.704907162	0.048307814
HOXB4	0.055	4.497692308	81.77622378	0.048345333
SLC20A2	1.611666667	39.54384615	24.53599555	0.048465284
MRPS5	18.02833333	51.36846154	2.849318381	0.048528808
TTI1	0	8.923076923	#DIV/0!	0.048560674
GRIA2	0	33.54692308	#DIV/0!	0.049032855
ABCB7	3.866666667	17.84153846	4.614190981	0.049129942
SLC18A2	3.061666667	55.74	18.20577028	0.049196079
SARS2	0.663333333	38.67	58.29648241	0.04921158
ALKBH3	2.72	32.07076923	11.79072398	0.049504769
HES6	0	35.96923077	#DIV/0!	0.049589735
MTMR9	0.065	28.03076923	431.2426036	0.049786268
IRF2BP1	0	2.435384615	#DIV/0!	0.049878388
VPS26B	2.686666667	12.53692308	4.66634854	0.050007937
CEP68	2.906666667	38.03923077	13.08689132	0.050119545
ANKIB1	2.796666667	19.03769231	6.807279729	0.050190369
UBA7	0.365	8.059230769	22.0800843	0.05025586
PDZD8	12.44333333	29.49	2.369943745	0.050457696
RNF14	15.77	60.10538462	3.811375055	0.050490229
PTMS	7.316666667	38.12538462	5.210758717	0.050512605
ARMC5	2.638333333	18.70230769	7.088682638	0.050531575
TET1	0.811666667	12.63384615	15.56531354	0.050672764
FZD10	0.533333333	3.283846154	6.157211538	0.050692702
SAMM50	8.068333333	42.92461538	5.320134111	0.050866203
TFAM	15.87	42.34923077	2.668508555	0.050951714
GPATCH2	1.16	19.14153846	16.50132626	0.051154694
REPIN1	2.006666667	14.55461538	7.25313059	0.051272867
2010012O05RIK	6.165	19.39153846	3.145423919	0.051402534
FAM78B	0.043333333	5.774615385	133.260355	0.051446212
TMEM165	8.953333333	40.03846154	4.471905607	0.05145031
NTS	0	85.65692308	#DIV/0!	0.051571632
ST7L	3.53	21.37230769	6.0544781	0.051624534
AMN1	0.195	21.10384615	108.2248521	0.051674438
NSUN6	0	1.520769231	#DIV/0!	0.051720659
ARFGAP1	3.646666667	45.61846154	12.50963296	0.052127566
SLC4A7	9.286666667	32.87307692	3.539814457	0.052423429
PELI2	1.78	19.47846154	10.94295592	0.052510364
NAGK	6.618333333	57.91769231	8.751099316	0.05266822
LGMN	11.48833333	50.06846154	4.358200627	0.052749415
GCDH	2.618333333	29.30769231	11.1932625	0.052854704
CPNE1	8.236666667	41.49307692	5.037605454	0.052940324
VMA21	2.865	13.77230769	4.8070882	0.052973039
D830031N03RIK	0.346666667	6.445384615	18.59245562	0.05308216
UCK2	4.32	27.92846154	6.464921652	0.053105139
SRP68	9.048333333	70.17	7.755019341	0.053137657
SLC25A30	0	21.66076923	#DIV/0!	0.053274355
ZFP558	0.768333333	20.02846154	26.06741198	0.053322948
PHTF1	0.896666667	31.32846154	34.93880469	0.05347776
HERC6	1.573333333	10.14153846	6.44589309	0.053846185
RCOR1	6.696666667	63.35615385	9.460849255	0.053868948
FBXL4	1.75	32.24461538	18.42549451	0.053889135
FBXL19	1.071666667	12.73076923	11.87941141	0.054084426
KDM6A	5.525	17.71076923	3.205569092	0.054545687
RAPH1	2.233333333	39.28076923	17.58840413	0.054784469
TANC2	0.118333333	10.27923077	86.86673889	0.05487343
ABCE1	55.66166667	200.5246154	3.602562183	0.054974313
RMND1	0.41	29.31615385	71.50281426	0.055302636
C1QTNF6	0	1.048461538	#DIV/0!	0.055399921
MAF1	17.76666667	100.4276923	5.652590561	0.055451124
ZFP869	6.383333333	55.8	8.74151436	0.055502064
FAM125B	0.116666667	3.541538462	30.35604396	0.055627209
RNF40	10.07833333	31.58076923	3.133530931	0.055641091
ZSCAN12	0.526666667	9.412307692	17.8714703	0.055665583
FAM49B	2.506666667	10.73923077	4.284267594	0.05577894
SUCLA2	73.88833333	148.5692308	2.010726512	0.055789996
CCDC126	0	20.03538462	#DIV/0!	0.055798969
SLC25A12	0.403333333	25.63384615	63.55499046	0.055851423
ERCC2	0.231666667	3.83	16.5323741	0.055878132
UBOX5	6.57	36.27461538	5.521250439	0.055904215
NOS3	0.686666667	12.66846154	18.44921583	0.055905624
CERCAM	0.83	3.07	3.698795181	0.055944772
NCOA7	3.856666667	56.52846154	14.65733661	0.055945052
BCL2	2.008333333	16.59230769	8.261729971	0.056008723
MAGEB18	0.2	1.033846154	5.169230769	0.05608565

