Electronic Supplementary Material (ESI) for Lab on a Chip.
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Supplementary Information

Microfluidic capture of chromatin fibres measures neutrophil extracellular traps (NETs) released in a drop of human blood

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Supplementary Video

Visualization of fluorescently tagged ligated-lambda DNA capture on the post-arrays.

Supplementary Figures

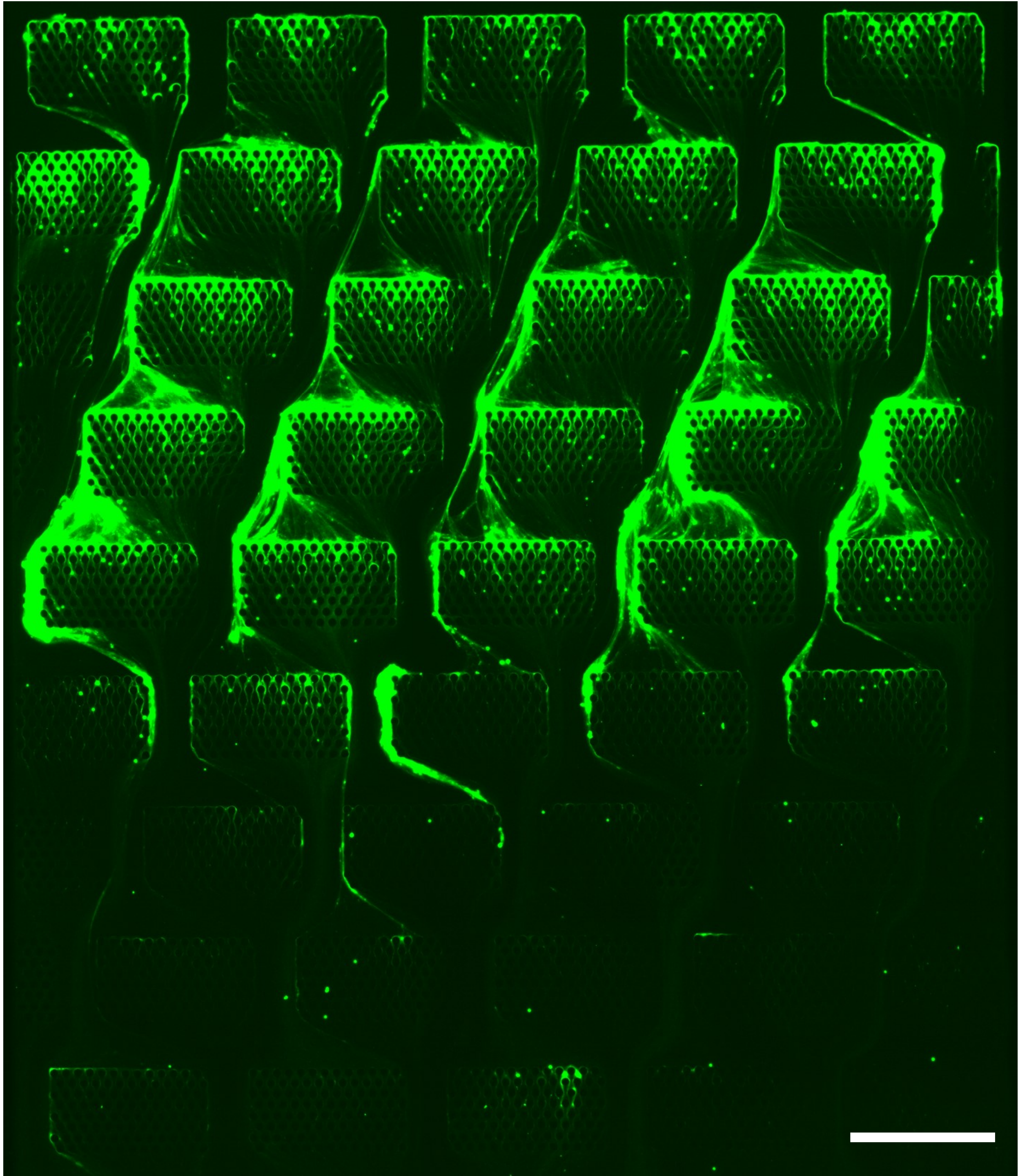
Supplementary Figure 1. Overview of NETs captured inside a microfluidic device from 10 µL of human blood.

