Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

Substrate stiffness reduces particle uptake through mechanoregulation in a size-dependent manner

*Aaron Lee, Mauro Sousa de Almeida, Daela Milinkovic, Dedy Septiadi, Patricia Taladriz-Blanco, Celine Loussert-Fonta, Sandor Balog, Amelie Bazzoni, Barbara Rothen-Rutishauser, Alke Petri-Fink**

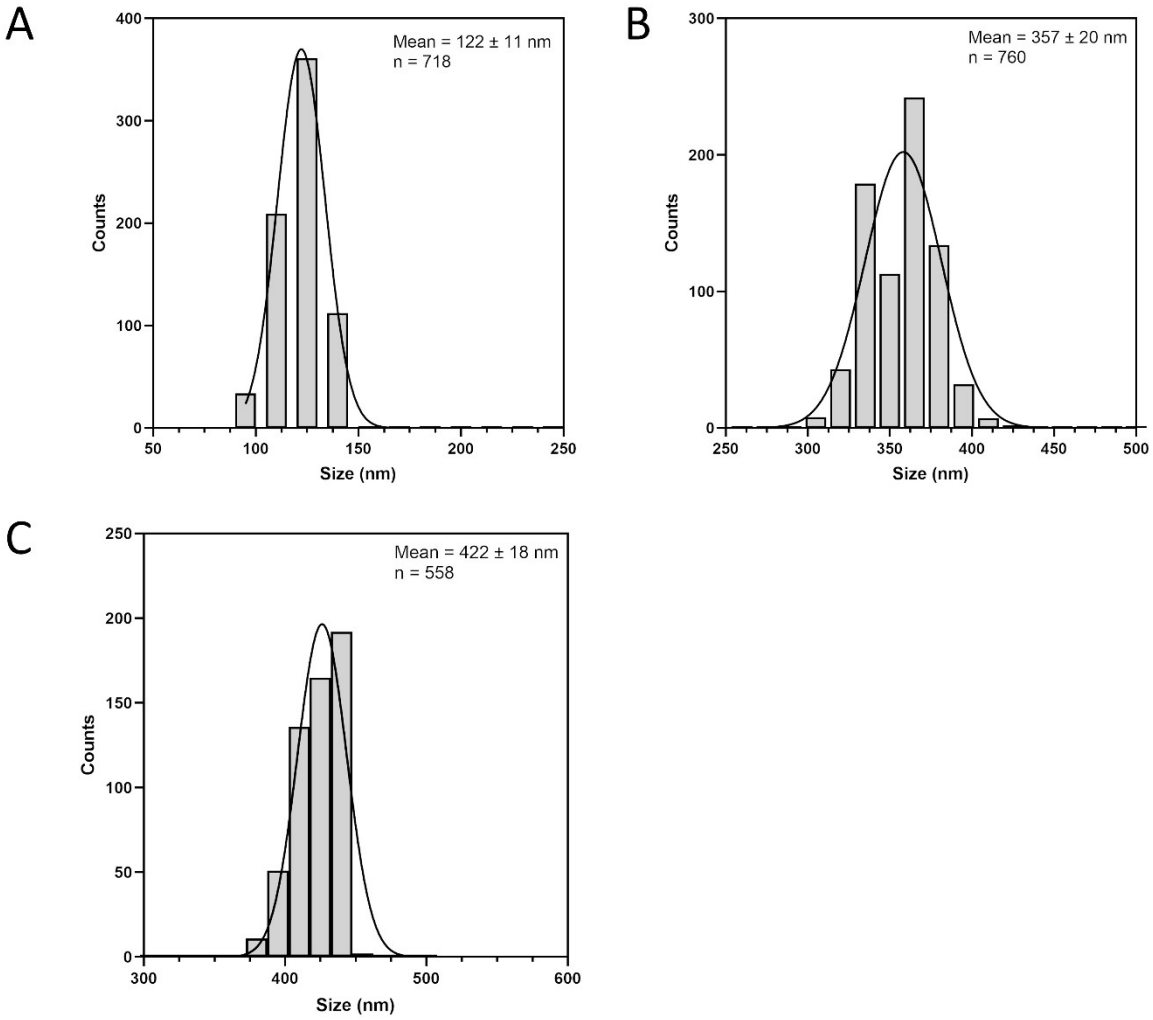


Figure S1. Determination of particle sizes by transmission electron microscopy. A) Size distribution of SiO_2 – 120 nm. B) Size distribution of SiO_2 – 360 nm C) Size distribution of SiO_2 – BDP. Data presented as mean ± standard deviation.