TABLE 4-continued

Genes Enriched in P1 (arterial endothelial cells) vs. P3 (venous cells)

SYMBOL	Average Venous	Average arterial	Arterial/Venous	p
GTPBP5	0.228333333	25.01846154	109.5699045	0.056248586
CPEB4	0.836666667	24.53692308	29.32699969	0.056407253
HAX1	30.27666667	126.9284615	4.19228652	0.056534504
GLCCI1	0.158333333	11.92076923	75.28906883	0.056835057
CCL24	0.786666667	2.102307692	2.672425033	0.056862408
ZFP808	12.35166667	132.1638462	10.700082	0.057000052
ADIPOR2	5.226666667	37.31615385	7.139570251	0.057117537
RAB35	0.408333333	26.87692308	65.82103611	0.05715073
DLL4	12.24833333	35	2.857531637	0.057278061
E130311K13RIK	3.908333333	14.12538462	3.614170904	0.057379198
APOBEC3	2.563333333	42.88615385	16.73061919	0.057549875
SLC25A24	18.32166667	54.09307692	2.952410275	0.057583665
2610101N10RIK	37.03666667	78.11538462	2.109136476	0.057642267
ZMYM5	5.008333333	24.55153846	4.902137463	0.057664765
CCDC43	43.24833333	111.7946154	2.584946211	0.057668463
VKORC1L1	0.063333333	8.742307692	138.0364372	0.057806041
IL13RA1	0.016666667	7.85	471	0.05788107
MSX1	0.05	8.469230769	169.3846154	0.057982811
HMCN1	2.816666667	8.090769231	2.872462449	0.057997308
TAF6L	0.068333333	5.810769231	85.03564728	0.058254094
STAT3	8.168333333	33.56615385	4.109302654	0.058254632
ETF1	27.52	79.39615385	2.88503466	0.058342227
MFSD5	7.451666667	41.13769231	5.520602859	0.05866687
YY2	0.06	6.092307692	101.5384615	0.058761486
SLC7A1	6.753333333	20.11769231	2.978927785	0.058828552
RNF185	7.015	80.98538462	11.54460223	0.05890966
CYP26B1	3.913333333	26.37230769	6.739090552	0.058910796
DDX3Y	7.835	46.02769231	5.874625693	0.05895258
IMP4	42.61166667	106.0707692	2.489242443	0.059341979
SERTAD1	7.193333333	52.86538462	7.349219363	0.059362584
PGCP	2.616666667	41.60230769	15.89897109	0.059751388
PFKP	6.793333333	30.24538462	4.452215596	0.059823579
IPMK	1.013333333	8.623076923	8.509615385	0.059838428
VEGFA	0.476666667	2.713076923	5.691769769	0.059863499
E2F2	0.098333333	11.65615385	118.5371578	0.060057838
ARRDC1	5.116666667	40.29538462	7.875319469	0.060236708
PLEKHG5	1.643333333	9.620769231	5.854423467	0.060363717
PHF13	0.523333333	19.32230769	36.92160706	0.060421909
COQ4	19.20166667	68.86692308	3.586507581	0.06045569
ALDH4A1	0.32	3.928461538	12.27644231	0.060509956
4933403F05RIK	9.585	27.94307692	2.915292324	0.06051212
PAPD4	3.011666667	62.82461538	20.86041463	0.060657403
ZFP647	0.058333333	33.44923077	573.4153846	0.06068685
LSM4	15.04	58.13923077	3.865640344	0.060767276
CAPG	2.843333333	72.77769231	25.59590585	0.060879157
RNF2	44.29333333	98.41384615	2.221865882	0.060931071
LIN54	0.211666667	16.89307692	79.80981224	0.060934324
CDC37L1	0.216666667	16.32538462	75.34792899	0.061058673
2610002M06RIK	0.818333333	6.697692308	8.184552718	0.061092923
ARMCX3	8.833333333	36.69461538	4.154107402	0.061330159
TTC37	5.881666667	29.13384615	4.953331735	0.061347448
PARP6	1.473333333	23.45230769	15.9178559	0.061450809
RBM7	70.67	157.5892308	2.229931099	0.061570105
ADAT2	0	28.44230769	#DIV/0!	0.061838009
PTPMT1	0	14.89461538	#DIV/0!	0.061985931
RTEL1	0.345	25.66	74.37681159	0.06221779
DCP1B	0.226666667	2.858461538	12.61085973	0.062376621
CRIM1	0.54	6.808461538	12.60826211	0.062729098
STK35	1.875	36.02230769	19.21189744	0.062962924
IL4RA	0.056666667	6.492307692	114.5701357	0.062976524
CCNT2	13.215	43.71923077	3.308303501	0.063205018
BAG2	20.57833333	94.91230769	4.612244644	0.063265705
SORBS2	0.226666667	9.810769231	43.28280543	0.063290001
PHF10	3.685	32.13153846	8.719549108	0.06337384
MOGAT2	0.05	52.81	1056.2	0.063468062
ARIH2	2.333333333	18.13153846	7.770659341	0.063470474
PACSIN2	13.56	64.47	4.754424779	0.063478507
YIPF1	3.13	37.29615385	11.9157041	0.063554766
COG6	3.126666667	38.22307692	12.22486469	0.063747439
SLC7A6	4.208333333	15.10076923	3.588301599	0.063782233
2310001A20RIK	2.845	55.87230769	19.63877248	0.064090499
D630042P16RIK	0	12.96307692	#DIV/0!	0.064146809
STK4	3.62	15.75769231	4.352953676	0.064174954
FXC1	35.825	94.07769231	2.626034677	0.064185248
ZFP369	2.483333333	10.37846154	4.179246257	0.064303184
KIF3B	0.043333333	4.393076923	101.3786982	0.06441931

