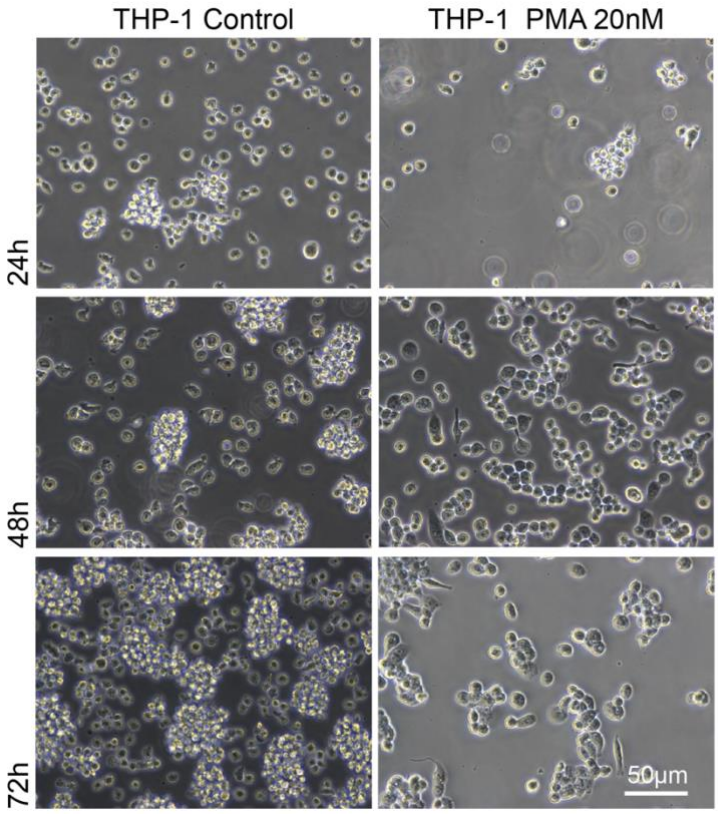
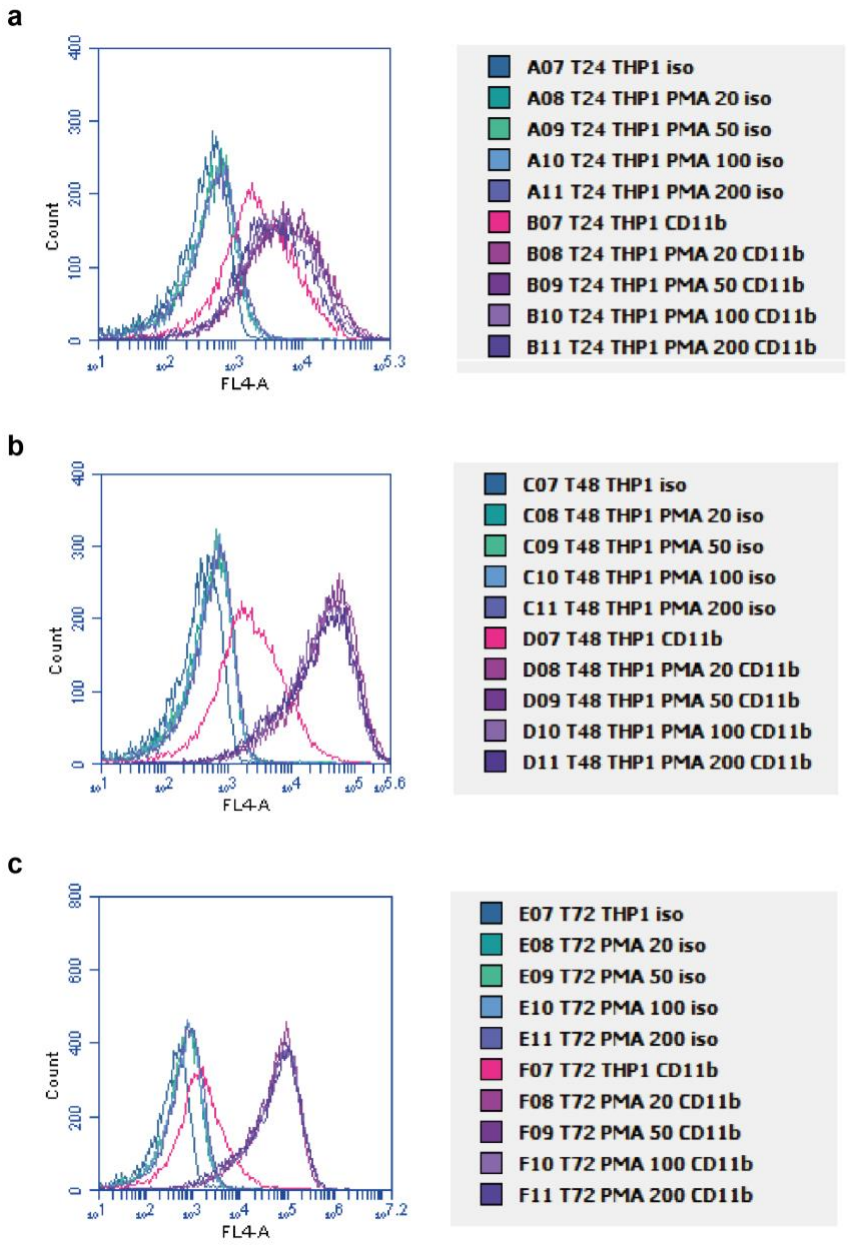
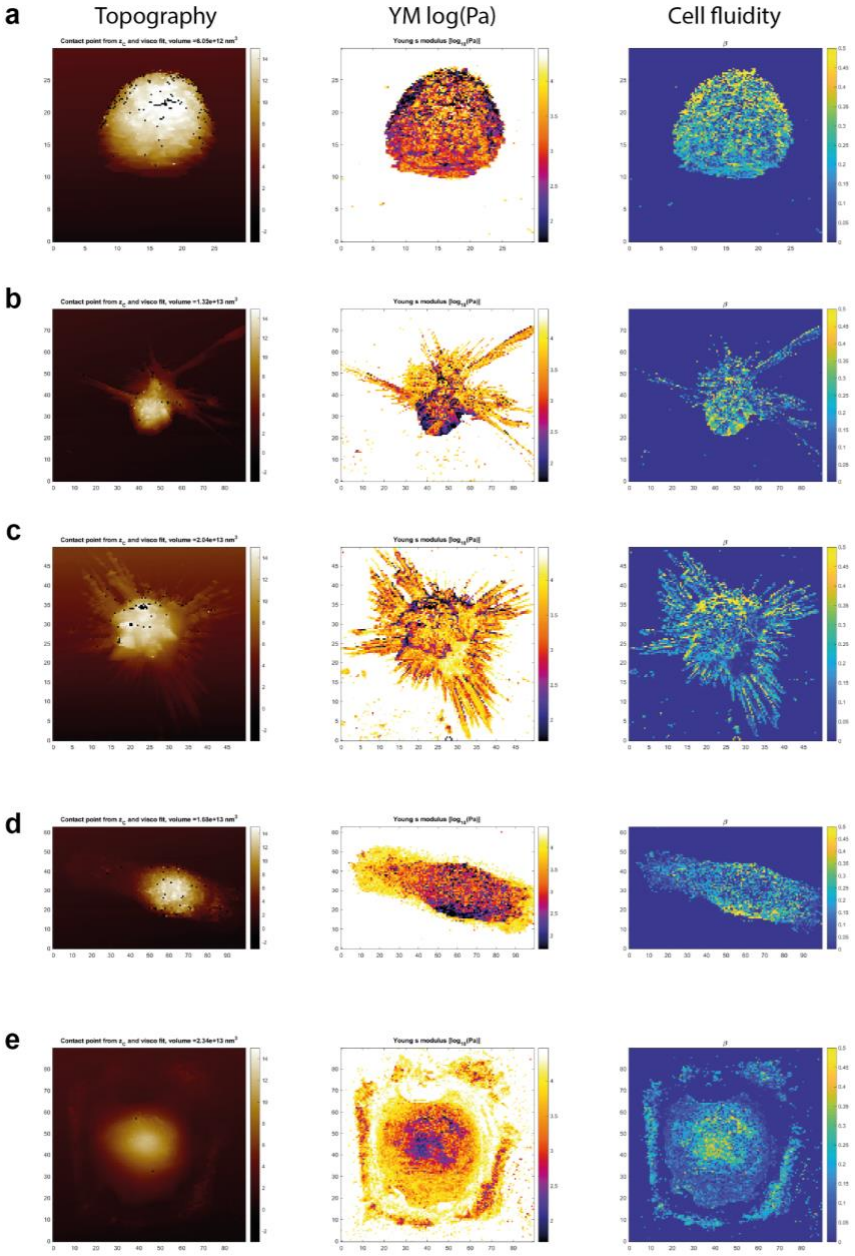
SUPPLEMENTARY INFORMATION



