SUPPLEMENTAL MATERIAL

Takayama et al., http://www.jem.org/cgi/content/full/jem.20100844/DC1

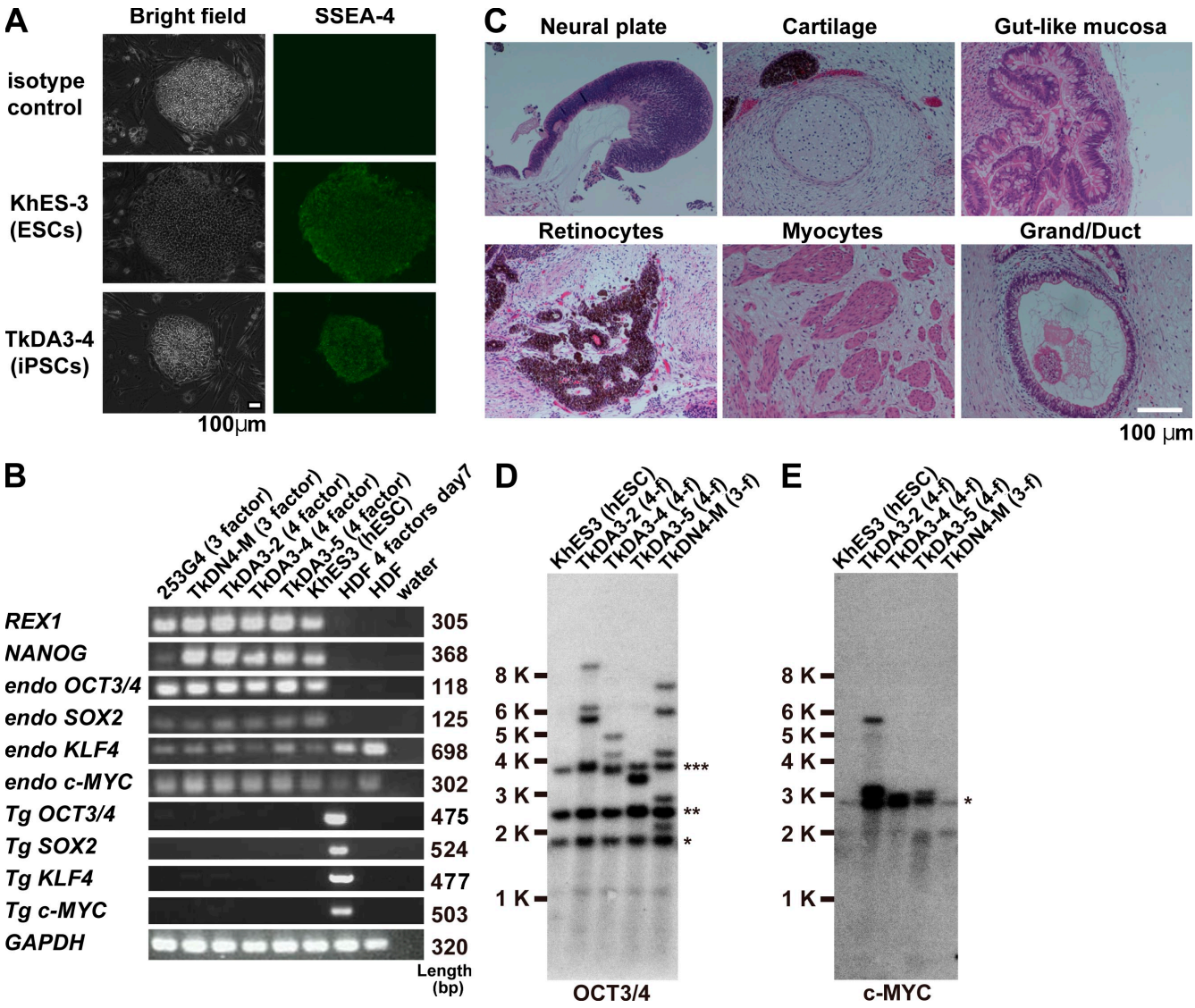


Figure S1. Characterization of hiPSCs derived from HDFs. (A) hESCs (clone KhES-3) or hiPSCs (clone TkDA3-4) were stained with SSEA-4 antibody. TkDA3-4 iPSCs were also treated with isotype IgG control (isotype control). (B) Extracted mRNAs were used for semi-qRT-PCR. HDFs were transduced with retrovirus encoding *OCT3/4*, *SOX2*, *KLF4*, or *c-MYC*, and individual cells harboring one Tg were used as a positive control. HDFs endogenously expressed *KLF4* and *c-MYC* but not *REX1*, *NANOG*, *OCT3/4*, or *SOX2*. (C) Hematoxylin and eosin–stained sections of a teratoma from a NOD/SCID mouse showing variable differentiation lineages derived from three germ layers. (D and E) Southern blot analyses using *OCT3/4* (D) and *c-MYC* (E) cDNA probes. Asterisks indicate the endogenous *OCT3/4* (1.7 kb) or *c-MYC* alleles (2.7 kb). Double asterisks indicate the POU5F1P3 (2.3 kb) and triple asterisks indicate the POU5F1B (3.5 kb). K, kb pairs.

