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Supplemental Information

Meso-Endothelial Bipotent Progenitors from Human Placenta Display Distinct Molecular and Cellular Identity

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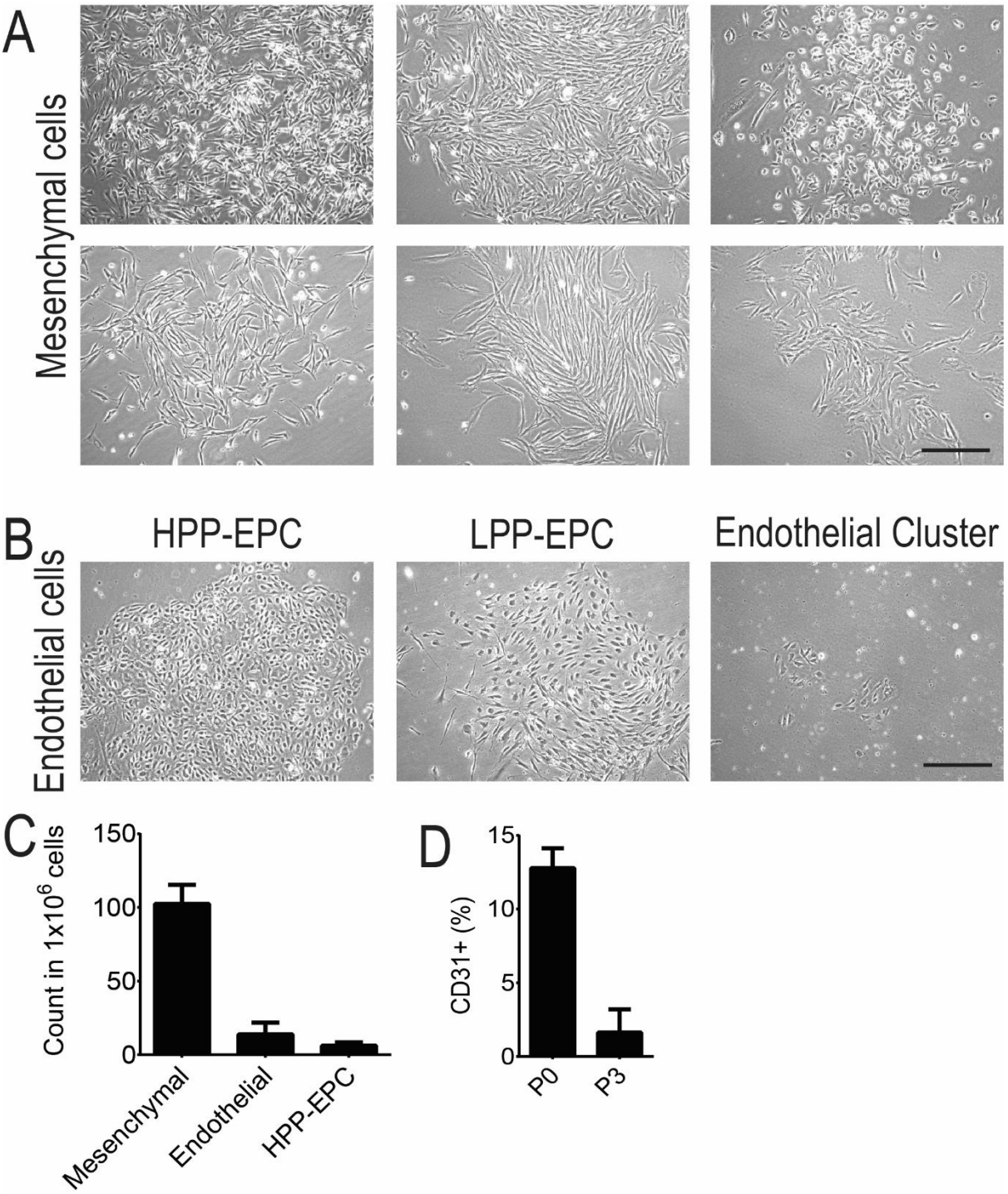
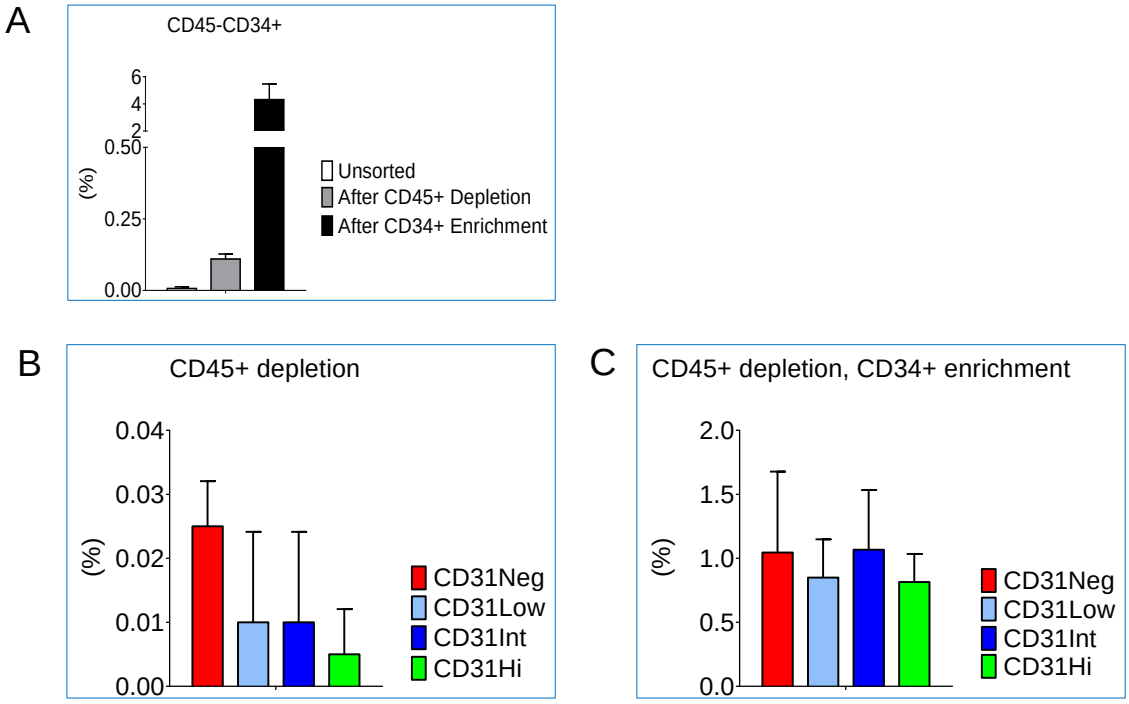
Supplemental Information List

