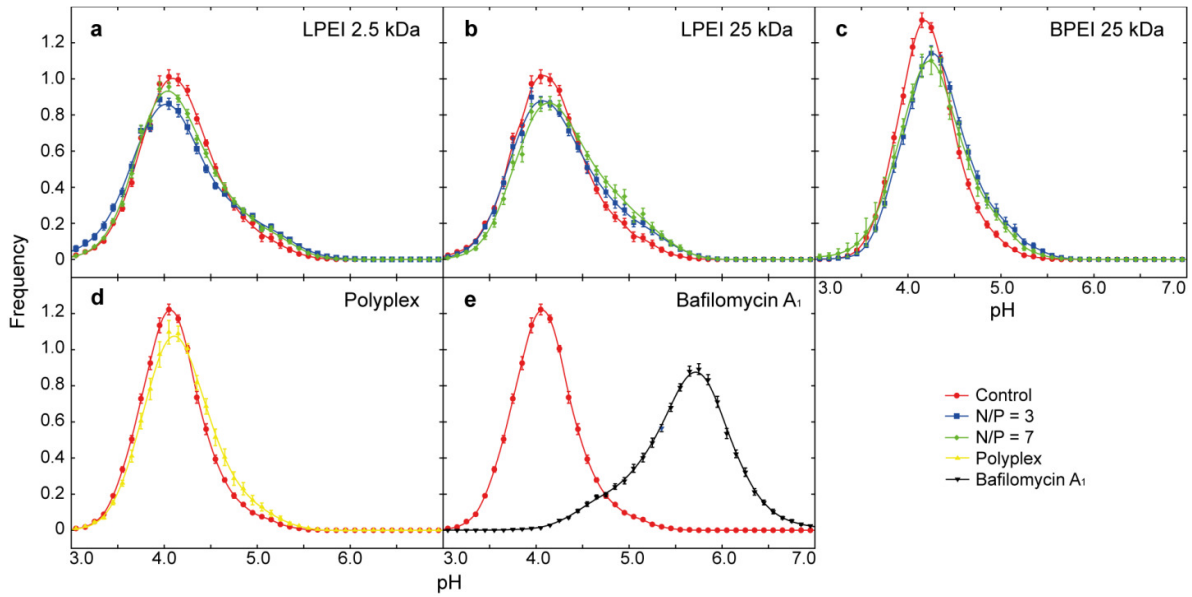
polyplexes of N/P = 6 and 10 respectively with addition of 0.8 µg DNA per well) as well as a polyplex of BPEI 25 kDa N/P = 6. Treatment was performed in full growth medium for 4 h where after cells were washed with the above mentioned procedure and kept in imaging medium for observation by confocal microscopy. Treatment with 200 nM bafilomycin A_1 was also performed in full growth medium without phenol red for 45 min. For imaging, cells were transferred to imaging medium with 200 nM bafilomycin A_1 without prior washing. Supplementary **Fig. S2** shows frequency distributions of pH measurements of nanosensor containing cells before and after treatment with free PEI, polyplex or bafilomycin A_1. No difference in pH distributions are observed compared to control cells for any of the PEIs and N/P ratios tested. Treatment with complete polyplexes neither revealed any difference in pH distribution. As a positive control is included bafilomycin A_1 treated cells, to verify that an increase in pH can be observed with this method.



Supplementary Figure S2: Measurements of lysosomal pH. Nanosensor internalized during 24 h by HeLa cells imaged by confocal microscopy before (control) and after treatment with LPEI 2.5 kDa, LPEI 25 kDa of BPEI 25 kDa at N/P = 3 and 7 or BPEI 25 kDa polyplex at N/P = 6 or bafilomycin A_1. Histograms show pH distributions of nanosensor allocated signals with mean ± SEM (n = 8 - 12 images). Representative of at least three independent experiments.

PEI content of lysosomes.

HeLa cells were seeded in 24-well plates on 9 mm round cover glasses for 24 h. They were then treated with 25 kDa BPEI-RhB at N/P = 7 (corresponding to polyplexes of N/P = 10 with 0.8 µg DNA per well) in full growth medium for 4 h at normal growth conditions. Cells were then washed washed three times with ice cold PBS supplemented with heparin (20 unites/mL), once with PBS and kept in imaging medium for observation by confocal microscopy. Supplementary **Fig. S3a**