Supplementary data

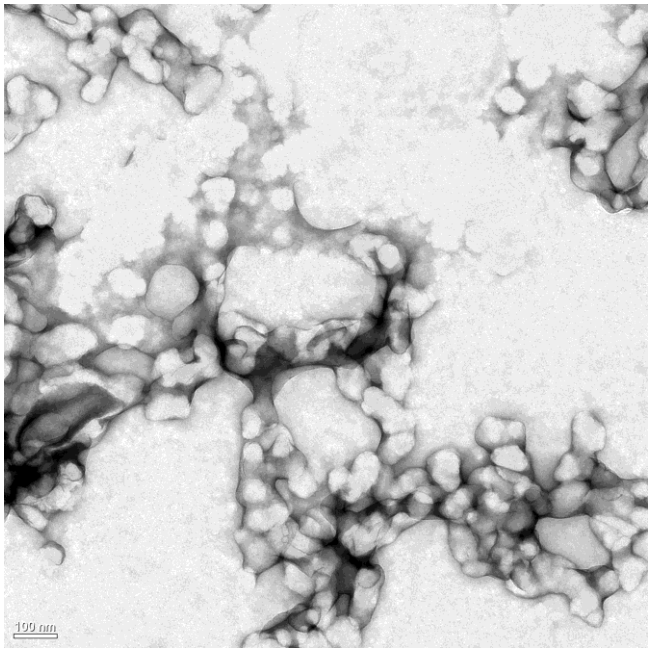

Figure S1. The TEM image of GMA-EGDMA displays a clustered array of spherical nanogels with an average diameter of 60 nm.

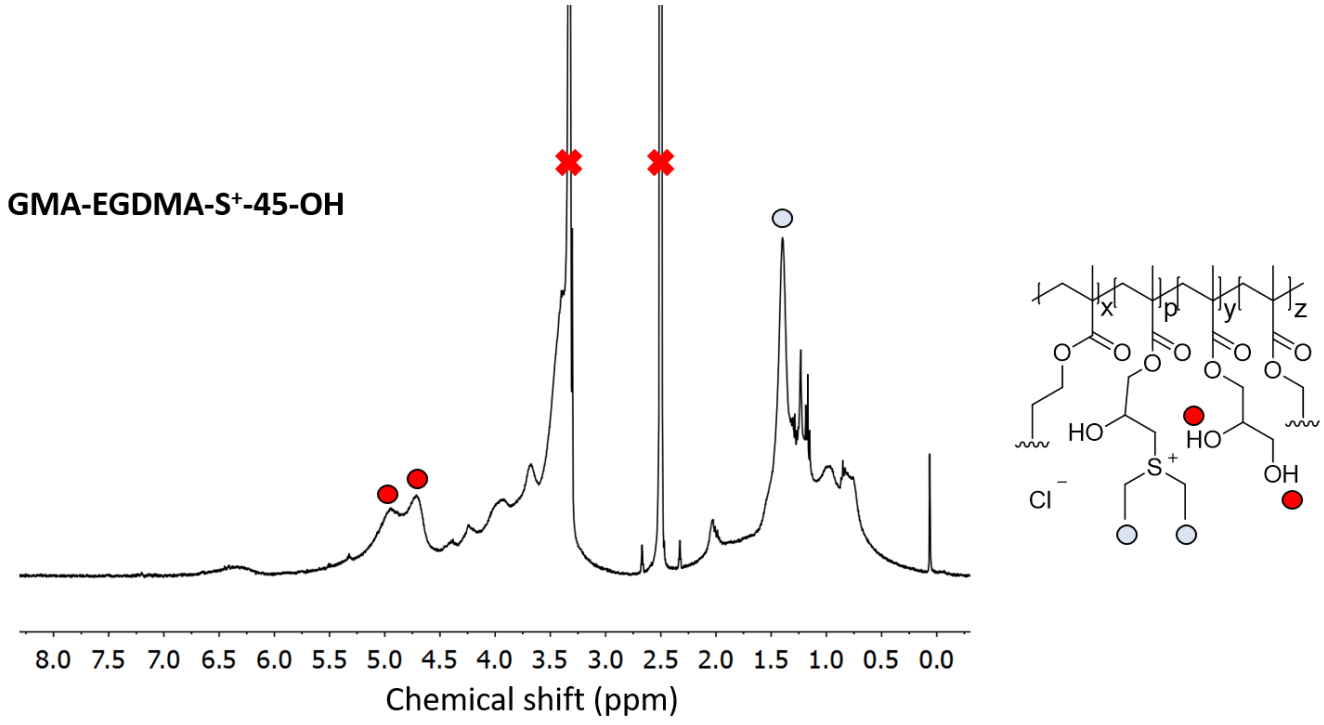

Figure S2. ^{1}H NMR of GMA-EGDMA-S^{+}-45-OH in DMSO-d_6. The characteristic hydroxyl groups are seen at δ = 4.9 ppm and δ = 4.7 ppm, along with the sulfonium CH_3 at δ = 1.4 ppm.

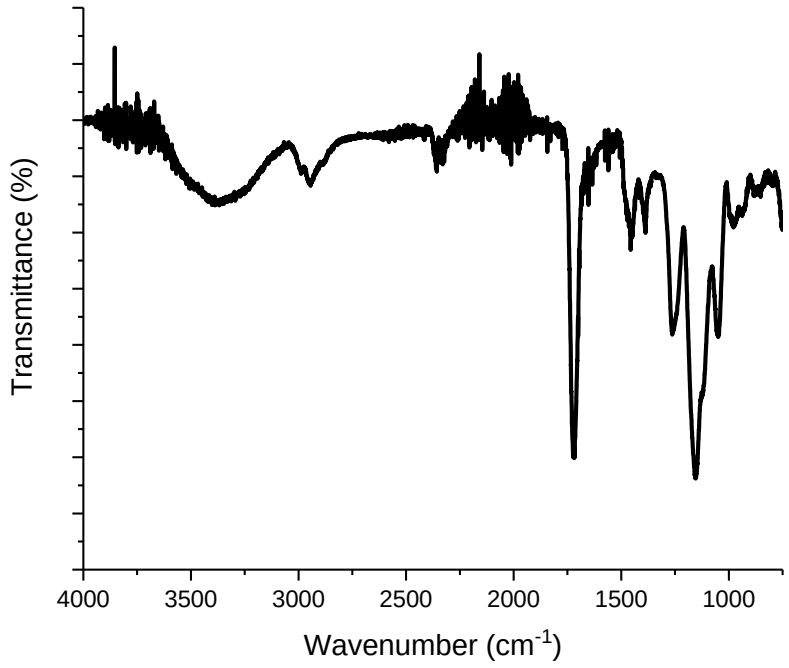

Figure S3. IR spectrum of GMA-EGDMA-S$^+$-45-OH after lyophilization. The epoxide peak at 907 cm^{-1} is no longer present meaning complete epoxide conversion.

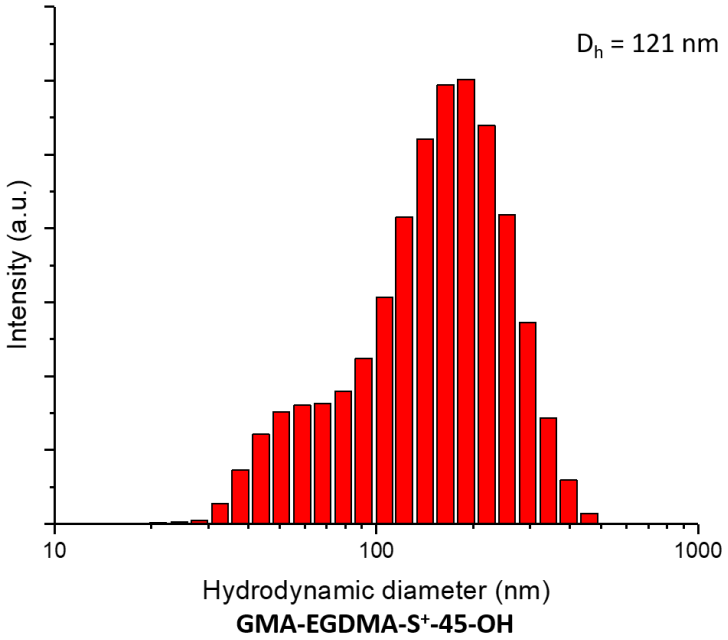

Figure S4. DLS of GMA-EGDMA-S$^+$-45-OH in water displays a semi-bimodal distribution with an average hydrodynamic diameter of 121 nm in intensity.

