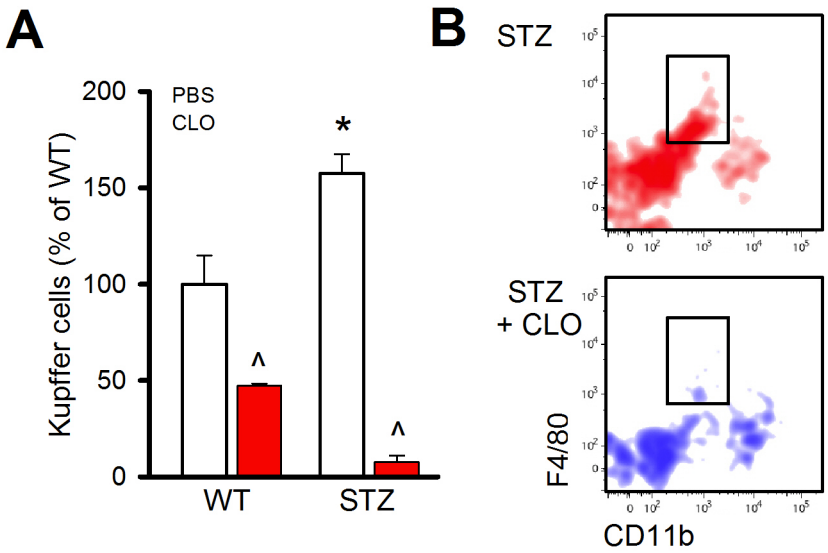
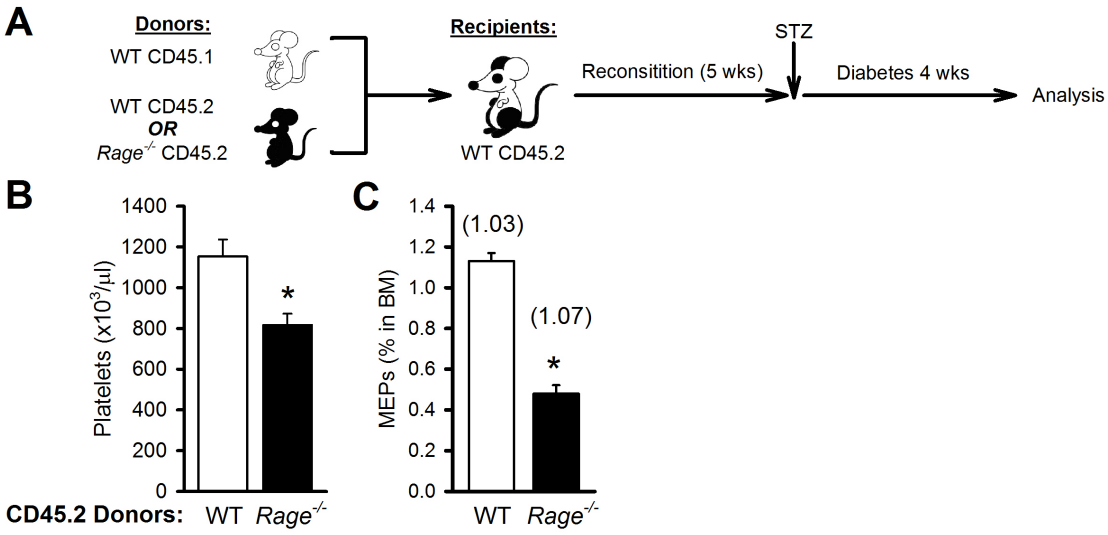
