Nanoparticles co-delivering mRNA and siRNA for simultaneous restoration and silencing of gene/protein expression *in vitro* and *in vivo*

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Supplementary information

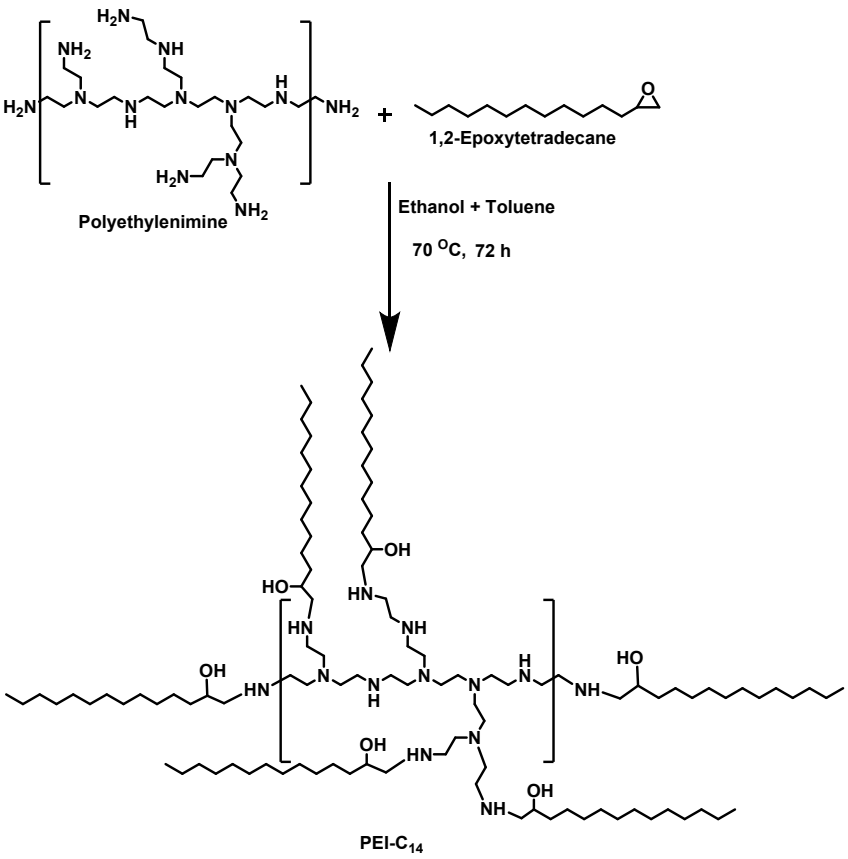

Figure S1. PEI-C_{14} lipid synthetic route: epoxide ring opening by polyethylenimine in presence of ethanol and toluene solvents at 70 °C.

