**Supplemental Methods**

**Cell Culture**
Human THP-1 monocytes (ATCC) were maintained in RPMI-1640 medium (Sigma-Aldrich) supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin, and 0.05 mM β-mercaptoethanol at 37°C in a 5% CO₂ atmosphere. All cells were maintained and all incubations were performed under these conditions unless mentioned otherwise. HUVEC (ATCC) were maintained in CS-C Serum Supplemented Medium (Sigma-Aldrich) in gelatin-coated flasks at 37°C in a 5% CO₂ atmosphere. After the cells were 80% confluent, they were passaged into new media. GFP-HUVEC were obtained through transfection with a EF1α-eGFP vector and were a kind gift of the Platt laboratory at the Georgia Institute of Technology. Primary AA PBMCs were isolated from whole blood via Ficoll centrifugation and cultured in THP-1 media supplemented with 100ng/mL macrophage colony stimulating factor (MCSF).

**Animals**
Wild-type C57BL/6 mice were obtained from The Jackson Laboratories; Heterozygous AS and homozygous SS mice were initially obtained from the sickle transgenic breeding colony at Georgia Institute of Technology. Townes’ model sickle transgenic mice were heterozygous (AS) or homozygous (SS) for the sickle mutation. To determine sickle status, whole blood was obtained from each animal via retro-orbital capillary draws. Whole blood was lysed with distilled water and diluted 1:1 in a 50% glycerol solution. Blood samples were then run on a Native PAGE gel and hemoglobin was visualized using a liquid tetramethylbenzidine stain (34% EtOH, 32% liquid 3,3',5,5’-tetramethylbenzidine, 2% acetic acid, 1% H2O2); sickle hemoglobin has a smaller electrophoretic migration distance than normal hemoglobin. Mice in all studies were male, 8–12 wk old, and weighed 18–25 g. Amitriptyline was injected at 4 different doses via intraperitoneal administration and blood was collected via retro-orbital capillary draws. RBC and microparticles in blood were quantified as described below with a BD FACS Aria flow cytometer. All surgical procedures and animal care protocols were approved by the Georgia Institute of Technology Animal Care and Use Committee.

**Sphingomyelinase (neutral, acidic and expression), ceramidase and sphingosine kinase expression quantification**
Neutral and acidic sphingomyelinase (SMase) activity were measured with a sphingomyelinase fluorometric assay kit (catalog # 10006964, Cayman Chemical Company). For neutral SMase activity, the reaction was performed in a neutral buffer at a pH of 7.4. For acidic SMase activity, the reaction was performed in an acidic buffer at a pH of 5. Briefly, 10µL of samples (plasma, whole blood or RBC) were combined with alkaline phosphatase and a fluorometric sphingomyelin substrate. The reaction involving SMase hydrolyzes sphingomyelin to form ceramide and phosphorylcholine. Alkaline phosphatase hydrolyzes phosphorylcholine to form choline. Choline is then oxidized by choline oxidase to produce betaine and H₂O₂. H₂O₂, in the presence of horseradish peroxidase, reacts with ADHP to yield fluorescent resorufin, which was measured on a plate reader at an excitation wavelength of 535nm and emission wavelength of 590nm after 30 minutes.

Acid sphingomyelinase expression was measured with a sphingomyelinase absorbance ELISA assay kit (catalog # SEB360Hu, USCN Life Science). 10µL sample was loaded into wells in a 96 well plate ELISA kit pre-coated with a monoclonal antibody against acid SMase (catalog #MAB360Hu22, USCN Life Science). Expression was measured by reading the absorbance at 450nm.

