Supplemental Methods

Microarray analysis

imMKCLs were cultured for 11 days in EBM medium supplemented with rhSCF (R&D Systems) and 1 μg/mL doxycycline (TAKARA) in the presence of rhTPO (50 ng/mL) or TA-316 (200 ng/mL). Total RNA was isolated as described above, and each RNA sample was analyzed on an Affymetrix Human Gene 2.0 ST Array (Affymetrix, California, USA and Kurabo, Osaka, Japan). The expression levels of genes in the samples treated with TPO (50 ng/mL) or TA-316 (200 ng/mL) were compared. Genes that were up- or down-regulated were identified using DNA Microarray Data Viewer Software (Kurabo). All data are MIAME compliant, and the raw data were deposited in Gene Expression Omnibus (accession number; GSE75338).

Transmission electron microscope (TEM) analysis

TEM was performed as described previously[8]. Samples were observed using a transmission electron microscope operating at 80 kV (HT-7700; Hitachi, Tokyo, Japan).

Supplemental Table

Table S1. Up-regulation of genes (top 10) related to growth factor activity in imMKCLs.

Gene Symbol	Signal		Fold Change
	rhTPO	TA-316	
FGF2	74.09	132.52	1.79
CSF1	41.84	71.94	1.72
FGF7	7.52	12.57	1.67
EREG	43.62	67.48	1.55
PDGFC	76.67	116.94	1.53
KGFLP1	7.78	11.24	1.44
AMELX	8.77	12.62	1.44
NRG4	8.96	12.84	1.43
VEGFA	73.96	102.87	1.39
LEFTY1	35.13	48.69	1.39

Supplemental Figures

Figure S1

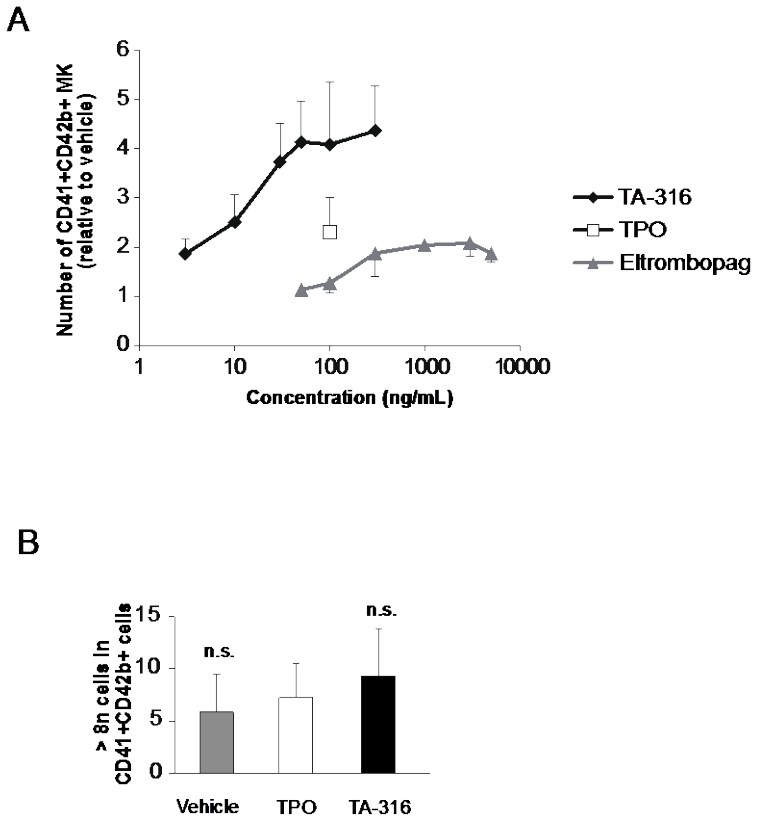

Figure S1. TA-316 induces MK-lineage cells from iHPCs.

Crude iHPCs from iPSC-Sacs were cultured for 10 days with rhTPO, TA-316 or Eltrombopag, and the $CD41^+/CD42b^+$ cell population was analyzed by FACS. (A) The number of $CD41^+/CD42b^+$ cells relative to that in control culture (vehicle, DMSO alone). (B) Percentages of multinucleated (≥8n) megakaryocytes were also analyzed by FACS. (rhTPO, 100 ng/mL; TA-316, 100 ng/mL). Data represent the mean and standard deviation.

