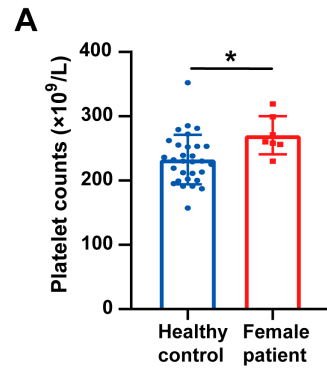
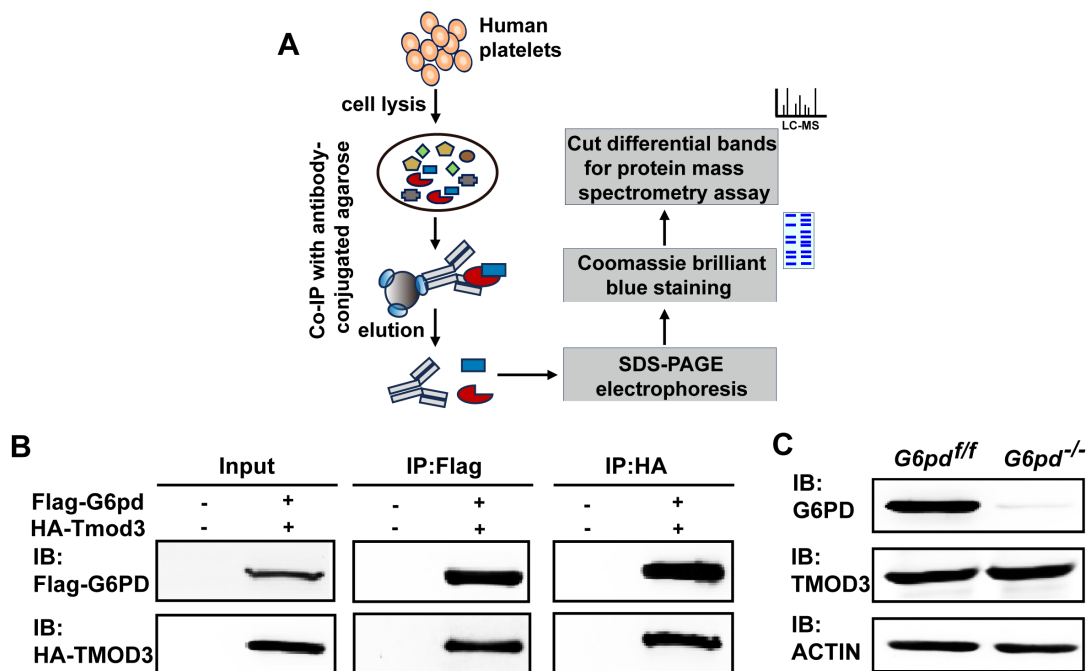


