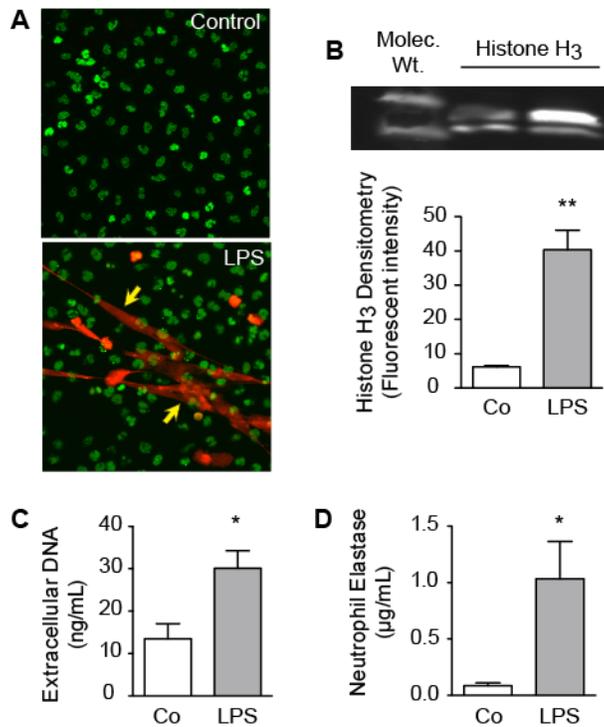
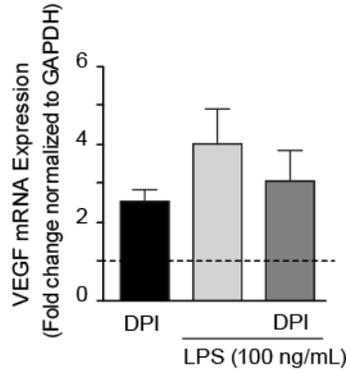


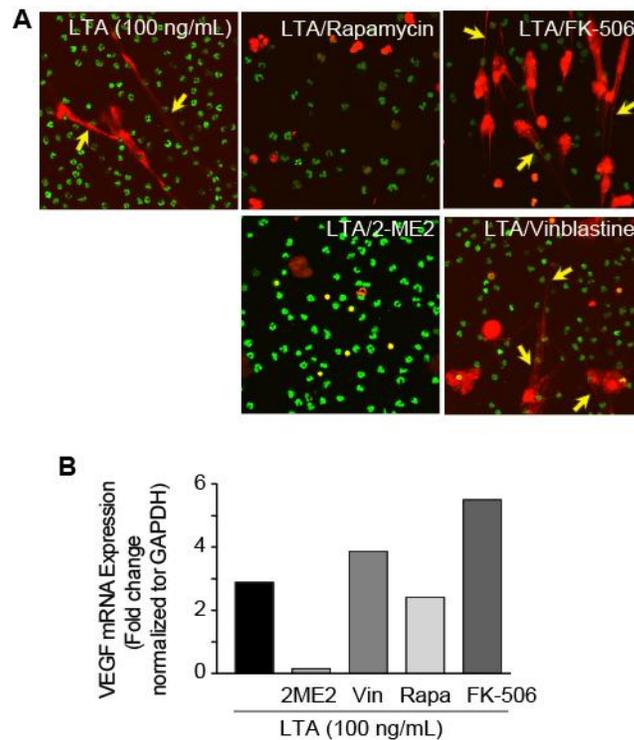
Supplemental Figure 1. Differentiated surrogate PMNs form NETs following LPS stimulation. NET formation was assessed by live cell imaging with confocal microscopic examination of surrogate PMNs. Undifferentiated (top images) and differentiated (bottom images) HL-60 cells were assessed for NET formation following stimulation with LPS (100 ng/mL) for 1 hour. Extracellular and intracellular DNA were detected using a combination of cell permeable (nuclear DNA; green) and cell impermeable (extracellular DNA; grayscale) DNA dyes. Yellow arrows highlight areas of NET formation.



