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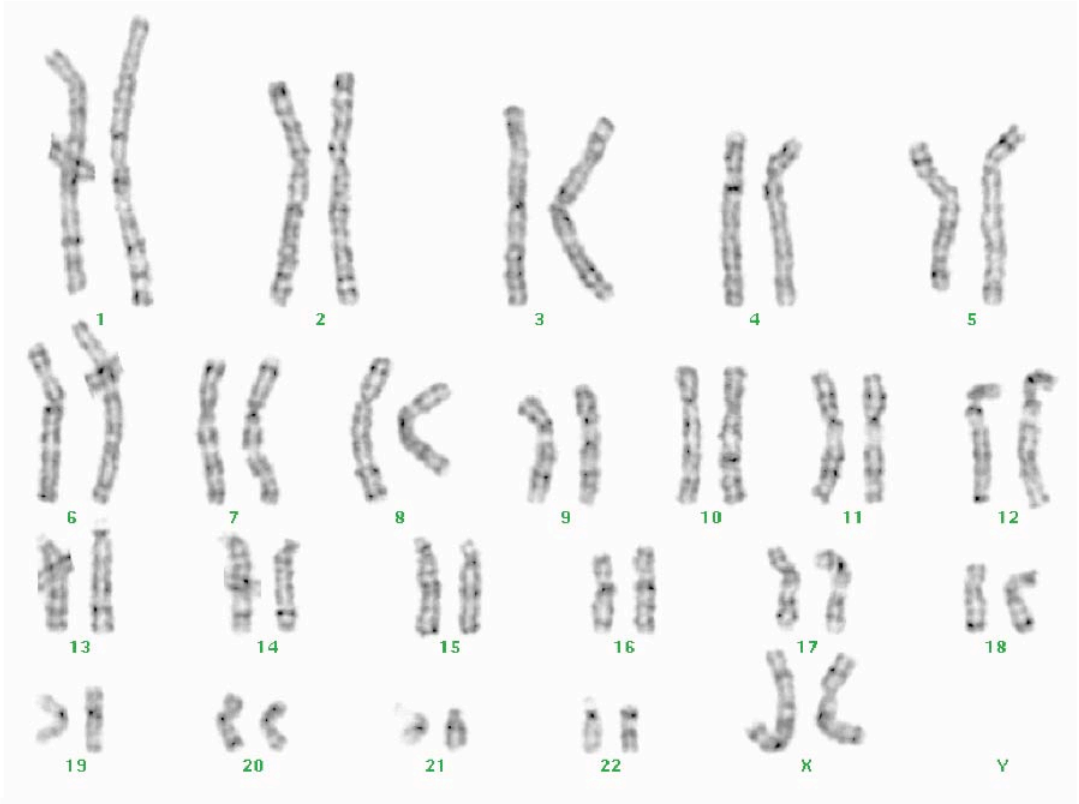
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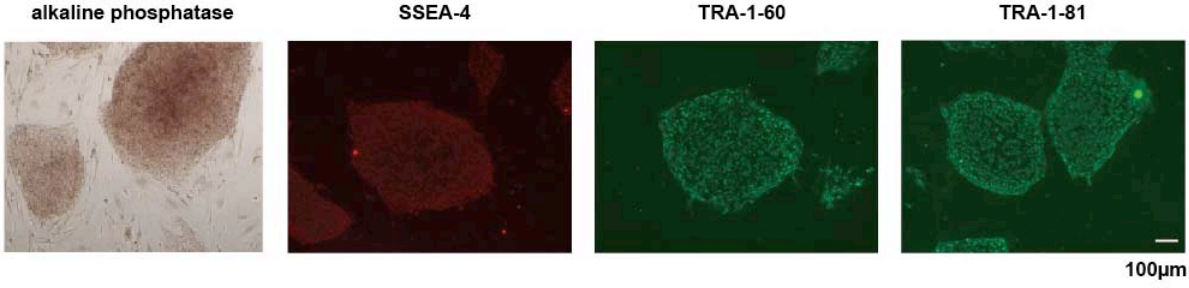
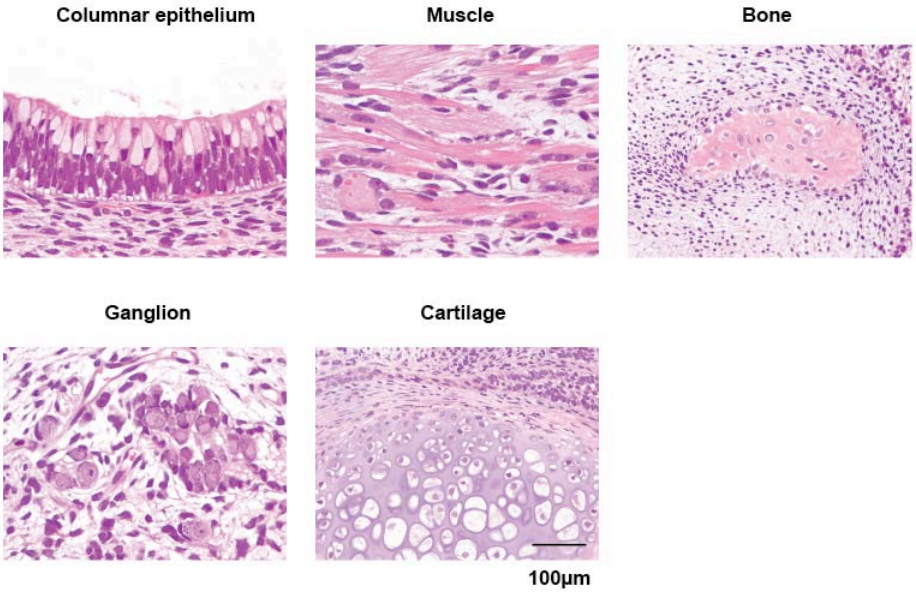

