Electronic Supplementary Material (ESI) for RSC Advances.

Electronic Supplementary Information:

Cellular binding of nanoparticles disrupts the membrane potential

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Nanoparticle (NP)		Supplier diameter (nm)	Hydrodynamic diameter (nm)
Surface modification	Emission		
Amine (NH_2)	red	50	60.8 ± 0.11
	yellow-green	50	53.6 ± 0.41
	dark	58	59.8 ± 1.26
	dark	200	271 ± 4.87
Carboxylate (COOH)	red	50	57.3 ± 0.37

Table S1 Hydrodynamic diameters of NPs used in experiments. n=3, error shows standard deviation.

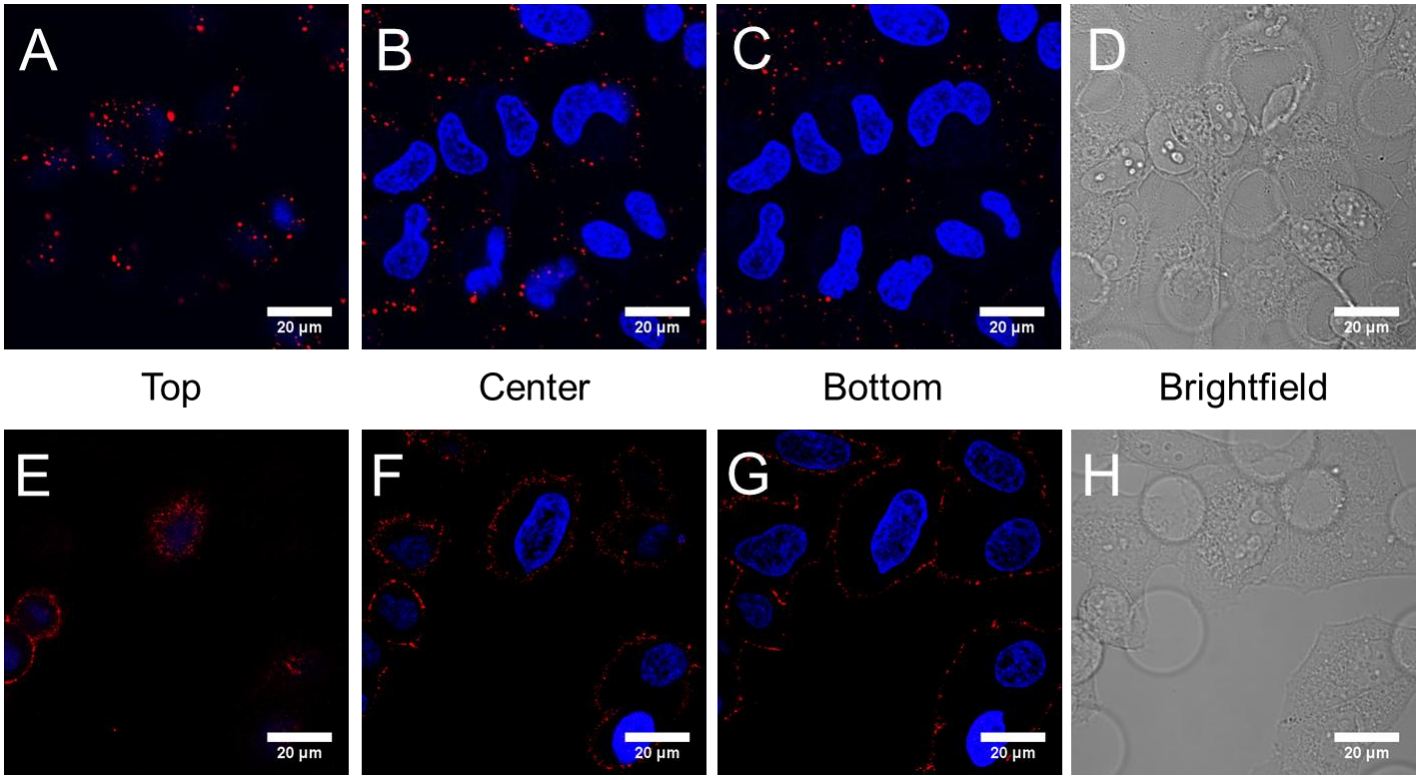

Fig. S1 (A-D) Confocal microscopy images of 50 nm amine-modified NPs (red, 100 pM) following cold binding (4 ºC, 10 min) to HeLa cells. (E-F) Confocal microscopy images of 50 nm carboxlyate-modified NPs (red, 100 pM) following cold binding to HeLa cells. For all images, nuclei are stained with DAPI (blue). Although confocal microscopy is not a perfect measure of NP internalization,[1] these z-slices show NPs accumulating at the edges of the cell rather than dispersed in the cytosol. At this temperature, even the additional 10 min incubation necessary for DiBAC staining would not lead to any significant internalization of NPs. Previous work in our lab with the 200 nm amine-modified NPs showed that these NPs are not internalized even after an 18 hr incubation at 37 ºC.[2] The scale bar is 20 μm.

