SUPPLEMENTARY APPENDIX

Thrombopoietin maintains cell numbers of hematopoietic stem and progenitor cells with megakaryopoietic potential

Aled O'Neill,[1] Desmond Chin,[1] Darren Tan,[1] A'Qilah Banu Bte Abdul Majeed,[1] Ayako Nakamura-Ishizu[1,2] and Toshio Suda[1,2]

[1]Cancer Science Institute, National University of Singapore, Singapore and [2]International Research Center for Medical Sciences, Kumamoto University, Kumamoto, Japan-

 doi:10.3324/haematol.2019.241406

Received: October 23, 2019.

Accepted: May 28, 2020.

Pre-published: June 11, 2020

Correspondence: *TOSHIO SUDA* - sudato@keio.jp

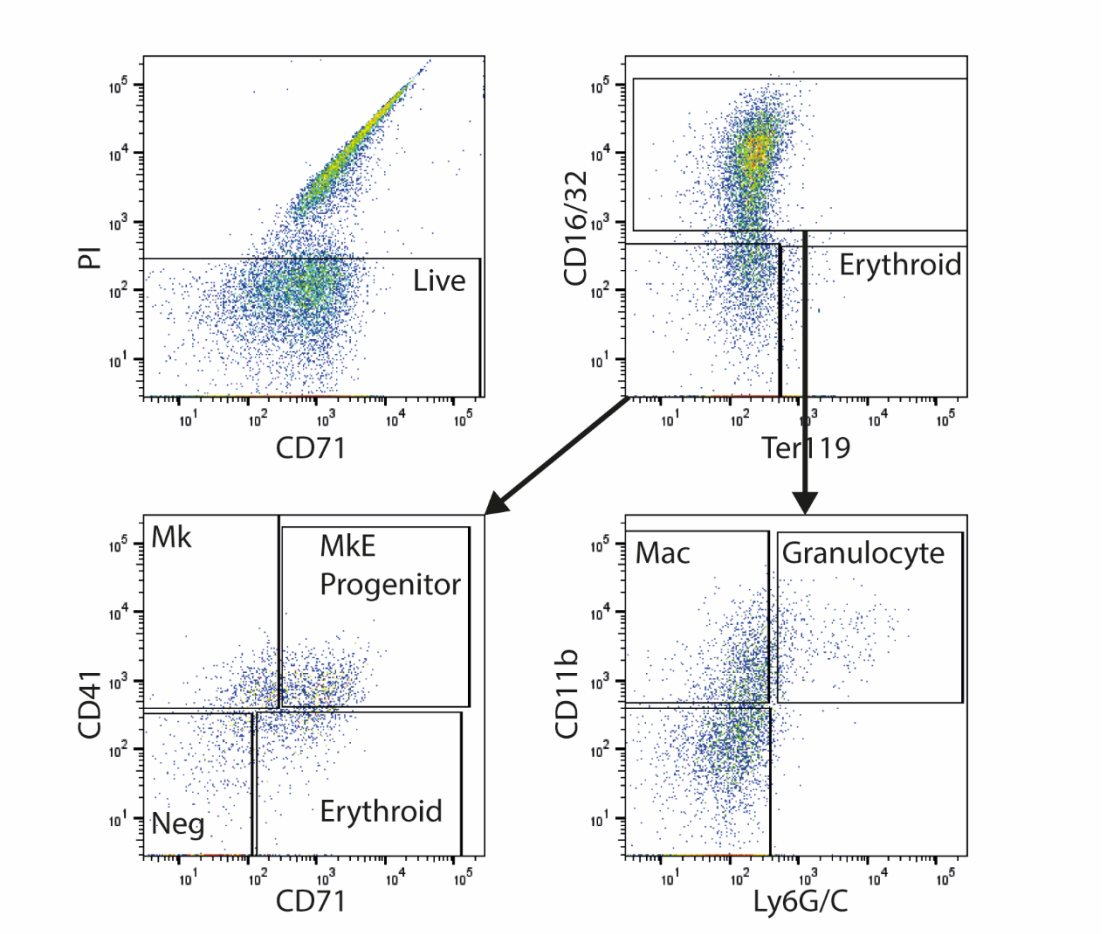
Supplemental Materials and Methods

Thpo$^{fl/fl}$ Mouse Generation

For gene editing by CRISPR/Cas9, two gRNA sequences were generated for target sites in Intron 1/2 and Intron 3/4 of the *Thpo* gene. Two single stranded oligo-deoxynucleotides (ssODNs) were generated with tails homologous to the target site sequences and containing a 34bp loxP sequence within the target cut site. These ssODNs were used to insert loxP sequences at the two target sites by homology directed repair. The gRNAs, recombinant Cas9 protein and ssODNs were microinjected into single cell zygotes and pups generated. A single pup containing both loxP sites flanking exons 2 and 3 of *Thpo* was identified and its genome analysed for off target mutation. No off target mutation was found and the mouse was bred with C57BL/6-NTac mice to produce a *Thpo*$^{fl/fl}$ strain.