alkaline phosphatase
SSEA-4
TRA-1-60
TRA-1-81
100μm

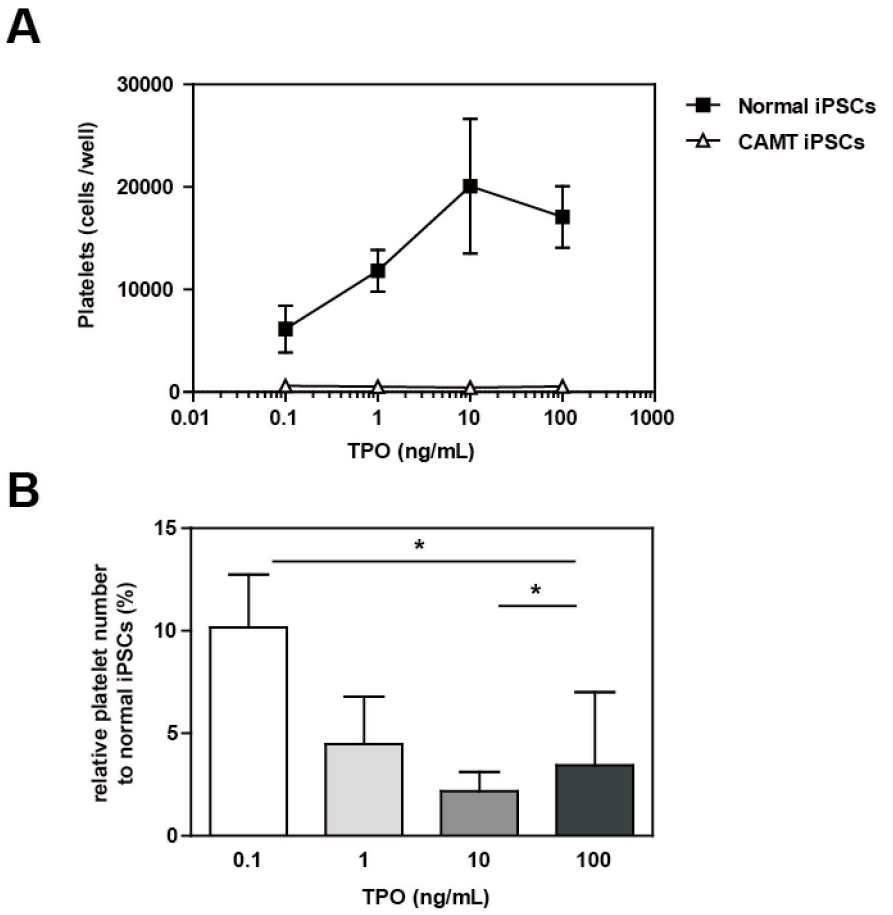
C


Columnar epithelium
Muscle
Bone
Ganglion
Cartilage
100μm

Supplemental Figure 1. Characterization of CAMT-iPSCs

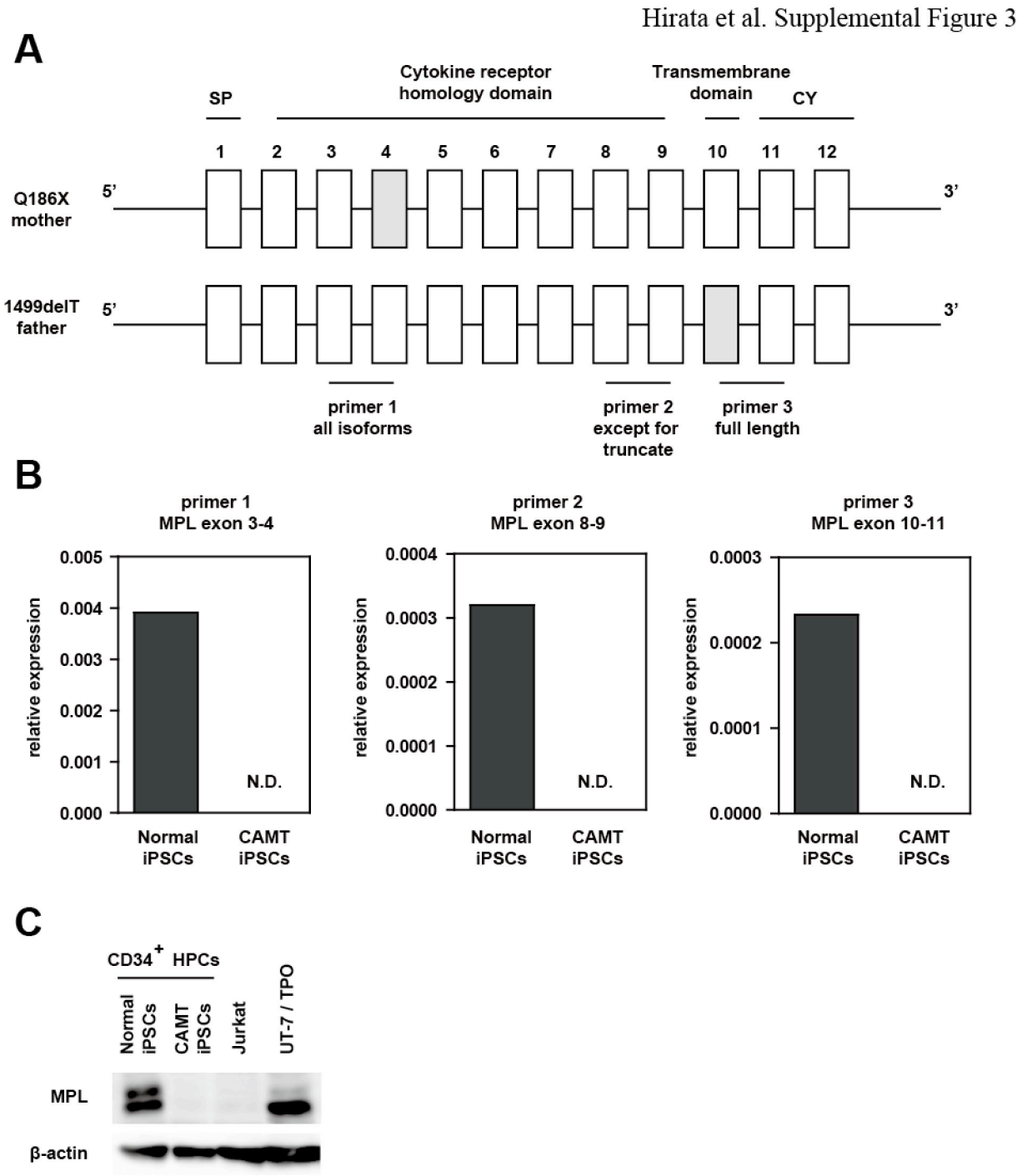
(A) Normal karyotype of CAMT-iPSCs. (B) Alkaline phosphatase staining and immunohistochemical staining for SSEA-4, TRA-1-60 and TRA-1-81 in CAMT-iPSC colonies. Scale bar: 100μm. (C) Hematoxylin and eosin-stained sections of a teratoma from a NOD/SCID mouse. Evaluation of two CAMT-iPSC clones showed the potential for pluripotency, as evidenced by the teratoma formation in NOD/SCID mice. Scale bar: 100μm.

Hirata et al. Supplemental Figure 2



Supplemental Figure 2. Numbers of platelets produced at physiological TPO concentrations.

(A) Numbers of platelets generated from normal- and CAMT-iPSC-derived $CD34^{+}$HPCs in the presence of 50 ng/mL SCF, 25 U/mL Heparin, and the TPO concentrations indicated on the X-axis of the graph. (B) Numbers of platelets generated from CAMT-iPSCs relative to those generated from normal iPSCs at the indicated TPO concentrations. Considering that the physiological TPO range in human serum is 0.1-1 ng/mL, the relative platelet numbers from CAMT-iPSCs produced in vitro equalizes at 5-10 % of normal-iPSCs, which is equivalent to the clinical manifestation in patients. $*P < 0.05$.

