Electronic Supplementary Information:

Membrane potential mediates the cellular binding of nanoparticles

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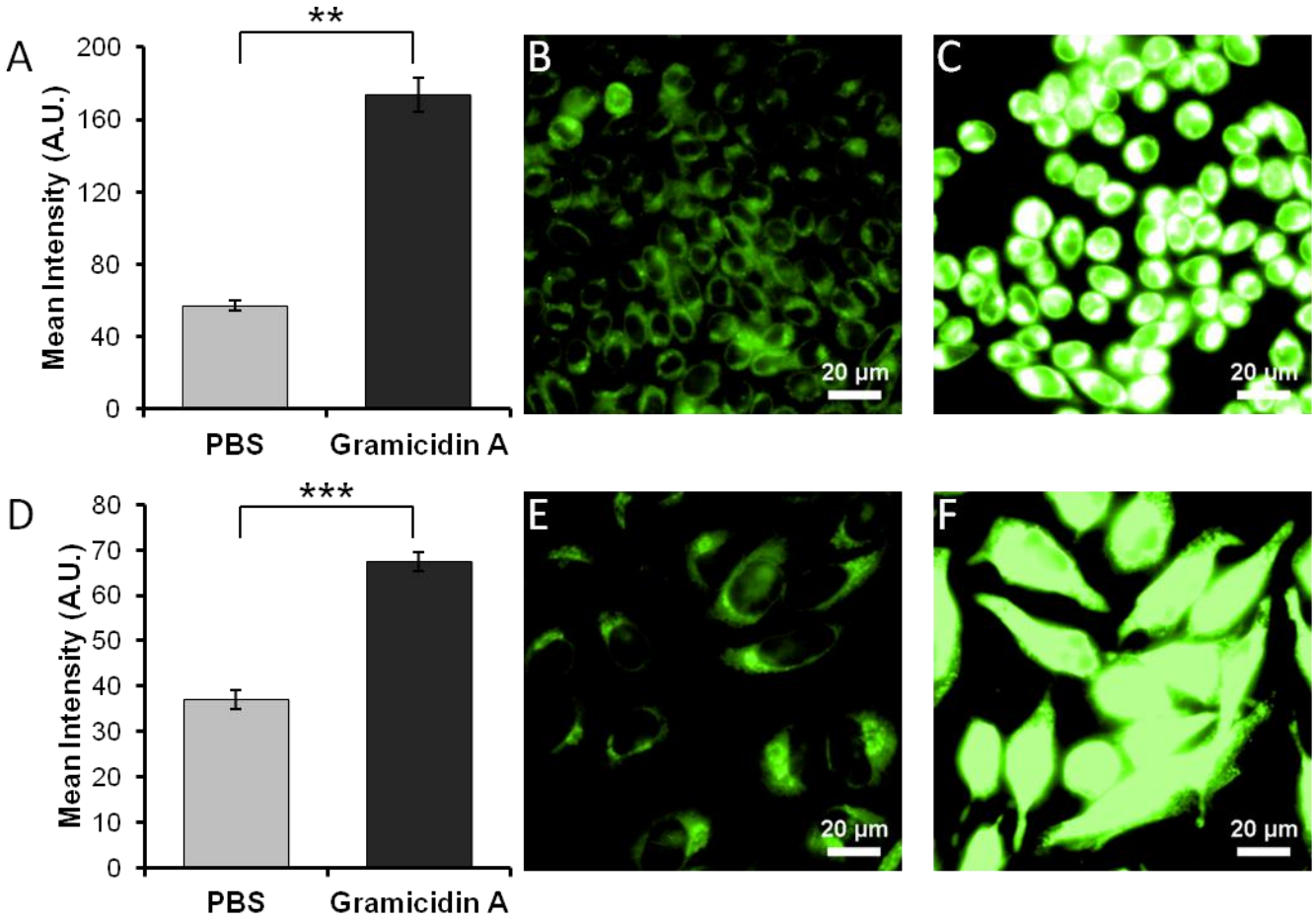

Fig. S1. Incubation of cells with gramicidin A leads to decreased membrane potential. (*A*) Flow cytometry was used to measure the depolarization of CHO cells following treatment with gramicidin A. Depolarization is observed as an increase in DiBAC signal, measured in the FITC channel. Mean intensity was used as a measure of DiBAC internalization Flow cytometry experiments were carried out in triplicate and error bars show standard deviation. P-values < 0.01 are indicated by **. (*B*) Fluorescence microscopy image of CHO cells incubated with DiBAC. (*C*) Treatment with gramicidin A leads to the increased cellular internalization of DiBAC. (*D-F*) HeLa cells show a similar depolarization following treatment with gramicidin A. P-values < 0.001 are indicated by ***.