Supplementary Figure 2. Image analysis protocols to automatically identify long chromatin fibres.

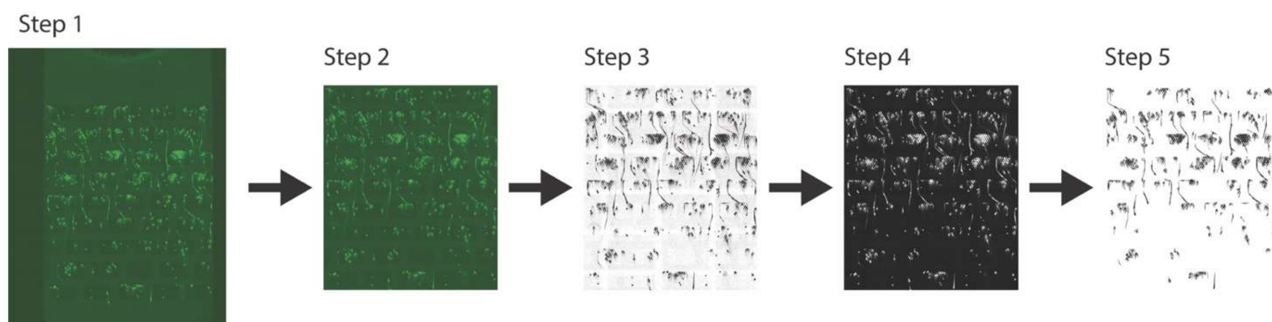
Supplementary Figure 3. The neutrophil origin of long chromatin fibres is confirmed by fluorescence overlap of Sytox green and fluorescently tagged secondary antibodies binding to anti-CitH3 antibodies.

Supplementary Figure 4. Lambda DNA is stained only by Sytox green dye and not by the CitH3 antibodies.

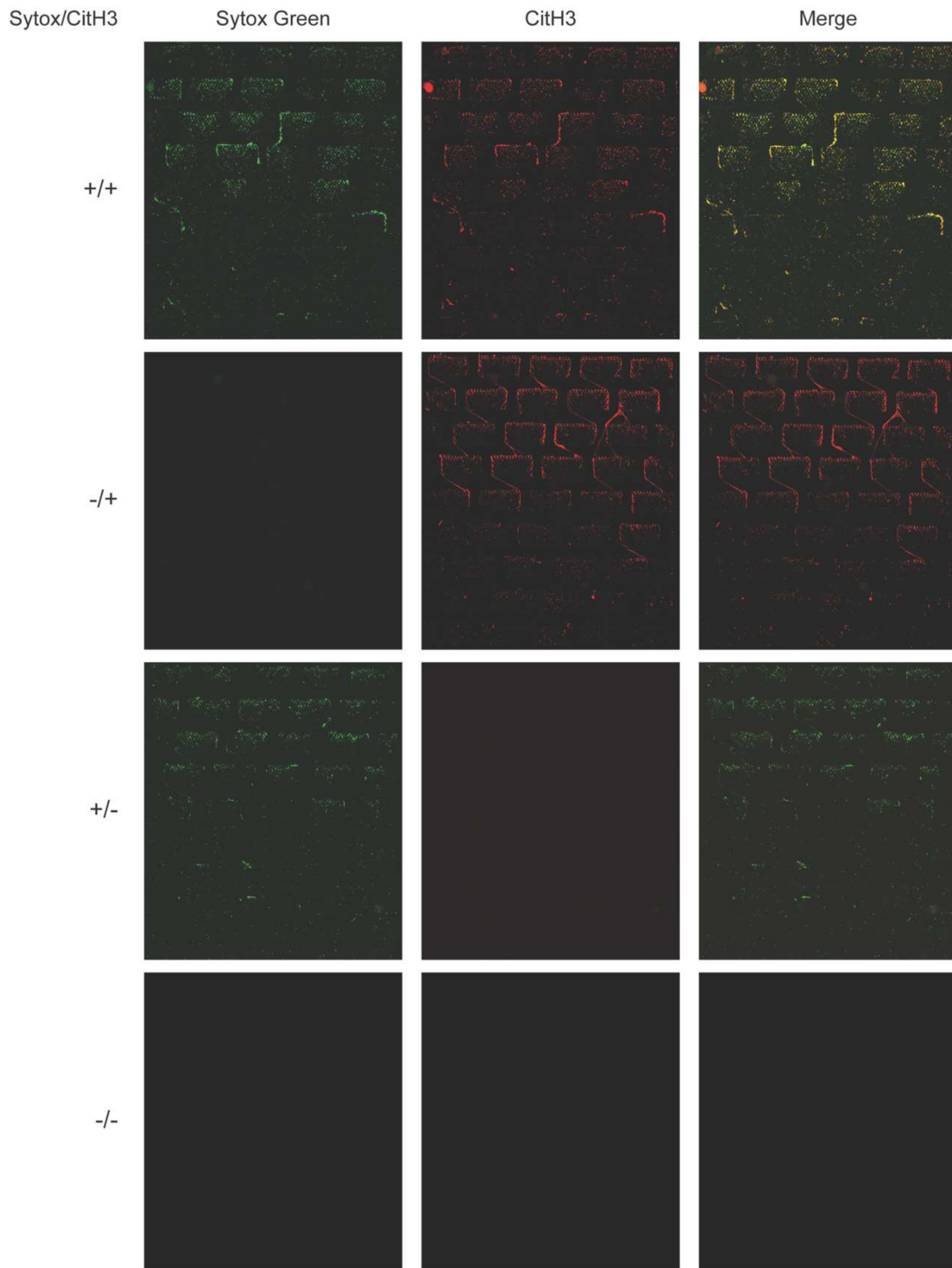
Supplementary Figure 5. The differences in NET capture in different devices are less than the differences between donors.