Figure S2

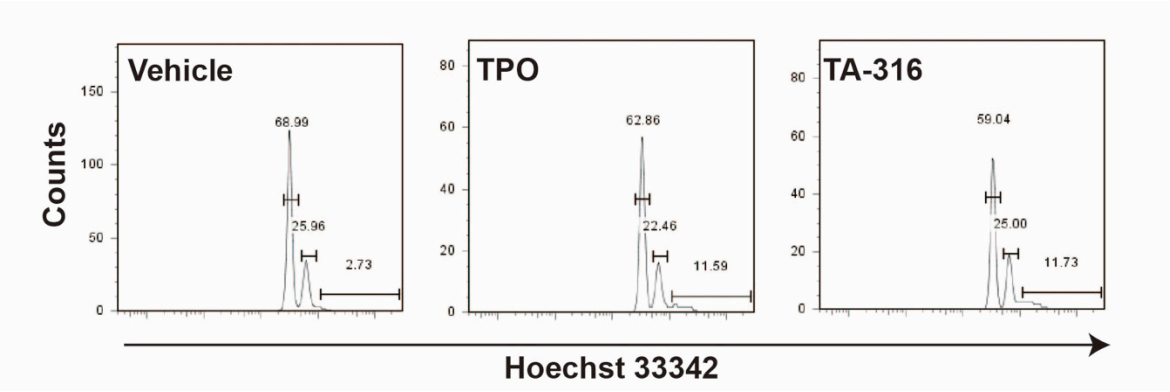

Figure S2. TA-316 induces maturation of imMKCLs and increases functional platelets.

imMKCLs were cultured for 15 days with rhTPO (50 ng/mL), TA-316 (200 ng/mL) or DMSO (0.05%). MK and platelets were collected. Representative histograms of DNA content in MKs cultured with DMSO, rhTPO or TA-316.

Figure S3

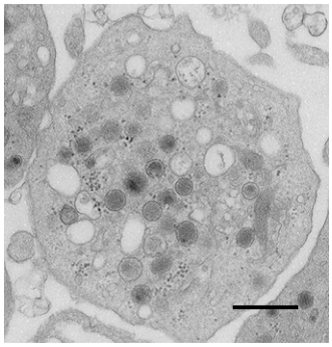

Figure S3. A transmission electron microscope image of imMKCL-derived platelets produced with TA-316. Scale bar: 1 μm.

Figure S4. Phosphorylation of signaling proteins in imMKCLs induced by rhTPO, TA-316 or Eltrombopag.

imMKCLs were stimulated with rhTPO, TA-316, Eltrombopag or DMSO after 24 h TPO starvation. Cells were stimulated for 15, 60, 120 or 240 min (p-STAT5 (A)) or 120 min (p-STAT3 (B), p-STAT1 (C), p-ERK1/2 (D), p-AKT (E)). The distributions on the right in (A) show STAT5 phosphorylation in cells cultured with DMSO, rhTPO (50 ng/mL), TA-316 (400 ng/mL) or Eltrombopag (1000 ng/mL) for 120 min. Data represent the mean and standard deviation.

