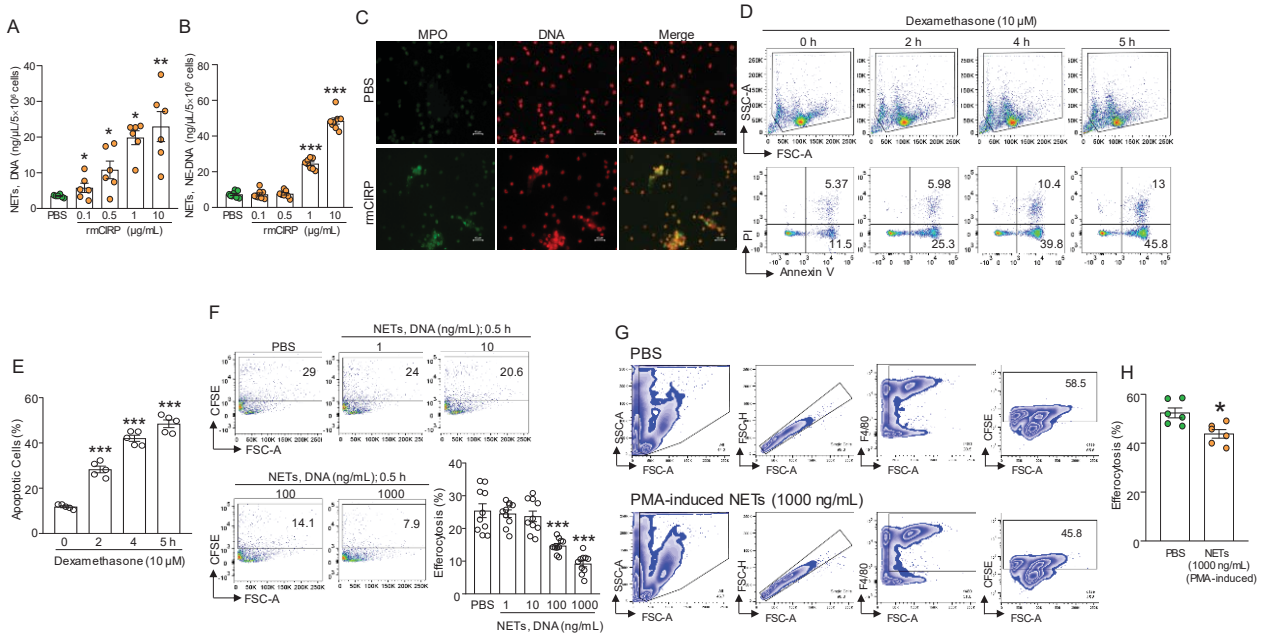