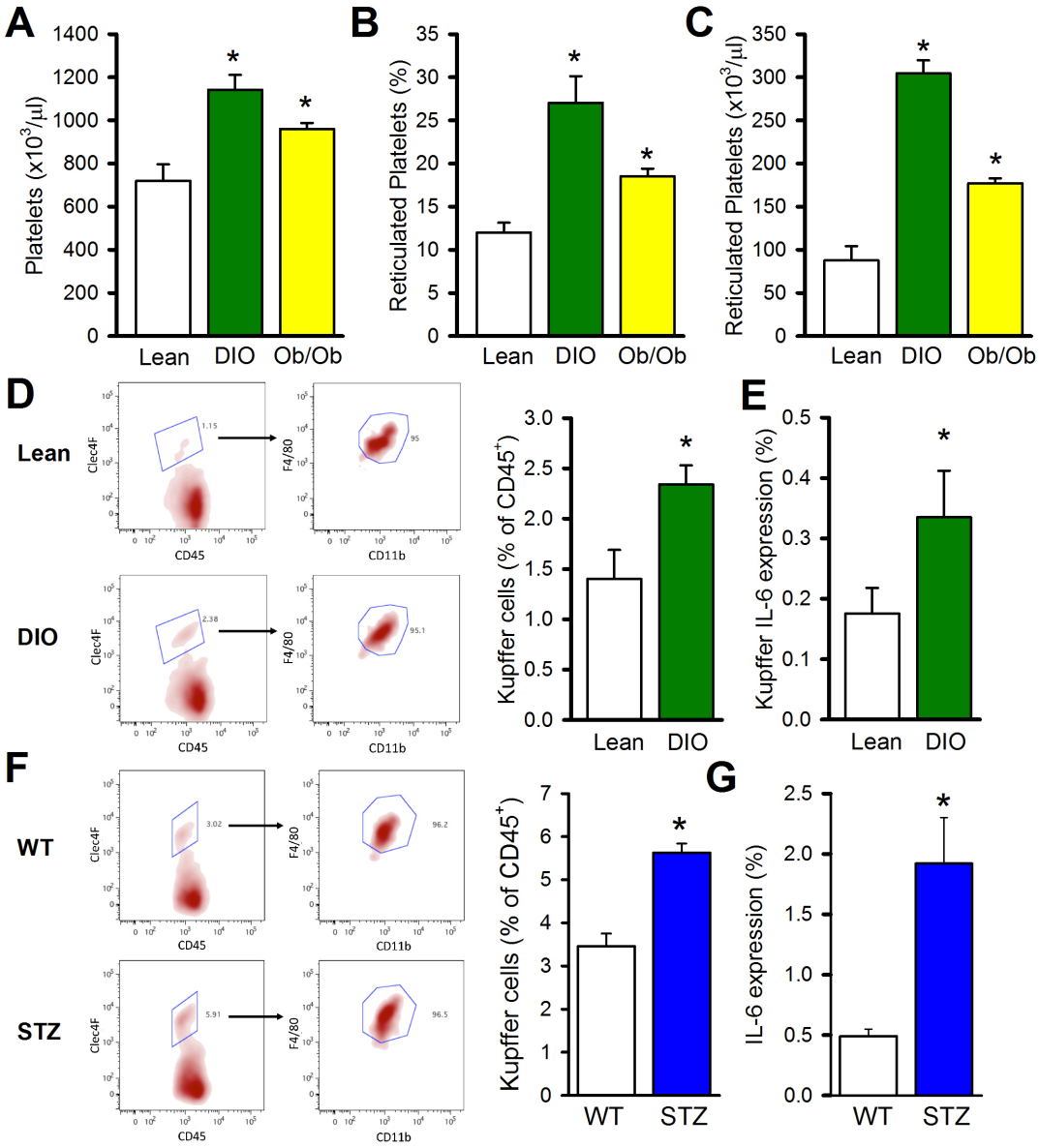


