Supplemental Methods

Generation of murine ES cells with a dox-inducible *Gata1* shRNA transgene.

A2lox.cre ES cells (16) were maintained on irradiated mouse embryonic fibroblasts (MEFs) in Knockout Dulbecco's modified Eagle's medium (DMEM), 1% penicillin/streptomycin (P/S, 100X), 1% L-glutamine (100X), 0.1 mM β-mercaptoethanol, 1% nonessential amino acids (100X, Gibco, Life technologies, Grand Island, NY), 15% fetal bovine serum (Tissue Culture Biologicals, Long Beach, CA), 1000 U/mL mouse (m) leukemia inhibitory factor (mLIF, Millipore, Billerica, MA). The ES cells were treated with 500 ng/mL dox (Clontech Laboratories, Mountain View, CA) for 24 hours to induce cre expression, then 5 x 10^6 cells were harvested and electroporated (250 V, 500 μF, 14 sec, Gene Pulser Xcell™ Electroporation Systems, Bio-rad, Hercules, CA) with 20 μg of the targeting vector p2Lox encoding either scrambled control shRNA or three tandem shRNAs targeting different regions of *Gata1* (Supplemental Figure 1). Cells were plated on CF6 Neo MEFs (GlobalStem, Rockville, MD) and 300 μg/mL G418 (Gibco) was added after 48 hours. ES cell colonies were harvested at day 10, expanded and analyzed by PCR of genomic DNA and cDNA to verify targeting of shRNAs into the *Hprt* locus exchange cassette (Supplemental Figure 1, A and B, and Supplemental Table 2).

Gata1 and control shRNA sequences.

<scrambled control shRNA>
5'-CTCGAGAAGGTATATTGCTGTTGACAGTGAGCGATCTCGCTTGGGCGAGAGTAAGTAGT
GAAGCCACAGATGTACTTACTCTCGCCCAAGCGAGAGTGCCTACTGCCTCGGAATTC-3'

<anti-*Gata1* shRNA >
5'-CTCGAGAAGGTATATTGCTGTTGACAGTGAGCGCCATCAACAAGCCCAGGTTCAATAGT
GAAGCCACAGATGTATTGAACCTGGGCTTGTTGATGCTGCCTACTGCCTCGGACTACGATG
GATCCGATCCTGCTGTTGACAGTGAGCGTAAGCGAATGATTGTCAGCAAATAGTGAAGCCA
CAGATGTATTTGCTGACAATCATTCGCTTCTGCCTACTGCCTCGGACTACGATTCTAGAGAT
CCTGCTGTTGACAGTGAGCGAGCTGGTATTTGGTTAAATAAATAGTGAAGCCACAGATGTA
TTTATTTAACCAAATACCAGCGTGCCTACTGCCTCGGAATTC-3'

Anti-Gata1 shRNA contains three tandem shRNAs designed from the mouse *Gata1* mRNA sequence (GenBank accession number NM_008089), flanked by miR30 processing sequences (38). The sequences were synthesized by GenScript (Piscataway, NJ), followed by being cloned into the targeting vector p2Lox. Sense and antisense sequences are underlined.

Generation and differentiation of G1ME2 cells.

ES cells containing *Gata1* or control shRNA transgenes were adapted to serum free ES cell maintenance medium composed of DMEM/F12, 0.5% of N2 and 1% B27 supplements (Gibco), L-glutamine, P/S, 0.05% bovine serum albumin (BSA), 1000 U/mL mLIF, 0.14 μM monothioglycerol (MTG, Sigma-Aldrich, St. Louis, MO), and 10 ng/mL human (h) bone morphogenetic protein 4 (hBMP4) (R&D Systems, Inc., Minneapolis, MN).

To generate EBs, trypsinized ES cells were plated on 0.1% gelatin-coated plate to deplete feeder cells and then suspended in serum-free differentiation medium (SFD) and 5 x 10^4 cells/well were placed into a low attachment 6-well plate (Costar, Corning Incorporated, Tewksbury, MA). SFD is composed of 75% Iscove's Modification of DMEM (IMDM), 25% Ham's F-12 (Cellgro, Manassas, VA), 0.5% N2 and 1% B27 supplement, L-glutamine, P/S, 0.05% BSA, 50 μg/mL ascorbic acid (Sigma-Aldrich) and 0.4 μM MTG. Cytokine mixtures were added sequentially as follows: **Days 0-3**: 2 ng/mL Activin A (R&D), 1 ng/mL hBMP4, 5 ng/mL human vascular endothelial growth factor (hVEGF) (R&D). **Days 4-5**: 5 ng/mL hVEGF, 100 ng/mL mScf, 1 ng/mL mIL-3, 5 ng/mL mIL-11 (Peprotech, Rocky Hill, NJ). On day 6, disaggregated EBs were counted and 5 x 10^5 cells in 2 mL/well were cultured in a 6 well tissue culture dish (Costar) with 100 ng/mL mScf, 20 ng/mL mTpo and ± 500 ng/mL dox. After 1-2 days, the cultures established an adherent stromal layer and non-adherent cells that were largely hematopoietic. Non-adherent cells were collected every 1-3 days, enumerated and analyzed for mRNA expression.