TABLE 4-continued

Genes Enriched in P1 (arterial endothelial cells) vs. P3 (venous cells)

SYMBOL	Average Venous	Average arterial	Arterial/Venous	p
SEC24D	5.013333333	32.95538462	6.573547463	0.06486327
CEP135	0.323333333	13.27769231	41.06502776	0.064872529
PCGF1	0	22.00692308	#DIV/0!	0.064899955
ARL13B	2.111666667	12.52076923	5.929330338	0.064987893
ZFP810	0.08	21.91153846	273.8942308	0.065100666
ESYT1	0	30.88384615	#DIV/0!	0.06518411
CCZ1	17.35666667	80.20538462	4.621013133	0.065245485
PLEKHJ1	10.81333333	37.43153846	3.461609599	0.065324885
NOL12	16.005	57.33538462	3.582342057	0.065352199
NEURL2	0	3.716153846	#DIV/0!	0.065511114
SLC35B2	11.93833333	56.23384615	4.710359862	0.065662923
STX3	0.106666667	15.75076923	147.6634615	0.065770952
ZFP524	1.28	27.75846154	21.68629808	0.065812321
CAPN7	8.455	55.77076923	6.596187963	0.065933189
REXO4	18.115	65.04538462	3.590691947	0.066064674
TMPPE	1.016666667	7.921538462	7.791677175	0.06625231
PGGT1B	10.61	47.86692308	4.511491336	0.066290551
CMKLR1	0.501666667	4.066923077	8.106823409	0.066320641
LEPR	10.915	25.52769231	2.338771627	0.066591229
MLL5	16.92333333	38.43615385	2.271192861	0.066632225
CPE	24.92833333	147.0984615	5.900854244	0.066656079
3110002H16RIK	10.475	55.47307692	5.295759133	0.066660336
FAM18B	35.45166667	106.0323077	2.990897683	0.066824071
MYLK	6.318333333	16.80461538	2.659659517	0.066907628
NPR2	0	12.93615385	#DIV/0!	0.067048221
MAPRE2	29.54	75.37307692	2.551559815	0.067252831
POLR3H	1.106666667	10.86384615	9.816728452	0.067397529
ZDHHC7	0.008333333	9.286923077	1114.430769	0.067424431
FAT1	2.761666667	11.12384615	4.027946706	0.067442384
D19ERTD386E	0.603333333	23.59461538	39.10709732	0.067464044
EBPL	1.13	11.62076923	10.28386658	0.067566323
ILKAP	0.436666667	33.9	77.63358779	0.067683201
PIK3C2B	6.925	21.25076923	3.068703138	0.067776036
NINJ1	16.98	91.41923077	5.383935852	0.067889903
APLP1	0.921666667	23.67692308	25.68924746	0.06789714
KBTBD7	0.371666667	9.033846154	24.30631252	0.067953384
MTPAP	13.205	43.51153846	3.295080535	0.067985483
CCDC111	0.971666667	18.11384615	18.64203721	0.068052146
AIFM1	12.335	93.59307692	7.587602507	0.068283362
CGRRF1	6.303333333	72.53692308	11.50770858	0.068447187
PLAU	39.32	123.4115385	3.138645434	0.068622405
TEX261	6.128333333	40.55307692	6.617309261	0.068722977
NNT	17.38833333	50.90615385	2.927603979	0.068756325
CTXN1	1.228333333	20.71230769	16.86212295	0.069009229
NGRN	0.335	36.52538462	109.0309989	0.069080224
AACS	3.008333333	41.58153846	13.82211805	0.069255291
NAMPT	2.465	27.80076923	11.27820253	0.069272222
KLHL5	4.145	31.6	7.623642943	0.069427954
FBXL17	4.44	9.891538462	2.227823978	0.06957291
SLC25A39	29.67333333	120.4469231	4.059096487	0.06964896
GGH	6.396666667	31.18846154	4.875736562	0.069772486
ZADH2	0.131666667	5.839230769	44.34858812	0.069834529
IGFBP3	37.60833333	113.7	3.02326612	0.069870412
PABPC1L	0.125	2.477692308	19.82153846	0.07007761
TMEM175	0.411666667	27.98923077	67.99003426	0.070133209
SSFA2	6.608333333	50.53307692	7.646871666	0.070436849
GM7616	4.268333333	11.64153846	2.727420178	0.0704728
DNAJC25	0	10.49923077	#DIV/0!	0.070482756
AATF	2.943333333	42.88615385	14.5706072	0.070515129
IKBKG	5.018333333	23.21230769	4.625501367	0.070677919
BEND4	0.03	10.33769231	344.5897436	0.070719978
WDFY2	0	3.26	#DIV/0!	0.070900974
SH3PXD2B	6.605	24.56615385	3.71932685	0.07098515
MPDU1	0.543333333	13.54923077	24.93723454	0.07119844
4933439F18RIK	27.53	61.72846154	2.242225265	0.071330049
ROCK2	22.725	55.42692308	2.439028518	0.071419743
PYGO2	2.42	51.32769231	21.20979021	0.071514864
PRMT3	4.151666667	30.64461538	7.381280301	0.071527724
PDCL3	62.66333333	138.4676923	2.209708372	0.071574233
ZFP229	0.11	2.258461538	20.53146853	0.071632948
ANKMY2	8.108333333	51.18769231	6.312973358	0.071726681
PIGH	5.813333333	37.59846154	6.467625265	0.07174143
SMAD2	16.53	63.96615385	3.869700777	0.071751137
RAD17	3.868333333	22.01153846	5.690186591	0.071818302
RPS19BP1	14.89	41.31307692	2.774551842	0.071825088
TSNAX	8.946666667	47.33692308	5.291012266	0.071872299