Table S1. Characterisation of different silica particles by transmission electron microscopy, dynamic light scattering and zeta potential analysis. Hydrodynamic diameter and zeta potential determined in ultrapure water at 25C. Data presented as mean ± standard deviation, n = 10.

	d_{TEM} (nm)	d_{DLS} (nm)	PDI	Zeta potential (mV)
Cy5-small	122 ± 11	145 ± 4	0.003	-48 ± 6.8
Cy5-large	357 ± 20	362 ± 8	0.005	-37 ± 4.5
BDP	422 ± 18	420 ± 13	0.006	-51 ± 2.6

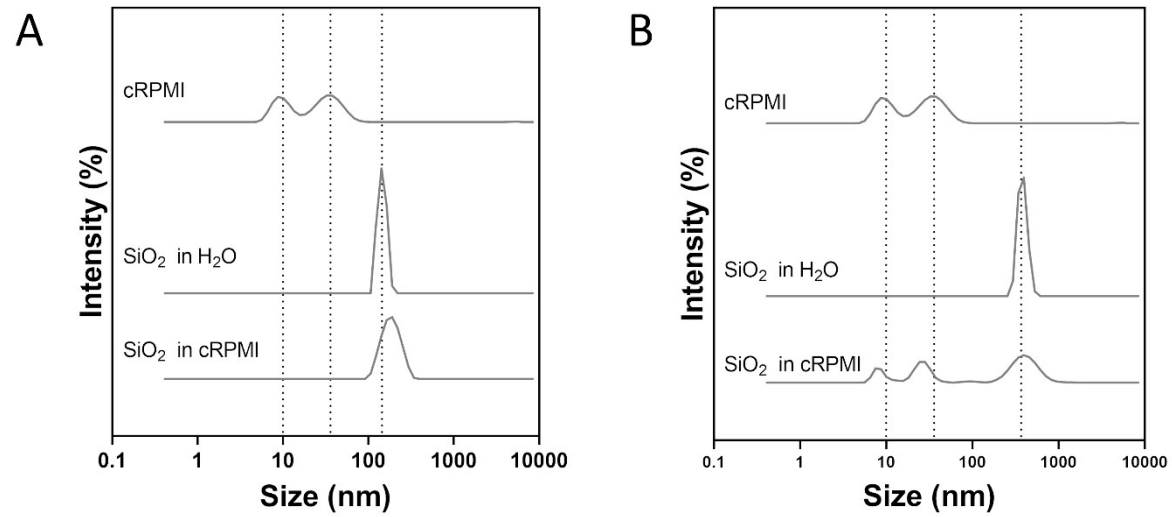
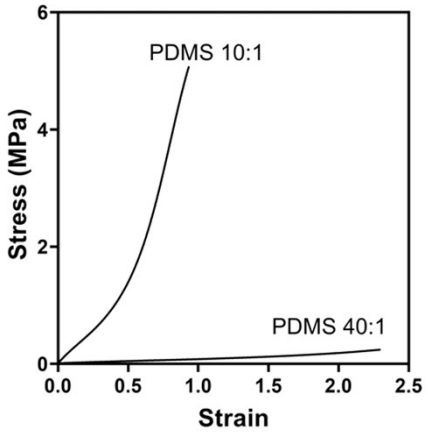


Figure S2. Stability assessment of SiO_2 particles in water and serum supplemented cell culture medium after six hours at 37 °C. A) Intensity-weighted size distribution of 120 nm SiO_2 (Cy5-small) B) Intensity-weighted size distribution of 360 nm SiO_2 (Cy5-large).



	E (MPa)	ε_f
10:1	2.68 + 0.04	0.91 + 0.1
40:1	0.13 + 0.03	2.09 + 0.4

Figure S3. Representative stress strain curves obtained for PDMS with different curing ratios, 10:1 and 40:1 respectively. Data presented as mean ± standard deviation. $N = 5$.