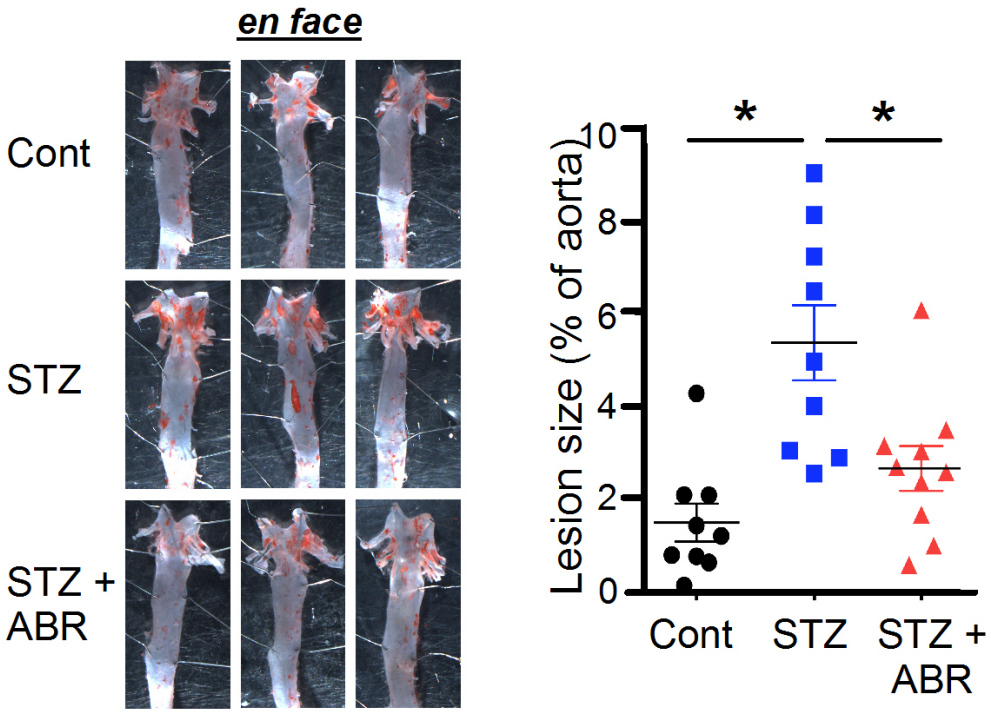
Supplemental Figure 1. WT and diabetic were treated with PBS or clodronate liposomes (250µl/mouse, i.v.) at day 1 and day 3 and the mice were assessed at day 7. (A) Quantification of Kupffer cells in the liver as a percentage of WT-PBS treated mice. (B) Representative flow plots of Kupffer cells from STZ mice treated with PBS or clodronate (CLO) liposomes. n=3/group, *P<0.05 for disease effect, ^P<0.05 for treatment (CLO) effect. All data mean ± SEM.