TABLE 4-continued

Genes Enriched in P1 (arterial endothelial cells) vs. P3 (venous cells)

SYMBOL	Average Venous	Average arterial	Arterial/Venous	p
MRPL2	51.55833333	123.6776923	2.398791511	0.071975114
FAM101B	5.973333333	20.17230769	3.37706044	0.072065154
DCAF4	0.515	21.47	41.68932039	0.072109056
AU041133	1.098333333	22.56	20.54021244	0.072256734
UTP3	40.45833333	92.13538462	2.27729066	0.072281619
GHITM	104.5366667	229.7469231	2.197764004	0.072408307
PWP1	11.47166667	53.19153846	4.636775109	0.072681631
NGLY1	1.963333333	27.76230769	14.14039441	0.072980173
LCMT1	7.988333333	48.44615385	6.064613459	0.073087799
GUSB	2.208333333	54.70923077	24.77399129	0.073104179
ERLIN1	0.821666667	28.54384615	34.73896084	0.073439545
PEX1	1.008333333	13.02615385	12.91849968	0.073596884
ZFP213	0	2.616153846	#DIV/0!	0.07364258
1810043G02RIK	3.758333333	42.48230769	11.3034965	0.073815133
PPM1M	9.055	55.05230769	6.079768933	0.074119249
FAF1	0.28	21.60384615	77.15659341	0.074282706
MPG	6.378333333	41.29230769	6.473839722	0.074284242
FEM1B	15.08166667	47.09230769	3.122486973	0.074313525
PRND	91.23333333	413.7092308	4.534628032	0.074340949
LGALS9	0	17.82230769	#DIV/0!	0.074396424
AFF4	5.818333333	21.55538462	3.704735253	0.074543688
SYT11	1.373333333	13.87846154	10.10567588	0.074599147
THAP7	1.876666667	28.75846154	15.32422462	0.074762579
FANCA	0.75	4.398461538	5.864615385	0.074842805
BRCA1	0.605	7.436923077	12.29243484	0.075035847
ZFP395	1.325	4.239230769	3.199419448	0.075184804
FASTKD2	2.106666667	34.70846154	16.47553554	0.075317103
AA960436	2.48	27.60076923	11.12934243	0.075450343
SCN2B	0.65	11.79076923	18.13964497	0.075488674
OIT3	2.066666667	50.04615385	24.21588089	0.075564105
FTSJ1	5.628333333	23.77	4.223275096	0.075633763
PRSS43	0.656666667	1.351538462	2.058180398	0.075737719
2310011J03RIK	0.973333333	12.27538462	12.61169652	0.075772075
MTERFD3	1.116666667	33.04538462	29.59288175	0.075936529
GCOM1	30.17166667	109.2938462	3.622400027	0.076142485
RHPN2	1.756666667	9.225384615	5.251642096	0.076437756
ERO1L	3.411666667	17.92461538	5.253917553	0.076475266
GLT25D1	2.668333333	28.42692308	10.65343776	0.07649072
HOXB5	0	4.008461538	#DIV/0!	0.076514631
ARHGAP6	0	4.700769231	#DIV/0!	0.076631422
2810408M09RIK	9.125	33.52076923	3.673508957	0.076681426
HHEX	3.306666667	37.29923077	11.28000931	0.076709174
NENF	0.396666667	7.282307692	18.35875889	0.076957706
CDAN1	1.221666667	7.300769231	5.97607304	0.077035418
GTPBP10	10.83166667	45.17153846	4.170322061	0.077134342
AKAP2	24.88833333	65.69692308	2.639667438	0.077150809
RFX1	0.378333333	9.636923077	25.47204338	0.077222052
CCT6B	0.416666667	2.223076923	5.335384615	0.07739899
MIB1	11.11333333	29.37076923	2.642840663	0.077413192
SFXN3	11.115	44.09615385	3.967265303	0.077614758
TPST1	0.446666667	7.065384615	15.81802526	0.077673955
TOMM22	99.79666667	229.6223077	2.300901577	0.077751857
GNG3	0.215	4.396923077	20.45080501	0.077757389
TTYH3	1.92	6.545384615	3.409054487	0.077901363
PXDN	30.36833333	69.64461538	2.293330181	0.078076924
TRMT5	0	24.12384615	#DIV/0!	0.078096776
PCDH1	0.78	25.93615385	33.25147929	0.078241262
TRF	4.176666667	38.07692308	9.116581742	0.078244798
PAPD7	0.121666667	10.72615385	88.1601686	0.078349373
FAM63A	5.275	47.83307692	9.067881881	0.078493926
DTX3	1.013333333	14.84461538	14.6492915	0.078495557
EGLN1	18.66	58.30538462	3.124618682	0.078604265
LAMB2	0.046666667	14.58538462	312.543956	0.078722122
JKAMP	25.19666667	84.03230769	3.33505653	0.078884402
UBE2G2	16.19333333	66.61384615	4.113658676	0.07927351
CD9	78.29166667	187.4415385	2.394144185	0.079393771
PAPD5	7.001666667	29.12538462	4.159778807	0.07947003
5830433M19RIK	2.936666667	14.77153846	5.030035798	0.079529283
CCDC115	4.416666667	31.77538462	7.194426705	0.079533801
SERINC3	20.18333333	58.57384615	2.902089818	0.079937391
TBC1D4	0.49	18.47538462	37.70486656	0.07997626
ZFP936	0.355	1.615384615	4.550379198	0.080109918
FAM158A	0	15.91230769	#DIV/0!	0.080445323
ETAA1	0.543333333	10.66692308	19.63237376	0.080705637
MYST1	0	35.48153846	#DIV/0!	0.080787758
MICAL2	1.843333333	7.543846154	4.092502434	0.081485709