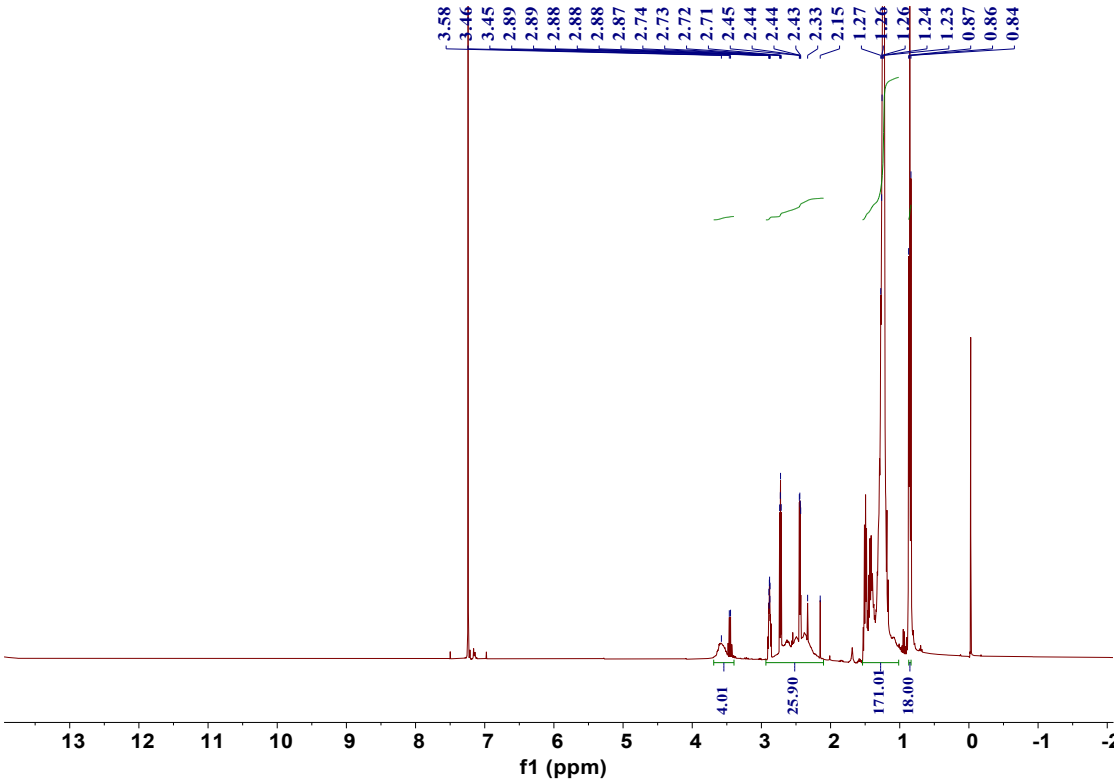

Figure S2. ^{1}H (400 MHz Bruker AVANCE) NMR Spectrum of PEI-C_{14} lipid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.71 – 3.30 (m, 4H), 2.91 – 2.10 (m, 26H), 1.51-1.24 (d, m 171H), 0.92 – 0.76 (m, 18H).