By days 8-14, the numbers of suspension cells were noticeably increased in *Gata1* shRNA cultures (+dox) compared to controls; the suspension cells were transferred to 75T flasks for further expansion and maintained at concentrations of 5 x 10^5 to 1.5 x 10^6 cells/mL. Continuously proliferating *Gata1* knockdown cells, termed G1ME2 cells, were maintained with 100 ng/mL Scf, 20 ng/mL Tpo and 500 ng/mL dox or cryopreserved in 50% medium, 40% FBS, and 10% dimethylsulfoxide (DMSO).

Culture and differentiation of FL cells and G1ME cells.

FL megakaryocytes were generated as described (20) with slight modifications. FLs were collected from embryonic day 14.5 CD1 mouse embryos (Charles River Laboratories, Wilmington, MA) and hematopoietic progenitors were isolated using the EasySep enrichment kit (StemCell Technologies, Vancouver, Canada) according to the manufacturer's protocol. The progenitors were cultured in expansion medium (IMDM, 10% fetal bovine serum, L-glutamine, P/S, 1% mScf conditioned medium, 0.5% mTpo conditioned medium) for 5 days, then switched to the same medium without Scf and cultured for an additional 6 days (differentiation). G1ME cells were maintained and manipulated as described (10).

Colony assays.

G1ME2 cells were washed to remove dox and plated in 1% methylcellulose (Sigma-Aldrich) in IMDM supplemented with 5% protein-free hybridoma medium II (PFHM II, Gibco), 10% plasma derived serum (PDS, Antech, Tyler, TX), 50 μg/mL ascorbic acid, 0.14 μM MTG, and multiple cytokines: 100 ng/mL m Scf, 2 U/mL hEpo, 20 ng/mL mTpo, 1 ng/mL mIL-3, 10 ng/mL mIL-6, 5 ng/mL mIL-11, 3 ng/mL mGM-CSF, 5 ng/mL mM-CSF. Six to eight days after plating of G1ME2 cells, erythroid (Ery), erythro-megakaryocyte (E-Meg), and megakaryocyte (Meg) colonies were counted using an Olympus CKX41 microscope (Olympus America, Center Valley, PA) and collected for further experiments. Isolated mouse fetal liver hematopoietic progenitors were

plated in methylcellulose for control experiments either with multiple cytokines or granulocyte-macrophage cytokines: 1 ng/mL mIL-3, 3 ng/mL mGM-CSF, and 5 ng/mL mM-CSF.

Semiquantitative Real-Time PCR.

RNA was purified using the RNeasy micro kit (Qiagen, Valencia, CA) and reverse transcribed using the iScript cDNA synthesis kit (Bio-rad). qPCR was conducted using the ViiA7™ Real-Time PCR System (Applied Biosystems (Life Technologies)) with SYBR Green dye (Applied Biosystems). Gene expression was assessed by standard curve methods and normalized to *Gapdh* and *β-Actin* expression. Primer sequences are provided in Supplemental Table 3. For time dependent maturation studies (Figure 2, A-C, main text) cells were collected at differentiation days 0, 2, 4, and 6. Mature megakaryocytes derived from G1ME2 and fetal liver were purified by a two-step BSA density gradient (20) after 5 days of differentiation and cultured for one day thereafter. G1ME cells expressing *Gata1* cDNA and GFP were purified by fluorescence activated cell sorting (FACSAria™ III, BD Biosciences, San Jose, CA) 24 hours after retroviral transduction and cultured for subsequent studies.

Western blotting.

Cells were lysed in RIPA buffer (Sigma-Aldrich) with protease inhibitors and dithiothreitol added according to the manufacturer's instructions. Protein concentration was determined using the BCA protein assay kit (Thermo Fisher Scientific, Waltham, MA). In general, 20 μg of protein were analyzed for each sample in Western blotting. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was conducted on a 4-12% gradient NuPAGE Bis-Tris Precast Gel (Life Technologies) and proteins were transferred to PVDF membrane (Bio-Rad). Primary and secondary antibodies were used as follows: monoclonal rat anti-mGATA-1 (1:500, sc-265, Santa Cruz Biotechnology, Santa Cruz, CA), polyclonal rabbit anti-VWF (1:2000, A0082, Dako, Carpinteria, CA), polyclonal rabbit anti-mPF4 (1:2000, Sigma-Genosys, Sigma-Aldrich) primary

antibodies. Secondary antibodies were horseradish peroxidase (HRP)-conjugated rabbit anti-rat IgG (A5795) or HRP-conjugated goat anti-rabbit IgG (A0545, Sigma-Aldrich). Monoclonal anti-β-actin-peroxidase antibody (A3854, Sigma-Aldrich) was used as a protein loading control.

Histological analysis.

Cells were centrifuged onto glass slides and stained with May-Grünwald-Giemsa (Sigma-Aldrich) or acetylcholinesterase (19). Light microscopy images were obtained with a Zeiss Axioskop 2 microscope, Axiocam camera, and AxioVision 4.8 software (Carl Zeiss, Jena, Germany). Phase contrast imaging for proplatelet formation was obtained using an Olympus CKX41 microscope and an Axiocam camera (Carl Zeiss). Immunofluorescence of proplatelet formation was performed as described (39). Briefly, proplatelet-enriched fractions were incubated with Alexa Fluor 488-conjugated polyclonal rabbit anti-detyrosinated mouse β1-tubulin antibody (39) and 4',6-diamidino-2-phenylindole (DAPI, Sigma-Aldrich) to label the megakaryocyte nucleus. Images were obtained using an Axiovert 200 microscope (Carl Zeiss) equipped with a 63x NA 1.4 oil immersion objective, a charge-coupled device camera (Hamamatsu Photonics, Hamamatsu City, Japan) and MetaMorph image analysis software (MDS Analytical Technologies, Sunnyvale, CA).