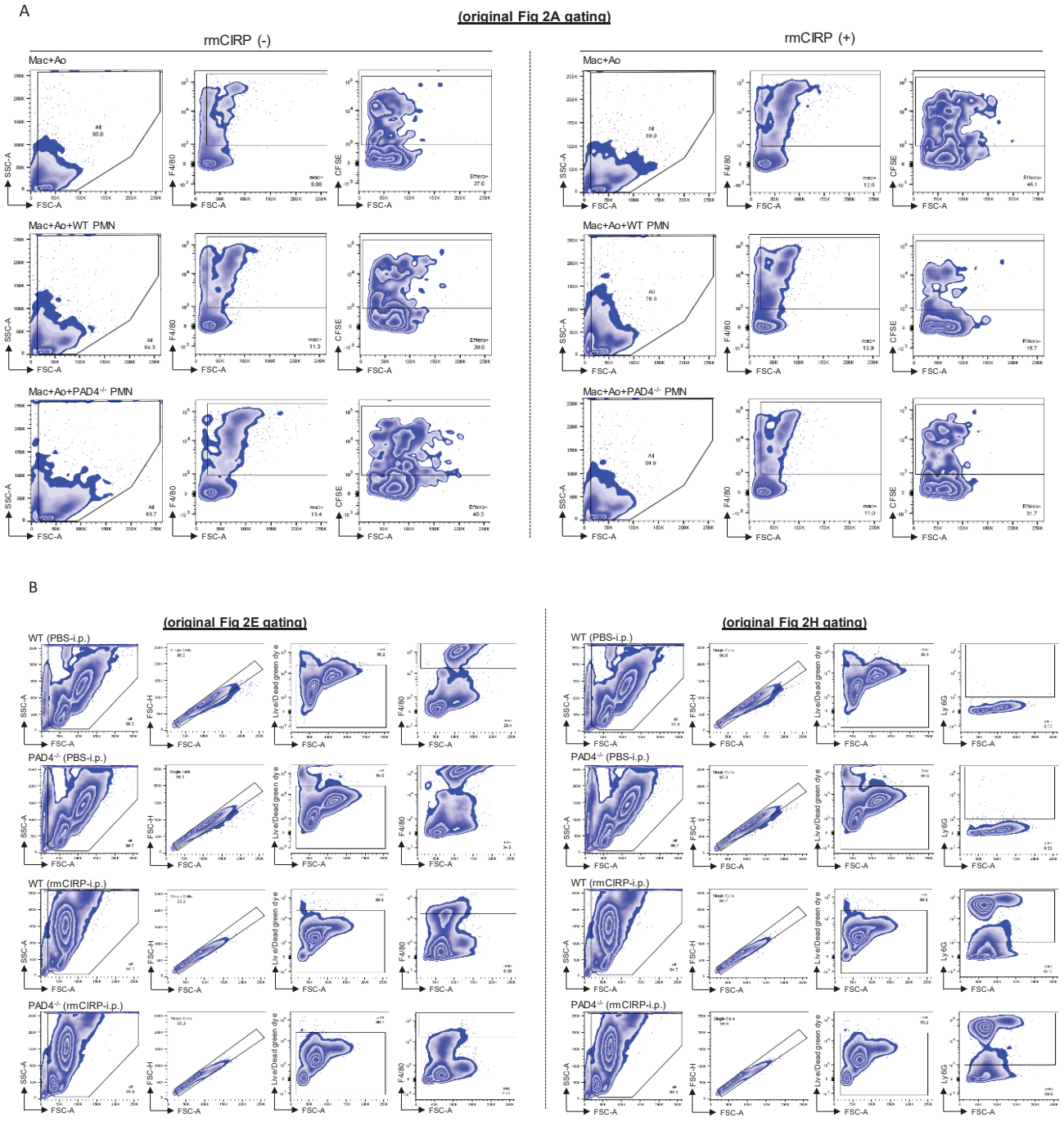
Supplemental Figure 1