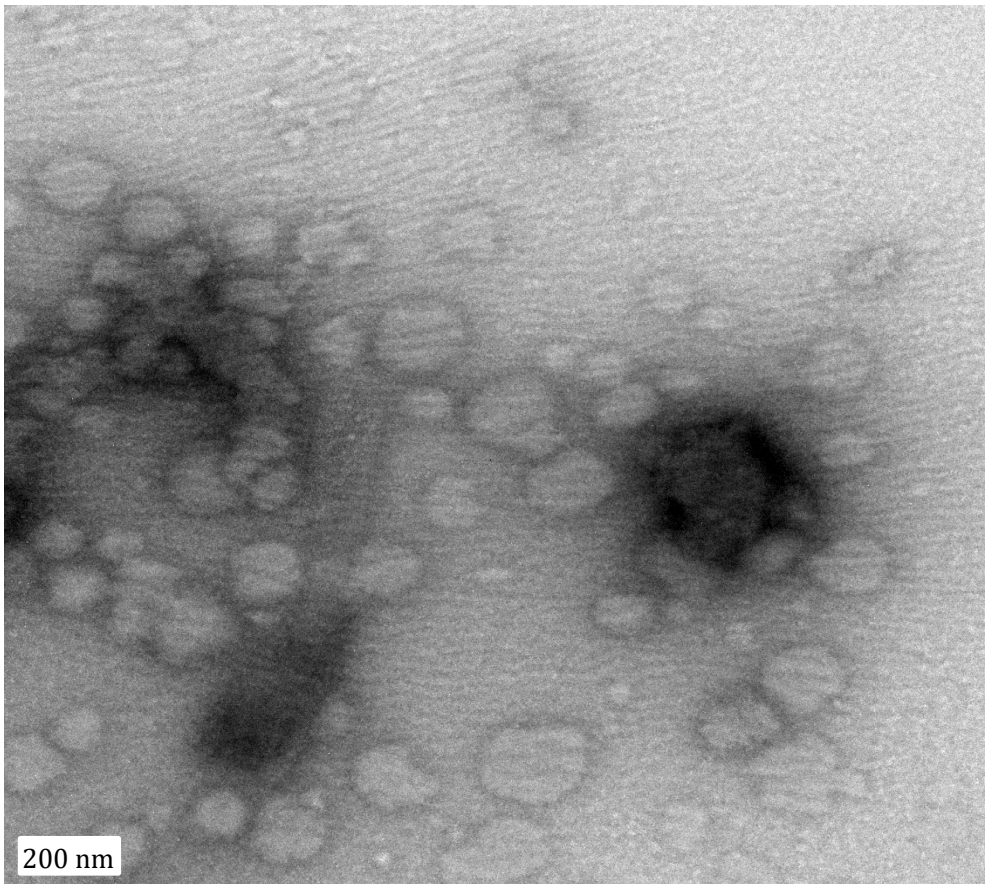

Figure S5. TEM micrograph of 50-THG polyplexed with pCMV-GFP at t=0 and S/P = 75. The mean diameter = 127 nm.

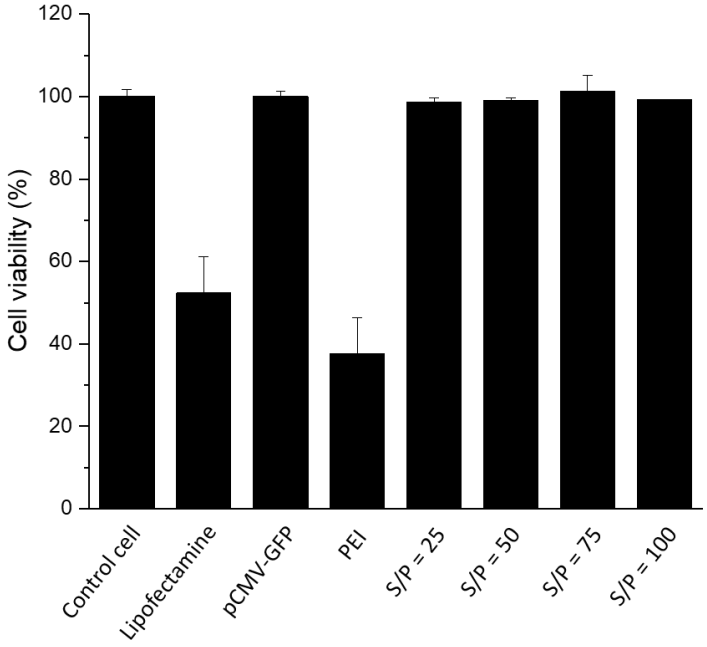

Figure S6. Cell viability of HeLa cells treated with polyplexes was evaluated by conducting an *alamarBlue*™ cytotoxicity experiment. Untreated cells served as negative control. Experiments were done in triplicate.

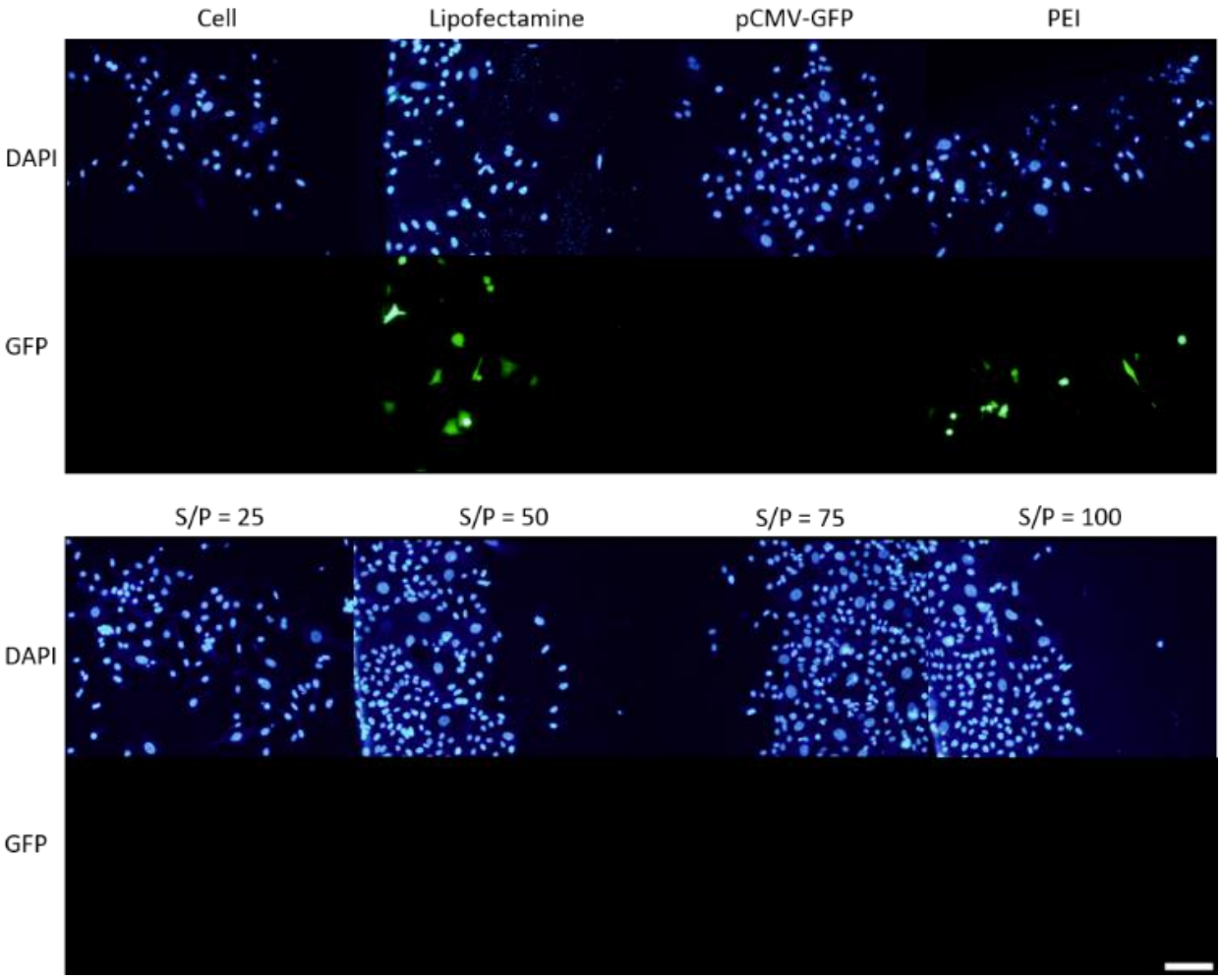

Figure S7. Fluorescence microscope images of HeLa cells treated for 20h with pCMV-GFP, at 0.25 µg/well. 100x magnification. Scale bar represents 200 µm.

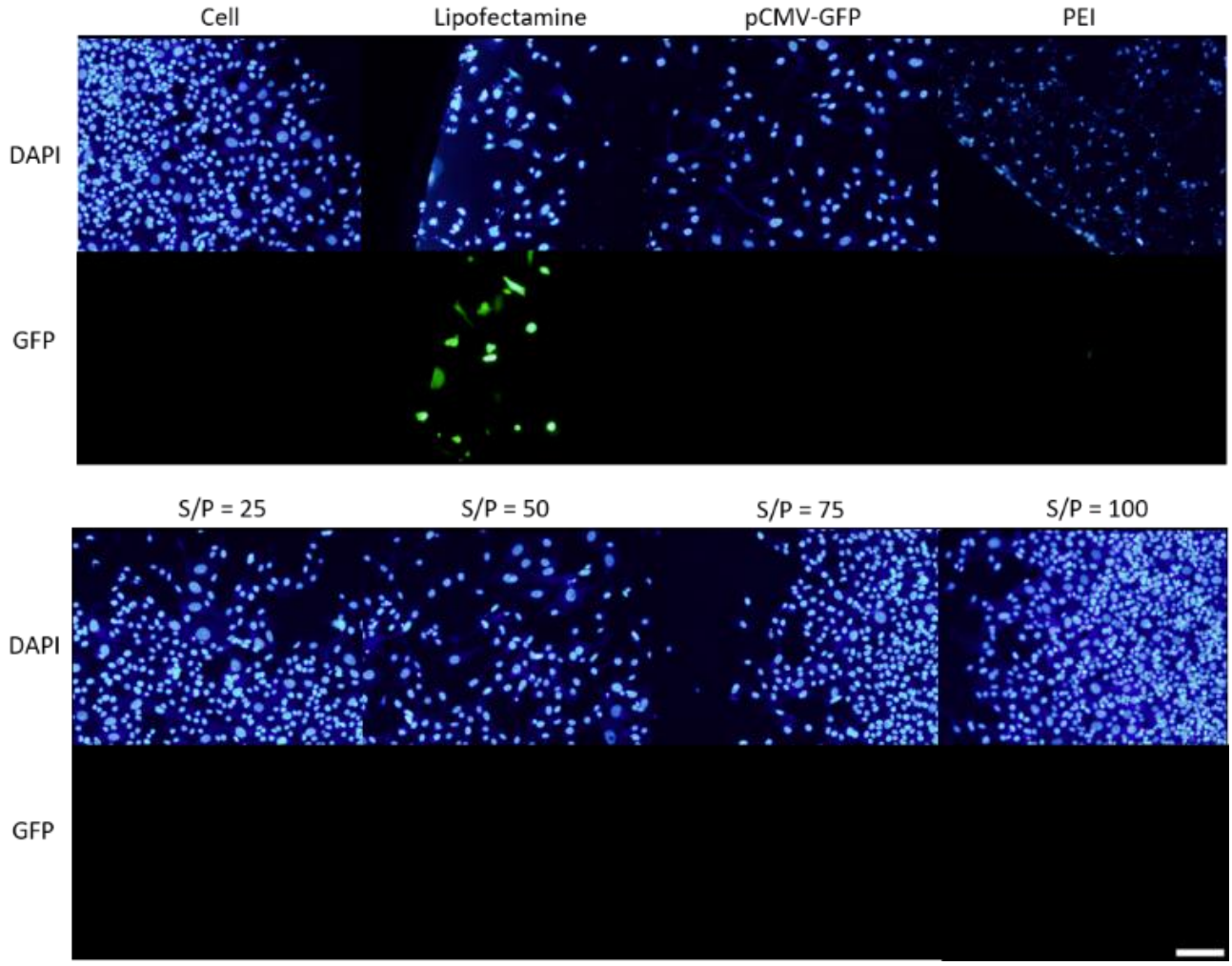

Figure S8. Fluorescence microscope images of HeLa cells transfected for 20h by pCMV-GFP (1 μg per well) via 50-THG. 100x magnification. Scale bar represents 200 μm.

