Figure S1: FACS analysis of the expression of adhesion molecules on human neutrophils

Purified human neutrophils were stimulated with C5a (1μg/mL), IL-8 (10 μg/mL) or fMLP (1 μM) in the presence of HSA (1 μM) or IAIP (100 μg/mL) for 1 h at 37℃. The neutrophils were stained by FITC-labeled anti-human CD 11b Ab, FITC-labeled anti-human CD 18 Ab, FITC-labeled anti-human CD 162 Ab or FITC-labeled anti-human CD 62L Ab for 25 min at 4℃. After washing, the Abs were fixed with 0.5 % PFA and the fluorescence intensity of the cells was analyzed on a FACS caliber. The data were representative of three independent experiments.

Figure S2: IAIP inhibits LPS- and calcium inophore (A 23187)-induced neutrophil extracellular traps (NETs) formation

Neutrophils isolated from peripheral blood of normal volunteers were stimulated with LPS 10 ng/mL or calcium inophore (A 23187) at 1 μM or 5 μM in the presence of HBSS, HSA 1μM or IAIP 100 μg/mL for 4 h at 37℃ in 5% CO_2 atmosphere. NETs were visualized by immunofluorescence of Sytox orange and was analyzed by the In Cell Analyzer 2000. Neutrophil′s cytoplasm was stained by calcein in green and nucleus was stained with Hoechst 33342 in blue. Typical pictures of fluorescence staining from each group were shown (A-C).

Scale bars, 100 μm. Quantitative analysis of NETs count (D-F) and total area of NETs were shown in (G-I). the results are the means ± SEM of three experiments. **P< 0.01 vs. HBSS, ## P< 0.01 vs. HSA and # P<0.05 vs. HSA.

Figure S3: Effects of IAIP on spontaneous death of isolated neutrophils

Neutrophils isolated from peripheral blood of normal volunteers were treated with HSA 1μM or IAIP 100 μg/mL for 4 h at 37℃ in 5% CO_2 atmosphere. The dead cells were visualized by Sytox orange using In Cell Analyzer 2000.Sytox orange is a high affinity nucleic acid stain that can easily penetrate the compromised plasma membranes but does not pass across the intact cell membranes. Typical pictures of fluorescence staining from each group were shown (A). Scale bars, 100 μm. Quantitative analysis of dead cell counts was shown in (B). The results are the means ± SEM of three experiments. *P<0.05 vs. HBSS and # P< 0.05 vs. HSA.

Supplemental video legend

Video 1-6

Neutrophils passage through microcapillaries

The purified human neutrophils were incubated with HBSS (video 1), HSA (1 μM) (video 2), IAIP (100 and 200 μg/mL) (video 3 and 4) or bikunin (100 and 200 μg/mL) (video 5 and 6) for 30 minutes at 37°C and applied to a MC-FAN. The passage of neutrophils through was monitored by a CCD camera.