TABLE 4-continued

Genes Enriched in P1 (arterial endothelial cells) vs. P3 (venous cells)

SYMBOL	Average Venous	Average arterial	Arterial/Venous	p
1700021F05RIK	45.13166667	130.0023077	2.880512006	0.081639691
AFTPH	0	10.92769231	#DIV/0!	0.081703171
BLM	9.335	26.98846154	2.89110461	0.081733816
VPS13C	0.305	3.502307692	11.48297604	0.081746041
FBXW2	6.831666667	37.81461538	5.535196202	0.081782896
EHD4	11.12666667	42.60692308	3.82926211	0.081782985
ZFP746	5.821666667	28.69461538	4.928934793	0.081868957
TPP2	40.015	107.2569231	2.68041792	0.082386499
3110040N11RIK	11.76333333	45.52846154	3.870370774	0.082478516
LRRK2	0.031666667	5.944615385	187.7246964	0.082509549
MTMR11	0	18.52615385	#DIV/0!	0.082659818
POLR2H	75.4	178.6461538	2.369312385	0.08301763
GRAP	41.45833333	100.7638462	2.430484731	0.083047879
DHX32	0.07	1.777692308	25.3956044	0.083171504
EXOG	2.391666667	32.61153846	13.63548646	0.083234252
FREM1	0	2.106153846	#DIV/0!	0.083400371
ABCA3	0.055	11.17384615	203.1608392	0.083492441
ZFP692	0.093333333	27.01230769	289.4175824	0.083511612
MTRF1L	0.38	11.79461538	31.03846154	0.083565319
TTC17	0.908333333	24.64538462	27.13253352	0.083591981
NDRG4	0.048333333	22.71538462	469.9734748	0.083742907
TPRGL	6.11	54.65769231	8.945612489	0.083826264
ATP9B	4.38	30.31538462	6.921320688	0.083929184
SDC3	0.91	10.60923077	11.65849535	0.084461087
JAGN1	19.50833333	74.27230769	3.807209279	0.084512063
HOXA3	2.305	14.26692308	6.18955448	0.084568934
ANKRD49	15.33666667	72.39538462	4.720411951	0.084669253
KDM5C	7.55	16.58153846	2.19623026	0.084685266
HIST1H2BK	0	10.31923077	#DIV/0!	0.084808162
HIST3H2A	0	3.484615385	#DIV/0!	0.084868951
TATDN2	0.095	4.943846154	52.04048583	0.08494456
NRBP1	67.615	177.4661538	2.624656568	0.084947479
EPB4.1L5	3.13	17.17307692	5.486606046	0.084988786
MTMR4	1.741666667	14.32153846	8.222892897	0.085019684
GCNT1	0.055	18.89153846	343.4825175	0.085118852
MOAP1	2.406666667	20.36692308	8.46271042	0.085146926
POLM	0.046666667	12.84076923	275.1593407	0.085249177
QSOX1	0.216666667	2.013846154	9.294674556	0.085259606