Supplementary Figure 1. PMA differentiation of THP-1 cells over time. a. Phase contrast images of THP-1 cells (control) and 20nM PMA treated cells at 24h, 48h and 72h.

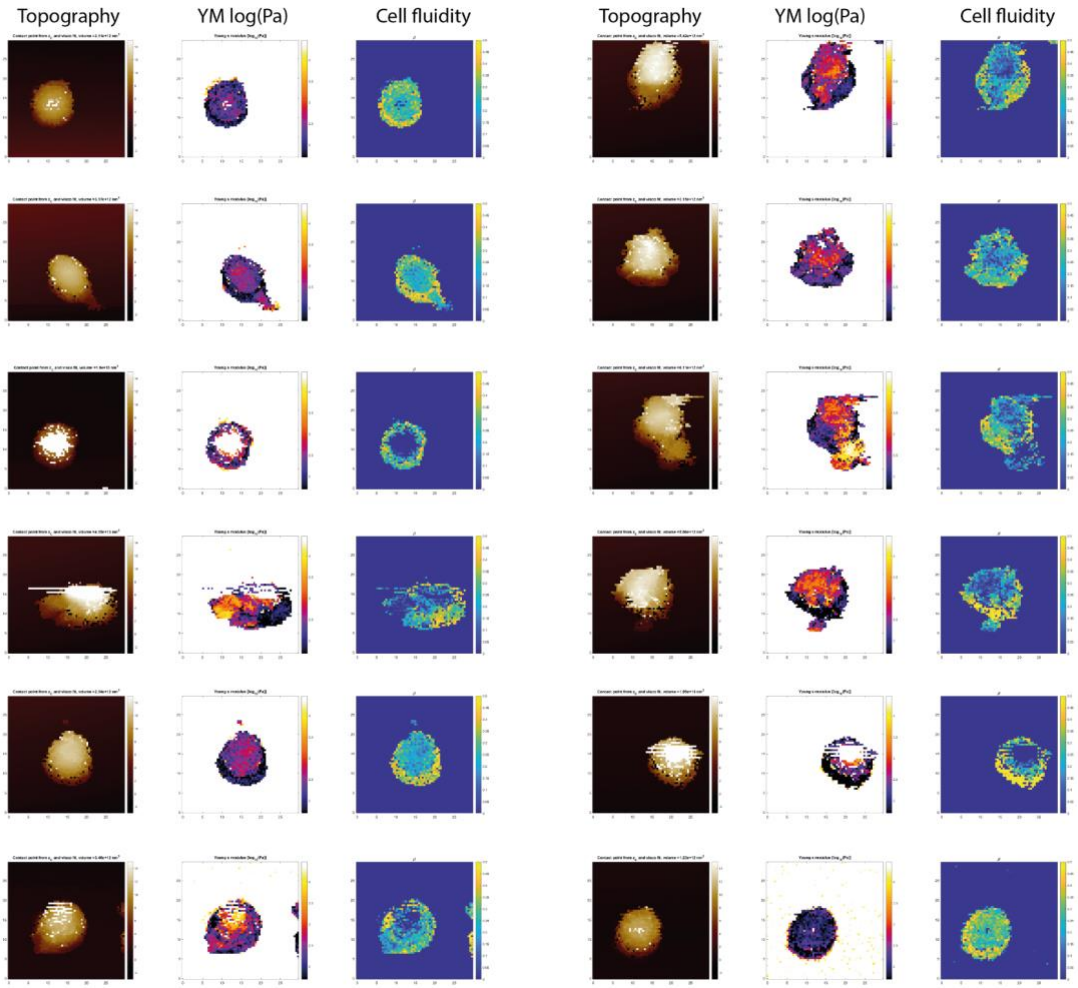


Supplementary Figure 2. Flow cytometry fluorescence intensity (F.I) for CD11b. a. F.I. for THP-1 treated with 20, 50, 100, and 200 nM PMA at 24 hours. b. F.I. for THP-1 treated with 20, 50, 100, and 200 nM PMA at 48 hours. c. F.I. for THP-1 treated with 20, 50, 100, and 200 nM PMA at 72 hours.



Supplementary Figure 3. a-e. Mechanical maps of THP-1 cells treated with 200nM for 5 days. New phenotypes are observed if the PMA concentration and time of treatment are increased. While we still find round and spread macrophage phenotypes, some stellate cells appear with very long protrusions or filopodia, similar to dendritic cells (b-c). Also, more macrophages migrate in a fused form (d).

