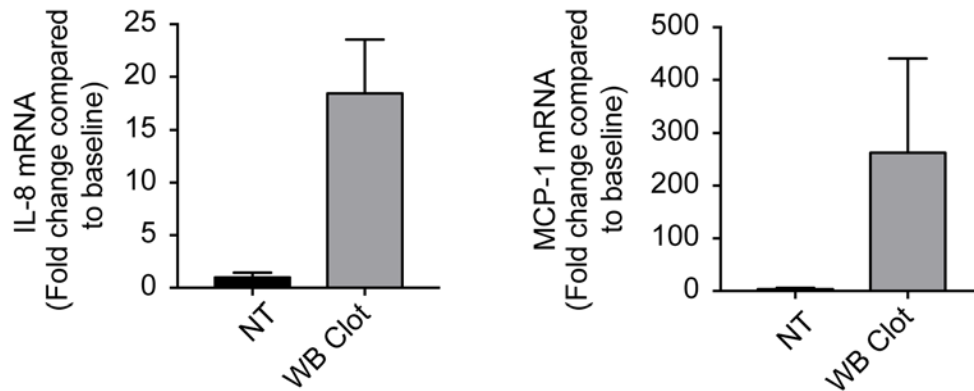
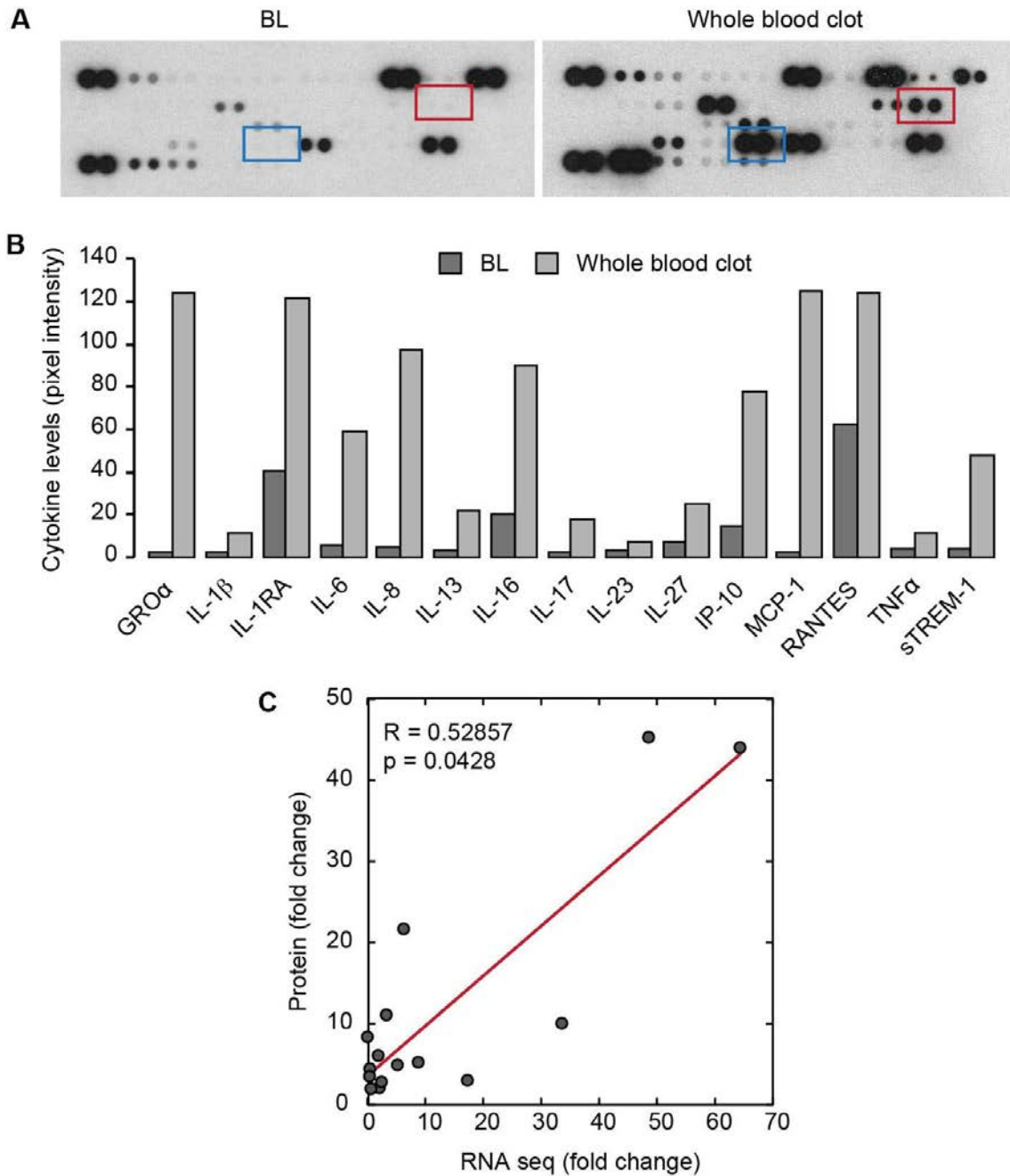


Supplemental Figure I. Whole blood clot formation does not alter CD14⁺CD16⁻ and CD14⁺CD16⁺ populations. Whole blood (10 mLs) was left untreated or allowed to clot (20 mM CaCl₂ final and 1:20000 Innovin, final) for two hours. Monocytes were isolated using CD14⁺ microbeads from miltenyi biotec as described in the material and methods. Roughly 2 million monocytes were isolated from untreated blood and clotted blood (data not shown). Monocytes were then stained for CD14 and CD16. CD14 and CD16 positivity was determined by flow cytometry compared to IgG controls. CD14⁺⁺CD16⁻, CD14⁺⁺CD16⁺, and CD14⁺CD16⁺⁺ populations were unchanged between the two groups (N=3).



