Electronic Supplementary Material (ESI) for Metallomics.

SUPPLEMENTARY MATERIAL

Table S1: Total metal content of neutrophils determined by SR-XRF. Neutrophils isolated freshly from healthy volunteers were seeded onto Si_3N_4 wafers, shock-frozen, freeze-dried and analyzed by SR-XRF (P06 beamline, PETRA III). Trace-level metal content was determined using the fundamental parameter approach and NIST SRM 613 standard for quantification. Average and standard deviation of three individual cells are displayed.

Element	Atoms per cell	Intracellular concentration (µM)
Zn	$6.94 \pm 0.86 \times 10^{7}$	317.35 ± 39.17
Fe	$9.38 \pm 1.11 \times 10^{6}$	42.91 ± 5.09
Cu	$1.38 \pm 0.18 \times 10^{7}$	63.27 ± 8.17
Mn	8.73 1.76×10^{5}	3.99 ± 0.80

Table S2: Comparison of areal concentrations in neutrophil nucleus and cytoplasm. Neutrophils isolated freshly from healthy volunteers were seeded onto Si_3N_4 wafers, shock-frozen, freeze-dried and analyzed by SR-XRF (P06 beamline, PETRA III). Trace-level metal content was determined using a NIST SRM 613 standard for quantification. The average and standard deviation of three individual cells are displayed.

Element	Nucleus (µg/cm²)	Cytoplasm (µg/cm²)
Zn	$1.04 \pm 0.03 \times 10^{-2}$	$8.24 \pm 0.08 \times 10^{-3}$
Fe	$1.51 \pm 0.15 \times 10^{-3}$	$8.88 \pm 1.00 \times 10^{-4}$
Cu	$1.88 \pm 0.04 \times 10^{-3}$	$1.63 \pm 0.01 \times 10^{-3}$
Mn	$6.99 \pm 5.44 \times 10^{-5}$	$8.87 \pm 3.91 \times 10^{-5}$