Monocytes viscoelastic maps



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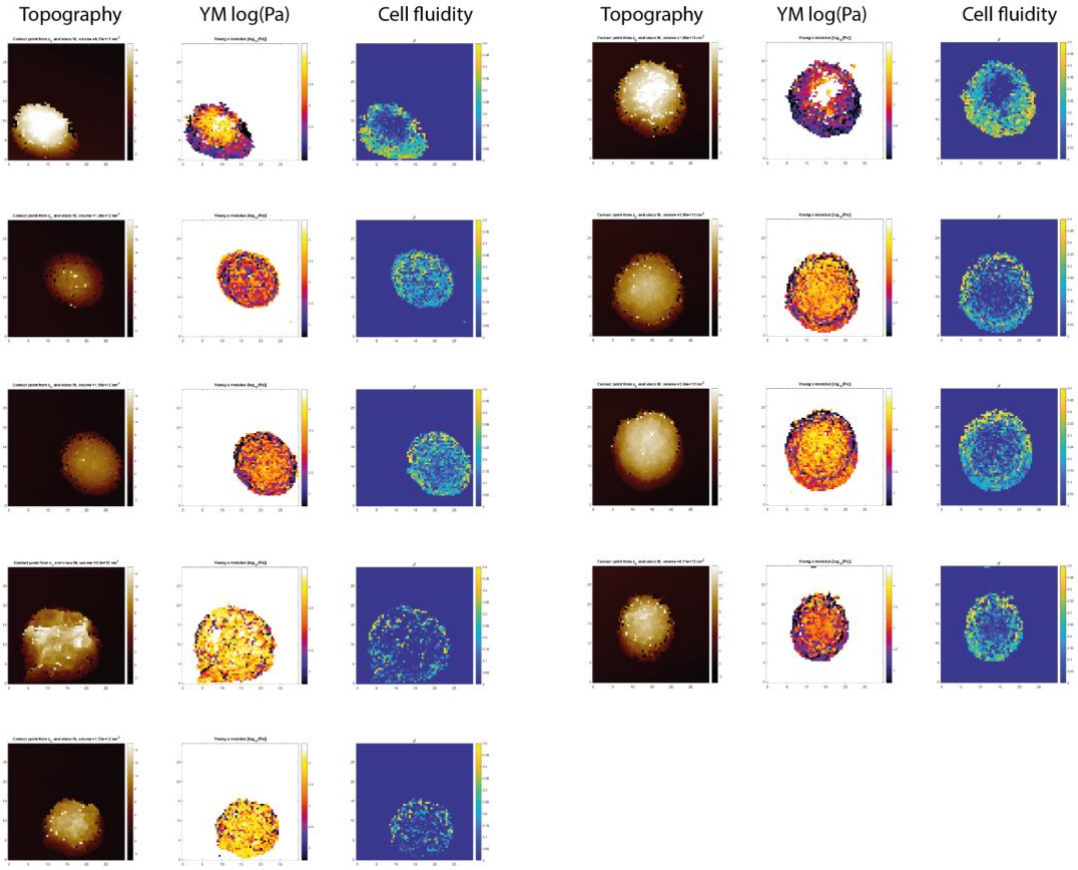
Round macrophages viscoelastic maps



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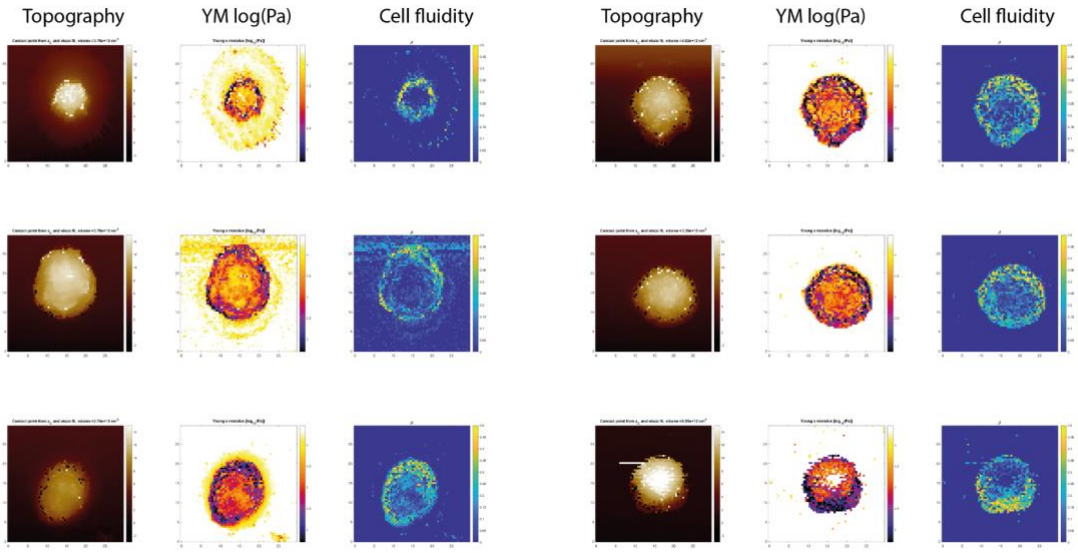
Spread macrophages viscoelastic maps