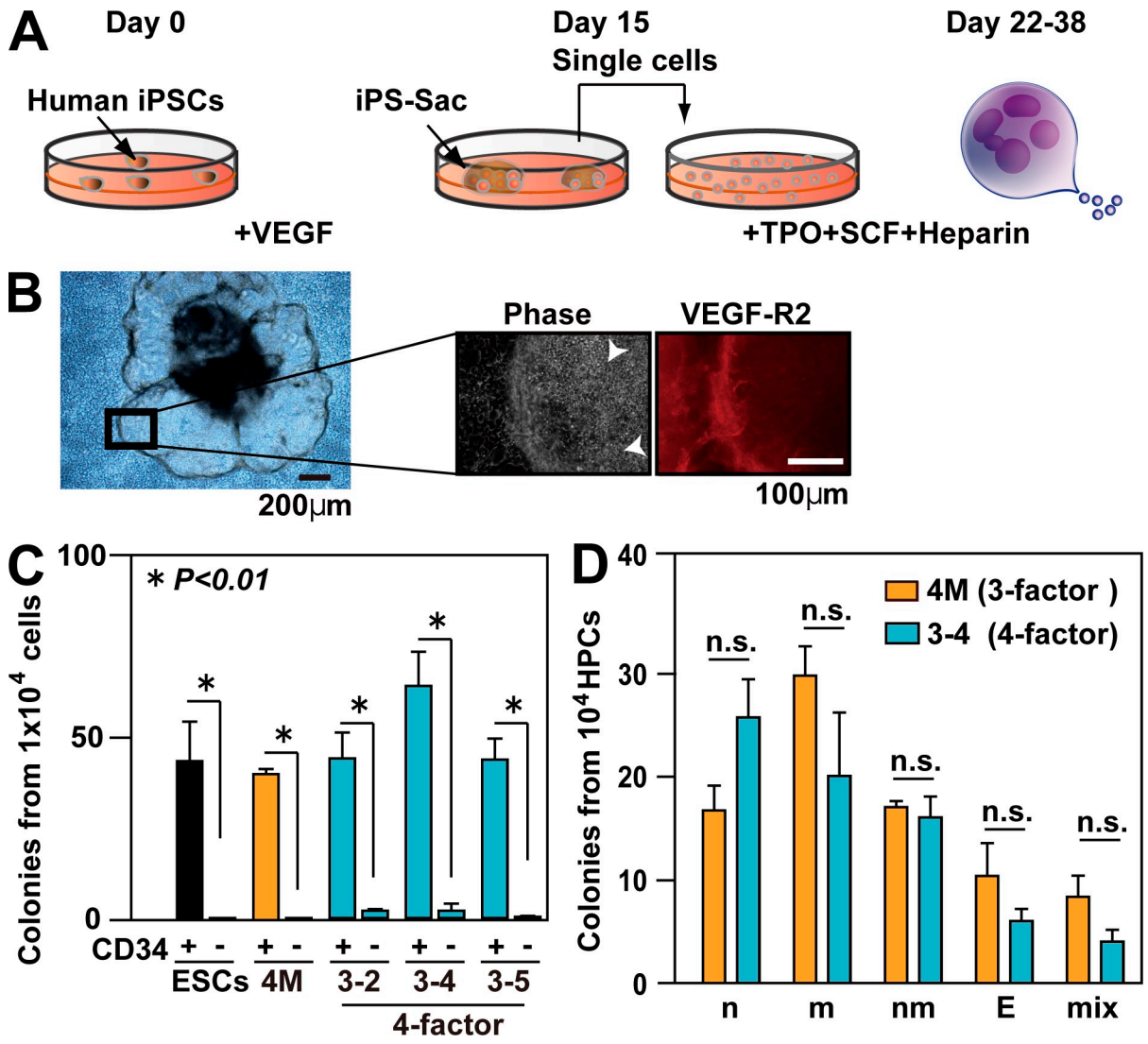


Figure S2. Human iPSC-derived sac-like structures (iPS-sacs) concentrate multipotent hematopoietic progenitors. (A) Schematic diagram of the in vitro differentiation protocol for hiPSC-derived hematopoietic cells. Mature hematopoietic cells were generated from cells within the iPS-Sacs on days 14–15. (B) Photomicrographs showing an iPS-Sac on day 15 of culture. Original magnification, 40×. A high-magnification view shows an iPS-Sac containing numerous bright, spherical hematopoietic progenitor cells (arrowheads; 200×). Immunohistochemical staining shows that VEGF-R2 is expressed on the wall of the iPS-Sac. (C) Numbers of colonies arising from 10^4 $CD34^+$ or $CD34^-$ cells derived from