Figure S4

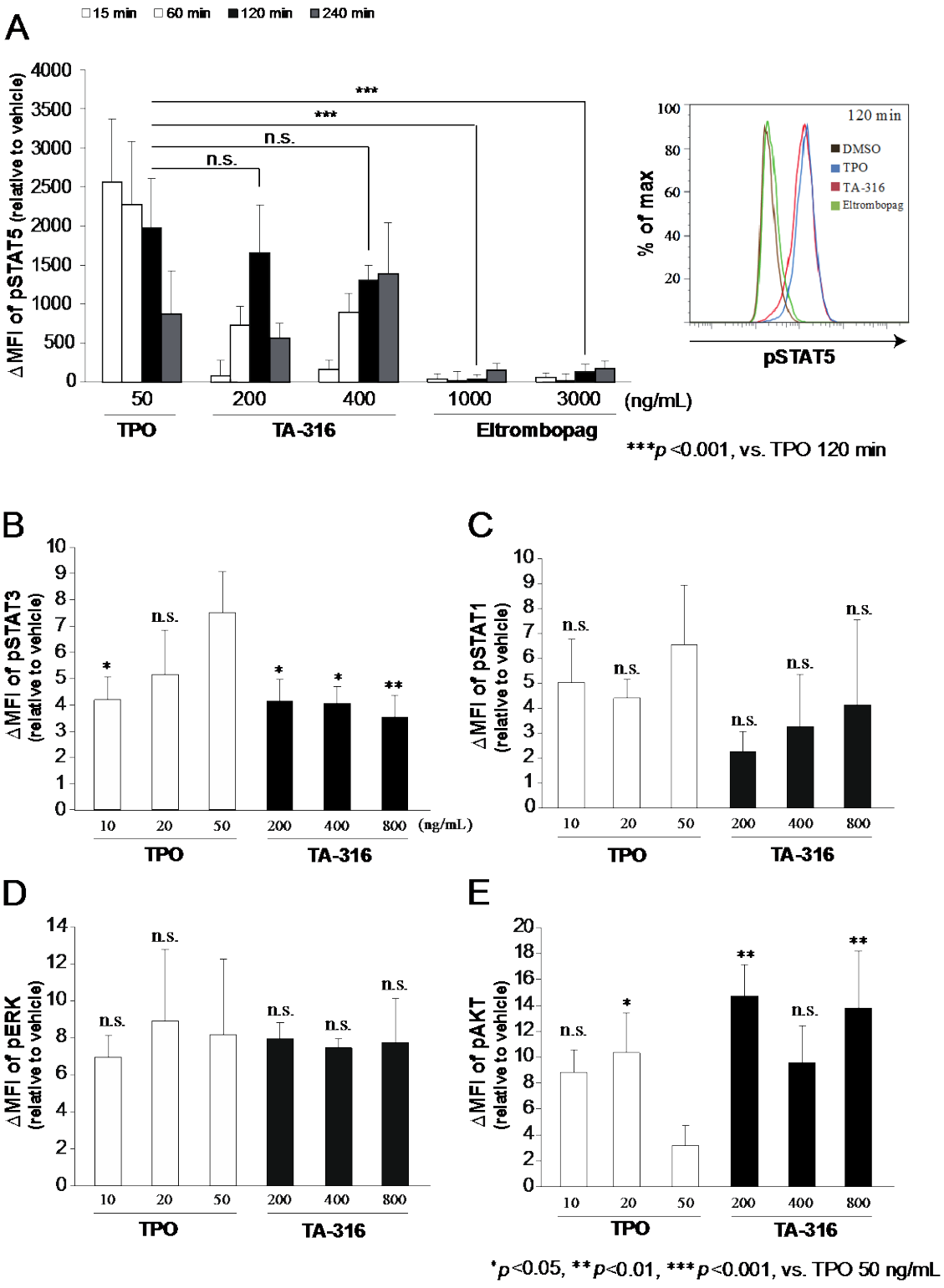
A
15 min
60 min
120 min
240 min
ΔMFI of pSTAT5 (relative to vehicle)
n.s.
***
***
n.s.
50
200
400
1000
3000
(ng/mL)
TPO
TA-316
Eltrombopag
120 min
DMSO
TPO
TA-316
Eltrombopag
% of max
pSTAT5
***p <0.001, vs. TPO 120 min
B
ΔMFI of pSTAT3 (relative to vehicle)
n.s.
10
20
50
200
400
800
(ng/mL)
TPO
TA-316
C
ΔMFI of pSTAT1 (relative to vehicle)
n.s.
D
ΔMFI of pERK (relative to vehicle)
n.s.
E
ΔMFI of pAKT (relative to vehicle)
n.s.
*p<0.05, **p<0.01, ***p<0.001, vs. TPO 50 ng/mL

