Supplemental Data:

Title: Human megakaryocytic microparticles induce de novo platelet biogenesis in a wild-type murine model

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Supplemental Materials and Methods

Chemicals and Reagents

Recombinant human interleukin 3 (IL-3), IL-6, IL-9, IL-11, stem cell factor (SCF), and thrombopoietin (TPO) were purchased from PeproTech Inc. BIT 9500 was purchased from Stemcell Tech. Anti-CD61 magnetic microbeads, MACS cell-separation tools, pre-separation filter, and lineage depletion kit were purchased from Miltenyi. RPMI 1640X, Antibiotic-Antimycotic were purchased from Thermo Fisher Scientific. LEAF rat anti-mouse CD41 antibody was purchased from Biolegend. Retic-Count kit was purchased from BD bioscience. All other chemicals were purchased from Sigma-Aldrich.

In vitro human megakaryocyte (Mk) culture derived from mobilized peripheral blood (MPB) $CD34^+$ HSPCs

$CD34^+$ cell-derived Mks were cultured as described[1]. Brief, frozen G-CSF-mobilized human peripheral blood $CD34^+$ cells (Fred Hutchinson Cancer Research Center) were cultured in megakaryocytic lineage growth media (Iscove modified Dulbecco medium, IMDM, Gibco) supplemented with 20% BIT 9500 (Stemcell Tech.), 100 ng/mL TPO, 100 ng/mL stem cell factor (SCF), 2.5 ng/mL interleukin-3 (IL-3), 10 ng/mL IL-6 & IL-11 and human LDL) under 5% O_2 for 5 days. From day 5 to day 7, IL-3 was increased to 10 ng/mL and IL-6 was substituted with IL-9. At day 7, dead cells were removed by using Dead Cell Removal Kit (Miltenyi), followed by the enrichment of $CD61^+$ cells using MACS separation with anti-CD61 magnetic microbeads (Miltenyi).

Isolation of human Megakaryocytic Microparticles (huMkMPs)

MkMPs were isolated as described [2, 3]. Briefly, cells, cell debris, and apoptotic bodies from day-12 $CD34^+$-derived Mk culture described above, were removed by centrifugation at 1000 × g for 10 min. MkMPs were then enriched from the supernatant via ultracentrifugation (Optima Max Ultracentrifuge and Rotor TLA-55, Beckman Coulter) under 25,000 rpm for 30 min at 4 °C. After that, MkMPs were resuspended in PBS or stored at -80 °C until used.

Supplemental Figures

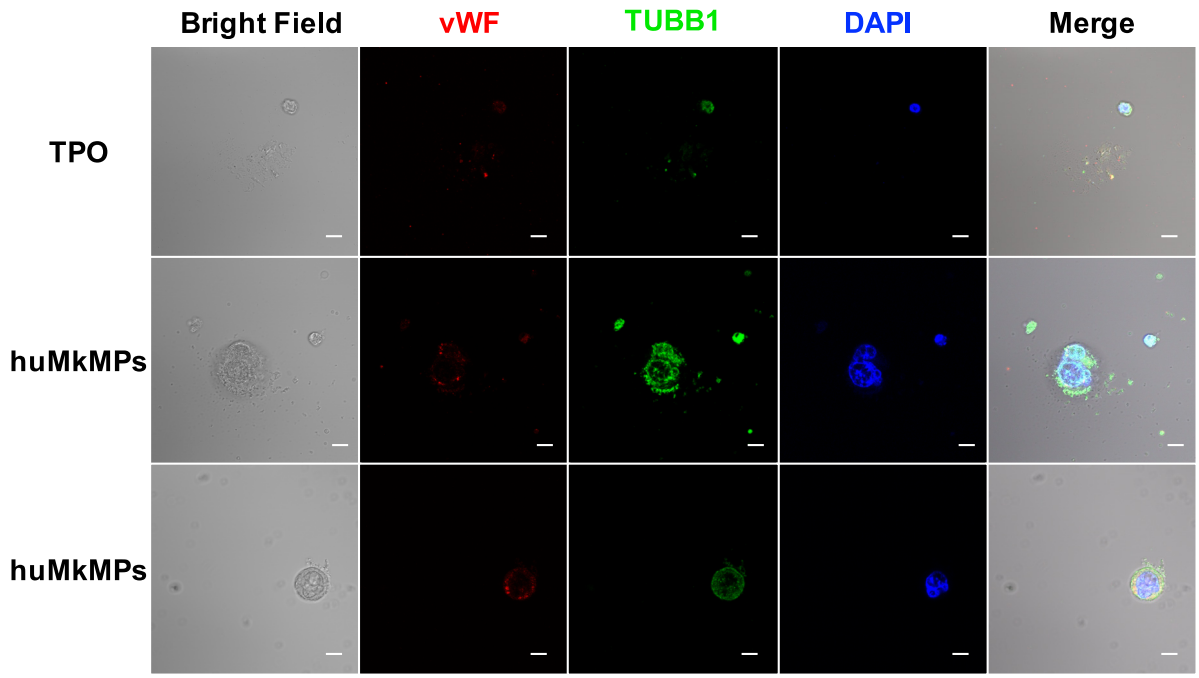

Figure S1. Co-culture of huMkMPs with muHSPCs promotes murine megakaryopoiesis. muHSPCs were co-cultured with either huMkMPs only, or 50 ng/ml thrombopoietin (TPO) for 5 days. Cells were harvested and fixed at day 5, stained for expression of von Willebrand Factor (vWF), beta-tubulin I (TUBB1), and nucleus (DAPI), and examined via confocal and bright-field microscopy. Scale bar = 10 μm.

