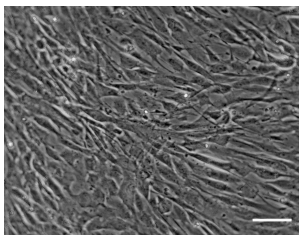
Figure S1

A



B

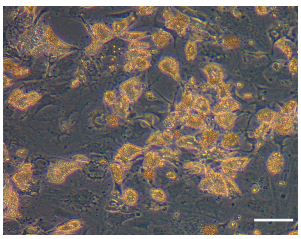


Figure S2

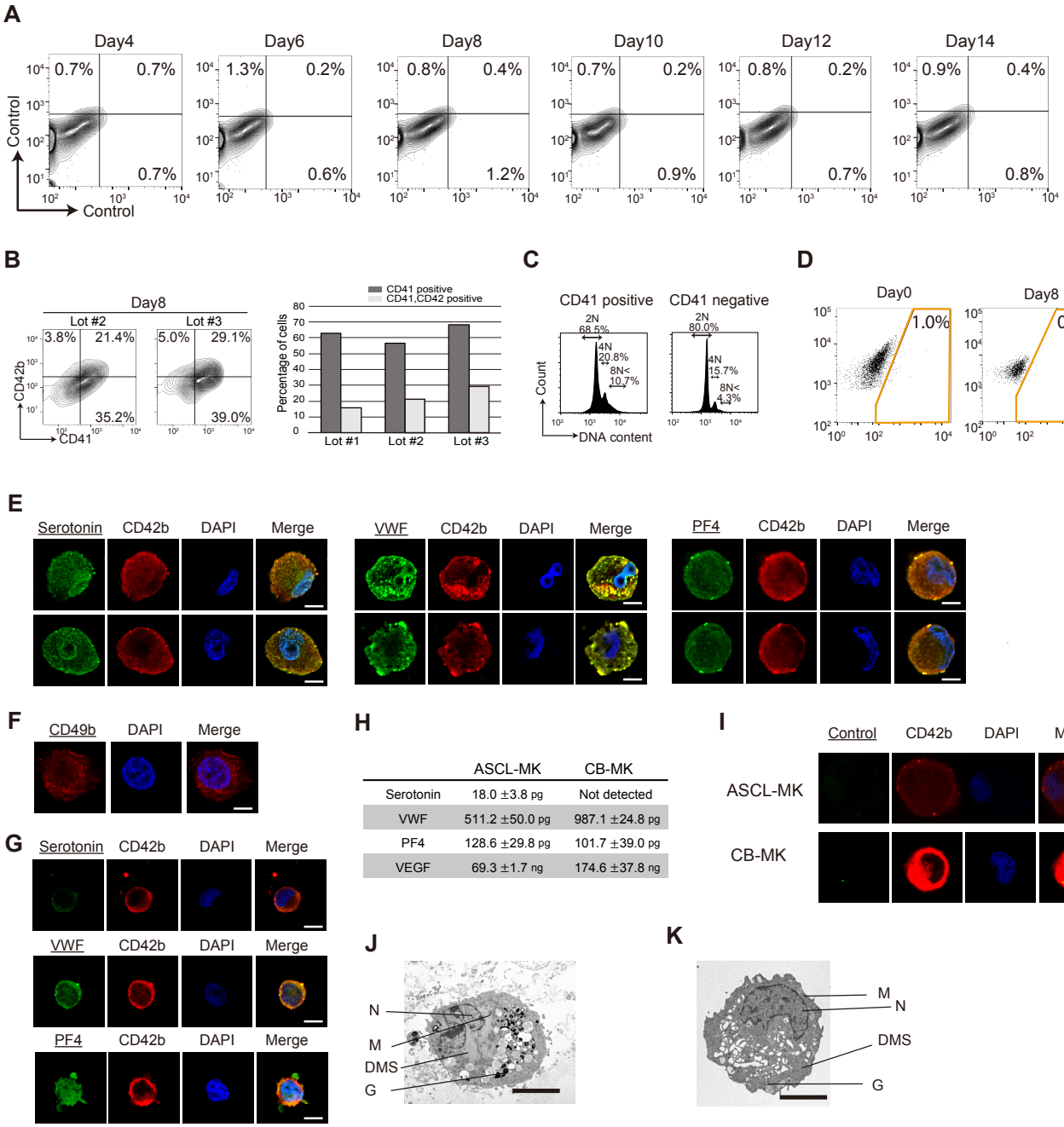

A
Day4
Day6
Day8
Day10
Day12
Day14
Control
0.7%
0.7%
0.7%
1.3%
0.2%
0.6%
0.8%
0.4%
1.2%
0.7%
0.2%
0.9%
0.8%
0.2%
0.7%
0.9%
0.4%
0.8%
B
Day8
Lot #2
Lot #3
3.8%
21.4%
35.2%
5.0%
29.1%
39.0%
CD42b
CD41
CD41 positive
CD41,CD42 positive
Percentage of cells
Lot #1
Lot #2
Lot #3
C
CD41 positive
CD41 negative
2N
68.5%
4N
20.8%
8N<
10.7%
2N
80.0%
4N
15.7%
8N<
4.3%
Count
DNA content
D
Day0
Day8
1.0%
0.8%
E
Serotonin
CD42b
DAPI
Merge
VWF
CD42b
DAPI
Merge
PF4
CD42b
DAPI
Merge
F
CD49b
DAPI
Merge
G
Serotonin
CD42b
DAPI
Merge
VWF
CD42b
DAPI