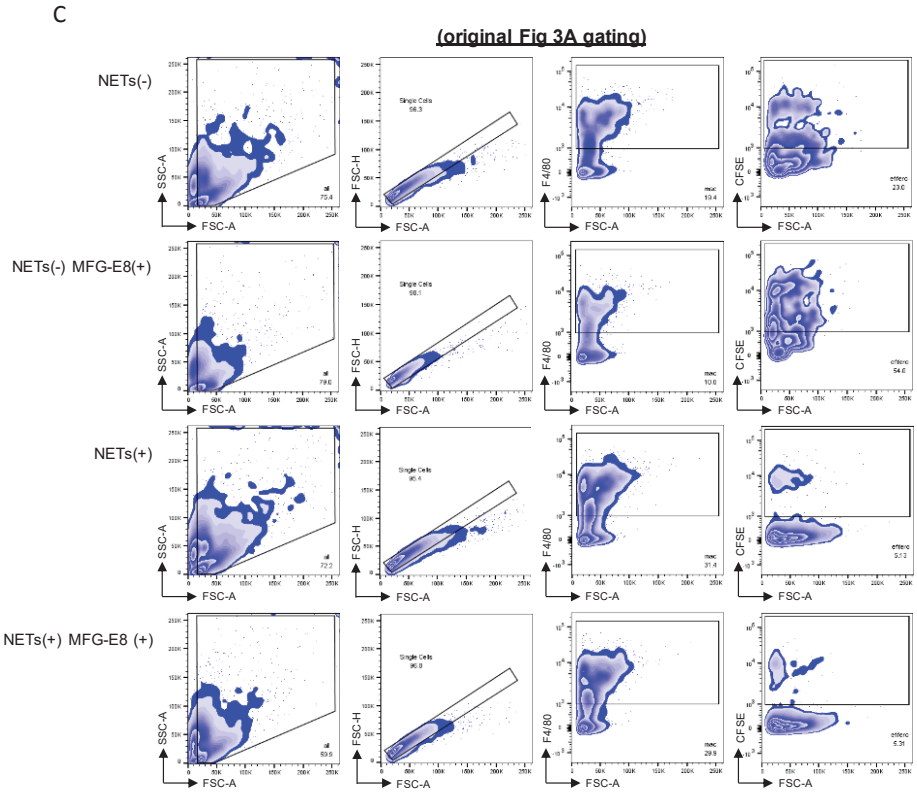



**Supplemental Figure 1: (A-C) eCIRP induces NET formation.** A total of 1 × $10^6$ BMDN were stimulated with various doses of rmCIRP for 4 h. **(A)** NETs were extracted and DNA concentrations in NETs were expressed as ng/μL/5×$10^6$ cells. **(B)** Extracted NETs were loaded in anti-NE-coated plates and reacted with anti-DNA-POD Abs. NETs contents in terms of the presence of NE-DNA complex were assessed by ELISA. Data were obtained from 3 independent experiments and expressed as means ± SE (n=6-8 samples/group). The groups were compared by one-way ANOVA and SNK method. *p<0.05, **p<0.01, ***p<0.001 vs. PBS group. **(C)** NETs assay by microscope. A total of 1 × $10^6$ BMDN were plated in 24-well plate and stimulated with PBS or rmCIRP (1 μg/mL). After 4 h of incubation, cells adhered into the plate were fixed with 2% PFA and stained with FITC-labeled anti-MPO Ab (green) and sytox orange (red) and viewed under fluorescent microscope at ×400 original magnification. Scale bar: 40 μm. **(D, E) Induction of thymocyte apoptosis.** A total of 1 × $10^7$ thymocytes were plated in 10-cm petri dish and stimulated with dexamethasone (10 μM) for various time-points. Apoptosis was determined by flow cytometry. At 5 h time point, 45.8% of the thymocytes were early apoptotic (Annexin $V^+$ $PI^-$), around 13% of the cells were late apoptotic (Annexin $V^+$ $PI^+$) giving rise to about 58.8% total apoptotic cells. Data were obtained from 3 independent experiments and expressed as means ± SE (n=5 samples/group). The groups were compared by one-way ANOVA and SNK method. ***p<0.001 vs. 0 h control. PI, propidium iodide. **(F) eCIRP-induced NETs inhibit efferocytosis at 0.5 h of co-culture.** A total of 5 × $10^5$ peritoneal macrophages were cultured with 1.5 × $10^6$ CFSE-labeled apoptotic cells in presence with PBS or various concentrations of NETs. After 0.5 h of incubation, cells were washed, fixed with 2% PFA and efferocytosis was assessed by using flow cytometry. Efferocytosis was determined as the percentage of CFSE-positive cells present in $F4/80^+$ macrophages. Data were obtained from 3 independent experiments and expressed as means ± SE (n=10 samples/group). The groups were compared by one-way ANOVA and SNK method (***p<0.001 vs. PBS-treated group). **(G, H) PMA-induced NETs inhibit efferocytosis. (G, H)** A total of 5 × $10^5$ peritoneal macrophages were cultured with 1.5 × $10^6$ CFSE-labeled apoptotic cells in presence with PBS or PMA (50 nM)-induced NETs (1000 ng/mL). After 1 h of incubation, cells were washed, fixed with 2% PFA, and efferocytosis was assessed by flow cytometry. Efferocytosis was determined as the percentage of CFSE-positive cells present in $F4/80^+$ macrophages. Data were expressed as means ± SE (n=6 samples/group). The groups were compared by Student's t-test (*p<0.05 vs. PBS-treated group). PMA, phorbol 12-myristate 13-acetate.