ESCs, TkDN4-M (three-factor clone), TkDA3-2, 3–4, and 3–5 (four-factor clones) on day 15 ($n = 3$, means ± SEM). (E) Numbers of colonies arising from 10^4 hematopoietic progenitors derived from TkDN4-M (three-factor clone) and TkDA3-4 four-factor clone) were counted ($n = 3$, means ± SEM). n, neutrophil; m, macrophage; nm, neutrophil and macrophage; E, erythrocyte; Mix, mixed lineages.

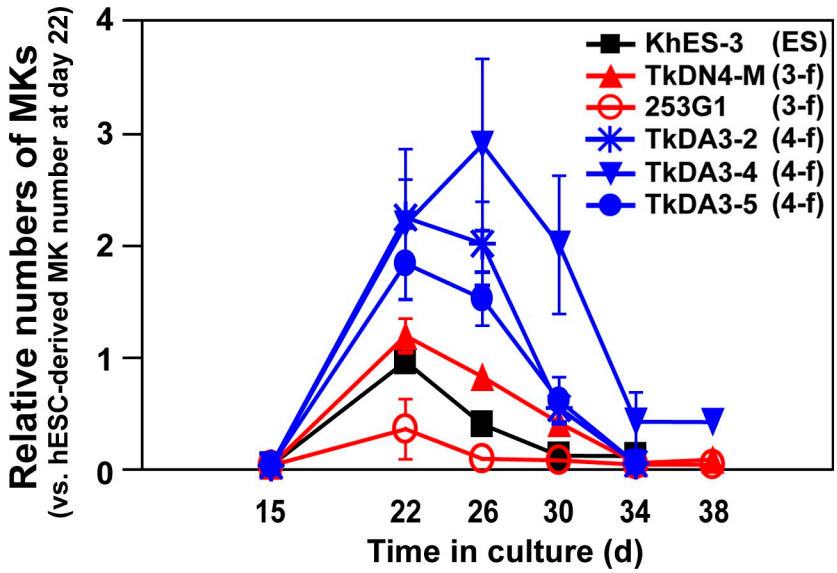


Figure S3. Time-dependent changes in the numbers of CD42b (GPIbα)$^+$ MKs, from days 15 (sac emergence) to 38. MKs were generated from 10^5 hematopoietic progenitors within ES- or iPS-Sacs. The number of MKs derived from hESCs on day 22 (7 d after replating) was assigned a value of 1.0 (n = 5, means ± SEM).