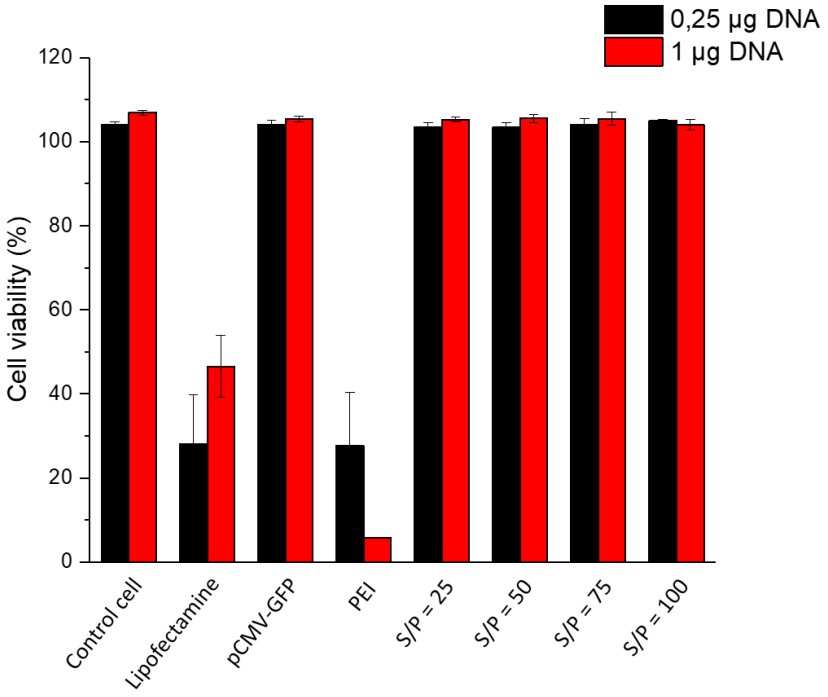

Figure S9. The cell viability of the HeLa cells treated with polyplexes is depicted above. Untreated cells served as negative control. Experiments were done in triplicate.

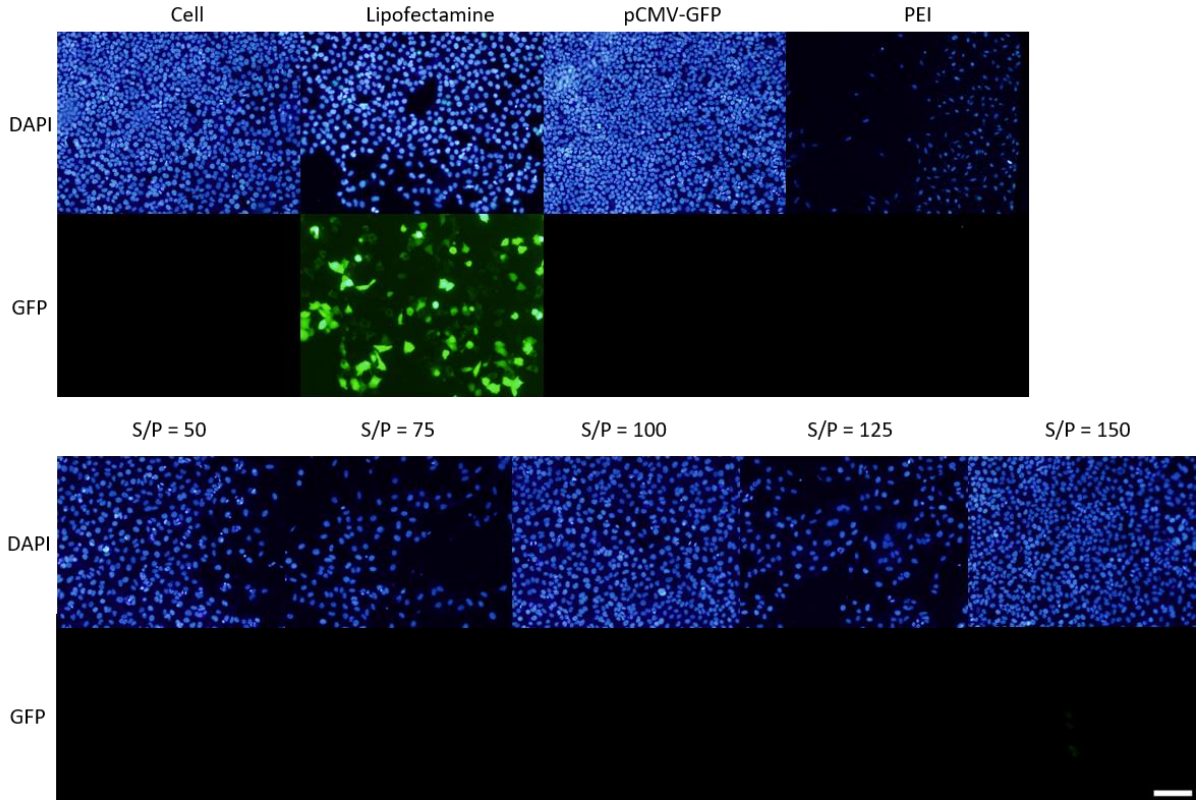

Figure S10. HeLa cells were imaged under a fluorescent microscope after transfection experiments with 40-THG polyplexes carrying pCMV-GFP. No expression was observed in the HeLa cells. The scale bar represents 200 µm.

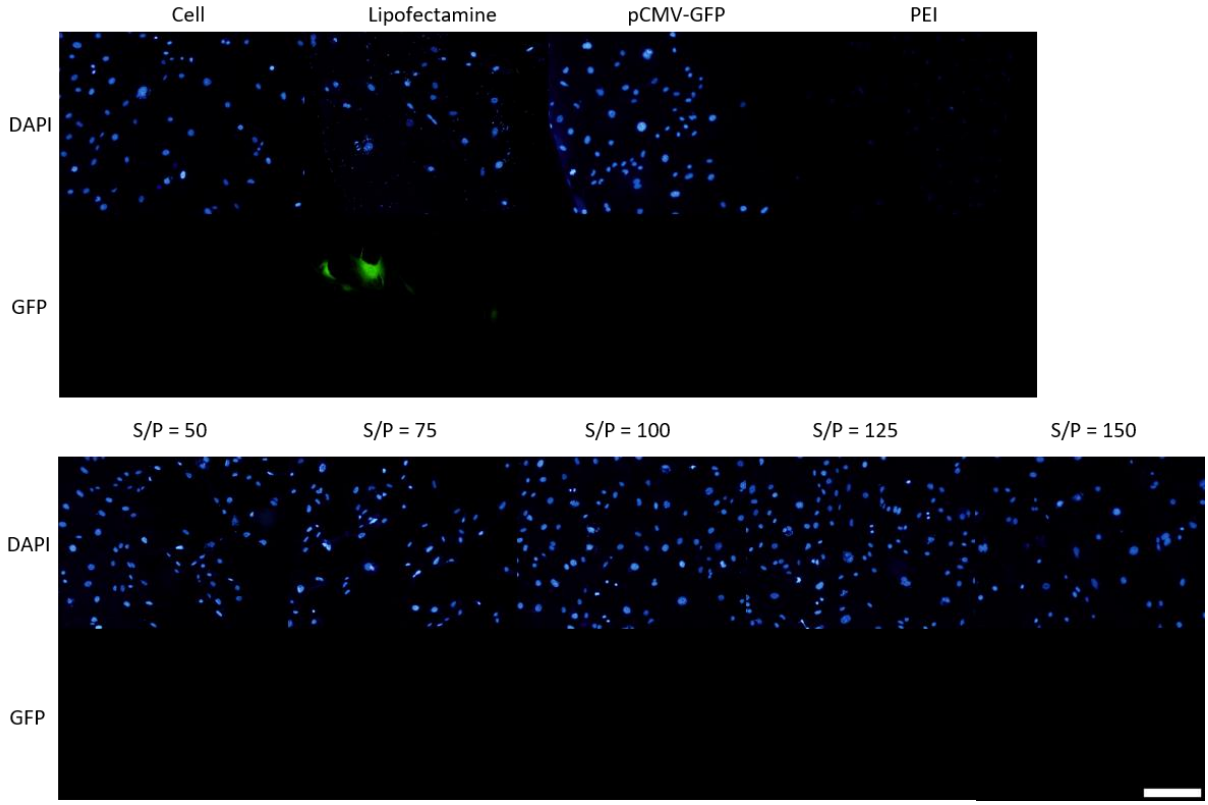

Figure S11. bEnd.3 cells were imaged under a fluorescent microscope after transfection experiments with 40-THG polyplexes carrying pCMV-GFP. No expression was observed in the bEnd.3 cells. The scale bar represents 200 µm.