**Appendix to “G6PD suppresses thrombopoiesis via interfering with
TMOD3-mediated actin dynamics”**

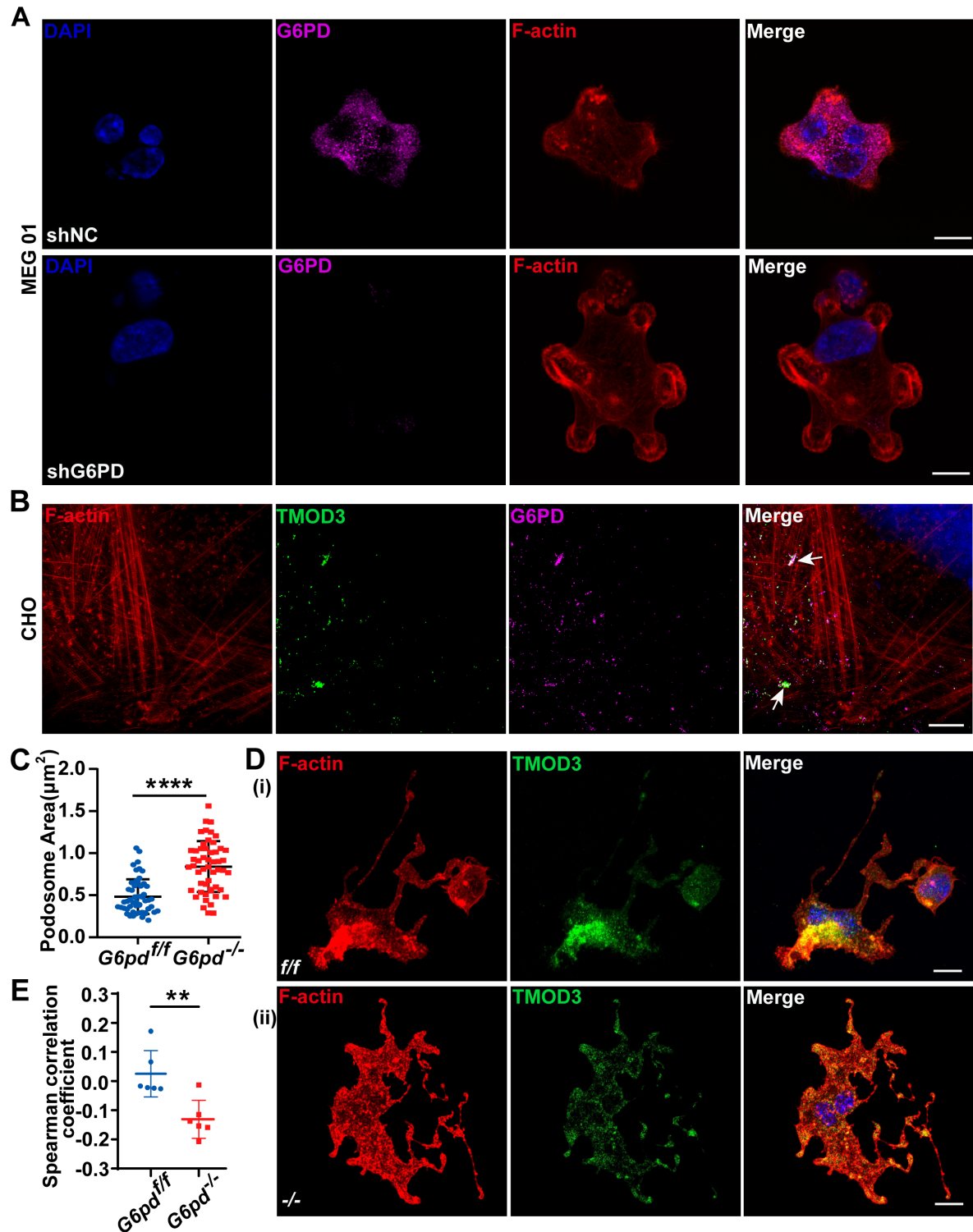
<i>Appendix Figure S1.....</i>	<i>2</i>
<i>Appendix Figure S2.....</i>	<i>2</i>
<i>Appendix Figure S3.....</i>	<i>3</i>
<i>Appendix Figure S4.....</i>	<i>5</i>
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Appendix Figure S1. (A) Platelet count of female healthy controls and G6PD-deficient patients (n=30 vs n=7, *P<0.05). All the enrolled subjects aged 18~40 years old. G6PD activity of the patient group: 1.929±0.822 IU/g Hb.