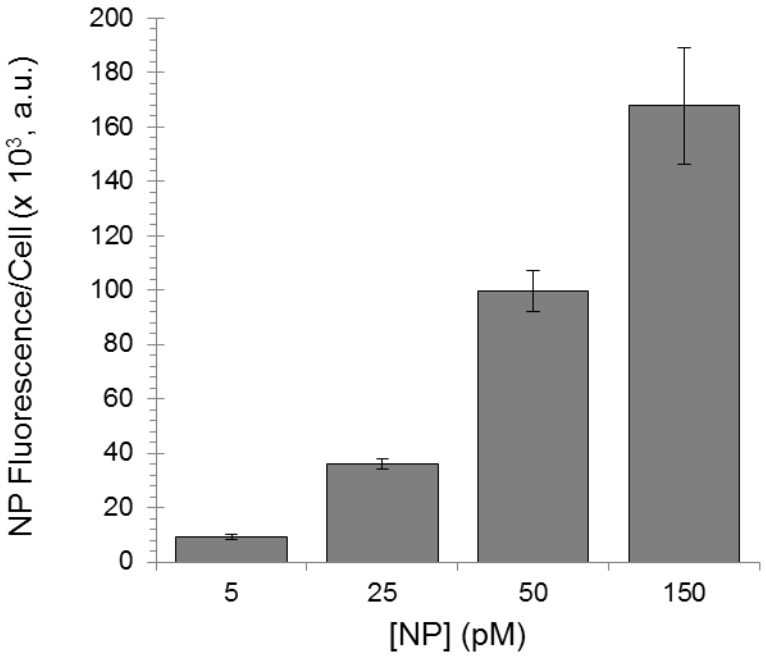

Fig. S2 Cellular binding of NPs is concentration dependent. Increased concentrations of 50 nm amine-modified NPs lead to increased fluorescence, measured with flow cytometry. Error bars show standard deviation, n=4.