Supplemental Figures

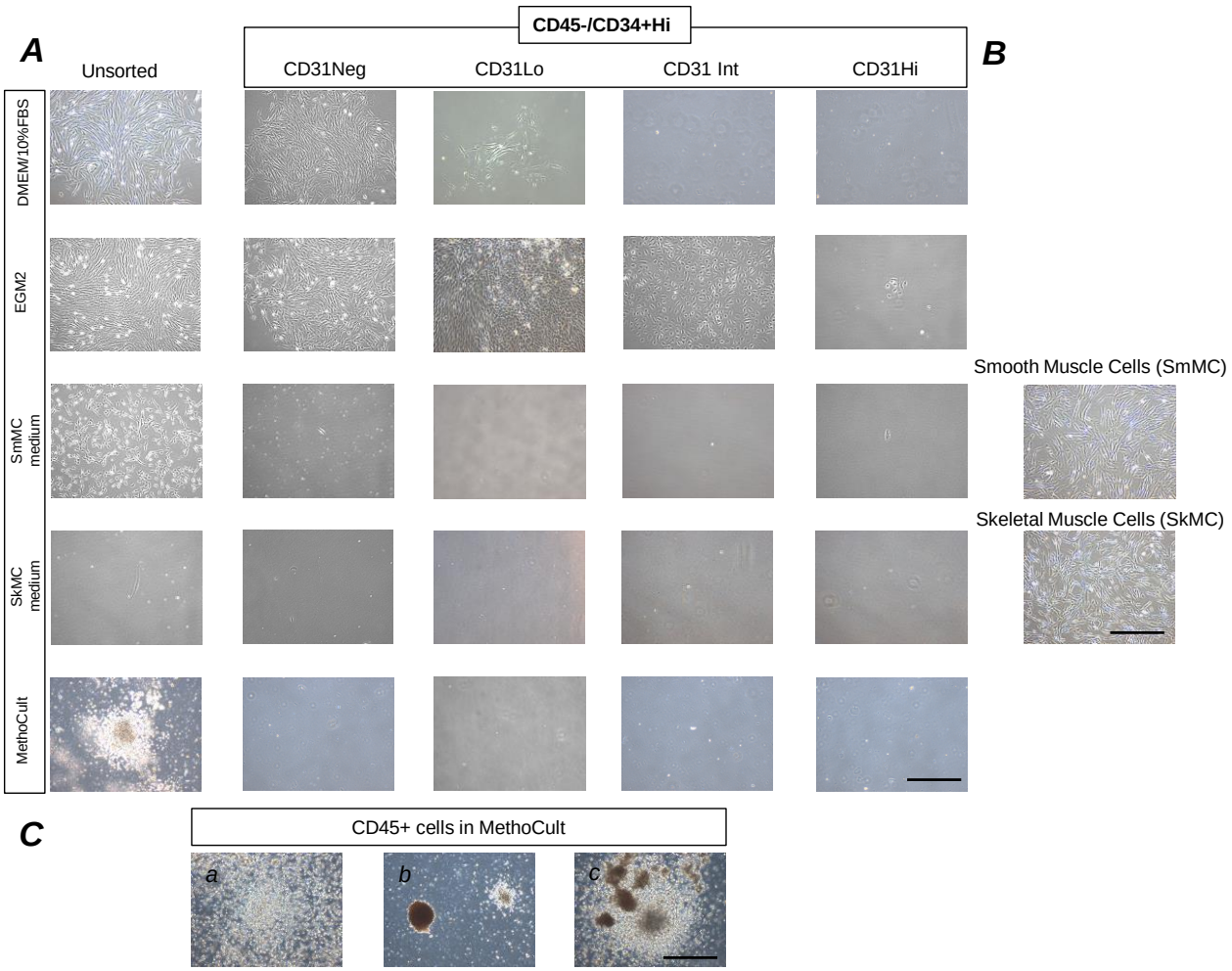


Supplemental Figure 1 (related to Figure 1): Persistent culturing unsorted placental cells resulted in maternal mesenchymal stem cells.

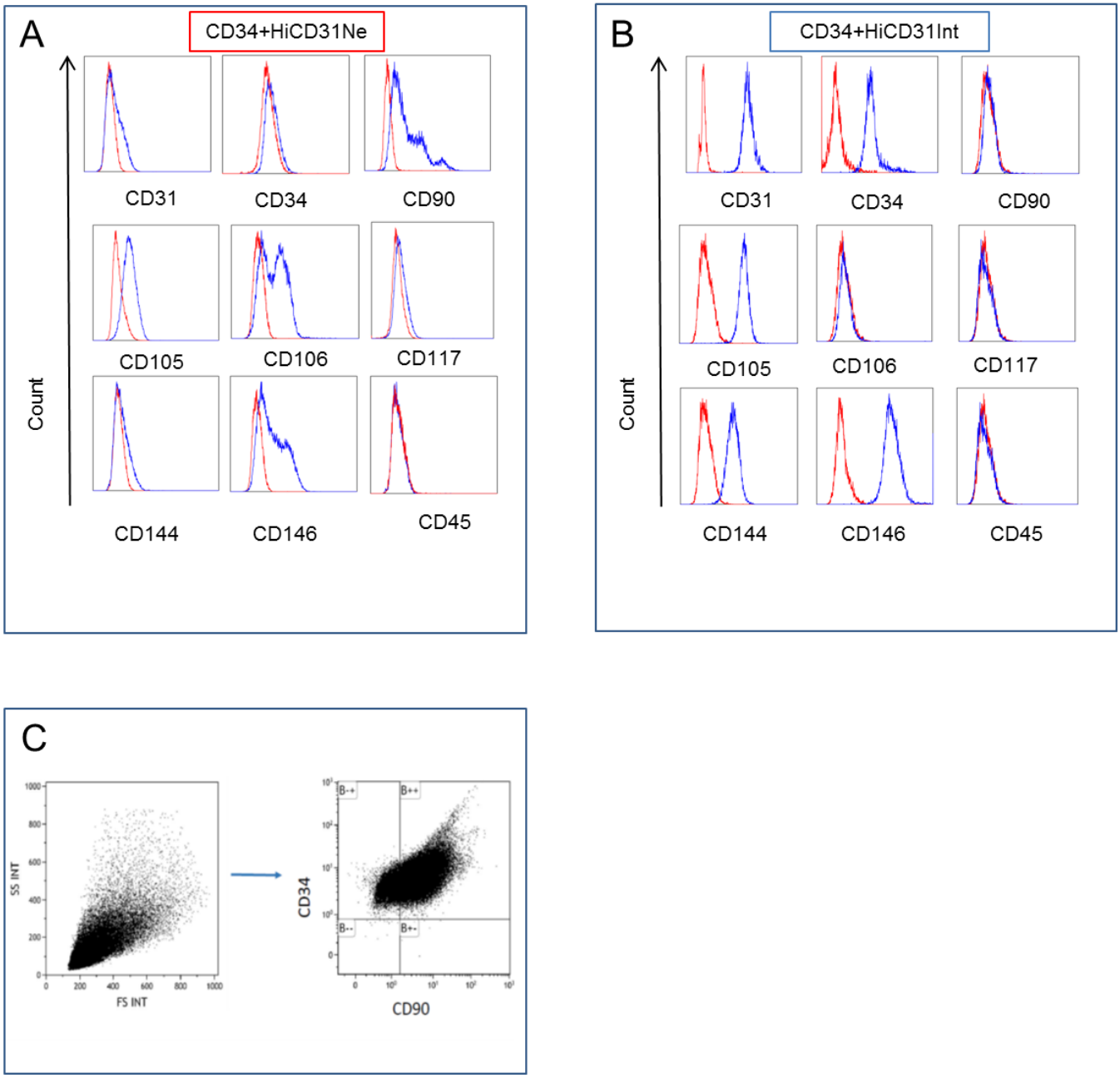
A-B) Unsorted term placental cells were cultured in endothelial growth medium, and this resulted in harvesting both A) mesenchymal and B) endothelial cells, including high proliferative potential-(HPP), low proliferative potential (LPP-) endothelial progenitor cells (EPC) and endothelial clusters at primary culture. C) Placental unsorted population harbours a small HPP-EPC fraction (0.0066 +/- 0.001%). D) Flow cytometry confirmed 12.36 +/- 3.92 (%) of unsorted placental cells could express CD31 at primary culture. Scale bar: 100µm.