Merge
PF4
CD42b
DAPI
Merge
H
ASCL-MK
CB-MK
Serotonin
18.0 ±3.8 pg
Not detected
VWF
511.2 ±50.0 pg
987.1 ±24.8 pg
PF4
128.6 ±29.8 pg
101.7 ±39.0 pg
VEGF
69.3 ±1.7 ng
174.6 ±37.8 ng
I
Control
CD42b
DAPI
Merge
ASCL-MK
CB-MK
J
N
M
DMS
G
K
M
N
DMS
G

Figure S3

Sample	C_T Values	
	GAPDH	GATA1
K562	21.1±0.1	24.9±0.1

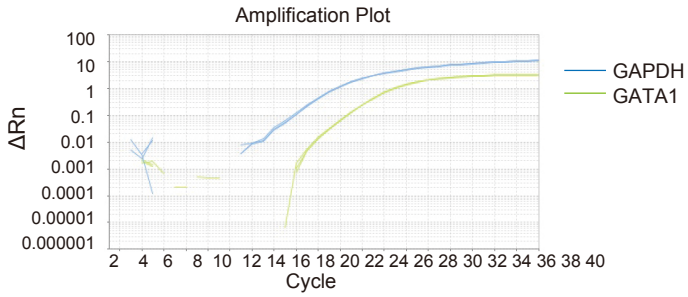


Figure S4

MSC gene set

FGF2	BMP2	MCAM	BMP7	IFNG	PIGS
INS	CASP3	NGFR	COL1A1	IGF1	PTPRC
LIF	CD44	NT5E	CSF2	IL10	SLC17A5
POU5F1	ENG	PDGFRB	CSF3	IL1B	TGFB3
SOX2	ERBB2	PROM1	CTNNB1	IL6	TNF
TERT	FUT4	THY1	EGF	ITGB1	VEGFA
WNT3A	FZD9	VCAM1	EUT1	KITLG	VIM
ZFP42	ITGA6	ANXA5	GTF3A	MMP2	VWF
ALCAM	ITGAV	BDNF	HGF	NES	
ANPEP	KDR	BGLAP	ICAM1	NUDT6	