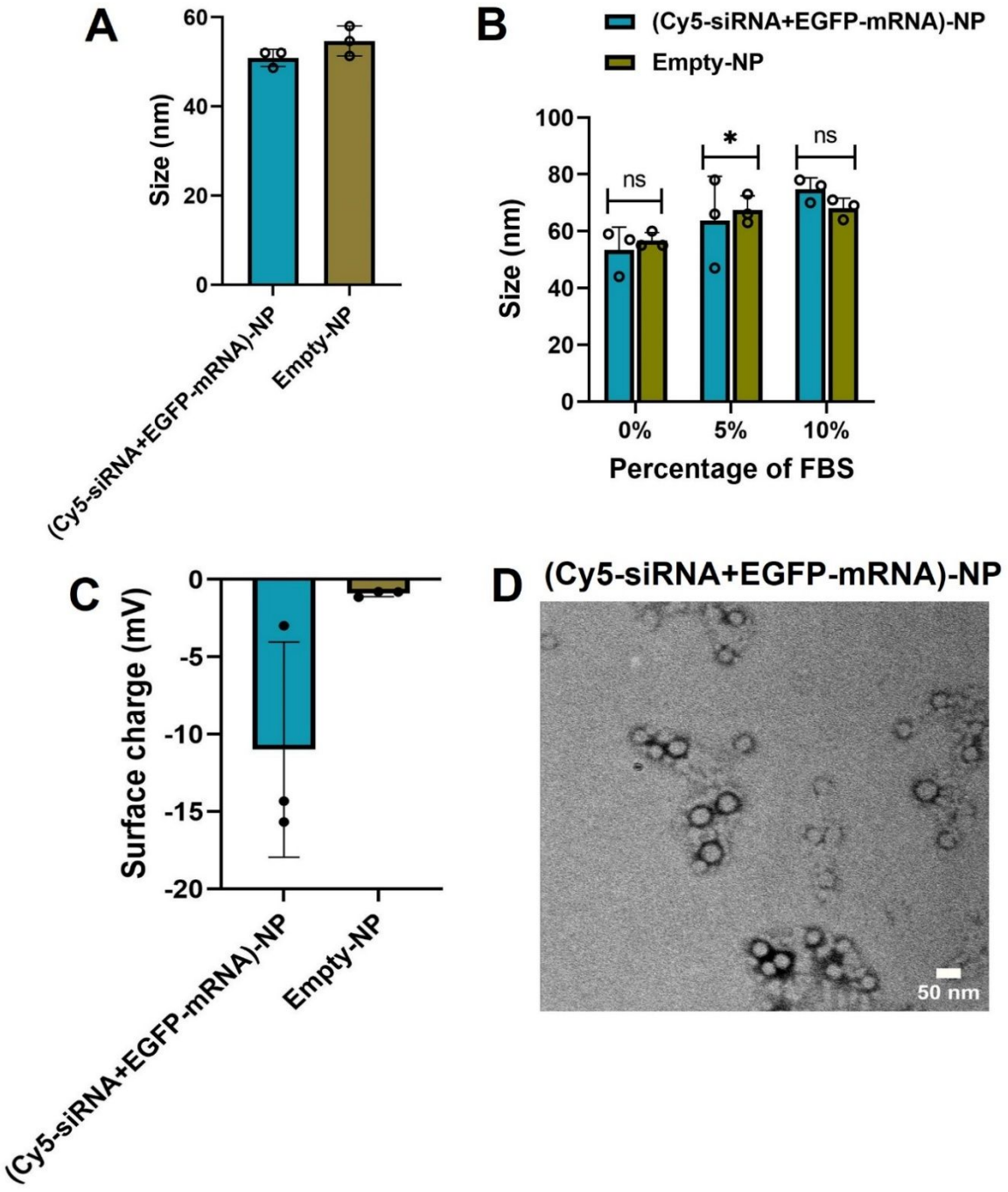

Figure S3. (A) Size of Cy5-siRNA+EGFP-mRNA-NPs along with control-NPs, measured by diluting 20 µL of NP in 980 µL sterile water using Dynamic light scattering n=3. (B) Stability of Cy5-siRNA+EGFP-mRNA-NPs in different percentages of FBS; NPs incubated for 6 hours and measured the size; n=3 (ns-no significant difference). (C) Surface of Cy5-siRNA+EGFP-mRNA-NPs along with control-NPs, measured by diluting 20 µL of NP in 980 µL sterile water using Dynamic light scattering n=3. (D) Size and morphology of Cy5-siRNA+EGFP-mRNA-NPs by transmission electron microscopy (scale bar-50 nm).

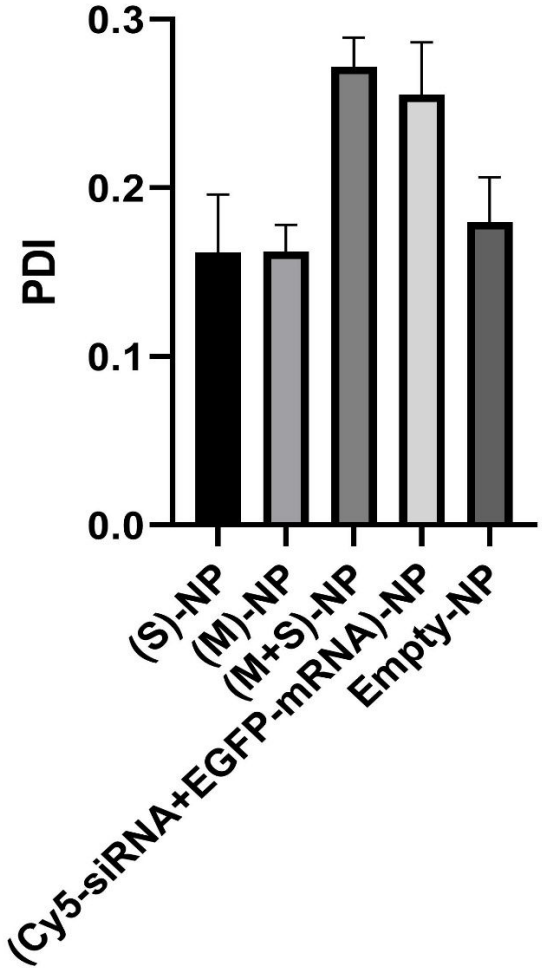

Figure S4. PDI (polydispersity index) of all single and dual NPs formulations analysed by DLS; n=3.