Supplemental Figure 2 (related to Figure 1): The percentage distribution for each subpopulation at each step of sorting. The percentage distribution for each subpopulation was calculated using flow cytometry. A) The percentage of CD45-CD34+ population after CD45+ depletion and CD45+ depletion, CD34+ enrichment. B) The percentage distribution for each subpopulation after CD45+ depletion and CD45+ depletion, CD34+ enrichment. Data presented as mean +/- SD.

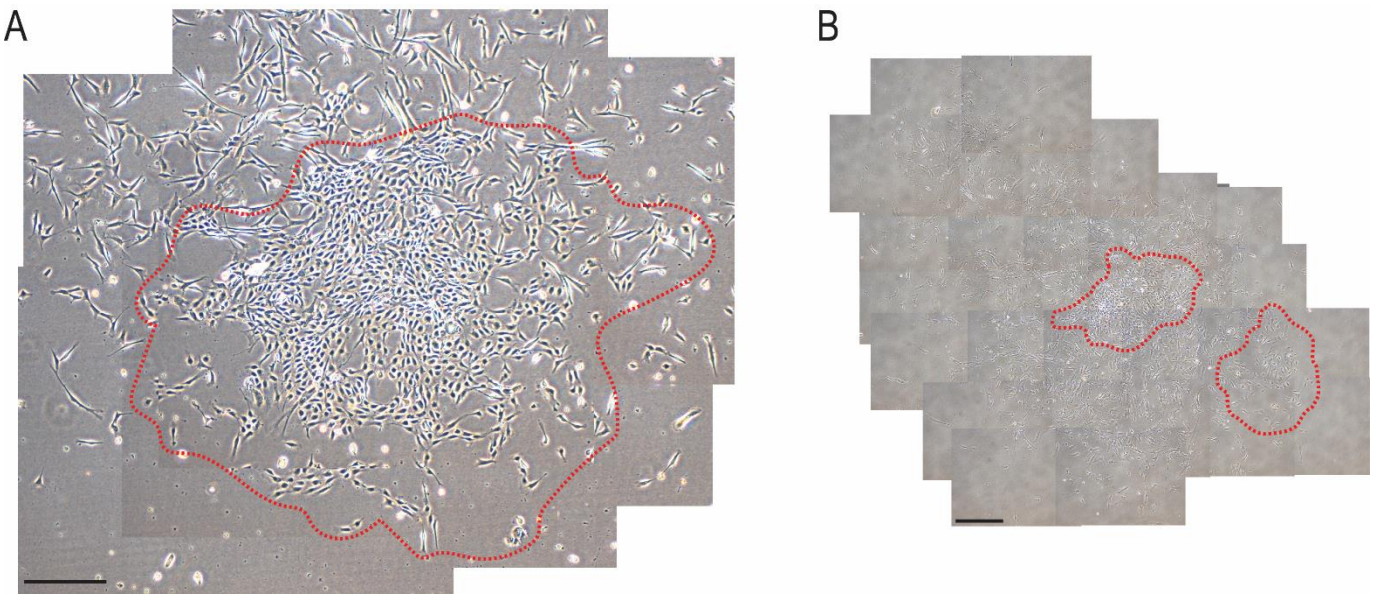


Supplemental Figure 3 (related to Figure 2): Characterization of placenta derived cells. A) Representative images for placental cells cultured in different culture media for 2-3 weeks. Placental cells cultured and monitored for 2-3 weeks. B) Primary skeletal and smooth muscle cells were cultured in their respective growth media. C) Placental CD45+ cells were used as positive control and gave rise to several types of hematopoietic colonies in Methocult, a) CFU-GM colony, b) Two BFU-E colonies one a compact and a small colony, c) A CFU-GM colony and several BFU-E colonies. EGM2: Endothelial growth medium, DMEM: Dulbecco's Modified Eagle Medium, FBS: fetal bovine serum, SmMC: Smooth muscle cell, SkMC: Skeletal muscle cell. Scale bar: 100µm.