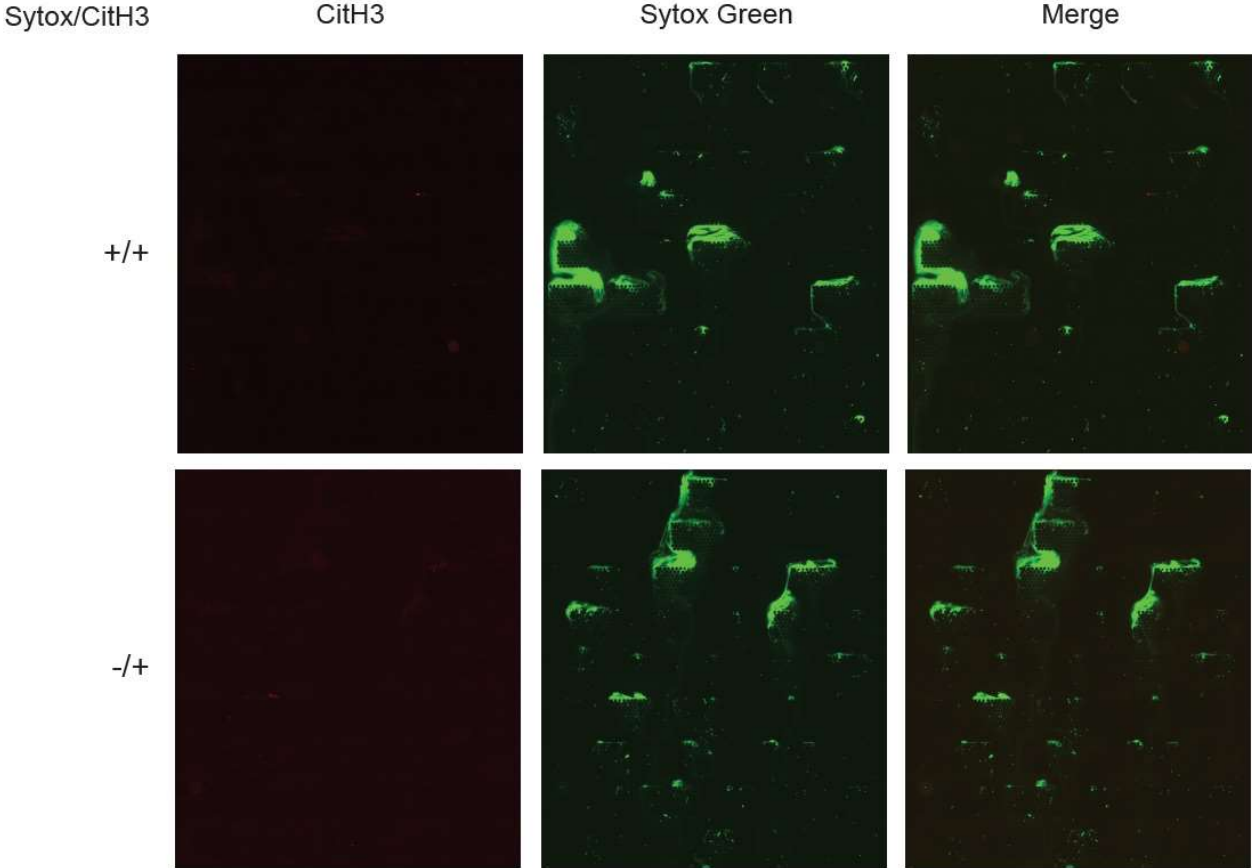
Supplementary Figure 1. Overview of NETs captured inside a microfluidic device from 10 µL of human blood. Blood samples were stimulated by 10 µg/mL PMA for four hours. NETs are stained using Sytox green fluorescent dye. Nine rows of five patches of posts could be identified by the exclusion of fluorescence at the posts location. Fluorescence microscopy. Scale bar is 1 mm.



