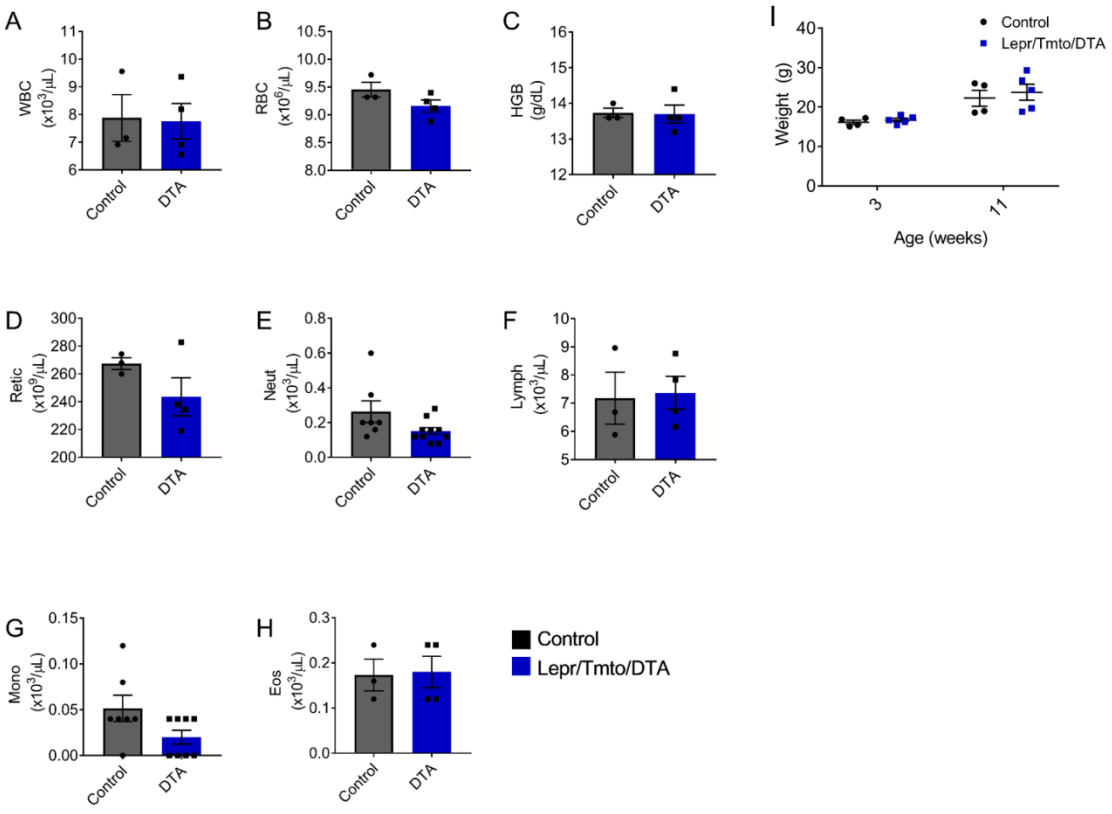
Supplemental Data

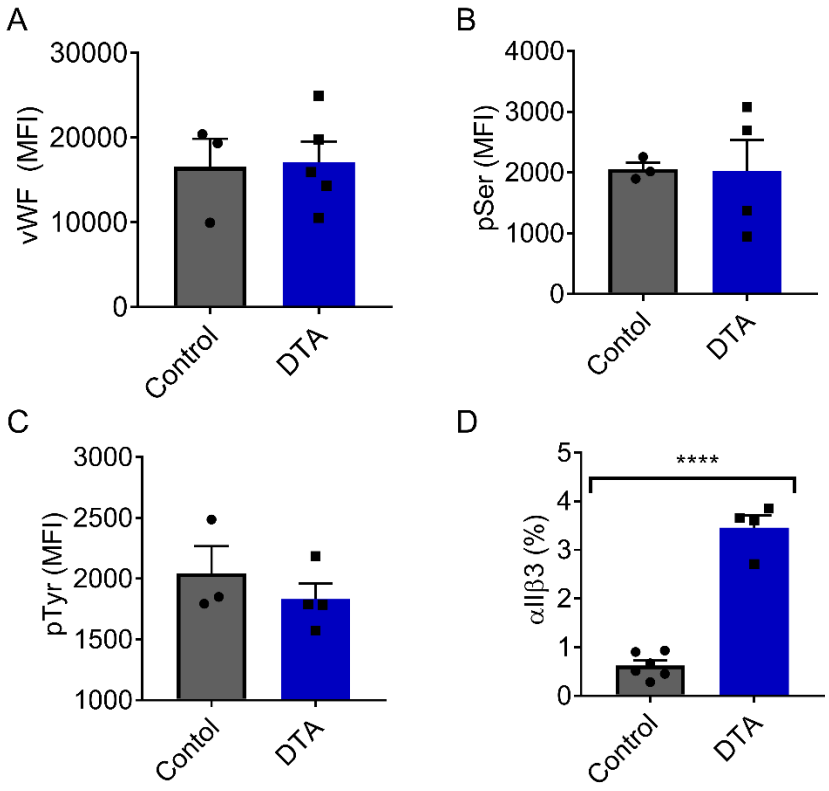
Table S1. Human primer sequences. Primer sequences used for performing RT-PCR.

Name	Sequence 5'-3'
GAPDH F	TCTGCTCCTCCTGTTCGACA
GAPDH R	AAAAGCAGCCCTGGTGACC
NESTIN F	GAAGGGCAATCACAACAGGTG
NESTIN R	GGGGCCACATCATCTTCCA
CXCL12 F	TGGGCTCCTACTGTAAGGGTT
CXCL12 R	TTGACCCGAAGCTAAAGTGG
VCAM F	GTCTCCAATCTGAGCAGCAA
VCAM R	TGAGGATGGAAGATTCTGGA
OPN F	AGATGGGTCAGGGTTTAGCC
OPN R	CATCACCTGTGCCATACCAG
SCF F	AATCCTCTCGTCAAAACTGAAGG
SCF R	CCATCTCGCTTATCCAACAATGA
RAP1B F	AGCAAGACAATGGAACAACTGT
RAP1B R	TGCCGCACTAGGTCATAAAAG
