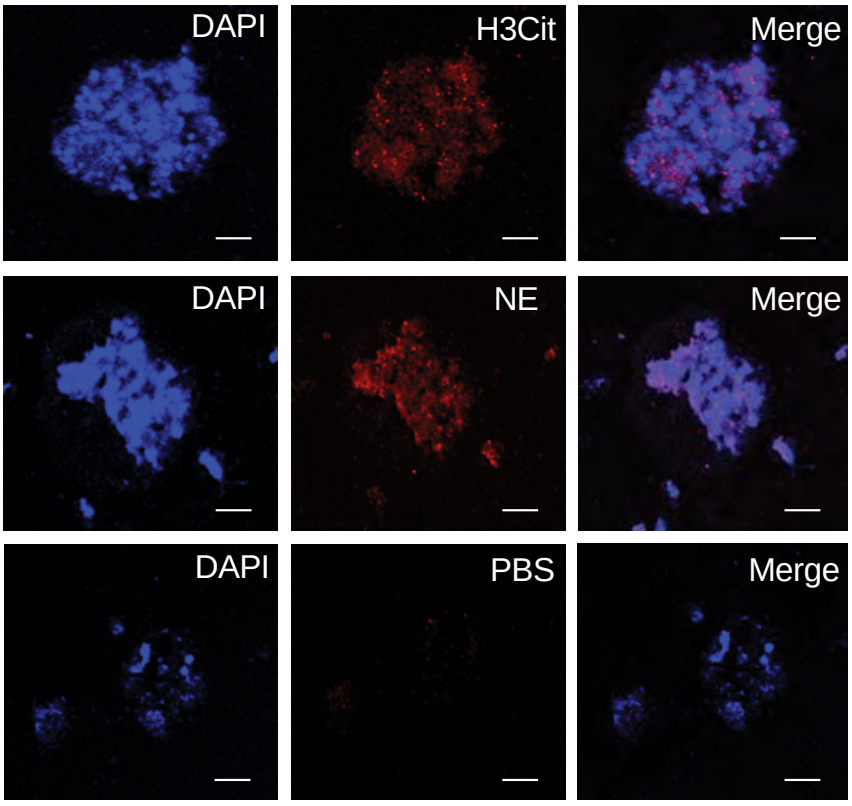
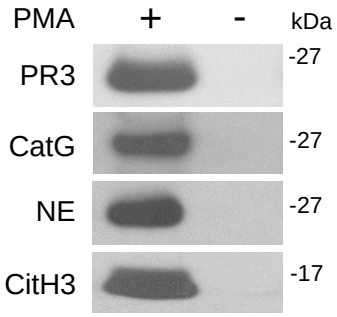
# SUPPLEMENTAL MATERIAL

## Supplemental Figure I

A)

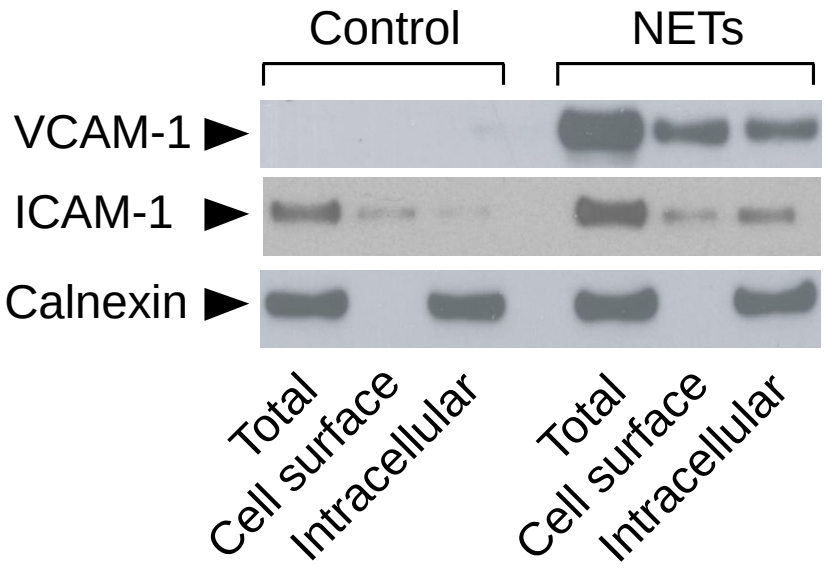



B)



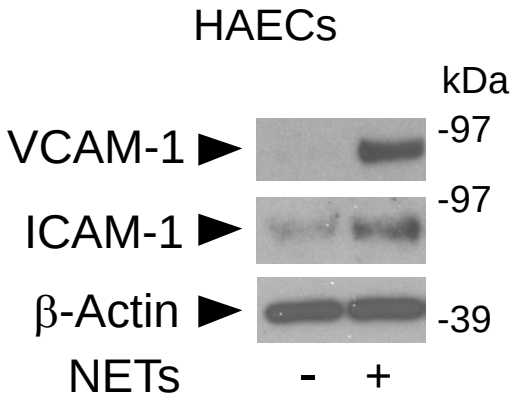

**Supplemental Figure I. Characterization of NETs. (A)** NETs were fixed and stained with anti-H3Cit or anti-NE and DAPI as described in the Materials and Methods section. Omission of the primary antibody was used as a negative control (PBS, bottom panels). Scale bar = 50 μm. **(B)** NETs were fractionated by SDS-PAGE and immunoblotted with antibodies to PR3, CatG, NE, and CitH3.