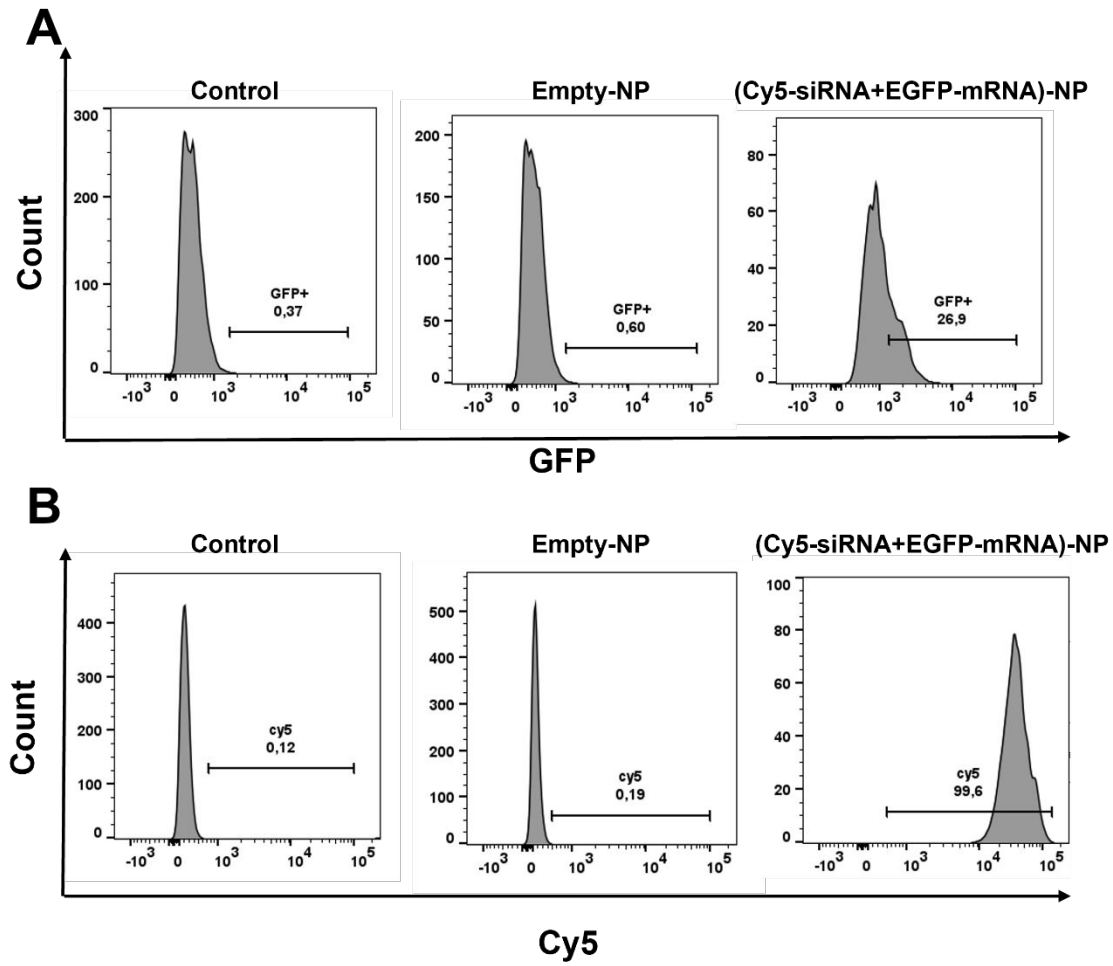

Figure S5. Representative flow cytometry histogram showing GFP (A) and Cy5 (B) expression in HT1080 cells after treated with (48 h post-treatment) Cy5-siRNA +EGFP-mRNA-NPs and Empty-NPs (control is non-treated).