Figure S5

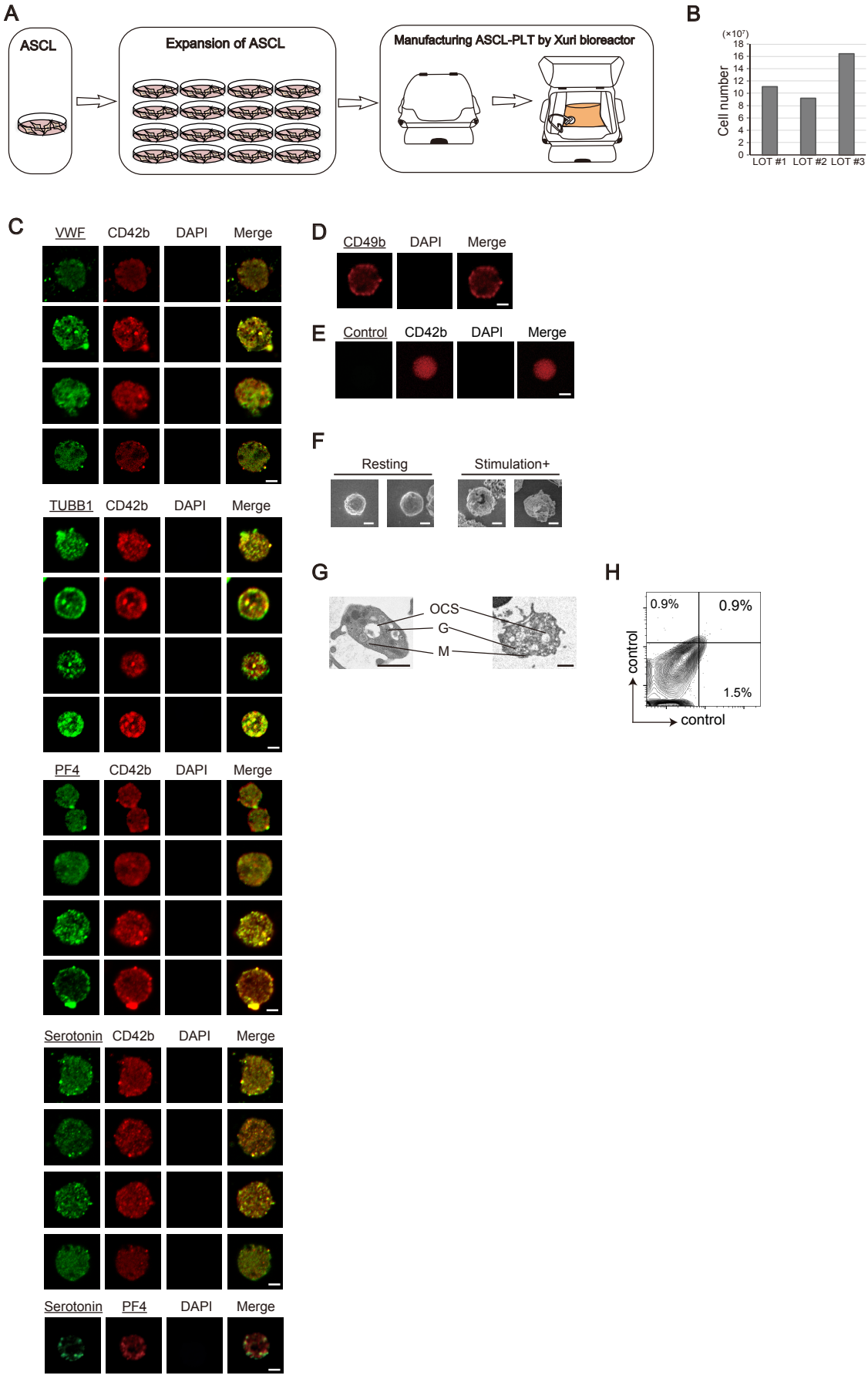
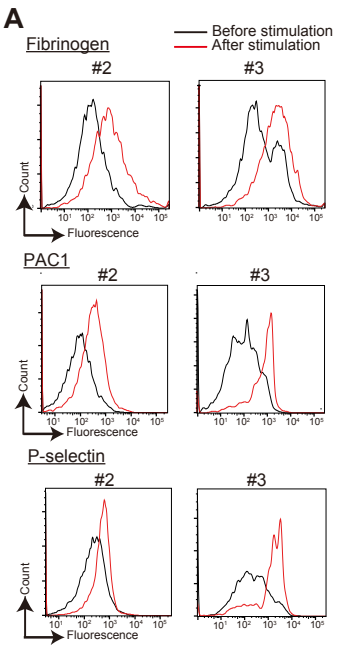
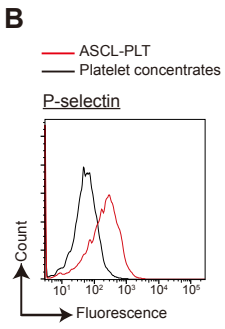

A
ASCL
Expansion of ASCL
Manufacturing ASCL-PLT by Xuri bioreactor
B
(×10⁷)
Cell number
LOT #1
LOT #2
LOT #3
C
VWF
CD42b
DAPI
Merge
TUBB1
CD42b
DAPI
Merge
PF4
CD42b
DAPI
Merge
Serotonin
CD42b
DAPI
Merge
Serotonin
PF4
DAPI
Merge
D
CD49b
DAPI
Merge
E
Control
CD42b
DAPI
Merge
F
Resting
Stimulation+
G
OCS
G
M
H
0.9%
0.9%
1.5%
control
control

Figure S6





Expression level (MFI)	
ASCL-PLT	Platelet concentrates
250.7 ±56.7	77.0 ± 11.9

Supplementary Materials

Supplemental Figure 1.