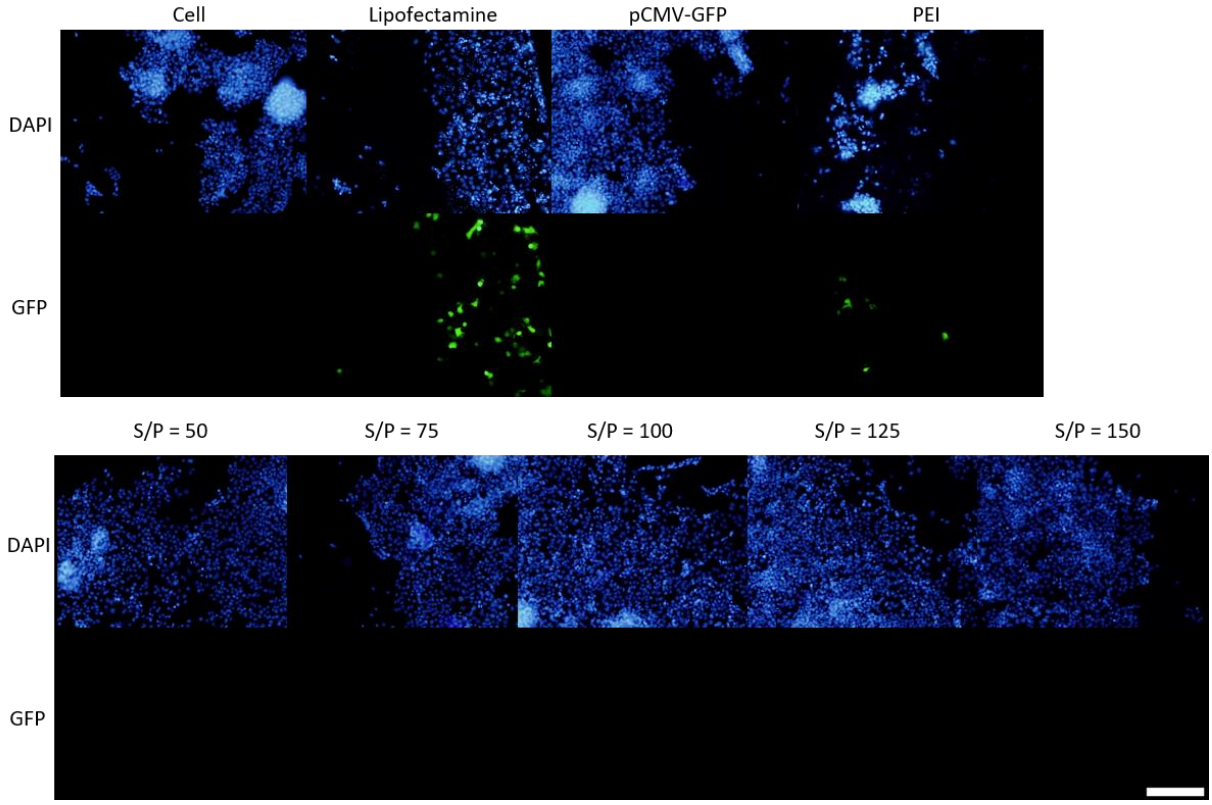

Figure S12. HepG2 cells were imaged under a fluorescent microscope after transfection experiments with 40-THG polyplexes carrying pCMV-GFP. No expression was observed in the HepG2 cells. Scale bar represents 200 µm.

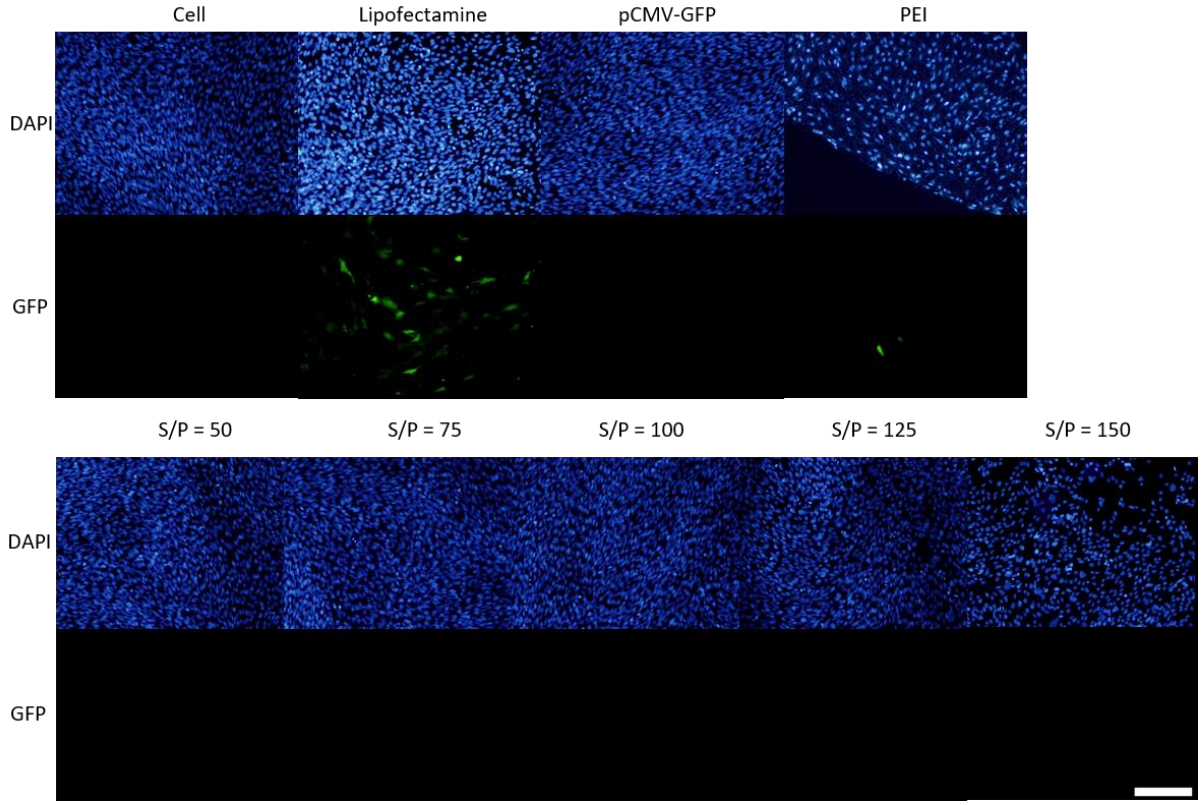

Figure S13. C2C12 cells were imaged under a fluorescent microscope after transfection experiments with 40-THG polyplexes carrying pCMV-GFP. No expression was observed in the C2C12 cells. The scale bar represents 200 µm.

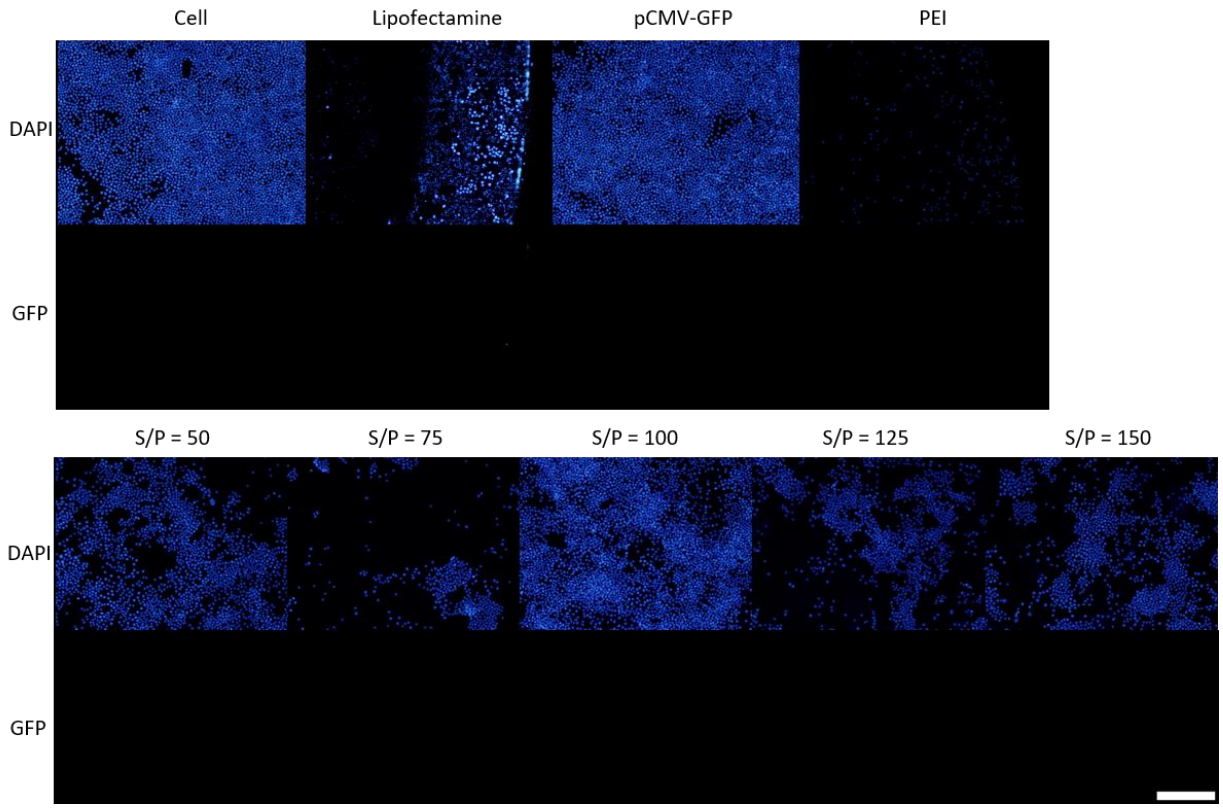

Figure S14. RAW264 cells were imaged under a fluorescent microscope after transfection experiments with polyplexes carrying pCMV-GFP. No expression was observed in the RAW264 cells. The scale bar represents 200 µm.

