Supporting information

Transport and programmed release of nanoscale cargo from cells by using NETosis

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Supplemental Movies

Suppl. Movie 1 (AVI)**:** SWCNT loaded neutrophils migrate in a 100 nM fMLP treated medium. nIR video with light bright field illumination to detect the cell shape.

Suppl. Movie 2 (AVI)**:** Multichannel live-cell images of PMA activated neutrophils (respectively: Phase Contrast, Chromatin, $(GT)_{15}$-SWCNT channels).

Suppl. Moview 3 (AVI): Migration and decondensation behavior of differently activated neutrophils. Video shows Hoechst 33342 stained nuclei of unactivated cells (left), activated with 100 nM PMA (middle) or 100 µg/ml LPS (right).

Suppl. Movie 4 (AVI)**:** Neutrophils under agarose migration in an 1 µg/ml LPS medium.

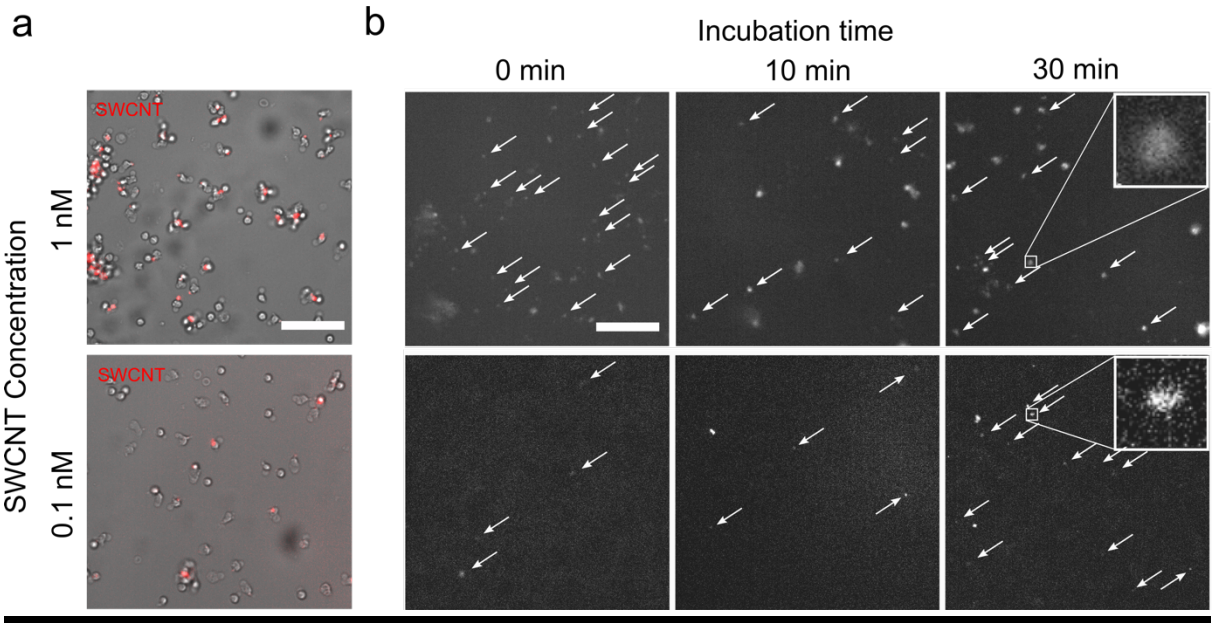
Suppl. Movie 5 (AVI)**:** nIR response of $(GT)_{15}$-SWCNT loaded in a neutrophil during addition of 100 nM dopamine (no cell activation/intact cell, cell & chromatin geometry shown in **Suppl. Fig. 7a**, grey dot marks timepoint of dopamine addition)

Suppl. Movie 6 (AVI)**:** nIR response of $(GT)_{15}$-SWCNT loaded in a neutrophil during addition of 100 nM dopamine (after rupture, cell & chromatin geometry shown in **Suppl. Fig. 7a**, grey dot marks timepoint of dopamine addition)

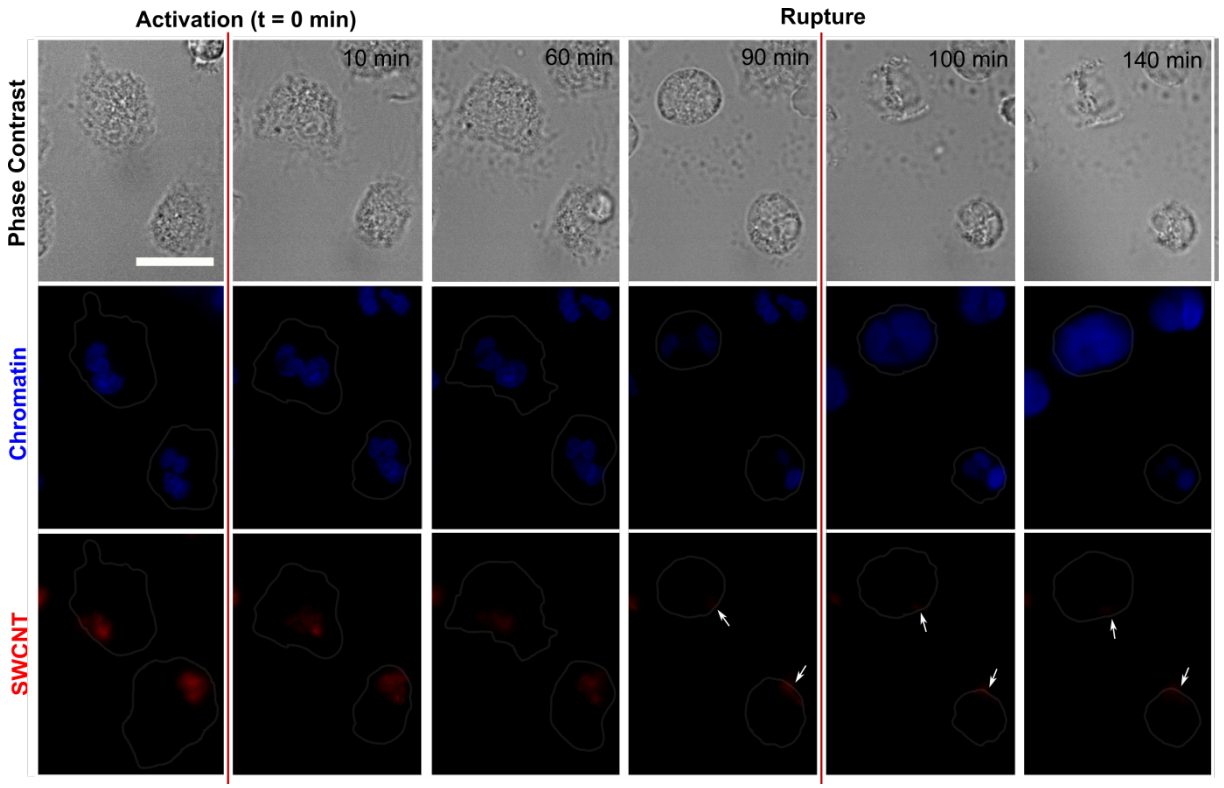
Suppl. Movie 7 (AVI)**:** nIR response of aptamer coated Hemin-SWCNTs loaded in an unactivated, intact neutrophil during addition of 100 µM H_2O_2 (cell & chromatin geometry shown in **Suppl. Fig. 7a**, grey dot marks timepoint of H_2O_2 addition)