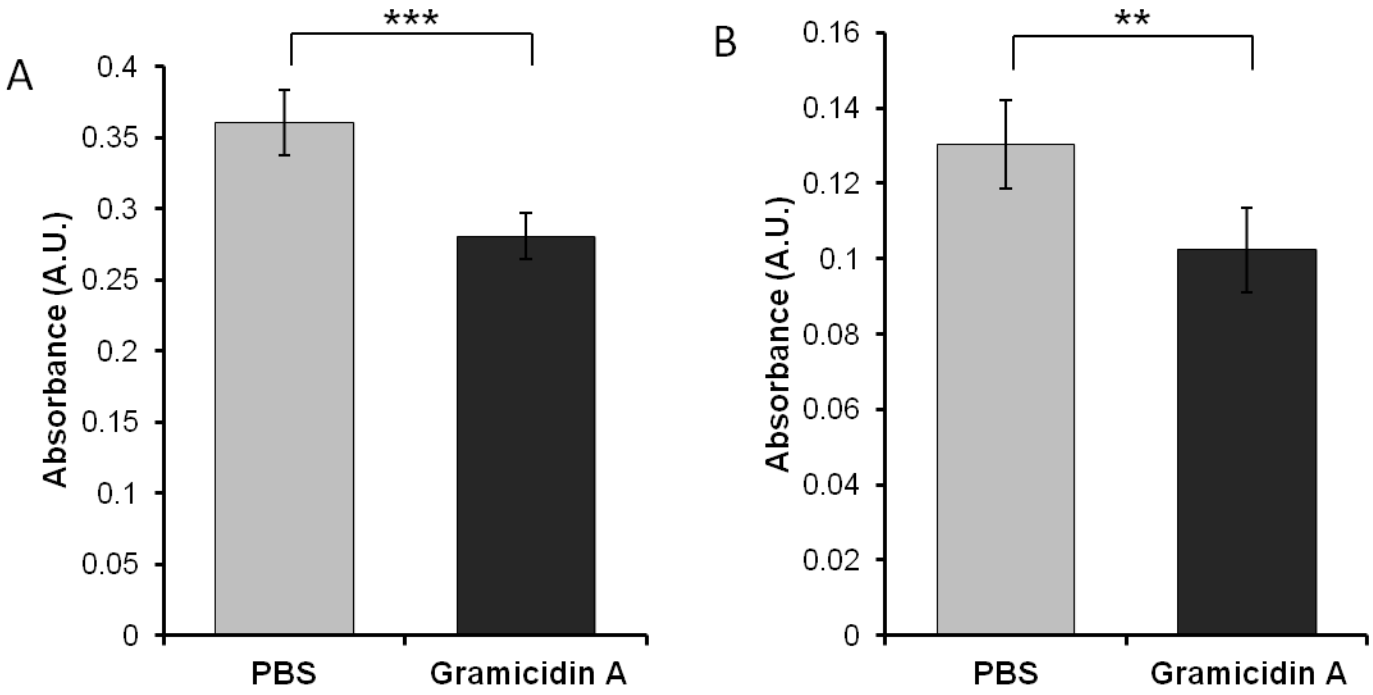

Fig. S2. MTT assays (V13154, Invitrogen) show a 20% decrease in cell viability following treatment with gramicidin A. Cells were incubated with 1 μM gramicidin A in PBS for 20 min at 4 ºC and then 10 min at room temperature to mimic experimental conditions. Experiments were carried out in triplicate and error bars show standard deviations. (*A*) CHO cells. (*B*) HeLa cells. P-values < 0.001 are indicated by ***, < 0.01 are indicated by **.