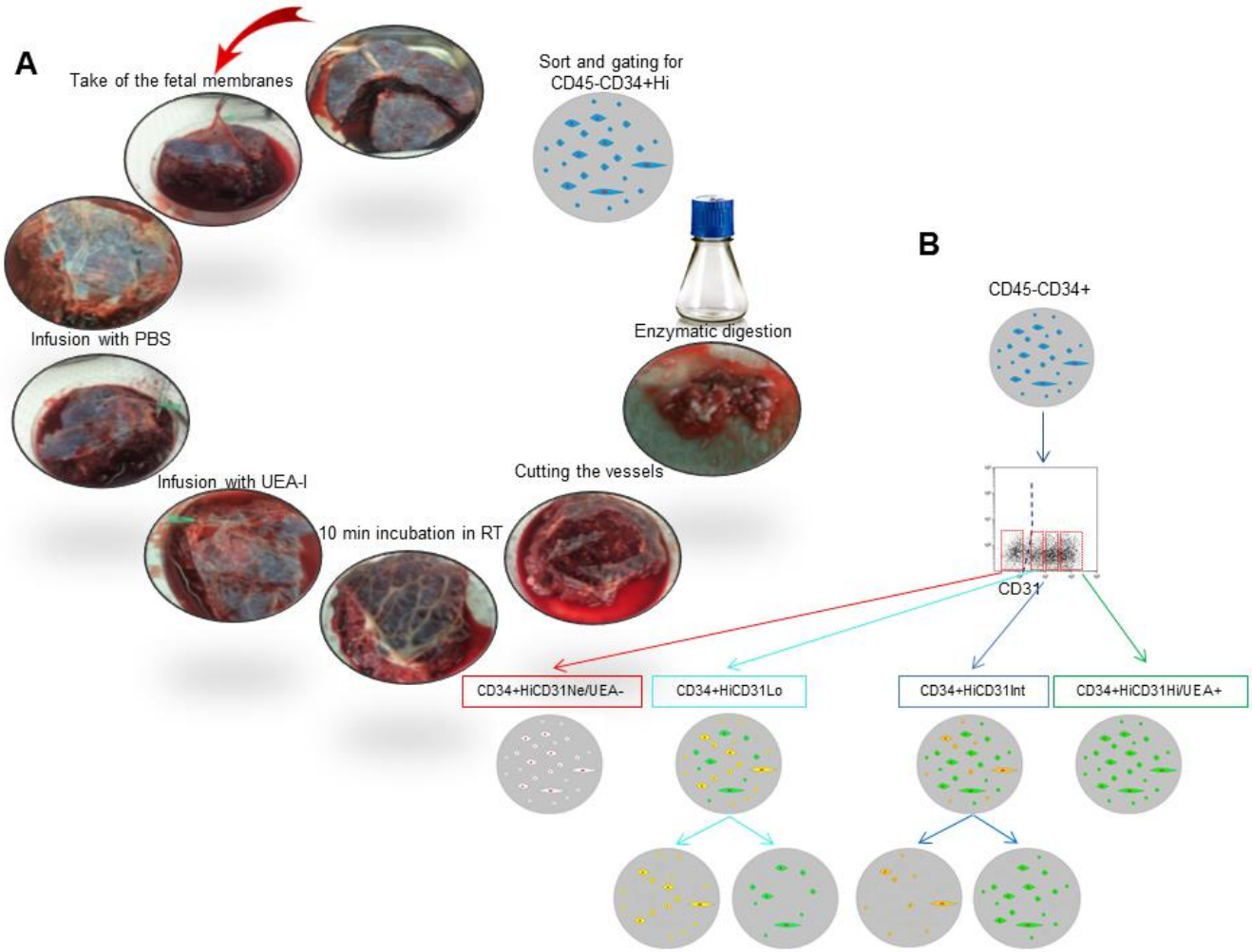


Supplemental Figure 4 (related to Figure 3): CD31Neg and CD31Int derived cells expressed mesenchymal and endothelial key markers respectively.

A) Flow cytometry analysis on colonies isolated from CD31Neg fraction using limiting dilatation assay at passage 1. B) Flow cytometry analysis on colonies isolated from CD31Int fraction using limiting dilution assay at passage 1. C) Dot blot graphs for flow cytometry analysis using multicolor approach (CD34 and CD90 antibodies) for CD31Lo subpopulation.

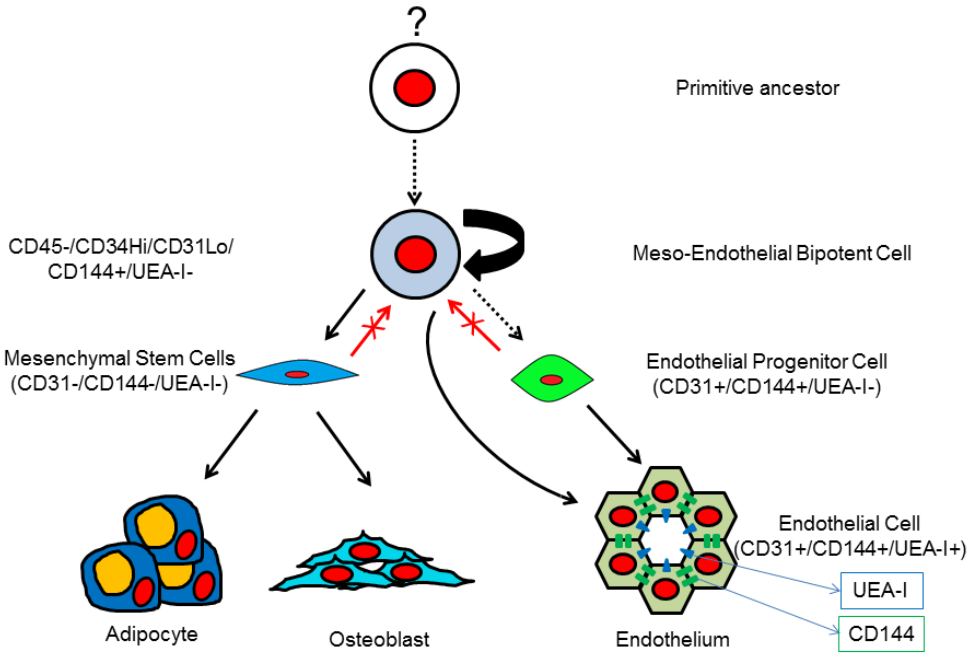


Supplemental Figure 5 (related to Figure 3) A, B) Representative images from CD31Low population at P1 derived from single colony, dashed lines define bipotential colonies surrounded by mesenchymal cells. (Scale bar: 100µm).