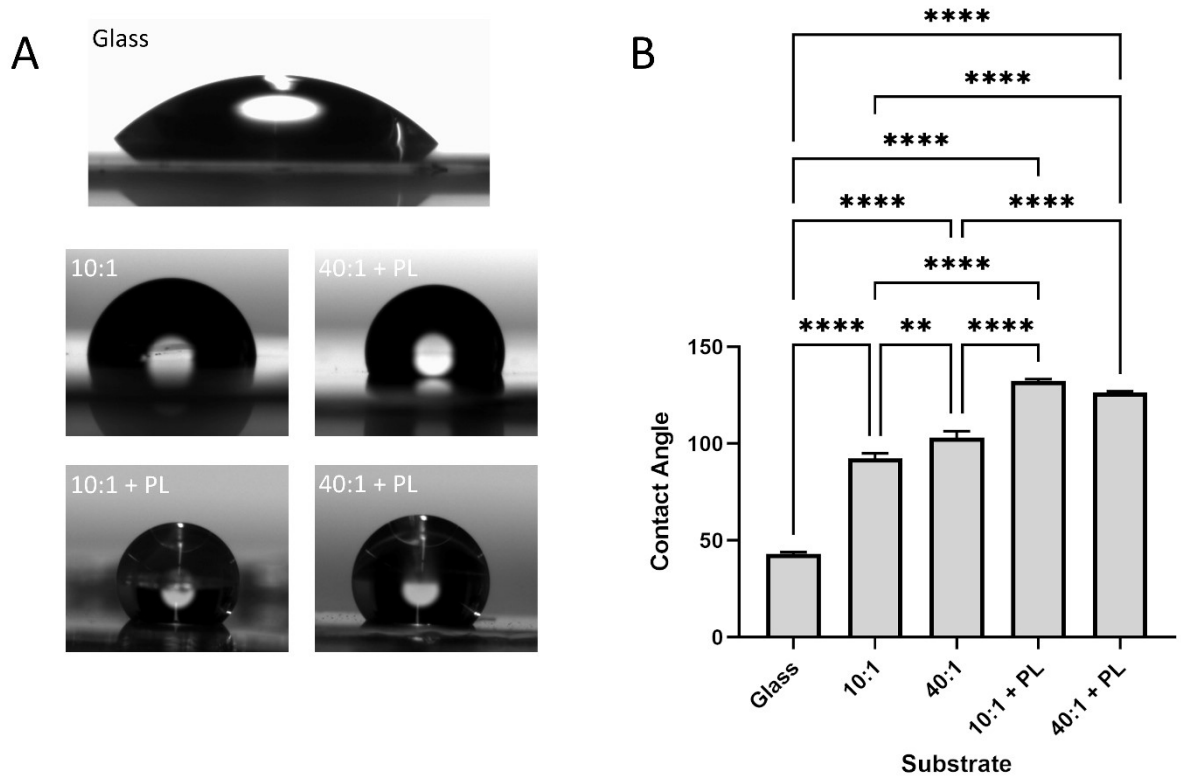


Figure S4. Water contact angle obtained on different culture surfaces obtained via sessile drop. A) representative images of substrate contact angles. B) Mean water contact angle + standard deviation, n = 5. Statistical significance assessed by one-way ANOVA with Tukey's test. ** $p < 0.01$, ****$p < 0.0001$