Figure S5

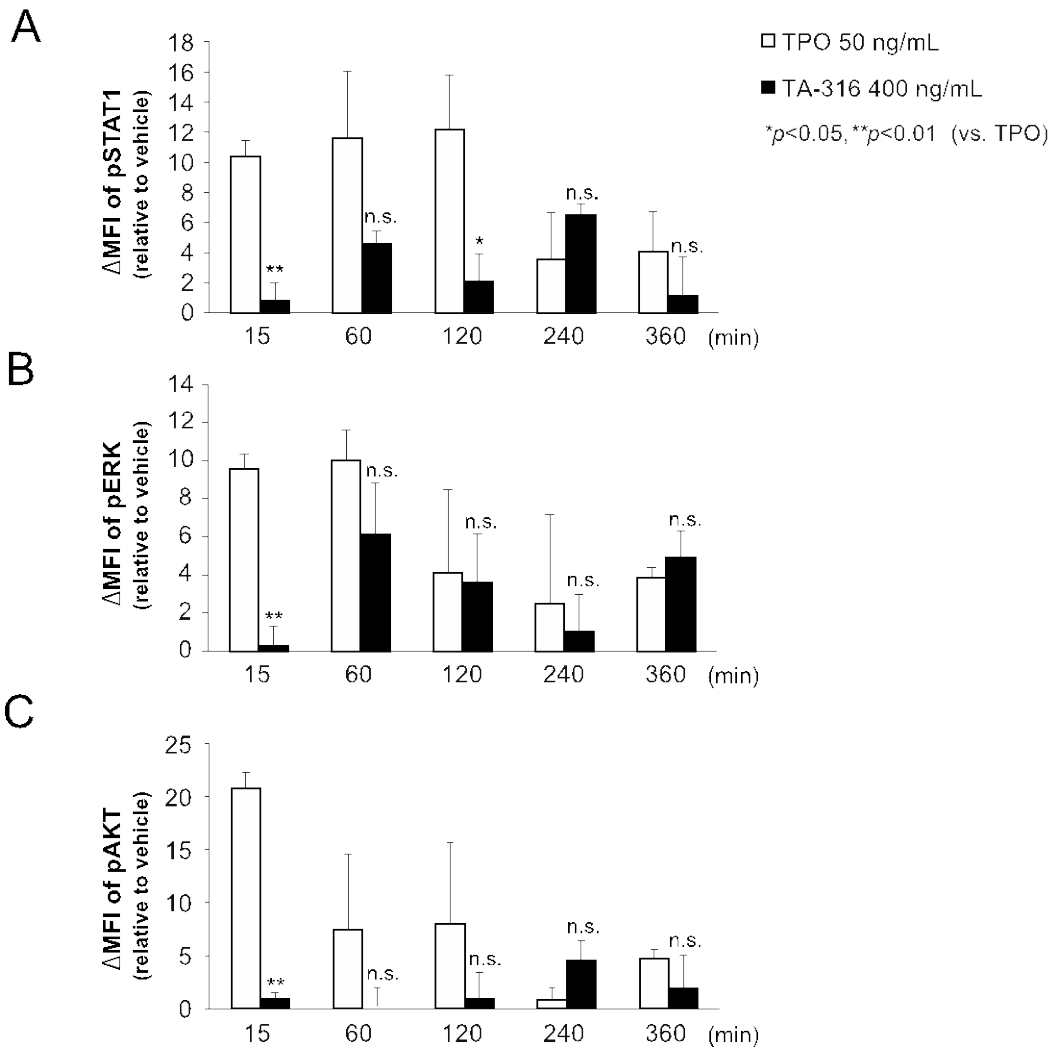

Figure S5. Phosphorylation kinetics of STAT1, ERK and AKT in imMKCLs induced by rhTPO or TA-316.

imMKCLs were stimulated with rhTPO (50 ng/mL), TA-316 (400 ng/mL) or DMSO (vehicle, 0.05%) after 24 h (STAT1, ERK1/2) or 40 h (AKT) TPO starvation. Cells were stimulated for 15, 60, 120, 240 or 360 min (p-STAT1 (A), p-ERK1/2 (B), and p-AKT (C)). Data represent the mean and standard deviation.