## Supplemental Figure II



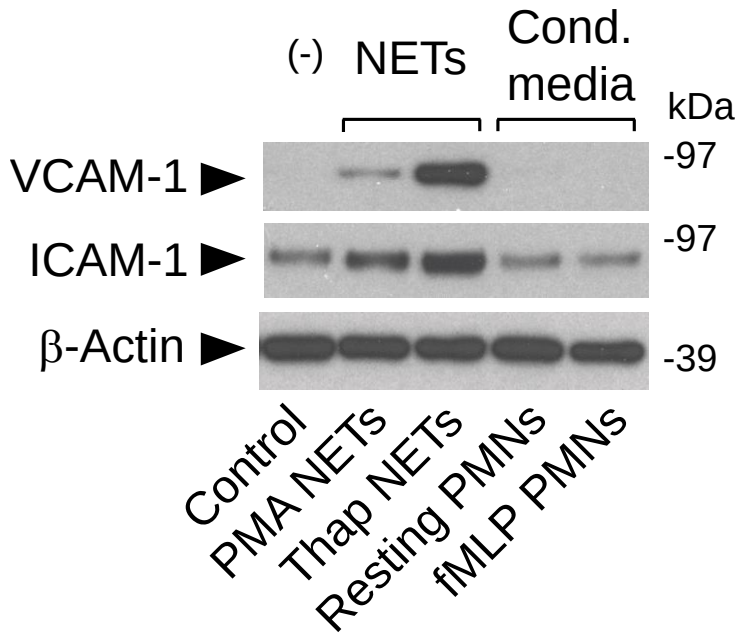

**Supplemental Figure II. NETs increase the expression of adhesion molecules on the HSVEC surface.** HSVECs were incubated with NETs (0.5 μg DNA/ml) for 6 hours, followed by biotinylation of cell surface glycoproteins. Biotinylated proteins were precipitated with streptavidin-agarose, fractionated by SDS-PAGE, and immunoblotted with antibodies to VCAM-1, ICAM-1, or calnexin, an endoplasmic-reticulum-resident protein.

## Supplemental Figure III



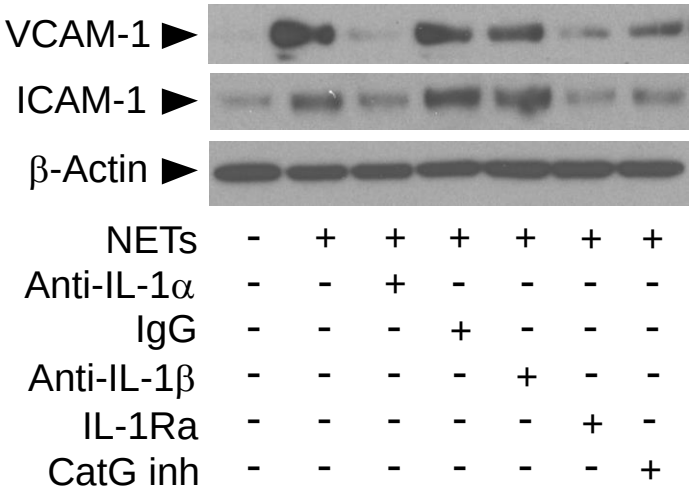

**Supplemental Figure III. NETs increase the expression of leukocyte adhesion molecules in HAECs.** HAECs were incubated with NETs (0.5 μg DNA/ml) for 6 hours. Whole-cell extracts were fractionated by SDS-PAGE and immunoblotted with antibodies to VCAM-1, ICAM-1, or β-actin.

## Supplemental Figure IV




**Supplemental Figure IV. Thapsigargin-induced NETs but not conditioned media from fMLP-activated neutrophils increase the expression of leukocyte adhesion molecules in HSVECs.** HSVECs were incubated for 6 hours with thapsigargin-induced or PMA-induced NETs (0.5 μg DNA/ml), or with media conditioned by neutrophils that had been treated with 1 μM fMLP or left unstimulated for 20 minutes. Whole-cell extracts were fractionated by SDS-PAGE and immunoblotted with antibodies to VCAM-1, ICAM-1, or β-actin. Thap NETs, thapsigargin-induced NETs; PMA NETs, PMA-induced NETs; PMNs, polymorphonuclear leukocytes; cond., conditioned.