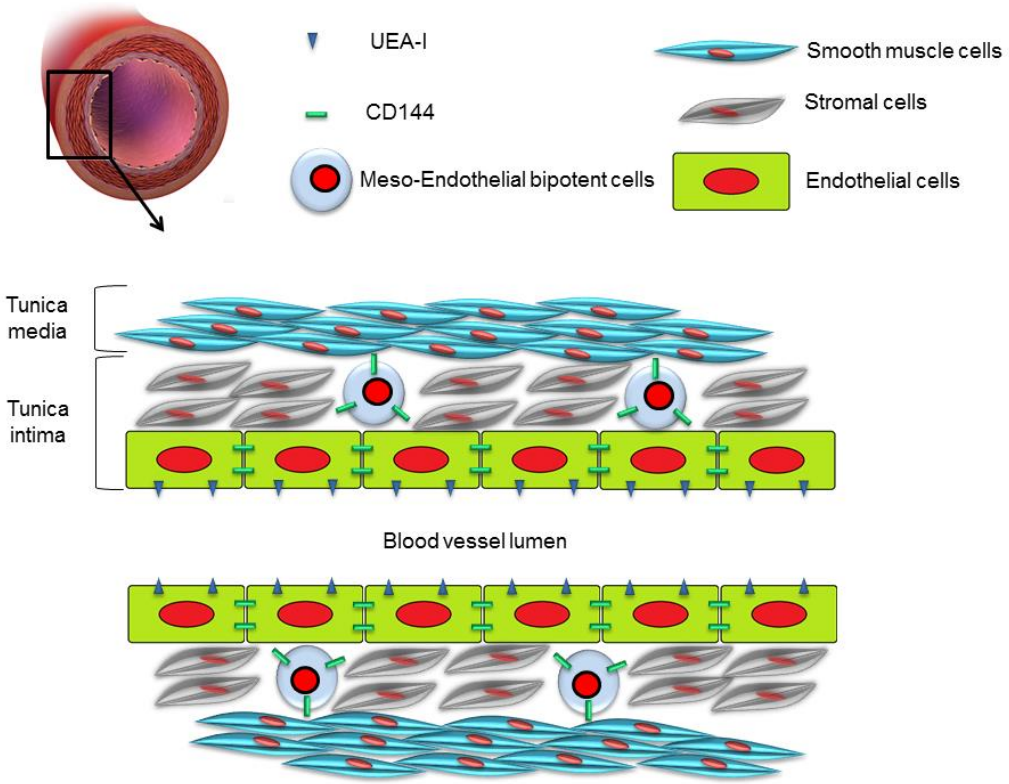


Supplemental Figure 6 (related to Figure 5). Perfusion of fetal placental arteries with FITC-conjugated UEA-I. A) Placental arteries firstly washed off with phosphate buffer saline and then perfused with FITC-conjugated UEA-I. After 10 minutes incubation, arteries were dissected from surrounding chorionic tissue and enzymatically digested and sorted as described. B) Schematic demonstration of CD45-CD34+ population stained for CD31 and UEA-I antibodies.

A


?
Primitive ancestor
CD45-/CD34Hi/CD31Lo/
CD144+/UEA-I-
Meso-Endothelial Bipotent Cell
Mesenchymal Stem Cells
(CD31-/CD144-/UEA-I-)
Endothelial Progenitor Cell
(CD31+/CD144+/UEA-I-)
Endothelial Cell
(CD31+/CD144+/UEA-I+)
UEA-I
CD144
Adipocyte
Osteoblast
Endothelium

B


UEA-I
CD144
Meso-Endothelial bipotent cells
Smooth muscle cells
Stromal cells
Endothelial cells
Tunica
media
Tunica
intima
Blood vessel lumen

Supplemental Figure 7 (related Figures 3, 4 and 5). A) Schematic image demonstrating an *in vivo* populations of foetal progenitors (Meso-Endothelial bipotent cell) that can give rise to endothelial, mesenchymal or both types of colonies. B) Schematic image demonstrating that the Meso-Endothelial bipotent progenitors are closely associated with the vasculature but not in contact with the circulation.

Supplemental Tables

Antibody	**Company/Catalogue No**
anti human CD31-V450	BD Biosciences (561653)
anti human CD34-phycoerythrin (PE)	AbD Serotec (MCA1578PE)
anti human CD45-fluorescein isothiocyanate (FITC)	Bio-Legend (304006)
anti human CD45-PE/CY7	Bio-Legend (304016)
anti human CD73-APC	BD Biosciences (560847)
anti human CD90-FITC	BD Biosciences (MCA90F)
anti human CD105-FITC	BD Biosciences (560839)
anti human CD106-FITC	AbD Serotec (MCA907F)
anti human CD117-PE	Abcam (12-1179)
anti human CD146-FITC	BD Biosciences (MCA2141F)
anti human CD144-FITC	BD Biosciences (560411)
7-amino-actinomycin D (7-AAD)	BD Biosciences (559925)

Supplemental Table 1 (related to Figures 1-7). Antibodies used for flow cytometry and FACS.

Endothelial Growth Medium-2 (EGM™-2 Medium)			
Company	Cat. No.	Product	Supplements
Lonza	CC-4176	EGM™-2 SingleQuots™ Kit	Epidermal Growth Factor, Vascular Endothelial Growth Factor, R3-Insulin-like Growth Factor-1, Ascorbic Acid, Hydrocortisone, Fibroblast Growth Factor-Beta, Heparin, Fetal Bovine Serum, Gentamicin/Amphotericin-B, EBM™-2 (Basal Medium).
Mesenchymal Stem Cell Medium			
Gibco	11995073	Dulbecco's modified Eagle's medium (DMEM)	Fetal Bovine Serum, L-Glutamine, High glucose, Pyruvate
Smooth Muscle Cell Medium			
Promocell	C-39262	Smooth Muscle Cell Growth Medium 2	Fetal Calf Serum, Epidermal Growth Factor, Basic Fibroblast Growth Factor, Insulin, Smooth Muscle Cell Basal Medium 2.
Skeletal Muscle Cell Medium			
Promocell	C-39360	Skeletal Muscle Cell Growth Medium	Fetal Calf Serum, Fetuin, Epidermal Growth Factor, Basic Fibroblast Growth Factor, Insulin, Dexamethasone, Skeletal Muscle Cell Basal Medium.
MethoCult™			
Stemcell technology	H4034	MethoCult™ H4034 Optimum	Methylcellulose in Iscove's MDM Fetal Bovine Serum Bovine Serum Albumin 2-Mercaptoethanol Stem Cell Factor GM-CSF G-CSF IL-3 Erythropoietin Other Supplements

Supplemental Table 2 (related to Figure 2). Specific media used to assess lineage differentiation potential.