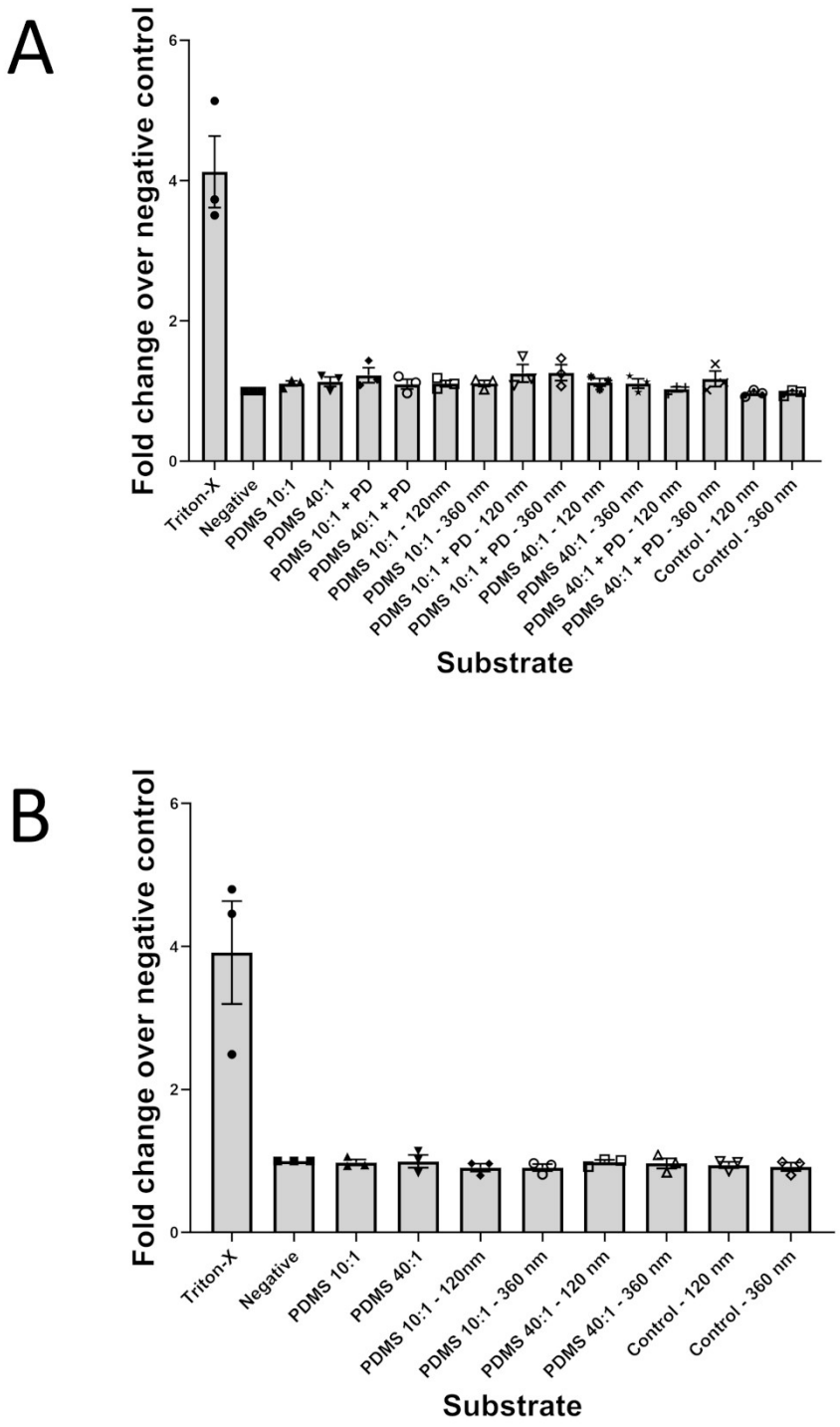


Figure S5. Cytotoxicity response of surfaces and particles assessed using the lactate dehydrodenase assay for A) A549 and B) J774A.1 after 24 hours determined over three biologically independent replicates. Data presented as mean ± standard error of the mean. PD – particle decorated surface, Control surface is TCPS.