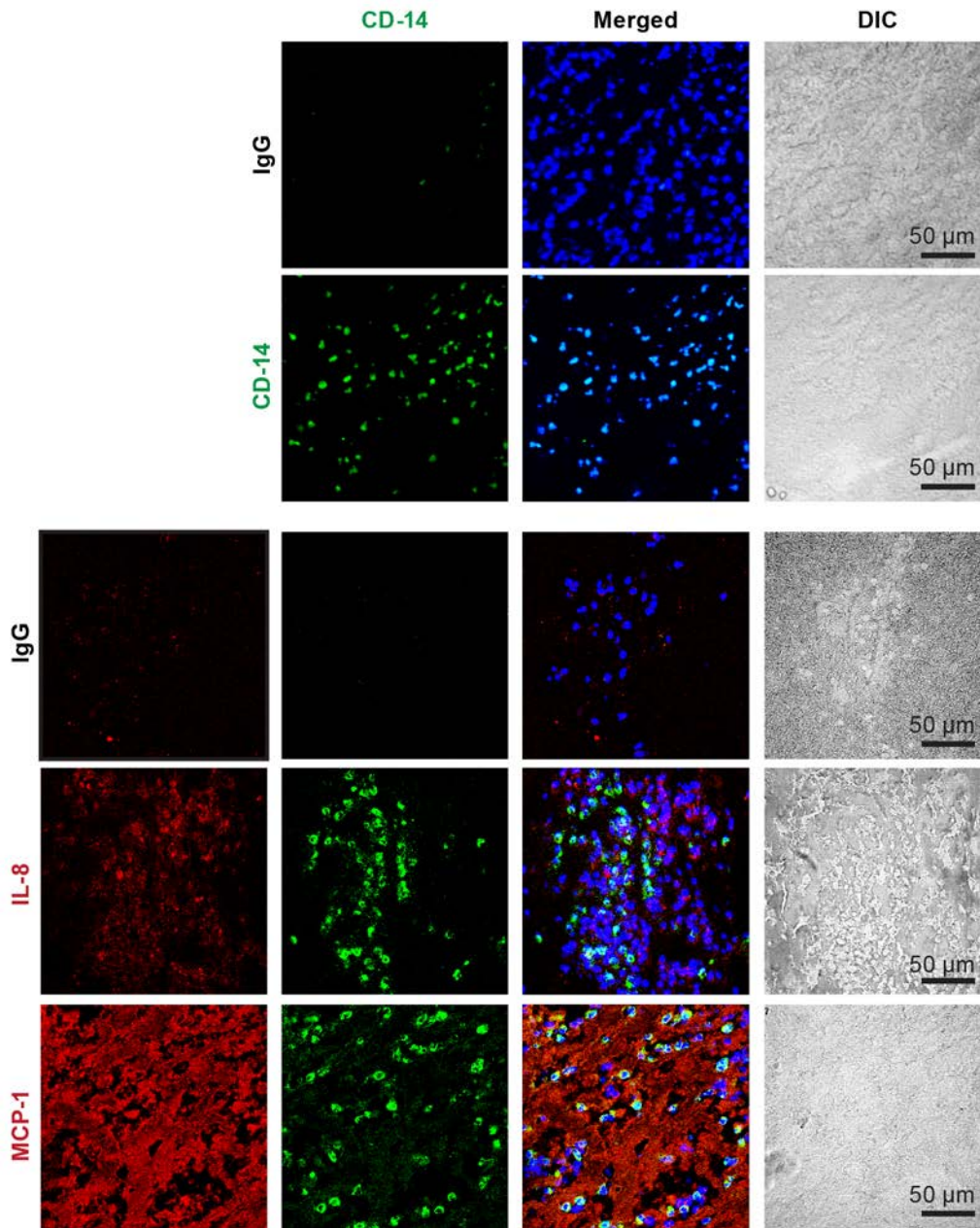
Supplemental Figure II. IL-8 and MCP-1 mRNA increase compared to untreated

whole blood. Monocytes were isolated from untreated whole blood (NT) or whole blood clots (WB Clot) after 2 hours at 37°C at 5% CO₂ and mRNA isolated. Real-time PCR to measure IL-8 and MCP-1 levels was then performed (n>3, *p<0.05).

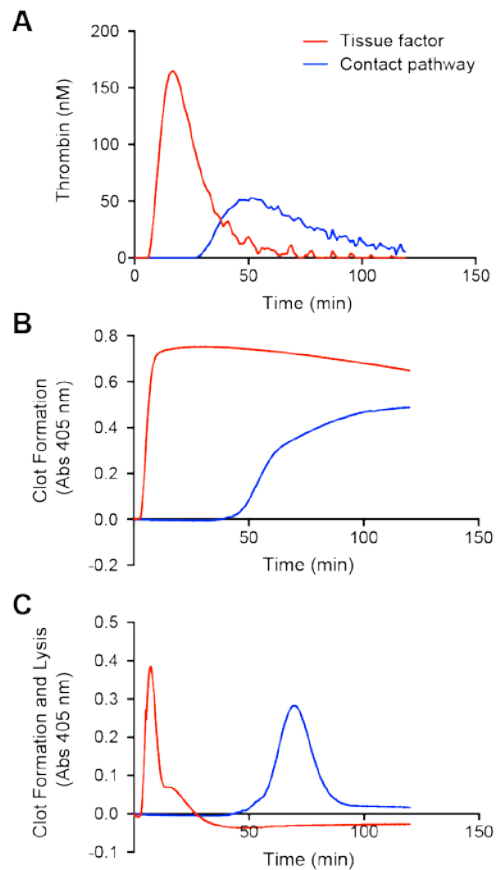


Supplemental Figure III. Clot induced changes in the expression of mRNAs and their corresponding proteins correlate with one another. (A, B) Whole blood clots were formed as described in the Supplemental Methods. Proteins were assessed by a dot blot cytokine protein array in freshly-isolated whole blood (BL- baseline) and compared to a whole blood