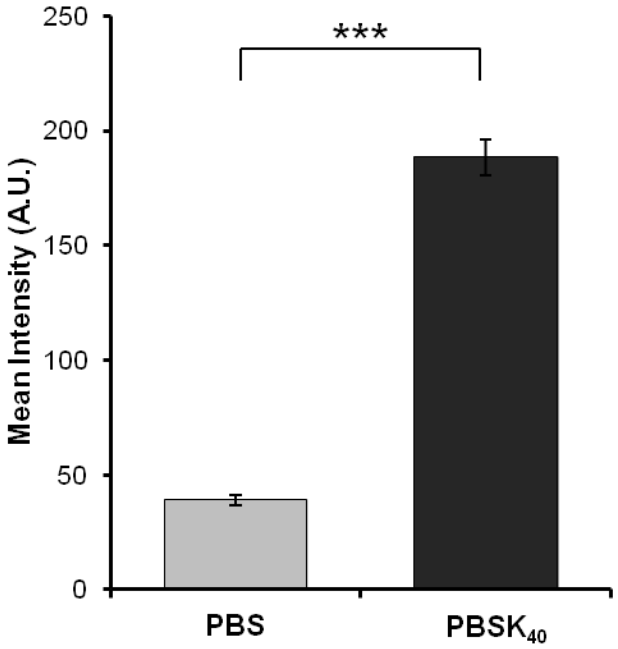

Fig. S3. Depolarization of CHO cells using $PBSK_{40}$ measured with flow cytometry. Depolarization is observed as an increase in DiBAC signal, measured in the FITC channel. Mean intensity was used as a measure of DiBAC internalization. Flow cytometry experiments were carried out in triplicate and error bars show standard deviation. P-values < 0.001 are indicated by ***.

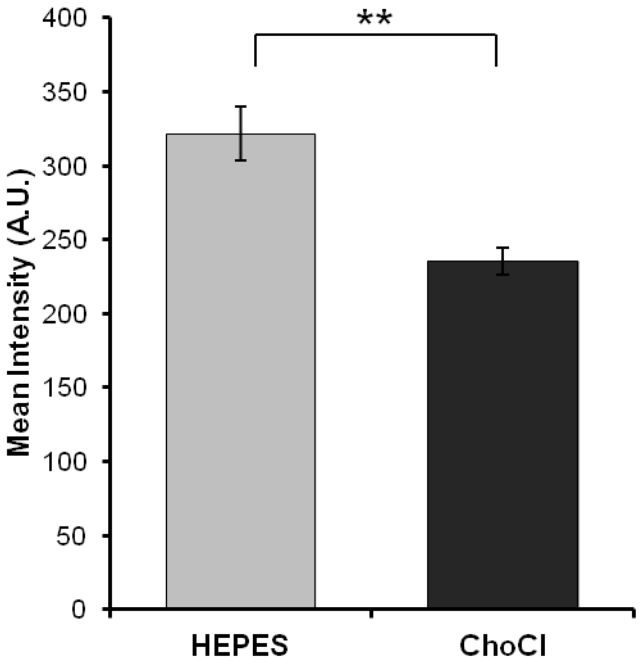

Fig. S4. Incubation of cells with choline chloride (ChoCl) leads to increased membrane potential measured using flow cytometry. Hyperpolarization is observed as a decrease in DiBAC signal, measured in the FITC channel. Mean intensity was used as a measure of DiBAC internalization. Flow cytometry experiments were carried out in triplicate and error bars show standard deviation. P-values < 0.01 are indicated by **.