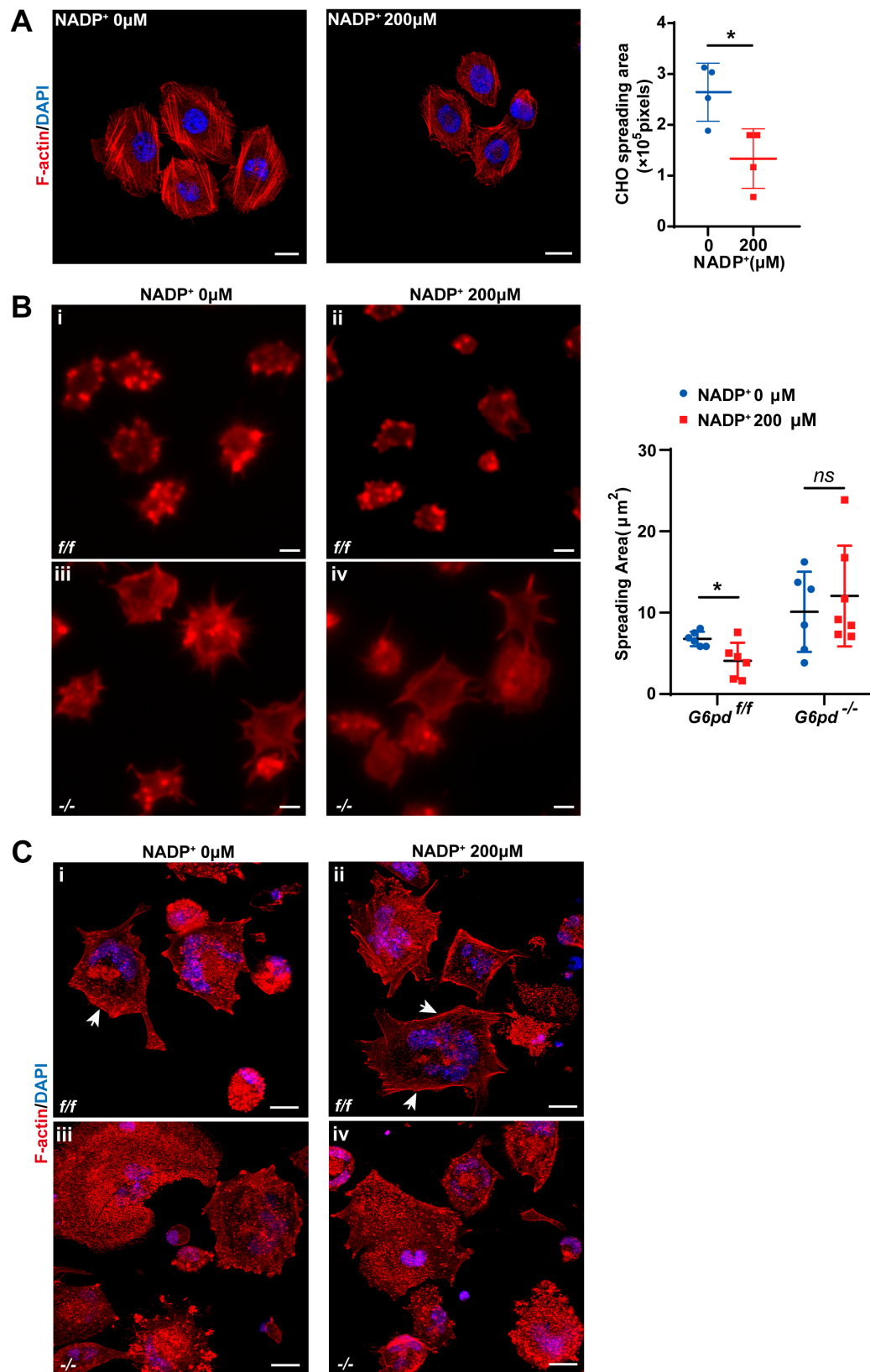


Appendix Figure S2. (A) A simple schematic for the process of Co-IP and mass spectrometry to find potential G6PD-interacting protein in human platelets. **(B)** Co-IP and immunoblotting were conducted to confirm the interaction between G6PD and TMOD3 in HEK 293T cells transfected with Flag-G6pd and HA-Tmod3 plasmids. **(C)** The protein levels of TMOD3 in *G6pd^{f/f}* and *G6pd^{-/-}* murine platelets were detected by immunoblotting.



Appendix Figure S3. G6PD interacted with TMOD3. (A) G6PD-knockdown (shG6PD) and control (shNC) Meg01 cells were stimulated with Phorbol 12-myristate 13-acetate (PMA). The cells were then allowed to spread on immobilized fibrinogen for 48 hours, followed by fixation and immunofluorescence staining. Immunofluorescence images via Leica SP8X displayed the distribution

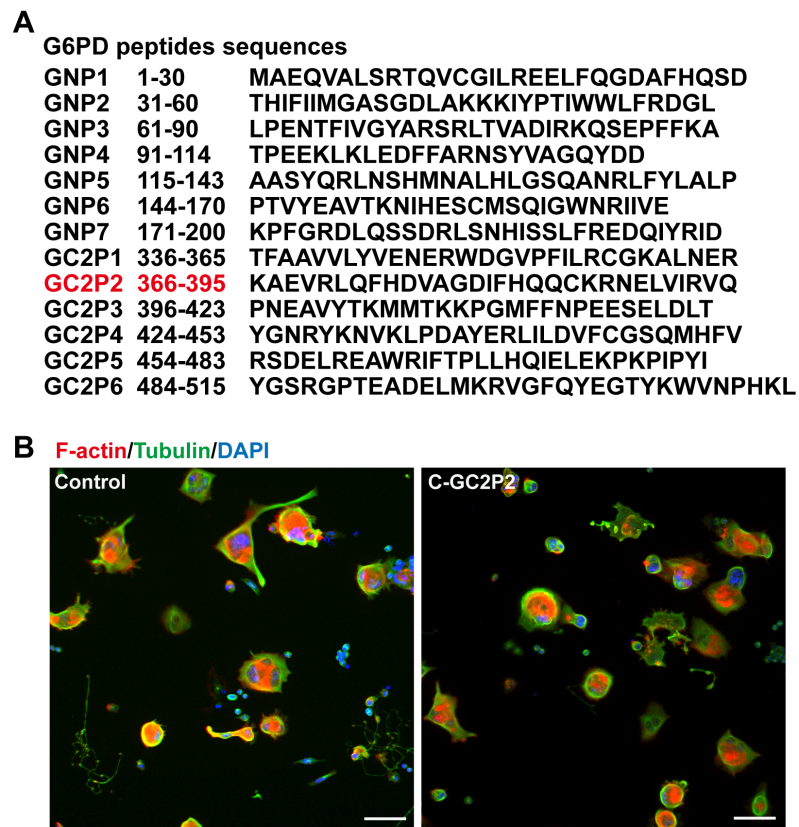
of G6PD (purple) and F-actin (red). Scale bars represented 10 μ m. **(B)** Immunofluorescence images via SIM displayed the colocalization among G6PD (purple), TMOD3 (green) and F-actin (red) in CHO cells. Arrows pointed to representative co-localizations of the three. Scale bars represented 5 μ m. **(C)** Statistical analysis of podosome area from Figure 5H (n=52). **(D)** Immunofluorescence images via ZEISS 880 displayed the distribution of TMOD3 (green) and F-actin (red) in *G6pd^{fl/fl}* and *G6pd^{-/-}* MKs at the PPF stage. Scale bars represented 20 μ m. **Note:** Representative images shown in this figure were acquired from the same original imaging dataset as those presented in the corresponding main-text Figure 3A. Different fluorescence channels were displayed for different purposes: F-actin and TMOD3 staining are shown here, whereas Tubulin and F-actin staining from the same fields are presented in the main-text Figure 3A. **(E)** Randomized delineations in MKs from Figure 5H were performed to investigate the correlation between TMOD3 and F-actin localization. The colocalization quantified analysis (Spearman correlation coefficient) of 6 randomized lines was performed (n=6, **P<0.01). ImageJ software and GraphPad Prism 8 was used to analyze the fluorescence colocalization.