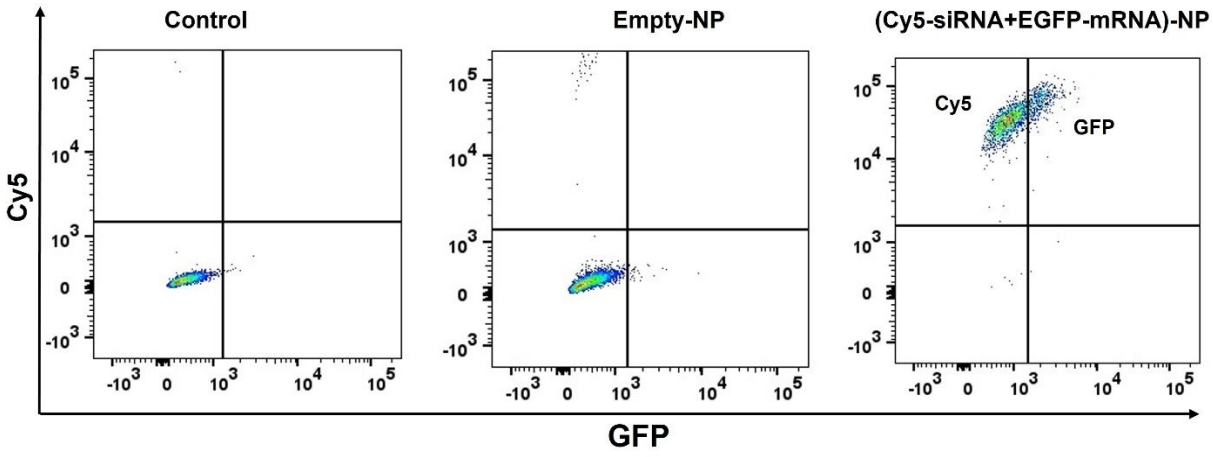

Figure S6. Cy5-siRNA+EGFP-mRNA-NPs or Empty-NPs treated to HT1080 cells. Representative flow cytometry histogram showing dual-drug in single plot, GFP population in x-axis and Cy5 population in y-axis; control is non-treated.

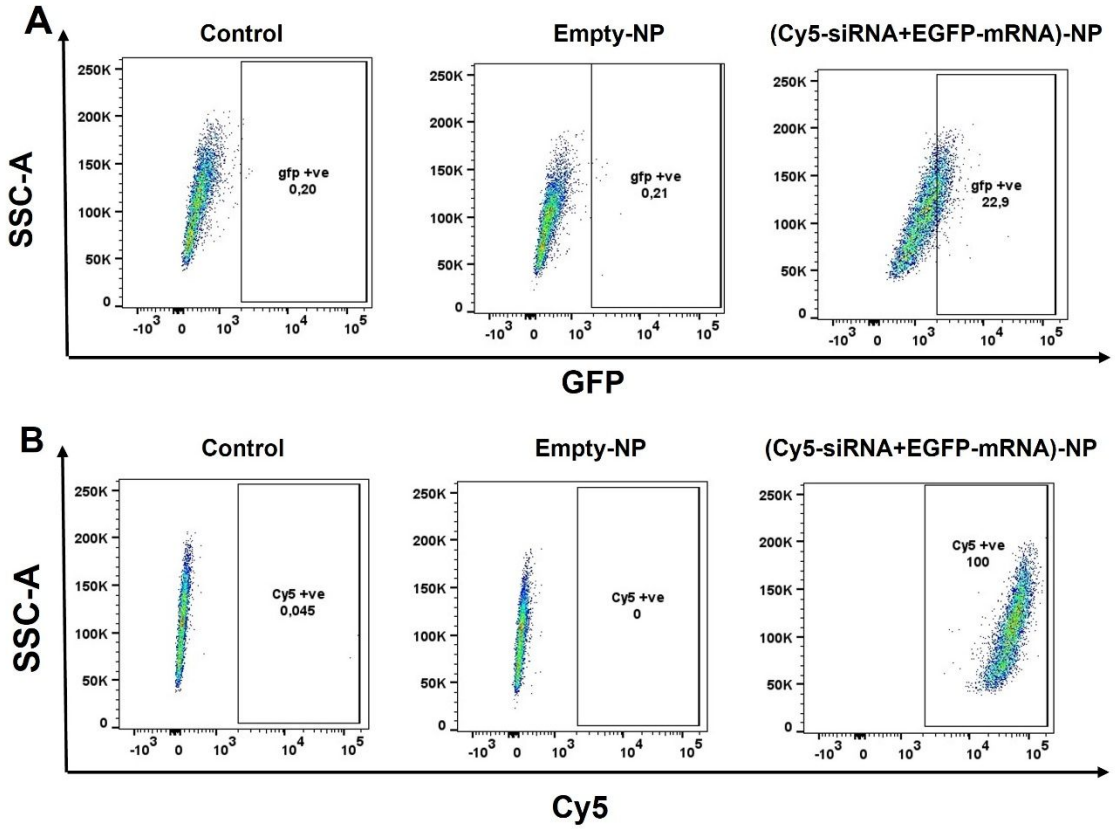

Figure S7. Representative flow cytometry SSC-A plots showing GFP (A), and Cy5 (B) expression in HT1080 cells 48 h post-treatment with Cy5-siRNA+EGFP-mRNA-NPs and Empty-NPs (control are non-treated).