Supplemental Figure 2.
(A) Experimental overview: Equal proportions of BM from either WT CD45.1 and WT CD45.2 mice, or WT CD45.1 and *Rage*$^{-/-}$ CD45.2 were transplanted into WT CD45.2 recipients and made diabetic with STZ. (B) Circulating platelets were quantified by CBC. (C) The abundance of BM MEPs and the ratio (in brackets) of CD45.1:CD45.2 derived MEPs were quantified by flow cytometry. n=5/group, *P<0.05. All data mean ± SEM.



Supplemental Figure 3.
(A) Circulating platelet counts, and (B) percentage and (C) count of reticulated platelet levels were measured in aged matched lean, DIO (diet induced obese; 16 week high fat diet fed mice) and Ob/Ob mice. *P<0.05 vs lean, n=6/group. (D) Flow cytometry plot and quantified data of Clec4f+ Kupffer cells and (E) IL-6 expression. *P<0.05 vs lean, n=6/group. (F&G) WT and STZ-diabetic mice (4weeks od diabetes); (F) Flow cytometry plot and quantified data of Clec4f+ Kupffer cells and (G) IL-6 expression. *P<0.05 vs WT, n=6/group. All data mean ± SEM.



Supplemental Figure 4.
8wk old *Apoe*$^{-/-}$ were divided into 3 groups; Vehicle (Cont), Vehicle diabetic (STZ) and ABR-215757 diabetic (ATZ+ABR; 10mg/kg/day in drinking water) and fed a chow diet for 12 weeks. Aortas were dissected and stain with ORO to quantify atherosclerotic lesions. 3 representative aortas per group and the quantified data. n=9 control and STZ and n=10 STZ+ABR. *P<0.05 as indicated, data mean ± SEM.

Experimental Procedures

Mice and treatments. *S100a9*$^{-/-}$ mice were provided by Karin Bornfeldt (University of Washington, Seattle, WA) originality created by Johannes Roth (University of Munster, Munster, Germany) (1). Ob/Ob were purchased from Jackson laboratories. Wild type C57Bl/6, *Rage*$^{-/-}$, *IL-6*$^{-/-}$ and *S100a9*$^{-/-}$ mice were bred and housed in the AMREP Precinct Animal Centre in a pathogen-free facility under controlled conditions and exposed to a 12:12 hour light dark cycle and were fed a standard chow (Specialty Feeds, Glen Forrest, AUS).

Treatments. To induce diabetes, streptozotocin (STZ) was administered to mice by i.p injection (50 mg/kg for 5 days) using the recommended protocol from the Animal Models of Diabetic Complications Consortium (AMDCC). Mice were considered diabetic if their non-fasted glucose level exceeded 17 mmol/L. The sodium glucose co-transporter 2 inhibitor (SGLT2i) (dapaglipflozin) was obtained from Bristol Myers Squibb (Princeton, NJ) and administered in the drinking water at a dose of 25 mg/kg/day.

Kupffer cell depletion: Liposomes containing either clodronate (CLO) or phosphate buffered solution (PBS) were injected via the tail vein (250µl/mouse) into a group of control (non-diabetic) and diabetic mice at day 1 and day 3 to deplete Kupffer cells.

ABR-215757: ABR-21575 was kindly provided by Active Biotech (Lund, Sweden). ABR-215757 was used to therapeutically inhibit the bioactivity of S100A8/A9. ABR-215757 was added to the drinking water to deliver an approximate dose of 10mg/kg/day.

Bone Marrow Transplantation (BMT). Recipient mice were given 100mg/L neomycin 2 weeks before and after the BMT procedure. Recipient mice were irradiated (from a cesium gamma source) with 2 doses of 5.5 Gy separated by at least 4h. For standard BMTs, mice were transplanted by tail vein injection with BM ($5x10^6$ cells) from WT, *Rage*$^{-/-}$, *IL-6*$^{-/-}$, or *S100a9*$^{-/-}$ donor mice. Following a 5-week reconstitution period, a group was made diabetic with STZ as previously described.

For competitive BMTs, following irradiation, WT mice (CD45.2) were transplanted by tail vein injection with equal portions of BM ($5x10^6$ cells in total) from 8-week old WT CD45.1 and WT CD45.2 mice or WT CD45.1 and *Rage*$^{-/-}$ CD45.2 mice. After a 5-week reconstitution period, a group was made diabetic with STZ as described above. Four weeks post STZ injections MkPs were analyzed to examine the contribution of each genotype. Total platelet counts were also recorded.

Complete Blood Count. Complete blood counts were measured in whole blood obtained from tail bleeding using an automated hematology analyzer (Sysmex XS-1000*i*; Kobe, Japan).

Blood glucose, plasma cholesterol and TPO. Blood glucose was determined by a glucometer (Accu-chek, Roche). Plasma cholesterol was determined by a calorimetric enzymatic assay (Wako Diagnostics, Japan). Plasma TPO was analysed by ELISA (R&D), performed according to manufacturers guidelines.