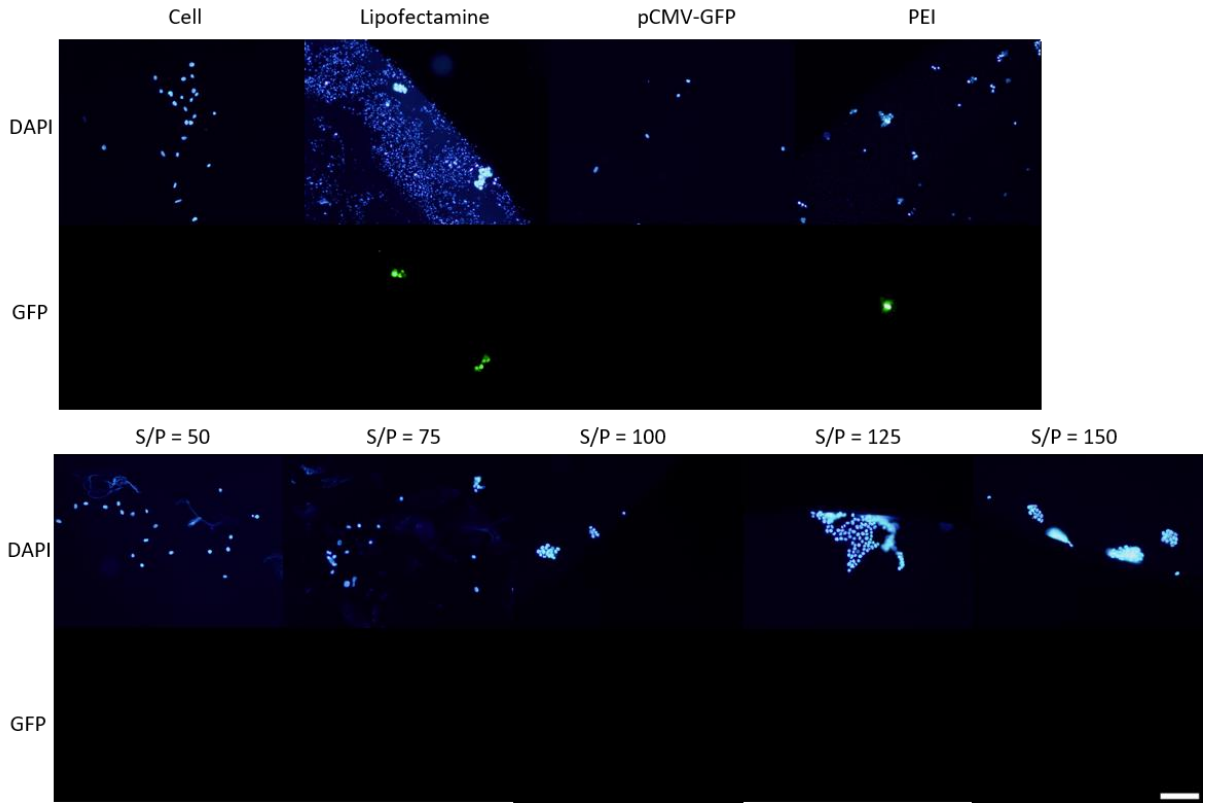

Figure S15. HEK293 cells were imaged under a fluorescent microscope after transfection experiments with polyplexes carrying pCMV-GFP. No expression was observed in the HEK293 cells. The scale bar represents 200 µm.

Although, HEK293 cells were seeded at 15.000 cells/well like the other cell lines, very few cells are seen in Figure 9. This is a result of HEK293 cells being semi-adherent and thus not adequately attaching to the well plate. As HEK293 cells are known to be easily transfected, optimization of their adherence properties is expected.[30] However, this set of experiments clearly indicate that **40-THG** is not adequate for gene transfection purposes. Optimization of cell adherence would most likely be time consuming and result in minor, if any, GFP expression.

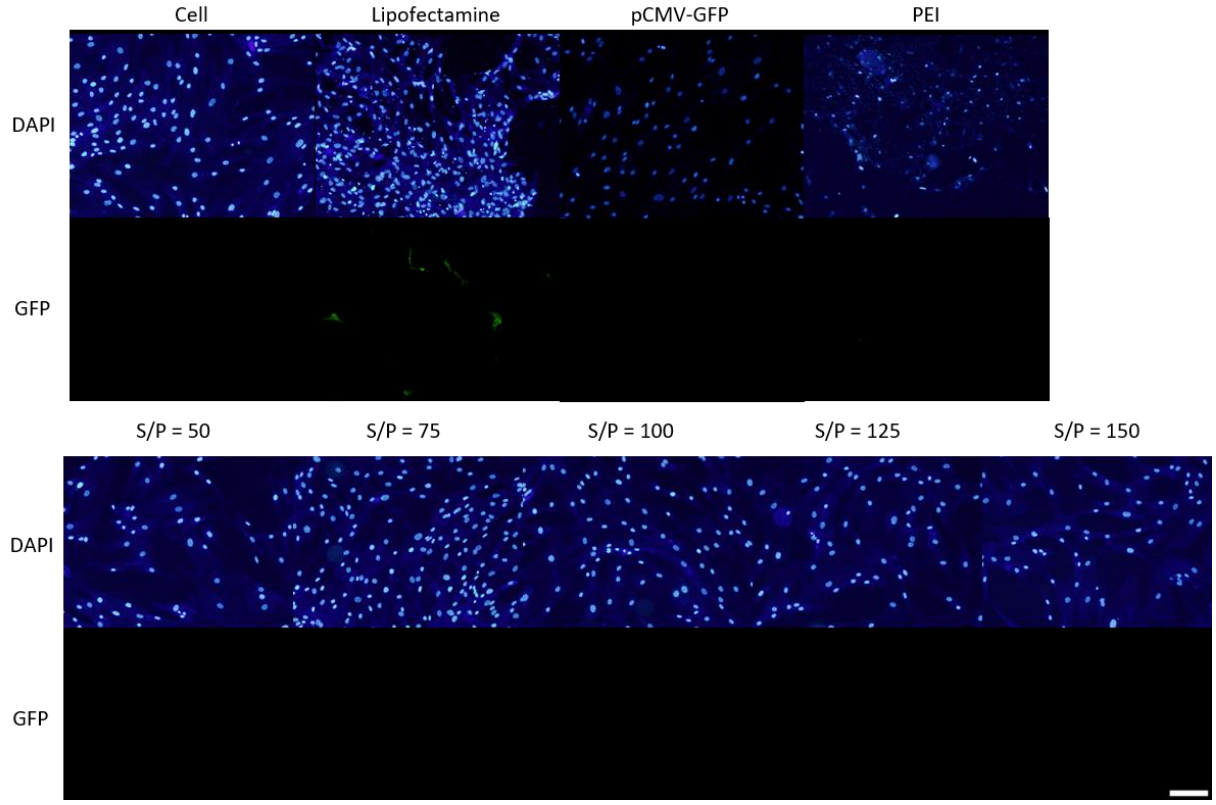

Figure S16. HFF cells were imaged under a fluorescent microscope after transfection experiments with polyplexes carrying pCMV-GFP. No expression was observed in the HFF cells. The scale bar represents 200 µm.

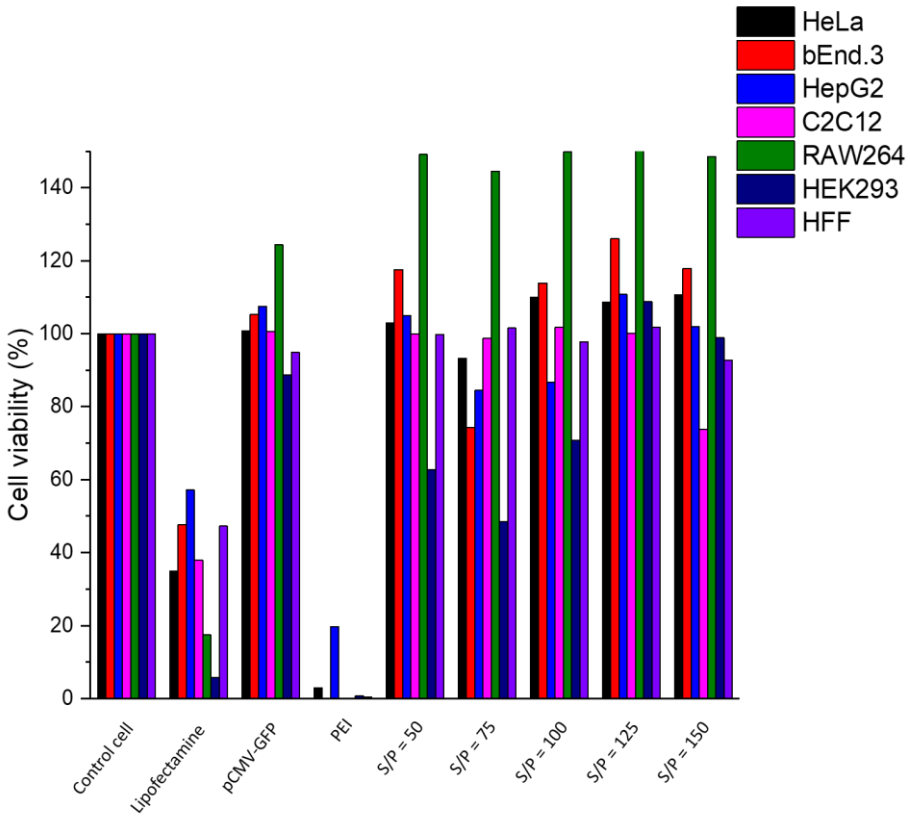

Figure S17. AlamarBlue cytotoxicity assay with resazurin. Significant cell death is only observed for Lipofectamine 3000 and *bPEI*$_{25k}$.