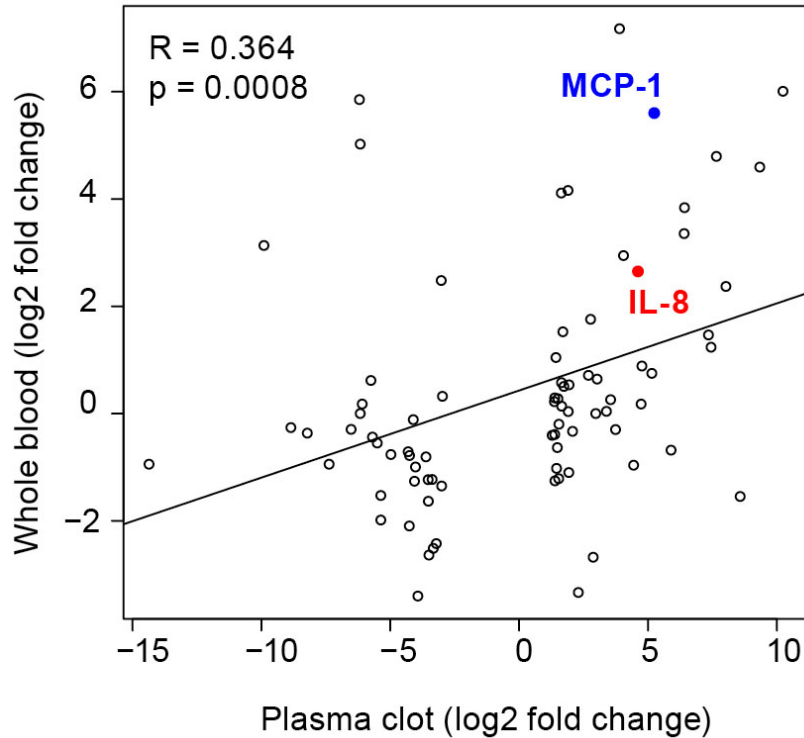
clot after 18 hours. IL-8 (red) and MCP-1 (blue) are indicated on the actual dot blot and the relative levels of proteins that were increased in response to clotting are shown in **panel B**. **(C)** Proteins with increased expression (**Panel B**) were correlated to their corresponding mRNA expression levels, as measured by RNA-sequencing.



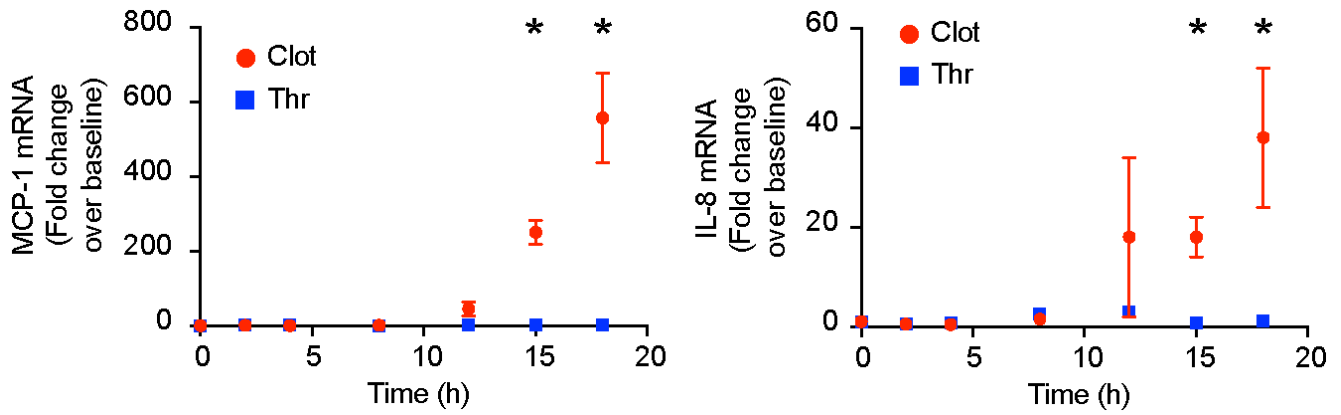
Supplemental Figure IV. IL-8 and MCP-1 are expressed in clots extracted from patients with renal disease. Thrombi were harvested from the iliac artery of a patient who developed thrombosis after undergoing renal transplantation. The clots were stained for CD14 (green), IL- 8 (red), or MCP-1 (red). IgG using isotype controls at similar concentrations are shown to assess background stain. Nucleated cells were stained with DAPI (blue). Differential contrast is shown in the far-right panels. The images are representative of three independent samples taken from patients with varying degrees of renal disease (see **Supplemental Table 1**).



Supplemental Figure V. Tissue factor driven thrombin generation induces greater levels of thrombin and clot formation compared to the contact pathway. Plasma was isolated as described in the material and methods. Plasma was recalcified (20 mM final) in the presence (TF pathway) or absence (contact pathway) of recombinant tissue factor (1:20000 final) and thrombin generation measured using a fluoregenic substrate as previously described (A). Clot formation was measured in parallel using a thermomax plate reader that monitored changes in optical density at 405 nm (B). Fibrinolysis was also measured with the addition tissue factor plasminogen (tPA) in parallel clot formation assays (C). All assays were monitored for 2 hours at 37°C. Tracings are from representative experiments (n=3).