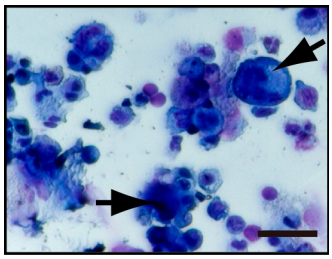


Figure S4. Hematopoietic progenitors within iPS-Sacs generate mature MKs. Floating cells on day 26 were stained with Write-Giemsa stain. Arrows indicate MKs with polyploidy. Bar, 50 μm.

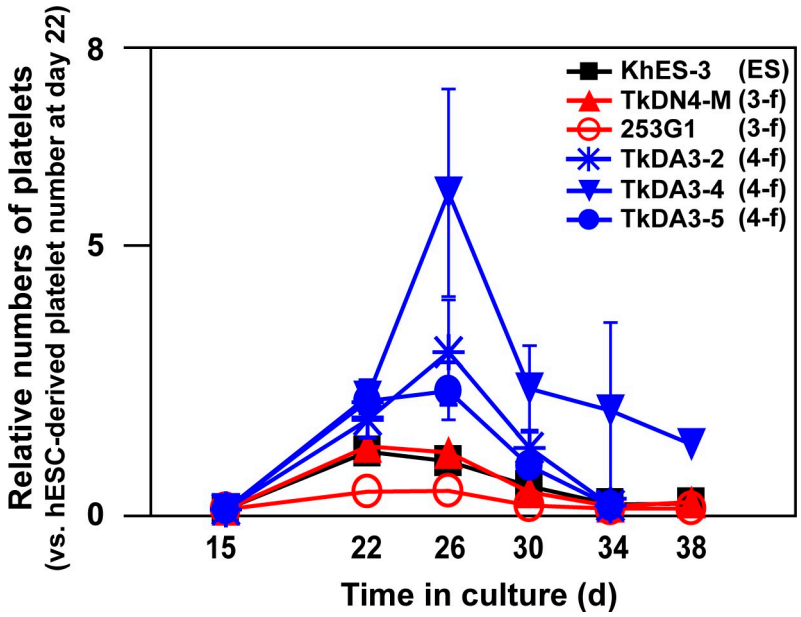


Figure S5. Time-dependent changes in the numbers of platelets generated from hESCs or hiPSCs from days 15 to 38. $CD41a^+CD42b^+$ platelets were generated from 10^5 hematopoietic progenitors within ES- or iPS-Sacs. The number of $CD41a^+CD42b^+$ platelets generated from hESCs on day 22 (7 d after replating) was assigned a value of 1.0 ($n = 5$, means ± SEM).