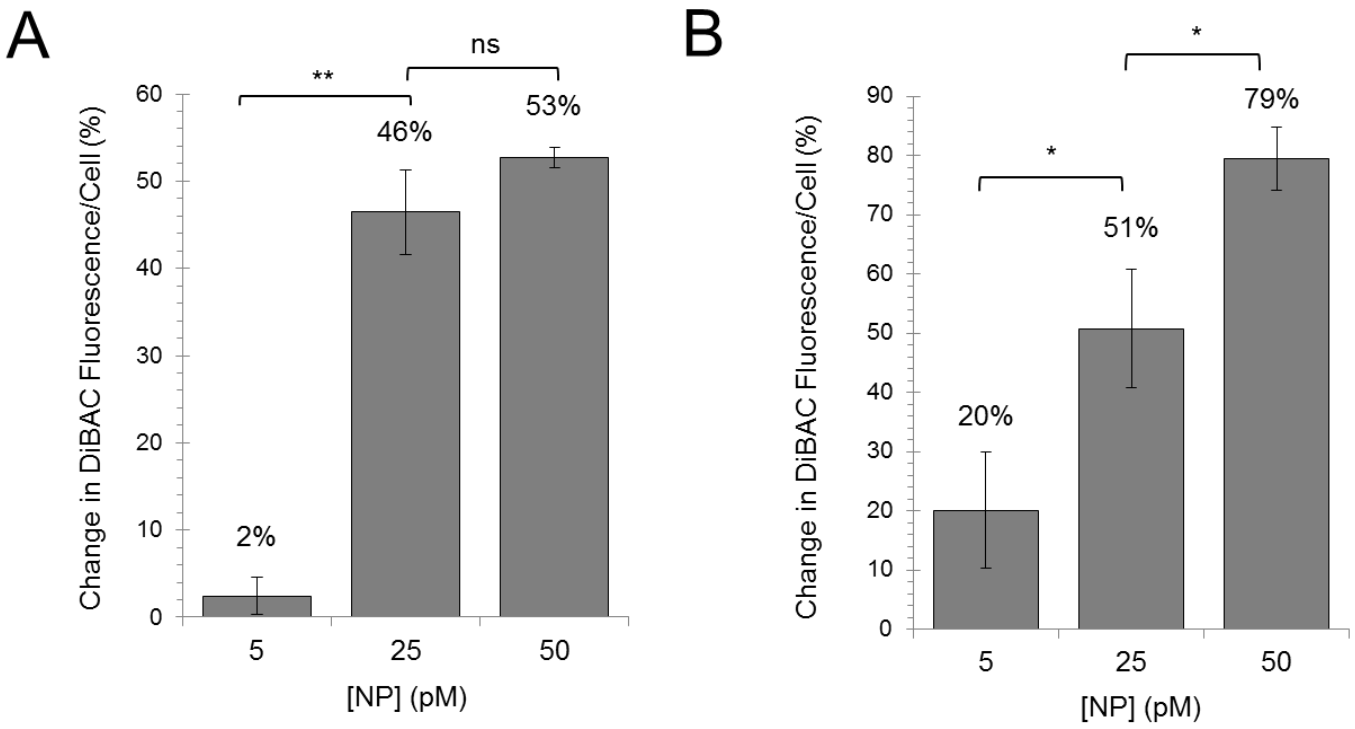

Fig. S3 Larger NPs (200 nm, amine-modified) also lead to depolarization. (A) CHO cells were incubated with 200 nm amine-modified NPs for 10 minutes at 4 ºC. Flow cytometry was used to measure the internalization of DiBAC (20 nM). Data show the percentage increase in DiBAC intensity per cell compared to a control in the absence of NPs. (B) Identical experiments were carried out with HeLa cells. For both cell types, values are the average of quadruplicate measurements. Error bars show standard deviation. ns=not significant, *$p < 0.05$, **$p<0.01$