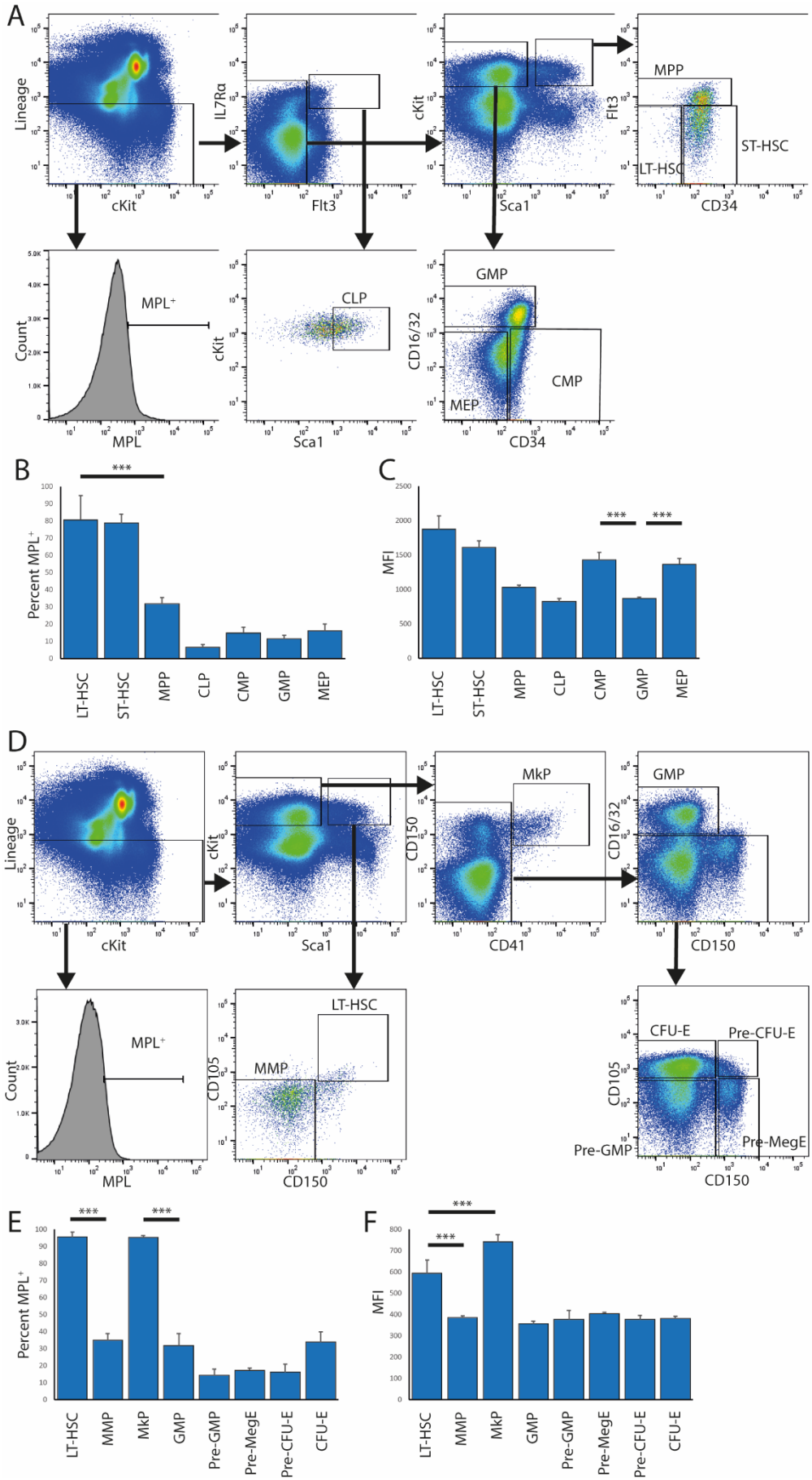
Flow Cytometric Antibody Panels

SLAM LSK cell analysis: BMMNCs were stained with a lineage cocktail of PerCP-Cy5.5 conjugated CD4 (RM4-5, BD Biosciences), CD8a (53-6.7, eBioscience), B220 (RA3-6B2, Biolegend), CD11b (M1/70, BD Biosciences), Ly6G/C (RB6-8C5, Biolegend) and Ter119 (TER-119, Biolegend), PE-Cy7 conjugated Sca1 (D7, Biolegend), biotinylated MPL (AMM2, Immuno-Biological Laboratories), PE conjugated CD41 (MWReg30, BD Biosciences), APC conjugated CD150 (TC15-12F12.2, Biolegend), BrilliantViolet421 conjugated cKit (2B8, Biolegend) and FITC conjugated CD34 (RAM34, eBioscience) antibodies. MPL was resolved with APC-eFluor780 conjugated Streptavidin (eBioscience) and PI was added prior to analysis to resolve live cells.
HSPC analysis: BMMNCs were stained with the PerCP-Cy5.5 lineage cocktail in addition to PE-Cy7 conjugated Sca1, biotinylated-MPL, PE conjugated IL7Rα (A7R34, eBioscience), APC conjugated Flt3 (A2F10, eBioscience), AlexaFlour700 conjugated CD16/32 (93, eBioscience), BrilliantViolet421 conjugated cKit and FITC conjugated CD34 antibodies. MPL was resolved with APC-eFluor780 conjugated Streptavidin and PI was added prior to analysis to resolve live cells.
Myeloid progenitor analysis: BMMNCs were stained with the PerCP-Cy5.5 lineage cocktail in addition to PE-Cy7 conjugated CD105 (MJ7/18, Biolegend), APC-Cy7 conjugated Sca1 (D7, Biolegend), PE conjugated CD41, APC conjugated CD150, AlexaFluor700 conjugated CD16/32, Brilliant Violet421 conjugated cKit and biotinylated MPL. MPL was resolved with FITC conjugated Streptavidin and PI was added prior to analysis to resolve live cells.
In vitro culture analysis: cKit enriched BMMNCs were stained with PerCP-Cy5.5 lineage cocktail, FITC conjugated CD34, PE conjugated CD41, BV510 conjugated Sca1, APC conjugated CD150 and PE-Cy7 conjugated cKit, AlexaFluor700 conjugated CD48 and Biotinylated MPL antibodies. MPL was resolved with APC-eFluor780 conjugated Streptavidin and PI was added to resolve live cells prior to sorting. On day 10 of culture colonies were stained with CD41 conjugated AlexaFluor700 (MWReg30, Biolegend), CD16/32 conjugated APC-Cy7 (93, Biolegend), CD71 conjugated PE (C2, BD Biosciences), Ter119 conjugated FITC (TER-119, Biolegend), CD11b conjugated PE-Cy7 (M1/70, Biolegend), Ly6C/G conjugated APC (RB6-8C5, Biolegend) and PI was added to resolve live cells.



