Electronic Supplementary Material (ESI) for Nanoscale Horizons.

Supplementary files

Nanoscale membrane architecture of healthy and pathological red blood cells

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Table S1. Young's modulus values of hRBCs and sRBCs before and after treatment with cytoskeleton disrupting drugs.

Cell type	Low load		Medium load		High load	
	Mean (kPa)	*SD (kPa)*	*Mean (kPa)*	*SD (kPa)*	*Mean (kPa)*	*SD (kPa)*
hRBCs (Control)	24.2	23.9	32.1	29.9	40	34.9
hRBCs LatrunculinA 0.5 µM	10.4	4.7	13.8	4.9	15	5.8
hRBCs Blebbistatin 50 µM	8.4	5.4	12.3	5.6	13.8	5.8
sRBCs (Control)	87	83.4	298	362.8	380	482.9
sRBCs LatrunculinA 0.5 µM	37.8	17.5	39.1	14.7	41	16.8
sRBCs Blebbistatin 50 µM	32	15.6	39.7	25.2	42	26.6

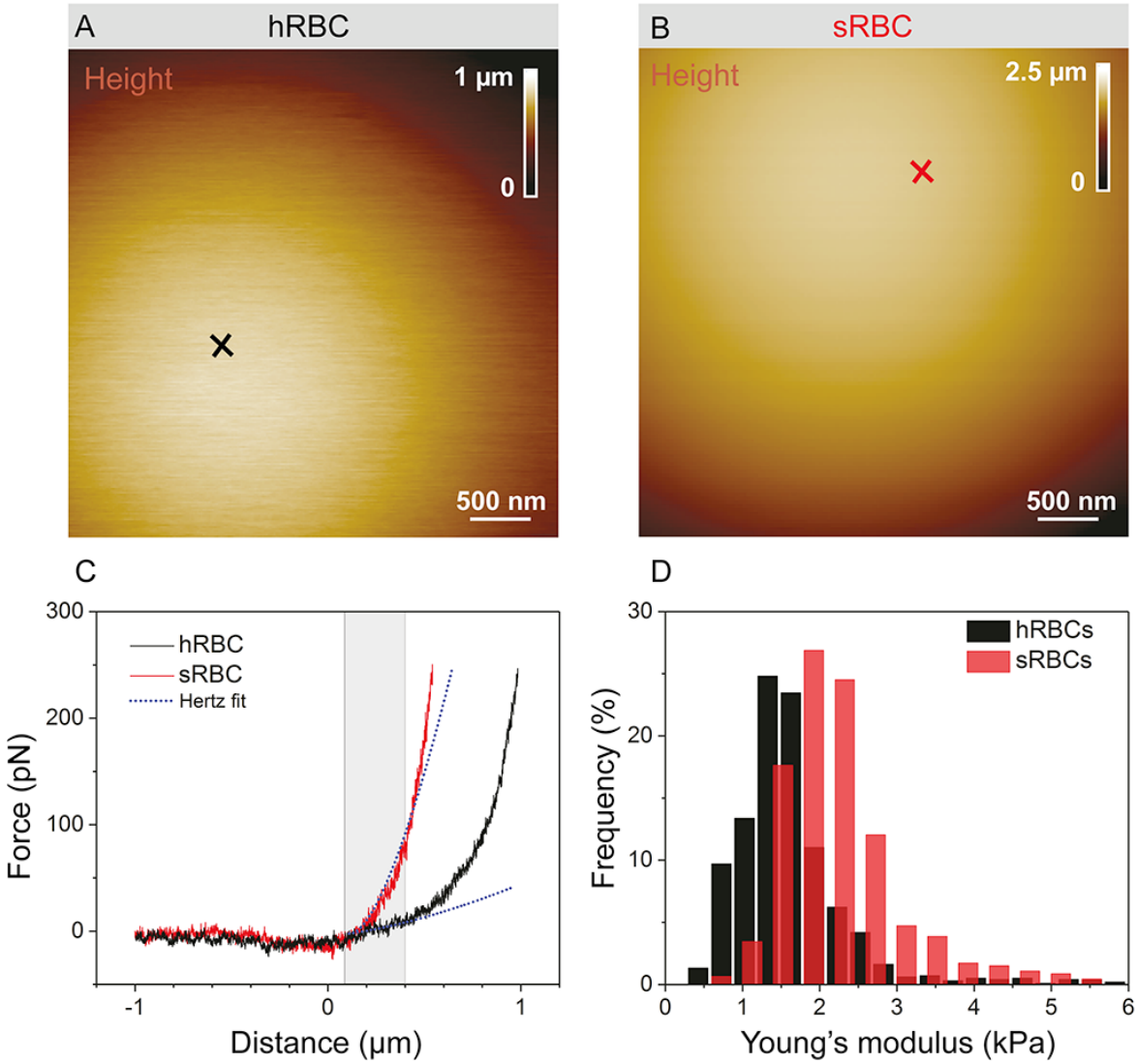

Figure S1. FD-based AFM of hRBCs and sRBCS. (A,B) Height images of a hRBC **(A)** and a sRBC **(B)** acquired in FD-based AFM. **(C)** Representative FD curves acquired in the central area of the cells, as indicated by crosses in (A-B) and corresponding Hertz fit of the 0-400 nm indentation part (blue dashed line). The slope of the FD curves is steeper on sRBCs. **(D)** Histograms showing the elastic Young's modulus of hRBCs and sRBCs at low indentation rates (~1 µm/s). The data are representative of 10 hRBCs from at least 3 patients and 9 sRBCs from a spherocytosis patient during at least 3 independent experiments per condition.