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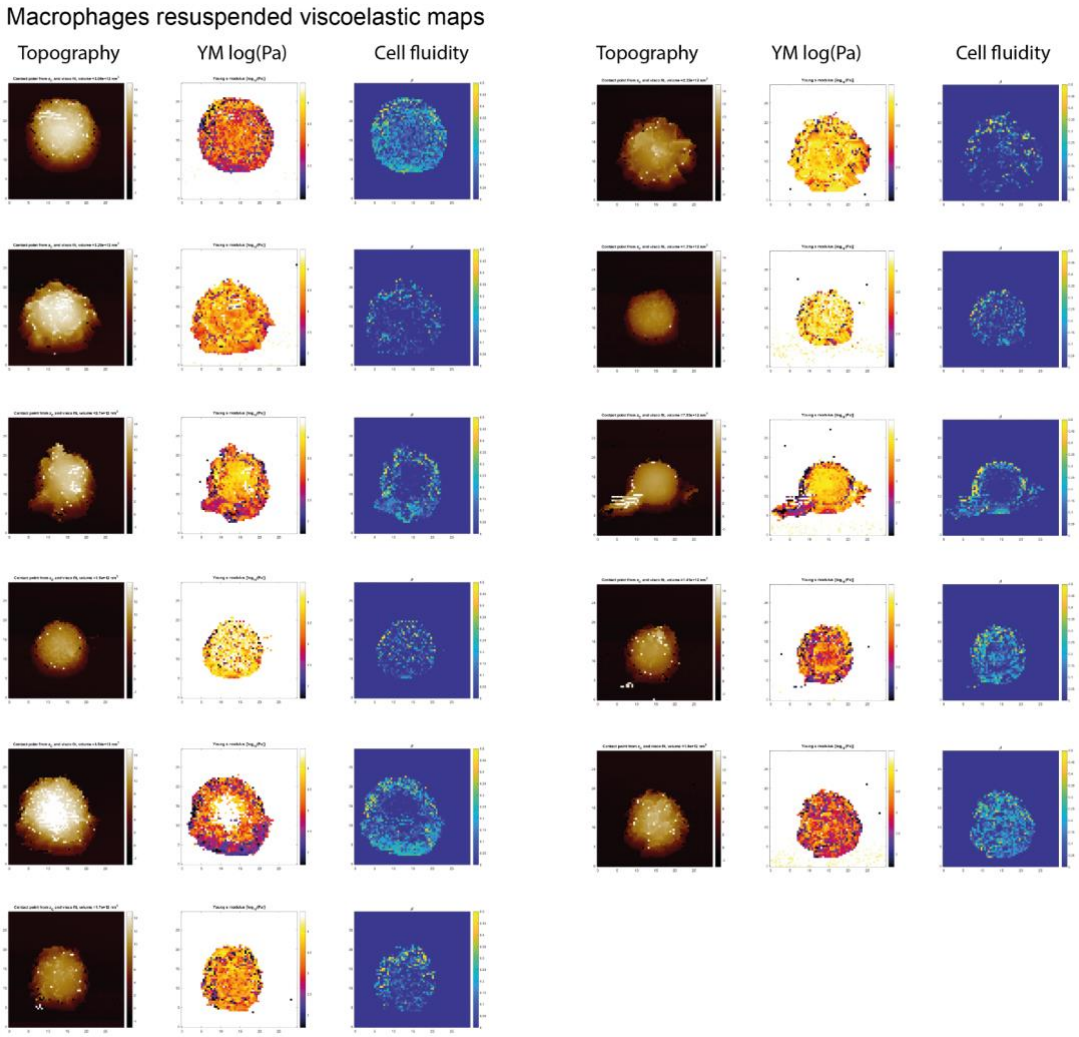
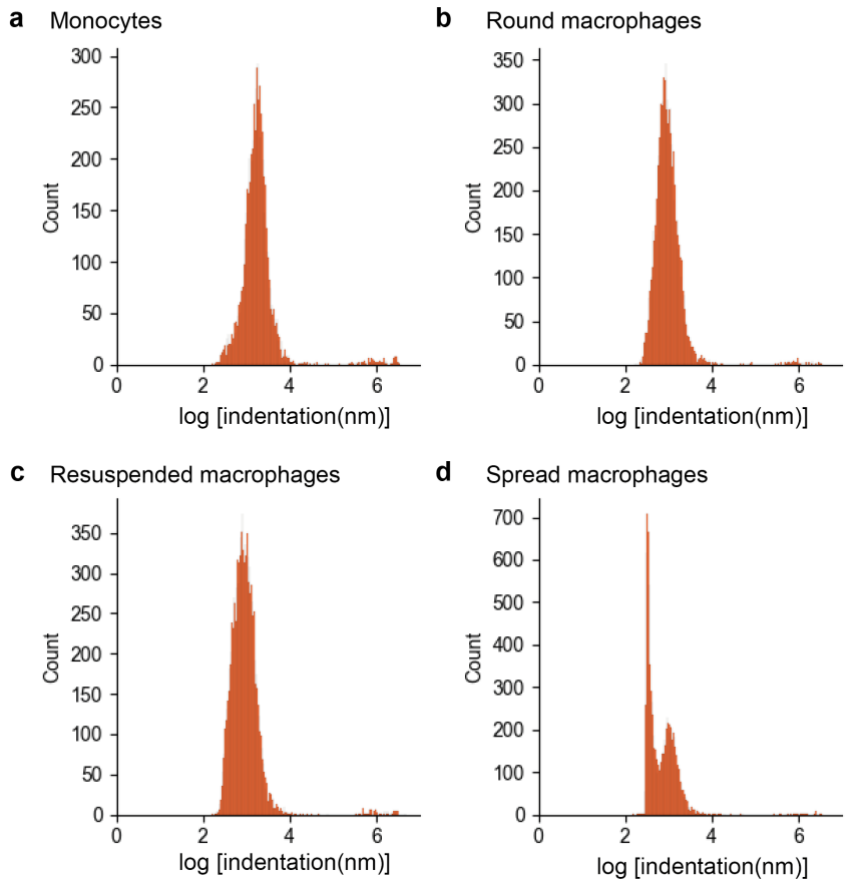
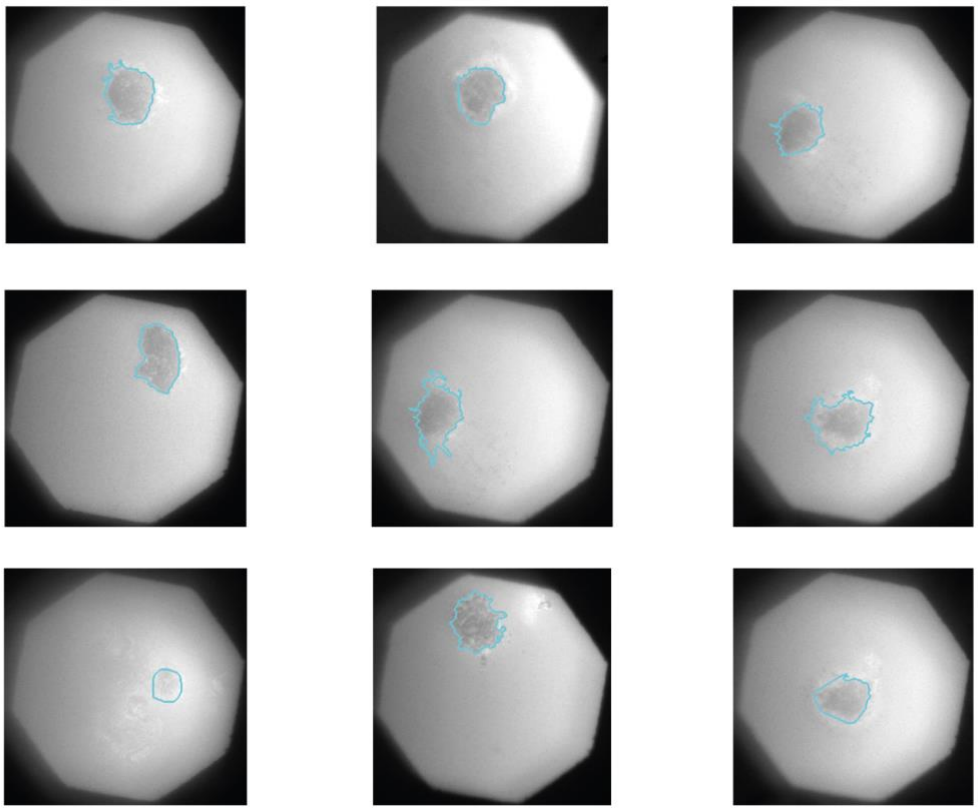


Supplementary Figure 4. Single cell elasticity and fluidity maps. All the cell maps used for the Figure 3 analysis.



Supplementary Figure 5. histograms of the maximum indentation reached during the mechanical analyses of the studied phenotypes.