Suppl. Movie 8 (AVI)**:** nIR response of Hemin coated-SWCNT loaded in a ruptured neutrophil during addition of 100 µM H_2O_2 (cell & chromatin geometry shown in **Suppl. Fig. 7a**, grey dot marks timepoint of H_2O_2 addition)

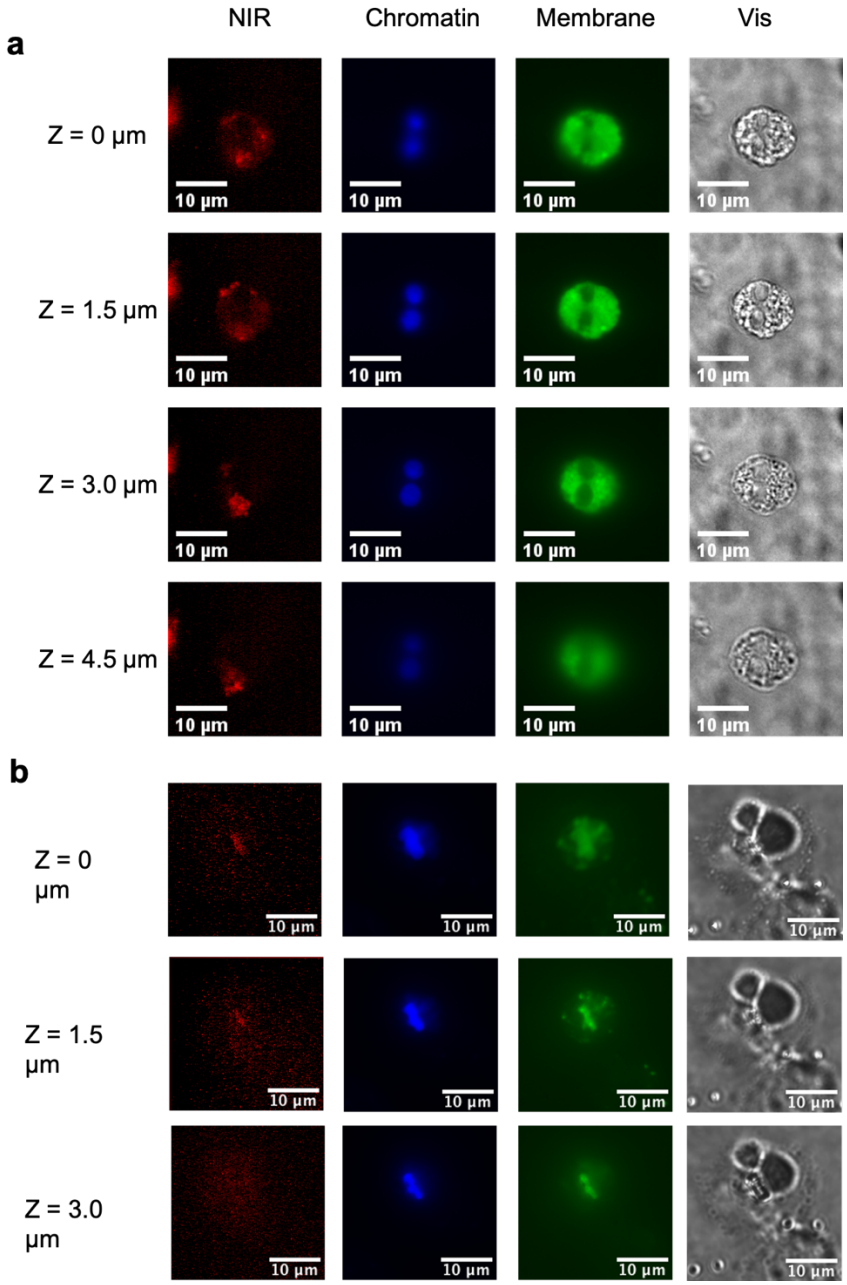
Suppl. Movie 9 (AVI)**:** Response of patterned $(GT)_{15}$-SWCNT to 100 nM dopamine - grey dot marks timepoint of addition