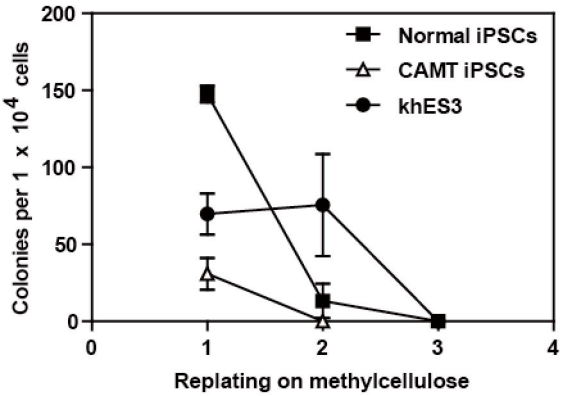


Supplemental Figure 3. CAMT-iPSCs harbors corresponding mutations in the patient

(A) Diagram of the maternal mutation in exon 4 and paternal mutation in exon 10 in the CAMT patient. (B) *MPL* expression in CD34$^+$ HPCs was confirmed by quantitative RT-PCR using the indicated primer sets (1 to 3), which respectively amplified all isoforms (primer set 1), all or a truncated isoform (primer set 2) and only the full-length isoform (primer set 3) (5). Any type of *MPL* was not detected in CD34$^+$ HPCs derived from CAMT-iPSCs by

indicated primer sets. N.D: not detected. (C) Western blotting analysis of $CD34^+$ HPCs derived from normal- or CAMT-iPSCs. Jurkat cells and UT-7/TPO cells were used as negative and positive controls, respectively.

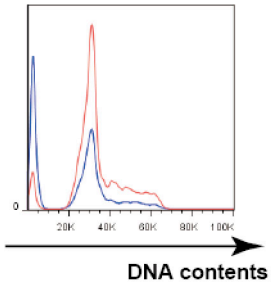
Hirata et al. Supplemental Figure 4



Supplemental Figure 4. Impaired self-replication of CD34$^+$ HPCs derived from CAMT iPSCs

Shown are the mean numbers (± SD) of colonies per 10^4 cells plated in MethoCult H4434 semisolid medium containing TPO. CD34$^+$ HPCs derived from normal-iPSCs, ESCs and CAMT-iPSCs were plated. Colony counts and replating were performed every 7 days.

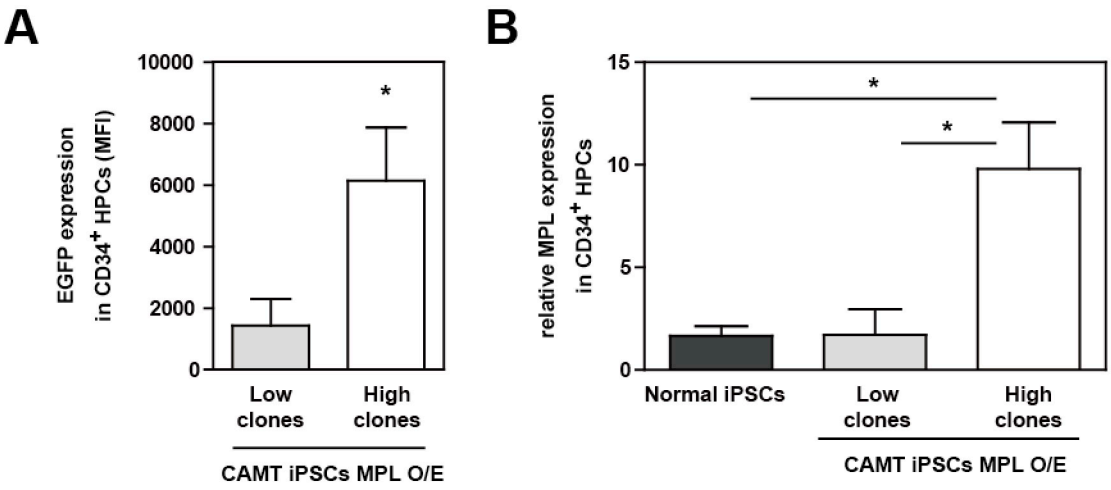
Hirata et al. Supplemental Figure 5



Supplemental Figure 5. Cell cycle distribution of MPPs on day +2

Representative flow cytometric analysis of the cell cycle of MPPs cultured in the presence of SCF, TPO and EPO at day +2. MPPs were derived from normal iPSCs (red line) or CAMT-iPSCs (blue line).