Monocytes ICM



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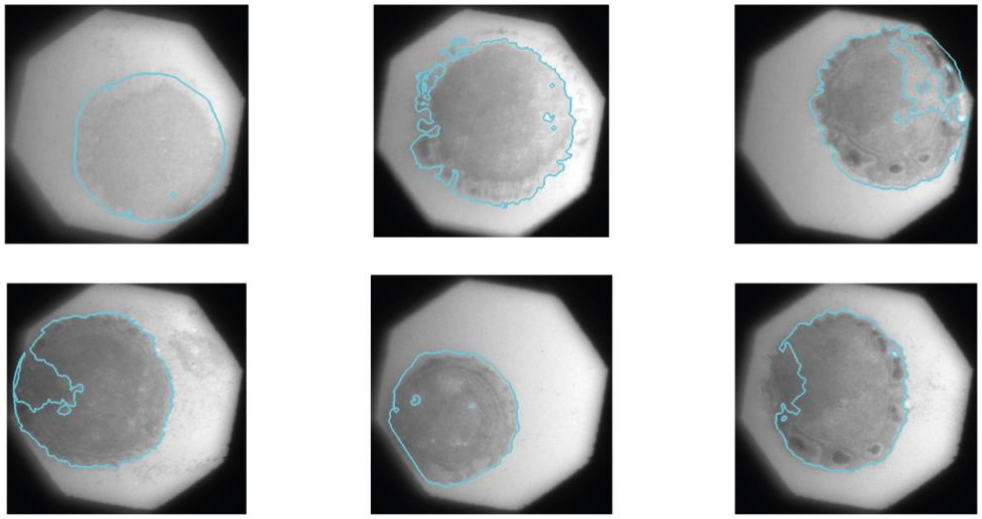
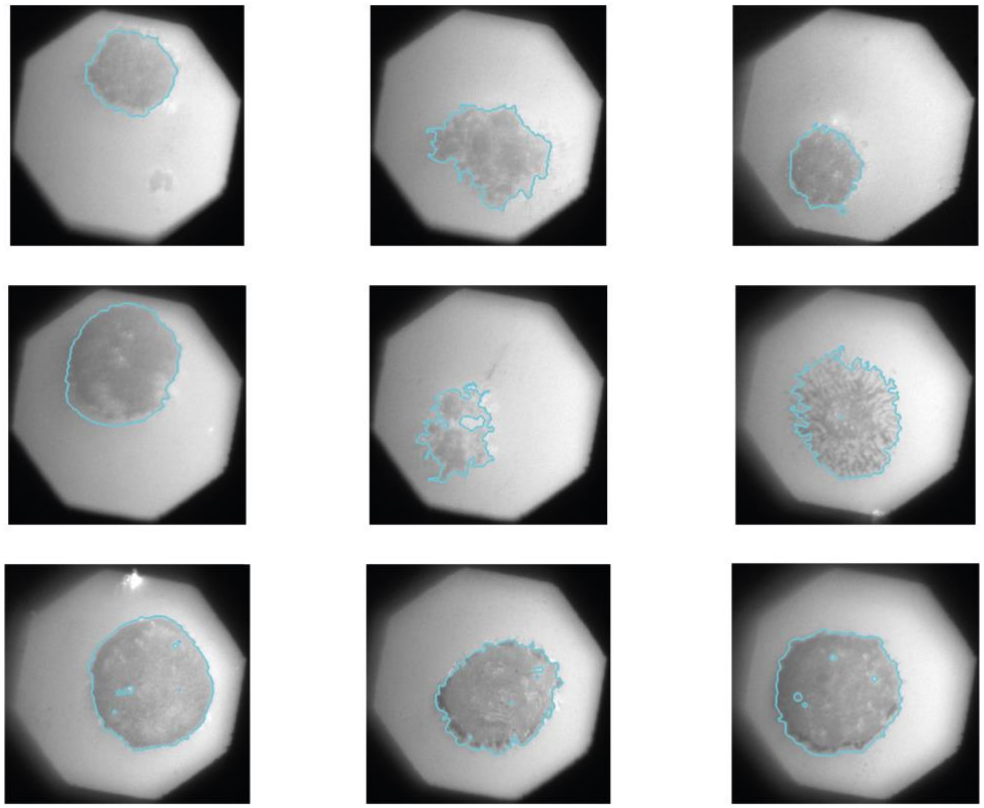
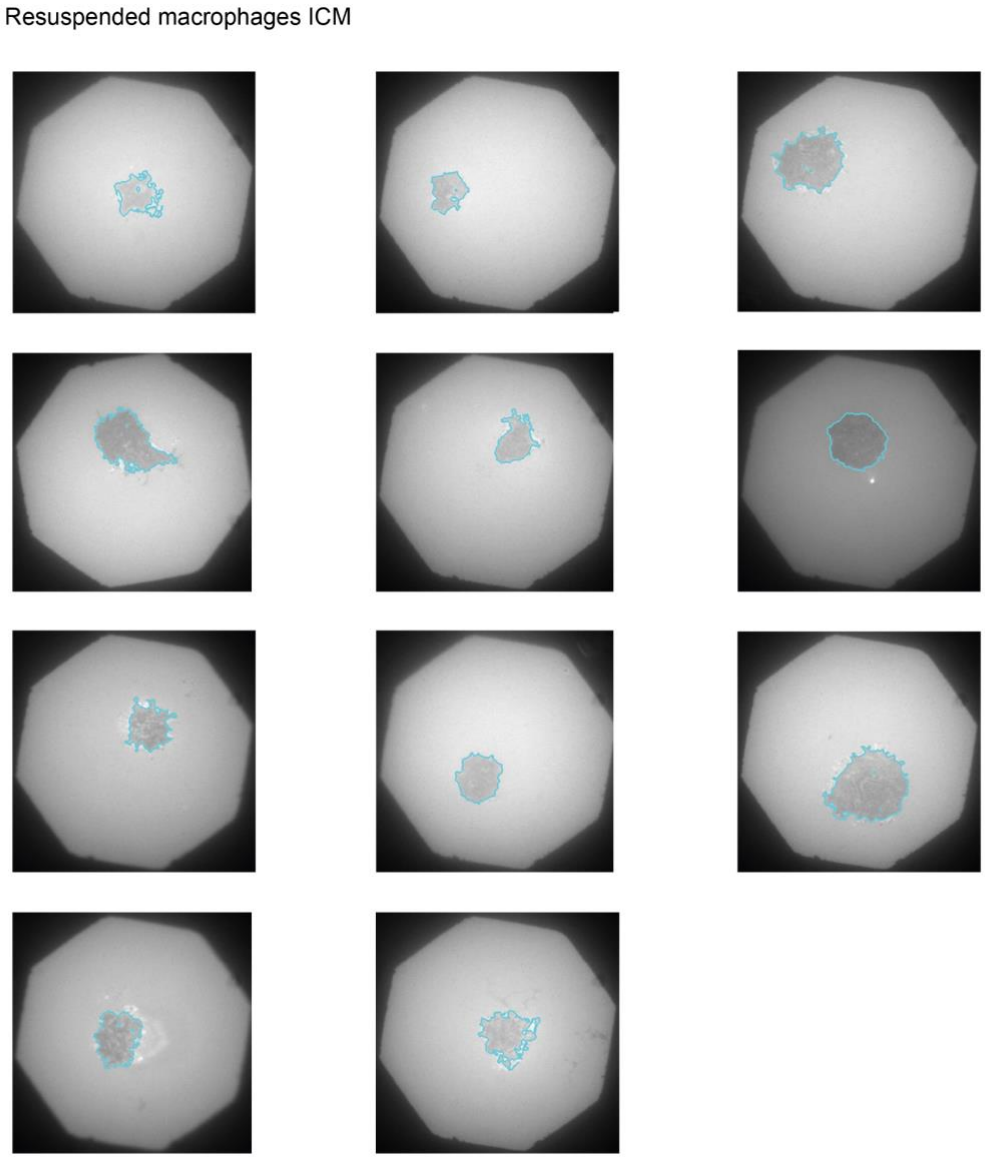
Spread macrophages ICM