Flow cytometry

Table S1: Antibodies details:

Antibodies	Cat #	Clone	Source	Fluorochrome
Thiazole Orange	349204	-	BD Bioscience	FITC
CD41	17-0411-82	eBioMWReg30	eBioscience	APC
CD62P	12-0626-82	Psel.KO2.3	eBioscience	PE
CCL5	149104	2E9/CCL5	BioLegend	PE
CD45	103126	30-F11	BioLegend	PB
Gr1	552093	RB6-8C5	BD Pharmingen	PerCP-Cy5.5
CD115	12-1152-82	AFS98	eBioscience	PE
CD11b	553310	M1/70	BD Bioscience	FITC
Gr1	553127	RB6-8C5	BD Bioscience	FITC
CD2	11-0021-85	RM2-5	eBioscience	FITC
CD3	11-0033-82	eBio500A2	eBioscience	FITC
CD19	101506	MP19-1	BioLegend	FITC
TER119	11-5921-85	TER-119	eBioscience	FITC
CD45R	11-0452-85	RA3-6B2	eBioscience	FITC
CD8a	553030	53-6.7	BD Bioscience	FITC
CD4	11-0042-85	RM4-5	eBioscience	FITC
Sca1	108120	D7	BioLegend	PB
ckit	105826	2B8	BioLegend	APC/CY7
Fcgamma	560540	2.4G2	BD Bioscience	PERCP-Cy5.5
CD71	113812	RI7217	BioLegend	PE/Cy7
DAPI	564907	-	BD Pharmingen	PB
p-STAT5	612599	47/STAT5(pY694)	BD Bioscience	APC
cMPL	-	-	Dr Wei Tong	PE
F4/80	123114	BMS	BioLegend	PE/Cy7
IL-6	504508	MP5-20F3	BioLegend	APC

Clec4F	AF2784	Polyclonal	R&D Systems	-
anti-goat IgG	F0124	-	R&D Systems	PerCP
RAGE	FAB11795V	697023	Novus Biologicals	PB

Quantification of reticulated platelets. Reticulated platelets were measured as previously described (2). In brief, undiluted EDTA-anticoagulated blood (5μL) within 30mins of collection was mixed with an anti-CD41-APC antibody and the fluorescent dye thiazole orange (final concentration 1μg/mL) and incubated at room temperature in the dark for 20 min. Reticulated platelets were identified as $CD41^{+}$thiazole $orange^{hi}$.

Haematopoietic stem and progenitor cells. BM cells were obtained from femurs and tibias and stained with a cocktail of antibodies before analysis by flow cytometry as previously described(2-4). Briefly, lineage committed cells were identified as (CD45R, CD19, CD11b, CD3e, TER-119, CD2, CD8, CD4 and Ly6-C/G all FITC positive, with antibodies against Sca1 and cKit to identify progenitor cell populations and LSK ($Lineage^{-}$,$Sca1^{+}$,$cKit^{+}$) cells and with antibodies to CD16/CD32 (FcγRII/III) and CD34 to separate CMP ($Lineage^{-}$, $Sca1^{-}$, $cKit^{+}$, $CD34^{int}$, FcγRII/III^{int}) and MEP ($Lineage^{-}$, $Sca1^{-}$, $cKit^{+}$, $CD34^{lo}$, FcγRII/III^{lo}) and MkPs ($Lineage^{-}$, $Sca1^{-}$, $cKit^{+}$, $CD34^{int}$, FcγRII/III^{int}, $CD41^{+}$).

c-MPL expression. After harvesting BM progenitor cells, RBCs were lysed, and the cells were resuspended in FACS buffer. BM cells were stained for MkPs as stated above with c-MPL or isotype control antibodies included. Cells were then washed and stained with a fluorescently conjugated secondary rabbit-specific antibody to detect the antibody to c-MPL for a further 30 min on ice. Following this, the cells were washed and resuspended in FACS buffer. MEPs and MkPs were identified as described above.

Flow cytometry–based proliferation studies.