Flow Cytometry.

Antibodies used were: anti-CD41 FITC (553848, 1:100), CD71 FITC (553266, 1:100), Ter119 PE-Cy7 (557853, 1:100), Ter119 APC (557909, 1:100), Kit PE-Cy7 (558163, 1:100), Kit APC (553356, 1:100), Mac1 PE-Cy7 (552850, 1:200), Gr1 APC-Cy7 (557661, 1:200) (BD Biosciences), CD41 APC (17-0411-82, 1:500), IL-7Rα FITC (11-1271-85, 1:200), B220 APC (17-0452-83, 1:200) (eBioscience, San Diego, CA), CD42b PE (M040-2, 1:50) (Emfret Analytics, Germany). Cells were resuspended in PBS with 0.1% BSA and 0.2 mM EDTA and incubated with antibodies for 30 mins at room temperature. DNA ploidy was determined by staining with

20 μg/mL propidium iodide (Invitrogen, Life Technologies) in 40 mM sodium citrate, pH 7.4, 0.25 M sucrose, 0.5% NP-40, 0.5 mM EDTA and 0.5 mg/mL RNase A (Sigma-Aldrich). Stained cells were analyzed on a LSRFortessa flow cytometer (BD Biosciences) with FlowJo software (Tree Star, Ashland, OR). The percentages of Megs with > 4N DNA is measured from the differentiation time points that exhibited the optimal megakaryocyte maturation: G1ME2 cells at 5 days after dox withdrawal, FL progenitors after 6 days culture in Tpo and G1ME cells 4 days after transduction with *Gata1* cDNA.

In vivo platelet generation.

Mature megakaryocytes were enriched on a two-step BSA density gradient (20). Megakaryocytes were BSA purified from G1ME2 cells at 5 days after dox withdrawal, G1ME cells at 4 days after retroviral transfer of *Gata1* cDNA, or FL hematopoietic progenitors after 6 days culture in TPO. These time points represent optimal megakaryocyte maturation, as assessed by CD42b expression and ploidy (see Figure 2, main text, panels C and D). Megakaryocytes were infused into hαIIb$^+$ transgenic mice and platelet progeny were quantified by flow cytometry (25). Venous blood samples were stained with monoclonal FITC-mouse anti-human CD41 (11-0419-42, 1:25) (eBioscience) and monoclonal PE-rat anti-mouse CD41 (558040, 1:100) (BD Biosciences) to detect circulating endogenous host platelets and donor megakaryocyte-derived platelets, respectively.

Platelet function studies using the cremaster laser injury animal model.

C57BL/6 WT mice were anesthetized with sodium pentobarbital (80 mg/kg IP) and a cannula was inserted into the jugular vein for infusion of additional anesthetic, antibodies, and cells. The cremaster muscle was exteriorized onto a coverslip on an intravital microscopy tray, superfused with physiological buffer (PBS containing 0.9 mM $CaCl_2$ and 0.49 mM $MgCl_2$) at 37°C.

Megakaryocytes derived from G1ME2 cells or fetal liver progenitors were enriched by two-step BSA gradient centrifugation, stained with 10 μg/mL Calcein AM (Invitrogen) for 30 mins at 37°C and infused. For platelet inhibitor studies, the megakaryocytes or control endogenous platelets were incubated in 1 μM prostaglandin E1 (PGE1) (Sigma-Aldrich) + 1 mM acetylsalicylic acid (ASA) (Sigma-Aldrich) for 1 hour prior to infusion. Washed mouse platelets were prepared as previously described (20), Calcein AM loaded and infused into mice.

The microcirculation of the cremaster muscle was visualized using an Olympus BX61WI upright microscope with a 60X (0.9 NA) water immersion objective. Time lapse videos of platelet incorporation, brightfield and fluorescent images were captured using a Cooke Sensicam CCD camera (Cooke, Auburn Hills, MI). For p-selectin expression studies, images were captured using a Yokogawa CSU-X1 spinning disk confocal scanner coupled to an Evolve EM-CCD camera (Photometrics, Tucson, AZ). Alexa-647 conjugated anti-p-selectin antibody (0.2 μg/g body weight, clone RB40.34, BD Biosciences) was infused after G1ME2-derived megakaryocytes and before laser injury. Vascular injury was induced with a pulsed nitrogen dye laser (SRS NL100, 440 nm) focused on the vessel wall through the microscope objective. Arterioles of 20-40 μm were selected and the laser was pulsed until adequate injury was observed. Data were acquired and analyzed using Slidebook 5.0 software (Intelligent Imaging Innovations, Denver, CO).

Transcriptome studies.