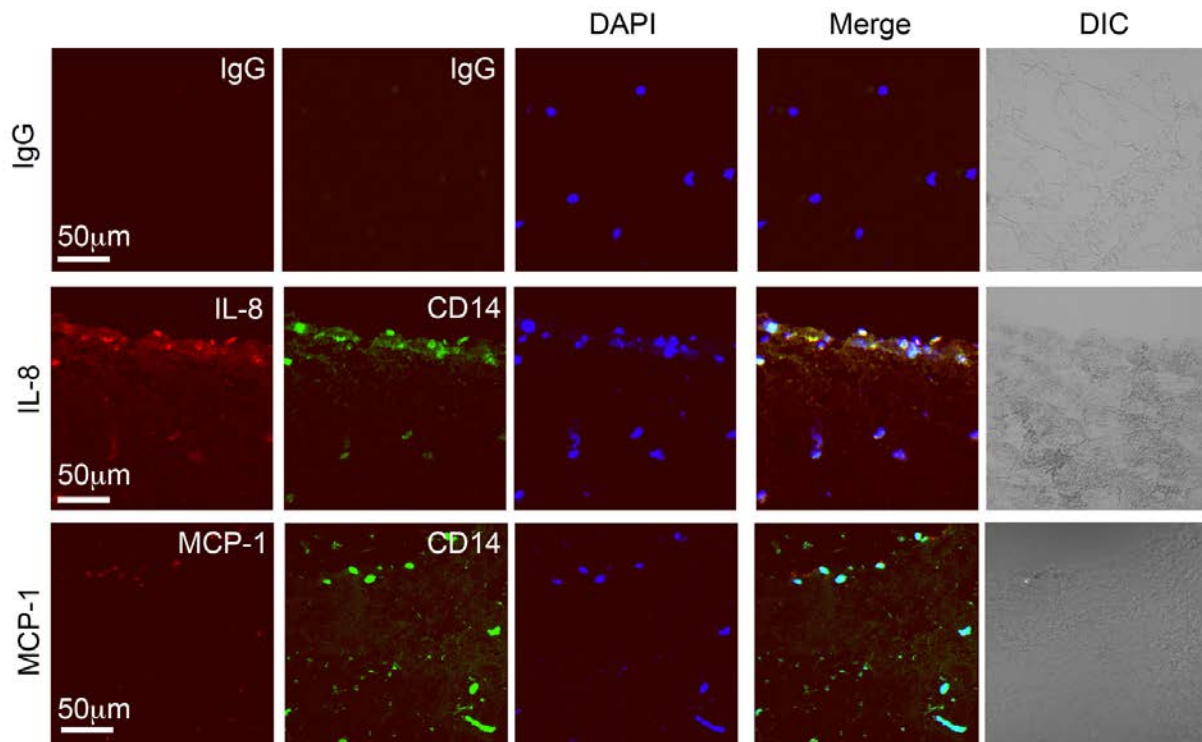


Supplemental Figure VI. Gene expression changes observed in whole blood and plasma fibrin clots correlate with one another. Inflammatory mRNA expression fold changes in monocytes from whole blood clots versus BL whole blood compared to fold changes in monocytes from plasma clot versus NT. Inflammatory genes selected according to the inflammation Gene Ontology (GO) (GO term: inflammation) Genes that did not significantly change ($p > 0.05$) in response to clotting in either group were excluded from the analysis. A Pearson correlation revealed a positive correlation ($p < 0.05$) between whole blood and plasma clots.

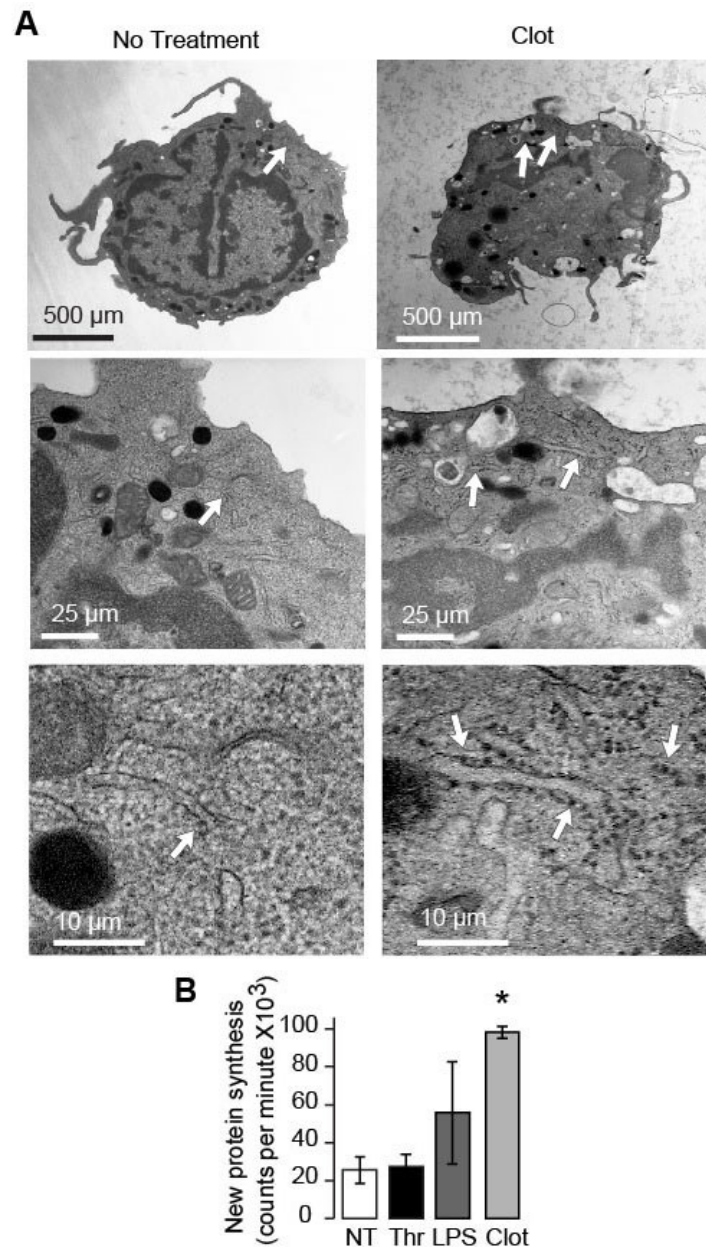


Supplemental Figure VII. IL-8 and MCP-1 mRNA increase over time in plasma

clots. Monocytes were embedded into plasma clots or stimulated with 0.1 U/mL thrombin and lysed in Trizol. IL-8 and MCP-1 mRNA was measured at various time point (n>3, *p<0.05).