Photograph of ASCL before differentiation. (A) Human adipose-derived mesenchymal stromal/stem cell line (ASCL) established from adipose-derived stromal cells (ASCs) was maintained and expanded by using Preadipocyte Basal Medium (Cell Applications, San Diego, CA. USA). ASCL before megakaryocyte (MK) differentiation shows adherence to plastic *in vitro* culture. Bar, 100 μm. (B) ASC was cultured in media to differentiate into mature adipocytes for 14 days. Bar, 100 μm.

Supplemental Figure 2.

ASCL was cultured in MK lineage induction (MKLI) media for 14 days. ASCL-derived cells were analyzed by flow cytometry. (A) Isotype controls were used instead of specific markers for CD41 and CD42b (Figure 2A). (B) MK yield from ASCL obtained from different individuals. (C) DNA ploidy in CD41-positive/negative ASCL-MK. (D) Isotype control were used instead of specific marker for c-MPL (Figure 2C). (E) Expression of serotonin, VWF, and PF4 in MKs derived from ASCL cultured in MKLI media for 8 days. Cells stained with antibody for CD42b (red), DAPI (blue), and either of serotonin, VWF, or PF4 (green). Bar, 5 μm. (F) Expression of CD49b in ASCL-MK. Bar, 5 μm. (G) Expression of serotonin, VWF, and PF4 in $CD34^{+}$CB-MK. Cells stained with antibody for CD42b (red), DAPI (blue), and either of serotonin, VWF, or PF4 (green). Bar, 5 μm.

(H) The levels of serotonin, VWF, PF4, and VEGF in ASCL-MK and $CD34^+$CB-MK. (I) Isotype control (serotonin, VWF, and PF4) for Figure 2D, Figure S2E, and Figure S2G.

(J) Electron microscopic observations of ASCL- MK. G indicates granule; N, nucleus; DMS, demarcation membrane system; and M, mitochondrion. Bar, 5 μm. (K) Electron microscopic observations of $CD34^+$CB-MK. G indicates granule; N, nucleus; DMS, demarcation membrane system; and M, mitochondrion. Bar, 5 μm.

Supplemental Figure 3.

Gene expression of *GATA1* in K562 cells. Total RNA sample was prepared from K562 cells as a control of *GATA1* expressing cells, and then cDNA sample was obtained. Gene expression was assessed by quantitative real-time PCR. *GAPDH* was used as an internal control.

Supplemental Figure 4.

List of mesenchymal genes used to perform GSEA in Fig 3E.

Supplemental Figure 5.

(A) A large-scale culture for ASCL-PLT. Expanded ASCL were cultured with a bioreactor, Xuri Cell Expansion System® with rocking technology. (B) ASCL-PLT yield derived from different individuals. (C) Expression of serotonin, VWF, and PF4 in ASCL-PLT. Cells stained

with antibody for CD42b (red), DAPI (blue), and either of serotonin, VWF, or PF4 (green). Bar, 2 μm. (D) Expression of CD49b in ASCL-PLT. Bar, 2 μm. (E) Data of isotype control for Figure 3B. ASCL was cultured in MKLI media for 12 days. Cells were stained with antibody for CD42b (red), DAPI (blue), and isotype control (green) instead of VWF, b-1 tubulin, PF4, and serotonin. Bar, 2μm. (F) Scanning electron microscopy in ASCL-PLT in the presence or absence of stimulation (5 μg/ml collagen for 5 min). (G) Electron microscopic images of $CD34^{+}$CB-derived platelets. OCS indicates open canalicular system; G, granule; and M, mitochondrion. Bar, 1 μm. (H) Data of isotype control for Figure 4F.

Supplemental Figure 6.

(A) Fibrinogen binding, PAC1 binding, and P-selectin surface exposure in ASCL-PLT from different individuals. (B) Baseline P-selectin surface exposure level of ASCL-PLT and platelet concentrates.