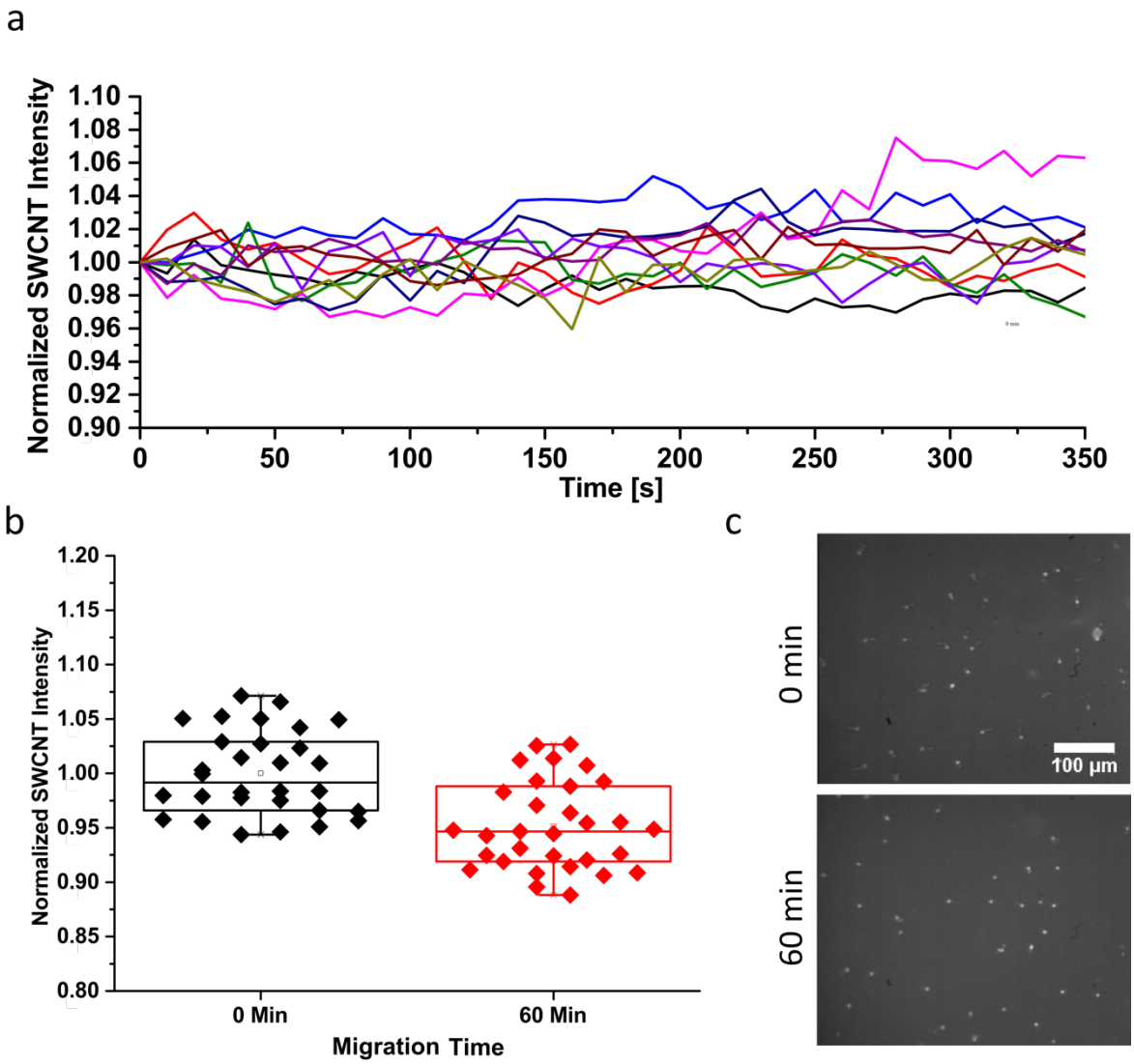
Suppl. Fig. S1 SWCNT uptake by neutrophils. a Phase contrast (grey) images of neutrophilic granulocytes after $(GT)_{15}$-SWCNT uptake. nIR SWCNT fluorescence (red) colocalized for higher concentrations(1 nM for 30 minutes, top) but there was also a tendency to cell aggregation. For lower SWCNT concentrations (0.1 nM, bottom) there was no cell aggregation but also less SWCNT signal. Normally, sensors could be found at the cell rear during migration, whereas in cell agglomerates SWCNT signals were located in the center of the cell bulk. Scale bar = 100 µm. **b** nIR images of uptaken $(GT)_{15}$-SWCNTs for different amounts of incubation time. SWCNT fluorescence signals increased with incubation time and starting concentration. Scale bar = 100 µm. The contrast of images at the bottom was increased to show SWCNT locations.



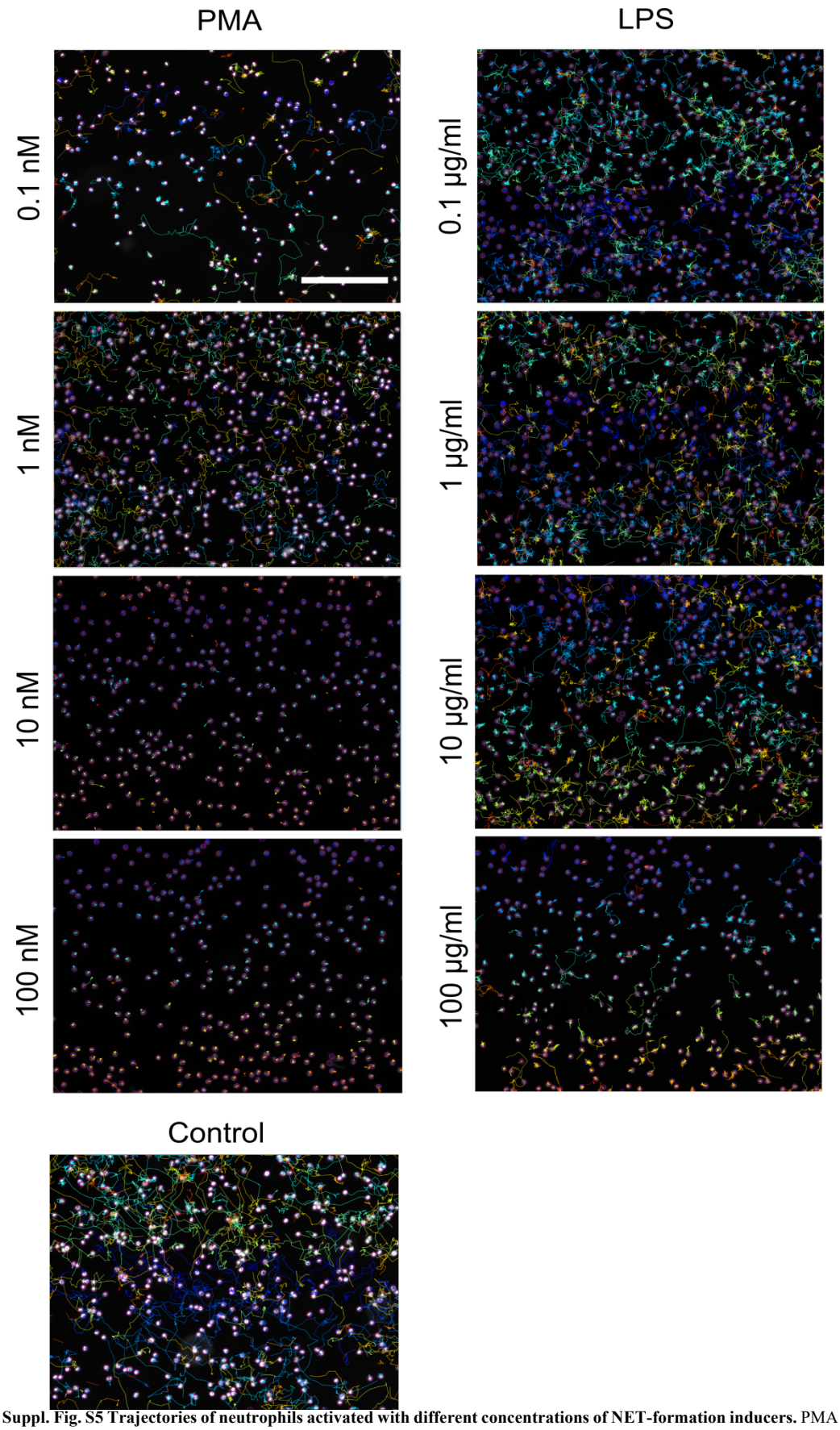
Suppl. Fig. S2 SWCNT and chromatin geometry during NETosis. Activated & SWCNT loaded cells show tight adhesion in the early phase of NETosis (phase contrast, top) while the uptaken SWCNTs (bot) and nuclei (mid) remain in their condensed compartments. In later stages, however, SWCNTs start to relocate to the cellular membrane resulting in a reduction of the sensor area and their overall fluorescence intensity. In the same time, the cells begin to deform while innercellular chromatin decondenses and mixes with the cytosolic content. Finally, in the last phase of NETosis, the cellular membrane ruptures and releases the decondensed chromatin as well as parts of the incorporated sensors. Scale bar = 10 µm, chromatin stained with Hoechst 33342.