LAT F	GCATCCATCCATCAGTGGC
LAT R	CCCATTCACGTAATCCGGTTC
SRC F	TGCAGCAGAATGTCTTCCAG
SRC R	AAAGTTGGCCTCACCTTGG
BAX F	TCCCCCCGAGAGGTCTTTT
BAX R	CGGCCCCAGTTGAAGTTG
DIAPH1 F	TGTCAGTTGGGTGCAAACAT
DIAPH1 R	GCTTGTTCCGGCTATCGTAA
THPO F	CAGGACTGAAAAGGGAATCA
THPO R	CGTTGGAAGGCCTTGAATTT

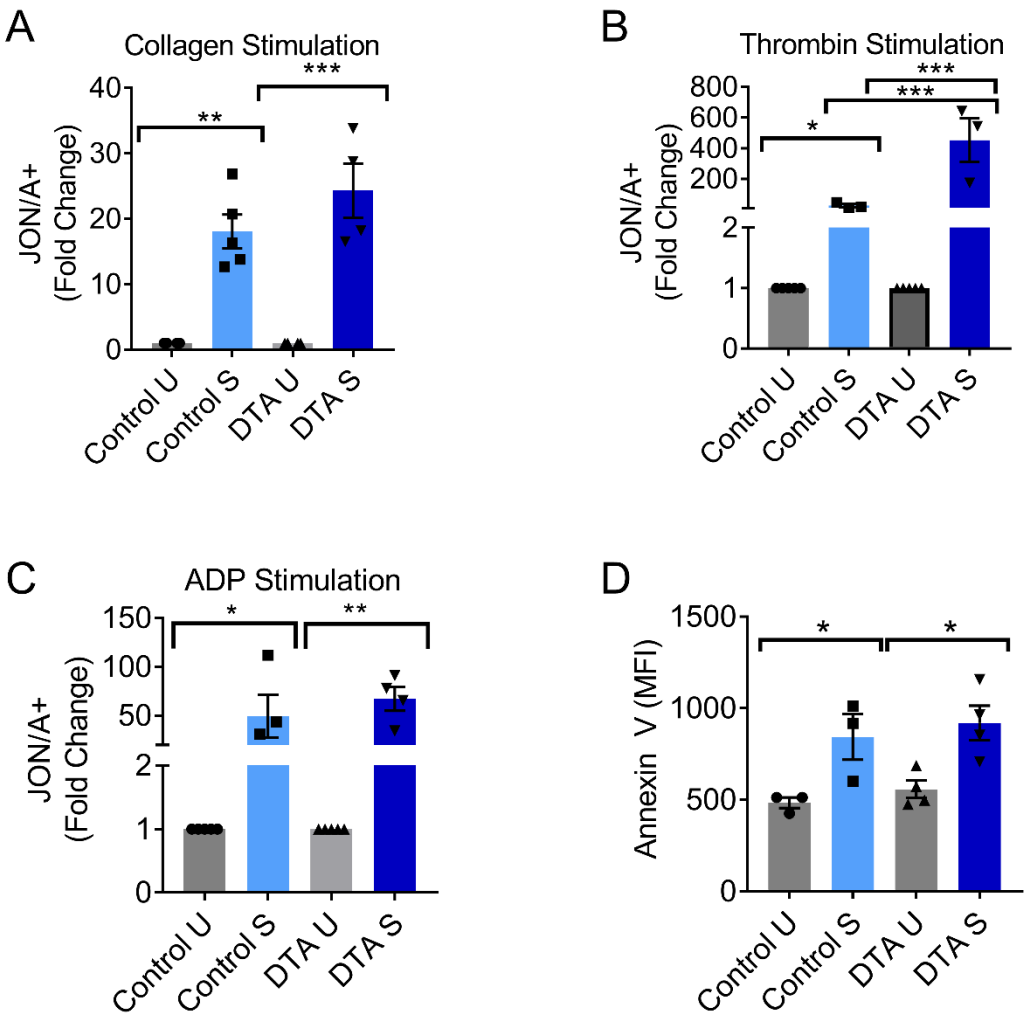
Table S2. RPPA Analysis Data Set. Entire data set of protein analysis from megakaryocytes cultured alone compared to those cultured with cMSCs.



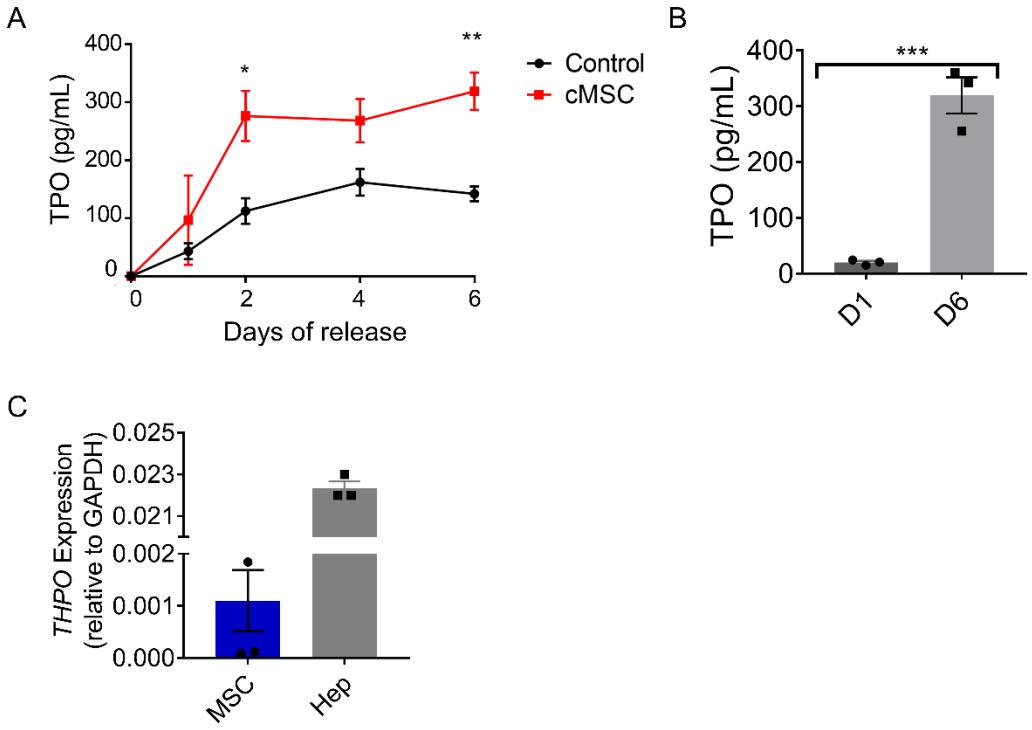
Supplemental Figure 1. Lepr/Tmto/DTA mouse complete blood count and weight analysis. Using an advia