Supplemental materials and methods

Cell culture

Subcutaneous adipose tissue was obtained from patients undergoing reconstruction surgery after tumorectomy at Keio University Hospital in Tokyo, Japan. After defect reconstruction was finished, the excess subcutaneous adipose tissue was trimmed. The specimens usually weighed between 0.5 and 1 g. Subcutaneous adipose tissues were digested with collagenase at 37 degrees C for 45 mins. Cell pellets, i.e., ASCs after centrifugation were maintained and expanded by using Preadipocyte Basal Medium (Cell Applications, San Diego, CA, USA).To obtain ASCL, ASCs cultured with Adipocyte Differentiation Medium (Cell Applications), and fat-drop from trypsinized cells underwent an upside-down culture (**Figure 1A**). ASCL was maintained and expanded by using Preadipocyte Basal Medium.

Human umbilical cord blood was purchased from Riken BioResource Center (Tsukuba, Japan). CD34-positive cells were purified by using CD34 MicroBead Kit MACS (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's instructions. Cells were cultured in StemSpan SFEM II media with StemSpan Megakaryocyte Expansion Supplement (STEMCELL Technologies, Vancouver, Canada).

ASCL was cultured in 10 L of MKLI media with a bioreactor, a Xuri Cell Expansion System® with rocking technology (GE Healthcare Life Sciences, Chicago, IL, USA). The rocking rate was 10 rocks/min, and rocking angle was 6 degrees. This system is in a low-shear environment, and rocking speed, dissolved oxygen, and pH were monitored. The MKLI media ware not changed during the culture time. To collect ASCL-PLT, culture media were passed through a 11 μm nylon mesh (SEFAR, Heiden, Switzerland), and the fraction < 11 μm was collected and centrifuged at 1200g for 10 min. We obtained the ASCL-PLT as a pellet.

Differentiation into osteoblast, mature adipocyte and chondrocyte

ASCL was differentiated into osteoblast in Osteogenic Differentiation Medium (PromoCell, Heidelberg, Germany). Alkaline phosphatase of differentiated cell was visualized by using Alkaline Phosphatase Staining Kit (Cosmo Bio, Tokyo, Japan), and calcified nodule was stained by using Calcified Nodule Staining Kit (Cosmo Bio) according to manufacturer's protocol. ASCL was also differentiated into mature adipocyte in Adipocyte Differentiation Medium (Cell Applications), and the lipid accumulation in adipocyte was visualized by using Lipid Assay Kit (Cosmo Bio) according to manufacturer's protocol. ASCL was differentiated into chondrocyte in Chondrogenic Differentiation Medium (PromoCell) and differentiated cell was stained with alcian blue.

Flow cytometry

We used antibodies against human CD11b, CD13, CD14, CD19, CD34, CD41, CD44, CD45, CD56, CD73, CD90, CD166, HLA-DR, and HLA-ABC (BioLegend, San Diego, CA, USA). We also used antibodies against CD29, CD42b, CD71 (BD Pharmingen, Franklin Lakes, NJ, USA), CD105 (Beckman Coulter, Brea, CA, USA), and c-MPL (BD Biosciences, San Jose, CA, USA). 7-aminoactinomycin D (BD Pharmingen) was used to assess dead cells. Expression of cell surface marker was examined by flow cytometry, as described previously.[1] DNA ploidy was examined as described.[1] We performed data analyses using FlowJo (FLOWJO, Ashland, OR, USA).

Karyotype analysis

ASCL after two months of culture was used for karyotype analysis. The samples were analyzed at Chromocenter (Tottori, Japan).

Measurement of TPO protein

ASCL was cultured in MKLI media (1 x 10^5/mL). TPO protein levels in supernatants were measured in cells on Day 0, 2, 4, 6 and 8. Human Thrombopoietin ELISA kit (Abcam, Cambridge, UK) was used according to manufacturer's protocol.

Immunohistochemical studies

Cell samples were fixed and permeabilized as previously described.[2] The samples were stained with either of unlabeled von Wiilebrand factor (VWF) (Abcam), beta-1 tubulin (Abgent, San Diego, CA, USA), platelet factor 4 (PF4) (PeproTech, Rocky Hill, NJ, USA), serotonin (Merck Millipore, Darmstadt, Germany), or CD49b (Abcam) followed by the staining with Alexa Fluor 488-conjugated anti-rabbit antibody (Thermo Fisher Scientific, Waltham, MA, USA). All cell samples were also stained with 4,6-diamidino-2-phenylindole blue (DAPI, Thermo Fisher Scientific), and unlabeled CD42b and Alexa Fluor 647-conjugated anti-mouse antibody (Abcam). Imaging was obtained using an LSM710 fluorescent microscope (Carl Zeiss, Oberkochen, Germany).

