

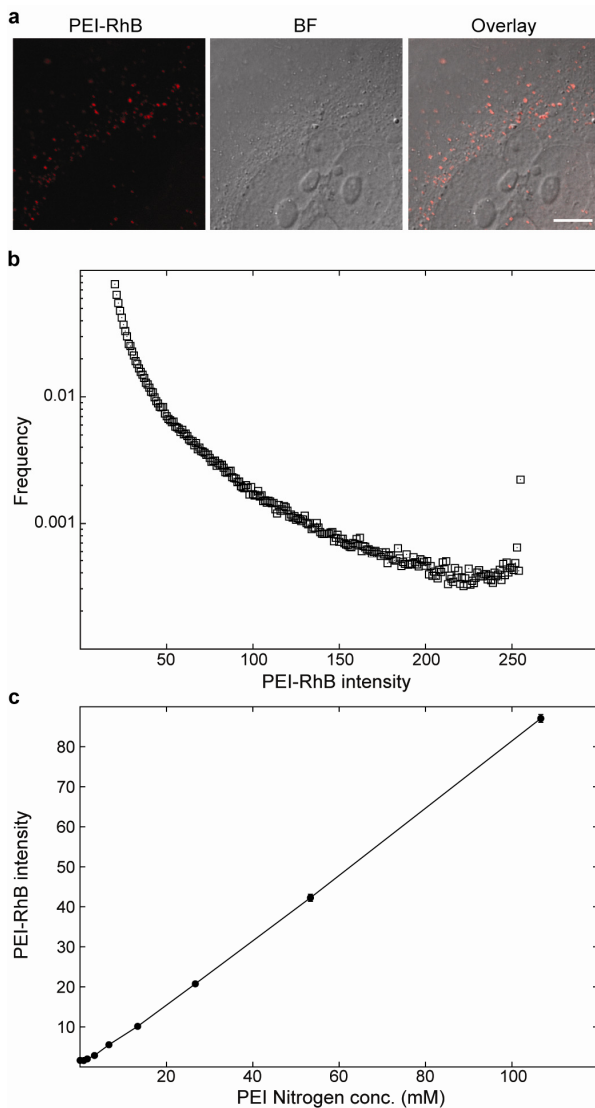
shows the localization of the red BPEI-RhB in cells. **Fig. S3b** illustrates the distribution of the amount of PEI-RhB in cells where the frequency of intensity is plotted. Pixels with an intensity larger than 255 is collected as a point at 256.

In order to determine the concentration of BPEI-RhB in the lysosomes a calibration curve was constructed by diluting free BPEI-RhB in water at descending concentrations of which the mean intensity was measured at the microscope. **Fig. S3c** shows the intensity plotted as a function of PEI nitrogen concentration which yields a straight line:

$$I = 0.81 \cdot c(\text{PEI}) + 0.28$$

Where  $I$  is the intensity and  $c(\text{PEI})$  the concentration of PEI nitrogen atoms,  $R^2 = 0.999$ .

The frequency distribution of intensities in **Fig. S3b** was converted to a frequency distribution of PEI nitrogen concentrations via the calibration curve in **Fig. S3c**, and presented in **Fig. 6c** of the main article along with the accumulated frequency distribution.



### Supplementary Figure S3: Lysosomal PEI content.

**a**, HeLa cells were treated with BPEI-RhB for 4 hours, washed and imaged by confocal microscopy. Scale bar, 10  $\mu\text{m}$ . **b**, Frequency distribution of intensities of PEI-RhB image in **a**. Pixels with an intensity larger than 255 is collected as a point at 256. **c**, Free PEI-RhB was diluted in water and images were collected at the confocal microscope. Mean intensity  $\pm$  SD is presented, SD is 1-4%. Representative of three independent experiments.