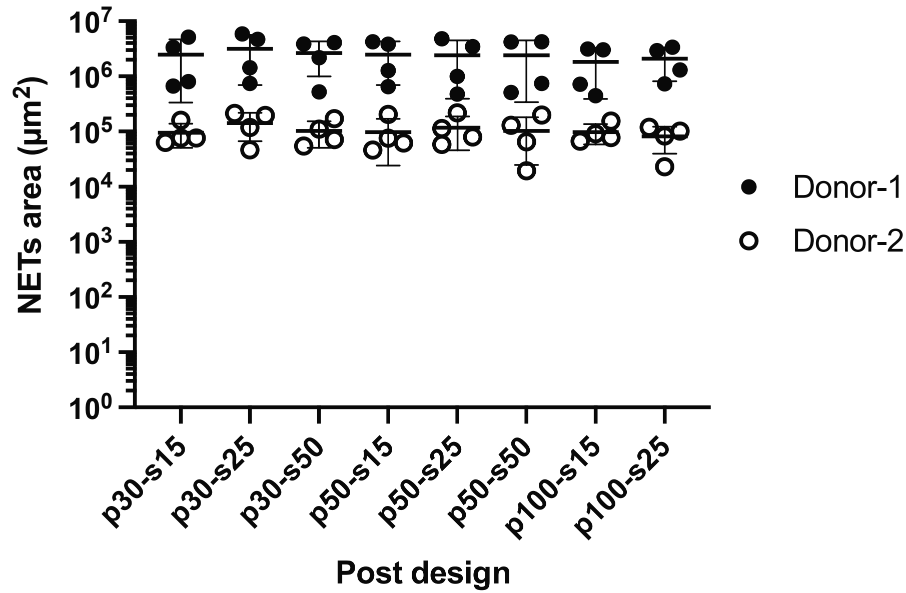
Supplementary Figure 2. Image analysis protocols to automatically identify long chromatin fibres. Step 1: adjusting the brightness, Step 2: cropping the post area, Step 3: making a binary with selected threshold, Step 4: inverting the image bright and dark areas, Step 5: despeckling and analyzing the objects using a threshold area of 100 μm^2 and a threshold shape-factor of 0.5. Larger and more elongated objects are identified as NETs with the thresholds.



Supplementary Figure 3. The neutrophil origin of long chromatin fibres is confirmed by fluorescence overlap of Sytox green and fluorescently tagged secondary antibodies binding to anti-CitH3 antibodies. To verify the specificity of each dye, the dyes were tested alone and in combination. NETs are generated in healthy donor blood samples by incubation with 1 µl/mL PMA for 4 hours at 37 °C.



Supplementary Figure 4. Lambda DNA is stained only by Sytox green dye and not by the CitH3 antibodies. Images from individual fluorescence channels and merged images that combine the red and green channels are presented.



Supplementary Figure 5. The differences in NET capture in different devices are less than the differences between donors. We compared NETs trapping on devices with 30, 50 and 100 µm diameter posts (p) and 15, 25, 50 µm spacing between posts (s). Blood samples were stimulated with PMA (1 µg/mL) for 4 hours. Blood was then diluted 10 x and flown through the devices at 10 µL/min. N=4 devices per donor sample, N=2 donors.