SLC17A5	9.885	35.82692308	3.624372593	0.085349793
PRKCE	1.345	8.452307692	6.284243637	0.085444387
CDK5RAP3	0.12	24.28461538	202.3717949	0.08607689
ZNHIT2-PS	0	7.737692308	#DIV/0!	0.08617829
FBXW11	27.075	71.24538462	2.631408481	0.086198362
TBC1D22B	1.285	5.722307692	4.453157737	0.086479697
RSBN1L	3.188333333	19.6	6.147412441	0.086603607
FAM168B	15.53333333	44.54153846	2.867481017	0.086632754
4930452B06RIK	0	2.956923077	#DIV/0!	0.08673539
RAB23	0.118333333	13.69692308	115.7486457	0.087126177
SEMA3A	0.26	11.02769231	42.41420118	0.087255104
PIBF1	4.38	35.08538462	8.010361784	0.08735497
UBE3A	27.88166667	60.75384615	2.178989043	0.08746605
WWOX	0	13.01923077	#DIV/0!	0.087513341
LIME1	3.16	22.71307692	7.187682571	0.087514088
2210021J22RIK	1.381666667	43.14153846	31.22427392	0.087561778
STXBP5	0.216666667	5.331538462	24.60710059	0.087791523
TMC7	0.151666667	14.85153846	97.92223161	0.088096566
GM2058	9.375	65.04538462	6.938174359	0.088319952
IFFO2	3.718333333	16.18846154	4.35368755	0.088509133
MRPL38	7.89	47.35230769	6.00155991	0.088573901
CDK2AP1	41.42333333	126.6184615	3.056694171	0.088830232
FILIP1	0.146666667	5.945384615	40.53671329	0.088900629
RABAC1	35.475	106.05	2.989429175	0.089018546
SMTNL2	6.01	19.89076923	3.309612185	0.089067395
CORO1B	66.88666667	158.0946154	2.363619287	0.089086084
RNPEPL1	5.7	31.52076923	5.529959514	0.089197406
CNTROB	0.043333333	5.869230769	135.443787	0.089204152
MAPK3	68.19166667	140.4184615	2.059173333	0.089290852
TRIM13	0	16.12769231	#DIV/0!	0.089447268
MFN1	0.593333333	14.25923077	24.03241141	0.089526593
ATXN1	0.158333333	6.222307692	39.29878543	0.089556559
TOR1AIP1	6.466666667	27.20846154	4.207494052	0.08972818
4930444A02RIK	1.983333333	5.843076923	2.946089205	0.089899132
6430527G18RIK	1.82	5.698461538	3.131022823	0.089965414
PTER	3.01	20.27769231	6.736774853	0.090184994
FBXO42	0.531666667	10.18769231	19.16180371	0.090275926
ELK4	4.016666667	24.07	5.99253112	0.090321702

TABLE 4-continued

Genes Enriched in P1 (arterial endothelial cells) vs. P3 (venous cells)