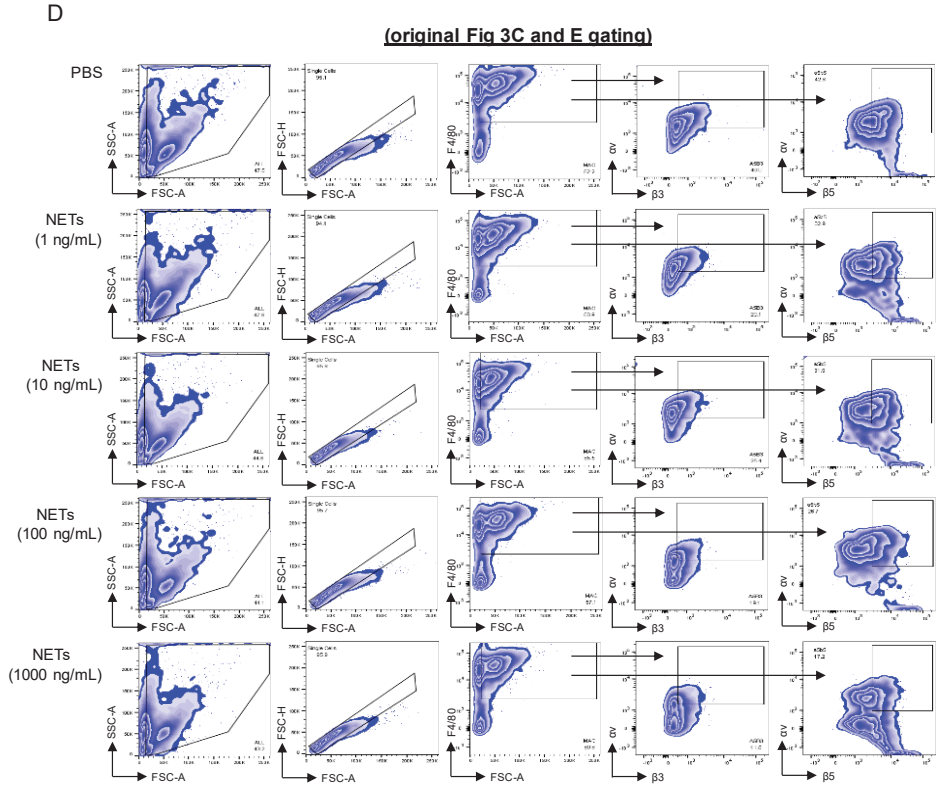
Supplemental Figure 2 Gating Strategies of Original Representative Figures



C


(original Fig 3A gating)
NETs(-)
NETs(-) MFG-E8(+)
NETs(+)
NETs(+) MFG-E8 (+)
SSC-A
FSC-A
FSC-H
F4/80
CFSE