Measurement of serotonin, VWF, PF4 and VEGF

We performed an ELISA to quantify the presence of serotonin, VWF, PF4 and VEGF in $1x10^6$ each of ASCL-MK and $CD34^+$CB-MK and also quantify the presence of PF4 and VEGF in $1x10^7$ each of ASCL-PLT and platelets. Serotonin ELISA Kit (Enzo Life Sciences, Farmingdale, NY, USA), Human von Willebrand Factor ELISA Kit (Abcam), Human PF4 ELISA Kit (Abcam) and VEGF ELISA Kit (Abcam) were used according to manufacturer's protocol.

Gene expression analysis by Quantitative real-time PCR

Samples were prepared from ASCL, ASCL cultured in MKLI media, and K562 cells cultured in 10% FBS and RPMI-1640 medium (Sigma Aldrich, St. Louis, MO, USA). Total RNA was extracted using TRIzol (Thermo Fisher Scientific) according to the manufacturer's protocol, and cDNA samples were obtained by QuantiTect Reverse Transcription Kit (Qiagen, Hilden, Germany) according to manufacturer's protocol. Quantitative real-time PCR (qRT-PCR) was performed with premade primers for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), TUBB1, GATA2, FOG1, FLI1, PF4, RUNX1, MEIS1, and GATA1 by TaqMan-based Gene Expression Assay (Applied Biosystems, Foster City, CA, USA). SYBR Green-based Gene Expression Assay (Applied Biosystems) was also used with following primers: GAPDH (forward, 5'-CAGTCCATGCCATCACTGC-3'; reverse, 5'-GGATGATGTTCTGGAGAG-3'),

MPL (forward, 5'-CACTACAGGAGACTGAGGC-3'; reverse, 5'-TTCACAGGTATCTGAGACT-3'), VWF (forward, 5'-ACCCTTTGTGCAGAAGGA-3'; reverse, 5'-TCAAAGGTGTTGACGAAGTCAC-3'), TPO (forward, 5'-ATGGAGGAGACCAAGGCACA-3'; reverse, 5'-CCATCACTCCCTCCAGCAGA-3'), NFE2 (forward, 5'-GCTGCAGGGTCTGAATGCTC-3'; reverse, 5'-TGAGCAGGGGCAGTAAGTTGT-3'). GAPDH was used as an internal control. The fold change of mRNA was calculated by use of the comparative Ct method.

Microarray analysis

ASCL was cultured in MKLI media for 8 days. CD34-positive cord blood was cultured in MKLI media in the presence of rTPO for 8 days. DNA-free total RNA was extracted from CD42b-positive cell fraction by BD IMag cell separation system (BD biosciences). Amplified and biotinylated complementary RNA (cRNA) was obtained using GeneChip 3' IVT PLUS Reagent Kit (Affymetrix, Santa Clara, CA, USA), and hybridized to Affymetrix Human Genome U133 Plus 2.0Array according to the manufacturer's instructions. The data have been deposited in NCBI Gene Expression Omnibus (GSE112820). Data were analyzed with Gene Spring GX, and significantly up- or down regulated genes ($P<0.05$, 2-fold changes) were used to perform Gene ontology enrichment analysis. Gene set enrichment analysis was performed using GSEA 3.0 software (http://www.broadinstitute.org/gsea/), and gene sets with a p value < 0.05 and a false discovery rate (FDR) < 0.05 were considered significant.

Electron microscopy

Cell samples were fixed with a mixture of 4% paraformaldehyde and 4% glutaraldehyde (GA), washed, and post-fixed with 2% osmium tetroxide. After being dehydrated in graded ethanol solutions, the samples were embedded and polymerized in resin (Quetol-812, Nisshin EM, Tokyo, Japan). The polymerized resins were ultra-thin sectioned at 70 nm and stained with 2% uranyl acetate and Lead stain solution (Sigma-Aldrich). Samples were observed by a transmission electron microscope (JEM-1400Plus, JEOL, Tokyo, Japan) at an acceleration

voltage of 80 kV. Samples for scanning electron microscopy were prepared in the presence or absence of stimulation (5 μg/ml collagen for 5 min).