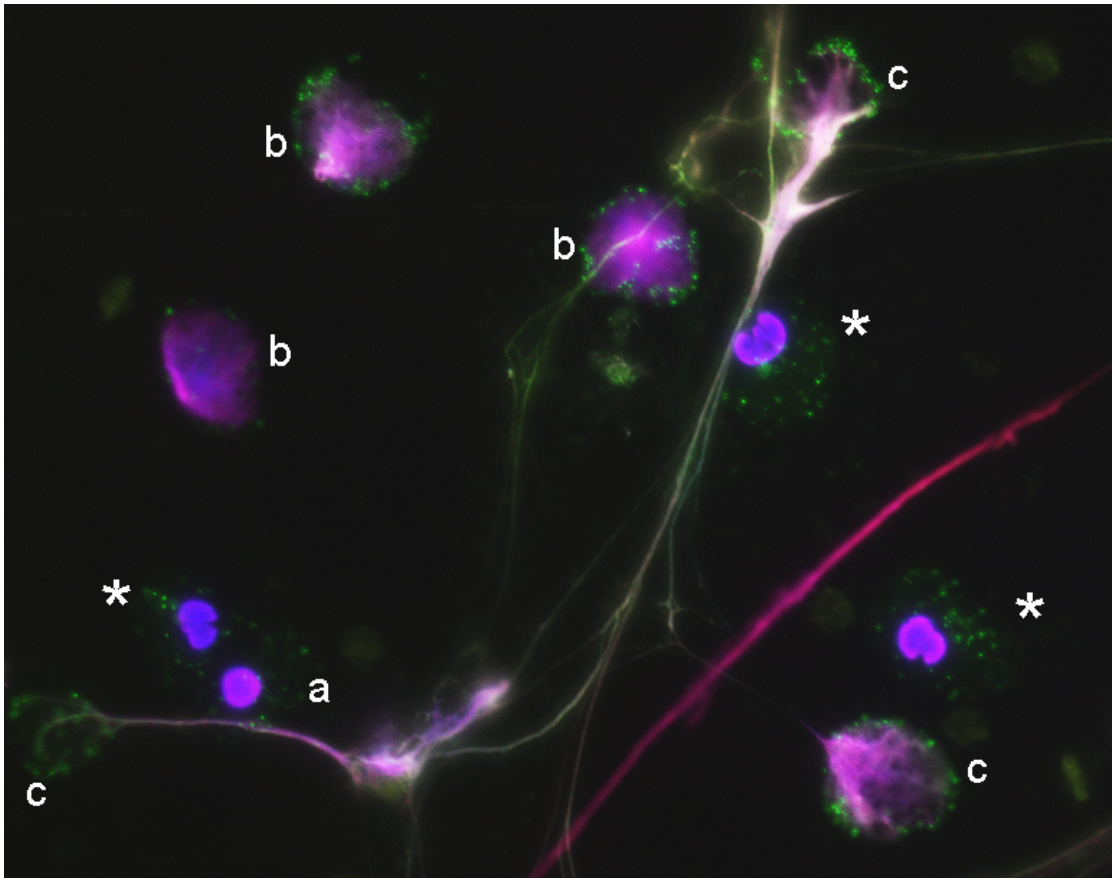

Figure S3: Typical morphological stages of NETosis. NET formation was induced in neutrophils with PMA for 3 h. Cells were fixed with paraformaldehyde and fluorescence-labelled. Blue: DAPI for DNA, red: histones, green: granular neutrophil elastase, white: colocalization of all three markers, indicating true NETs. Neutrophils in different stages of NETosis are displayed: * -unaffected with lobulated nucleus, a – early stage with condensed nucleus, b – later stage with decondensed nucleus, c – final stage with released NETs. A 40x objective was used.

Table S4: Comparison of measurements at PETRAIII and ID22NI

	PETRAIII	**ID22NI**
Cells	Freshly isolated neutrophils	Freshly isolated neutrophils
Conditions	Unstimulated, 2 h and 4 h PMA stimulation	Unstimulated, 1 h, 2 h, 3 h and 4 h PMA stimulation
Sample preparation	Plunge-freezing in liquid ethane and liquid nitrogen	High-pressure freezing and embedment in Spurr's resin
Cell condition	Intact, collapsed	2 µm thin section, not collapsed
Beam size (vertical x horizontal) / Step size of SR-XRF	500 nm x 400 nm / 1 µm	64 nm x 54 nm / 50 nm
Spatial distribution of P *	Enriched in nucleus, very low in cytoplasm / present in NETs	Low in nucleus
Spatial distribution of S	Low in cytoplasm / present in NETs	Below background in nucleus
Spatial distribution of Ca	Background level / present in NETs	Enriched in nucleus, very low in cytoplasm
Spatial distribution of Zn	Enriched in nucleus, low in cytoplasm / not present in NETs	Enriched in nucleus, low in cytoplasm
Spatial distribution of Fe	Very low in cytoplasm / present in NETs	Low in nucleus, low in cytoplasm
Spatial distribution of Cu	Background level / not present in NETs	Below background in nucleus
Spatial distribution of Mn	Background level / not present in NETs	Low in nucleus