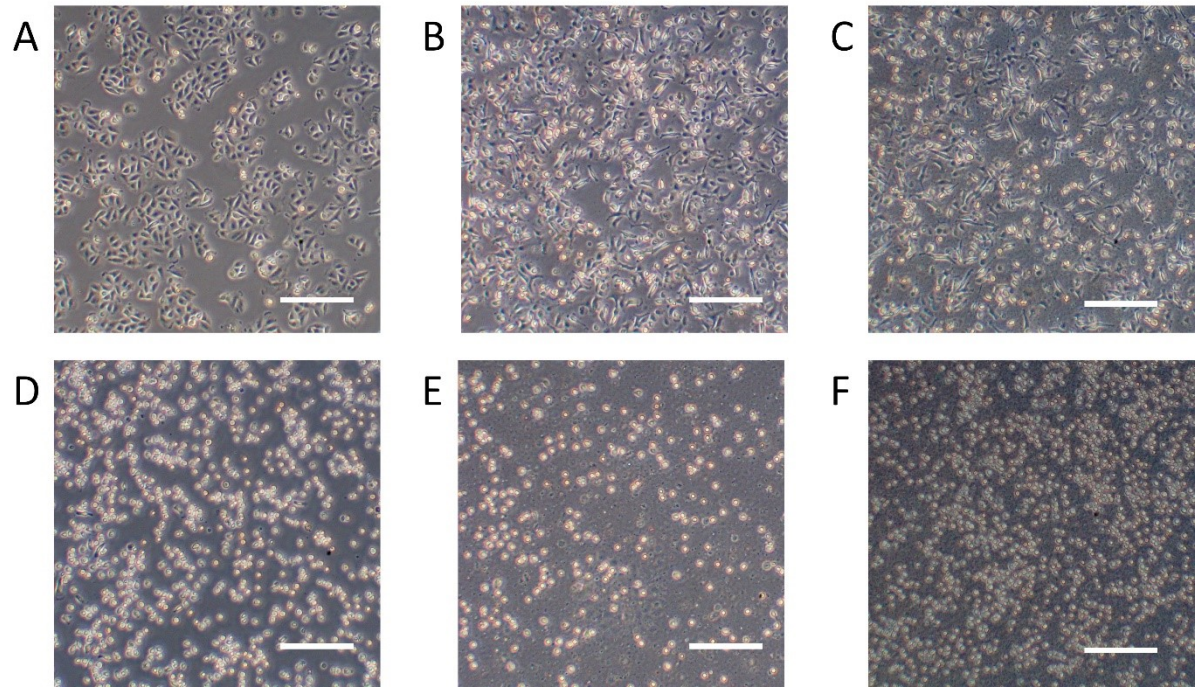


Figure S6. Phase contrast images of A549 cultured on A) TCPS, B) PDMS 10:1 and C) PDMS 40:1 for 24 hours. Phase contrast images of J774A.1 cultured on D) TCPS, E) PDMS 10:1 and F) PDMS 40:1 for 24 hours. Scale bar represents 100 μm.

Table S2. Summary of A549 spreading characteristics on different substrates following 24 hours of culture. For three biologically independent replicates, where n > 100. Data presented as mean ± standard error of the mean.

	Area (μm^2)	Length (μm)	Aspect Ratio	*n*
Glass	1557 ± 57	52 ± 0.9	1.44	344
10:1	1138 ± 39	47 ± 0.8	1.61	429
40:1	1051 ±36	45 ± 0.7	1.60	442

Table S3. Summary of J774A.1 spreading characteristics on different substrates following 24 hours of culture. For three biologically independent replicates, where n > 100. Data presented as mean ± standard error of the mean.

	Area (μm^2)	Length (μm)	Aspect Ratio	*n*
Glass	378 ± 12	26 ± 0.5	1.46	403
10:1	648 ± 25	35 ± 1.0	1.54	200
40:1	562 ± 19	33 ± 0.9	1.54	234

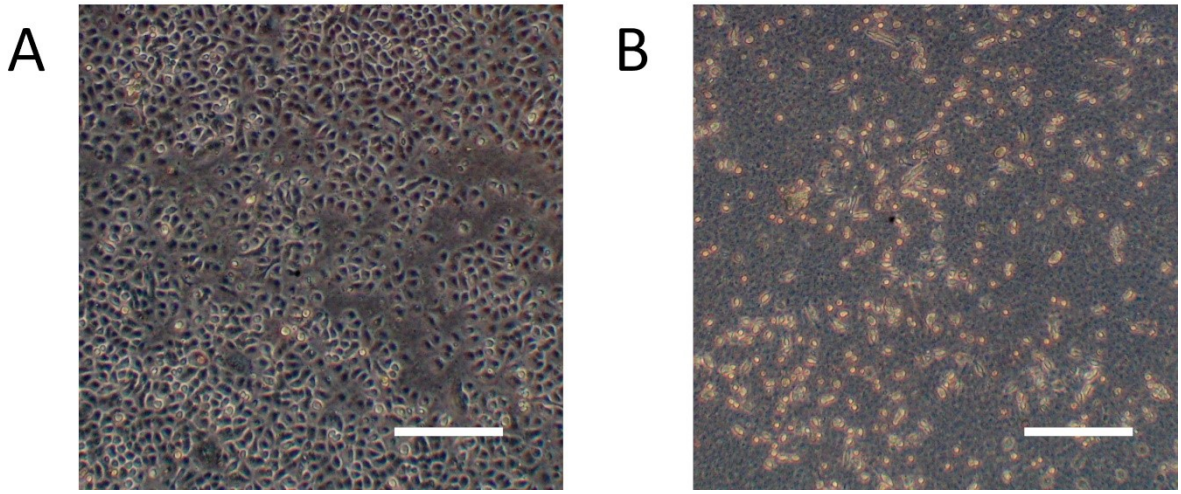


Figure S7. Phase contrast images of A549 cultured on A) PDMS 10:1 with an attached layer of 420 nm silica particles. B) PDMS 40:1 with an attached layer of 420 nm silica particles. Scale bar represents 100 μm.