coulter counter, we determined the number of A) white blood cells, B) red blood cells, C) hemoglobin, D) reticulocytes, E) neutrophils, F) lymphocytes, G) monocytes, and H) eosinophils (n=3-6) in the Lepr/Tmto/DTA mice compared to control mice. I) Analysis of the mouse weight at 3 and 11 weeks after birth did not reveal any significant differences at each time point between Lepr/Tmto/DTA mice and control mice (n=3-7). A-H was analyzed with Student's T-test and I by ANOVA with Tukey post-hoc test.



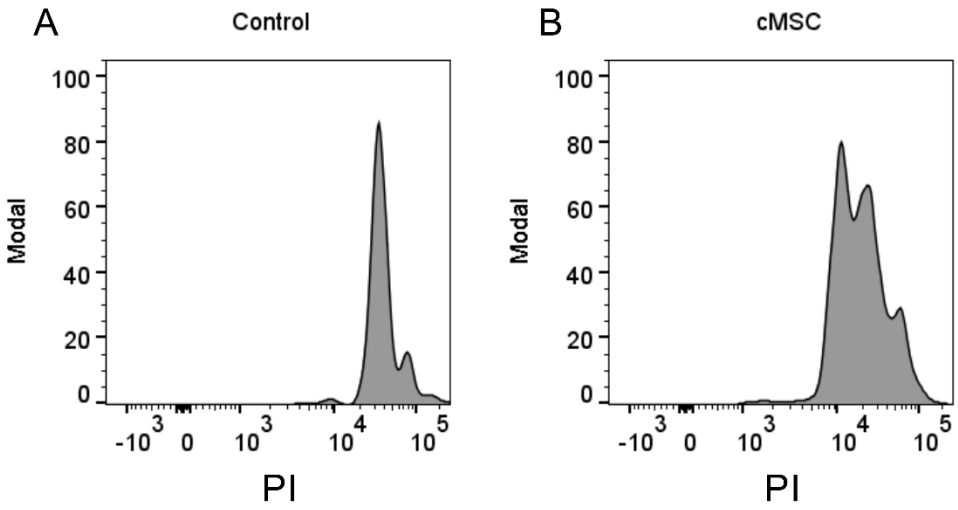
Supplemental Figure 2. Baseline evaluation of platelets from Lepr-DTA mice. A) Assessment of α-granule numbers by measuring intracellular von-Willebrand Factor levels with flow cytometry in Lepr/DTA mice compared to control mice (n=3-5). B) phosphorylated-Serine levels of platelets measured by flow cytometry in Lepr/DTA mice compared to control mice (n=3-4). C) phosphorylated-Tyrosine levels of platelets measured by flow cytometry in Lepr/DTA mice compared to control mice (n=3-4). D) Increased levels of integrin αIIβ3 was detected in Lepr-DTA mice compared to control mice suggesting increased baseline activation (n=4-5). **** $p \leq 0.0001$ by Student's T-test.