Figure S1

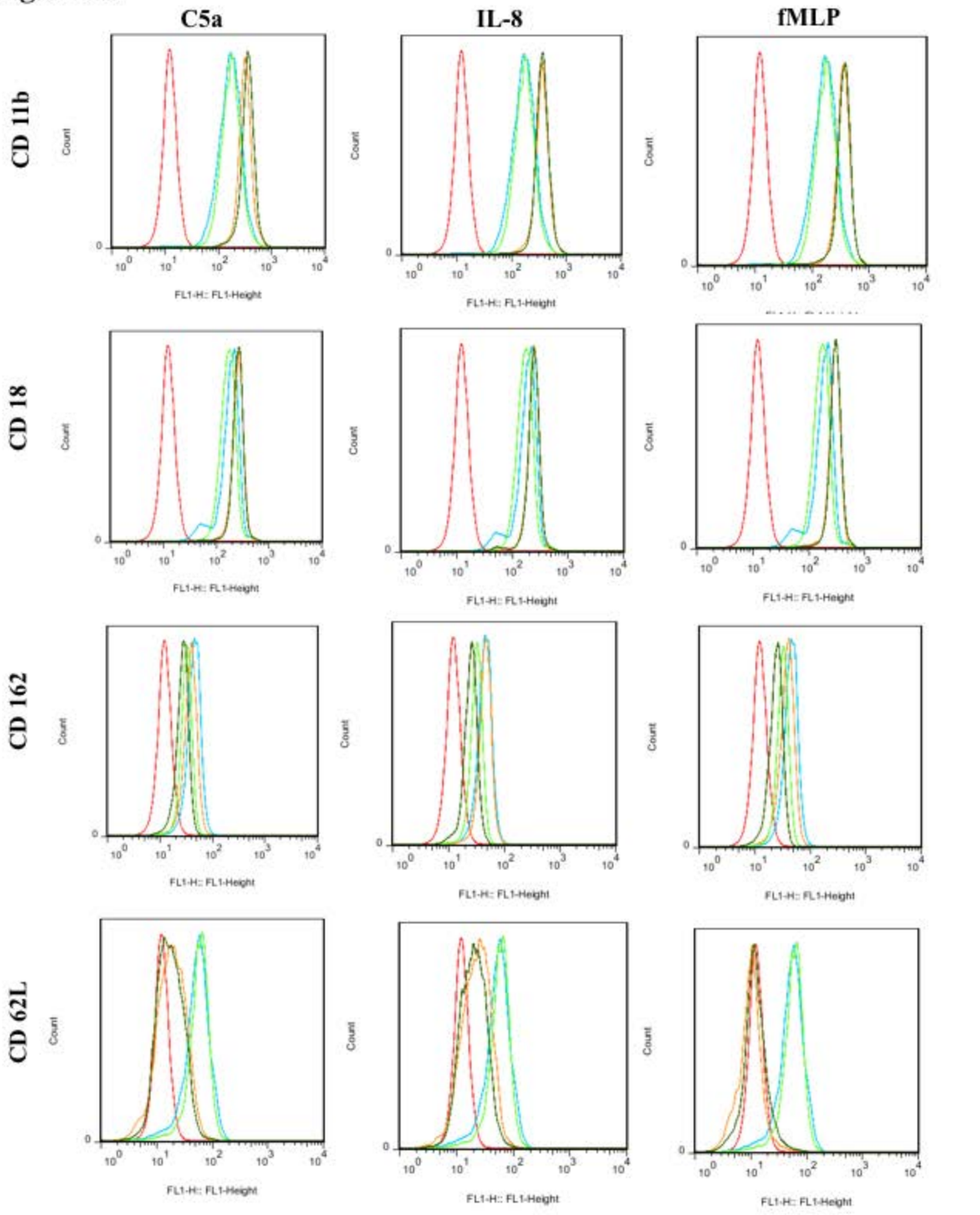
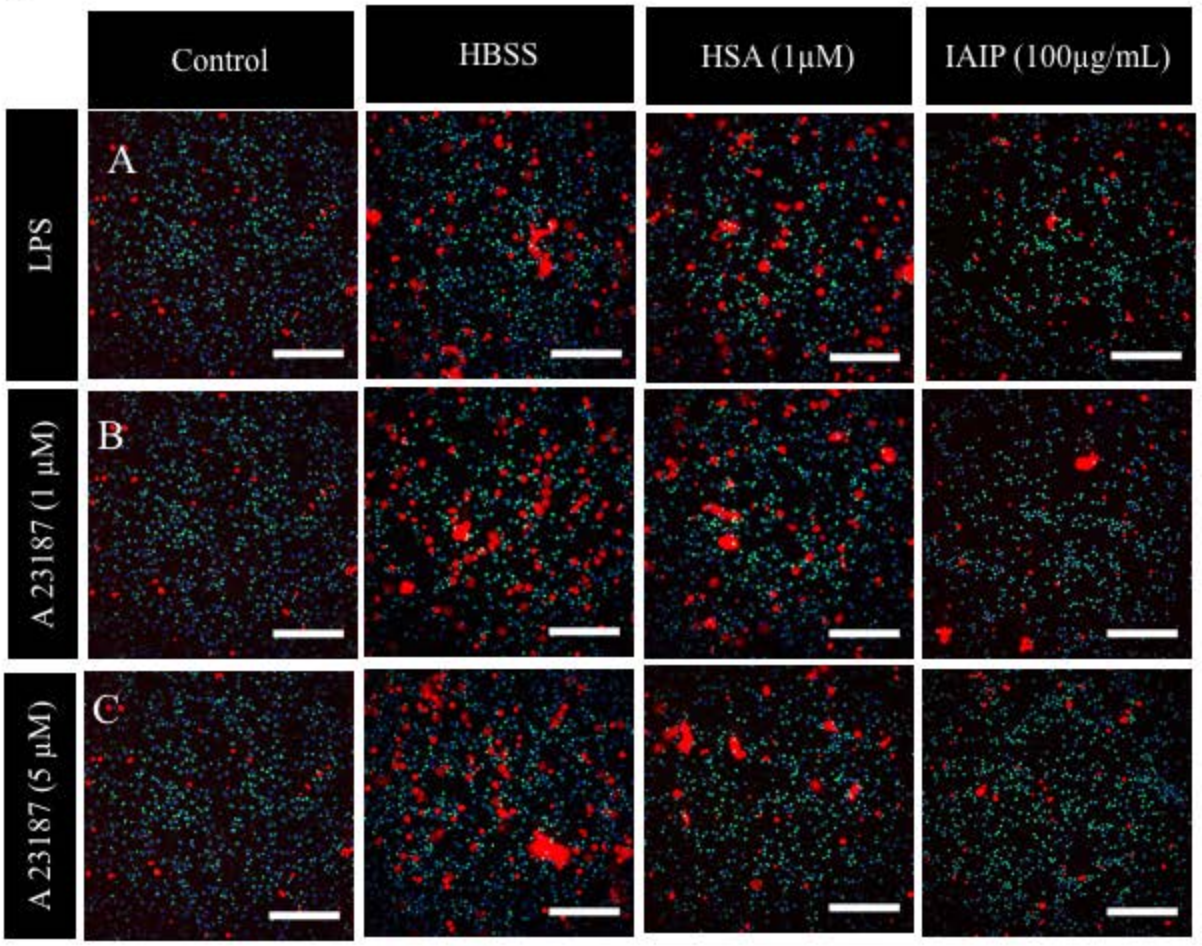

