Supplement Material

Methods

Assessment of platelet activation in whole blood

Mouse blood was collected from the retro-orbital plexus and mixed with heparin (20 U/mL). In some experiments, platelets in whole blood were activated with 1 μM ADP or 50 nM convulxin and then fixed with 1% (w/v) paraformaldehyde (PFA) before the addition of fluorescent antibodies. Platelet activation was assessed by 2-color flow cytometry. Platelets in whole blood were identified by characteristic light scattering and membrane expression of the platelet specific glycoprotein IX (GPIX) detected with rat anti-mouse GPIX antibody (Emfret Analytics, Wurzburg, Germany). Surface exposure of platelet activation antigens was measured with PE-labeled rat anti-mouse CD62P (Emfret Analytics) or rat anti-mouse CD40L (BD Biosciences PharMingen, San Jose, CA). The activation of $\alpha_{IIb}\beta_3$ integrin was assessed by binding of PE-labeled JON/A antibody. Appropriate rat IgGs were used to determine non-specific binding. Blood samples were incubated with the antibodies for 30 min at 20^{o}C and analyzed with a FACSCalibur (Becton Dickinson, San Jose, CA). Data analysis utilized FlowJo Software (Tree Star Inc, Ashland, OR).

Platelet-eosinophil aggregates

To detect platelet-leukocyte aggregates, blood was stained to detect leukocytes, monocytes, eosinophils, neutrophils, and platelets, respectively, anti–CD45-PerCP-Cy5.5, anti–mouse Siglec-F-PE (BD Biosciences PharMingen), anti–CD11b-APC, anti-GR1-PE-Cy7 and anti-GPIX-FITC antibodies. Blood samples were subjected to flow cytometry analysis and data collected on a CYAN flow cytometer.

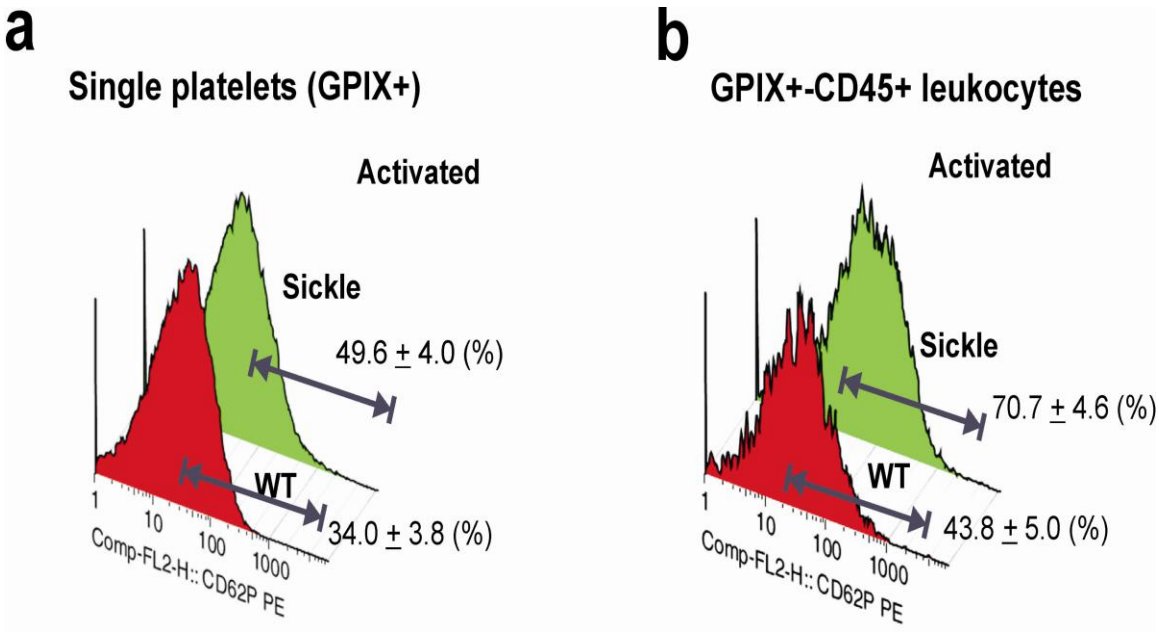
Lung vascular permeability

Pulmonary vascular permeability was evaluated by measurement of Evans blue dye extravasation (EBD, 30 mg/kg body weight, 200 µl) injected intravenously into anesthetized mice 30 min prior to lung removal. The chest was opened, the inferior vena cava transected, and the pulmonary vasculature flushed with 10 ml of saline via the right ventricle to remove excess intravascular dye. The lung lobes were removed, weighed, homogenized, and incubated in 100 % formamide at 37^{o}C for 24 hours to extract EBD. The concentration of dye extracted was analyzed by spectrophotometry. Correction of optical densities (*E*) for contaminating heme pigments was performed using the equation: E_{620}(corrected) = E_{620} – (1.426 X E_{740} + 0.03). Results are presented as µg EBD per g of lung tissue.