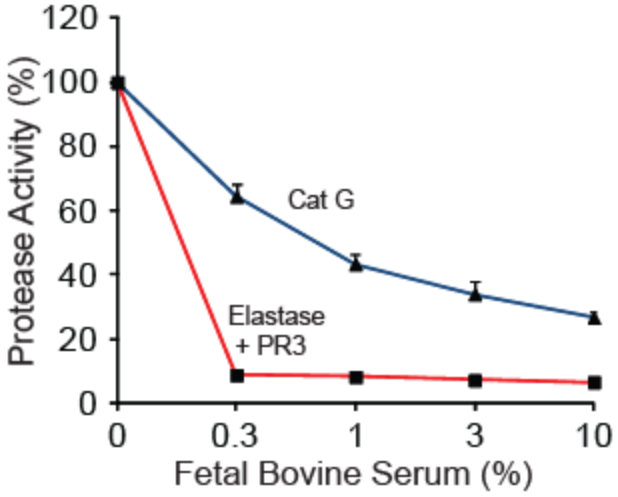
## Supplemental Figure V



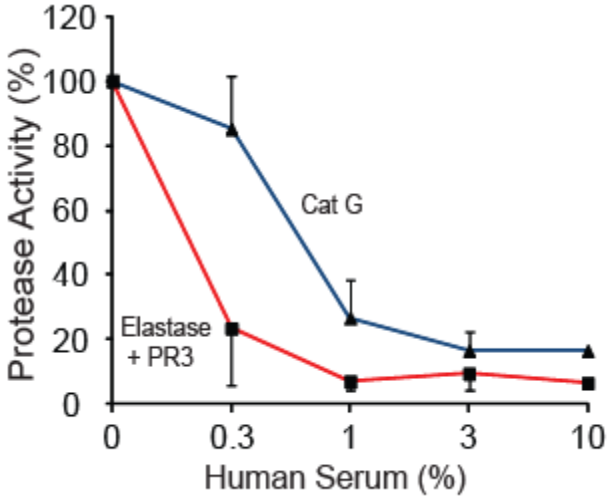

**Supplemental Figure V. IL-1α and CatG mediate NET-induced expression of leukocyte adhesion molecules in HUVECs.** Cells were incubated with NETs (0.5 μg DNA/ml) for 6 hours in the presence of the indicated antibodies (20 ng/ml), IL-1Ra (1 μg/ml), or CatG inhibitor I (50 μM), followed by fractionation of whole-cell extracts by SDS-PAGE and immunoblotting with antibodies to VCAM-1, ICAM-1, or β-actin.

## Supplemental Figure VI

### A)

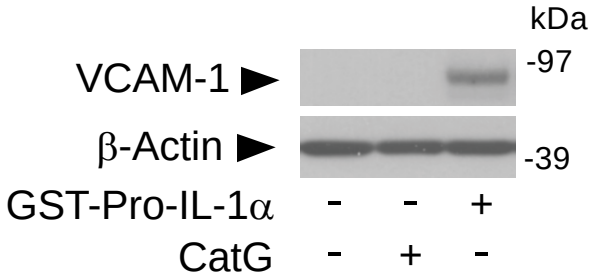



### B)



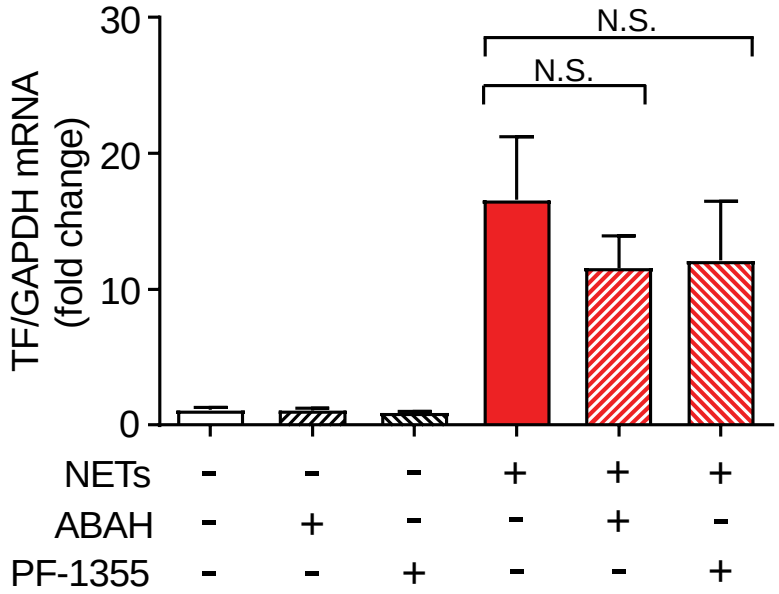

**Supplemental Figure VI. Serum inhibits NET serine proteases differentially.** CatG and the combined elastase + PR3 activities of NETs were determined as described in Materials and Methods in the presence of various concentrations of fetal bovine serum (A) or human serum (B). Activity is expressed as % of activity in the absence of serum, defined as 100%. N = 3.