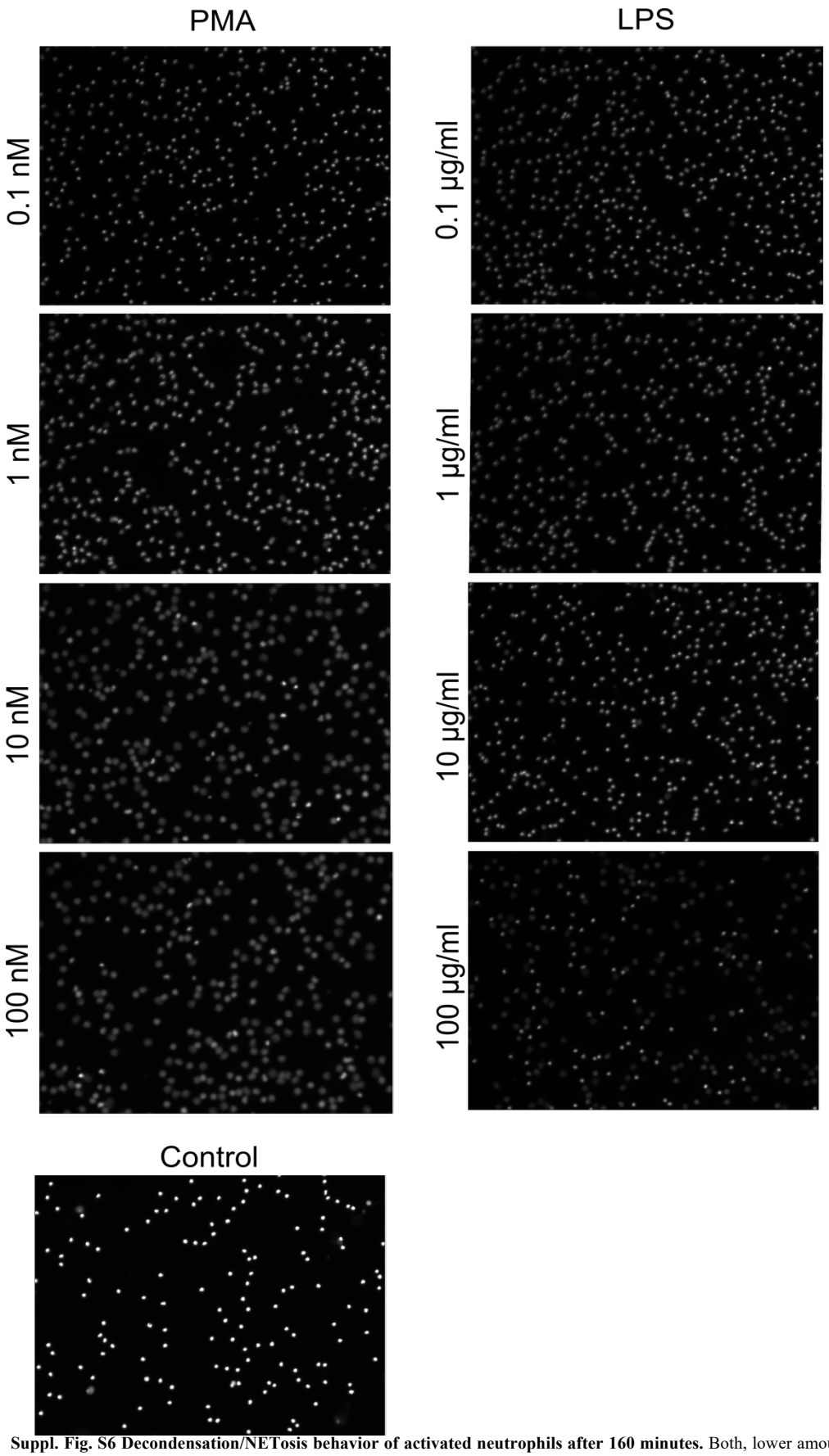
Suppl. Fig. S3 Near-infrared (NIR) confocal imaging of internalized (GT)15 SWCNTs in pre-activated (a) and activated (b) neutrophils. Fluorescence intensity was recorded for SWCNTs in confocal mode (laser excitation 640 nm, emission >800 nm, red), cell chromatin (LED excitation at 395 nm, emission filter 417-477 nm, blue), and cell membrane in widefield mode (LED excitation at 470 nm, emission filter 502-538 nm, green), as well as obtaining visible images of the cells (grey). Scale bar = 10 µm. All images were processed using ImageJ Fiji software. A Gaussian blur filter (sigma = 50) was applied to generate the images used for background subtraction.
Images were collected at different heights (Z) within the cells, with Z=0 corresponding to the lowest slice. The custom-built, NIR confocal setup used to image internalized SWCNTs consists of a 500 mW laser coupled to an inverted microscope body (Eclipse Ti-U, Nikon AG Instruments). The spinning-disc confocal head contains transmission lenses with an anti-reflective NIR coating (CREST Optics). An indium gallium arsenide (InGaAs) camera (NIRvana 640 ST, Princeton Instruments) was used for imaging NIR fluorescence. A visible camera (Andor) was used for imaging visible fluorescence and for collecting bright-field images.