D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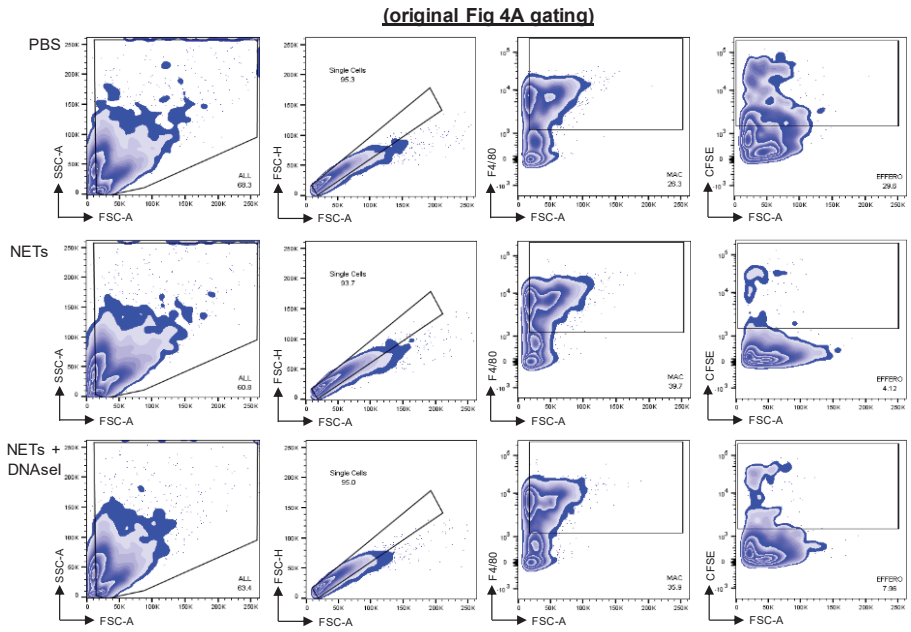
PBS
NETs (1 ng/mL)
NETs (10 ng/mL)
NETs (100 ng/mL)
NETs (1000 ng/mL)
SSC-A
FSC-A
FSC-H
F4/80
CV
β3
β5

E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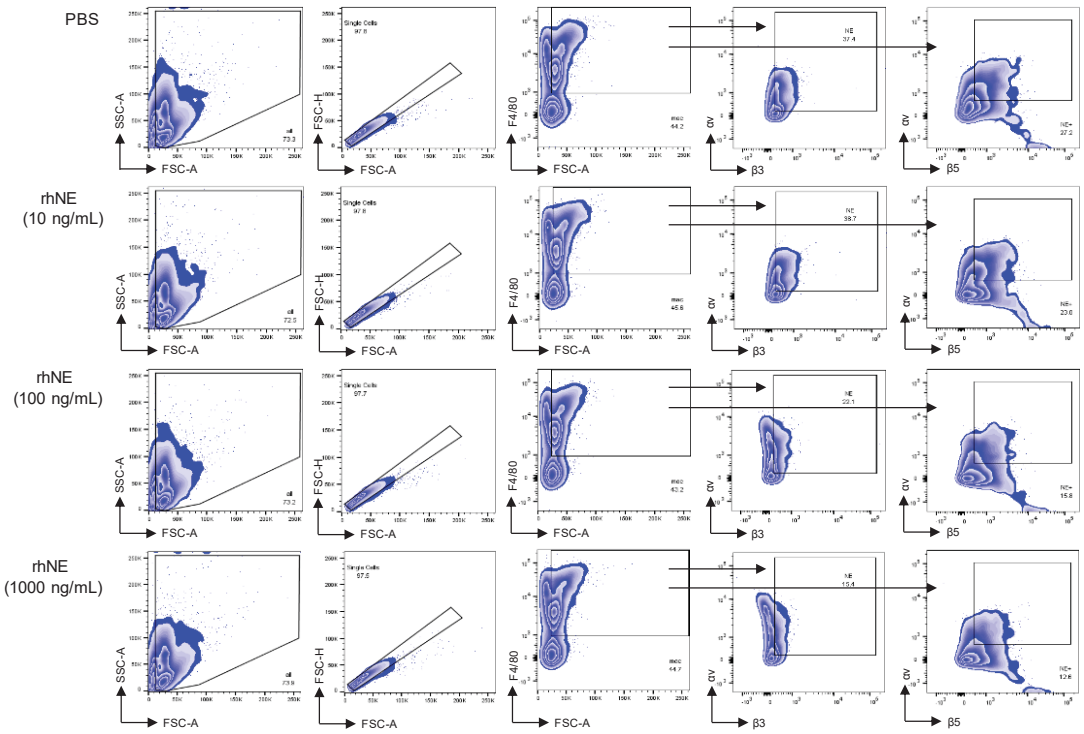
PBS
NETs
NETs + DNAseI
SSC-A
FSC-A
FSC-H
Single Cells
F4/80
MAC
CFSE
EFFERO