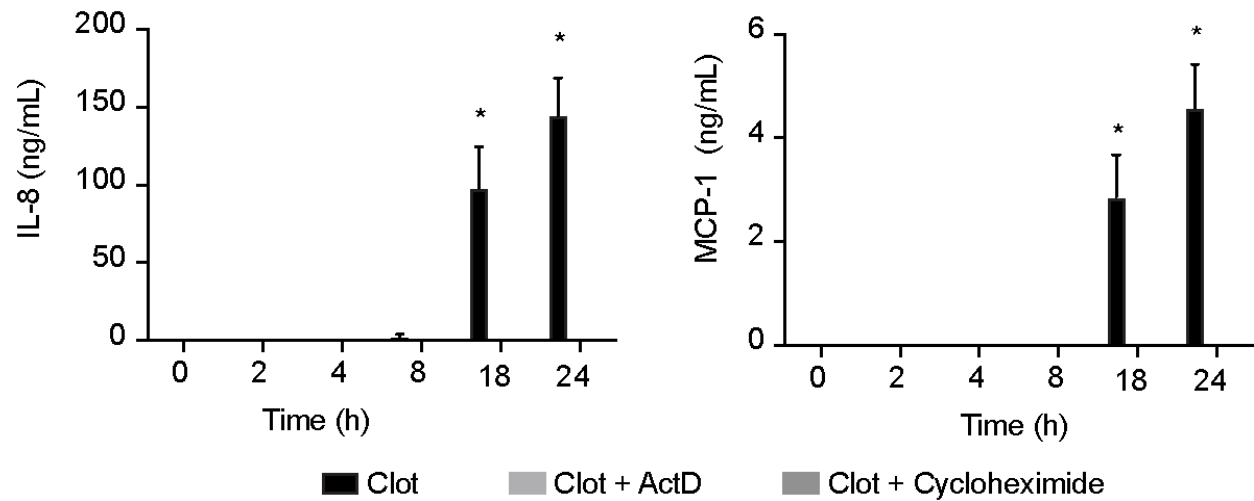


Supplemental Figure VIII. IL-8 and MCP-1 is expressed by monocytes embedded into plasma clots. Monocytes embedded into plasma clots after 18 hours were processed similar to human thrombi isolation for cytokine staining (see material and methods). Cryosectioned plasma clots were stained for IL-8 (red), MCP-1 (red) and CD14 (green). DAPI was used to stain the nucleus. IgGs for IL-8 and MCP-1 are the same. Light transmission images were taken to examine plasma clot morphology (DIC, far right). IL-8 and MCP-1 staining co-localized with CD14, a specific monocyte marker. The images are representative of three independent experiments.

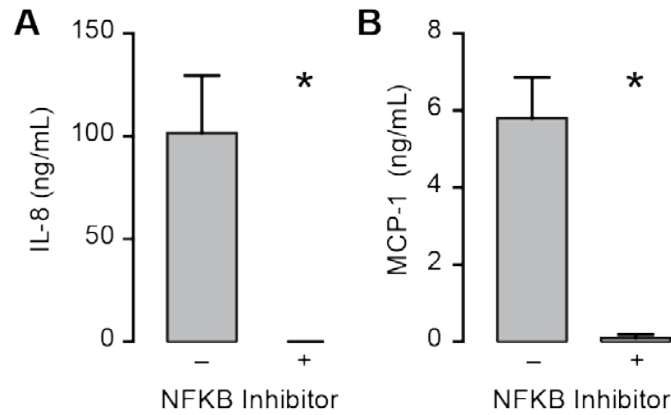


Supplemental Figure IX. Monocytes embedded in plasma fibrin clots have increased frequency of ribosomes tracking along endoplasmic reticulum. (A) Electron micrographs depicting monocytes at baseline compared to monocytes embedded in plasma fibrin clots. Monocytes embedded in plasma fibrin clots have increased cytoplasm to nuclear ratio as well as increased frequency of ribosomes attached to endoplasmic reticulum (white arrow). These images are representative of 3 independent experiments. (B) Global protein synthesis, as assessed by incorporation of S³⁵-labeled methionine/cysteine into proteins, in clot retrieved

monocytes compared to monocytes left in suspension, monocytes treated with thrombin, or monocytes stimulated with LPS. The bars represent the mean \pm SEM of 3 independent experiments for each group using 3 different donors. The asterisk indicates $p < 0.05$ compared to BL, NT and Thr samples.

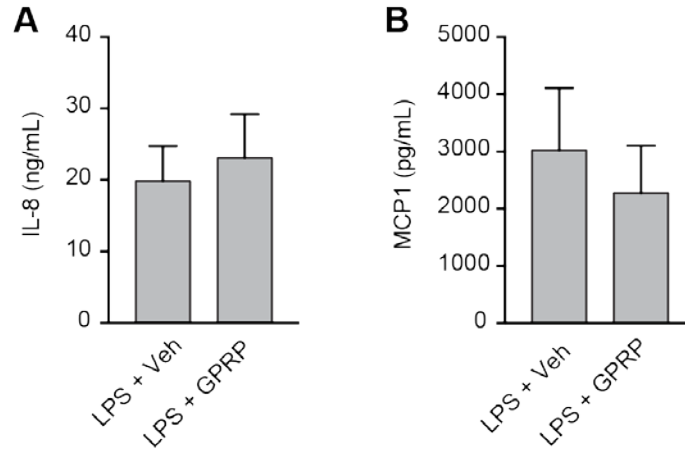


Supplemental Figure X. Production of IL-8 and MCP-1 is transcriptionally and translationally controlled. Monocytes were treated with vehicle, actinomycin D (ActD; 5 μ g/mL), or cycloheximide (5 μ g/mL) for one hour before being embedded into plasma clots. Supernatant were harvested at the indicated time points and IL-8 and MCP-1 were measured (N>3, *p<0.05).