* resting neutrophils / NETs

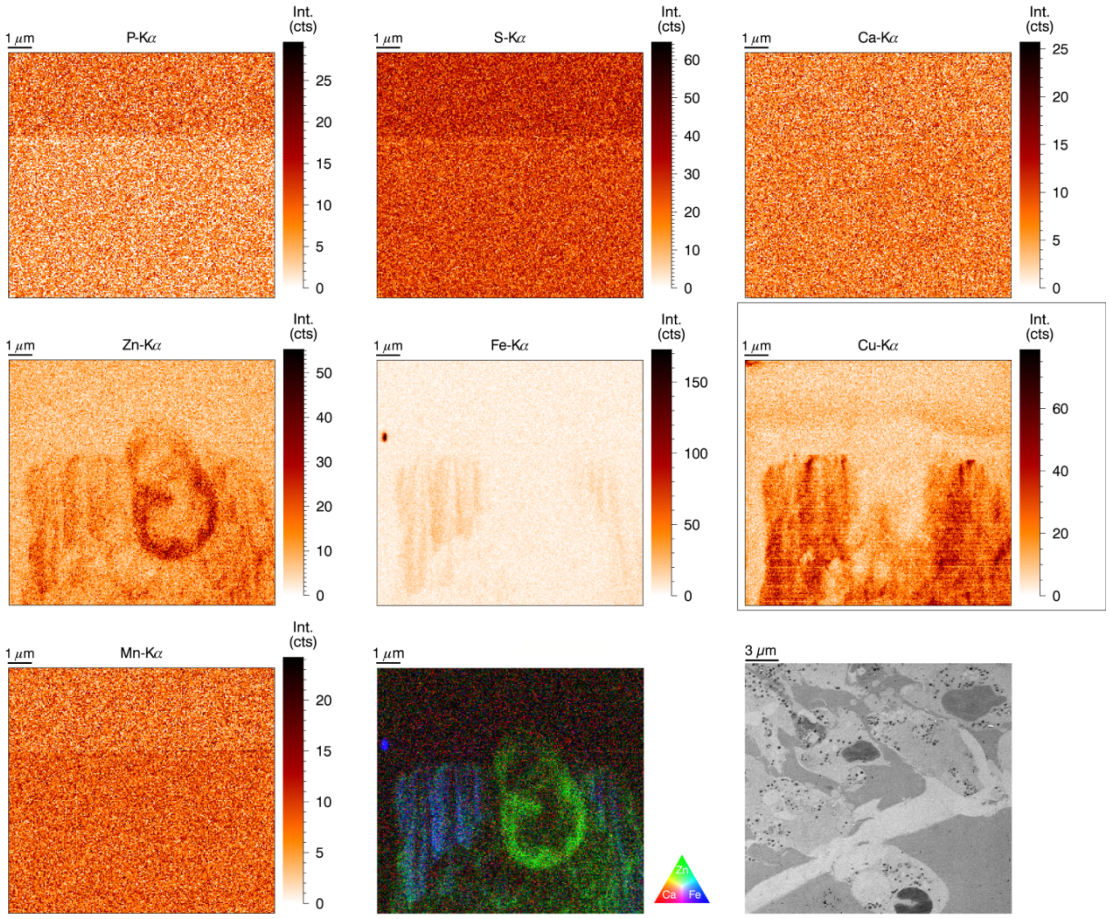

Figure S5: Normalized elemental distributions (P, S, Ca, Zn, Fe, Cu, and Mn) of an activated human neutrophil (4 h stimulation) obtained at ID22NI beamline (ESRF, Grenoble). Freshly isolated neutrophils were stimulated with PMA for 4 h, high pressure frozen, cryosubstitued in resin, sliced in 2 µm thin sections before deposition onto a Si_3N_4 wafer. Image area is 11.8 µm (hor.) x 10.8 µm (vert.), step size is 50 nm and 300 ms dwell time/point. The RGB image represents a colored overlay of the elements Ca (red) – Zn (green) and Fe (blue). The additional TEM image (lower right panel) was derived from the same sample and selected by similarity in morphology (1500x magnification).