F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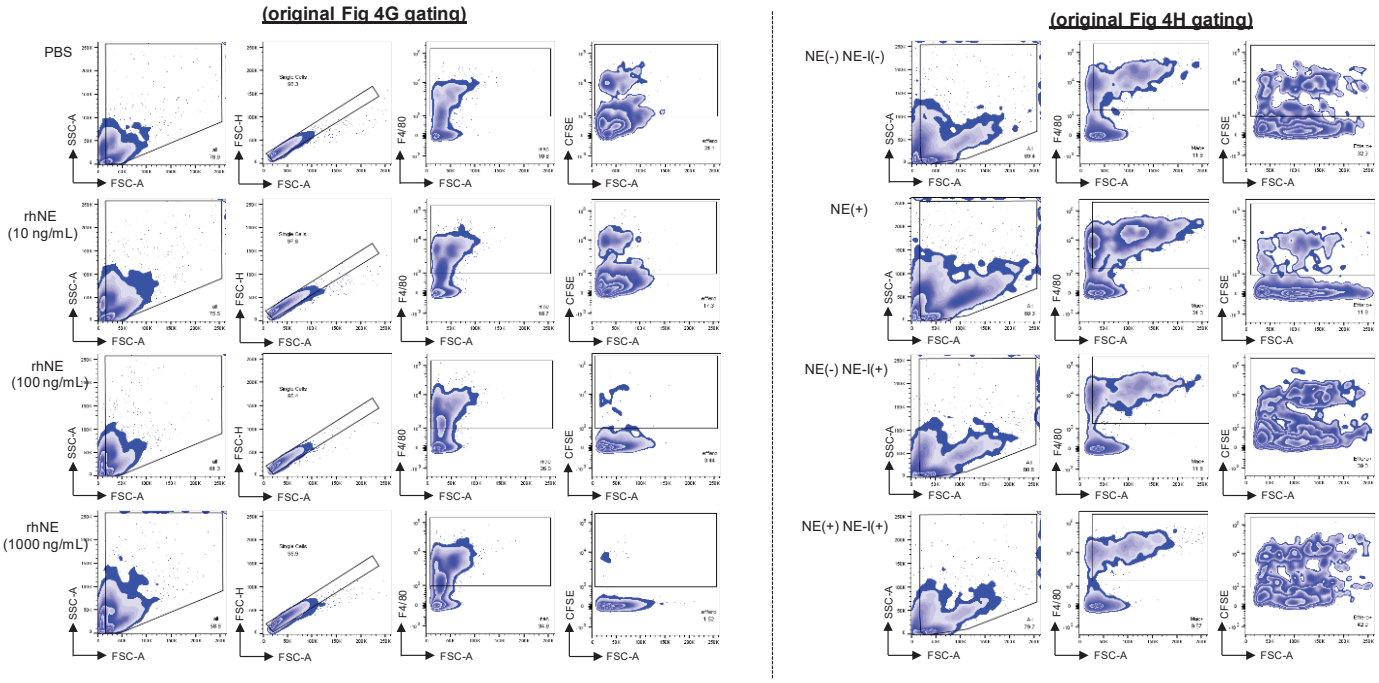
PBS
rhNE (10 ng/mL)
rhNE (100 ng/mL)
rhNE (1000 ng/mL)
SSC-A
FSC-A
FSC-H
Single Cells
F4/80
MAC
CD
β3
β5
NE

