Supplemental Methods

Study Population. Patients were excluded if they had any comorbid condition known to promote CVD or systemic inflammation, such as known CVD, uncontrolled hypertension, internal malignancy within 5 years, human immunodeficiency virus, active infection within the past 72 hours of baseline, and major surgery within 3 months. Inclusion criteria for psoriasis patients also included no previous history of biologic therapy use and was determined prospectively. A health care provider confirmed the onset, duration of psoriasis, and assessed psoriasis severity using the Psoriasis Area Severity Index (PASI) score. Clinical parameters including complete blood count, blood pressure, height, weight, waist and hip circumferences were measured. Laboratory parameters including fasting blood glucose, fasting lipid panel, white blood count with differential, and systemic inflammatory markers including Hs-CRP were evaluated in a clinical laboratory.

Primary Outcome. The primary outcome in the cohort was coronary plaque burden, both total burden and non-calcified burden by Coronary Computed Tomography Angiography (CCTA) (320-detector row Aquilion ONE ViSION, Toshiba, Japan). All patients underwent CCTA to assess coronary plaque burdens as described previously(1). CCTA measurements were blinded to time and clinical status.

Acquisition of CCTA. All patients underwent CCTA on the same day as the blood draw, using the same CT scanner (320-detector row Aquilion ONE ViSION, Toshiba, Japan). Guidelines implemented by the NIH Radiation Exposure Committee were followed. Scans were performed with prospective EKG gating, 100 or 120kV tube potential, tube current of 100-850 mA adjusted to the patient's body size, with a gantry rotation time of 275ms. Images were acquired at a slice thickness of 0.5 mm with a slice increment of 0.25 mm.

Analysis of CCTA. A single, blinded reader (blinded to treatment and time of scan) evaluated coronary plaque characteristics across each of the main coronary arteries > 2 mm using the dedicated software (QAngio CT, Medis; The Netherlands) (2,3). Automated longitudinal contouring of the inner lumen and outer wall was performed, and results were manually adjusted when clear deviations were present (4). Results of the automated contouring were also reviewed on transverse reconstructed cross-sections of the artery on a section-by-section basis at 0.5-mm increments. Lumen attenuation was adaptively corrected on an individual scan basis using gradient filters and intensity values within the artery. The inter-and intra-reader variability for scans were less than 5%. Quantitative as well as qualitative coronary plaque burden evaluation was performed in 98% of the available coronary segments.

Covariates. Baseline treatment status for the cohort was defined by up to 12 months of any of the following therapy before inclusion in the study: systemic or biologic therapy (steroids, methotrexate, apremilast, adalimumab, etanercept, and ustekinumab), statins, psoralen plus ultraviolet A (PUVA) or ultraviolet B (UVB), and topical treatments. Patients were asked to complete survey-based questionnaires regarding smoking, previous CVD, family history of CVD, and previous established diagnoses of hypertension and diabetes. Patient responses were then confirmed during history and physical by the study provider. CVD included acute coronary syndrome comprising both MI and unstable angina pectoris, angina pectoris, cerebrovascular event, transient ischemic attack, peripheral vascular disease and revascularization procedures that comprised of coronary artery bypass grafting and percutaneous interventional procedures. Diabetes and hypertension were defined either by an established diagnosis or by use of glucose lowering and blood pressure lowering drugs, respectively.

Laboratory Procedures.

Whole Blood Processing. Heparinized whole blood was centrifuged at 1400 rpm for 7 minutes to remove plasma and transferred to a 50 ml Falcon tube. Whole blood was treated with 20 ml of ACK Lysis Buffer (Quality Biologicals, Gaithersburg, MD) and placed on the rocker for 10 minutes. RBC lysed whole blood was centrifuged at 1400 rpm for 5 minutes and the supernatant discarded. Red blood cell lysis was repeated and the whole blood cells were then washed with 25 ml of FACs Buffer (1X PBS, 0.1% BSA, 0.02% EDTA). To retrieve the peripheral blood mononuclear cells (PBMCs), heparinized whole blood was mixed with 1X PBS at a 1:1 ratio and layered on a Ficoll gradient. Whole blood was centrifuged at 1600 rpm for 25 minutes and the PBMC layer was collected. Red blood cells were lysed using the hypotonic/hypertonic method. Cells were washed with 1X PBS twice for further experimentation.

Immunophenotyping. RBC lysed whole blood leukocytes and ficoll-separated PBMCs were incubated for 30 minutes in a 10-color antibody cocktail in FACs buffer on ice (Table S1). LIVE/DEAD Aqua fixable viability dye (Life Technologies, Carlsbad, CA) was added for 10 additional minutes. Whole blood and PBMCs were washed with FACs buffer twice and resuspended in 300 μl of 1% PFA in 1X PBS. Antibody stained cells were acquired on a BD Biosciences LSRII flow cytometer using DIVA 6.1.2 software (BD Bioscience, San Jose, CA) and the data were analyzed using Flowjo Legacy software (Flowjo, LLC, Ashland, OR).