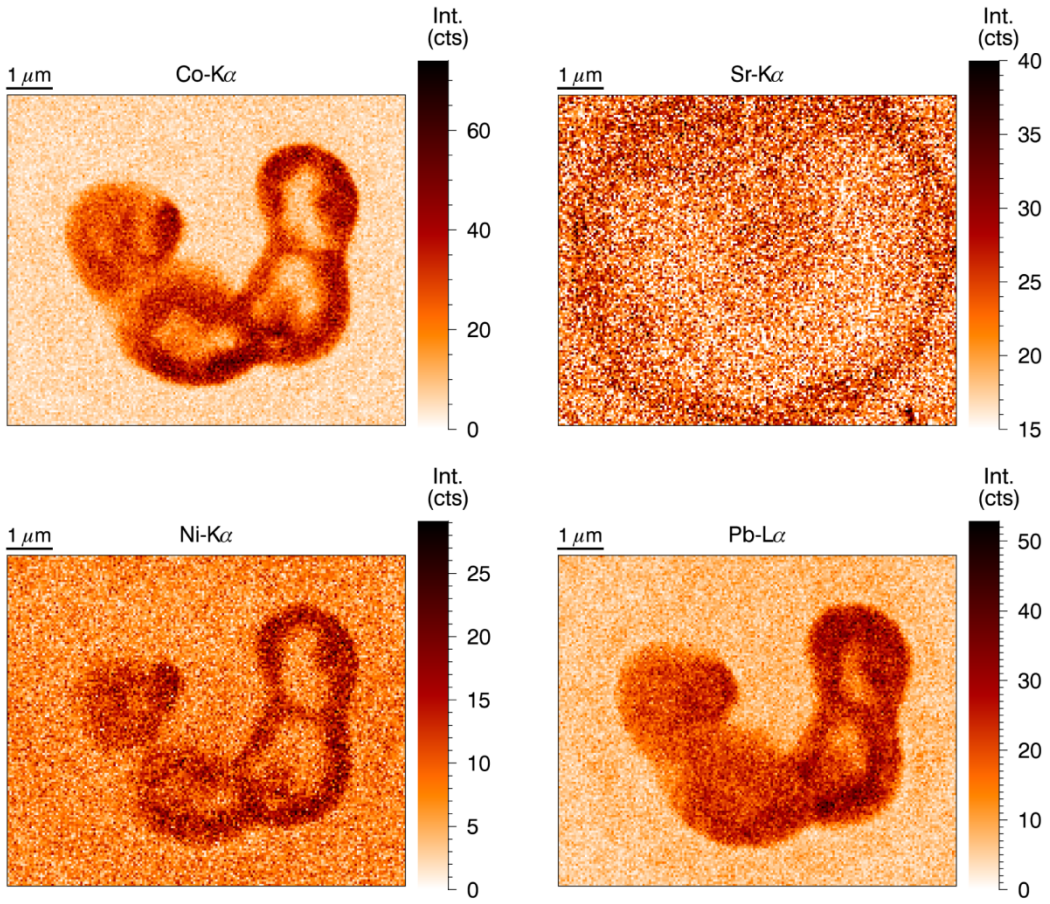

Figure S6: Normalized elemental distributions (Co, Sr, Ni, and Pb) of a resting human neutrophil obtained at ID22NI beamline (ESRF, Grenoble). Freshly isolated neutrophils were shock-frozen, cryosubstituted and sliced in 2 µm thin sections before deposition onto a Si_3N_4 wafer. Image area is 9 µm (hor.) x 7.4 µm (vert.), step size is 50nm and 300 ms dwell time/point.

Table S7: NET-mediated reduction of metal availability. NET formation was induced in neutrophils with PMA for 4 h in culture medium. The concentration of Fe, Cu, and Mn was quantified by ICP-MS. Plotted are the average and standard deviation of three independent experiments and a total of 8 healthy donors.

Element	prior NETosis (µM)	after NETosis (µM)
Fe	5.39 ± 0.79	4.89 ± 0.69
Cu	0.19 ± 0.03	0.18 ± 0.02
Mn	0.074 ± 0.024	0.069 ± 0.023

Video V1: FluoZin-labeled neutrophils activated by PMA. Human neutrophils were stained with FluoZin to visualize the labile Zn pool (green) and stimulated with PMA. Cells were followed for 3 h every 2 min. Incubation occurred under standard cell culture conditions.