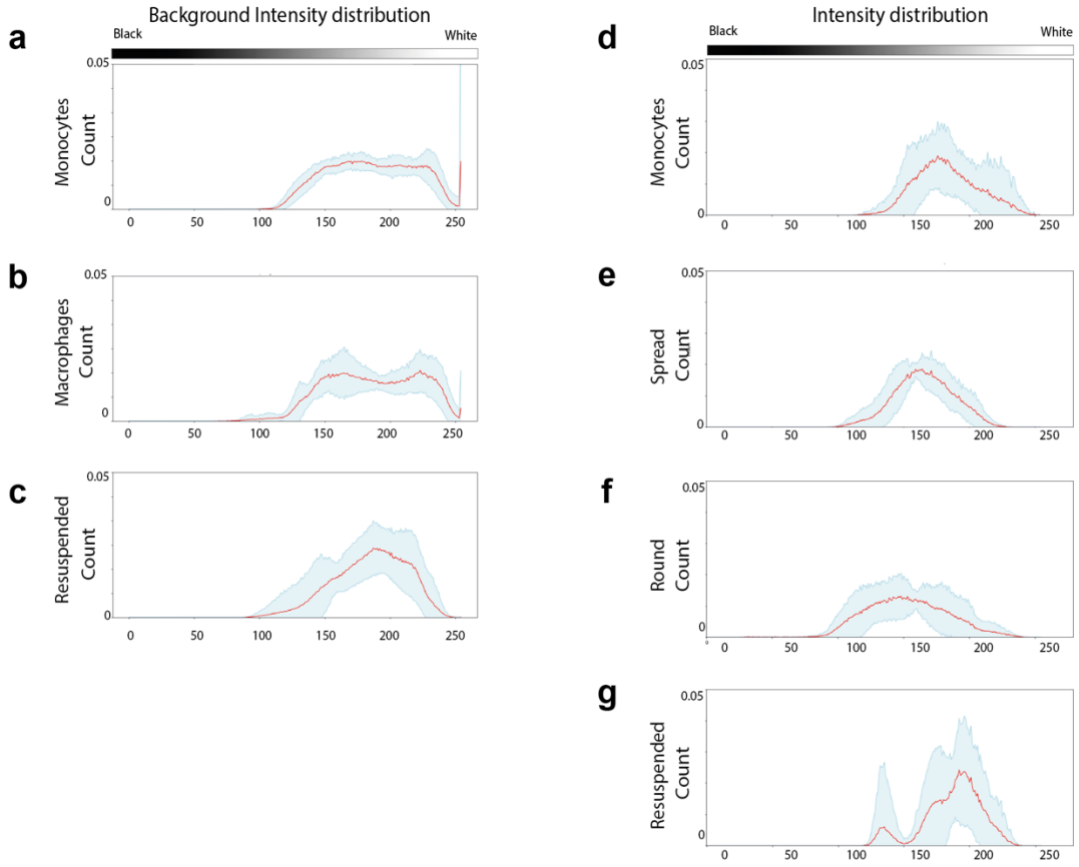


Round macrophages ICM

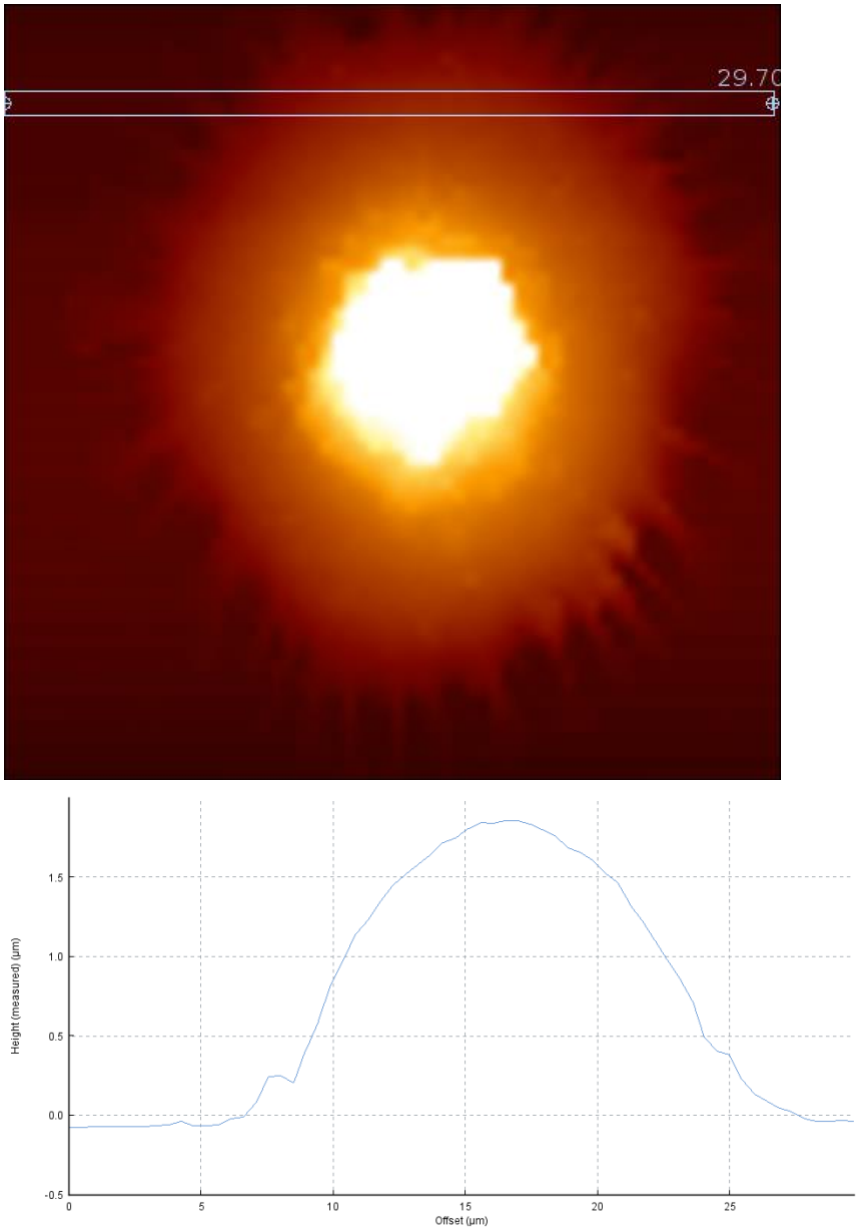




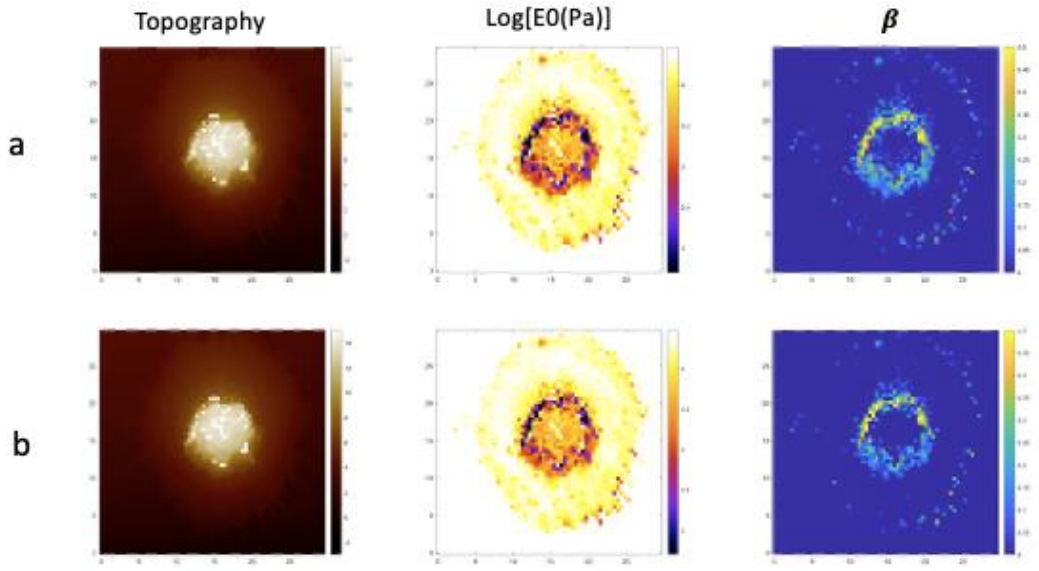
Supplementary Figure 6. ICM single cells images. All the ICM images used in the Figure 4 analysis.