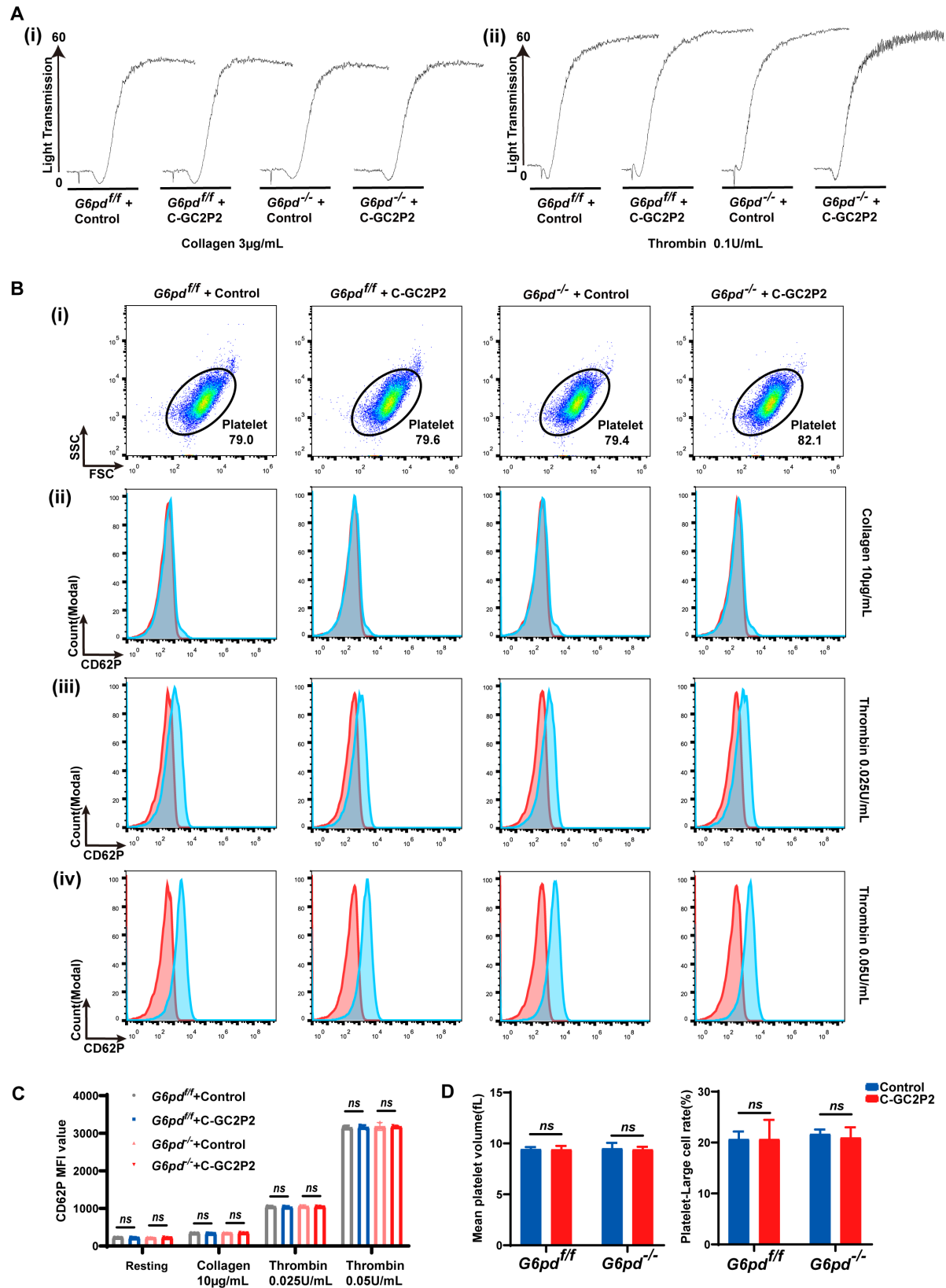
Appendix Figure S4. NADP⁺ was harmful for actin cytoskeleton organization. (A)