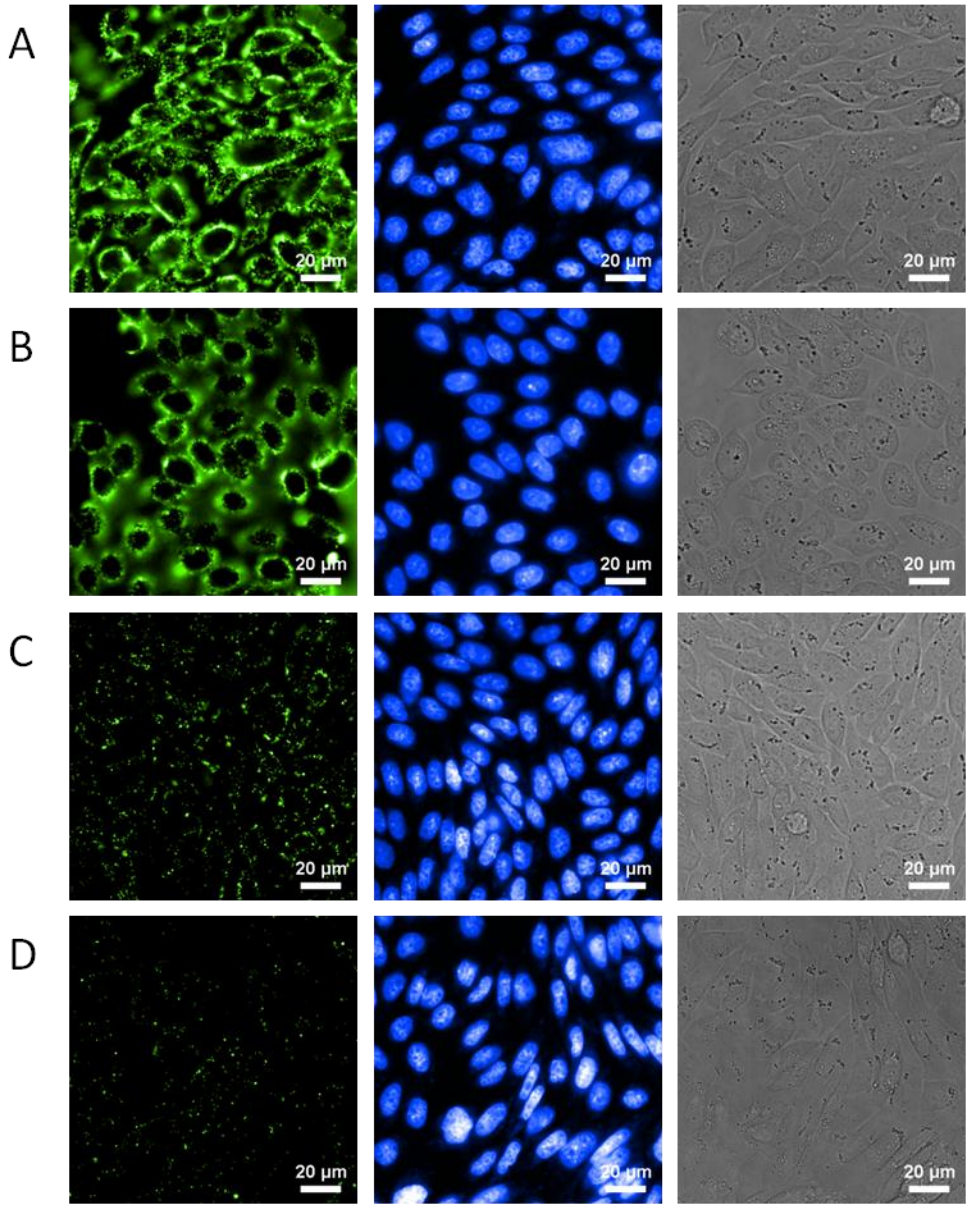

Fig. S5. Fluorescence microscopy images show the decreased binding of anionic NPs (green) following depolarization of CHO cells with gramicidin A. (*A*) Polarized, 40 nm NPs. (*B*) Depolarized, 40 nm NPs. (*C*) Polarized, 93 nm NPs (*D*) Depolarized, 93 nm NPs. Nuclei were stained with DAPI (blue). Bright field images are included to show cell morphology.