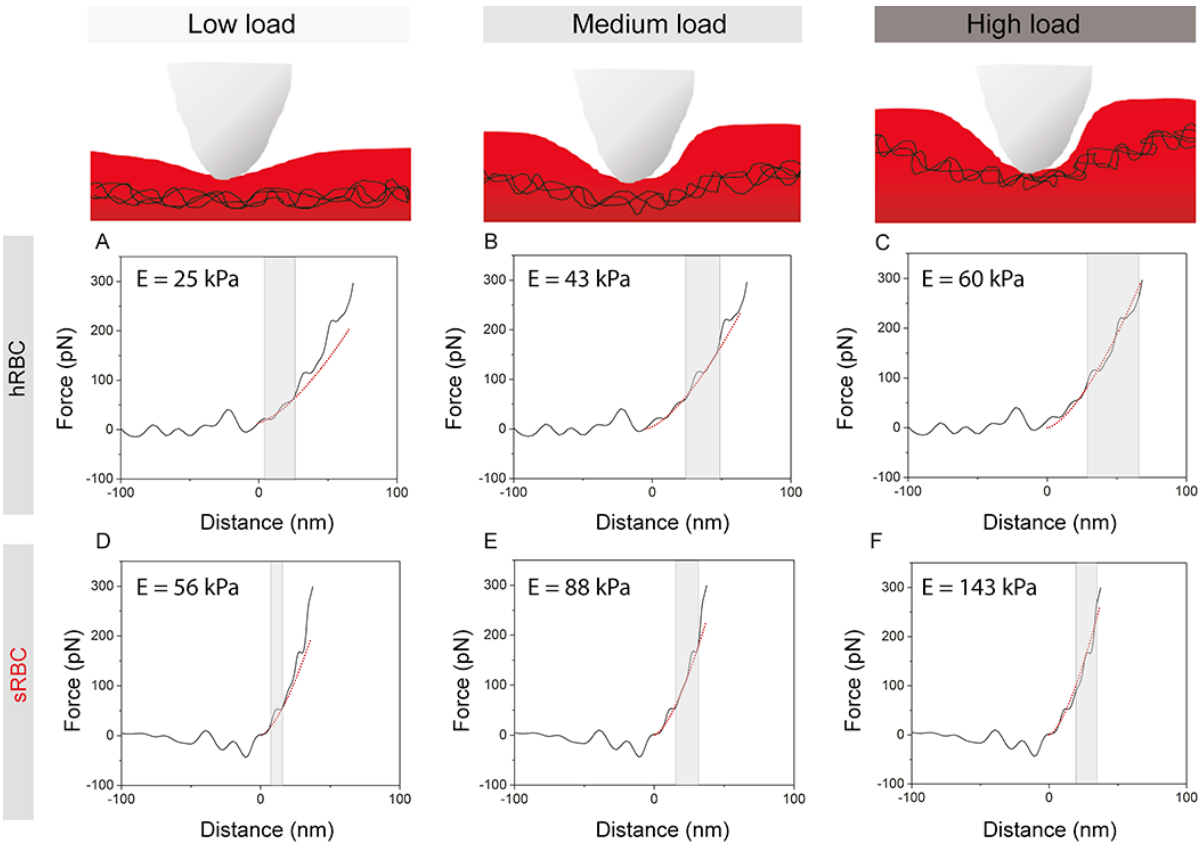

Figure S2. Representative FD-curves and fits for the different loads. (A-C) Representative FD curve obtained on a hRBC and corresponding best Hertz fit obtained for the low load (A), medium load (B) and high load (C). (D-F) Representative FD curve obtained on a sRBC and corresponding best Hertz fit obtained for the low load (D), medium load (E) and high load (F).

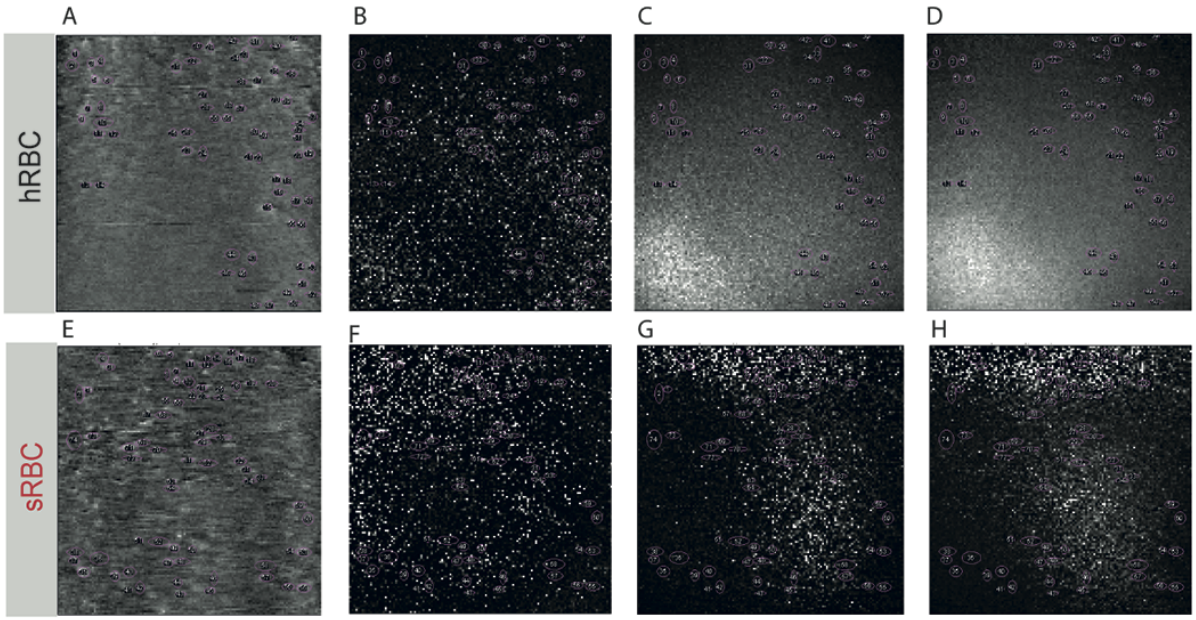

Figure S3. Analysis of correlation between height and Young's Modulus maps. Multiparametric height and Young's Modulus maps (at low, medium and high loads) were binarized into black and white images and loaded in the Image J software. First row shows results obtained on a healthy cell and the second row on a spherocyte. **(A)** and **(E)** Protrusions in the height image were selected manually and the corresponding areas in the Young's Modulus maps were marked automatically by the software for low load **(B,F)**, for medium load **(C, G)** and for high load **(D,H)**. The intensities of the peaks in both Height and Young's modulus images were saved in separate tables and further converted into real height and elastic moduli.