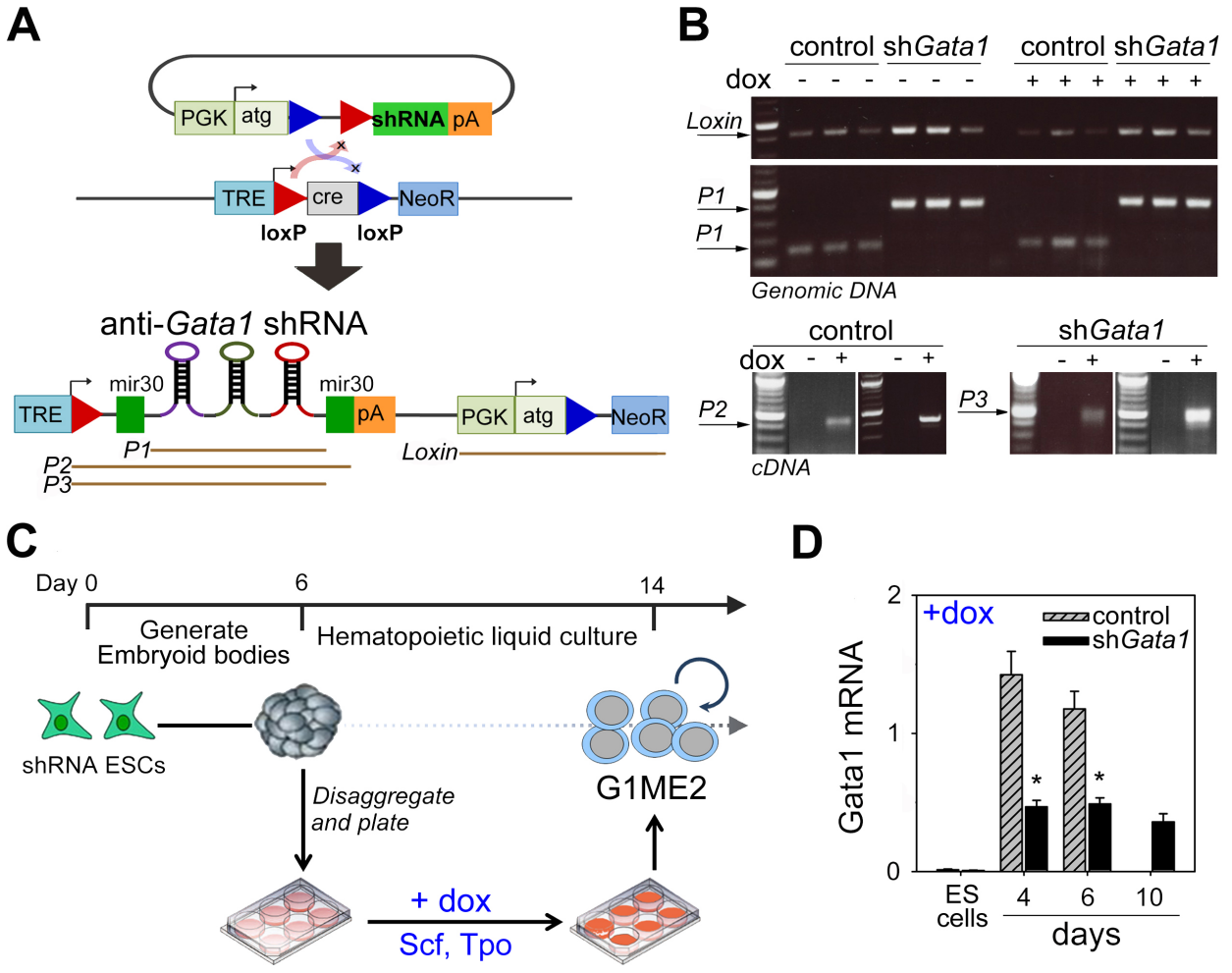
G1ME2 cells were deprived of dox and cultured for 5 days in Tpo. G1ME cells were infected with MIGR1 retrovirus expressing Gata1 cDNA and GFP (10). One day after infection, GFP expressing cells were purified by flow cytometry and cultured for an additional 3 days in TPO.

FL-derived megakaryocytes were generated from embryonic day 14.5 embryos by culturing lineage-depleted hematopoietic progenitors in Tpo for 6 days.