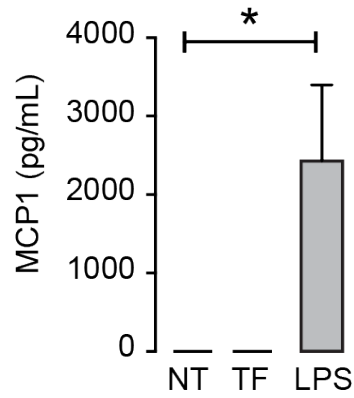


Supplemental Figure XI. Production of IL-8 and MCP-1 is NF κ B-dependent.

Monocytes were treated with vehicle, or the NF κ B inhibitor (BAY 11-7082, 5 μ M final) for one hour before being embedded into plasma clots. Supernatants were harvested at the indicated time points and IL-8 and MCP-1 protein was assessed (N>3, *p<0.05).

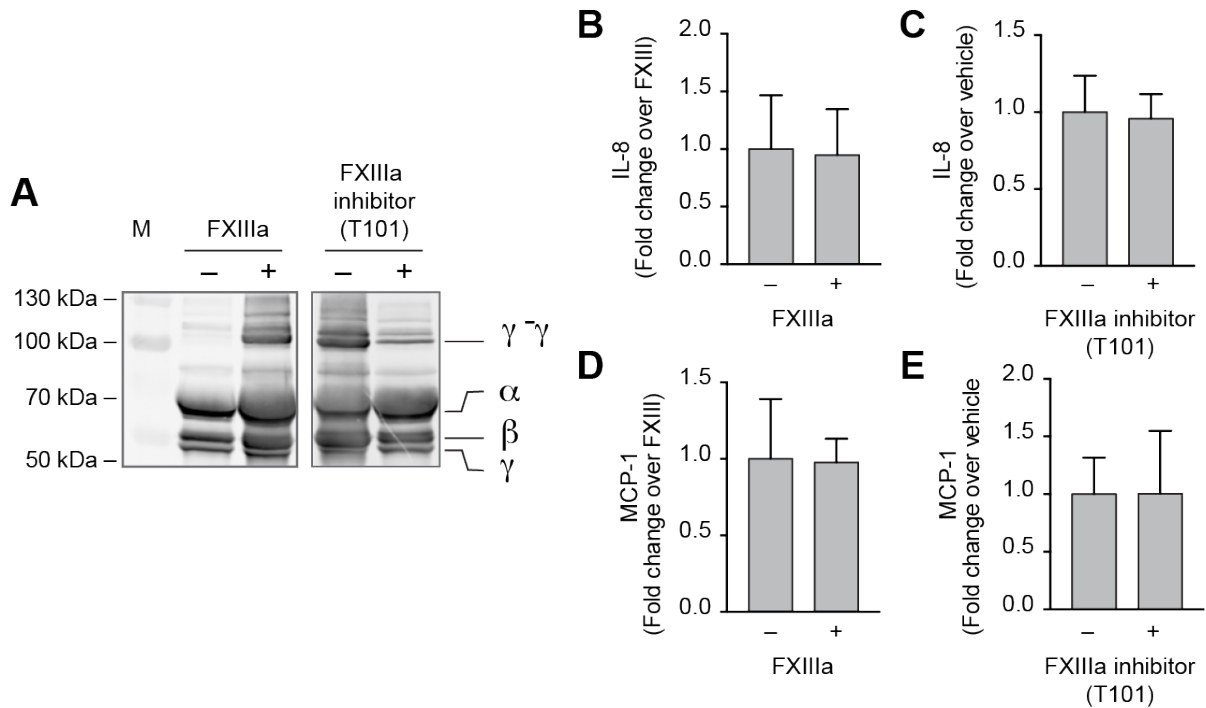


Supplemental Figure XII. The fibrin inhibitor GPRP does not affect LPS-induced cytokine synthesis. Monocytes were treated with 40 mg/mL GPRP or vehicle control (water) for 1 hour before addition of LPS (100 ng/mL). Supernatants were collected at 18 hours and cytokine synthesis was measured by ELISA (N>3).

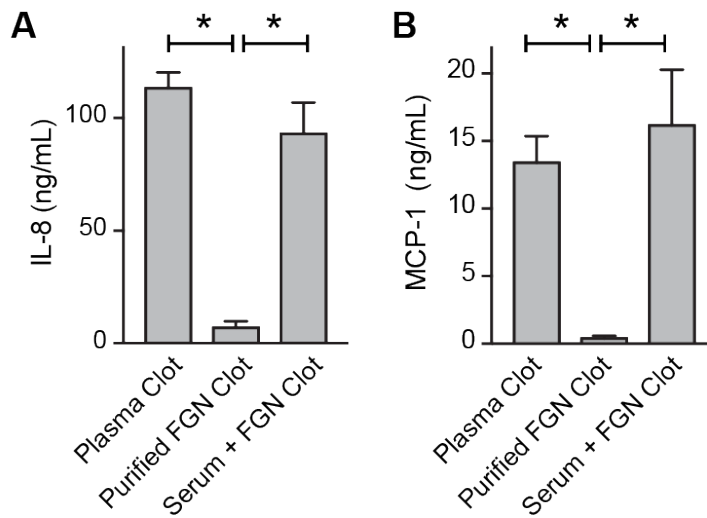


Supplemental Figure XIII. Tissue factor does not directly induce MCP-1 synthesis.