## Supplemental Figure VII




**Supplemental Figure VII. CatG alone does not induce VCAM-1 expression in HSVECs.** Cells were incubated with CatG (0.032 units/ml) or GST-Pro-IL-1α (3.6 μg/ml) for 6 hours, followed by fractionation of whole-cell extracts by SDS-PAGE and immunoblotting with antibodies to VCAM-1 or β-actin.

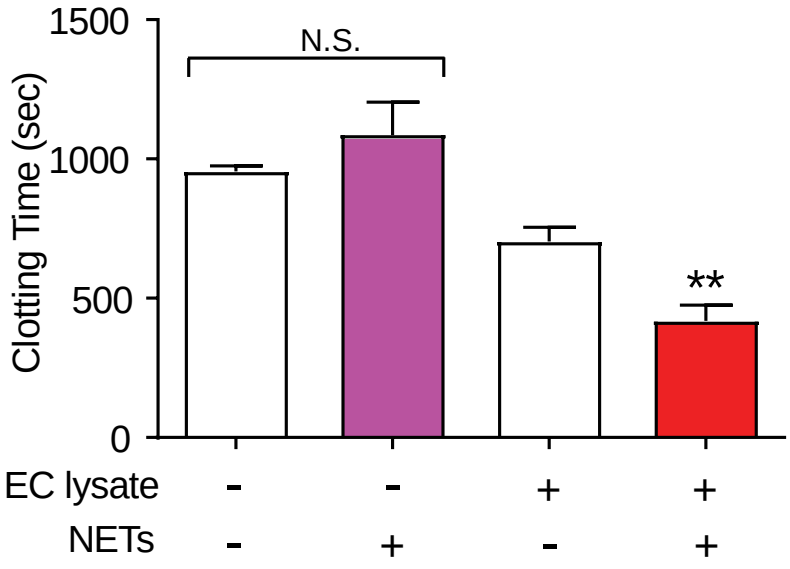
## Supplemental Figure VIII



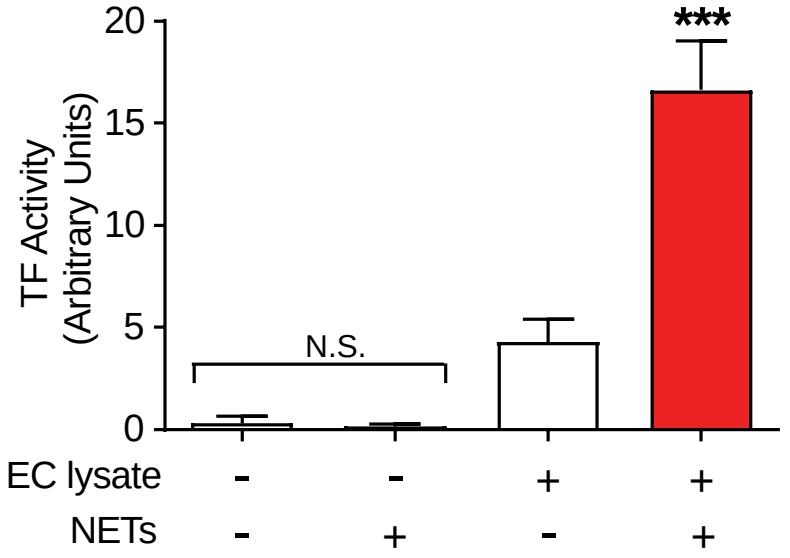

**Supplemental Figure VIII. MPO inhibition does not abrogate NET-induced TF expression in HSVECs.** Cells were incubated with NETs (0.5 μg DNA/ml) for 3 hours in the presence or absence of 10 μM MPO inhibitors, followed by RNA extraction and determination of TF mRNA levels by RT-qPCR. Levels of GAPDH mRNA served as an internal control for adjustment between samples. N = 3. N.S., not significant.

## Supplemental Figure IX

**A)**




**B)**




**Supplemental Figure IX. NETs alone do not exhibit TF pro-coagulant activity or accelerate clotting in cell-free assays.** Plasma clotting time (A, n = 8) and TF activity (B, n = 5-7) of NETs alone and of control or NET-treated HSVEC lysates were determined as described in the Materials and Methods section. P values: **<0.01, ***<0.001 vs. the respective controls.