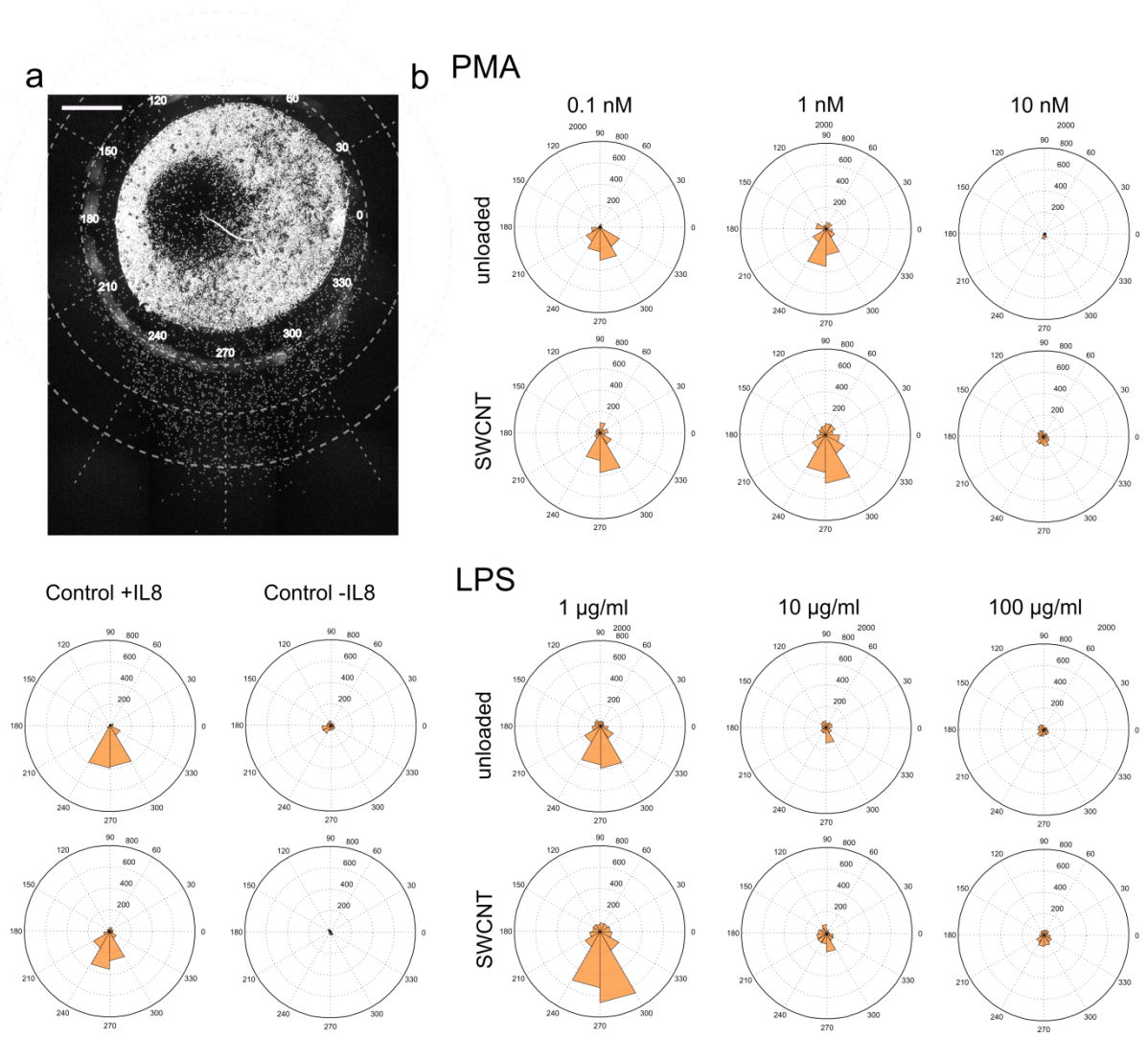
Suppl. Fig. S4 Intensity analysis of SWCNT loaded cells during migration. a Normalized SWCNT intensity change on short time scales (examplary extracted from the sample shown in Suppl. Movie 1). Values generally fluctuate with a average deviation of around 2% of the mean intensity. **b** Normalized SWCNT intensity change on long time scales. The emission strength of loaded SWCNTs were measured at the beginning (0 s) and after 60 minutes of free cell migration. Comparing both data sets reveals a very small intensity decrease (5%), which is attributed to changes in cell morphology. Data from n = 2 independent donors, squares define mean values, block height equals standard deviation and error bars symbolize 25% or 75% data intervals. **c** Exemplary SWCNT (nIR) image used to calculate the values shown in **b.** Even though cells were allowed to move for 60 minutes consecutively, SWCNT intensity values remained nearly the same.



Suppl. Fig. S5 Trajectories of neutrophils activated with different concentrations of NET-formation inducers. PMA (0.1 – 100 nM) and LPS (0.1 – 100 µg/ml) stimulate different NET-formation pathways. Images show chromatin stained neutrophils and their trajectories (colorcoded to improve trajecory visualization). Low amounts of PMA led to similar speed and stopping time of migrating cells compared to control samples. On the contrary, 10 – 100 nM PMA forced nearly all cells to instantly stop. Lower amounts of LPS, did not change the movement pattern of the cells, however increasing concentrations lowered the cells speed and locomotion duration. Hoechst stain plus migration traces generated by TrackMate ImageJ plugin. Scale bar = 100 µm. Traces show migration patterns after/during 160 minutes. The track's color indicates only the cell index in the image.