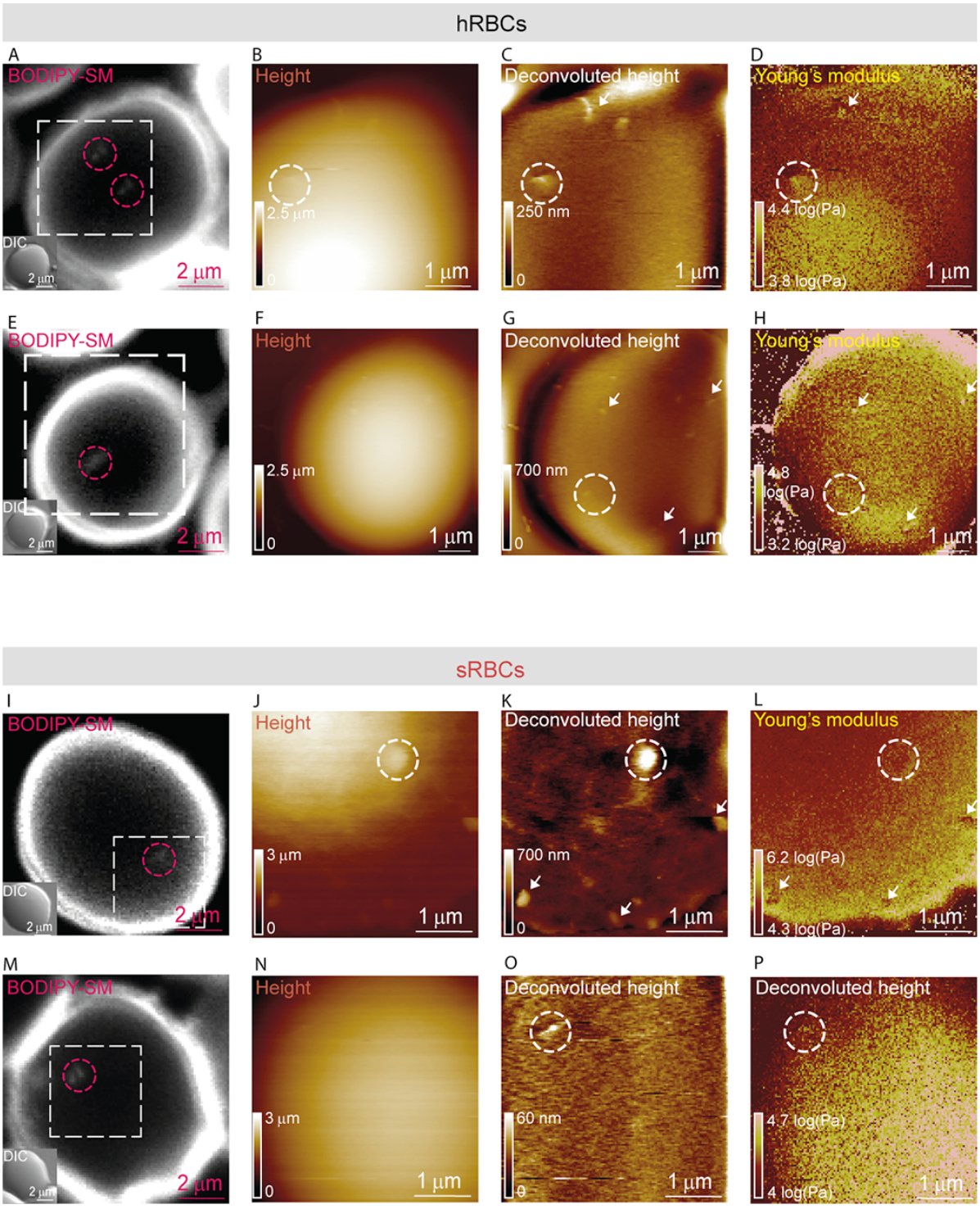

Figure S4. Correlation between FD-based AFM and fluorescence imaging on hRBCs (upper panel) and sRBCs (lower panel). Fluorescence images of hRBCs **(A),(E)** and **(I),(M)** sRBCs labeled with BODIPY-SM, along with corresponding DIC images in inset. The dashed square on the fluorescence image shows where the AFM image was recorded and circles indicate submicrometric SM domains. **(B),(F)** and **(J),(N)** FD-based AFM height images of hRBCs and sRBCs, respectively. **(C),(G)** and **(K),(O)** Deconvoluted height images of hRBCs and sRBCs. and corresponding Young's modulus maps in **(D),(H)** and **(L),(P)**. The images are representative of 28 cells from 3 healthy and 3 spherocytosis patients imaged in the same conditions during 3 independent experiments.