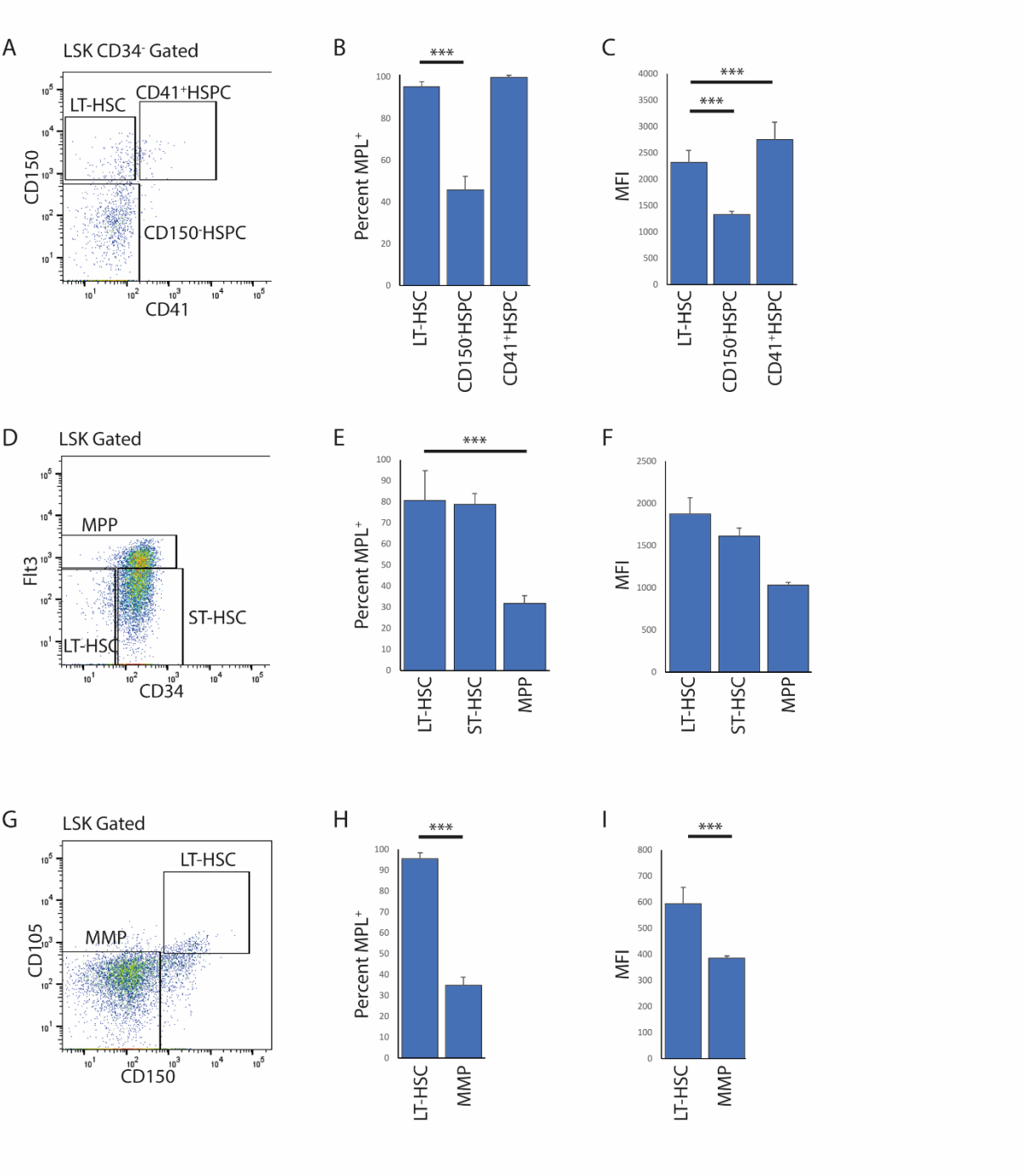
Supplemental Figure 1: In Vitro Colony Analysis

Colonies were analysed by flow cytometry at day 10. Gating