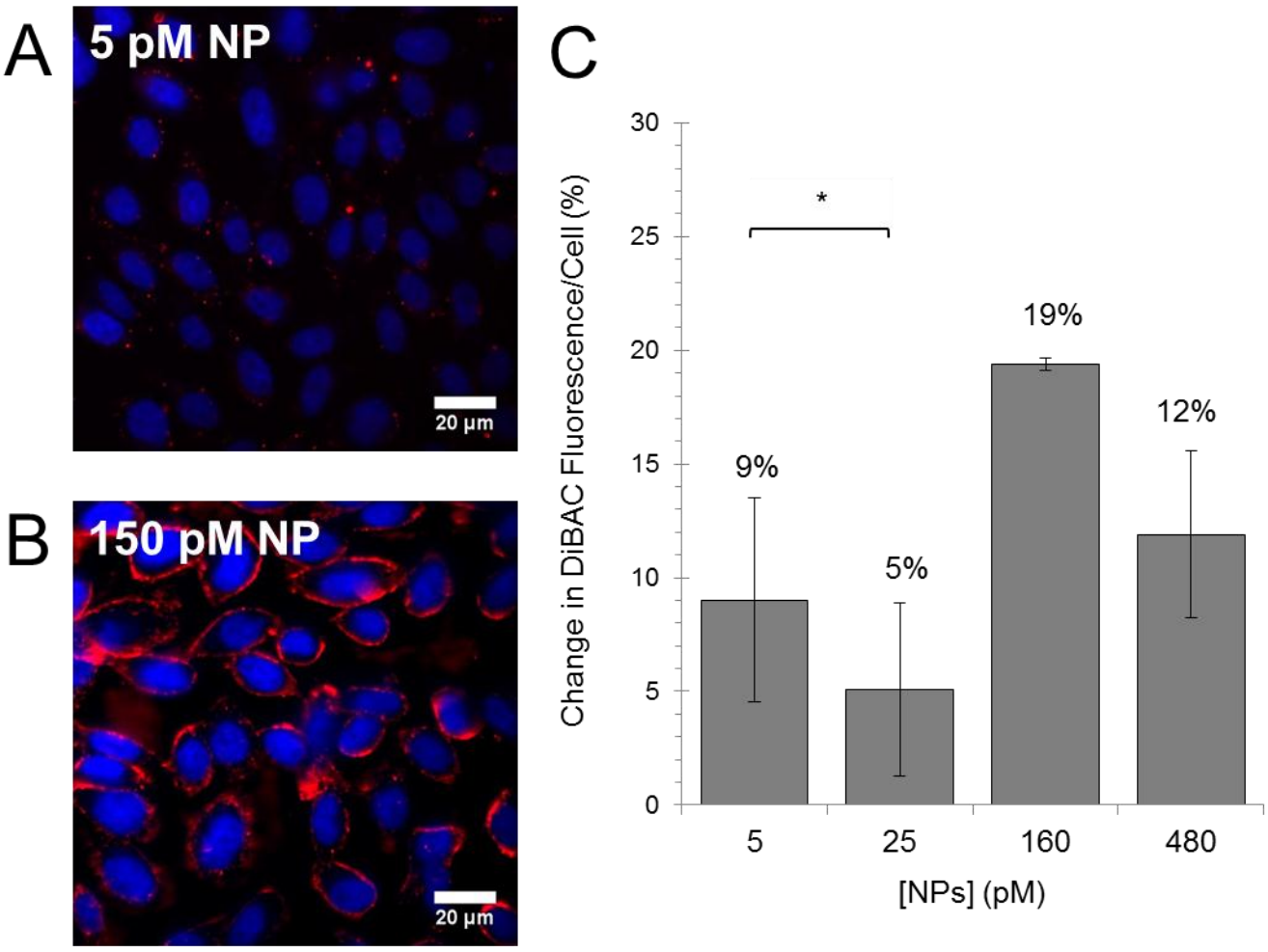

Fig. S4 Carboxylate-modified, 50 nm NPs do not lead to a concentration-dependent trend in depolarization. (A) Fluorescence microscopy image of CHO cells incubated with 5 pM of 50 nm carboxylate-modified NPs (red) for 10 minutes at 4 ^{o}C. Nuclei were stained with DAPI (blue). (B) Increased concentrations of NPs lead to increased binding. (C) Flow cytometry was used to measure the internalization of DiBAC (20 nM). Data show the percentage increase in DiBAC intensity per cell compared to a control in the absence of NPs. Values are the average of quadruplicate measurements. Error bars show standard deviation. *$p<0.05$