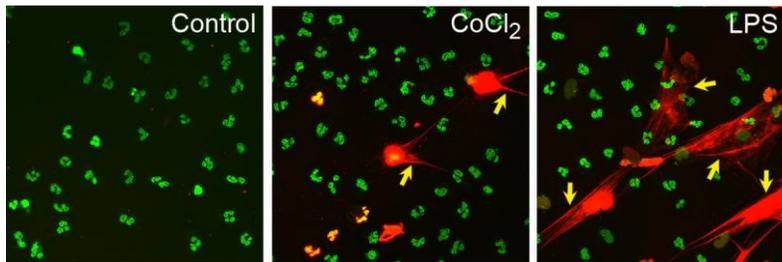
Supplemental Figure 2. NET quantification techniques. We compared two different surrogate markers of NET formation used in the literature with live cell imaging and with Histone H3 quantification in PMNs assayed in parallel. We employed Student's t-test to compare control and LPS-stimulated PMNs in each surrogate assay of NET formation. For all three surrogate measures of NET formation, the * or ** indicates a significant difference ($p < 0.05$ or $p < 0.01$) between samples from control PMNs and LPS-stimulated PMNs. A. NET formation was assessed by live cell imaging with confocal microscopy. Extracellular and intracellular DNA were detected using a combination of cell permeable (green fluorescence) and cell impermeable (red fluorescence) DNA dyes. Yellow arrows highlight areas of NET formation. Human PMNs were stimulated with LPS (100 ng/mL) for one hour. These images are representative of visual fields selected from randomly captured fields from assays performed with PMNs from 3 different adult donors. B. We quantified extracellular histone H3 content in DNase-treated supernatants by western blotting as a surrogate for NET formation by PMNs. PMNs were stimulated as above. The image is representative of assays performed using PMNs isolated from 3 different healthy adult donors with densitometry analysis ($n=3$) indicated in the graph below. Columns represent mean supernatant histone H3-associated fluorescence \pm SEM. C. Supernatant DNA content was determined using the 260 nm wavelength absorbance method for samples from control and LPS-stimulated PMNs isolated from 3 separate healthy adult donors. The data are expressed as mean \pm SEM values for extracellular DNA. D. NET-associated neutrophil elastase activity was determined in DNase-treated incubations of control and LPS-stimulated PMNs isolated from 5 separate healthy adult donors. Mean \pm SEM neutrophil elastase concentrations are shown.



Supplemental Figure 3. *Inhibition of ROS generation fails to alter HIF-1 induction of VEGF transcription.* Human PMNs were stimulated with LPS (100 ng/mL) for one hour \pm pretreatment for 1 hour with DPI to inhibit endogenous ROS generation. *VEGF* transcript expression was assessed via realtime RT-PCR. The columns represent the mean fold change over baseline \pm SEM. Control *VEGF* transcript expression was arbitrarily set at 1 (dashed line). We employed the single tailed Student's t test for statistical analysis. No significant differences were found. Three separate experiments were performed in PMNs isolated from 3 different healthy adult donors.



Supplemental Figure 4. *mTOR* and *HIF-1 α* regulate NET formation by lipoteichoic acid (LTA)-stimulated human PMNs. A. Human PMNs were treated with LTA, a cell wall component of gram + bacteria (100 ng/mL), for one hour \pm a one hour pretreatment with rapamycin (200 nM) or 2-ME2 (2 μ M). Pretreatment with FK-506 (200 nM) or Vinblastine (10 μ M) served as controls for rapamycin and 2-ME2, respectively. NET formation was assessed by live cell imaging with confocal microscopy (60x magnification). Extracellular and intracellular DNA were detected using a combination of cell permeable (green fluorescence) and cell impermeable DNA dyes (red fluorescence). Yellow arrows highlight areas of NET formation. These images are representative of visual fields selected from randomly captured fields from assays performed with PMNs from 3 different adult donors. B. We assessed mRNA expression of *VEGF*, an mRNA transcript with expression dependent on HIF-1 transcription factor activity, in human PMNs using realtime RT-PCR. Human PMNs were stimulated with LTA (100 ng/mL) for 2 hours following a 1 hour pretreatment \pm 2-ME2 (2 μ M) or rapamycin (200 nM), to inhibit HIF-1 α or mTOR activity, respectively. Pretreatment with Vinblastine (10 μ M) or FK-506 (200 nM) served as respective controls. This experiment was performed using PMNs isolated from a single healthy adult donor. The columns represent the mean fold change in mRNA expression over baseline normalized to GAPDH \pm SEM. Control mRNA expression was arbitrarily set at 1 (dashed line).



Supplemental Figure 5. *HIF-1 α* stabilization via prolyl hydroxylase inhibition induces NET formation by human PMNs. Human PMNs were treated with CoCl₂, a hypoxia mimetic known to inhibit prolyl hydroxylase activity (200 μ M) or LPS (100 ng/mL) for one hour. NET formation was assessed by live cell imaging with confocal microscopy (60x magnification). Extracellular and intracellular DNA were detected using a combination of cell permeable (green fluorescence) and cell impermeable DNA dyes (red fluorescence). Yellow arrows highlight areas of NET formation. These images are representative of visual fields selected from randomly captured fields from assays performed with PMNs from 2 different adult donors.