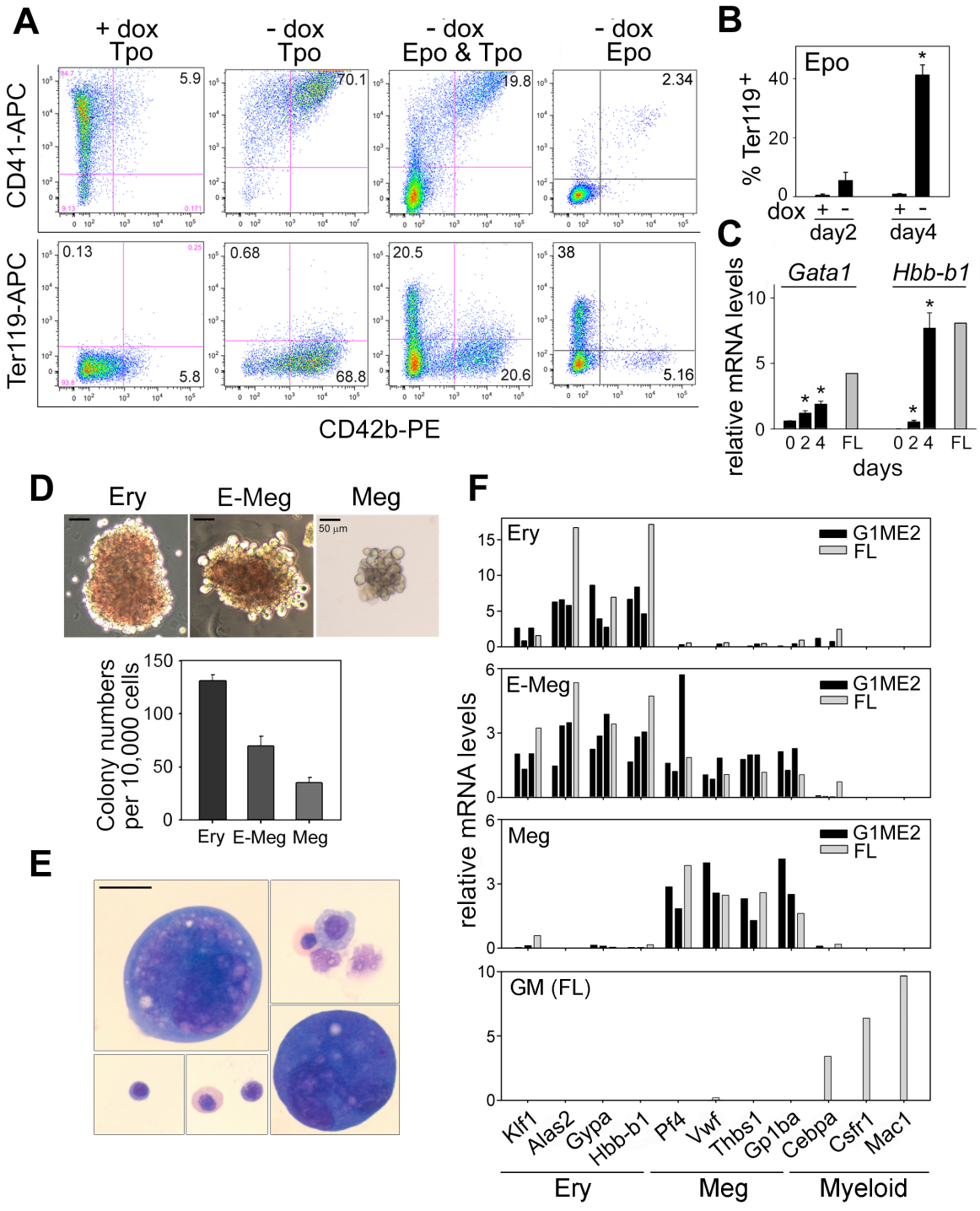
All megakaryocytes were enriched on BSA gradients. RNA was isolated with the RNeasy micro kit (Qiagen, Valencia, CA). Transcriptome analysis was performed at the University of Pennsylvania Molecular Profiling Core using the Affymetrix Mouse Gene 2.0 ST array.

Microarray data were processed in R using the oligo package (40). Expression profiles were either normalized with RMA or first batch-normalized with COMBAT and then with RMA (41). Differentially expression profiles were then analyzed using limma (42). Transcriptome data were visualized by PCA using Partek Genomics Suite 6.6. To generate megakaryocyte (MEG)-specific gene expression signatures, raw files were downloaded from the DMAP (43) website (http://www.broadinstitute.org/dmap/home) for human megakaryocyte-erythroid progenitors (MEPs: $CD34^+$, $CD38^+$, IL-$3R\alpha^-$, $CD45RA^-$), early MEGs (MEG1; $CD34^+$, $CD41^+$, $CD61^+$, $CD45^-$) and mature MEGs (MEG2; $CD34^-$, $CD41^+$, $CD61^+$, $CD45^-$). For hierarchical clustering (Supplemental Figure 3B), we generated COMBAT- and RMA-normalized genesets. Four top 100 lists were generated based on effect size. The top 100 up-regulated and top 100 down-downregulated genes for MEG1 vs. MEP used a p value threshold of < 0.01. For the MEG2 vs. MEP comparison, the p-value threshold was < 0.05. These four lists were joined, mapped to the mouse RMA microarray data, de-duplicated for gene symbol based on the highest expressing transcript and hierarchically clustered after z score transformation (color scale -2 SDs blue to 2 yellow SDs) in Spotfire Decision Site. For Geneset Enrichment Analysis (GSEA, Supplemental Figure 3C), we generated COMBAT- and RMA-normalized genesets consisting of the top or bottom most differentially expressed genes (n=100 for each group) in MEP vs. MEG2. GSEA (44) was downloaded from http://www.broadinstitute.org/gsea/index.jsp and performed on the above genesets with standard parameters except: 1) gene set permutation was used

rather than phenotype because the number of samples in each group was n=3, 2) no minimum for gene set size was required, and 3) MoGene 2.0 ST symbols for the microarrays were collapsed to human gene symbols using the GSEA platform data.