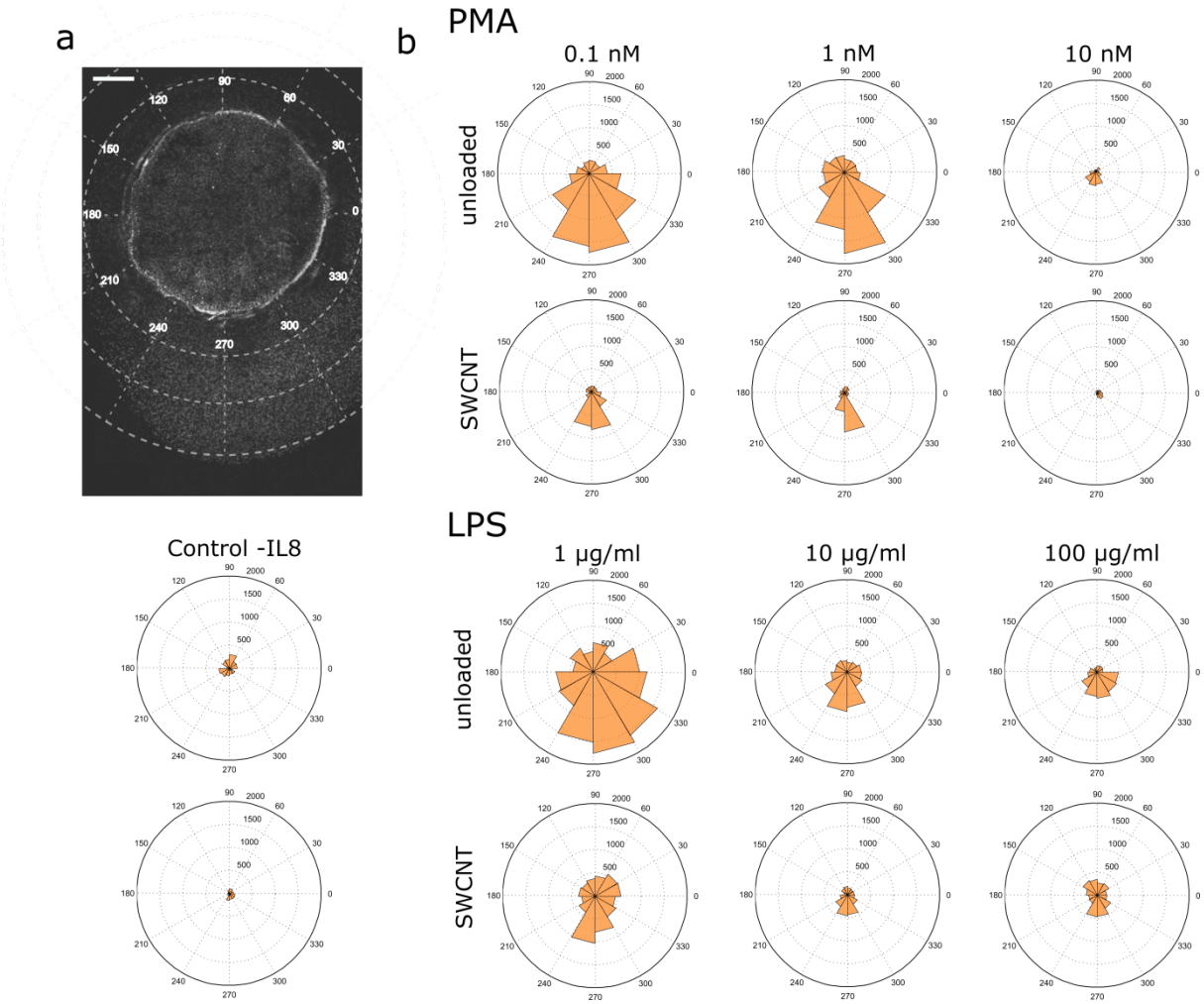
Suppl. Fig. S6 Decondensation/NETosis behavior of activated neutrophils after 160 minutes. Both, lower amounts of LPS and PMA (*i.e.* 0.1 – 1nM PMA and 0.1 – 1µg/ml LPS) did not show any significant decondensation compared to the control samples. In contrast, 10 – 100 nM PMA resulted in a nearly complete decondensation of all cells comparable to 100 µg/ml LPS.



Suppl. Fig. S7 Migration behavior of $(GT)_{15}$-loaded and unloaded neutrophils in a gradient(migration under agarose assay) NET formation inducers at 0.5% FCS concentration. a Exemplaric image of a typical under agarose gradient experiment and the description of the coordinate system. Cells were loaded in one well (diameter around d = 3 mm) and activated shortly before the chemoattractant (IL-8, 0.1 µM) was poured in a second well (distance d = 2.2 mm), which generated a consistent gradient within the gel. Neutrophils were allowed to move freely for 3 hours and were imaged (Hoechst 33342 staining). Images were arranged in such a way that the 270° faces towards the IL-8 well, scale bar = 400µm. **b** Analysis of the migratory distance of $(GT)_{15}$-loaded neutrophils and untreated ones after 3 hours within an under agarose sample. Cells with SWCNTs showed enhanced movement compared to those without SWCNTs. Increased LPS or PMA concentrations reduced movement in both cases. Data was generated by measuring the maximal distance between the cell bulk and the well's edge for the respective angles. Experiments were performed three times with three independent donors (n = 3) and results were averaged to show mean values in µm (values are presented in **Suppl. Table T1**).

