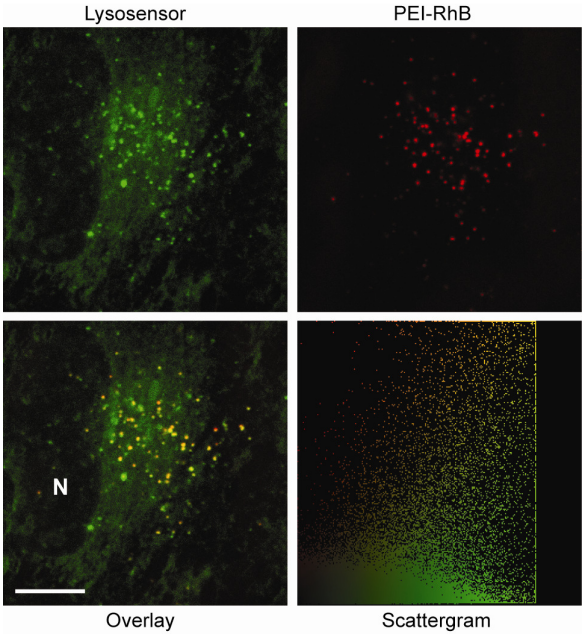
Colocalisation of PEI-RhB with Lysosensor

2×10^4 HeLa cells per well 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. After three hours Lysosensor Green DND-189 was added to a final concentration 1 µM. Cells were then washed three times with ice cold PBS supplemented with heparin (20 unites/mL), once with PBS and kept in imaging medium (DMEM without phenol red and bicarbonate, but supplemented with 30 mM HEPES, 10% FBS and 100 UI/mL penicillin and streptomycin) for observation by confocal microscopy. Supplementary **Fig. S1** shows the colocalization of the green lysosensor with red PEI-RhB. Most PEI has reached a lysosome with lysosensor whereas there are still lysosomes containing only lysosensor.



Supplementary Figure S1: Colocalization of RhB-PEI with Lysosensor Green DND-189. HeLa cells were treated with PEI-RhB for four hours and lysosensor for one hour. Top left: lysosensor signal; top right: PEI-RhB; bottom left: overlay and bottom right: scattergram relating the intensity of red to green of corresponding pixels. N: nucleus. Scale bar, 10 µm.

Lysosomal pH measurements in response to different PEI chains and polyplexes.

2×10^4 HeLa cells per well were seeded in 24-well plates on 9 mm cover glasses for 24 h. Cells were then treated with 10 µg/mL nanosensor in full growth medium for 20 h. Cells were then washed three times with ice cold PBS supplemented with heparin (20 unites/mL) and once with PBS. Cells were then kept in imaging medium or treated with PEI, polyplex or bafilomycin A_1. Different free PEI chains were tested: BPEI 25 kDa, LPEI 25 kDa or 2.5 kDa at N/P = 3 and 7 (corresponding to