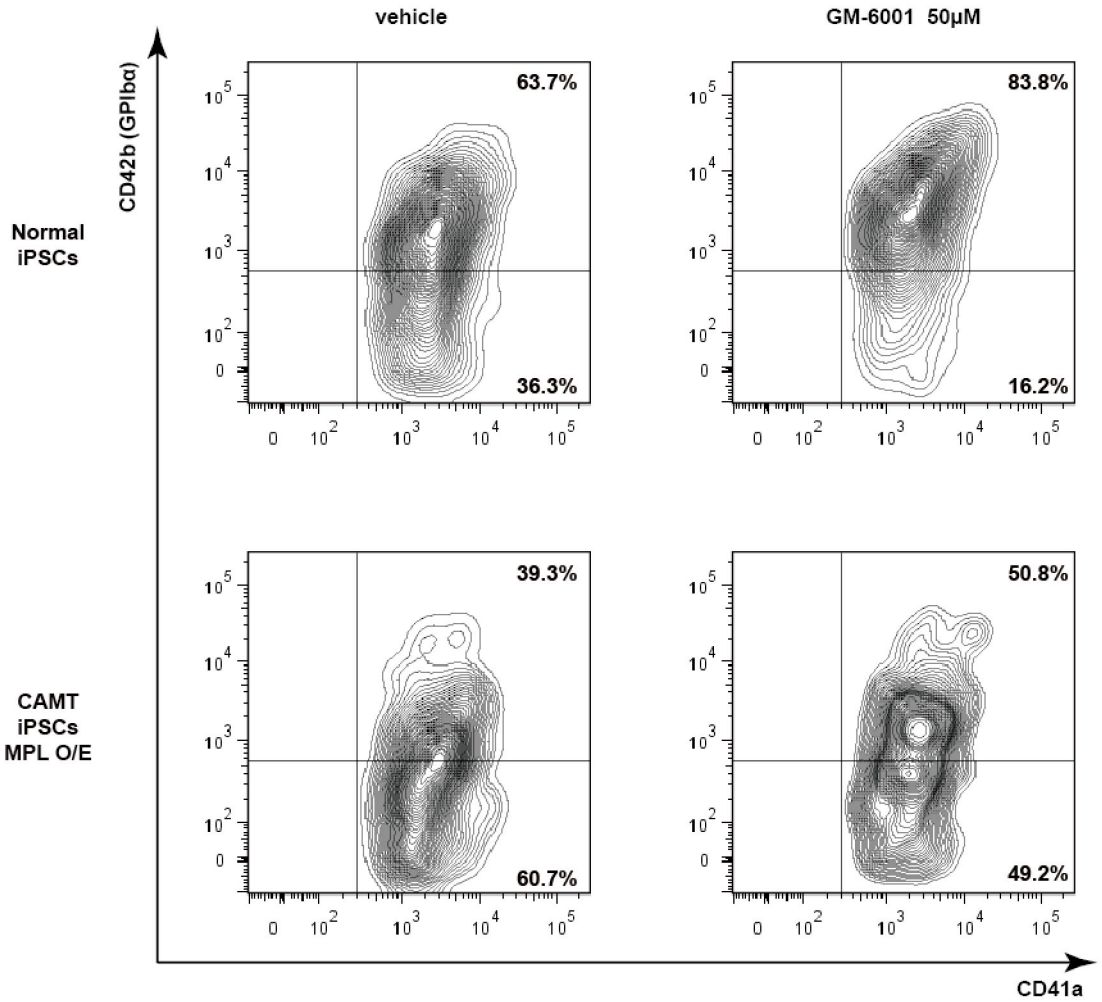
Hirata et al. Supplemental Figure 6



Supplemental Figure 6. Correlation of EGFP fluorescence intensity and *MPL* mRNA expression in CD34⁺ HPCs.

(A) MFI of EGFP reflecting expression achieved through retroviral transduction. (B) Expression of *MPL* mRNA in CD34⁺ HPCs derived from normal iPSCs and CAMT-iPSCs overexpressing MPL. The MFI of EGFP was well correlated with the level of *MPL* mRNA expression in samples. *$P < 0.05$.

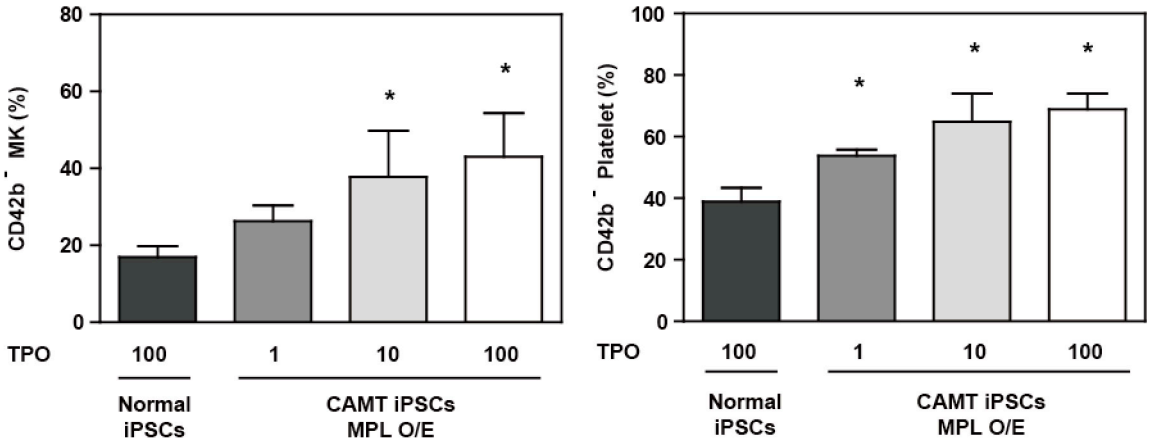
Hirata et al. Supplemental Figure 7



Supplemental Figure 7. CD42-null platelets were generated from CAMT-iPSCs with excessive MPL even in the presence of GM6001, inhibitor of CD42b (GPIbα) shedding