LDG Frequency. We determined the frequency of LDGs by quantitating the percentage of CD14^{lo}CD15^{hi}CD10^{hi} cells in the PBMC fraction by flow cytometry. Then, we calculated the percentage of LDGs in whole blood by the following equation:

> %LDGs WB %Monocytes WB $=$ %LDGs PBMCs $^-$ %Monocytes PBMC

Finally, we multiplied the percentage of LDGs in whole blood, determined by flow cytometry, by their respective complete blood count acquired at the National Institutes of Health Clinical Center, to determine the frequency of LDGs per μl.

LDG and NDG purification. NDGs were separated using a previously established method(5). Briefly, the remaining red blood cell and neutrophil layer from the PBMC isolation process was incubated with a 5% dextran solution for 45 minutes at room temperature. The NDG layer was then collected and transferred to a separate 50 ml Falcon tube. The red bloods cells were lysed with ACK lysis buffer (Quality Biologicals, Gaithersburg, MD). The cells were washed and purity was > 95%, confirmed by flow cytometry. NDGs were then stained and sorted as described below to account for any activation induced during the sorting process. LDGs were sorted from the PBMC cell layer. Briefly, PBMCs or dextran-purified NDGs were blocked with normal mouse serum and then stained with a 5-color antibody cocktail, and Zombie Green Viability Dye (Biolegend, San Diego, CA) for 30 minutes at room temperature (Supplemental Table 1). The PBMCs were washed 3 times with FACs buffer (1X PBS, 0.1% BSA, 0.02% EDTA) and sorted for SSC^{hi}CD14^{lo}CD15^{hi}CD10^{hi} cells on a Fusion Aria sorter (BD Bioscience, San Jose, CA)(Figure 2C).

Harvesting NET-associated proteins. Purified human normal-density granulocytes (NDGs) or low-density granulocytes (LDGs) were plated at $1.0x10^6$ cells/ml in a 12-well plate in phenol free RPMI. NDG NETosis was induced by adding phorbol 12-myristate 13-acetate (500 nM, Sigma-Aldrich, St. Louis, MO) for 4 hours at 37° C. LDG NETosis was induced by incubating for 4 hours at 37C. The NET-associated proteins were harvested by adding micrococcal nuclease (ThermoFisher, Waltham, MA) for 10 minutes at 37° C and the supernatant was collected in an Eppendorf tube. Cellular debris were removed by centrifuging at 5000 RPM for 5 minutes at 4 °C.

The supernatant was collected and stored at -20°C. The protein concentration was determined using the Pierce BCA Protein Assay Kit (ThermoFisher, Waltman, MA).

Endothelial Cell Cytotoxicity Assay. Human aortic endothelial cells (HAoECs) (Promocell, cat. C12272, Germany), a human primary cell line purchased from Promocell, were grown to confluency and co-cultured with purified NDGs, LDGs, LDGs plus 40 units/ml DNase, or 50 µg NET proteins from NDGs or LDGs for 18 hours in Complete RPMI supplemented with 10% FCS and 5% penicillin/streptomycin. HAoECs were harvested with 1 mL of TrypleE (ThermoFisher, Baltimore, MD) and washed with Cell Staining Buffer (Biolegend, San Diego, CA). HAoECs were incubated in Annexin V binding buffer (Biolegend, San Diego, CA) for 15 minutes. HAoECs were stained with anti-human CD146-APC (Biolegend, cat. 348507, San Diego, CA) for 15 minutes, washed and stained with Annexin V-FITC (Biolegend, cat. 640906, San Diego, CA) and Propidium Iodide (Biolegend, cat. 421301, San Diego, CA) for 15 minutes. HAoECs were washed with Annexin V binding buffer and apoptosis was measured using the BD Biosciences LSRII flow cytometer (BD, San Jose, CA).

Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). NDGs plus PMA or LDGs were seeded on poly-l-lysine coated glass coverslips (Thermo Scientific, Rochester, NY) for 30 mins, 2 hours and 4 hours to observe NET-formation. For SEM processing, specimens were fixed with 4% glutaraldehyde and 0.1M calcium chloride in 0.1M sodium cacodylate buffer, pH 7.2 for 24 hours and washed in 0.1M cacodylate for 3 x 10 minutes, followed by post-fixation with 1% OsO4 in 0.1M cacodylate buffer for 1 hour on ice in the dark. Cells were dehydrated in a graded ethanol series (30%, 50%, 70%, 85%, 95%, 100%) and dried using a Samdri-795 critical point dryer (Tousimis Research Corp, Rockville, MD). Afterwards, the samples were mounted on aluminum stubs, sputter coated with 5 nm of gold in a EMS 575-X sputter coater (Electron Microscopy Sciences, Hatfield, PA), and imaged with a Hitachi S-3400N1 scanning electron microscope at 7.5 kV (Hitachi High Technologies America, Inc., Pleasanton, CA). For TEM, specimens were pelleted in an Eppendorf tube, fixed, and processed as described above through ethanol dehydration and embedded in resin (6). Ultra-thin section samples on coated mesh grids were imaged using a JEM1400 transmission electron microscope.