Primary Antibodies		
Antibody	**Company/Catalogue No**	**Dilution**
anti-human CD34	BD Biosciences, USA (MCA1578PE)	(1:250)
anti-human CD144	BD Biosciences, USA (560411)	(1:200)
anti-human CD31	BD Biosciences, USA (561653)	(1:250)
anti-human CD146	EBioscience, USA (MCA2141F)	(1:200)
anti-human CD45	Biolegend, USA (304016)	(1:200)
anti-human Vimentin	Abcam, USA (ab24525)	(1:200)
anti-human alpha smooth muscle Actin(α-SMA)	Abcam, USA (ab5694)	(1:200)
FITC–conjugated Ulex europaeus agglutinin I (UEA-I)	Vector Laboratories, USA (B-1065)	(1:100)
anti-human fibroblast surface protein1 (FSP1)	Abcam, USA (ab11333)	(1:100)
Anti-HEY1	Abcam, USA (ab154077)	(1:200)
Anti-NOTCH1	Abcam, USA (ab52627)	(1:200)
Secondary Antibodies		
Alexa 488 goat anti-rabbit	Invitrogen, USA (A11034)	(1:1,000)
Alexa 488 goat anti-mouse	Invitrogen, USA (A11029)	(1:1,000)
Alexa 568 goat anti-rabbit	Invitrogen, USA (A11036)	(1:1,000)
Alexa 568 goat anti-rat	Invitrogen, USA (A11077)	(1:1,000)
Alexa 568 goat anti-mouse	Invitrogen, USA (A11004)	(1:1,000)

Supplemental Table 3 (related to figures 2, 3, 5, and 6). List of antibodies used for immunohistochemistry and immunocytochemistry.

A)

Primer	Forward (5'-3')	Reverse (5'-3')
CD34	CCTAAGTGACATCAAGGCAGAA	GCACAGCTGGAGGTCTTATT
CD31	TTGGCGGTGGGAAGCTTAC	GCTCTCTGTTAGTCTGCATCTC
CD144	AAGCGTGAGTCGCAAGAATG	TCTCCAGGTTTTCGCCAGTG
ACTA2	AAAAGACAGCTACGTGGGTGA	GCCATGTTCTATCGGGTACTTC
PDGFRB	AGCACCTTCGTTCTGACCTG	TATTCTCCCGTGTCTAGCCCA
HES1	GTCAACACGACACCGGATAA	TTCAGCTGGCTCAGACTTTC
HEY1	TGGAGAGGCGCCGCTGTAGTTA	CAAGGGCGTGCGCGTCAAAGTA
RUNX2	TGGTTACTGTCATGGCGGGTA	TCTCAGATCGTTGAACCTTGCTA
THY1	ATCGCTCTCCTGCTAACAGTC	CTCGTACTGGATGGGTGAACT
GREM2	ATCCCCTCGCCTTACAAGGA	TCTTGCACCAGTCACTCTTGA
SCUBE3	CAGAACACCCCGAGGTCATAC	GCCAGGGATGTTGACACAGTC
SPP1	GAAGTTTCGCAGACCTGACAT	GTATGCACCATTCAACTCCTCG
SOX9	AGCGAACGCACATCAAGAC	CTGTAGGCGATCTGTTGGGG
SOX17	GTGGACCGCACGGAATTTG	GGAGATTCACACCGGAGTCA
MUC12	CCAGTTCAAGCGACCCTTTTA	CGCTGTGGGATACTGTTGATT
DES	TCGGCTCTAAGGGCTCCTC	CGTGGTCAGAAACTCCTGGTT
CSPG4	AGGACGAAGGAACCCTAGAGT	CACAGGCACACTGTTGTGGA
GAPDH	GGTGAAGGTCGGAGT	CAAAGTTGTCATGGA

B)

	CSPG4			*MUC12*		
Population	CD31Neg	CD31Low	CD31Int	CD31Neg	CD31Low	CD31Int
Placenta 1	+	-	-	+	-	-
Placenta 2	+	-	-	+	-	-
Placenta 3	+	-	-	+	-	-

Supplemental Table 4 (related to Figure 4): A) Sequence of primers used for qRT-PCR. B) Only CD31Neg population could highly express pericyte key markers, chondroitin sulfate proteoglycan 4 (*CSPG4*) and *MUC12* as confirmed by qRT-PCR.

Supplemental Table 5 (related to Figure 4). List of genes differentially expressed between CD31Neg and CD31Int populations.

Supplemental Table 6 (related to Figure 4). List of genes differentially expressed between CD31Int and CD31Lo populations.

Supplemental Table 7 (related to Figure 4). List of genes differentially expressed between CD31Lo and CD31Neg populations.

Supplemental methods

Ethics statement

Human term placentas were obtained after written informed consent from mothers with term healthy singleton pregnancies undergoing elective caesarean section at the Royal Brisbane and Women's Hospital (RBWH, Brisbane, Queensland). This protocol was approved by Metro North Human Research Ethics Committee and The University of Queensland Ethics Committee.

Immunocytochemistry (ICC) and immunohistochemistry (IHC)

For ICC assay, placental isolated populations at passage 2 were cultured in the Nunc® Lab-Tek® Chamber Slides (Sigma) in EGM2 and then fixed with 1% paraformaldehyde (PFA) for 15 minutes at 4°C and stained with anti-human CD34, vimentin, alpha smooth muscle actin (alpha-SMA), FITC–conjugated Ulex europaeus agglutinin I (UEA-I), CD45, CD31, and CD144 (Supp. Table 3). Slides were incubated for 2 hours with primary antibodies, then washed with PBS/0.5%Tween and incubated with secondary antibodies (Supp. Table 3) at room temperature (RT) for 45 minutes. Finally, slides were washed again with PBS/0.5%Tween and mounted with Prolong Gold reagent with DAPI (Invitrogen, CA) and observed under a Zeiss Axio microscope (Carl Zeiss, Germany).

Expression of CD144, SNAIL/SLUG (Abcam, Cat No: ab63371) Osteopontin (OPN; Abcam; Cat No: ab8448), and UEA-I in placental tissues was assessed using IHC. A piece of fresh placenta was washed with PBS and fixed in 4% PFA for 2h and then immersed in 20% sucrose (Sigma-Aldrich, USA) and finally embedded in Optimum Cutting Medium (OCT, Tissue-Tek, USA). 8-µm cryosections were prepared using a cryostat (Microm, Thermo Fisher, Germany). Slides were washed with PBS and incubated with 10% normal bovine serum for 20 min to block nonspecific binding. Then slides were stained with anti-human CD34 (1:100 dilution), SNAIL/SLUG (1:200), Osteopontin (OPN; 1:400) and CD144 (1:100 dilution) antibodies by standard IHC protocol. In independent experiments slides were stained with FITC–conjugated UEA-I. Finally, slides were mounted in Prolong Gold anti-fade reagent with DAPI and observed under a Zeiss Axio microscope or Nikon A1R confocal (Nikon, Japan).

