Materials and Methods

Micellar peptide preparation. Myristoylated peptides MPαC (C13H27CONH-SIRYSGHpSL) and MCsC (C13H27CONH-LSISYGSHR) were synthesized in the Protein Research Laboratory, University of Illinois at Chicago. PEG2000-DSPE (Avanti Polar Lipids Inc. Alabaster, AL), L-α-phosphatidylcholine (egg PC, Type XI-E, Sigma-Aldrich, St. Louis, MO) and peptides were mixed at a molar ratio of 45:5:1 (in some experiments, 45:5:2). Micelles were prepared using a film rehydration method as previously described.

Survival analysis in transgenic GPIbα mice. The generation and characterization of mouse colonies expressing GPIbα variants has been previously described. Briefly, the IL-4R/Ibα variant lacks the gene for endogenous mouse GPIbα but instead expresses a fusion protein where the extracellular sequences of human GPIbα have been replaced by an extracellular domain from the interleukin-4 receptor. The mice have a significant bleeding phenotype similar to the mouse model of the human Bernard-Soulier syndrome. LPS purified from E. coli (0111:B4, Product #62630, Sigma, St. Louis, MO) and dissolved in isotonic saline was injected intraperitoneally. The referenced LPS dosage, i.e., 100 µg, is based on the amount per 30 g of mouse bodyweight. Male mice of approximately 12 weeks of age were used. Kaplan Meier survival curves and log-rank analysis of the data are presented.

Platelet preparation. Human blood was drawn by venipuncture from healthy volunteers. Institutional Review Board approval was obtained from the University of Illinois at Chicago, and informed consent from volunteers was obtained in accordance with the Declaration of Helsinki. To prepare platelet-rich plasma (PRP), whole blood was anticoagulated with 3.8% trisodium
citrate. To prepare human platelets, one-seventh volume of ACD was used as anticoagulant \(^4\).
Platelets were washed twice and resuspended in modified Tyrode’s buffer as previously described \(^4\).

**Coimmunoprecipitation of GPIb-IX with 14-3-3ζ.** Micellar MPαC or control peptides (100 μM) were preincubated with washed human platelets (1X10\(^9\)/mL) for 30 minutes. Platelets were then solubilized with a solubilization buffer \(^5\), and lysates were immunoprecipitated with an anti-GPIb monoclonal antibody LJ-P3 (kindly provided by Dr Zaverio Ruggeri, The Scripps Research Institute) \(^6\) or IgG control. The immunoprecipitates were then immunoblotted with anti-GPIbα and anti-14-3-3ζ antibodies, respectively \(^7\).

**Platelet adhesion to HUVECs under shear stress.** Human umbilical vein endothelial cell (HUVEC) monolayers were stimulated with 20 μM LPS (Sigma-Aldrich, St. Louis, MO) or vehicle control for various lengths of time. Washed platelets (3X10\(^8\)/ml) were preincubated with micellar peptides (25 μM) or antibodies (10 μg/ml) for 15 minutes and then loaded onto HUVECs. In some experiments, platelet adhesion was analyzed in the presence of isolated human leukocytes (1X10\(^8\)/ml). A cone-plate rheometer (Rheostress 1, Thermo-HAAKE, Paramus, NY) was used to introduce constant shear rate (800s\(^{-1}\)) to the platelets for 5 minutes \(^8,9\). Dishes were then quickly rinsed with 5 ml PBS three times. Cells were fixed with 4% paraformaldehyde and visualized under a Leica DMI RB microscope using a 40X/0.55 NA objective. Adherent platelets were counted in 10 randomly selected fields. Statistical significance was analyzed using \(t\)-test.

**In vivo bleeding time, arterial thrombosis, and thrombocytopenia.** All animal studies are approved by the Institutional Animal Care Committee. C57BL/6 mice were anesthetized and retro-orbitally injected with micellar MPαC, scrambled peptide (2 μmol/kg) or Integrilin.
(Integrilin, 5 mg/kg). After 15 minutes, tail bleeding time was determined using the previously described method \textsuperscript{10}. The bleeding time is defined as the time to stable cessation of bleeding with no re-bleeding within 1 minute. FeCl\textsubscript{3}-induced carotid arterial thrombosis was studied as previously described \textsuperscript{11,12}. For thrombocytopenia, mice were treated with LPS (12 µg /g, I.P.). Approximately 1 mm tail end was snipped at 0, 2, and 4 hour time points and blood was collected. Platelet counts were analyzed by a Hemavet (HV950FS, Drew Scientific, Dallas, TX) multispecies hematology analyzer.

**Histologic analysis.** LPS (22 mg/kg) was administered intraperitoneally into C57BL/6 mice 15 minutes after retro-orbital injection of MP\alphaC or MCsC (2 µmol/kg). After 24 hours, kidneys were perfused with phosphate-buffered saline and fixed in 10% formalin, detached, embedded in paraffin and sectioned at 5 µm. Slides were then stained with Mallory’s phosphotungstic acid hematoxylin method (PTAH) (Rowley Biochemical, Danvers, MA) to identify platelets and fibrin, and viewed with a Leica DMI RB microscope using a 40X/0.55 NA objective. Total thrombotic area/gomerulus was quantitated by analyzing 20 glomeruli from wild type mice and 50 glomeruli each from micellar MP\alphaC and scrambled peptide treated mice (6 mice for each group). Platelets in thrombi were detected with a rat anti-mouse integrin α\textsubscript{IIb} monoclonal antibody (Santa Cruz, Santa Cruz, CA) using Vectastain ABC system and a peroxidase substrate DAB.

**Survival Assay.** C57BL/6 mice were retro-orbitally injected with MP\alphaC or control peptide (2 µmol/kg) or Integrilin (Integrilin, 5 mg/kg) 15 minutes prior to LPS (22 mg/kg) intraperitoneal injection and then monitored for 48 hours. In some experiments, C57BL/6 mice were first injected intraperitoneally with LPS (22mg/kg) 1 hour before the retro-orbitally injection of MP\alphaC or control peptide (10µmol/kg). Survival rate (the percentage of living mice in the group at each time point) data were analyzed using Kaplan-Meier plot.