RNA Sequencing and Analysis. 50,000 NDGs or LDGs were sorted (Supplemental Figure 2) directly into QIAzol (Qiagen, Frederick, MD) and total RNA was purified as previously described(7). Rigorous quality controls of pair-end reads were assessed using FastQC tools(8), and adapter sequences and low quality bases were trimmed by using trimmomatic(9). We aligned reads to the human reference genome (GRCh37) by STAR(10), and only uniquely mapped reads were used for subsequent analyses. We quantified gene expression using HTSeq (11), using GENCODE $v24$ as gene annotations (12). We then performed quantile normalization and used limma (13) for differential expression analysis to identify genes that were dys-regulated between the NDG and LDG conditions, controlling for the individual and batch effects. False discovery rate (FDR) was used for multiple testing; and significant differentially expressed genes have $FDR \leq 0.1$ and $|log2(Fold Change)|>=1.5$. We then identified functions or gene ontologies that are enriched among differentially expressed genes, and FDR<=0.1 was used to declare significance. All graphical illustrations and RNA-seq analyses were conducted using custom scripts and libraries implemented in R.

Platelet and Neutrophil Aggregation. We measured the aggregation of platelets with LDGs (PBMC layer) or NDGs (whole blood) by quantitating the percentage of CD15hiCD41hi cells within the CD15^{hi} population by flow cytometry. Antibodies are described in Supplemental Table 1.

 At a second site, neutrophil platelet aggregation was confirmed in a subset of psoriasis and healthy controls. Patients with a history of moderate to severe psoriasis were recruited at New York University Langone Health between July 2017 and May 2018 as part of an on-going study to evaluate platelet activity in patients with psoriatic disease (IRB protocol 17-00692). Age- and sex-matched controls were sequentially recruited. After informed consent, blood sampling was performed in the brachial vein through a 20 gauge IV. For all samples, the first 2ml was discarded and remaining blood was collected in a vacutainer containing 3.2% sodium citrate. Samples were processed within 30 minutes of blood draw.

Neutrophil-platelet aggregates were acquired on an Accuri C6 flow cytometer (BD Biosciences, San Jose, CA). Immediately following phlebotomy, whole blood was fixed with formaldehyde in 1.4X Hanks balanced saline solution. Blood was labeled with monoclonal antibodies to CD61-FITC (BD Pharmingen); diluted 4-fold with distilled water to lyse the erythrocytes; and further diluted in an equal volume of HEPES–Tyrode's buffer, pH 7.4. Neutrophils were identified by their forward- and side-scatter characteristics. The percentage of neutrophil-platelet aggregates was identified in single parameter histograms of CD61-FITC fluorescence displaying events from the neutrophil gate. The positive analysis region was determined using an IgG-FITC conjugated isotypic control.

NETs Quantification. For the NET quantification assay, LDGs were isolated by negative selection as previously described (14) by a second site. The formation of NETs was quantitated as previously described (15). Briefly, purified NDGs or LDGs were seeded on poly-l-lysine coated coverslips at 1×10^6 cells /ml in RPMI. The coverslips were incubated for 4 hours with or without 2.5 µM A23187 and then fixed with 4% PFA overnight. Cells were stained with polyclonal rabbit anti-human anti-myeloperoxidase (1:1000, Dako, cat. A039829-2, Carpinteria, CA) for 1 hour followed by a 30-minute incubation with the secondary antibody, donkey anti-rabbit Alexa Fluor 555 (1:400, Life Technologies, cat. A-31572, Carlsbad, CA). Hoechst 33342 (1:1000, Life Technologies, Carlsbad, CA) was used to detect nuclear DNA by incubating the cells for 10 minutes at room temperature. NET formation was imaged using a Leica DMI 4000B fluorescent microscope and the NETs were manually quantified. Extracellular strands expressing both Hoechst and myeloperoxidase were quantified as NETs and the percentage determined as the average of five to six fields normalized to the total number of cells.

Supplemental Table 1. List of antibodies used for sorting and immunophenotyping.

Supplemental Figure 1. Patient Recruitment Scheme.

Supplemental Figure 2. LDGs from the peripheral blood mononuclear layer and normal density granulocytes (NDGs) were sorted, mounted by cytospin and stained with Wright's stain to confirm sample purity.

Supplemental Table 2. A comprehensive list of the 1,076 differentially expressed genes in LDGs relative to NDGs.

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