Differentiation assay

Upon sorting, cells were cultured in EGM2 until passage 3. The media for mesenchymal cells isolated from CD45-CD34+CD31Neg and CD45-CD34+CD31Lo populations at passage 3 were switched to DMEM/10% FBS and when culture reached 85% confluence, media were changed to adipogenic, or osteogenic media as previously described (Chen et al., 2012). After 2 weeks, bone or adipocyte differentiated cells were fixed in 4% PFA at RT, washed and stained with Alizarin Red S (Sigma) or Oil Red O (Sigma) for the detection of mineralization or lipid droplets respectively. Control cells were cultured in DMEM/10% FBS and stained. To calculate the cell number after *in vitro* culture, 1.0 x 10^4 cells from each proliferative potential CD45+CD34+ subpopulation after isolation were flow sorted and cultured in EGM2 and numbers were calculated after 3 weeks.

Limiting dilution clonal analysis of sorted populations

To assess the population's characteristics at single colony level, limiting dilution clonogenic assays were performed. Cells freshly isolated upon flow-sorting were cultured in EGM2 media in the pre-coated collagen type-I 96-well plate with 10 cells per well density allowing single clones to grow. On day 14 of culture, positive wells were observed microscopically (Nikon Instruments, Japan), scored and then passaged for further expansion. Colonies were examined based on morphology and categorized into mesenchymal-like or cobblestone-like (endothelial) colonies. Endothelial colonies with fewer than 50 cells and no proliferation potential on passage were scored as endothelial clusters (EC), while colonies with 50-2000 cells and no proliferation potential were considered low proliferative potential-EPC (LPP-EPC). Lastly, colonies with more than 2000 cells and highly proliferation potential were deemed to be highly proliferative potential-EPC (HPP-ECFC).We calculated the probability of monoclonality (PoM) as previously described for limiting dilution experiments (Lietzke and Unsicker, 1985). PoM was calculated by using following equation:

$$\text{PoM} = \frac{Sln(S)}{S-1}$$

S = proportion of empty wells.

To assess the expression of endothelial and mesenchymal markers on cells at passage 0 using ICC, freshly isolated and sorted populations were cultured in the Nunc® Lab-Tek® Chambers with low confluency in EGM2. After 2 weeks slides were fixed with 1%PFA for 15 minutes at

4°C and stained with anti-UEA-1, CD31, anti-vimentin, anti- fibroblast surface protein1 (FSP), anti-HEY1, and anti-NOTCH 1 antibodies (Supp. Table. 3) as described above.

Flow cytometry

CD45-CD34+ cells upon MACS or cultured cells at different passages were suspended in MACS buffer and stained with following directly conjugated anti-human CD31, CD34, CD45, CD90, CD105 and CD146 antibodies. Samples were incubated for 20 minutes. Then cells were washed twice with MACS buffer, and about 50,000 events were acquired on a Gallios flow cytometer (Beckman Coulter, USA) with Kaluza flow cytometry analysis software (Beckman Coulter).

RNA-sequencing (RNA-seq) and quantitative real-time PCR (q-PCR) analysis

RNA-seq analysis was performed to assess the gene expression signature of isolated placental populations at transcript level Freshly sorted populations were cultured in EGM2 medium for two weeks. Then total RNA from CD45-CD34+CD31Neg, CD45-CD34+CD31Lo and CD45-CD34+CD31Int colonies at passage 0 was extracted using the RNA easy Plus Mini Kit (Qiagen, CA) according to manufacturer's instruction. The quality and quantity of isolated RNA was analysed with Agilent 2100 Bioanalyzer (Agilent Technologies, Mulgrave, Victoria).

RNA-seq libraries were prepared using the manufacturer's instructions followed by sequencing on Illumina's HiSeq 2500 (Illumina, CA) performed at the Garvan Institute (Sydney, Australia). Sequence data were aligned to GRCm38 with STAR (version 2.5.0c). The read counts were measured using the GENCODE gene annotation. Differential gene expression was measured with DESeq2 (Love et al., 2014). Genes with significant expression levels were selected by applied a false discovery rate-adjusted P value cut-off of $P < 0.05$, and a log fold change cut-off of 2.

Validation of differential expression of selected genes was carried out using q-PCR. Total RNA was extracted using the RNA easy Mini Kit and reverse transcribed to cDNA using the Superscript III Reverse Transcription Kit (Invitrogen, Mount Waverley, Australia). qPCR was conducted using 0.5 μM of forward and reverse primers (Supp. Table 4) with FastStart SYBR Green Master-mix per reaction (Roche Applied Sciences, CA) in a ABI 7700 Sequence Detection System with SDS v1.9 software (Applied Biosystems, Foster City, CA). All PCR

samples were run in triplicate from at least 3 independent placentas. GAPDH primers were used as housekeeping gene control.

Growth and proliferation of sorted populations in specified media

In independent experiments, cell sorted populations were cultured in specific media: DMEM with 10% FBS as a popular cell culture medium for MSC culture, (n=3 independent placentas), Smooth Muscle Cell Medium (n=3 independent placentas, Promocell, Germany), or Skeletal Muscle Cell Medium (n=3 independent placentas, Promocell, Germany) (Supp. Table 2). Human primary skeletal muscle (SkMC) and umbilical artery smooth muscle cells (HUASMC) used as positive control were purchased from Promocell (Germany). The hematopoietic capability of each population was also assessed by culturing each population in MethoCult for 2 weeks (n=3 independent placentas, Stem Cell Technologies, Canada) (Supp. Table 2). The $CD45^+$ population harvested from the same placenta served as a positive control for hematopoietic potential evaluation. Fluorescent In Situ Hybridisation (FISH) analysis was applied in male fetus pregnancies to assess the origin of cells as reported in a previous study (Patel et al., 2014).

The immunocytochemistry (ICC) and immunohistochemistry (IHC), differentiation assay, limiting dilution clonal analysis of sorted populations, RNA-sequencing (RNA-seq) and quantitative real-time PCR (q-PCR) analysis, and perfusion of placental vessels with FITC–conjugated UEA-I sections are available at Supplemental Experimental Procedures.

Assessment of NOTCH and transforming growth factor-β (TGF-β) pathway impact on ECFC function

To assess the impact of the NOTCH pathway on different cell's population isolated from placenta, populations were freshly isolated as indicated and incubated with DAPT (*N*-[(3,5-Difluorophenyl)acetyl]-L-alanyl-2-phenyl]glycine-1,1-dimethylethyl ester, Abcam, CA), a well described γ-secretase inhibitor, or DMSO (control). In current study, DAPT with 50μM concentration was used based on recent research in our laboratory as the optimum concentration to block the NOTCH pathway (Shafiee et al., 2016). In two weeks culture in EGM2 with DAPT or DMSO cells were observed under microscope (Nikon Instruments, Japan), scored and then passaged for further expansion. DAPT treatment assay was performed in 3 independent placentas.

