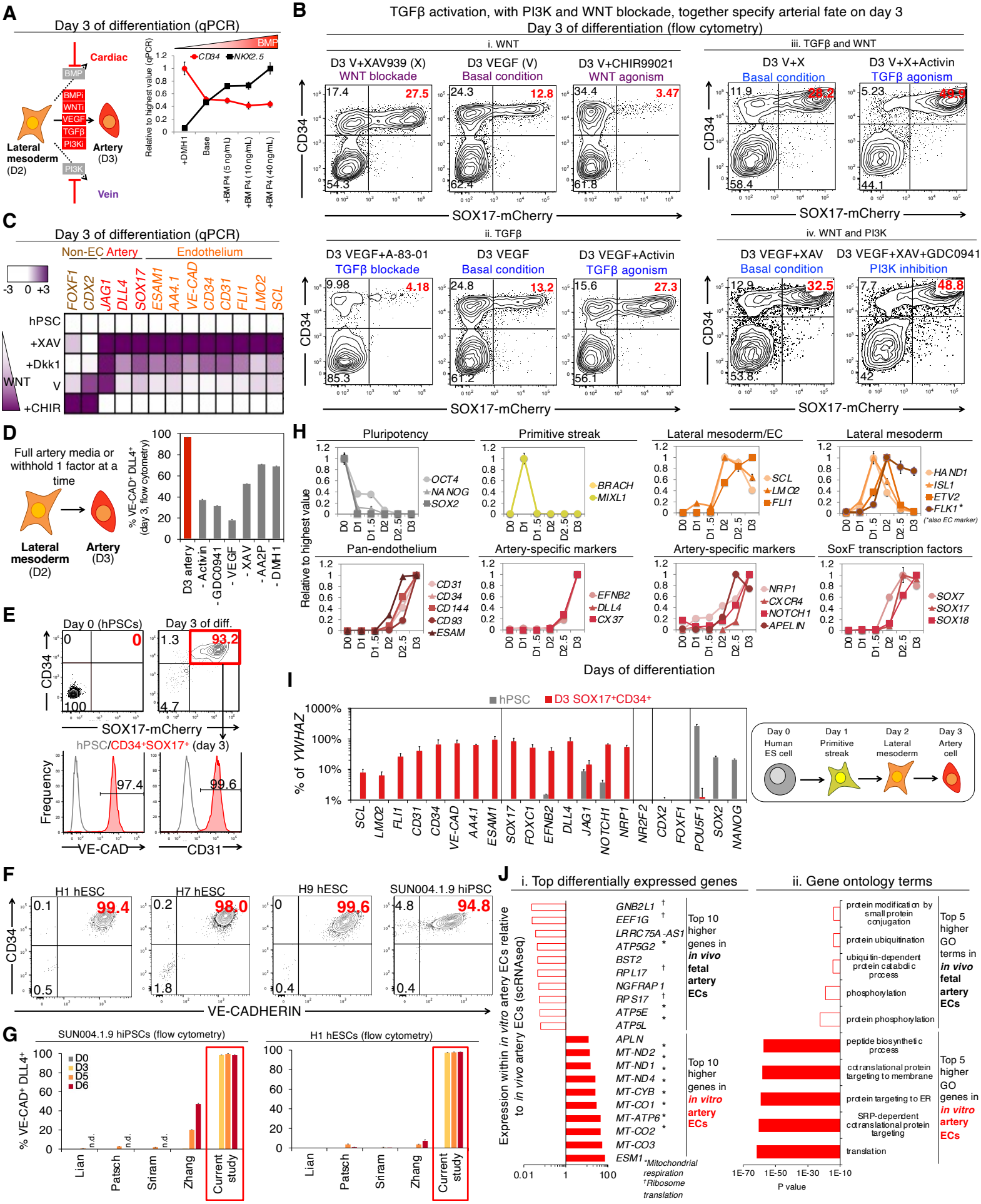
Figure S2


A
Day 3 of differentiation (qPCR)
Cardiac
BMP
BMPi
WNTi
VEGF
TGFβ
PI3Ki
PI3K
Lateral mesoderm (D2)
Artery (D3)
Vein
Relative to highest value (qPCR)
CD34
NKX2.5
+DMH1
Base
+BMP4 (5 ng/mL)
+BMP4 (10 ng/mL)
+BMP4 (40 ng/mL)
B
TGFβ activation, with PI3K and WNT blockade, together specify arterial fate on day 3
Day 3 of differentiation (flow cytometry)
i. WNT
D3 V+XAV939 (X)
WNT blockade
D3 VEGF (V)
Basal condition
D3 V+CHIR99021
WNT agonism
ii. TGFβ
D3 VEGF+A-83-01
TGFβ blockade
D3 VEGF
Basal condition
D3 VEGF+Activin
TGFβ agonism
iii. TGFβ and WNT
D3 V+X
Basal condition
D3 V+X+Activin
TGFβ agonism
iv. WNT and PI3K
D3 VEGF+XAV
Basal condition
D3 VEGF+XAV+GDC0941
PI3K inhibition
CD34
SOX17-mCherry
C
Day 3 of differentiation (qPCR)
Non-EC Artery
Endothelium
FOXF1
CDX2
JAG1
DLL4
SOX17
ESAM1
AA4.1
VE-CAD
CD34
CD31
FLI1
LMO2
SCL
hPSC
+XAV
+Dkk1
V
+CHIR
WNT
D
Full artery media or withhold 1 factor at a time
Lateral mesoderm (D2)
Artery (D3)
% VE-CAD+ DLL4+ (day 3, flow cytometry)
D3 artery
- Activin
- GDC0941
- VEGF
- XAV
- AA2P
- DMH1
E
Day 0 (hPSCs)
Day 3 of diff.
CD34
SOX17-mCherry
hPSC/CD34+SOX17+ (day 3)
Frequency
VE-CAD
CD31
F
H1 hESC
H7 hESC
H9 hESC
SUN004.1.9 hiPSC
CD34
VE-CADHERIN
G
SUN004.1.9 hiPSCs (flow cytometry)
H1 hESCs (flow cytometry)
% VE-CAD+ DLL4+
D0
D3
D5
D6
Lian
Patsch
Sriram
Zhang
Current study
H
Pluripotency
Primitive streak