Immunofluorescence images of CHO cells (untreated or treated with NADP⁺) spreading on

immobilized fibrinogen. Statistical analysis of the spreading areas in the images was presented (n=7, *P<0.05). Scale bars represented 20 μ m. **(B)** Immunofluorescence images of actin cytoskeleton in *G6pd^{fl/fl}* and *G6pd^{-/-}* murine platelets (untreated or treated with NADP⁺) spreading on immobilized fibrinogen. Statistical analysis of the platelet spreading areas in the images was presented (n=6 vs n=6, *P<0.05; n=6 vs n=7). Actin cytoskeleton in platelets was stained with rhodamine-labeled phalloidin. Scale bars represented 2 μ m. **(C)** Immunofluorescence images of mature *G6pd^{fl/fl}* and *G6pd^{-/-}* MKs that had not yet undergone the PPF stage, untreated or treated with NADP⁺. Scale bars represented 20 μ m.

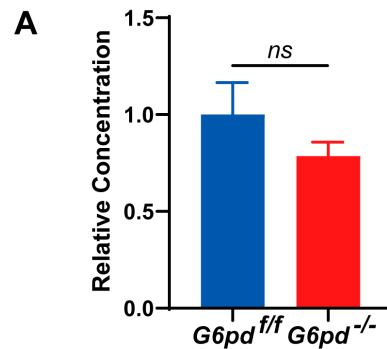


Appendix Figure S5. Peptide C-GC2P2 inhibited platelet biogenesis. **(A)** Sequences of the thirteen G6PD peptides derived from G6PD-N and G6PD-C2 domains. **(B)** Low-magnification immunofluorescence images of PPF-MKs (derived from wildtype murine fetal liver) treated with 50 μ g/mL control peptide CPP-Scramble or peptide C-GC2P2. Scale bars represented 20 μ m.



Appendix Figure S6. Peptide C-GC2P2 showed no impact on size, *in-vitro* aggregation or secretion of *G6pd^{+/+}* and *G6pd^{-/-}* platelets. (A) Aggregation of washed *G6pd^{+/+}* and *G6pd^{-/-}* platelets (treated with

50 $\mu\text{g/mL}$ control peptide CPP-Scramble and peptide C-GC2P2) in response to collagen (3 $\mu\text{g/mL}$) and thrombin (0.1 U/mL). **(B-C)** Flow cytometry was used to detect the CD62P (P-selectin) exposure of washed $G6pd^{ff}$ and $G6pd^{-/-}$ platelets (treated with control peptide CPP-Scramble and peptide C-GC2P2) stimulated with collagen (10 $\mu\text{g/mL}$) and thrombin (0.025 U/mL, 0.05U/mL). **(B)** The gating strategy is presented (i), along with overlaid histograms depicting platelet activation in response to various stimuli (ii-iv). In figure ii-iv, resting and activated platelets are shown in red and blue, respectively. **(C)** Platelet activation was assessed by determining the MFI of CD62P following exposure to different stimuli (n=3). **(D)** MPV and the P-LCR at 48 hours after peptide injection (i.e., Day 4) in both $G6pd^{ff}$ and $G6pd^{-/-}$ mice (n=3).



Appendix Figure S7. (A) $G6pd^{ff}$ and $G6pd^{-/-}$ platelets were isolated and pelleted after washing, with equal cell numbers (3×10^8) used for each sample. The pellets were lysed in RIPA buffer, and glucose levels were measured using an automated biochemical analyzer. Statistical analysis of relative glucose content is shown in the figure (n=3).