For TGF-β pathway analysis, freshly isolated cells were incubated with SB431542 (10 μM), selective inhibitor of the TGF-β type I receptor (James et al., 2010). After two weeks culture in EGM2 with SB431542 or DMSO, cells were observed under the microscope (Nikon Instruments, Japan), scored and then passaged for further expansion, or RNA was harvested for qRT-PCR analysis. Colonies were examined based on morphology and categorized into mesenchymal-like or cobblestone-like (endothelial) colonies. TGF-β pathway assay was performed in 3 independent placentas.

Supplemental Results

Bipotential cells display vascular and mesenchymal molecular signature

In comparison to CD31Neg population, the CD31Int population had significantly higher expression of endothelial markers, VE-cadherin (vascular endothelial cadherin) (*CDH5*, 10.3x), CD31 (*PECAM1*, 9.5x), *CD34* (8.6x), Von Willebrand factor (*VWF*, 7.6x), and Tyrosine kinase with immunoglobulin-like and EGF-like domains 1 (*TIE1*, 7.2x) (Supp. Table 5). Many genes involved in endothelial function were also upregulated in the CD31Int population, such as *ERG1* (9.9x), *SEMA3G* (9.0x), Plasmalemma vesicle associated protein (*PLVAP*, 8.5x), Apelin (*APLN*, 7.4X), *SOX18* (6.8x), *SOX17* (6.6x), and *SEMA6B* (6.6x) (Supp. Table 5). Compared to the CD31Neg population, CD31Lo again displayed higher expression of genes involved in endothelium definition and function, such as *CDH5* (10.0x), *PECAM1* (9.2x), *CD34* (8.4x), *VWF* (6.8x), *TIE1* (6.6x), and Protocadherin-17 (*PCDH17*, 6.5), Protocadherin 12 (*PCDH12*, 5.9x) (Supp. Table 7). Higher expression of top genes was confirmed using qRT-PCR revealing much larger differences.

CD31Neg cells displayed higher expression of MSC markers: smooth muscle alpha (α)-2 actin (*ACTA2*, 2.3x), Runt-related transcription factor 2 (*RUNX2*, 2.2x), and membrane metallo-endopeptidase (*MME*, 4.4x) (Granéli et al., 2014) (Supp. Table 7). In addition, the CD31Neg population expressed high levels of MSC precursor genes(Barberi et al., 2005; Lian et al., 2007): platelet-derived growth factor receptor alpha (*PDGFRA*, 5.1x), *PDGFRB* (2.7x), fibroblast activation protein (*FAP*, 3.1), *WNT5A* (4.4x) (Barberi et al., 2005) and early chondrogenic and osteogenic marker genes Decorin (*DCN*, 6.8x)(Friedl et al., 2007), Lumican (*LUM*, 6.1x) (Friedl et al., 2007), and Inhibin Beta E Subunit (*INHBE*, 6.3x) (Martin et al., 2010) (Supp. Table 7).

Of interest, only CD31Neg population showed highly expressed key pericyte markers: chondroitin sulfate proteoglycan 4 (*CSPG4*) and *MUC12*, as confirmed by RNA-seq and qRT-PCR, *CSPG4* and *MUC12* expressions only were detectable in CD31Neg population (Supp Table 4). However, in comparison to the CD31Neg component, CD31Lo cells displayed higher expression of mesodermal progenitor genes, ISL LIM Homeobox 1(*ISL1*, 5.2x) (Moretti et al., 2006), Mesenchyme Homeobox 2 (*MEOX2* also known as *MOX2*, 9.9x) (Mankoo et al., 1999), Apelin Receptor (*APLNR*, 4.3x) (Vodyanik et al., 2010), and Endomucin (*EMCN*, 9x) (Vodyanik et al., 2010) (Supp. Table 7). We next examined specific pathways that have

previously been shown of importance in determination of progenitor function and the transition from progenitor to differentiated cells in the endothelium compartment(Orlova et al., 2014) (Patel et al., 2016). Of interest, our cell populations displayed differential expression of SOX genes as confirmed using qRT-PCR (Fig 4G).

Supplemental Discussion

During development, both endothelial cells and mesenchymal cells derive from mesodermal progenitors (Pardanaud et al., 1996). Using positive selection of Flk1 cells, Minasi et al. isolated a precursor from E9.5 mouse dorsal aorta which showed potential to differentiate into mesenchymal, blood, and endothelial lineage cells (Minasi et al., 2002). Our findings corroborate these observations in human term placenta. We demonstrated that bipotent progenitors as well as EPCs can be found in vascular structures *in vivo* as demonstrated by their expression of CD144 and through dissection of fetal arteries. A mesodermal progenitor with potential to acquire either a MSC and/or endothelial phenotype has also been characterized using human embryonic stem cells. In colony-forming assays the mesodermal precursors could express MSC markers and had multi-lineage capability upon differentiation including endothelial potential (Vodyanik et al., 2010). Orlova et al. derived functional ECs and pericytes from the same progenitor using human-induced pluripotent stem cells (Orlova et al., 2014). Although, the existence of an equivalent of a mesenchymoangioblast has not been reported *in vivo,* gene expression profiling suggested a lateral plate mesodermal or extra-embryonic mesodermal origin in accordance with our findings in placenta. Furthermore we show that this bipotential population's plasticity can persist in term placenta. Whether this population is similar to mesodermal precursors with endothelial capacity (MAPCs) (Reyes et al., 2002; Roobrouck et al., 2011)or meso-angioblasts isolated from muscle remains to be addressed. Recently, existence of a population with similar surface marker characteristics in adult human adipose stromal vascular tissue has been reported (SundarRaj et al., 2015).

A limitation of our study is the inability to perform fate mapping given the *in vivo* human setting of our experiments. Whether Meso-Endothelial bipotent progenitors give rise to both EPCs and mesenchymal progenitors *in vivo* is supported by their capacity to give rise to both types of colonies generated by each of their monopotent progenitors. It could thus be argued that our data are supportive of a hierarchy of plasticity with Meso-Endothelial progenitors at the top. However, colony formation and self-renewal of these colonies was not as robust as with the other progenitors. Although this may be due to the culture conditions used herein, our results cannot prove a hierarchy or a lineage relationship between bipotent progenitors and EPCs. In particular, we cannot fully rule out endothelial to mesenchymal transition. Indeed, the Meso-Endothelial progenitors entirely differentiated into MSCs after 3 passages. It could however be argued that EndoMT is highly unlikely in EGM2 medium and blocking TGFβ

signalling, known to be important in EndoMT(Birket et al., 2015; James et al., 2010), did not prevent the derivation of mesenchymal cells from bipotent progenitors.

Supplemental Reference

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