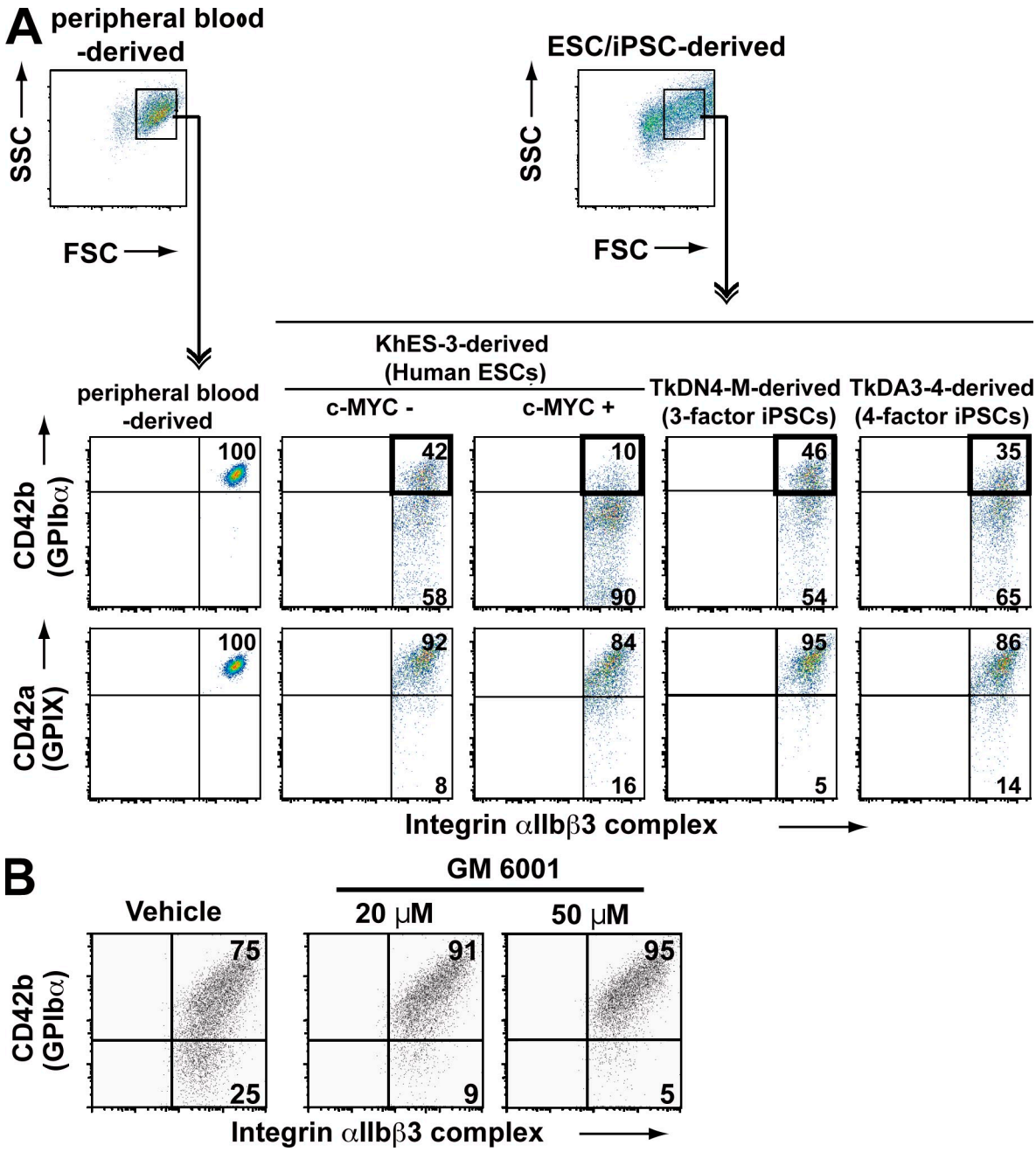


Figure S6. Flow cytometric analysis of platelets derived from hESCs and hiPSCs. (A) To confirm platelet generation from ESCs or iPSCs, particles in culture dishes were subjected to flow cytometry using the same forward- and side-scatter gates as those used for human plasma-derived adult platelets. Using flow cytometry, adult PB, ESC, and iPSC platelets or *c-MYC*-transduced ESC platelets were examined for CD42b(GPIbα) and CD42a(GPIX), along with CD41a. We repeated five times to confirm the gate and expression of CD42b or CD42a. (B) Metalloproteinase inhibition improves in vitro generation of intact $CD42b^+$ platelets from human iPSCs. Administration of GM6001, a nonspecific metalloprotease inhibitor, acted in a concentration-dependent manner to enable restoration of GPIbα expression in human iPSC-platelets. Vehicle, DMSO alone.