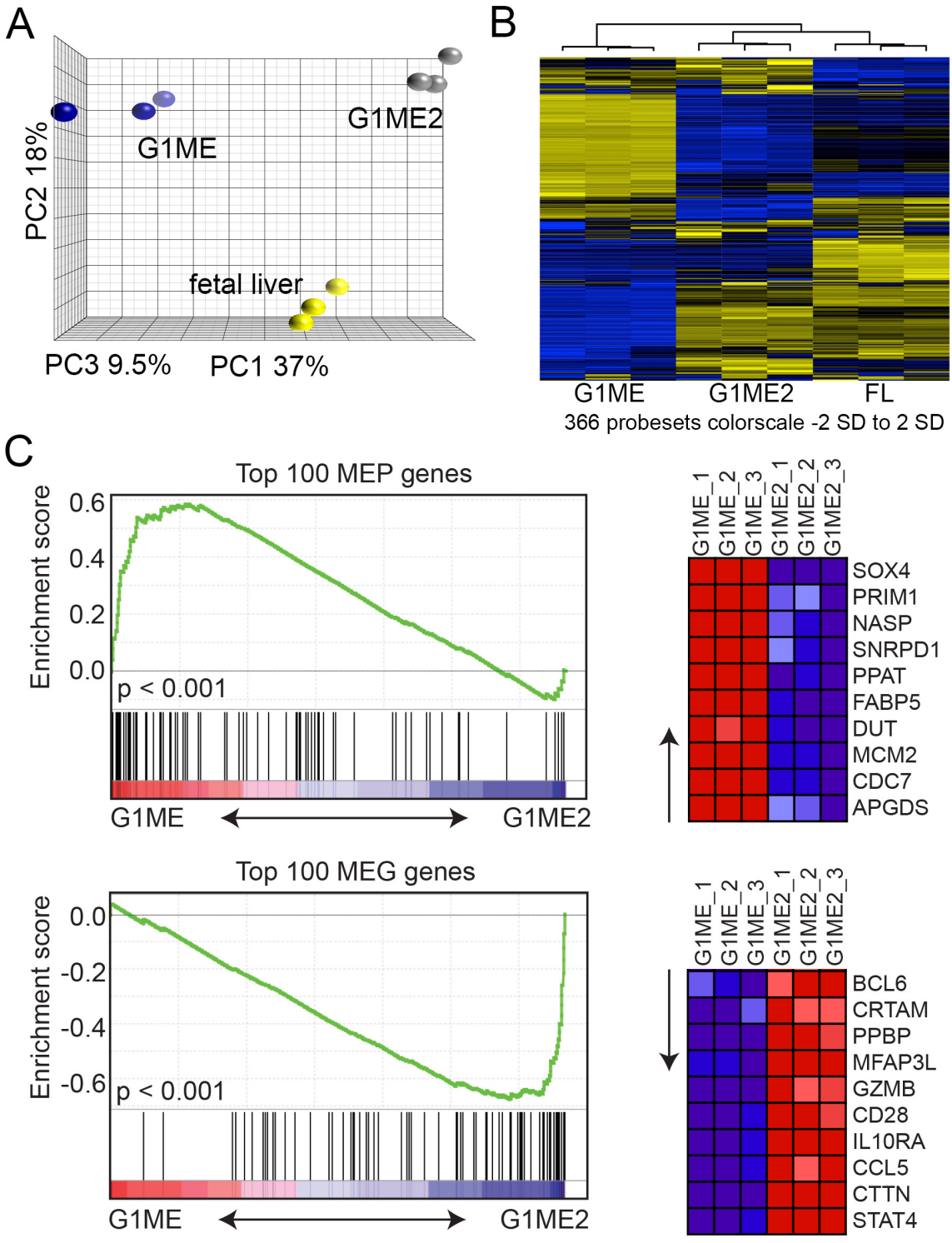


Supplemental Figure 1. Generation and analysis of murine ES cells expressing dox-regulated *Gata1* shRNAs. (**A**) The inducible cassette exchange method was used to generate modified ES cell lines. Top: targeting vector containing a phosphoglycerate kinase promoter (PGK) linked to an atg start codon, self-incompatible loxP sites (triangles), *Gata1* shRNA or scrambled (control) shRNA cassettes and a polyadenylation signal (pA). Middle: modified *Hprt* locus in A2lox.cre ES cells containing a tetracycline/dox response element (TRE), a dox-inducible *cre* transgene flanked by loxP sites and a neomycin resistance cassette (NeoR). Bottom: the modified locus after cre-mediated recombination. Three tandem *Gata1* shRNAs are flanked by microRNA (mir) 30 processing sequences. The PCR products used to assess modification of the locus are shown: Loxin, 420 bp; P1, 160 bp for scrambled shRNA and 380 bp for *Gata1* shRNA; P2, 430 bp for scrambled shRNA; P3, 509 bp for *Gata1* shRNA. PCR primers are described in Supplemental Table 2. (**B**) Confirmation of homologous recombination in ES cells by PCR. Genomic DNA (upper panels) or cDNA (lower panels) from 2-3 different ES cell clones expressing scrambled shRNA (control) or triple anti-*Gata1* shRNAs (shGata1) ± dox treatment for 24 hours. (**C**) Generation of G1ME2 cells. Six day old embryoid bodies (EBs) were disaggregated and replated onto tissue culture dishes with dox, Scf and Tpo. (**D**) Semiquantitative real-time PCR analysis for *Gata1* expression in EB-derived cells, as described in panel (**C**). (n = 3 experiments) *, $p < 0.05$ vs. control group by Student's *t*-test. Mean ± S.E.M.