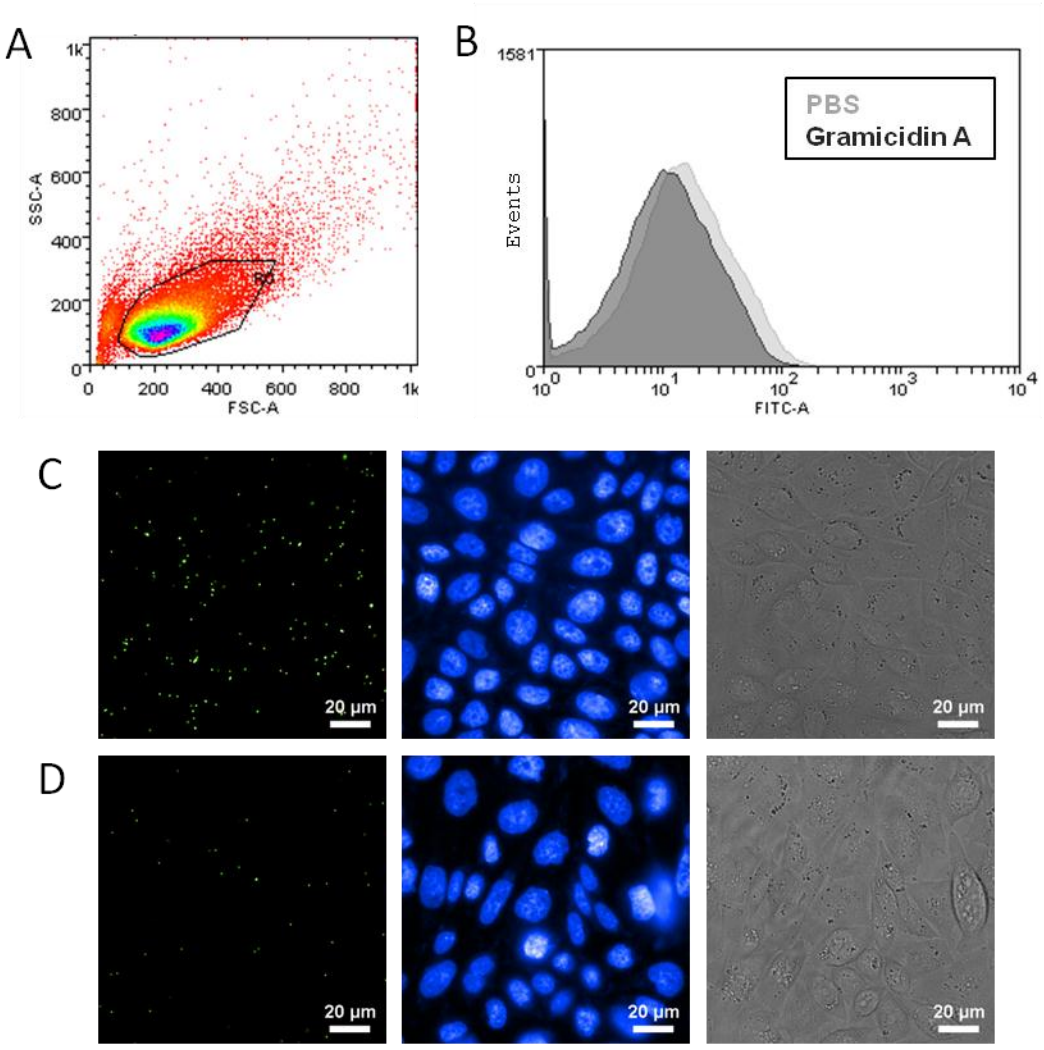

Fig. S6. Binding of anionic 190 nm NPs to CHO cells decreased following depolarization. (*A*) Forward and side scatter of cells measured using flow cytometry. The gate (R0) shows cells used for analysis. (*B*) Histogram obtained from flow cytometry data. (*C*) Binding of NPs (green) to polarized CHO cells. Nuclei are stained with DAPI. (*D*) Binding of NPs (green) to depolarized CHO cells. These individual fluorescence microscoy images were used to construct the overlaid images in Fig. 2.

Table S1. Summary of flow cytometry results for the binding of anionic and cationic NPs to HeLa cells. Results for CHO cells are shown in Table 2.

Charge	**Size**	*PBS* **Intensity**	*Gramicidin A* **Intensity**	**% Change**
Anionic	*40 nm*	52.0 ± 2.8	42.6 ± 4.5	**- 18** ± 10
	93 nm	78.1 ± 5.1	55.8 ± 2.5	**- 29** ± 8
	190 nm	33.1 ± 0.3	21.2 ± 2.1	**- 36** ± 6
Cationic	*87 nm*	43.9 ± 1.1	47.8 ± 0.7	**+ 9** ± 3