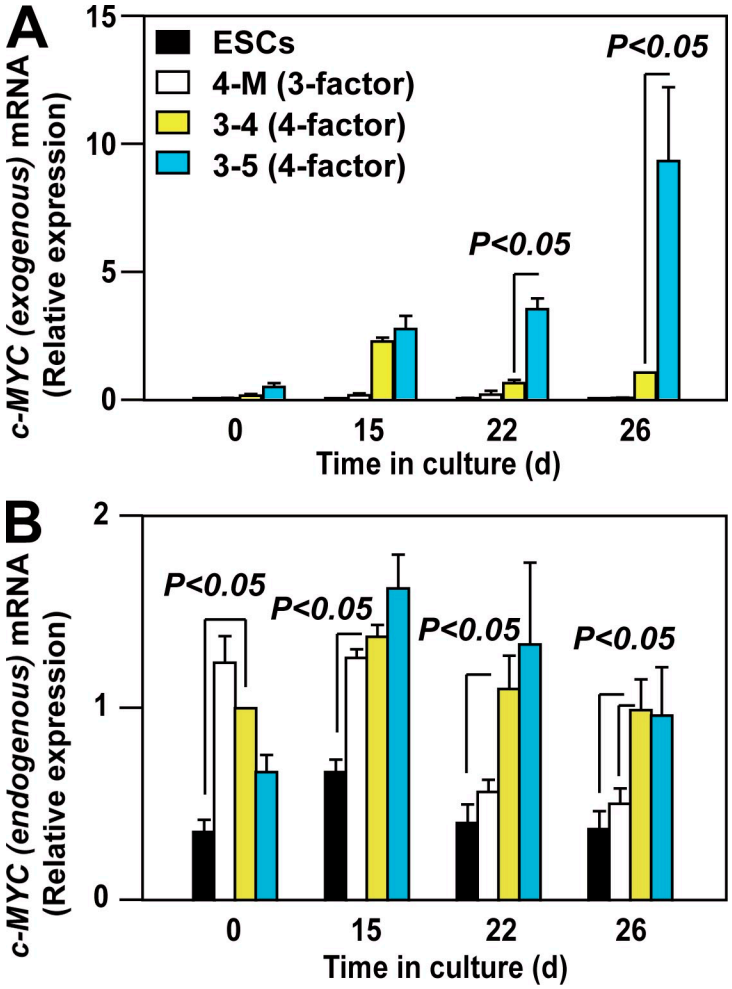


Figure S7. Changes in c-MYC mRNA in several iPSCs. (A and B) Total RNA was isolated from iPS cells on days 0, 15, 22, and 26 and analyzed using qPCR. Primers used for c-MYC specifically detect the transcripts from the exogenous (A) or endogenous (B) genes. All expression levels were normalized to the level of *GAPDH* expression. Levels of exogenous *c-MYC* (A) expression in TkDA3-4–derived mature MKs (day 26) or endogenous *c-MYC* (B) in undifferentiated TkDA3-4 iPSC clone was assigned a value of 1.0 ($n = 4$, means ± SEM).