Functional assay for ASCL-PLT and platelet concentrates

To assess platelet functions, we used ASCL-PLT and platelet concentrates obtained from the Japanese Red Cross Society. Fibrinogen binding, P-selectin surface exposure, and PAC1 binding in the presence or absence of stimulation (thrombin (1 U/mL), epinephrine (1 μg/mL), calcium (1.5 mM) and magnesium (2 mM)) were essentially determined as described previously [27]. These tests with flow cytometry were performed on CD42b-positive platelet-sized cells. Alexa Fluor 488-labeled fibrinogen (Thermo Fisher Scientific), anti-human CD62P antibody and anti-human PAC1 antibody (BioLegend) were used. We also performed aggregation study using light transmission aggregometry. Sample was prepared for 2 x 10^7 per assay according to the protocol of aggregometer, PRP313® (TAIYO Instruments, Inc. Osaka, Japan). As agonist, 20 μM ADP, 5 μg/mL collagen, 1.5 mg/mL ristrocetin, and 10 mM epinephrine were used with 10 mM calcium. Agonist-induced platelet aggregation was recorded. ASCL was cultured in MKLI media for 12 days, and ASCL-PLT were collected. Their spreading on fibrinogen-coated glass upon stimulation was observed using an LSM710 fluorescent microscope (Carl Zeiss) as previously described.[2] Platelet concentrates to use in the present study ware approved by the Japanese Red Cross Society. Studies with human platelets were approved by the ethics review committee for Keio University.

Thromboxane B_2 secretion

Thromboxane (TX) B_2 levels in 2 x 10^7 of ASCL-PLT and 2 x 10^7 of platelet concentrates under calcium (10 mM) stimulation in the presence or absence of 100 μM aspirin were analyzed using ELISA Kit (Enzo Life Sciences) according to the manufacture's protocol.

Infusion studies

Six-week-old male immunodeficient NSG mice were irradiated with 2.0 Gy to induce mild thrombocytopenia (7~8 x 10^4/μL). A week later, these mice were used for the *in vivo* kinetics assay and *ex vivo* thrombus formation under flow conditions. To investigate the *in vivo* kinetics of ASCL-derived platelets and platelet concentrates, these cells (1 x 10^7 cells) were infused into irradiated NSG mice. Blood samples from recipient mouse were examined for the presence of human platelets 0 min, 30 min, 2 h, and 24 h after infusion, based on flow cytometry analysis.

To examine *ex vivo* thrombus formation under flow conditions, we obtained blood samples from ASCL-derived platelet- or platelet concentrate-infused mildly thrombocytopenic NSG mice 24 h after infusion. Each blood sample was stained with FITC-conjugated anti-human CD41 antibody (clone SZ22, Beckman Coulter) and perfused on a type I collagen-coated chip under flow condition (1000 $seconds^{-1}$) using a microchip flow chamber system, the Total Thrombus-formation Analysis System® (T-TAS®), an automated microchip flow chamber system (ZACROS/Fujimori Kogyo, Tokyo, Japan). After perfusion for 10 mins, the collagen-coated chip was followed by fluorescence microscopy to examine incorporation of ASCL-derived platelets or platelet concentrates. We also performed perfusion study in the presence of 50 μg/mL (final concentration) of anti-human CD42b blocking antibody (HIP1; BD Biosciences).

NSG mice were purchased by Oriental Yeast (Tokyo, Japan) and maintained in the animal care facility at Keio University. All animal studies were approved by the ethics review committee for animal experiments of Keio University.

Statistical analysis

We used the Student's *t*-test to compare mean values of two groups. One-way analysis of variance was performed to compare mean values in multiple groups. Statistical analysis was performed with JMP (SAS Institute). A p value of < 0.05 was considered to be statistically significant.

References

1. Matsubara Y, Murata M, Ikeda Y. Culture of megakaryocytes and platelets from subcutaneous adipose tissue and a preadipocyte cell line. *Methods Mol Biol*. 2012;788:249-258.

2. Ono Y, Wang Y, Suzuki H, et al. Induction of functional platelets from mouse and human fibroblasts by p45NF-E2/Maf. *Blood*. 2012;120(18):3812-3821.