system of blood cell lineages is shown.



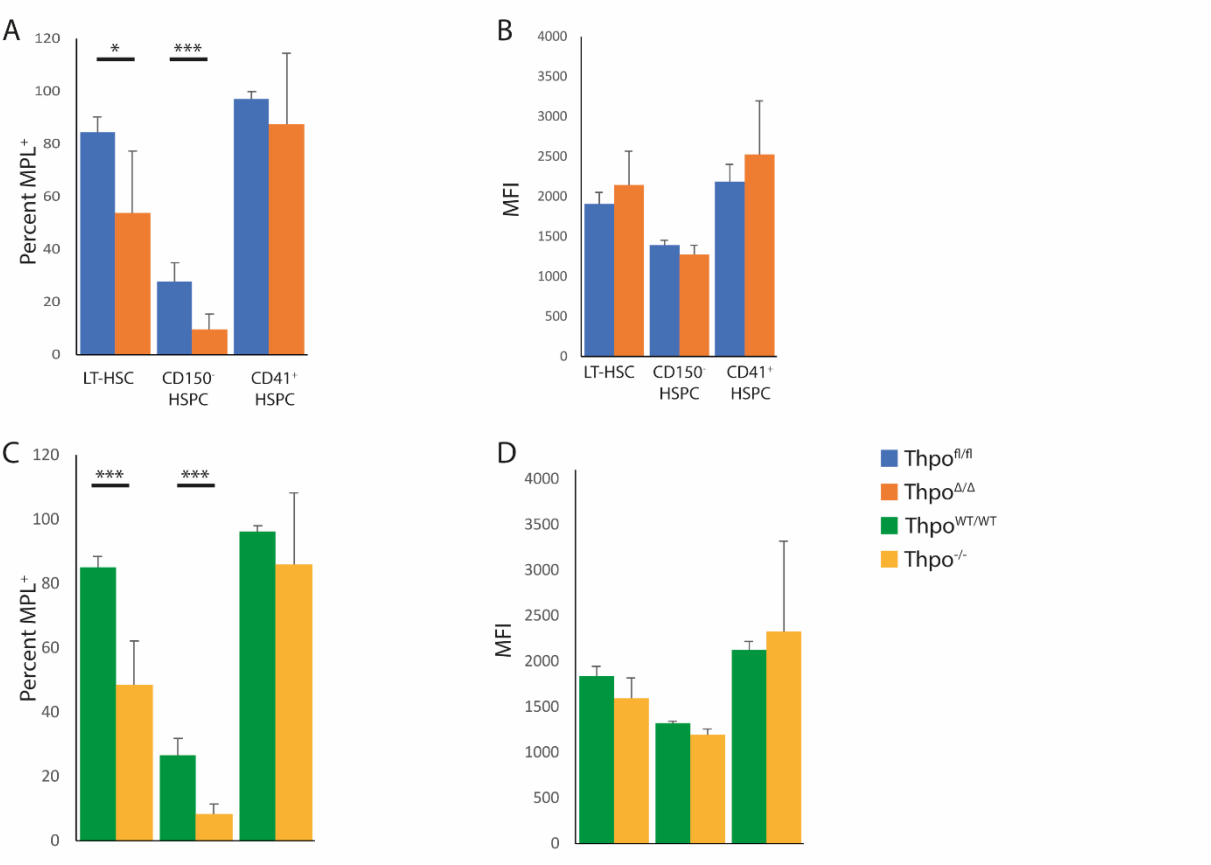
Supplemental Figure 2: MPL Expression in Progenitor Populations