G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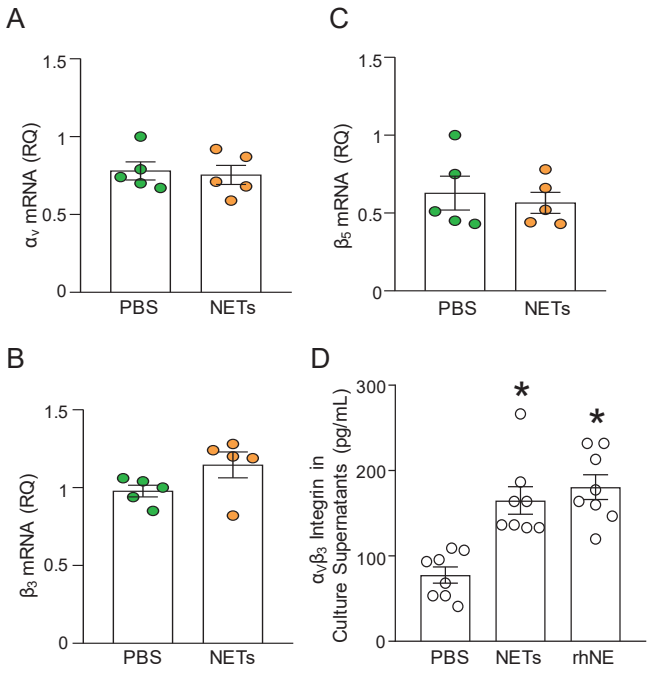
PBS
rhNE (10 ng/mL)
rhNE (100 ng/mL)
rhNE (1000 ng/mL)
SSC-A
FSC-A
FSC-H
F4/80
CFSE
(original Fig 4H gating)
NE(-) NE-I(-)
NE(+)
NE(-) NE-I(+)
NE(+) NE-I(+)

Supplemental Figure 3




**Supplemental Figure 3: Treatment of macrophages with NETs or rhNE do not alter integrins expression at mRNA levels. (A-C)** A total of $5 \times 10^5$ peritoneal macrophages from each mouse were stimulated with NETs (1000 ng/mL) or rhNE (1000 ng/mL) for 1 h. After stimulation of the cells with NETs or rhNE, mRNA was isolated and assessed the expression of integrins $\alpha_v$, $\beta_3$, $\beta_5$ using RTqPCR. Primers used in the PCR were shown in **Supplemental Table-1**. Amplification and analysis was conducted in a Step One Plus real-time PCR machine (Applied Biosystems). Mouse β-actin mRNA was used as an internal control for amplification and relative gene expression levels were calculated using the ΔΔCT method. Relative quantity (RQ) of mRNA was expressed. Data were expressed as means ± SE (n=5 mice/group, where each dot represents macrophages of one mouse). The groups were compared by one-way ANOVA and SNK method. p values between the groups were non-significant. **(D) Treatment of macrophages with NETs or rhNE increases soluble $\alpha_v\beta_3$ integrin.** Macrophages ($5 \times 10^5$) were treated with NETs (1000 ng/mL) and rhNE (1000 ng/mL) for 20 h. Soluble $\alpha_v\beta_3$ integrin in the culture supernatant were determined by ELISA (MBS9300860, MyBioSource, San Diego, CA). Data were obtained from 3 independent experiments and expressed as means ± SE (n=8 samples/group). The groups were compared by one-way ANOVA and SNK method (*$p<0.05$ vs. PBS-treated group).

**Supplemental Table 1: Primer sequences for RTqPCR.**

| | |
|---|---|
| **Mouse $\alpha_v$-Integrin (NM_008402)** | |
| Mu-αv-Integrin-F: | 5'-ATTCGCCGTGGACTTCTTC-3' |
| Mu-αv-Integrin-R: | 5'-GACCTCACAGAGGCTCCAAA-3' |
| **Mouse $\beta_3$-Integrin (NM_016780)** | |
| Mu-β3-Integrin-F: | 5'-GCTCATTGGCCTTGCTACTC-3' |
| Mu-β3-Integrin-R: | 5'-CCCGGTAGGTGATATTGGTG-3' |
| **Mouse $\beta_5$-Integrin (NM_001145884)** | |
| Mu-β5-integrin-F: | 5'-AGTGTGGGATCAGCCAGAAG-3' |
| Mu-β5-integrin-R: | 5'-GGCCTCAAGGTGAAAGACTG-3' |
| **Mouse β-actin (NM_007393)** | |
| Mu-β-actin-F: | 5'-CGTGAAAAGATGACCCAGATCA-3' |
| Mu-β-actin-R: | 5'-TGGTACGACCAGAGGCATACAG-3' |