Suppl. Table T1 Average distances traveled by the migrating front for 0.5% FCS conditions. Table shows the mean distances reached by migrating front (extracted from the 255° or 285° data shown in **Suppl. Figure S5**). Distances are in µm, n = 3, Data ± SEM

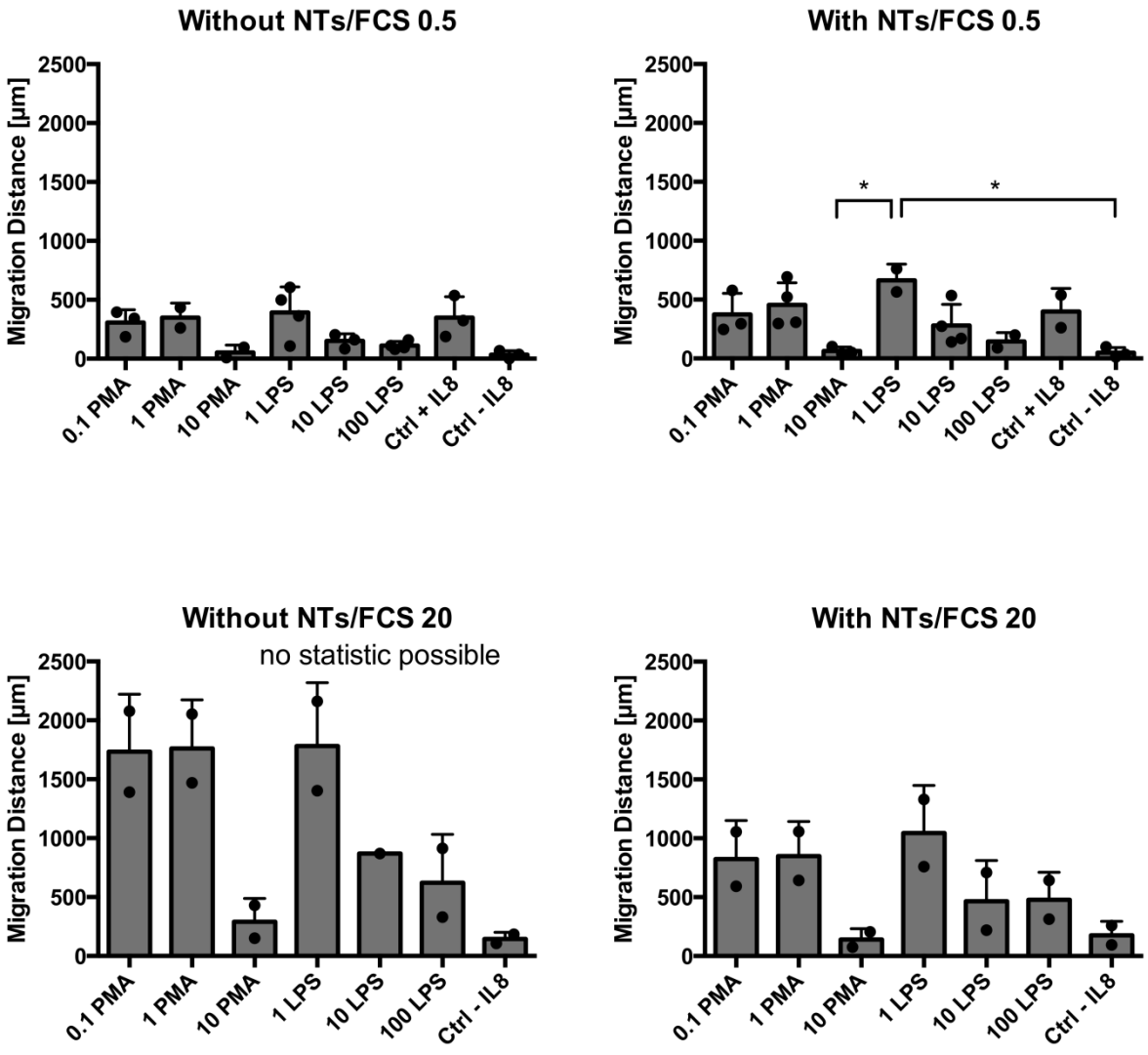
Maximal distance [µm]	Unloaded	SWCNT
PMA (0.1 nM)	308 ± 62	373 ± 104
PMA (1 nM)	349 ± 71	454 ± 109
PMA (10 nM)	54 ± 36	70 ± 15
LPS (1 µg/ml)	394 ± 124	663 ± 80
LPS (10 µg/ml)	151 ± 35	224 ± 80
LPS (100 µg/ml)	111 ± 20	145 ± 44
Control + IL8	395 ± 101	399 ± 113
Control – IL8	35 ± 19	48 ± 26