SYMBOL	Average Venous	Average arterial	Arterial/Venous	p
DHX40	4.04	24.59076923	6.086824067	0.090549444
ATP9A	0.948333333	27.15230769	28.63160741	0.090560867
PXMP3	16.93666667	61.61846154	3.638169349	0.090575993
GM5918	0.256666667	18.81384615	73.3006993	0.09066458
SEL1L	9.126666667	35.30923077	3.868798112	0.090703125
COG1	3.73	34.38230769	9.217776861	0.091096262
UTP20	2.581666667	7.744615385	2.999851021	0.091162217
METTL13	0	1.809230769	#DIV/0!	0.091314321
DTNBP1	6.423333333	31.12307692	4.845315556	0.09137728
DCBLD1	3.488333333	37.20307692	10.66500055	0.091420667
TRIP11	8.813333333	23.98076923	2.720964739	0.091457917
EDN1	2.101666667	27.69923077	13.17964985	0.09145804
TTPA	0.061666667	7.979230769	129.3929314	0.091463134
GPR180	1.023333333	10.13307692	9.902029567	0.091478453
FBXO11	23.225	57.61615385	2.480781651	0.091670491
TRP53INP1	0	19.60076923	#DIV/0!	0.091851979
TMEM147	60.07666667	168.2623077	2.800793004	0.091857268
FGFR2	0.073333333	15.16846154	206.8426573	0.091862636
SMC5	15.54666667	35.28769231	2.269791529	0.091894325
SALL4	0	59.29384615	#DIV/0!	0.091924063
ETV3	0.871666667	20.86461538	23.93646124	0.09194535
FNIP2	2.345	15.99846154	6.822371658	0.092117245
HSPA13	30.865	76.65692308	2.483619734	0.092160083
PLXNA2	1.018333333	6.062307692	5.95316631	0.092203574
TMEM115	2.766666667	19.09	6.9	0.09221924
SOLH	1.17	5.256923077	4.493096647	0.092247217
GSTZ1	2.51	64.62538462	25.74716519	0.092258383
MFSD6	0.423333333	2.280769231	5.387643852	0.092479056
2310022A10RIK	0.406666667	6.086153846	14.96595208	0.092553602
TMEM66	5.94	65.58615385	11.04144004	0.092857173
GCAT	9.278333333	42.12230769	4.539857125	0.093123416
CXCR7	11.72	53.78461538	4.589131006	0.09314384
TATDN1	3.045	24.37923077	8.006315524	0.09316526
BIN1	5.986666667	29.13615385	4.866840843	0.093255424
E2F3	0.36	5.876153846	16.32264957	0.093659226
PPP2R5A	1.905	22.96230769	12.05370483	0.093767868
DAG1	8.025	29.70769231	3.701893122	0.093858638
EZH1	0.881666667	9.670769231	10.96873637	0.093877551
REEP1	0.11	21.07153846	191.5594406	0.093879695
PHPT1	169.3266667	346.5476923	2.046622066	0.093969581
AMBRA1	1.726666667	10.70615385	6.2004752	0.094060067
SBF2	10.61666667	33.22769231	3.129766936	0.094061511
PDDC1	23.91333333	98.18	4.105659325	0.094109405
ZBTB39	0.445	7.649230769	17.18928263	0.094264899
ALG11	0.296666667	10.02307692	33.78565255	0.094288288
SGIP1	0.291666667	3.756153846	12.87824176	0.094575454
SIGIRR	0	10.66230769	#DIV/0!	0.094598079
EMP3	4.958333333	54.22846154	10.93683258	0.094824097
DGKH	0.081666667	6.36	77.87755102	0.094868476
CTNNBIP1	0.03	7.819230769	260.6410256	0.095187154
RNF141	0.951666667	42.28615385	44.43378688	0.095319
SDC2	5.173333333	51.55692308	9.965900079	0.095348192
CDYL	2.603333333	28.02076923	10.76341968	0.095410278
MTMR3	1.261666667	22.02461538	17.45676252	0.095537086
2310003H01RIK	0.46	45.96615385	99.9264214	0.09571754
MREG	0.04	3.934615385	98.36538462	0.095767806
DOT1L	4.913333333	12.80461538	2.606095397	0.095898107
PLEKHA1	20.235	77.14384615	3.812396647	0.096096971
VPS8	0.211666667	5.752307692	27.17625681	0.09615618
H2AFJ	3.13	8.043846154	2.569918899	0.09616433
GTF3C3	1.79	10.75769231	6.009883971	0.096236639
FZD7	0.318333333	1.48	4.64921466	0.096282298
HAUS2	16.255	55.17692308	3.39445851	0.096282605
LRRC42	14.73	56.98	3.868295995	0.096307002
PUS7L	0	19.13615385	#DIV/0!	0.096501578
DDX49	7.923333333	58.56307692	7.391217113	0.096688134
ARRB2	0.415	2.435384615	5.868396664	0.096710155
CUTC	0.468333333	47.42076923	101.2543115	0.096772584
RRAGA	0.716666667	28.74	40.10232558	0.096773662
HAS2	0	7.902307692	#DIV/0!	0.097054884
CUX1	3.91	31.22923077	7.987015542	0.09705562
ACOT8	0	9.064615385	#DIV/0!	0.097142277
ZFP58	0.86	19.77076923	22.98926655	0.097225082
EFNA1	152.66	317.8	2.081750295	0.097431704
PACS2	4.711666667	16.33846154	3.467660744	0.097437511
5730403B10RIK	19.50666667	57.61307692	2.953507019	0.097515148

TABLE 4-continued

Genes Enriched in P1 (arterial endothelial cells) vs. P3 (venous cells)