Supplemental Figure 2. G1ME2 cells undergo erythro-megakaryocytic differentiation. (**A**) Flow cytometry for CD42b and Ter119 before and 4 days after dox removal with the indicated cytokines. Scf was present continuously in experiments represented by the 1st, 3rd, and 4th panels and for 2 days after dox withdrawal in the study shown in the 2nd panel. Representative of at least 5 experiments. (**B**) Ter119 expression before and after dox withdrawal with Scf and Epo present (see also the lower 4th panel in

Supplemental Figure 2A). The Ter119^{-} cells remaining at 4 days after dox withdrawal were CD41^{-}, CD71^{high} and negative for the lineage markers CD42b, Gr1, B220, IL7-Ra and Mac1 (not shown), indicating that they are immature erythroblasts. Consistent with that interpretation, histological examination of the unfractionated population (containing Ter119^{-} CD71^{high} *and* Ter119^{+} CD71^{high} cells) revealed immature and mature erythroblasts (not shown). (n = 4 experiments) *, $p < 0.05$ vs. +dox group by Student's *t*-test. Mean ± S.E.M. (**C**) Semiquantitative real-time PCR analysis of samples from (B) showing induction of *Gata1* and *Hbb-b1* (β-globin) expression. Relative mRNA levels are normalized to *Gapdh* and *β-Actin*. FL, fetal liver-derived erythroblasts. (n = 3 experiments) *, $p < 0.05$ vs. +dox group by Student's *t*-test. Mean ± S.E.M. (**D**) Erythroid (Ery), erythroid-megakaryocyte (E-Meg) and megakaryocyte (Meg) colonies generated from G1ME2 cells plated in methylcellulose with no dox and a multilineage cytokine mix (Scf, Epo, Tpo, IL-3, IL-6, IL-11, GM-CSF, M-CSF). Original magnification 100X. Scale bars, 50 μm. Representative of 3 experiments. The graph shows the frequencies of Ery, E-Meg and Meg progenitors in a bulk population of G1ME2 cells. The bars represent mean ± S.E.M of two independent experiments, each performed in triplicate. (**E**) May-Grünwald-Giemsa-stained megakaryocytes and erythroblasts within a single E-Meg colony. Original magnification 400X. Scale bar, 20 μm. (**F**) Semiquantitative real-time PCR analysis of erythroid (Ery), megakaryocyte (Meg) and myeloid-specific mRNAs in single Ery, E-Meg and Meg colonies from G1ME2 cells (first three panels). Each black bar represents a single G1ME2 cell-derived colony. (n = 3 experiments) The grey bars represent similar type colonies generated from fetal liver (FL) hematopoietic progenitors. (n = 1 experiment) The bottom panel shows analysis of FL-derived granulocyte-macrophage (GM) colonies, as a control for myeloid gene expression.


A
G1ME
G1ME2
fetal liver
PC2 18%
PC3 9.5%
PC1 37%
B
G1ME
G1ME2
FL
366 probesets colorscale -2 SD to 2 SD
C
Top 100 MEP genes
Enrichment score
0.6
0.4
0.2
0.0
p < 0.001
G1ME
G1ME2
G1ME_1
G1ME_2
G1ME_3
G1ME2_1
G1ME2_2
G1ME2_3
SOX4
PRIM1
NASP
SNRPD1
PPAT
FABP5
DUT
MCM2
CDC7
APGDS
Top 100 MEG genes
Enrichment score
0.0
-0.2
-0.4
-0.6
p < 0.001
G1ME
G1ME2
G1ME_1
G1ME_2
G1ME_3
G1ME2_1
G1ME2_2
G1ME2_3
BCL6
CRTAM
PPBP
MFAP3L
GZMB
CD28
IL10RA
CCL5
CTTN
STAT4

Supplemental Figure 3. Transcriptome analysis of BSA gradient-purified megakaryocytes derived from G1ME cells (4 days after retroviral transfer of *Gata1* cDNA)**, G1ME2 cells** (5 days after dox withdrawal) **and fetal liver** (FL, 6 days after culture of progenitors in Tpo)**.** (**A**) Principal component analysis of all probesets. The signature from FL megakaryocytes more closely associates with G1ME2 megakaryocytes, particularly in the PC1 axis, which accounts for 37% of the variability (> 2-fold more than PC2 or PC3). (**B**) Hierarchical clustering of 407 transcripts that are differentially expressed between mature megakaryocytes and MEPs in human bone marrow. (**C**) Gene Set Enrichment analysis (GSEA) comparing genes that are differentially expressed between G1ME2- and G1ME- derived megakaryocytes and sets of 100 genes that are most repressed (Top 100 MEP) or induced (Top 100 MEG) during the differentiation of MEPs into mature megakaryocytes. Each black bar corresponds to one gene in these genesets. The heat maps on the right show expression of the top 10 most differentially expressed genes in each geneset. Red represents high expression and blue represents low expression.

Supplemental Table 1. **Platelet production from donor megakaryocytes (Megs).** The percentage of donor Meg-derived platelets was obtained 30 minutes (for G1ME2- and G1ME-derived Megs) or 8 hours (for FL-derived Megs) after intravenous injection of donor megakaryocytes into hαIIb$^+$ transgenic host mice in which the endogenous *Itgab2* genes were ablated (Figure 2, G and H, main text). The number of platelets per recipient mouse was calculated according to a mean platelet count of 0.51 x 10^9 platelets/mL before injection of Megs and a total blood volume of 2 mL per mouse. Each time the number of injected CD41$^+$CD42b$^+$ Megs was analyzed by flow cytometer.