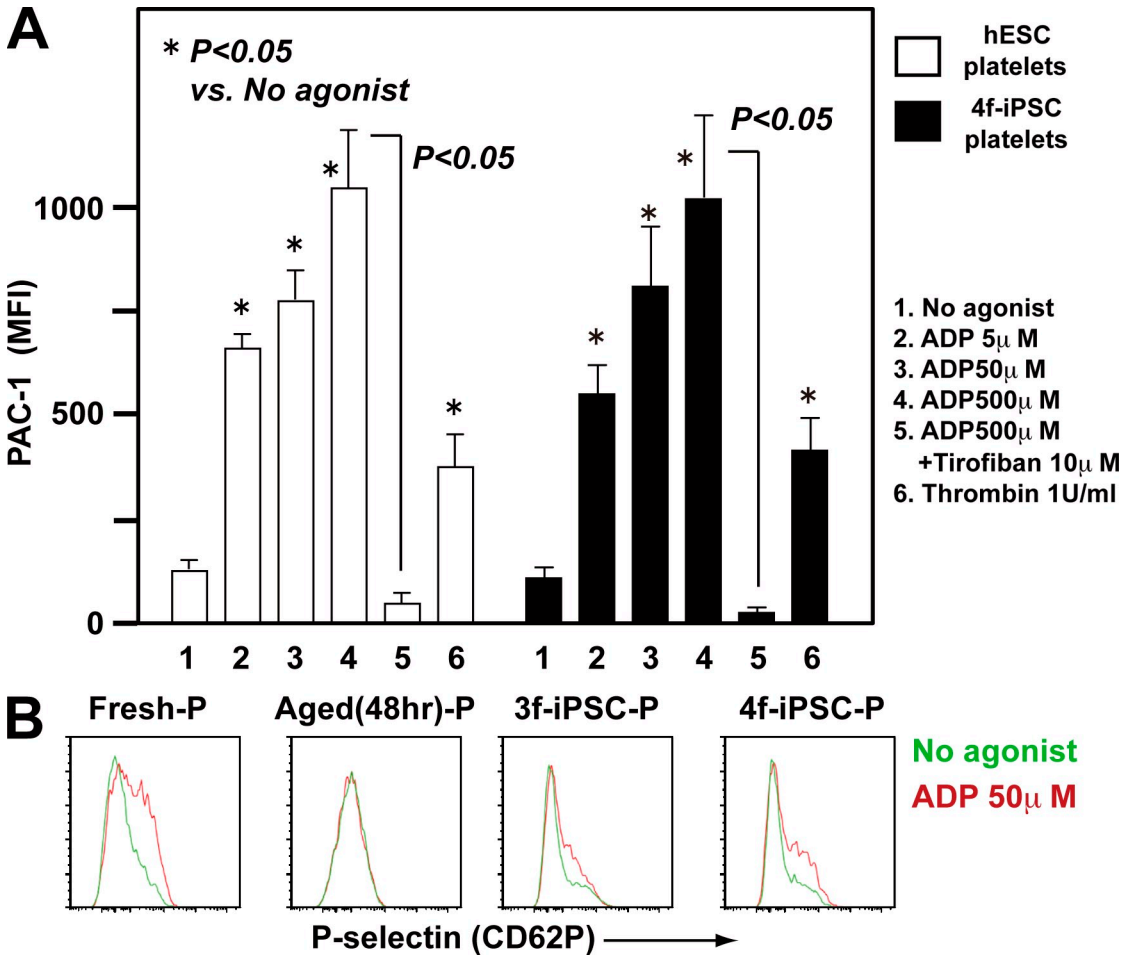
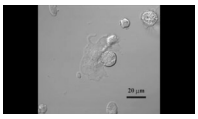
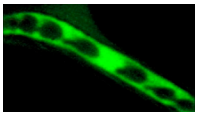


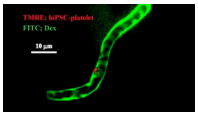
Figure S8. PAC1 binding and P-selectin expression in platelets derived from ESCs and iPSCs. (A) Binding of FITC-conjugated PAC-1 to hESC- and hiPSC (four-factor clone)-derived platelets was quantified in the absence or presence of ADP or thrombin using flow cytometry. Some specimens were also incubated with tirofiban to block PAC-1 binding to the integrin αIIbβ3 complex. Administration of ADP induced an increase in PAC-1 binding to both hESC- and hiPSC-derived platelets in a concentration-dependent fashion (n = 4, means ± SEM). (B) Representative P-selectin (CD62P) expression on platelets in the absence or presence of 50 µM ADP. We repeated and confirmed similar tendency of P-selectin expression (n = 3).



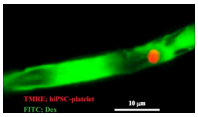
Video 1. Time-dependent changes in proplatelet formation from iPSC-derived MKs. 10 µg/ml of vascular cell adhesion molecule 1 (R&D Systems) was applied to MKs to facilitate platelet generation through α4β1 integrin ligation (Takizawa, H., K. Eto, A. Yoshikawa, H. Nakauchi, K. Takatsu, and S. Takaki. 2008. *Exp. Hematol.* 36:897–906). Images were taken every 15 min for up to 24 h.