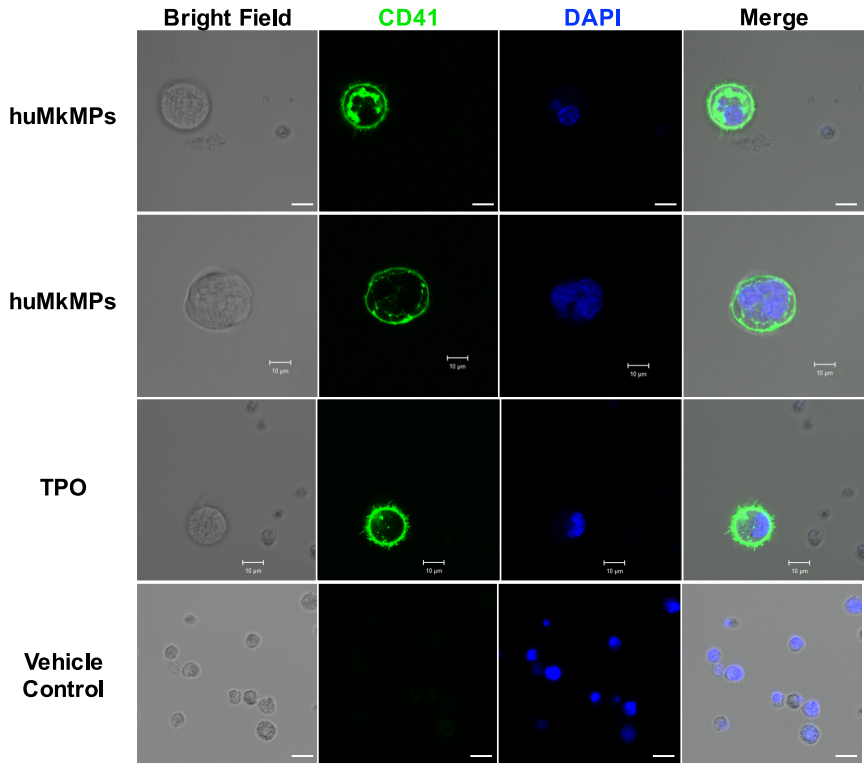

Figure S2. Co-culture of huMkMPs with muHSPCs promotes the biogenesis of murine CD41^{+}-cells (megakaryocytes). muHSPCs were co-cultured with either huMkMPs only, or 50 ng/ml thrombopoietin (TPO), or without any supplements (vehicle control) for 5 days. Cells were harvested, stained with anti-CD41 antibody and examined via confocal and bright-field microscopy. Scale bar = 10 μm.

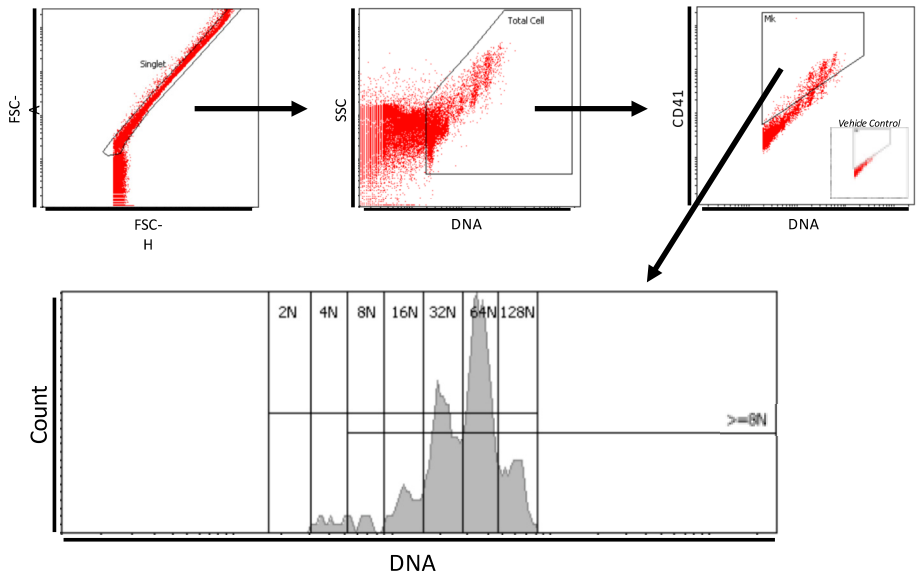

Figure S3. Representative flow-cytometry plots for megakaryocytic ploidy analysis. muHSPCs were cultured with 50 ng/ml thrombopoietin (TPO) for 5 day and were harvested for flow-cytometric analysis**.** Singlet events were first gated on the FSC-A (Forward Scatter, Area) vs. FSC-H (FSC, Height) plot, followed by gating the total cell population on the SSC (Side Scatter) vs. DNA-content plot. Megakaryocytes (Mks) were next selected based on CD41 expression on the $CD41^{+}$ vs. DNA-content plot. The various ploidy classes (from 2N up to 128N) of the Mk population can be visualized on a histogram of Mk-cell count vs. DNA content.

Supplemental References

[1] Panuganti, S., Schlinker, A. C., Lindholm, P. F., Papoutsakis, E. T., and Miller, W. M. (2013) Three-stage ex vivo expansion of high-ploidy megakaryocytic cells: toward large-scale platelet production, *Tissue Eng Part A 19*, 998-1014.

[2] Jiang, J., Woulfe, D. S., and Papoutsakis, E. T. (2014) Shear enhances thrombopoiesis and formation of microparticles that induce megakaryocytic differentiation of stem cells, *Blood 124*, 2094-2103.

[3] Jiang, J., Kao, C. Y., and Papoutsakis, E. T. (2017) How do megakaryocytic microparticles target and deliver cargo to alter the fate of hematopoietic stem cells?, *J Control Release 247*, 1-18.