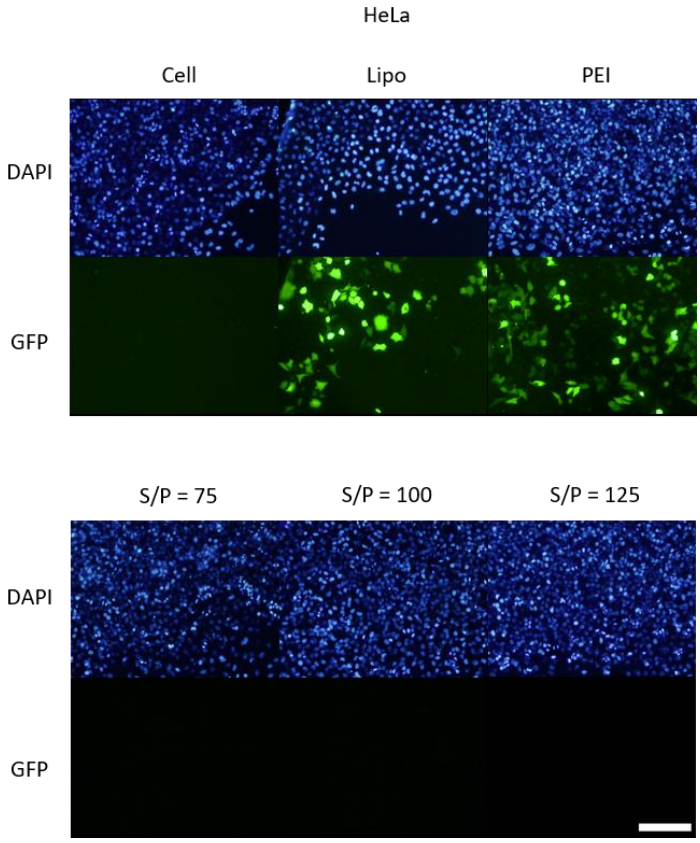

Figure S18. Transfection with 50-THG at S/P = 75, 100 and 125 on HeLa cells was repeated, along with Lipofectamine™ 3000 and $bPEI_{25k}$ as positive controls. The scale bar represents 200 μm.

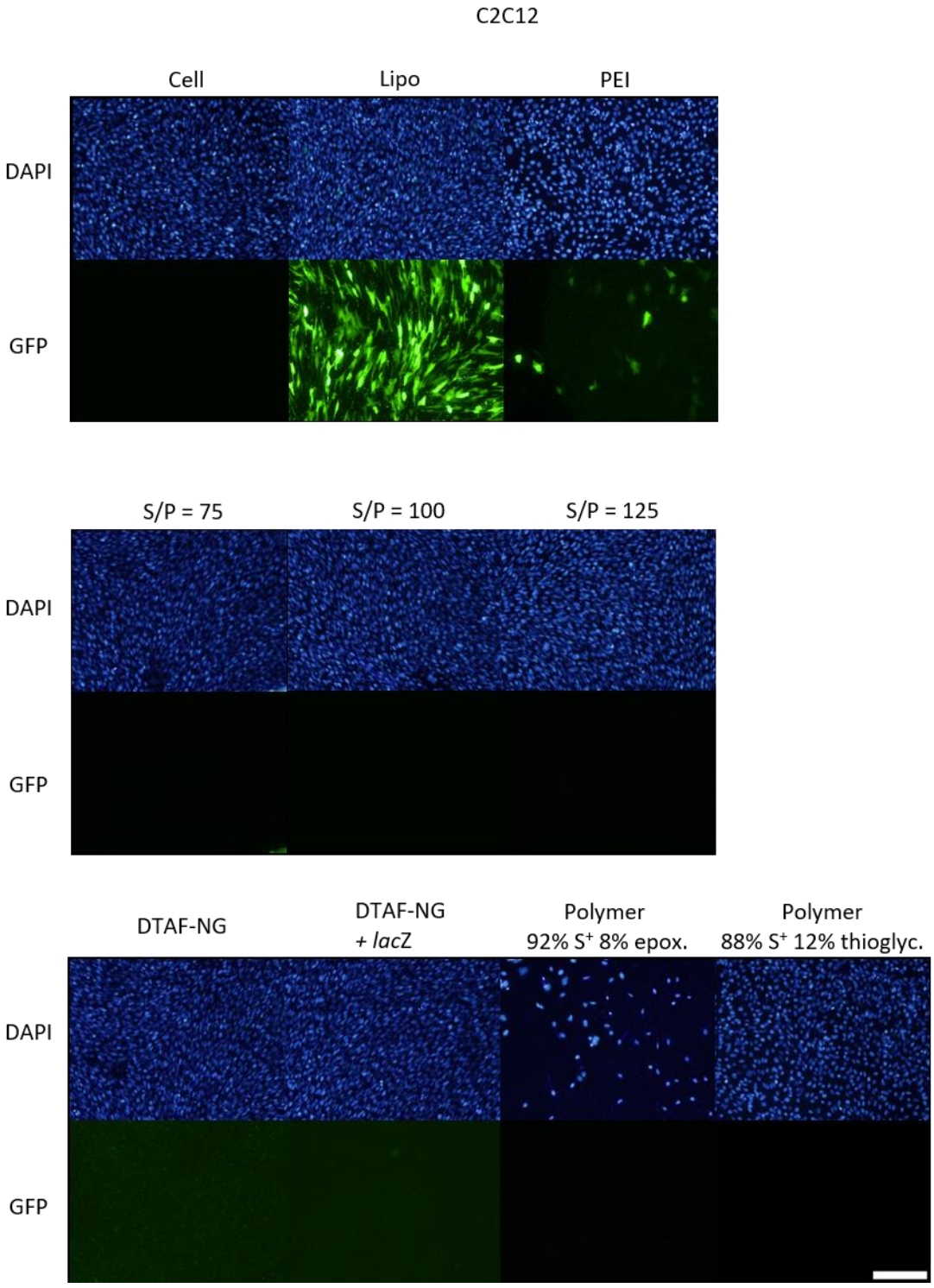

Figure S19. Transfection with 50-THG at S/P = 75, 100 and 125 on C2C12 cells was repeated, along with positive controls with Lipofectamine™ 3000 and $bPEI_{25k}$. Uptake was evaluated for *lacZ* polyplexes in dyed nanogel and empty dyed nanogels. Transfection efficiency was also monitored for GFP polyplexes formed from sulfonium polymers. The scale bar represents 200 μm.

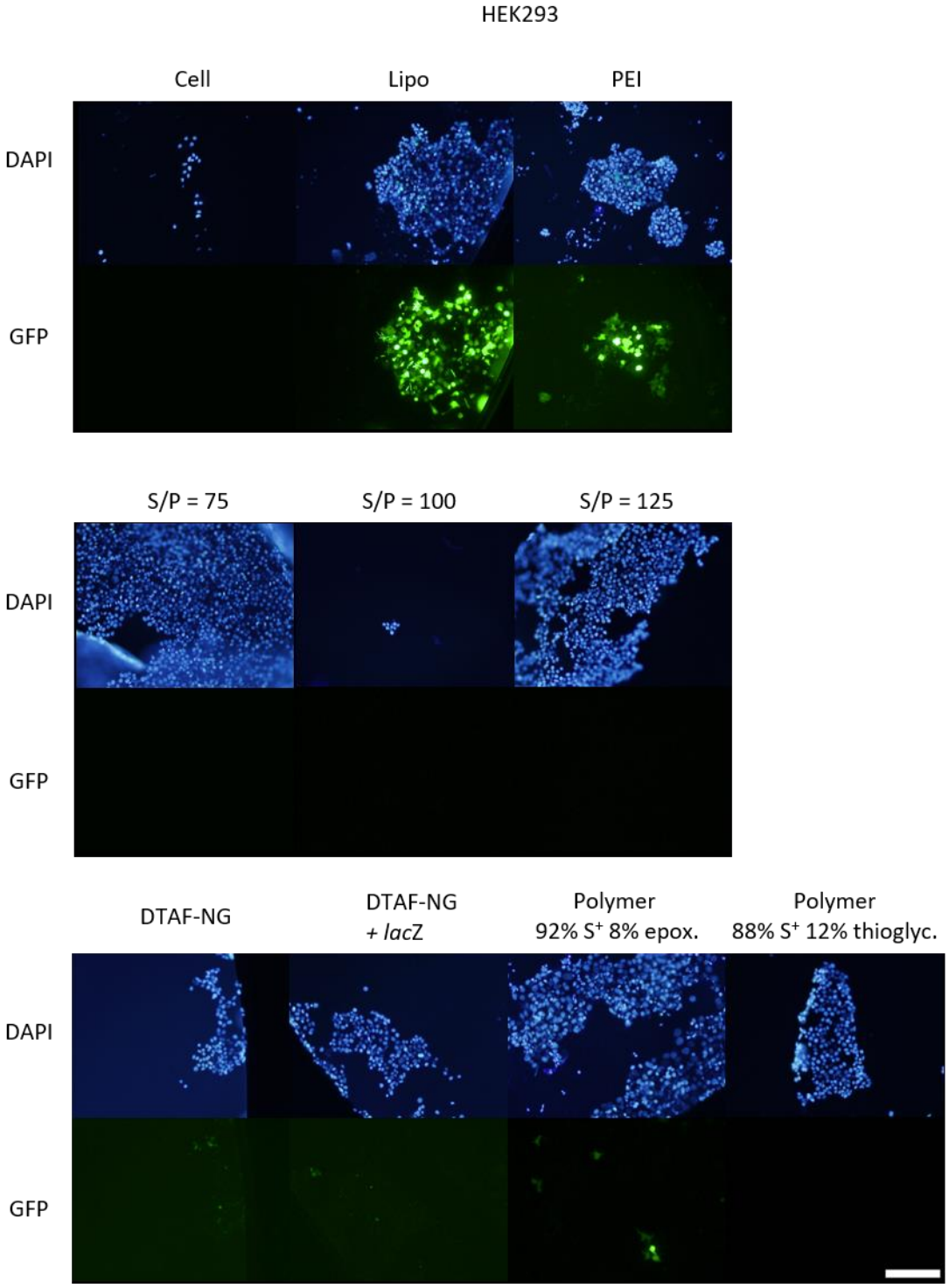

Figure S20. Transfection with 50-THG at S/P = 75, 100 and 125 on HEK293 cells was repeated, along with positive control. Uptake was evaluated for *lac*Z polyplexes in dyed nanogel and empty dyed nanogels. Transfection efficiency was also monitored for GFP polyplexes formed from sulfonium polymers. The scale bar represents 200 µm.