SYMBOL	Average Venous	Average arterial	Arterial/Venous	p
RND3	22.19166667	111.0853846	5.00572518	0.097576798
CCDC28A	0	2.475384615	#DIV/0!	0.097626798
1500009L16RIK	2.046666667	18.86615385	9.217990479	0.097677532
SOX18	26.20333333	69.22769231	2.641942207	0.097679365
1700025G04RIK	2.005	5.525384615	2.755802801	0.097747959
KLF4	9.098333333	38.94846154	4.280834754	0.097779938
PDLIM1	4.161666667	54.08384615	12.99571794	0.097813795
RELN	0	2.663846154	#DIV/0!	0.098090859
LETMD1	0.741666667	42.64461538	57.49835782	0.098178385
PYCR2	13.975	51.68	3.6980322	0.098386231
BCAS3	0.095	10.15615385	106.9068826	0.098499825
CHN1	6.918333333	28.86538462	4.172303245	0.09863188
LDLR	1.35	5.981538462	4.430769231	0.098711275
PKD2L2	0.35	2.766153846	7.903296703	0.098945217
MTAP4	25.88333333	60.14076923	2.323532617	0.098988923
ROBO2	0	4.482307692	#DIV/0!	0.099108054
COPZ2	0.541666667	22.39692308	41.34816568	0.09915995
INTS7	36.29333333	85.40307692	2.353134008	0.099393182
AGPAT4	17.75	47.84769231	2.695644637	0.099419301
CFL2	44.70166667	105.5246154	2.360641633	0.099522782
CD2AP	39.62166667	93.75153846	2.366168472	0.099591675
ZBTB10	3.166666667	28.44923077	8.983967611	0.099729084
1810008A18RIK	0.483333333	21.95	45.4137931	0.099791657
TRIOBP	2.751666667	29.92	10.87341005	0.099835126
9030025P20RIK	7.903333333	17.32538462	2.192161697	0.099870069
CRY2	2.626666667	14.22153846	5.414291292	0.099894165
DDX26B	9.481666667	25.61538462	2.701569831	0.099983822

We claim:

1. A method of obtaining human arterial endothelial cells, the method comprising

culturing human mesodermal cells in a serum-free, albumin-free, chemically defined culture medium that is free of insulin and comprises a fibroblast growth factor (FGF), a vascular endothelial growth factor (VEGF), a Notch agonist, a TGF-beta inhibitor, and an inhibitor of inositol monophosphatase for about 6 days until a cell population comprising at least 80% human Ephrin B2 (EFNB2)-positive arterial endothelial cells and comprising fewer than 20% EphB4+ cells is obtained.

2. The method of claim **1**, wherein the mesodermal cells express one or more mesodermal markers selected from the group consisting of Brachyury (T), EMOS, FOXA2, MIXL1, MSX1, and MSX2.

3. The method of claim **1**, wherein the human mesodermal cells are obtained by culturing human pluripotent stem cells for a period of about two days in a serum-free, albumin-free, chemically defined cell culture medium comprising a Bone Morphogenetic Protein (BMP), Activin A, and an activator of Wnt/β-catenin signaling to obtain a cell population comprising mesodermal cells.

4. The method of claim **3**, wherein the mesodermal cells express one or more mesodermal markers selected from the group consisting of Brachyury (T), EMOS, FOXA2, MIXL1, MSX1, and MSX2.

5. The method of claim **3**, wherein the pluripotent stem cells are human embryonic stem cells or human induced pluripotent stem cells.

6. The method of claim **3**, wherein the activator of Wnt/β-catenin signaling is a Gsk3 inhibitor.

7. The method of claim **6**, wherein the Gsk3 inhibitor is selected from the group consisting of CHIR 99021, CHIR 98014, BIO-acetoxime, BIO, LiCl, SB 216763, SB 415286, AR A014418, 1-Azakenpaullone, and Bis-7-indolylmaleimide.

8. The method of claim **1**, wherein the Notch agonist is selected from the group consisting of Resveratrol (3,4',5-trihydroxystilbene), valproic acid, and suberoyl bishydroxamic acid.

9. The method of claim **1**, wherein the TGF-beta inhibitor is SB431542.

10. The method of claim **1**, wherein the inhibitor of inositol monophosphatase is L-690,330.

11. A kit for obtaining a cell population comprising at least 80% arterial endothelial cells, the kit comprising: (i) a serum-free, albumin-free, chemically defined culture medium suitable for differentiation of mesodermal cells into arterial endothelial cells, wherein the culture medium is free of insulin and comprises a fibroblast growth factor (FGF), a vascular endothelial growth factor (VEGF), a Notch agonist, a TGF-beta inhibitor, and an inhibitor of inositol monophosphatase; and (ii) instructions describing a method for differentiating human mesodermal cells into a cell population comprising at least 80% arterial endothelial cells, the method comprising human mesodermal cells for about six days in the serum-free, albumin-free, chemically defined culture medium until a cell population comprising, at least 80% EFNB2+ human arterial endothelial cells and comprising fewer than 20% EphB4+ cells.

12. The kit of claim **11**, further comprising:

(a) a serum-free, albumin-free, chemically defined culture medium suitable for differentiation of human pluripotent stem cells into human mesodermal cells, the culture medium comprising a BMP, Activin A, and an activator of Wnt/β-catenin signaling; and

(b) instructions describing a method for differentiating human pluripotent stem cells into human mesodermal cells, the method employing the culture medium of (a).

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