(A) Gating system for CLP and myeloid progenitors. Percentage of each population gated as MPL positive is shown (B). Mean fluorescence intensity of MPL+ cells in each population is shown (C). (D) Gating system for MkP and myeloid progenitors. (E) percentage of each population gated as MPL positive. (F) mean fluorescence intensity of MPL+ cells from each population.



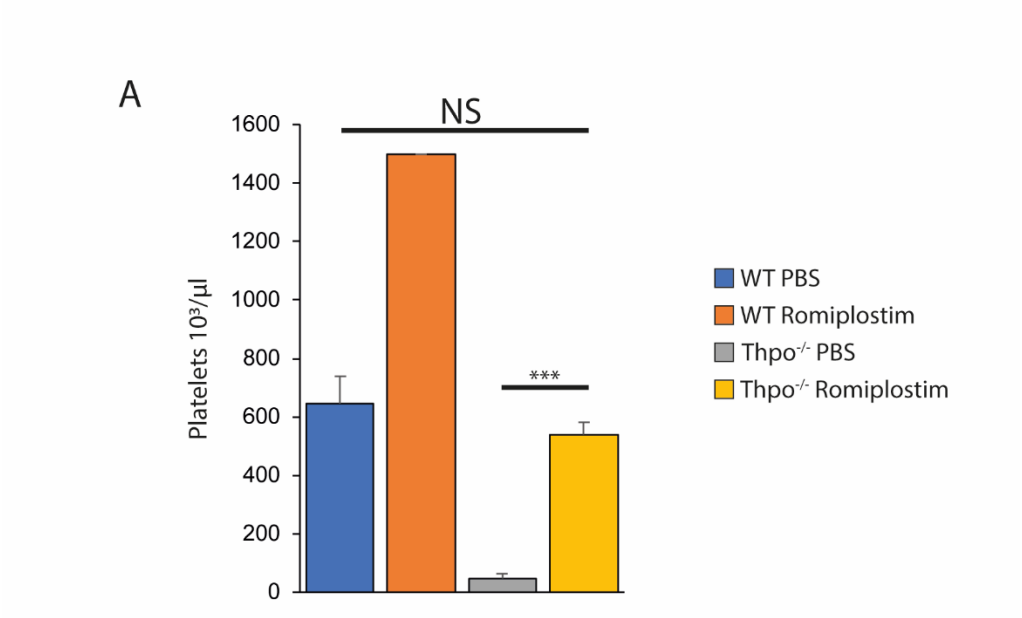
Supplemental Figure 3: LSK MPL expression

SLAM gating system is shown (A) with MPL expression (B) and mean fluorescence intensity (MFI) of defined populations (C). HSPC gating system is shown (D) with MPL expression (E) and MFI (F) of each population. Myeloid progenitor gating system is shown (G) with MPL expression (H) and MFI (I) of each population.



Supplemental Figure 4: MPL Expression of HSPCs in Thpo-KO Models

(A) Percentage of MPL+ cells in each HSPC population of Alb-Cre mice. (B) Mean fluorescence intensity of MPL+ cells from each HSPC population of Alb-Cre mice. (C) Percentage of MPL+ cells in each HSPC population of *Thpo*$^{-/-}$ mice. (D)) Mean fluorescence intensity of MPL+ cells from each HSPC population of *Thpo*$^{-/-}$ mice.



Supplemental Figure 5: Platelet Counts in Mice Treated with MPL Agonist

(A) Peripheral blood platelet counts in WT and Thpo$^{-/-}$ mice on day 5 after daily treatment with MPL agonist Romiplostim or PBS control.