Ex vivo G_2M Phase: Cell cycle analysis was performed using DAPI (Sigma) in cells that had been stained with the above markers and then incubated in cytofix/cytoperm buffer (BD Biosciences).

In vitro: BM cells were resuspended in IMDM (Invitrogen) containing 10% FCS (StemCell Technologies) and cultured for 2hrs in tissue culture flasks to enrich progenitor cells (non-adherent cells). Suspended cells were then cultured for 16hrs either in high glucose (25mmol) in the presence of cytokines essential for hematopoietic growth; stem cell factor (CSF, kit-ligand; 100ng/ml, R&D Systems), IL-3 (6ng/ml, R&D Systems) and GM-CSF (2ng/ml, R&D Systems) are treated with our without S100A8/A9 (R&D). For proliferation measurements, cells were incubated with 10μM of 5-(ethynyl-2´-deoxyuridine) (EdU) for 16hrs. In preparation for flow cytometry, cell populations were immunostained as described above to identify MkPs. They were then fixed and permeabilized (cytofix/cytoperm buffer; BD Biosciences) for 30 min. Cells were then washed and stained with Alexa Flour 488-conjugated azide using the Click-iT system (Invitrogen). Proliferation was quantified and expressed as percentage of EdU^+ cells.

Neutrophil and monocyte platelet aggregates. Blood was collected through the tail vein into EDTA-lined tubes on ice to prevent leukocyte activation. Red blood cells (RBCs) were lysed, and the washed cells were then stained with CD45, CD115, Gr1 (Ly6-C/G), CD11b and CD41 at 1:200 dilution for 30 min on ice. The cells were carefully washed, resuspended in FACS buffer and run on an LSRII flow cytometer to detect leukocyte platelet interactions and leukocyte activation. Viable cells were selected on the basis of forward and side scatter characteristics, and then $CD45^+$ leukocytes were selected. Ly6-C^{hi} monocyte platelet aggregates were identified as $CD115^+Gr1^{hi}$ (Ly6-C^{hi}) and $CD41^+$. Neutrophil-platelet aggregates were identified as $CD115^-Gr1^+$ (Ly6-G^+) and $CD41^+$. Platelet-dependent activation of Ly6-C^{hi} monocytes and neutrophils was measured by CD11b expression (MFI).

Neutrophil depletion. Neutrophils were depleted in WT and diabetic (STZ) mice after the onset of diabetes for 4 weeks, by i.p injection of the neutrophil specific anti-Ly6-G antibody (clone 1A8; 1mg/injection) every 3 days. This antibody is specific to neutrophils and does not affect monocytes(5).

Bone Marrow Derived Macrophages (BMDMs). BMDMs were generated from the BM of hind limb bones. Following 7 days of culture in RPMI media containing 15% fetal bovine serum (FBS) and 20% L-cell conditioned media. BMDMs were then changed into fresh RPMI media containing 5% FBS and 2% bovine serum albumin (BSA) prior to treatment in high glucose (25mmol/L).

RNA Isolation, cDNA synthesis and qRT-PCR. Total RNA from cells was extracted using QIAGEN RNeasy mini or micro kits and cDNA synthesized using Superscript Vilo (Invitrogen). qRT-PCR was monitored in real time with an Mx3000 sequence detection system (Stratagene) using SYBR Green PCR Core Reagents (Agilent Technologies) and normalized to *m36b4*.

BM Megakaryocytes: Bones were fixed in 4% paraformaldehyde for 24 hrs and then decalcified with EDTA solution and sectioned. H&E staining was performed. Megakaryocytes were identified as large multinucleated cells within the marrow.

Atherosclerosis study: *Apoe*$^{-/-}$ mice were fed a chow diet and a portion of mice were made diabetic with STZ. A group of STZ mice were treated with ABR-215757 (10mg/kg/day in drinking water). After 12 weeks of diabetes the mice were sacrificed for atherosclerotic lesion analysis.