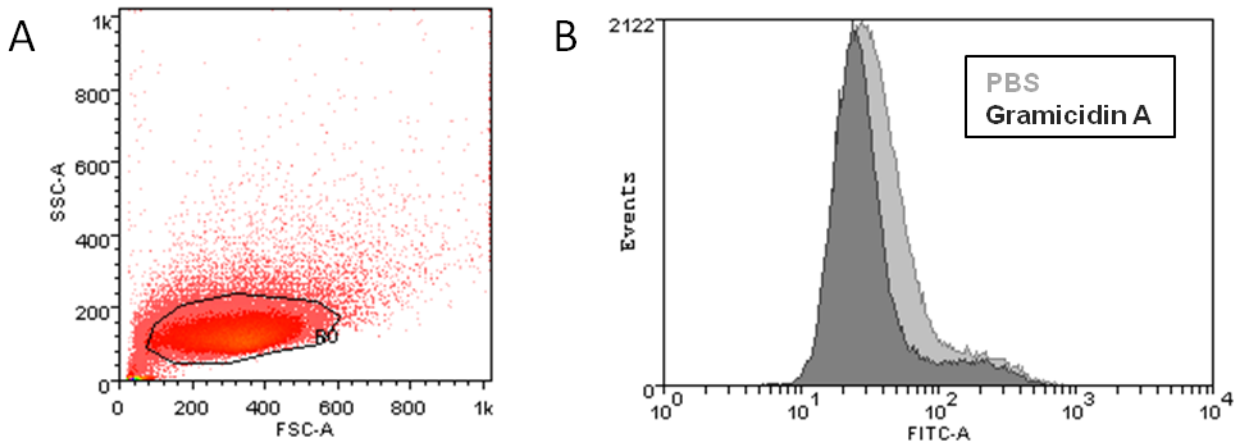

Fig. S7. Binding of anionic 40 nm NPs to HeLa cells was decreased following depolarization. (*A*) Forward and side scatter of cells measured using flow cytometry. The gate (R0) shows cells used for analysis. (*B*) Histogram obtained from flow cytometry data.