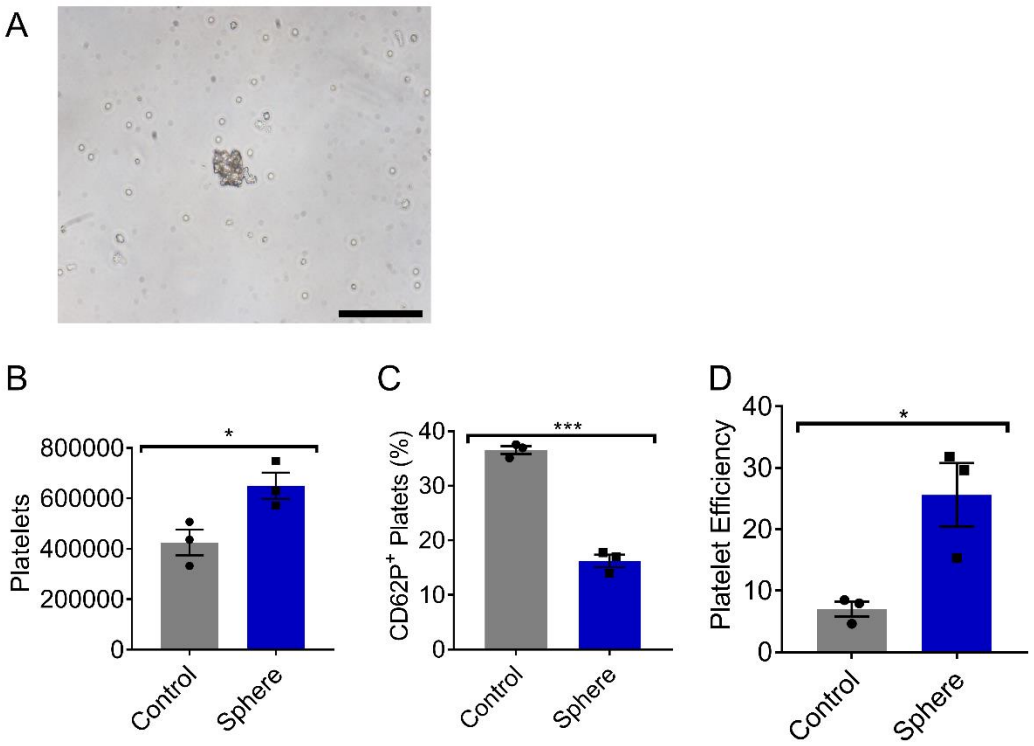
Supplemental Figure 3. Platelets from Lepr/DTA mice activation in response to agonist stimulation. Assessment of platelet activation in Lepr/DTA mice by flow cytometry in response to A) Collagen (80µg/mL), B) Thrombin (1U/mL), and C) ADP (20µM) stimulation revealed increased expression of JON/A (integrin αIIβ3) in stimulated (S) samples compared to unstimulated (U) samples. Data is represented as fold change in stimulated samples relative to unstimulated samples of the same group. D) To assess phosphatidyl serine exposure, levels of Annexin V expression was measured by flow cytometry after stimulation with collagen (80µg/mL) (n=3-5 for all panels). * p = 0.05 ** p≤0.01 *** p≤0.001 by ANOVA with Tukey post-hoc test.