C5a
IL-8
fMLP
CD 11b
CD 18
CD 162
CD 62L
Count
FL1-H:: FL1-Height
Isotype control
C5a (0 μg/mL) + HSA 1 μM
C5a (1 μg/mL) + HSA 1 μM
C5a (0 μg/mL) + IAIP 100 μg/mL
C5a (1 μg/mL) + IAIP 100 μg/mL
Isotype control
IL-8 (0 μg/mL) + HSA 1 μM
IL-8 (10 μg/mL) + HSA 1 μM
IL-8 (0 μg/mL) + IAIP 100 μg/mL
IL-8(10 μg/mL) + IAIP 100 μg/mL
Isotype control
fMLP (0 μM) + HSA 1 μM
fMLP (1 μM) + HSA 1 μM
fMLP (0 μM) + IAIP 100 μg/mL
fMLP(1μM) + IAIP 100 μg/mL

Figure S2


Control
HBSS
HSA (1μM)
IAIP (100μg/mL)
LPS
A 23187 (1 μM)
A 23187 (5 μM)
A
B
C

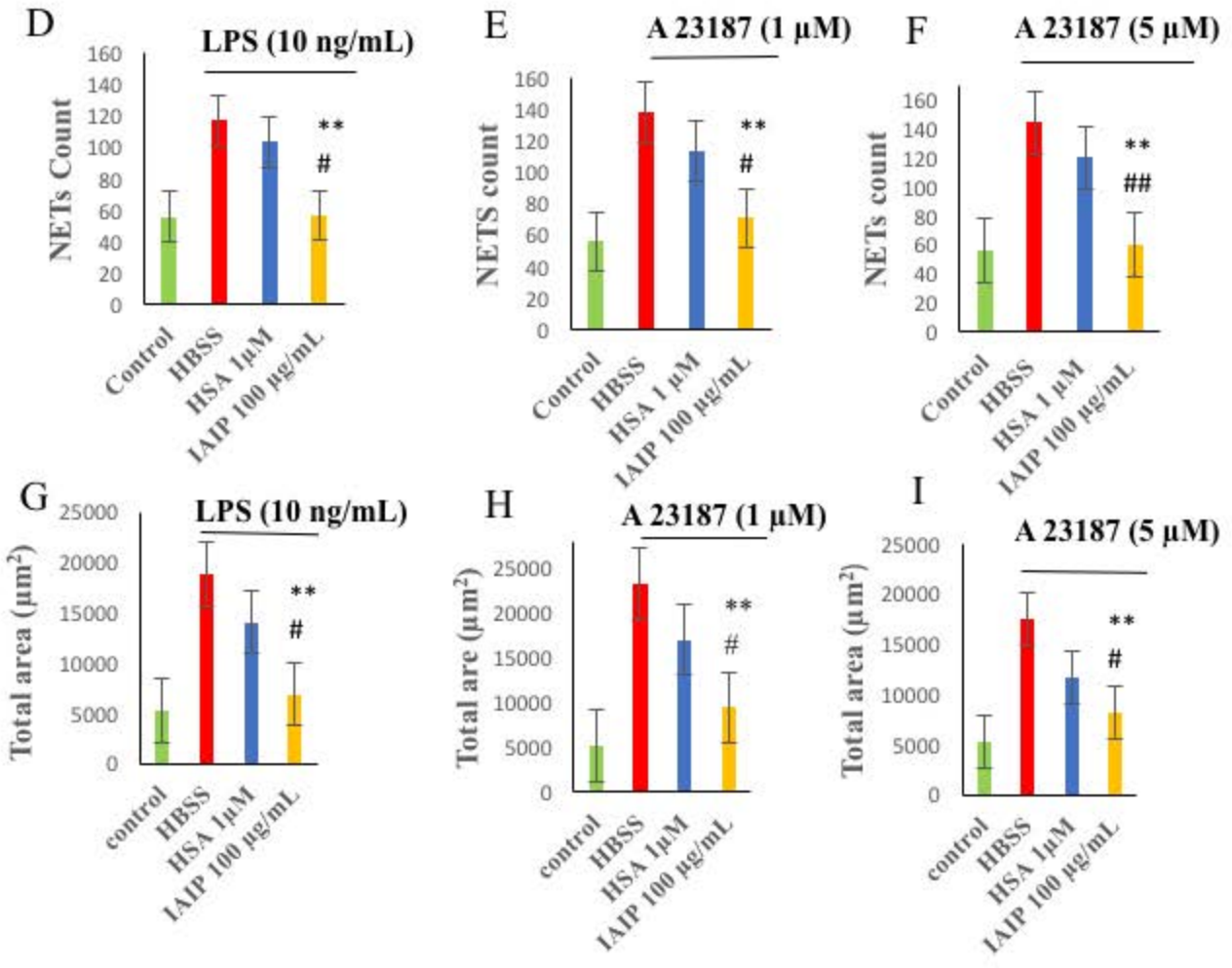
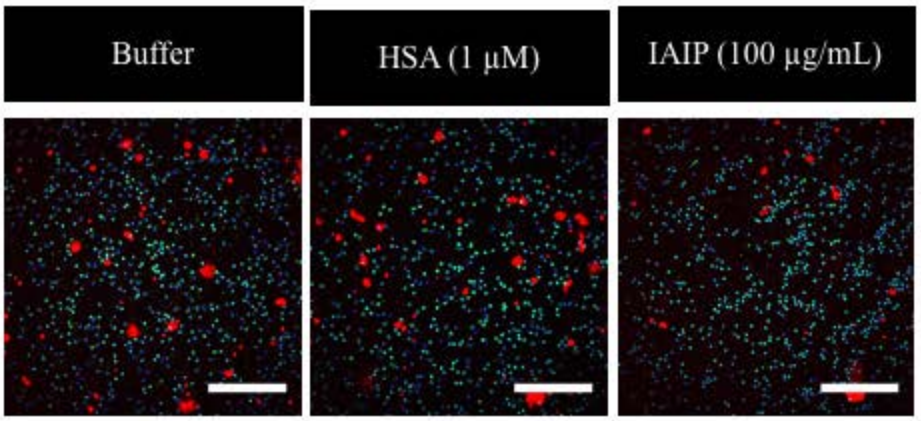
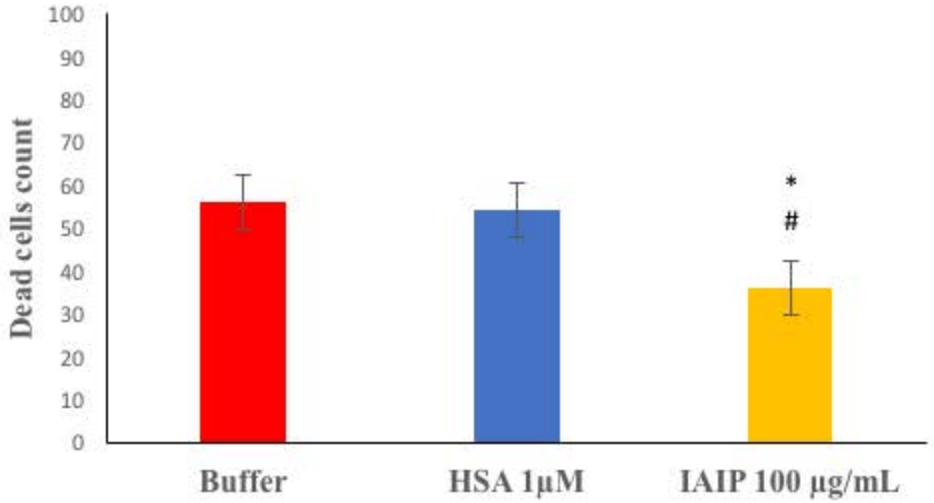

D
LPS (10 ng/mL)
NETs Count
E
A 23187 (1 μM)
NETS count
F
A 23187 (5 μM)
NETs count
G
LPS (10 ng/mL)
Total area (μm²)
H
A 23187 (1 μM)
Total are (μm²)
I
A 23187 (5 μM)
Total area (μm²)
Control
HBSS
HSA 1μM
IAIP 100 μg/mL
control
**
#
##

Figure S3

A


Buffer
HSA (1 µM)
IAIP (100 µg/mL)

B


Dead cells count
100
90
80
70
60
50
40
30
20
10
0
*
#
Buffer
HSA 1µM
IAIP 100 µg/mL