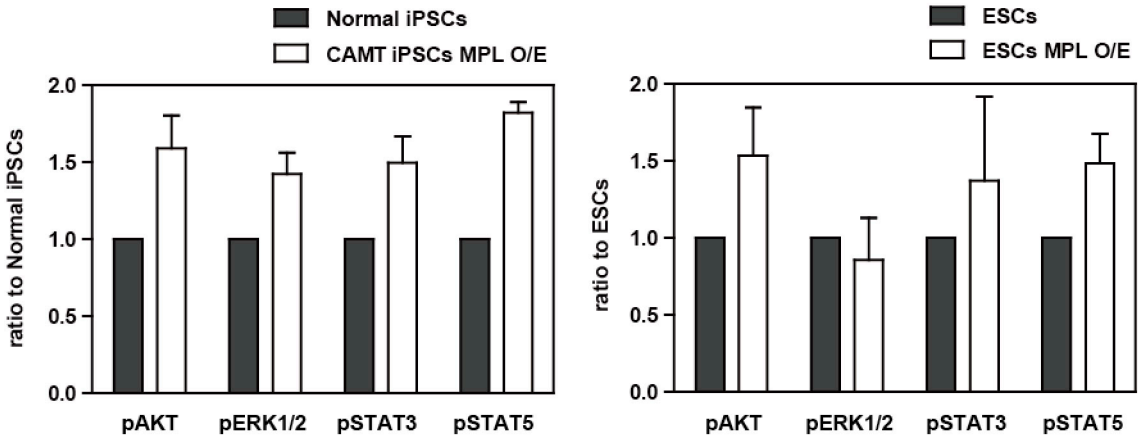
Representative contour plots showed platelets generated with or without the metalloproteinase inhibitor GM-6001 (50 μM) on days 22-24 of culture.

Hirata et al. Supplemental Figure 8



Supplemental Figure 8. Excessive MPL signaling impairs normal megakaryopoiesis

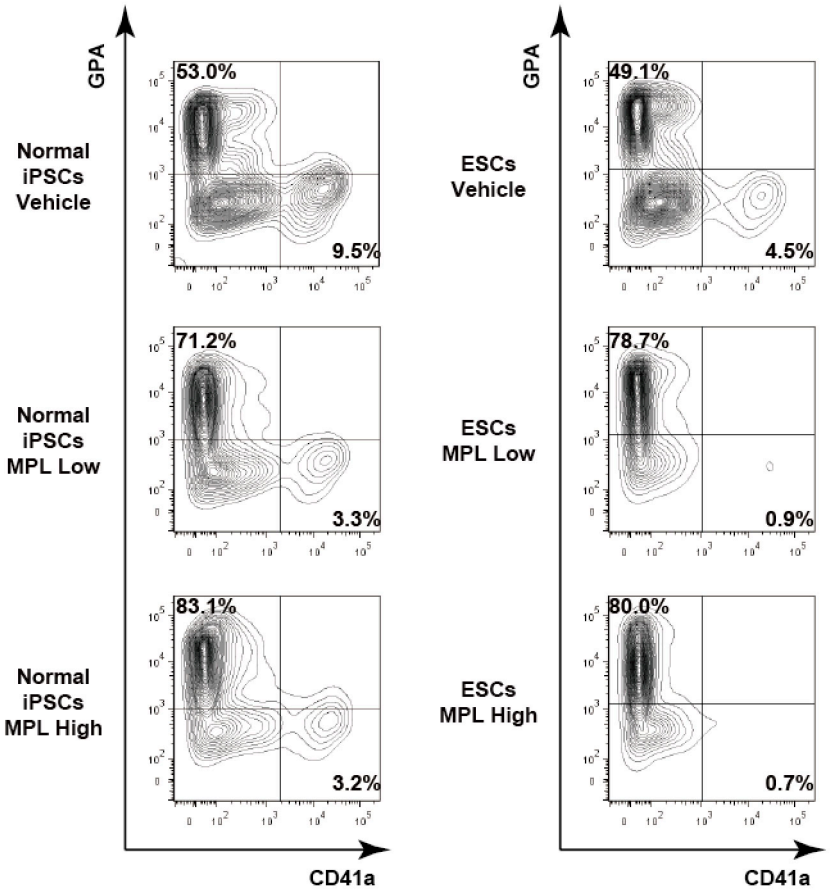
Percentages of $CD42b^-$ MKs and platelets derived from normal iPSCs or CAMT-iPSCs overexpressing MPL (O/E) on days 22-24 of culture in the presence of various concentrations of TPO (ng/mL). * $P < 0.05$.