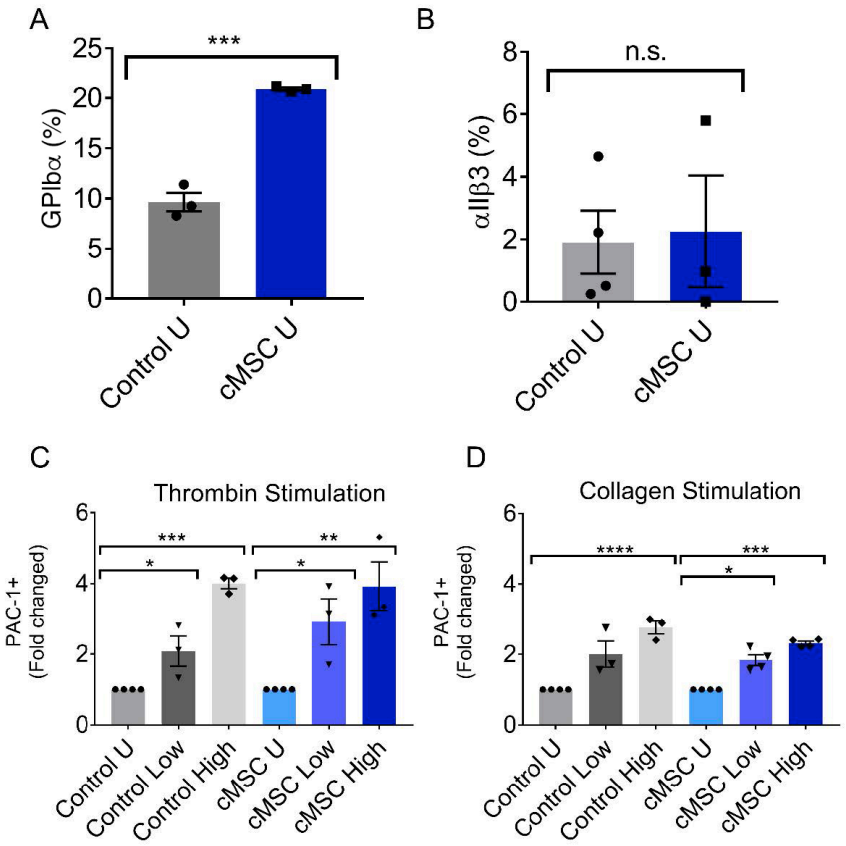
Supplemental Figure 4. Secreted TPO and gene expression measured from the co-culture group and control groups. A) An ELISA assay detected increased TPO presence in the cMSC co-culture group compared to the control group containing megakaryocytes alone (n=3-4). B) A significant increase in TPO presence was detected in the cMSC co-culture group supernatant at day 6 of culture compared to day 1 (n=3). C) rt-PCR analysis of *THPO* gene expression from cMSCs compared to primary human hepatocytes (n=3-4). * p = 0.05 ** p≤0.01 by ANOVA with Tukey post-hoc test *** p≤0.001 by Student's T-test



Supplemental Figure 5. cMSCs induce increased polyploidy in cultured megakaryocytes compared to those cultured alone. Using flow cytometry, megakaryocytes stained with propidium iodide (PI) were evaluated for their DNA content. Representative FACS plots are displayed for A) megakaryocytes cultured alone and B) megakaryocytes cultured with cMSCs.



Supplemental Figure 6. Cord tissue MSC spheroids enhance platelet formation and protect against platelet activation. A) cMSCs grown as spheroids were co-cultured with megakaryocytes (Scale = 1mm). B) Significantly greater amounts of platelets were generated in the spheroid co-culture group compared to the control group (n=3). C) Platelets generated from the spheroid co-cultures were less activated and had significantly lower expression of CD62P (n=3). D) The number of platelets produced per megakaryocyte was significantly increased in the spheroid co-culture group compared to the control group (n=3). * p = 0.05 *** p≤0.001 by Student's T-test.



Supplemental Figure 7. Baseline evaluation of platelets from co-cultures and response to agonist stimulation. A) Increased expression of GP1Bα was found in platelets from cMSC group compared megakaryocytes cultured alone (n=3). B) Very low baseline levels of integrin αIIβ3 was detected among all platelets generated in culture. N.S. = no significant difference (n=3). Platelets from co-cultures were able to activate in response to C) collagen low (20μg/mL) and collagen high (80μg/mL) and D) thrombin low (0.1U/mL) and thrombin high (1U/mL) stimulation. Data is represented as fold change in stimulated samples relative to unstimulated samples of the same group (n=3-4). * p = 0.05 ** p≤0.01 *** p≤0.001 and **** p≤0.0001 by Student's T-test in A and B and ANOVA with Tukey post-hoc test in C and D.