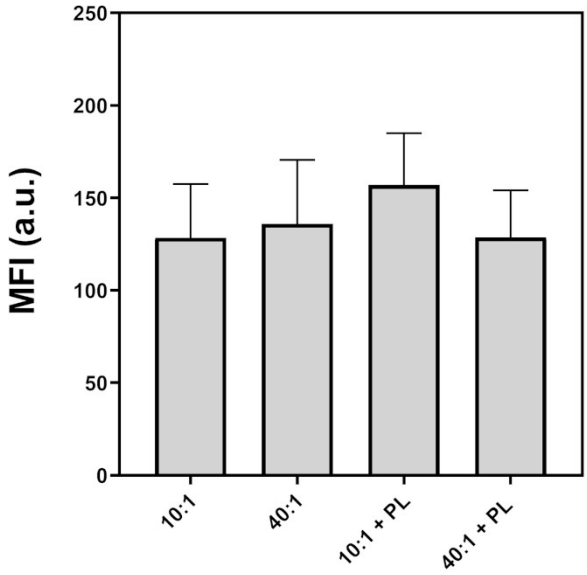


Figure S8. Median fluorescence intensity of A549 on different substrates in flow cytometry showing BDP channel from three biologically independent replicates. Fluorescence of cells in contact with BDP-labelled silica particles do not show any significant difference with cells not in contact with BDP-labelled particles indicating that no substantial removal of BDP-silica particles from the surface occurs. Data presented as mean ± standard error of the mean.

Table S4. Summary of A549 spreading characteristics on different substrates following 24 hours of culture. For three biologically independent replicates, where n > 100. Data presented as mean ± standard error of the mean.

	Area (μm^2)	Length (μm)	Aspect Ratio	*n*
10:1 + PD	804 ± 25	42 ± 0.8	1.73	416
40:1 + PD	917 ± 42	44 ± 0.9	1.65	208

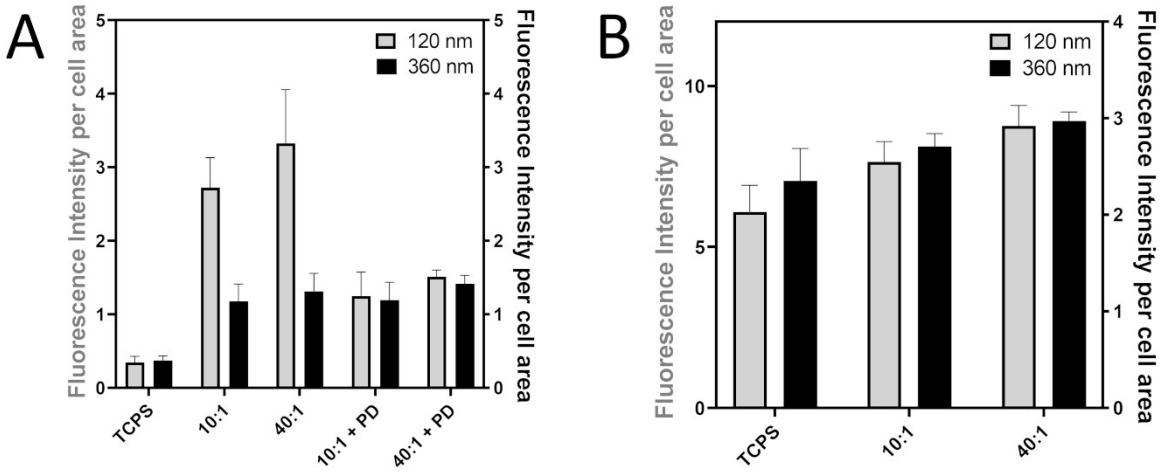


Figure S9. Uptake of silica particles determined by fluorescence intensity in flow cytometry normalised by cell area for A) A549 and B) J774A.1 after six hours at a concentration of 20 μg mL^{-1}. Data presented as mean ± standard error of the mean.