Lateral mesoderm/EC
Lateral mesoderm
Pan-endothelium
Artery-specific markers
Artery-specific markers
SoxF transcription factors
Relative to highest value
OCT4
NANOG
SOX2
BRACH
MIXL1
SCL
LMO2
FLI1
HAND1
ISL1
ETV2
FLK1*
(*also EC marker)
CD31
CD34
CD144
CD93
ESAM
EFNB2
DLL4
CX37
NRP1
CXCR4
NOTCH1
APELIN
SOX7
SOX17
SOX18
Days of differentiation
I
hPSC
D3 SOX17+CD34+
% of YWHAZ
SCL
LMO2
FLI1
CD31
CD34
VE-CAD
AA4.1
ESAM1
SOX17
FOXC1
EFNB2
DLL4
JAG1
NOTCH1
NRP1
NR2F2
CDX2
FOXF1
POU5F1
SOX2
NANOG
Day 0 Human ES cell
Day 1 Primitive streak
Day 2 Lateral mesoderm
Day 3 Artery cell
J
i. Top differentially expressed genes
Expression within in vitro artery ECs relative to in vivo artery ECs (scRNAseq)
GNB2L1
EEF1G
LRRC75A-AS1
ATP5G2
BST2
RPL17
NGFRAP1
RPS17
ATP5E
ATP5L
APLN
MT-ND2
MT-ND1
MT-ND4
MT-CYB
MT-CO1
MT-ATP6
MT-CO2
MT-CO3
ESM1
Top 10 higher genes in in vivo fetal artery ECs
Top 10 higher genes in in vitro artery ECs
*Mitochondrial respiration
†Ribosome translation
ii. Gene ontology terms
protein modification by small protein conjugation
protein ubiquitination
ubiquitin-dependent protein catabolic process
phosphorylation
protein phosphorylation
peptide biosynthetic process
cotranslational protein targeting to membrane
protein targeting to ER
SRP-dependent cotranslational protein targeting
translation
Top 5 higher GO terms in in vivo fetal artery ECs
Top 5 higher GO genes in in vitro artery ECs
P value