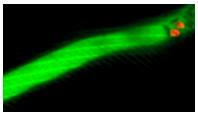
Video 2. In vivo imaging video of circulating iPSC platelets. FITC-dextran (green) and 10^7 TMRE-stained iPSC platelets (red) were injected, after which mesenteric capillaries were visualized using an in vivo imaging technique. Note the circulating iPSC-platelets (red). Images were reconstructed at 0.16× speed.



Video 3. In vivo imaging video of thrombus formation by an iPSC platelet or platelets within blood vessels. FITC-dextran (green), 10^7 TMRE-stained iPSC-platelets (red), and hematoporphyrin were administered. Thrombus formation induced by laser-induced injury in a mesenteric blood vessel is shown. Note the iPS-derived platelets in the developing thrombus (red). Images were reconstructed at 2× speed.



Video 4. In vivo imaging video of thrombus formation by an iPSC platelet or platelets within blood vessels. FITC-dextran (green), 10^7 TMRE-stained iPSC-platelets (red), and hematoporphyrin were administered. Thrombus formation induced by laser-induced injury in a mesenteric blood vessel is shown. Note the iPS-derived platelets in the developing thrombus (red). Images were reconstructed at 2× speed.



Video 5. In vivo imaging video of thrombus formation by an iPSC platelet or platelets within blood vessels. FITC-dextran (green), 10^7 TMRE-stained iPSC-platelets (red), and hematoporphyrin were administered. Thrombus formation induced by laser-induced injury in a mesenteric blood vessel is shown. Note the iPS-derived platelets in the developing thrombus (red). Images were reconstructed at 2× speed.

Table S1. Primers used in this study

Gene	Primer sequence (5′–3′)
c-MYC (Tg)	CAACAACCGAAAATGCACCAGCCCCAG; TACAGGTGGGGTCTTTCATTC
KLF4 (Tg)	TGCGGCAAAACCTACACAAAG; TACAGGTGGGGTCTTTCATTC
OCT3/4 (Tg)	CAACGAGAGGATTTTGAGGCT; TACAGGTGGGGTCTTTCATTC
SOX2 (Tg)	TGCAGTACAACTCCATGACCA; TACAGGTGGGGTCTTTCATTC
NANOG	CAGCCCCGATTCTTCCACCAGTCCC; CGGAAGATTCCCAGTCGGGTTCACC
REX1	CAGATCCTAAACAGCTCGCAGAAT; TATGACTCACTTCCAGGGGGCACT
c-MYC (endogenous)	AAGTTTGAGGCAGTTAAAATTATGGCTGAAGC; TGACCTAACTCGAGGAGGAGCTGGAATC
KLF4 (endogenous)	TCGCTTCCTCTTCCTCCGACACA; GCGAACTCACACAGGCGAGAAACC
OCT3/4 (endogenous)	TGCGGGCGGACATGGGGAGATCC; TCTTTCCACCAGGCCCCCGGCTC
SOX2 (endogenous)	TTGCCTTAAACAAGACCACGAAA; TAGAGCTAGACTCCGGGCGATCC
c-MYC (Tg) for qPCR	TTGCGGAAACGACGAGAACAG; CCCTTTTTCTGGAGACTAAATAAA
OCT3/4 (Tg) for qPCR	CCCCAGGGCCCCATTTTGGTACC; CCCTTTTTCTGGAGACTAAATAAA
SOX2 (Tg) for qPCR	ACTTCACATGTCCCAGCACTA; CCCTTTTTCTGGAGACTAAATAAA
KLF4 (Tg) for qPCR	ATGCGACCGAGCATTTTCCAG; CCCTTTTTCTGGAGACTAAATAAA
c-MYC (endogenous) for qPCR	TATTCTGCCCATTTGGGGACA; TTGGTGAAGCTAACGTTGAGG