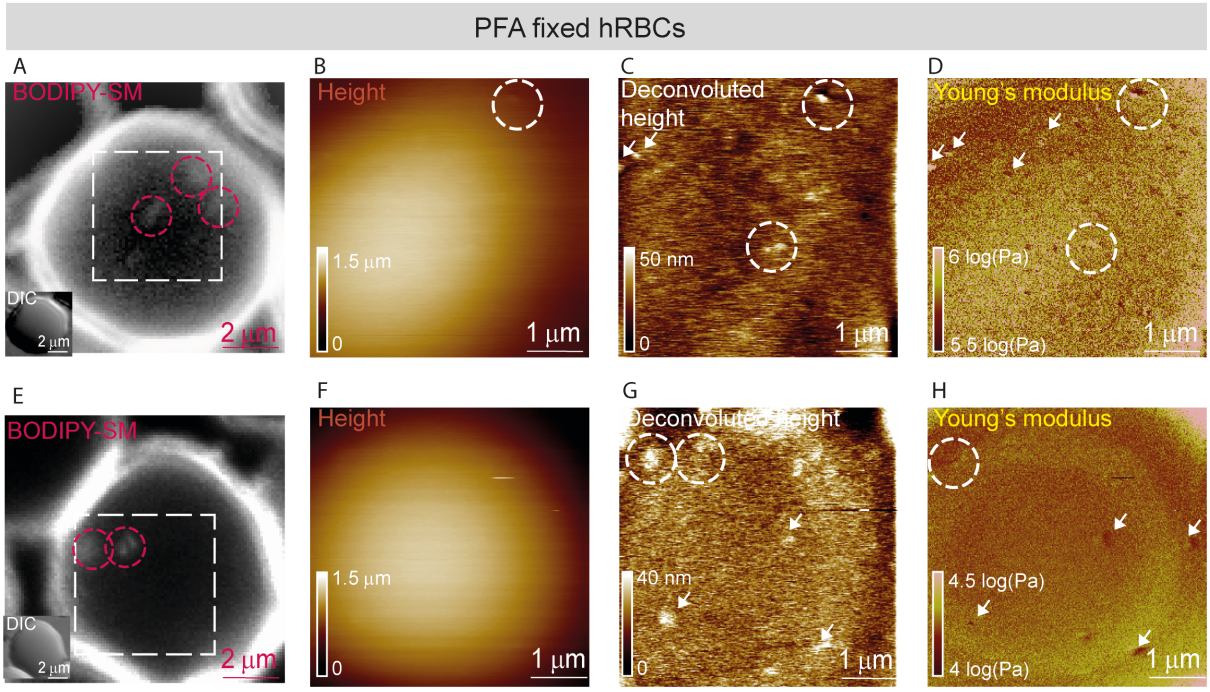

Figure S5. Correlation between FD-based AFM and fluorescence imaging on 4% PFA fixed hRBCs Fluorescence images of hRBCs **(A),(E)** labeled with BODIPY-SM, along with corresponding DIC images in inset. The dashed square on the fluorescence image shows where the AFM image was recorded and circles indicate submicrometric SM domains. **(B),(F)** FD-based AFM height images of fixed hRBCs , respectively. **(C),(G)** Deconvoluted height images of fixed hRBCs and corresponding Young's modulus maps in **(D),(H)**. The images are representative of 12 cells from 3 healthy individuals imaged in the same conditions during 3 independent experiments.