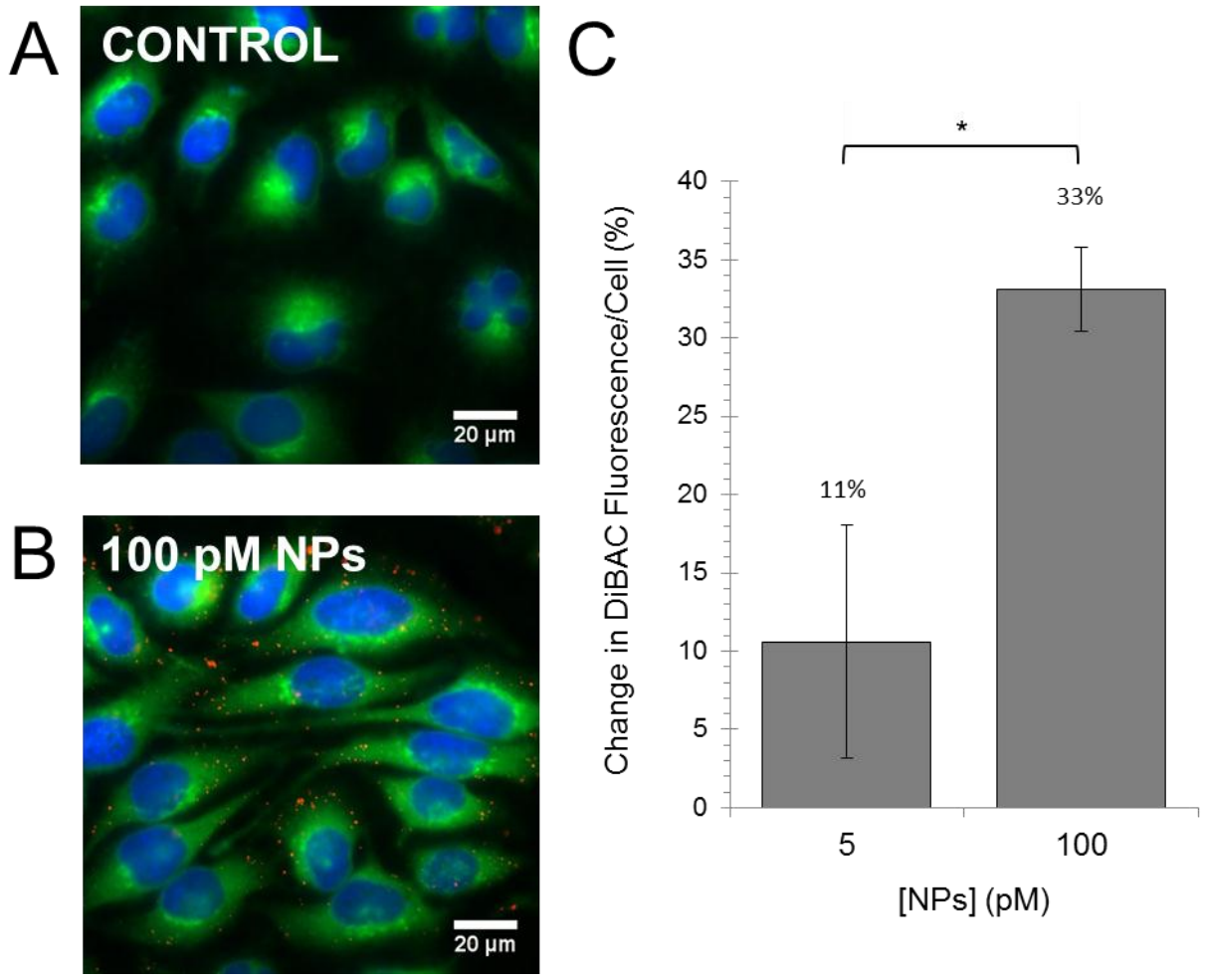

Fig. S5 NP-induced depolarization is also observed for HeLa cells. (A) Cellular internalization of DiBAC (green) in HeLa cells. Nuclei are stained with DAPI (blue). (B) The addition of 50 nm amine-modified NPs (red) leads to increased internalization of DiBAC (green). (C) Flow cytometry was used to measure the internalization of DiBAC (20 nM). Data show the percentage increase in DiBAC intensity per cell compared to a control in the absence of NPs. Values are the average of quadruplicate measurements. Error bars show standard deviation. *$p<0.05$

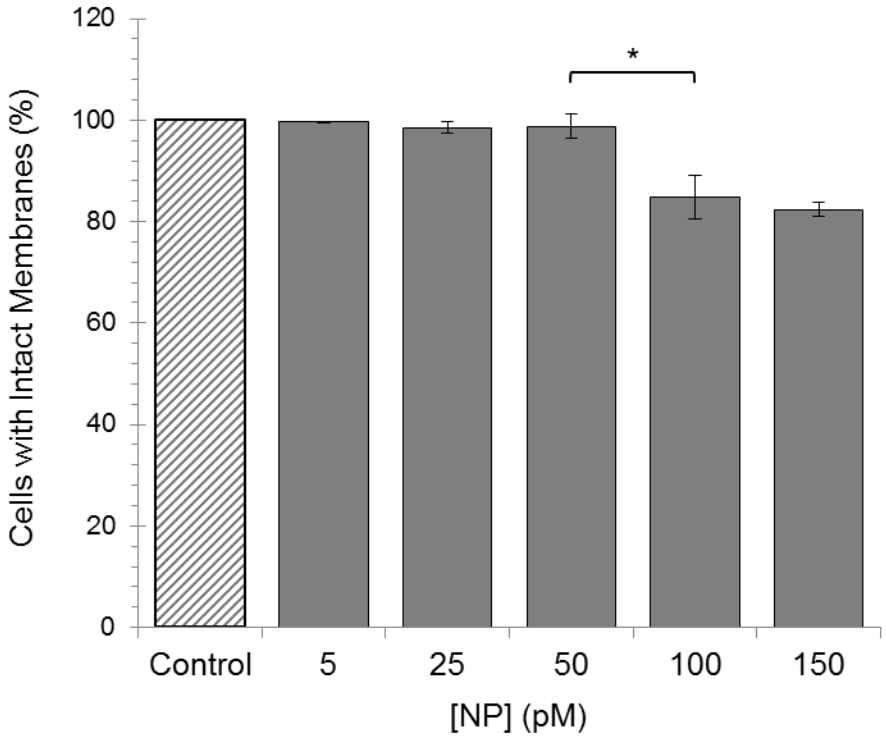

Fig. S6 PI staining (2 mM), measured with flow cytometry, was used to test for possible permeabilization of the plasma membrane induced by incubation with 200 nm NPs. At the NP concentrations used for experiments (5 pm – 50 pM, amine-modified), minimal internalization of PI was observed. Above 50 pM, signifcant PI internalization reflected substantial damage to the plasma membrane. Experiments were carried out in duplicate and normalized against a control in the absence of NPs. *$p<0.05$

References

1 A. E. Jablonski, T. Kawakami, A. Y. Ting and C. K. Payne, *J. Phys. Chem. Lett.*, 2010, 1, 1312.
2 G. W. Doorley and C. K. Payne, *Chem. Commun.*, 2011, 47, 466.