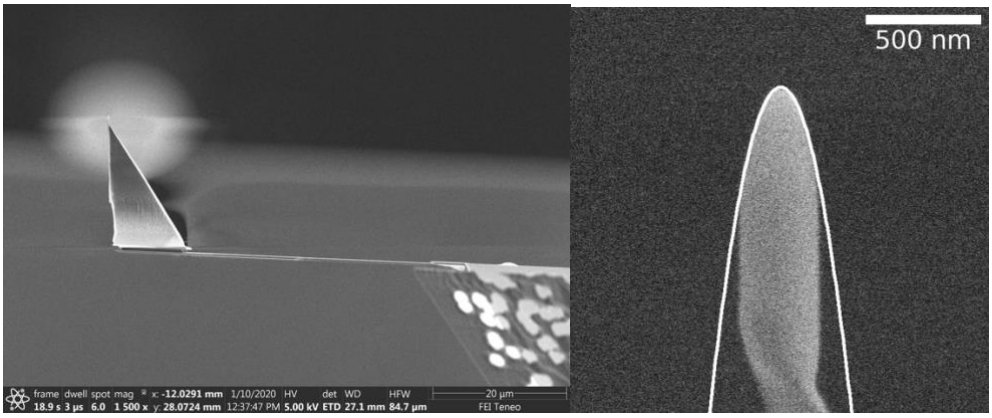
Supplementary Figure 7. a. ICM background intensity for the plates with THP-1 cells (monocytes). b. ICM background intensity for the plates with THP-1 cells treated 48 hours with 20nM PMA (spread and round macrophages). c. ICM background intensity for the plates with THP-1 cells treated 48 hours with 20nM PMA, resuspended with trypsin and immobilized with PLL (resuspended macrophages). d-g. The pixels values inside the mask for all cells at each state were pooled and an average histogram (red line) was generated, with values ranging from 0 (black) and 255 (white) (blue shading represents the standard deviation). Macrophages showed lower pixel values, suggesting that macrophages of any state had stronger adhesion compared to monocytes. In addition, ICM images on macrophages revealed more variability in the grey values, often showing small, circular dark areas. Adhesion decreased in resuspended macrophages whose intensity histogram showed a peak in a similar range as monocytes, with a second peak at lower values suggesting punctual strong adhesion areas.



Supplementary figure 8. Topography image of a spread macrophage. The horizontal rectangle (3 pixels width) was used to compute the average cross-section profile shown below .



Supplementary figure 9. Example of analysis done on the same cell (macrophage spread) with two different viscous drag coefficients. The first one (a, top row) was calculated from an average among regions (0.7 pN·s/µm), and the second (b, low row) was calculated from the region of the spread membrane only (0.9 pN·s/µm).



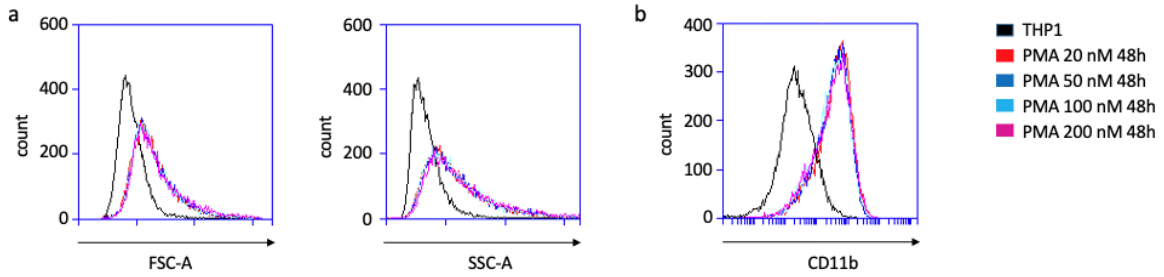
Supplementary figure 10. Scanning Electron Microscopy micrograph of the whole PFQNM-LC cantilever featuring the pyramidal base (lateral view, left) with the protruding tip revealing the parabolic shape of the apex (right). The solid line represents a fit to the top ~500 nm of a parabola of equivalent radius of ~30 nm.

Supplementary table 1. Mean LogE_0(Pa) +/- standard deviation (SD).

	Mean Log E0 (linear)	SD log E0 (+/- linear)	Mean Beta	SD Beta
Monocytes	2.40 (240 Pa)	0.63 ($_{-184\ Pa}^{+783\ Pa}$)	0.24	0.12
Macrophages round	3.08 (1202 Pa)	0.69 ($_{-957\ Pa}^{+4686\ Pa}$)	0.15	0.13
Macrophages spread	3.43 (2691 Pa)	0.73 ($_{-2190Pa}^{+11763\ Pa}$)	0.09	0.12
Macrophages resuspended	3.28 (1905 Pa)	0.61 ($_{-1438\ Pa}^{+5857\ Pa}$)	0.08	0.10

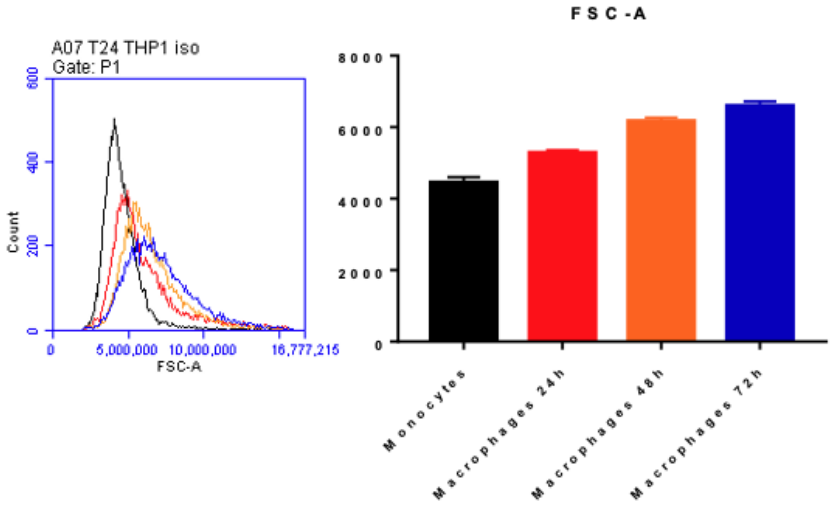
Supplementary table 2. Mean Log [indentation] +/- standard deviation (SD).

	Mean Log Indentation (linear)	SD log indentation (+/- linear)
Monocytes	3.27 (1862 nm)	0.45 ($_{-1201\ nm}^{+3386\ nm}$)
Macrophages round	3.00 (1000 nm)	0.37 ($_{-573\ nm}^{+1344\ nm}$)
Macrophages spread	2.84 (691 nm)	0.36 ($_{-381\ nm}^{+873\ nm}$)
Macrophages resuspended	2.98 (954 nm)	0.39 ($_{-566\ nm}^{+1389\ nm}$)



Supplementary figure 11. a. Morphological characteristics obtained by flow cytometry, forward scatter (FSC-A) and side scatter (SSC-A) for THP-1 cells non-treated or treated with increasing dose of PMA for 48h. b. Quantification of the expression of CD11b by flow cytometry on THP-1 and PMA differentiated macrophages for increasing dose of PMA after 48h of differentiation.