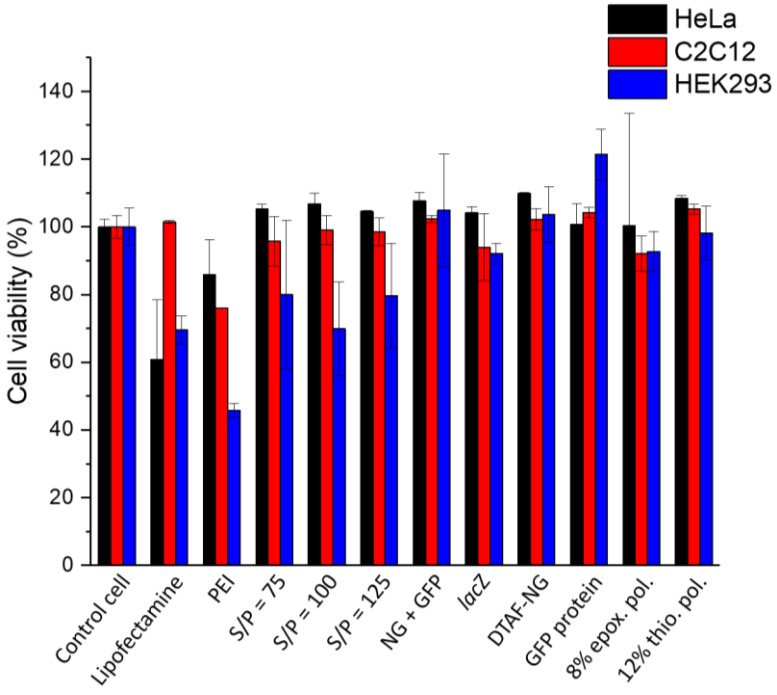

Figure S21. AlamarBlue cytotoxicity assay with resazurin gave the cell viability of the cells after 2h exposure to polyplexes, DTAF-labelled 40-THG, and free GFP protein. Significant cell death is only observed for Lipofectamine 3000 and *bPEI*$_{25k}$. Experiments were done in duplo.

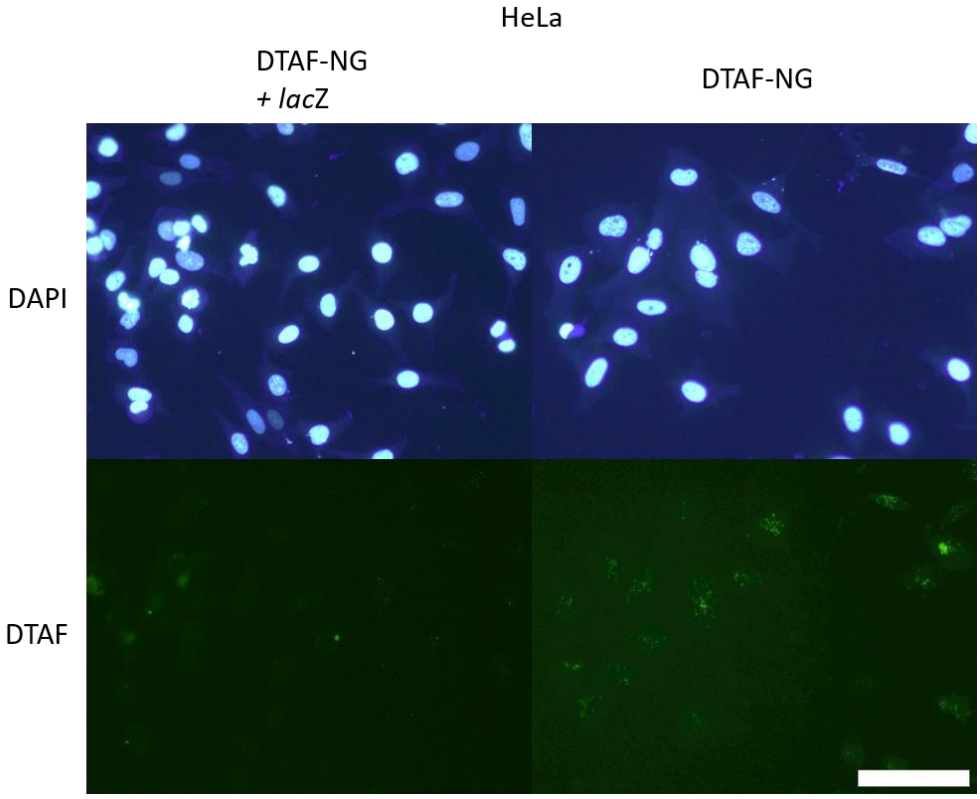

Figure S22. Fluorescence images taken of HeLa cells treated with DTAF-labelled 50-THG with or without plasmid DNA. 200x magnification. The scale bar represents 100 µm.

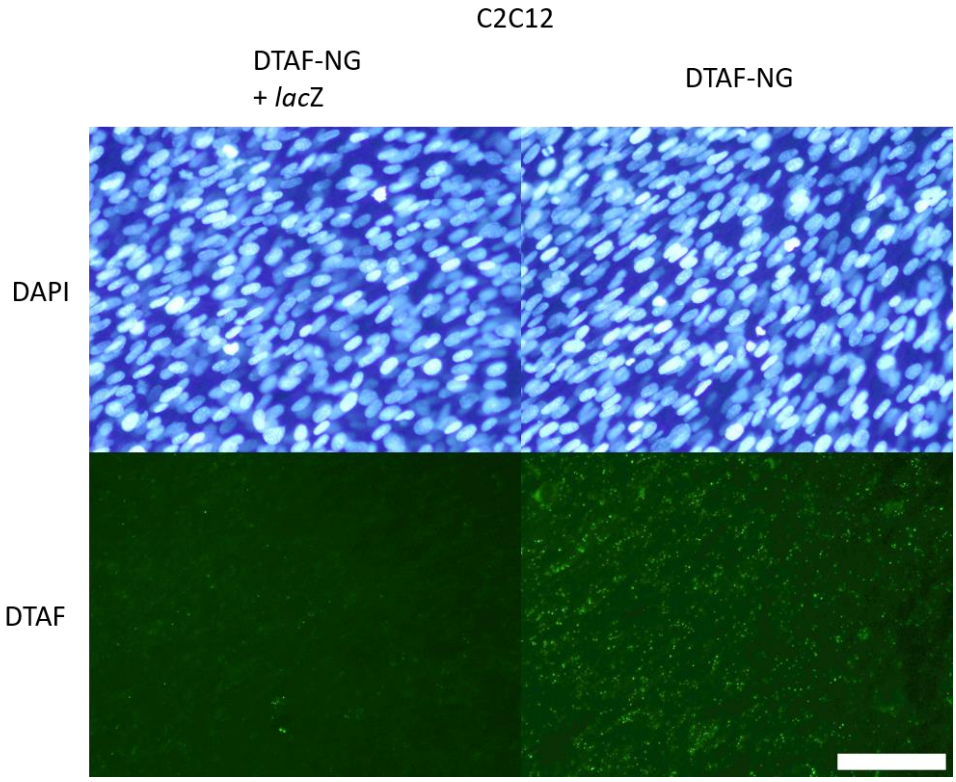

Figure S23. Fluorescence images taken of C2C12 cells treated with DTAF-labelled 50-THG with or without plasmid DNA. 200x magnification. The scale bar represents 100 μm.

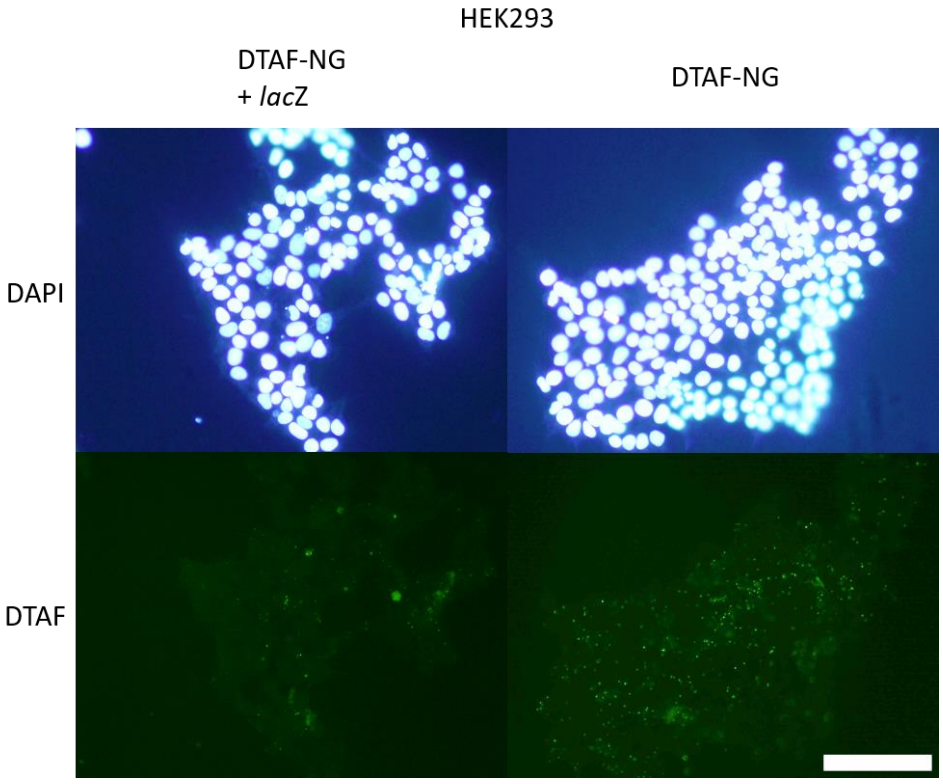

Figure S24. Fluorescence images taken of HEK293 cells treated with DTAF-labelled 50-THG with or without plasmid DNA. 200x magnification. The scale bar represents 100 μm.