	Exp.	platelets /mouse	# injected Megs	% donor Meg derived platelets (peak)	# donor Meg derived platelets /mouse	# platelets /donor Meg	# platelets /donor Meg (Mean ± S.E.M)
G1ME2	1	1 x 10^9	1.94 x 10^6	1.98	20.3 x 10^6	10.4	37.1 ± 21.5
	2	1 x 10^9	1 x 10^6	7.8	79.8 x 10^6	79.8	
	3	1 x 10^9	1.29 x 10^6	2.7	27.6 x 10^6	21.3	
FL	1	1 x 10^9	1.1 x 10^6	3.82	38.9 x 10^6	35.3	53.1 ± 8.94
	2	1 x 10^9	1.1 x 10^6	6.76	69.2 x 10^6	62.9	
	3	1 x 10^9	1.15 x 10^6	6.89	70.5 x 10^6	61.3	
G1ME	1	1 x 10^9	1.13 x 10^6	0.25	2.56 x 10^6	2.26	2.68 ± 0.57
	2	1 x 10^9	1.68 x 10^6	0.63	6.45 x 10^6	3.83	
	3	1 x 10^9	1.56 x 10^6	0.3	3.07 x 10^6	1.96	

Supplemental Table 2. Oligonucleotide primers used to analyze integrations of *Gata1* and control shRNAs into ES cells via inducible cassette-mediated exchange. The sizes of PCR products are shown in Supplemental Figure 1, A and B.

Primer	Sense (5'-3')	Antisense (5'-3')
Loxin	CTAGATCTCGAAGGATCTGGAG	ATACTTTCTCGGCAGGAGCA
P1	GTTAACCCAACAGAAGGCTCG	TGCTCCTAAAGTAGCCCCTTGAA
P2	ATACTTTATACGAAGTTATCTCGAG	ACTAGTGGATCCCCCAAACGCA
P3	ATACTTTATACGAAGTTATCTCGAG	TGCTCCTAAAGTAGCCCCTTGAA

Supplemental Table 3. Oligonucleotide primers for semiquantitative real-time PCR analysis of hematopoietic differentiation.

Gene	Sense (5'-3')	Antisense (5'-3')
Gata1	TTATGCTAGCTGGGCCTATGGCAA	TTGTTGCTCTTCCCTTCCTGGTCT
Gata2	CACCCCTAAGCAGAGAAGCAA	TGGCACCACAGTTGACACACT
Vwf	TCATCGCTCCAGCCACATTCCATA	AGCCACGCTCACAGTGGTTATACA
Pf4	TTCTGGGCCTGTTGTTTCTG	GATCTCCATCGCTTTCTTCG
Gp1ba	CTTGTTGCCAACGACCAAGCTGAA	AAGCCCTTTGGTATTGTGCGAAGC
Selp	AGCTACTCATTGCTCCACAGTCCT	AAGCAGAAGGGAACTGGAAGGTCA
Ppbp	GCGCAGTTCGATATATGGGT	ACCTCCAGATCTTGCTGCTG
Thbs1	TAGCTGAGGCGGATCAGCAAATCT	GGGAAGCCAAAGGAGTCCAAATCA
Klf1	CACGCACACGGGAGAGAAG	CGTCAGTTCGTCTGAGCGAG
Alas2	TATGTGCAGGCCATCAACTACCCA	TTTCCATCATCTGAGGGCTGTGGT
Gypa	TCACACGGCCCCTACTGAAGTGT	TCCCTGCCATCACGCGGAAAAT
Hbb-b1	AACGATGGCCTGAATCACTTG	AGCCTGAAGTTCTCAGGATCC
Cebpa	CAGACCAGAAAGCTGAGTTGTGA	ACCCCACAAAGCCCAGAAAC
Csfr1	GCGATGTGTGAGCAATGGCAGT	AGACCGTTTTGCGTAAGACCTG
Mac1	AGAACACCAAGGACCGTCTGC	TCTAAAGCCAGGTCATAAGTG
Gapdh	AGGTTGTCTCCTGCGACTTCA	CCAGGAAATGAGCTTGACAAAG
β-Actin	ACACCCGCCACCAGTTC	TACAGCCCGGGGAGCAT

Supplemental Video 1. G1ME2 cell-derived platelets participate in new thrombus formation.
Calcein-labeled G1ME2-derived megakaryocytes were infused intravenously 10 mins prior to laser injury. The micro arterial circulation of the cremaster muscle is visualized at the site of the laser pulse. Endogenous and G1ME2 donor-derived platelets (green) adhered to the injured vessel wall and initiated thrombus formation within 30 seconds. Images were visualized at 60X magnification and frames were acquired every 200 ms for 3 mins after laser injury.

Supplemental Video 2. G1ME2 cell-derived platelets become activated within newly formed thrombi.
To visualize p-selectin expression on the platelet surface, the laser injury was performed on the vessel wall 10 mins after intravenous infusion of Calcein-labeled (green) G1ME2-derived megakaryocytes and alexa-647 anti-p-selectin antibody (blue). Both endogenous and donor cell-derived platelets expressed p-selectin only after adhering to the vessel wall and thrombus. The 2-dimensional frames were captured every 310 ms using a time-lapse confocal scanner over a total time of 5 mins.

Supplemental references

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