Alkaline ceramidase (ACER1) expression was measured with a ceramidase absorbance ELISA assay kit (catalog # CSB-EL001151HU, CUSABio). 10µL sample was loaded into wells in a 96 well plate ELISA kit pre-coated with a monoclonal antibody against alkaline ceramidase (ACER1). Expression was measured by reading the absorbance at 450nm.
Sphingosine Kinase 1 and 2 expression
Sphingosine kinase 1 and 2 expression was measured by western blotting using antibodies against sphingosine kinase 1 (catalog #1000-6822, Caymen Chemical Company) or sphingosine kinase 2 (catalog #AB37977, Abcam). An equal amount of protein was loaded onto the gels and near IR dyes were used to stain the relevant bands. Expression is expressed as relative fluorescent units.

RBC density Fractionation
RBCs were separated into high and low density fractions according to a protocol by D’Alessandro et al. (http://www.ncbi.nlm.nih.gov/pubmed/22871816). Briefly, 8 Percoll solutions of varying density were made with Percoll, BSA, Hepes, NaCl and KCl and stacked in order of decreasing density, from bottom to top, in Beckman ultra-clear tubes 9/16 x 3 1/2 in. (14 x 89 mm). The stacked gradient was pre-centrifuged at 20,000 RCF for 20 minutes. Packed RBCs were harvested as described above. 300μL of packed RBCs were added to 1.5mL of 5mM Na2HPO4, 154mM NaCl, 5mM Glucose and 1mM PMSF and centrifuged at 200 RCF for 10 minutes at 4°C. The supernatant was discarded and this was repeated twice and the cells were resuspended in 1.5mL of 95% glucose. The RBC sample was loaded slowly on top of the Percoll gradient and centrifuged at 41,000rpm for 30 minutes using a Beckman SW 41 Ti rotor. The highest and lowest density RBC fractions were collected using a syringe and washed in PBS with 0.8% NaCl.

SEM/ preparation and imaging
Cells fixed with 2.5% glutaraldehyde fixative in 0.1 M cacodylate buffer (pH 7.4) were placed on a Poly-L-Lysine coated silicon wafer (5 x 5 mm), washed with same buffer and then post fixed in 1% osmium tetroxide with 1.5% potassium ferrocyanide in 0.1 M cacodylate buffer for one hour. Cells were subsequently rinsed 2 or 3 exchanges of de-ionized water. This was followed by dehydration through an ethanol series ending with three exchanges of 100% absolute ethanol. The samples were then placed into individual ventilated processing vessels in fresh absolute ethanol and placed into a Polaron E3000 critical point drying unit wherein the ethanol was exchanged for liquid CO2. The liquid CO2 was eventually brought to its critical point of 1073 psi at 31°C and allowed to slowly vent. The dried samples were then secured to labeled aluminum SEM stubs and coated with approximately 20 nm of chromium using an Denton DV-602 turbo magnetron sputter coater (Denton Vaccum, LLC., Moorestown, NJ). Samples were then viewed with a Topcon DS130F field emission scanning electron microscope using 5 kV accelerating voltage.

TEM preparation and imaging
5 µl microparticle suspension was placed on a 400 mesh carbon coated copper grid that had been made hydrophilic by glow discharge. After 5 minutes, the grid was rinsed by briefly touching the sample side with one drop of distilled water. The residual water on grid was then removed by dragging the side of grid on a piece of filter paper. For negative staining, 5 µl 1% aqueous phosphotungstic acid (pH 6.5) was applied onto grid immediately after water removal, and then removed as described above after 30 seconds. The grid was let completely dry before viewing on a JEOL JEM-1400 transmission electron microscope (JEOL Ltd, Tokyo, Japan) equipped with a Gatan US1000 2k x2k CCD camera (Gatan, Inc., Pleasanton, CA).