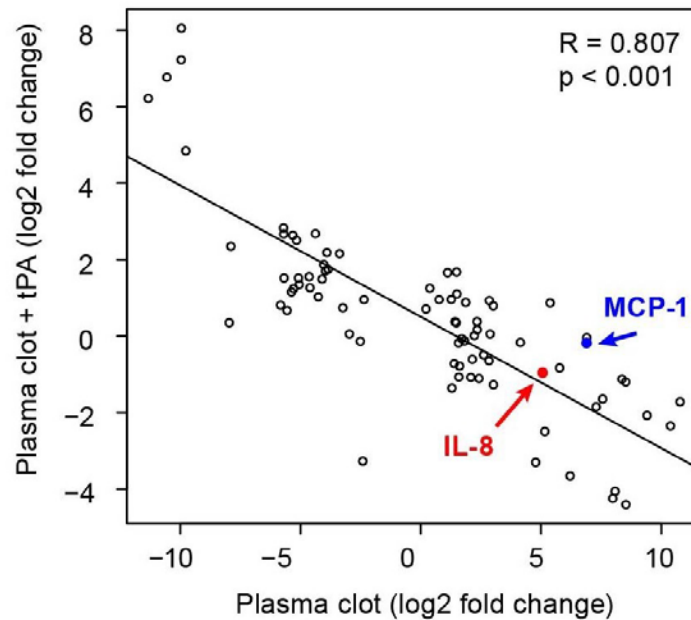
Monocytes were left untreated, stimulated with recombinant tissue factor (1:20000 dilution, final) or LPS (100 ng/mL, final). After 18 hours, supernatants were harvested and MCP-1 was measured (n = 3, *p<0.05).



Supplemental Figure XIV. FXIII does not influence IL-8 or MCP-1 production from monocytes embedded in plasma clots. Plasma clots using FXIII deficient plasma were formed in the presence or absence of recombinant FXIIIa (10 μ g/mL, final). Plasma clots were also formed in the presence of 5 μ M T101, a specific FXIII inhibitor. After 60 minutes, reactions were stopped by the addition of 50 mM DTT, 12.5 mM EDTA, and 8 M urea. Samples were then incubated at 60°C for 1 hour with occasional agitation. Samples were reduced, boiled, and probed with a rabbit anti-human fibrinogen polyclonal antibodies to determine the presence of γ - γ dimers. Monocytes were embedded into clots formed in the presence or absence of FXIII or in the presence of T101 or vehicle and cytokine production was measured after 18 hours.

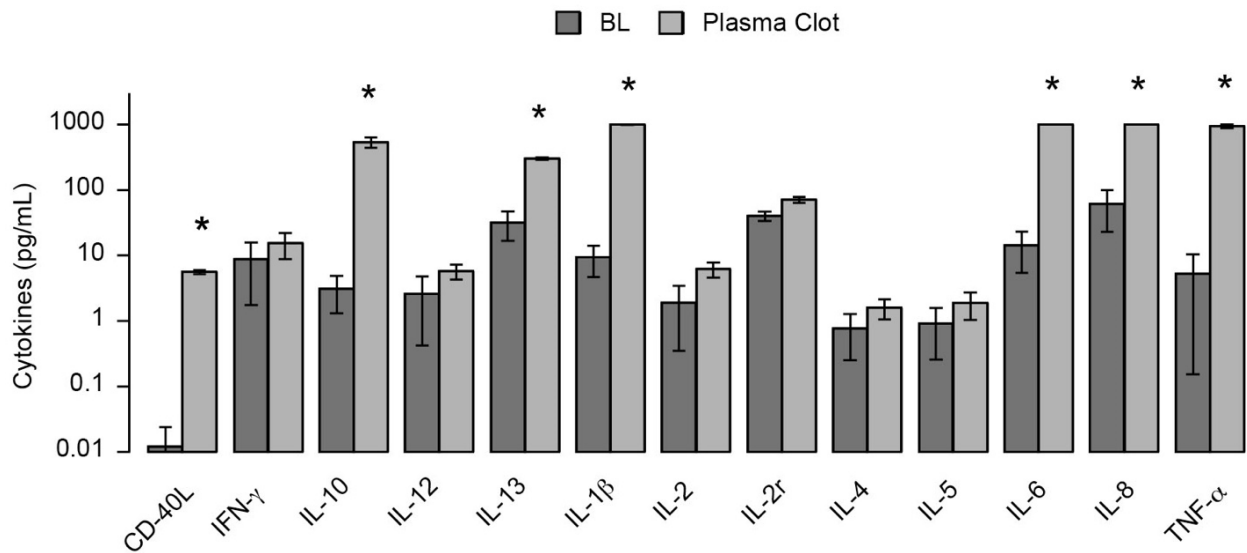


Supplemental Figure XV. Monocytes embedded in fibrinogen clots combined with serum synthesize IL-8 and MCP-1. Monocytes were embedded in plasma clots as described in the materials and methods or clots formed from purified fibrinogen (2 mg/mL, final) and thrombin (1 U/mL, final). To some purified fibrin clots, serum from autologous donors was added to recapitulate plasma fibrin clots (N=5, $p < 0.05$).