Human antibodies

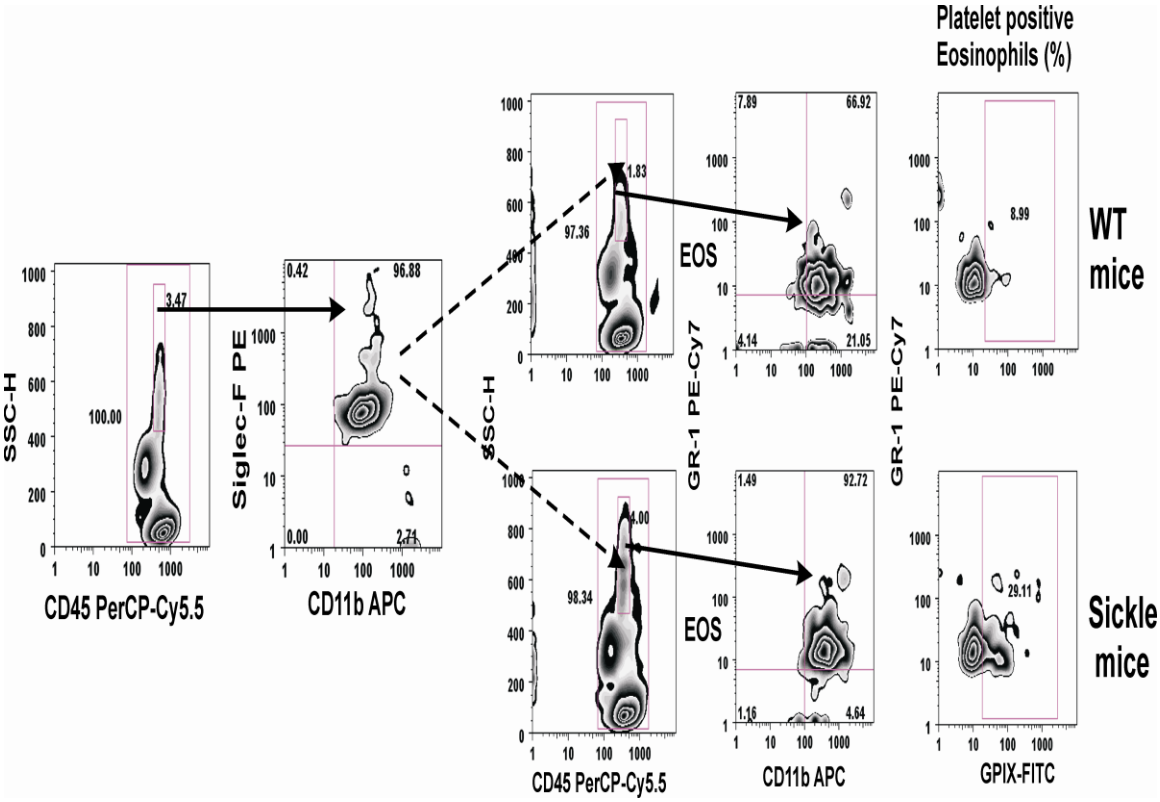
Monoclonal mouse antibodies (MAbs) against human antigens were purchased from the following sources: Pacific Blue-conjugated CD45 (HI30), PerCP-Cy5.5-CD45 (HI30), FITC-CD14 (61D3), PE-CD16 (eBioCB160), PE-CD11b (ICRF44), PE-Cy7-CD11b (ICRF44) and appropriate IgG1 and IgG2a controls were from eBiosciences (San Diego, CA). APC-conjugated CD41 and FITC-conjugated CD62P were obtained from BD Biosciences Pharmingen (San Jose, CA).

Supplemental Figure I



Supplemental Figure I. **Rapid increase in expression of P-selectin on platelet-leukocyte aggregates after activation with convulxin**. Blood samples were activated with 50 nM convulxin for 30 s on orbital shaker. To stop reaction the Lyse/Fix buffer was added (BD) and samples were washed. After incubation with antibodies, samples were analyzed by flow cytometry. **a**) P-selectin exposure on single platelets **b**) P-selectin exposure on platelet-leukocyte aggregates.

Supplemental Figure II



Supplemental Figure II. **Platelet-eosinophil aggregates are increased in SCD**. A distinctive population of blood CD45+ leukocytes with a high content of granules (SSC ^{High}) that is CD11b+ and GR-1^{dim} (dashed arrow) was confirmed to be ~98% eosinophils based on expression of Siglec-F (CD33rSiglec), a marker present on mature circulating mouse eosinophils. Platelet (CD41+)-eosinophil aggregates are more abundant in sickle (29%) than in WT (9%) mice as shown by whole blood flow cytometry.