Lipid Extraction and S1P/Sph Quantification
Lipids were extracted following a protocol from Shaner et al. (http://www.ncbi.nlm.nih.gov/pubmed/19036716). Briefly, 15-150µL of whole blood, plasma or RBC was transferred into 13 × 100 mm borosilicate tubes with a Teflon-lined caps (catalog #60827-453, VWR, West Chester, PA). 500µL of CH3OH, 250µL of CHCl3 and 10µL of C17 Sph+C17 S1P (Cayman Chemicals) internal standard were added to the sample and the samples were sonicated at room temperature for 30 seconds. The single phase mixture was incubated at 48°C overnight in a water bath and cooled before 75 µl of 1 M KOH in CH3OH was added. Samples were sonicated for 5 minutes and
shaken in a 37°C water bath for 2 hours. Samples were cooled and glacial acetic acid was added to bring the extract to neutral pH. Samples were centrifuged to remove the insoluble residue and the supernatant was collected. The supernatant was placed in glass vials and dried for 6 hours using a nitrogen blow down system. The dried residue was reconstituted in 300µL of methanol and analyzed using a Shimadzu LC-10 AD VP binary pump system coupled to a Perkin Elmer Series 200 autoinjector coupled to a 4000 quadrupole linear-ion trap (QTrap) LC-MS/MS system for S1P and sphingosine quantification.
Supplementary Figure 1. Sphingolipid metabolism and microparticle generation. Membrane stresses activate A-SMase on the outer leaflet of the plasma membrane (1), which hydrolyzes sphingomyelin to form ceramide, which can be further metabolized into sphingosine and S1P with CDase and SK1/2, respectively (2). Membrane-derived microparticles (3) are internalized into the cell and subsequently released into circulation (4).
Supplementary Figure 2. Scanning electron microscopy images of AA and SS RBCs. Donor blood was harvested and fractionated in a Ficoll density gradient to separate plasma and RBCs. RBCs were fixed, dehydrated and prepared for SEM imaging. While AA RBCs (top) display a normal, discoid shape, SS (bottom) RBCs are sickled and misshapen. SS RBCs also appear to have spindle-like protrusions from hemoglobin polymerizations. These alterations significantly enhance membrane forces. Scale bars = 5 μm.
Supplementary Figure 3. Platelet- and RBC-derived Microparticles in AS and SS mice. A) Blood was drawn via retro-orbital bleed from AS (left) or SS (right) Townes mice and stained with antibodies against PS, CD41 and Glycophorin A. B) Analysis reveals a significant increase in Glycophorin A+ (RBC-derived) microparticles in SS mice relative to AS mice (left). There were significantly more CD41+ (platelet-derived particles) in both mice but no significant differences in their concentration (right). * p<0.05 compared to AS.
Supplementary Figure 4. Diameter, acid SMase activity and macrophage internalization of AA and SS microparticles. Donor blood was harvested and fractionated in a Ficoll density gradient to separate plasma and RBCs. P3 microparticles were harvested from packed RBC by ultracentrifugation at 37,000xg for one hour. A) Quantification of P2, P3 and P4 MP diameter revealed that P2 MPs were around 300nm and P3 & P4 MPs were around 200nm. B) A plate-based assay for acid sphingomyelinase activity was performed. Acid sphingomyelinase activity was largely undetectable in both AA and SS microparticles, suggesting that the enzyme is not present in these particles. C) Quantification of internalized particles at 30 and 120 minutes suggests that MPs can be internalized and subsequently secreted but that there are no differences between the internalization of AA and SS MPs.
Supplementary Figure 5. S1P enhances THP-1 adhesion to endothelial cells. A-B) GFP HUVECs (green) were grown to confluence and co-incubated with Dil-labeled THP-1 monocytes (red) for 10 minutes or 4 hours after 1 hour vehicle treatment or 1 μM S1P treatment of monocytes. S1P treatment of monocytes significantly enhanced the proportion of adherent monocyte after 4 hours. * p<0.05 relative to vehicle.
Supplementary Figure 6. Pro-inflammatory cytokines in plasma of wild type mice after. Wild type mice were conditioned normoxia/hypoxia with intraperitoneal injections of saline or amitriptyline for 24 hours. The fold changes in IL-6, TNF-α and IL-1β were quantified. Amitriptyline significantly reduced the expression of IL-6 and IL-1β during normoxic conditioning, relative to saline-injected mice. p<0.05 in ANOVA relative to normoxia saline or hypoxia saline.