Supplemental Figure 9. Enhanced phosphorylation of MPL downstream effector molecules in MPL-overexpressing CAMT-iPSCs and ESCs.

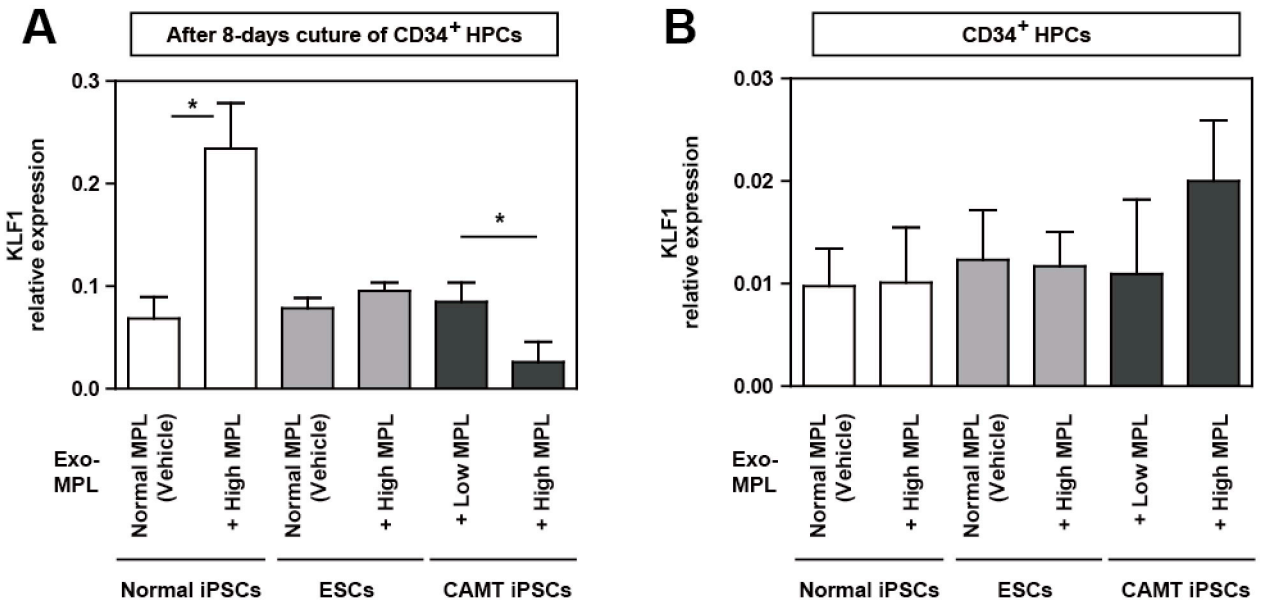
MPL-mediated downstream signaling in $CD41a^+$ MKs on day 24 of culture. $CD41a^+$ MKs were fixed and analyzed for phosphorylation of the indicated molecules (pAKT, pERK1/2, pSTAT3 or pSTAT5) using flow cytometry. The white bars depict the mean ratios of the fluorescence intensity of CAMT-iPSCs and ESCs overexpressing MPL (O/E); the black bars, normal iPSCs and ESCs (black).

Hirata et al. Supplemental Figure 10



Supplemental Figure 10. Strong MPL signaling in normal PSCs enhances erythropoiesis but not megakaryopoiesis.

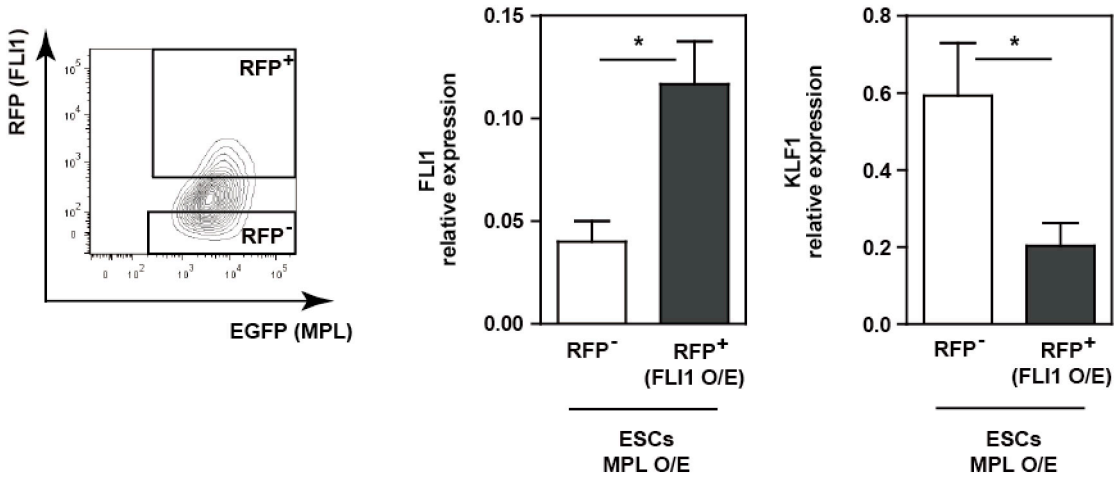
EGFP^{low} or EGFP^{high} CD34^{+} HPCs derived from normal iPSCs and ESCs transduced with a vehicle or MPL expression vector were cultured for 8 days in the presence of SCF, TPO and EPO. Erythropoiesis but not megakaryopoiesis was enhanced in a MPL signal intensity-dependent manner.