Total Cholesterol: Cholesterol levels in the plasma was quantified using the Wako total cholesterol kit as previously described (6).

Lesion Analysis: Hearts were dissected after the mice were perfused with saline and frozen in optimal cutting temperature compound (OCT). Serial 6 μm sections of the proximal aorta were prepared. H&E staining was performed as previously described (7). Lipid content was assessed by Oil Red O staining and macrophage content was quantified by staining for CD68 (6). Acellular regions were identified as nuclei free regions and collagen was quantified by staining with picrosirius red and capturing images under polarized light. Images were captured on an Olympus BX43 microscope and quantification of all images was performed using Adobe Photoshop CS5.

Aortic Arch Lipid Analysis: Lipid content in the aortic arch was measured by *en face* analysis. Dissected aortas were fixed in paraformaldehyde and prior to staining, all fat and connective tissue was removed from the outer layers of the vessel. The Aorta was cut longitudinally, stained with Oil Red O followed by washing and mounting on a silicone coated dish. Aortas were viewed on an Olympus SZX10 and captured using Q-Capture Pro 7 (QImaging) software. Quantification of ORO staining was performed off-line using Adobe Photoshop CS5.

Human Study. All studies were conducted in accordance with policies of the New York University Langone Medical Center Institutional Review Board. From ongoing studies, we identified subjects on aspirin in an ongoing registry and ongoing studies (NCT02106429 and NCT01897103) measuring platelet activity. Exclusion criteria were use of other antiplatelet therapy including use of anticoagulant and NSAIDs within 72 hours, platelet count $<100 \times 10^9$/L or $>500 \times 10^9$/L, hemoglobin <9, any hemorrhagic diathesis, or severe kidney disease.

Peripheral blood was drawn from subjects >18 years of age on at least 81-mg daily dose of aspirin for 7 days. Subjects were asked to fast overnight and refrain from intensive exercise and tobacco use for 4 hours before an early-morning phlebotomy to avoid circadian variation in platelet response. Blood was drawn using a 19-gauge needle. A subset of patients with cardiovascular disease were scheduled to undergo revascularization and had blood samples collected prior to their procedure. The first 5 mL was discarded, and the remaining blood was collected into tubes containing 3.2% sodium citrate for subsequent determination of platelet activity. Samples were processed within 30 minutes of collection.

Monocyte-Platelet Aggregates: Monocyte-platelet aggregates were identified using whole blood flow cytometry as previously described(8, 9). Briefly, whole blood was fixed with 1% formaldehyde (Sigma) 15 minutes after blood collection. Blood was labeled with CD61-FITC (Dako) to identify platelets, and CD14-APC (BD Biosciences) to identify monocytes. After lysis of red blood cells, monocytes were collected based off of side-scatter properties and

positive staining for CD14 using an Accuri C6 flow cytometer (BD Biosciences, San Jose, CA). Monocyte with adherent platelets were identified by CD61 positivity, and were recorded as a percent of 2,000 total monocytes collected.

Reticulated Platelets: Reticulated Platelets (RP) were measured with a previously described flow cytometry assay(10). Whole blood was labeled with CD42b (BD Biosciences) to identify platelets and thiazole orange (ReticCount, BD Biosciences) for 10 minutes in the dark. Samples were analyzed on the Accuri C6 flow cytometer (BD Biosciences, San Jose, CA). Platelets were identified and gated by their characteristic forward- and side-scatter properties, and 10,000 platelets were analyzed from each sample. Reticulated platelets were identified by mean fluorescent intensity and as a percent of total platelets positive for thiazole orange.

Statistics

Data are presented as mean ± SEM and were analyzed using the two-tailed Student t-test or one-way ANOVA followed by Newman-Kuel's post hoc test. A $P<0.05$ was considered significant. All tests were performed using the Prism software (GraphPad Software, Inc., La Jolla, CA).

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