a



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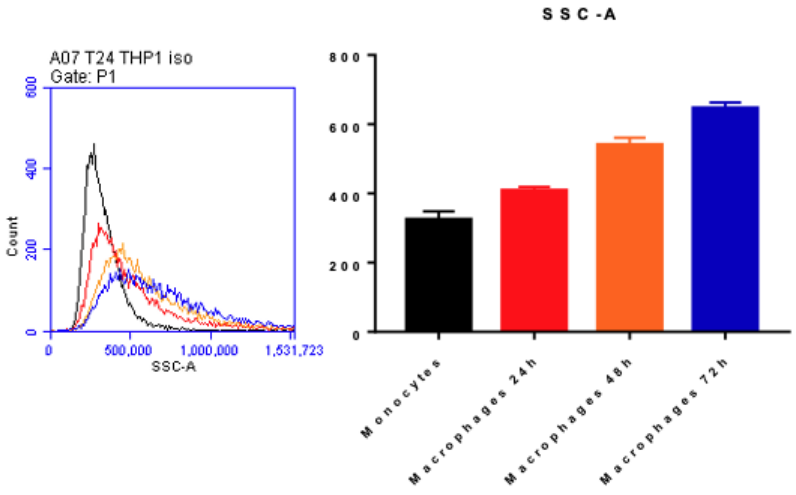
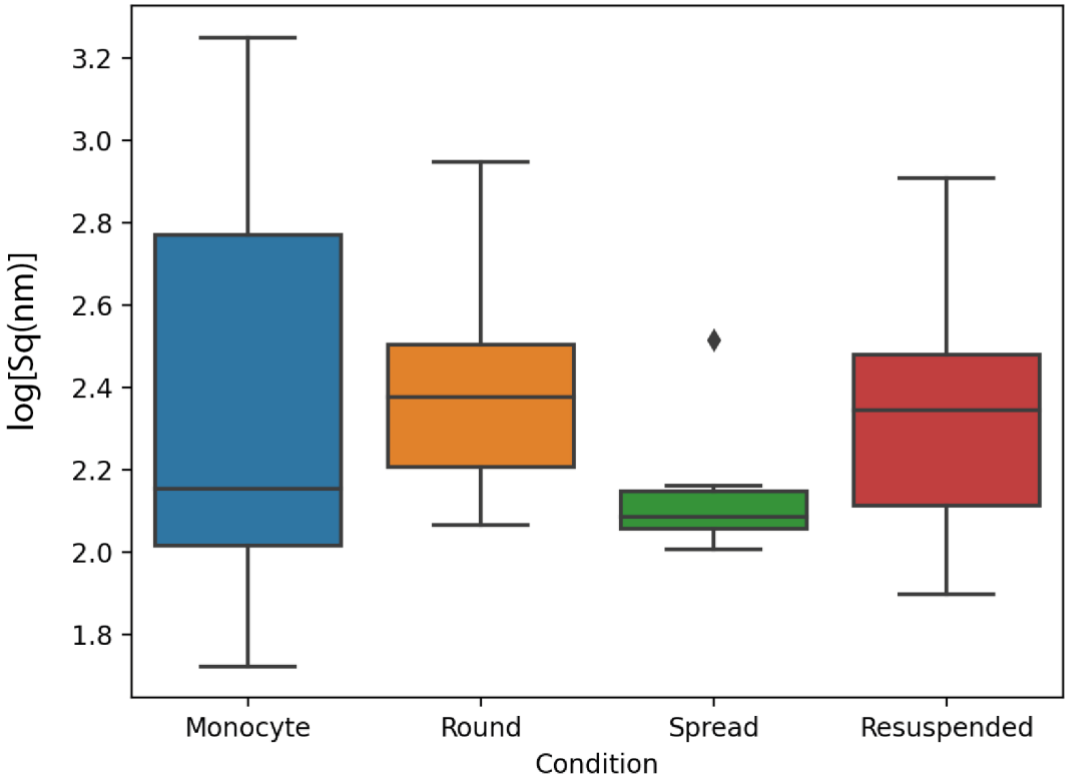
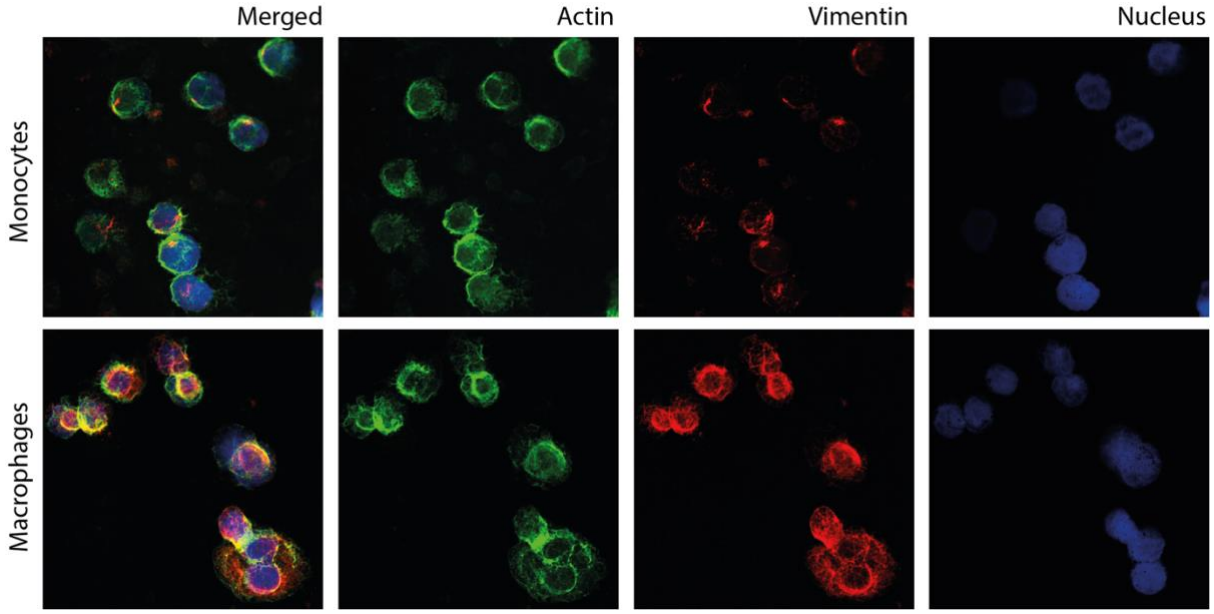
b



92
93 Supplementary figure 12. a-b. Morphological properties obtained by flow cytometry, forward scatter
94 (FSC-A), and side scatter (SSC-A) for THP-1 cells non-treated or treated with 20nM PMA at after 24h,
95 48h, and 72h. Quantification of FSC and SSC by flow cytometry on THP-1 and PMA differentiated
96 macrophages for 20nM PMA at time 0, 24h, 48h, and 72h.
97
98



Supplementary figure 13. RMS roughness (Sq) from the central area (10 px x 10px) in monocytes, round, spread, and resuspended macrophages. Non-statistical significance was found in macrophage groups (round, spread, and resuspended) against monocytes (t-test, $p>0.05$).



Supplementary figure 14. Fluorescence microscopy confocal images of THP-1 monocytes and differentiated macrophages (48h, 20nM PMA). Actin cytoskeleton (F-actin) in green (CellMask 488), vimentin in red (RV202), and the cell nucleus in blue (dapi).

Supplementary table 3. Statistical analysis of morphological (HTI), adhesive (ICM), and mechanical (AFM) parameters of macrophages studied groups against undifferentiated monocytes. Unpaired Student's *t*-test p values for each phenotype against monocytes.

	Compactness (HTI)	log[Volume] (HTI)	log[Surface] (HTI)	Area (ICM)	Perimeter (ICM)	Ellipse a/b (ICM)	Log[E_0] (AFM)	β (AFM)	Sq (AFM)
Round	0.4	0.003	0.016	$5*10^{-5}$	0.009	0.02	$6*10^{-5}$	0.0003	0.38
Spread	0.01	$5*10^{-7}$	0.00012	$1*10^{-10}$	0.003	0.015	0.0001	0.0001	0.11
Resuspended	0.5	0.08	0.08	0.14	0.24	0.1	$2.6*10^{-7}$	$5*10^{-9}$	0.30