Supplemental Figure 11. Expression of *KLF1* mRNA in CD34$^+$ HPCs and cells cultured for an additional 8 days.

EGFP^{low} (+ Low MPL) or EGFP^{high} (+ High MPL) populations among CD34$^+$ HPCs from normal PSCs and CAMT-iPSCs overexpressing MPL or vehicle were sorted (B) and then cultured for an additional 8 days in the presence of SCF, TPO and EPO (A). *KLF1* expression was analyzed using quantitative RT-PCR. Expression of *KLF1* at the CD34$^+$HPCs stage was not correlated with biased differentiation of the cell lines. * $P < 0.05$.

Hirata et al. Supplemental Figure 12



Supplemental Figure 12. Expression of *FLI1* mRNA was positively correlated and *KLF1* mRNA was negatively correlated with red fluorescent protein (RFP) expression in PSCs overexpressing MPL (O/E).

$CD34^+$ HPCs derived from normal PSCs overexpressing MPL-EGFP (O/E) were transfected with a FLI1-RFP expression vector and cultivated for an additional 8 days. The RFP^+ and RFP^- populations shown in the contour plot were sorted, and *FLI1* and *KLF1* expression were analyzed using quantitative RT-PCR. *FLI1* expression was positively correlated with RFP expression, while *KLF1* expression was negatively correlated. $* P < 0.05$.

Supplemental methods

Immunohistochemistry of human iPSCs

Human iPSCs were fixed with 4% paraformaldehyde in PBS (Sigma-Aldrich), after which they were labeled first with antibodies against human TRA-1-60 and TRA-1-81 (Invitrogen) and then with a secondary antibody (Invitrogen), or with an antibody against human SSEA-4–PE (R&D Systems). They were then observed using an epifluorescence microscope (DMIRBE; Leica, Solms, Germany).

Alkaline phosphatase staining

Human iPSCs were fixed with 4% paraformaldehyde in PBS at room temperature for 15 min, washed with 0.1M Tris HCl buffer, and then incubated with alkaline phosphatase substrate (VECTOR Laboratories, Burlingame, USA) for 15 min at room temperature. Cells positive for alkaline phosphatase activity stained red.

Teratoma formation and histological analysis

Human iPSCs were prepared from 10^7 cells/mL in PBS. Male NOD/SCID mice were anesthetized with diethyl ether, after which aliquots of suspended cells (1-3 $\times$ 10^6 cells) were injected into their testes. Eight weeks after injection, the mice were sacrificed and the resultant tumors dissected. The collected tumor samples were fixed in 4% paraformaldehyde, embedded in paraffin, sectioned and stained with hematoxylin and eosin.

Western blotting analysis

Experiments were performed as described previously (Eto et al., 2007; Nishiki et al., 2008). In brief, cell lysates treated with TNE buffer (10 mM Tris-HCl, pH7.8, 150 mM NaCl, 1% NP-40, and 1 mM EDTA) supplemented

with proteasome inhibitor cocktail (Roche) were separated by electrophoresis on a 10-20% SDS-polyacrylamide gradient gel (Bio-Rad Laboratories) and transferred to a polyvinylidine difluoride membrane (Millipore). The separated proteins were visualized using SuperSignal West Pico Chemiluminescent Substrate (Thermo Fisher Scientific). Anti-TPOR/ Mpl (Millipore) or anti-β-actin (Sigma-Aldrich) was used as the primary antibody. Horseradish peroxidase-conjugated anti-rabbit antibody (GE Healthcare) or anti-mouse antibody (GE Healthcare) was used as the secondary antibody. Proteins were detected using an ECL kit (GE Healthcare).

Serial replating assay

$CD34^+$ HPCs (8×10^3 cells/dish) were cultivated for 7 days in MethoCult H4434 semisolid medium containing TPO, after which the colony numbers were counted. Secondary colony-forming cell assays were performed by harvesting all of the cells from the primary culture dishes and replating 5×10^3 cells each into new plates. Secondary and tertiary assays were conducted in the same way. Colony numbers obtained from each assay were normalized to 1×10^4 replated cells.