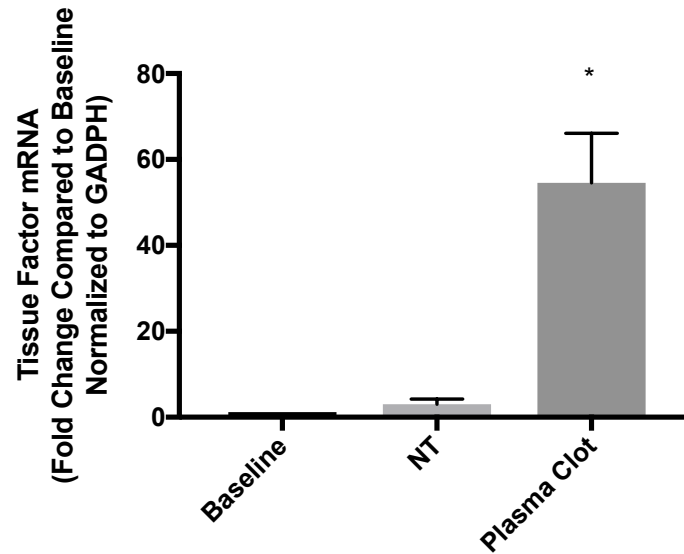


Supplemental Figure XVI. Fibrinolysis blunts clot-induced changes in

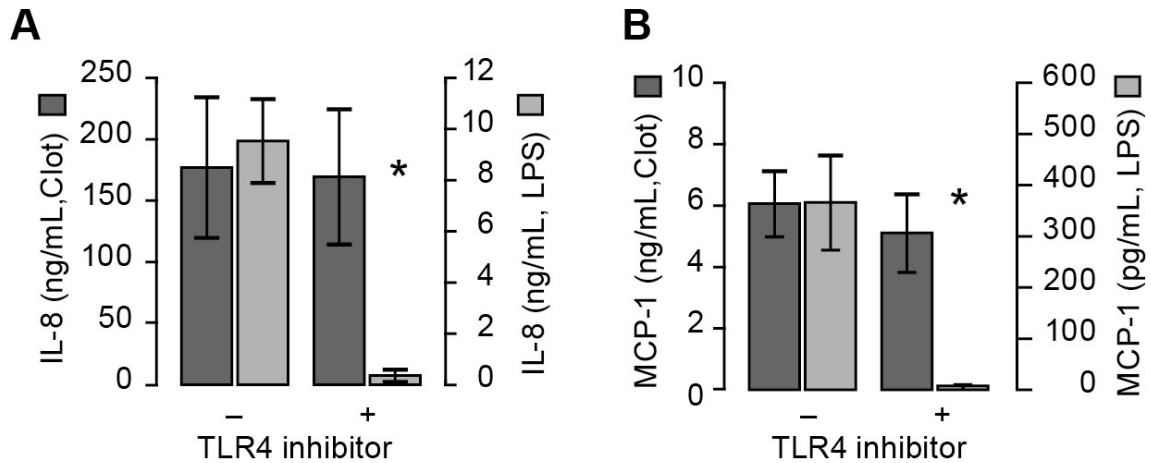
inflammatory gene expression. Using genes selected according to the inflammation GO term, a correlation analysis was performed between monocyte-derived mRNAs that displayed altered expression ($p < 0.05$) when embedded in plasma clots (compared to NT) versus their expression level when the plasma clots were lysed with tPA (compared to plasma clot) (lysis = 30 minutes post-clot formation). A Pearson correlation revealed a negative correlation ($p < 0.05$) in mRNA expression levels with and without lysis.



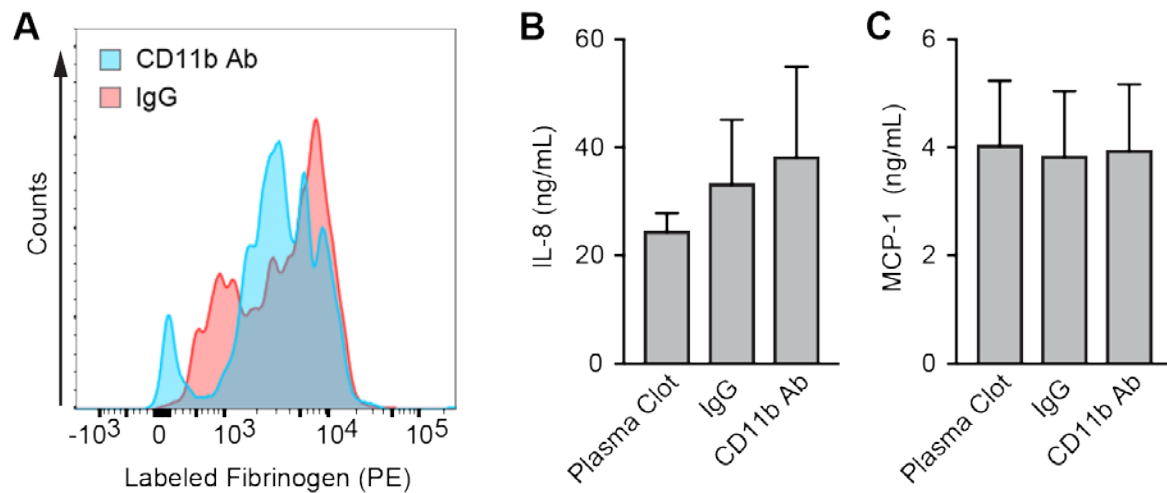
Supplemental Figure XVII. Plasma fibrin clots induce robust cytokine protein expression. Baseline plasma and supernatants from monocytes stimulated by plasma clots after 18 hours were harvested for multiplex cytokine analysis that was performed at ARUP Laboratories as described in the Supplemental Methods. N=3 for all experiments. Significance indicated by * with $p < 0.05$.



Supplemental Figure XVIII. Plasma fibrin clots induce tissue factor expression in monocytes. Tissue factor (TF) mRNA expression was assessed in monocytes left untreated (NT) or embedded in plasma clot for 2 hours compared to baseline monocytes. TF express levels were normalized to GADPH. Significance indicated by * with $p < 0.05$ compared to baseline and NT (N=3).



Supplemental Figure XIX. Plasma clots do not induce inflammatory gene expression via toll-like receptor-4 (TLR4). Monocytes were pretreated with the TLR4 inhibitor CLI-095 (1 μ M) and then embedded in plasma clots or stimulated with LPS (100 ng/mL). IL-8 (left panel) and MCP-1 (right panel) protein levels were assessed after 18 hours. The bars in this figure represent the mean \pm SEM of 3 independent experiments. The asterisk indicates $p > 0.05$ in untreated clot or LPS-treated samples versus the TLR4 inhibitor.



Supplemental Figure XX. Inhibition of monocyte CD11b does reduce IL-8 or MCP-1 synthesis in response to plasma clot stimulation. Monocytes were pretreated with 5 $\mu\text{g/mL}$ anti-CD11b antibody (red) or isotype control (blue) before the addition of 10 μg total Alexa-fluor 555 labeled fibrinogen. Fibrinogen binding was assessed by flow cytometry (**A**). Monocytes were left alone or pretreated with 5 $\mu\text{g/mL}$ anti-CD11b antibody or isotype control before cells were added to plasma clots (**B**). After 18 hours, supernatants were harvested and IL-8 and MCP-1 were measured ($n>3$).