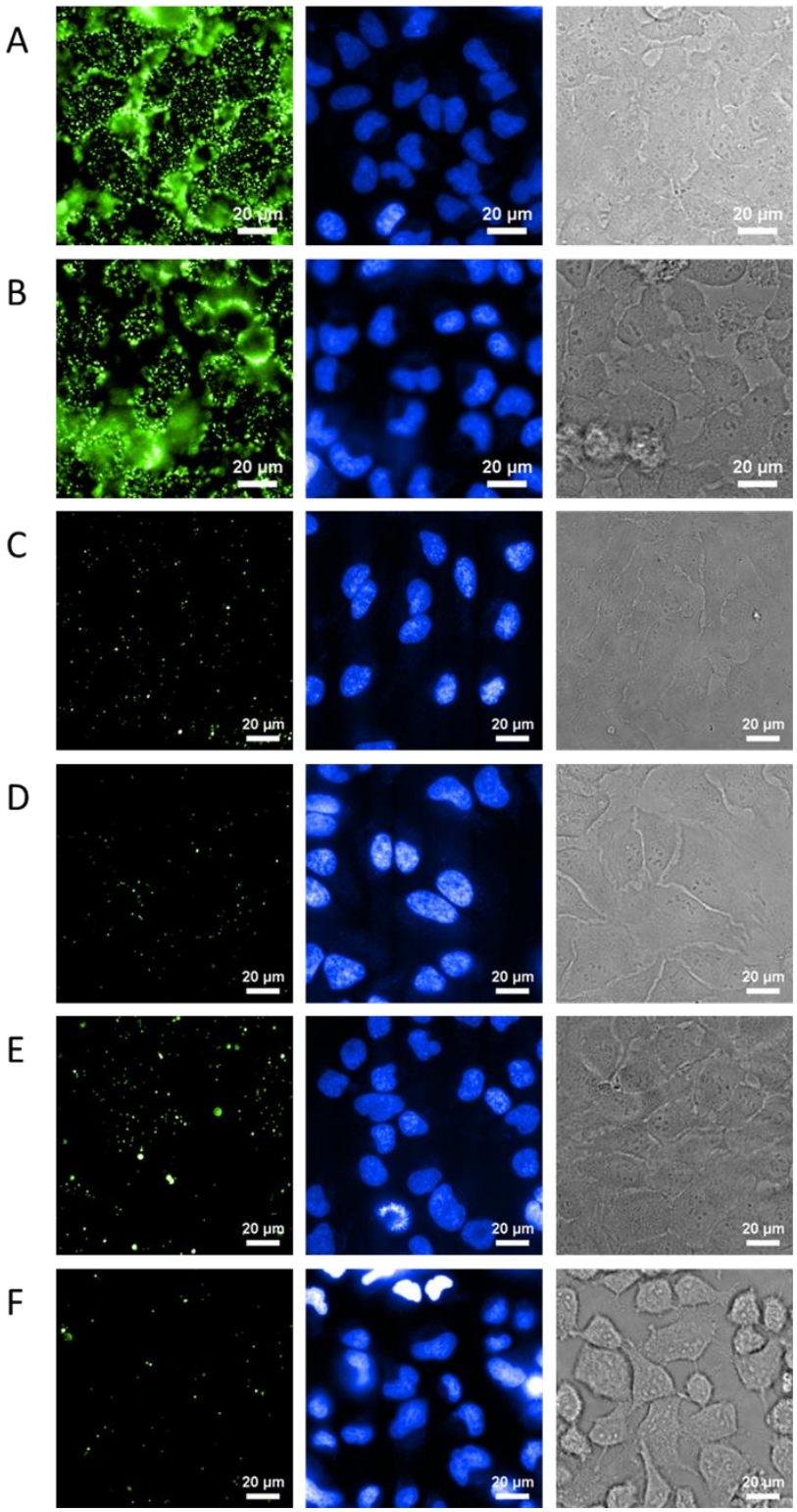

Fig. S8. Fluorescence microscopy images show the decreased binding of anionic NPs (green) following depolarization of HeLa cells with gramicidin A. (*A*) Polarized, 40 nm NPs. (*B*) Depolarized, 40 nm NPs. (*C*) Polarized, 93 nm NPs. (*D*) Depolarized, 93 nm NPs. (*E*) Polarized, 190 nm NPs (*F*) Depolarized, 190 nm NPs. Nuclei were stained with DAPI (blue).

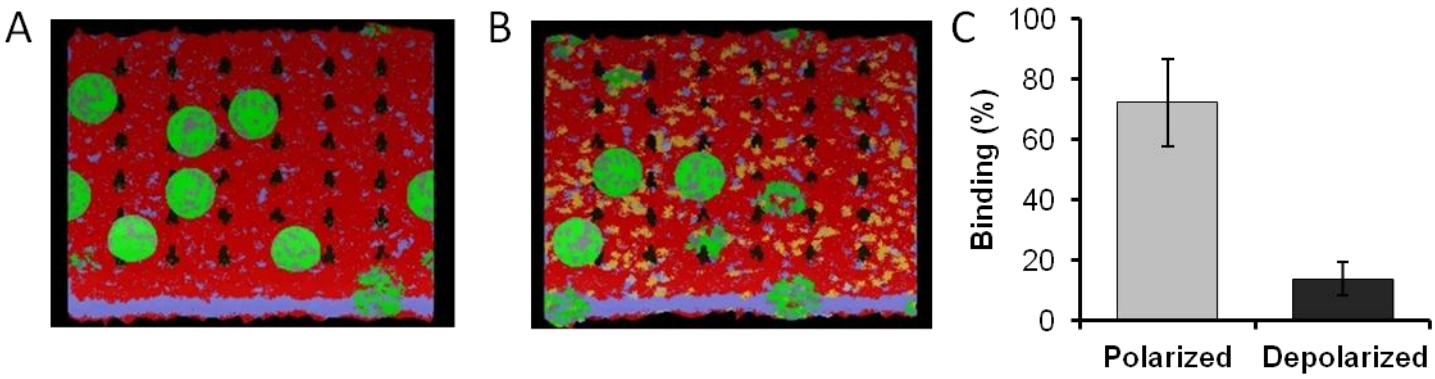

Fig. S9. Dissipative particle dynamics simulations that include anionic proteins arrayed on the membrane show decreased cellular binding of anionic NPs to a neutral membrane, similar to the effect of gramicidin A. The overall result is similar to that of a membrane lacking proteins (Fig. 5), with less total binding. (*A*) Snapshot from a simulation showing anionic NPs and a charged membrane. The NP core is shown in green, anionic ligands in gray, cationic lipids in yellow, and neutral lipids in red. Anionic proteins are shown in black. Counterions and water are not shown. (*B*) Snapshot from a simulation of NPs and a neutral membrane shows the reduced binding of anionic NPs. (*C*) Binding is measured as the fraction of NPs bound to the membrane after a 640 ns simulation. A 80% decrease in binding was observed for the neutral membrane, a model of depolarized cells.