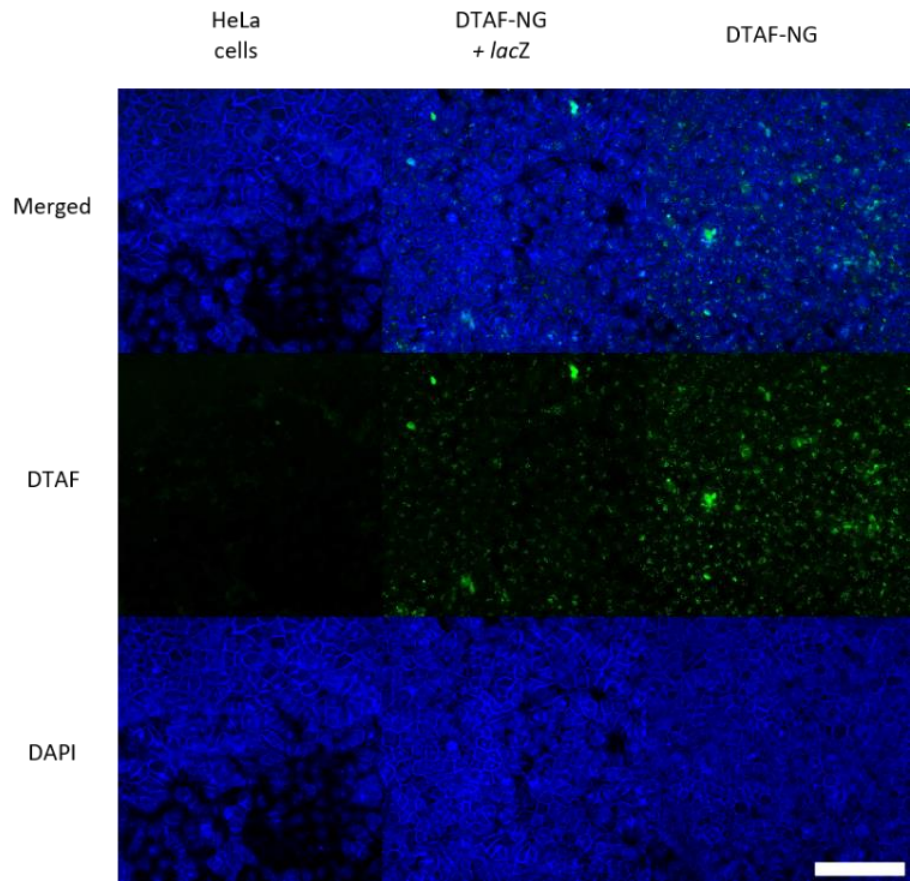

Figure S25. Confocal images were taken of HeLa cells treated with polyplexes formed from DTAF-labelled 50-THG and pCMV-*lac*Z (middle) or with empty DTAF-labelled 50-THG (right). Examining the DTAF fluorescence emitted at λ = 548 nm reveals significantly higher fluorescence, and thus cellular uptake, for 50-THG than for pCMV-*lac*Z. 200x magnification. Scale bar represents 200 µm.

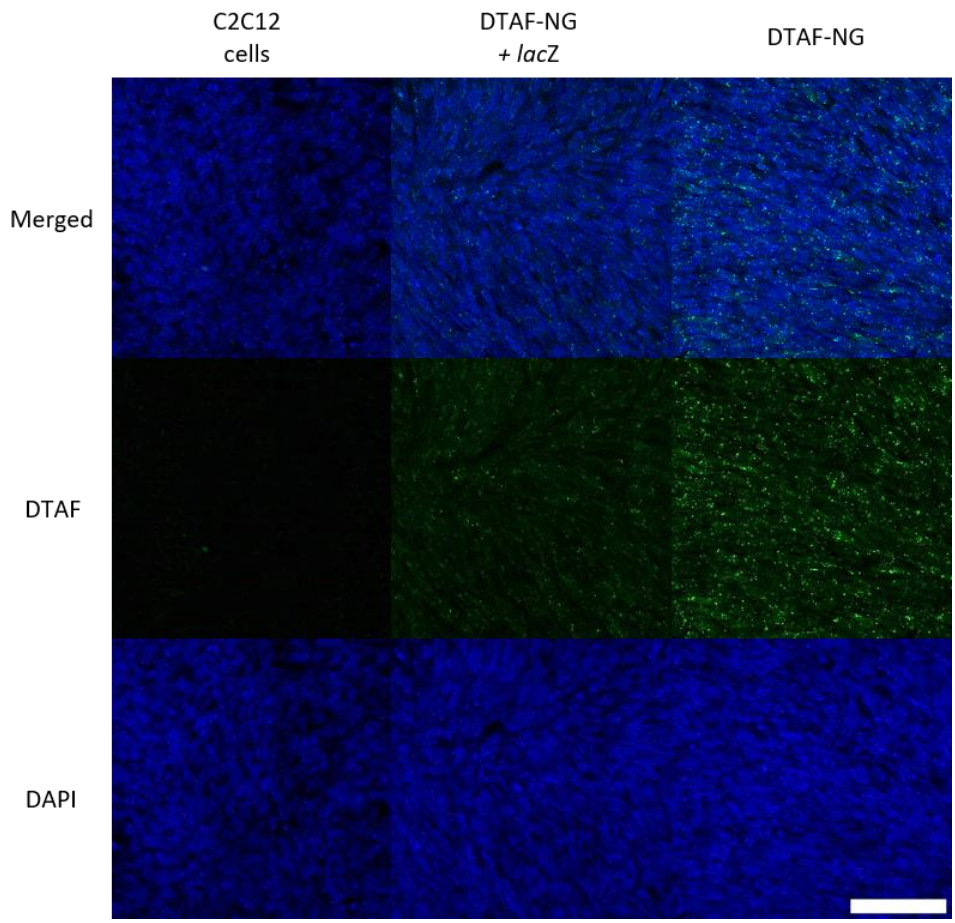

Figure S26. Confocal images were taken of C2C12 cells treated with polyplexes formed from DTAF-labelled 50-THG with pCMV-*lac*Z or with empty DTAF-labelled 50-THG. Examining the DTAF fluorescence emitted at λ = 548 nm reveals significantly higher fluorescence for 50-THG than for pCMV-*lac*Z. 200x magnification. Scale bar represents 200 μm.

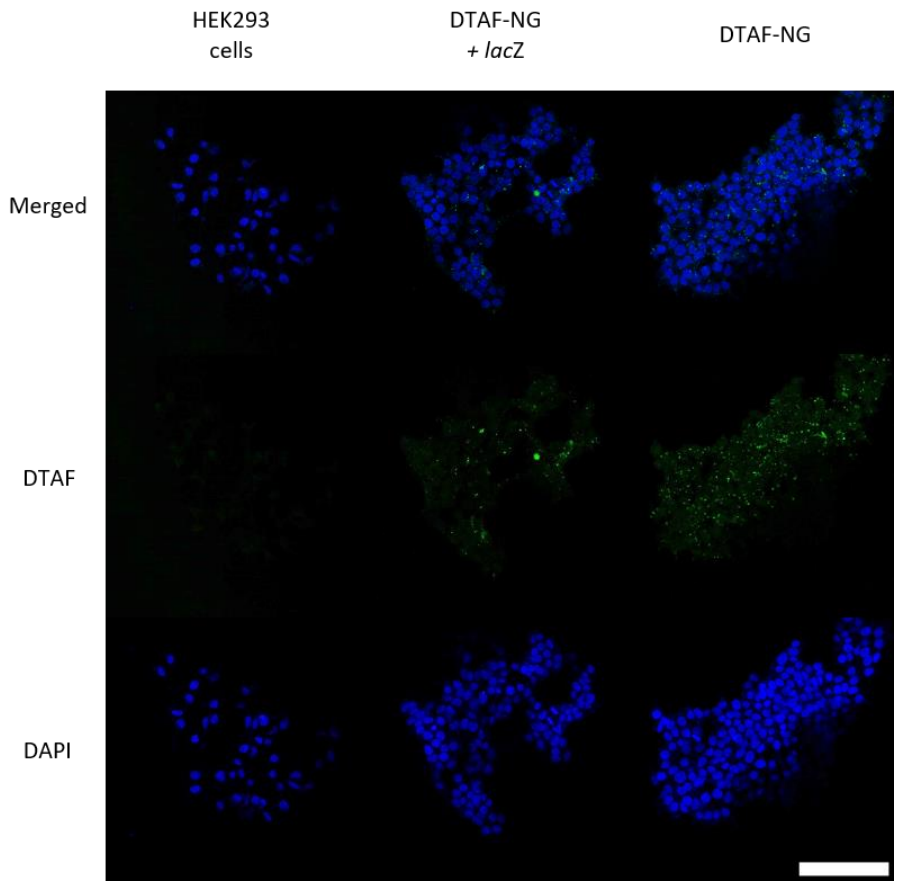

Figure S27. Confocal images were taken of HEK293 cells treated with polyplexes formed from DTAF-labelled 50-THG with pCMV-*lac*Z or with empty DTAF-labelled 50-THG. Examining the DTAF fluorescence emitted at λ = 548 nm reveals higher cellular uptake for 50-THG than for pCMV-*lac*Z. 200x magnification. Scale bar represents 200 µm.