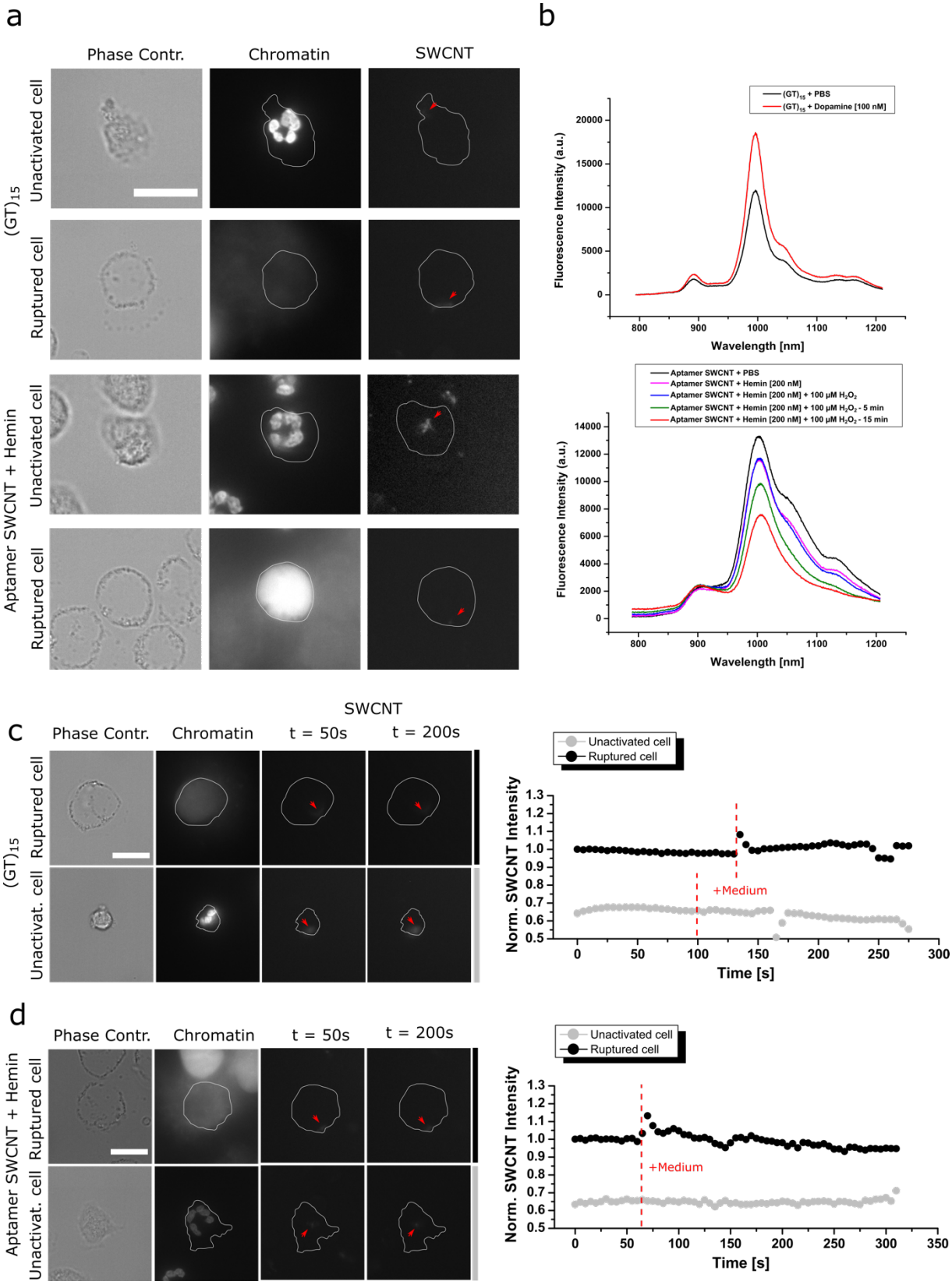
Suppl. Fig. S8 Migration behavior of $(GT)_{15}$-loaded and unloaded neutrophils in an migration under agarose experiment using different amounts of NETosis activators and 20% FCS inherited agarose gels. a Exemplaric image of a typical under agarose experiment plus decribtion of the used coordinate system. Cells were loaded in one well (diameter around d = 3 mm) and activated shortly before the chemoattractant (IL-8, 0.1 µM) was poured in a second well 2.2 mm away from the cells which generated a consistent gradient within the gel. Neutrophils were allowed to move freely for 3 hours and were imaged using Hoechst 33342 stain afterwards. Pictures were arranged so that the 270° faces towards the IL-8 well, scale bar = 500µm. **b** Analysis of the migratory distance of $(GT)_{15}$-loaded neutrophils and untreated ones after 3 hours within an under agarose sample. Cells without SWCNTs showed enhanced movement compared to those which came in contact with the sensors and even reached the other well sometimes. Increasing the concentrations of LPS or PMA resulted in reduced locomotion in both cases. Data was generated by measuring the maximal distance between the cell bulk and the well's edge for the respective angles. Furthermore, experiments were performed two times with two independent donors (n = 2) and results were averaged to show mean values in µm (exact values are presented in **Suppl. Table T2**).

Suppl. Table T2 Average distances traveled by the migrating front for 20% FCS conditions Table shows the mean distances reached by migrating front (extracted from the 255° or 285° data shown in **Suppl. Figure S6**). Distances described in µm, n = 2, Data ± SEM

Maximal distance [µm]	Unloaded	SWCNT
PMA (0.1 nM)	1724 ± 343	823 ± 247
PMA (1 nM)	1762 ± 388	850 ± 237
PMA (10 nM)	317 ± 127	141 ± 62
LPS (1 µg/ml)	1781 ± 481	1032 ± 235
LPS (10 µg/ml)	869	465 ± 101
LPS (100 µg/ml)	578 ± 183	478 ± 162
Control – IL8	145 ± 32	170 ± 51



Suppl. Fig. S9 Statistical analysis of the data listed in Suppl. Table T1 & T2. The number of blood donors is low (n = 2-4) and therefore statistical test could not performed for every condition. However, for every blood donor >100 cells were observed. Two-way ANOVA test, significance * p = 0.95, ** p = 0.99, *** p = 0.999. Histograms show mean ± standard deviation values.



Suppl. Fig. S10 Optical response of $(GT)_{15}$- and Aptamer/Hemin SWCNTs. a Exemplaric cell and nIR sensor images used to depict the SWCNT responses shown in **Fig. 5 c-d** (respective SWCNT movies can be seen in **Suppl. movie 6-9**). Contrast of nIR images were enhanced to show sensor geometry and position. Scale bar = 10 µm. **b** Excitation spectrum of $(GT)_{15}$-SWCNT (top) and Aptamer/Hemin-SWCNT (bottom) in PBS. 100 nM dopamine increase the nIR fluorescence of $(GT)_{15}$-SWCNT (dopamine sensor). 100 µM of H_2O_2 decreased the nIR fluorescence of Aptamer/Hemin-SWCNTs (H_2O_2) sensor. **c** $(GT)_{15}$-SWCNTs loaded activated/non-activated cells show no significant fluorescence change when RPMI-medium is added as a control. **d** Similar control for Aptamer/Hemin SWCNT loaded activated/non-activated cells. Scale bar = 10 µm.

Supplementary methods

nIR fluorescence spectroscopy

nIR fluorescence spectra were recorded with a Shamrock 193i spectrometer (Andor Technology Ltd., Belfast, Northern Ireland) connected to an IX53 microscope (Olympus, Tokyo, Japan). Excitation was performed with a 561 nm Cobolt Jive™ laser (Cobolt AB, Solna, Sweden) for $(GT)_{15}$-SWCNT samples or with an 785 nm iBeam smart laser (Toptica Photonics, Germany, Munich) in case of Aptamer-SWCNTs.
To test $(GT)_{15}$-SWCNTs responses to dopamine, 180 µl of a 0.1 nM ssDNA/SWCNT solution in PBS was placed in a 96-well plate, measured and 20 µl of 1 µM dopamine was added subsequently to yield a final concentration of 100 nM. The fluorescence response of the Aptamer/Hemin-SWCNTs was studied by adding 20 µl of 1 mM H_2O_2 solution in PBS to 180 µl of 2 nM Aptamer/Hemin-SWCNT with 200 nM hemin in it.