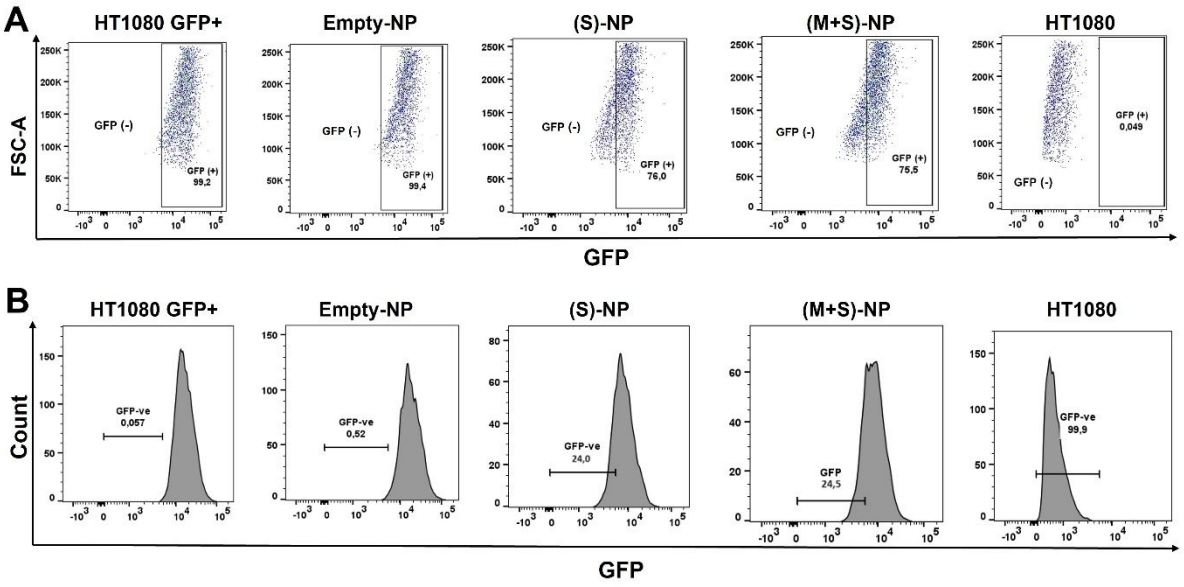

Figure S8. Single or dual-NPs along with Empty-NPs treated to HT1080 GFP (+) cells and 48 h post-treatment, the count of GFP knockdown was quantified by flow cytometry; (A) FSC-

A plots; (B) histograms representing count of GFP knockdown cells**.** HT1080 cells used as positive control.

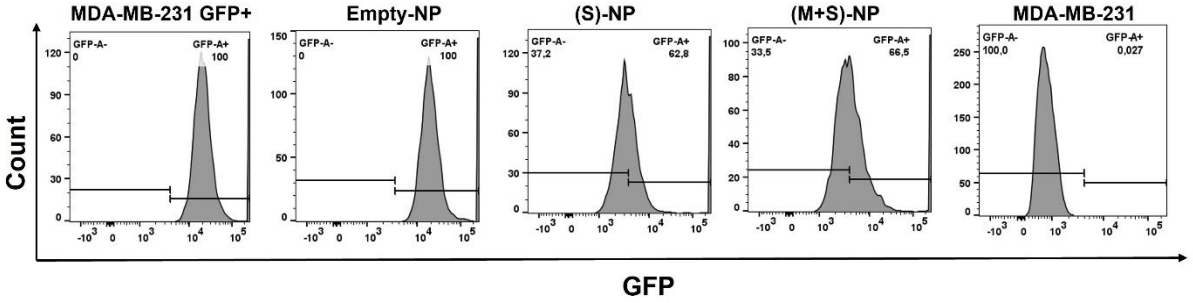

Figure S9. Single or dual-NPs along with Empty-NPs treated to MDA-MB-231 GFP (+) cells and 48 h post-treatment, the count of GFP knockdown was quantified by flow cytometry; histograms representing count of GFP knockdown cells**.** MDA-MB-231 cells used as positive control.

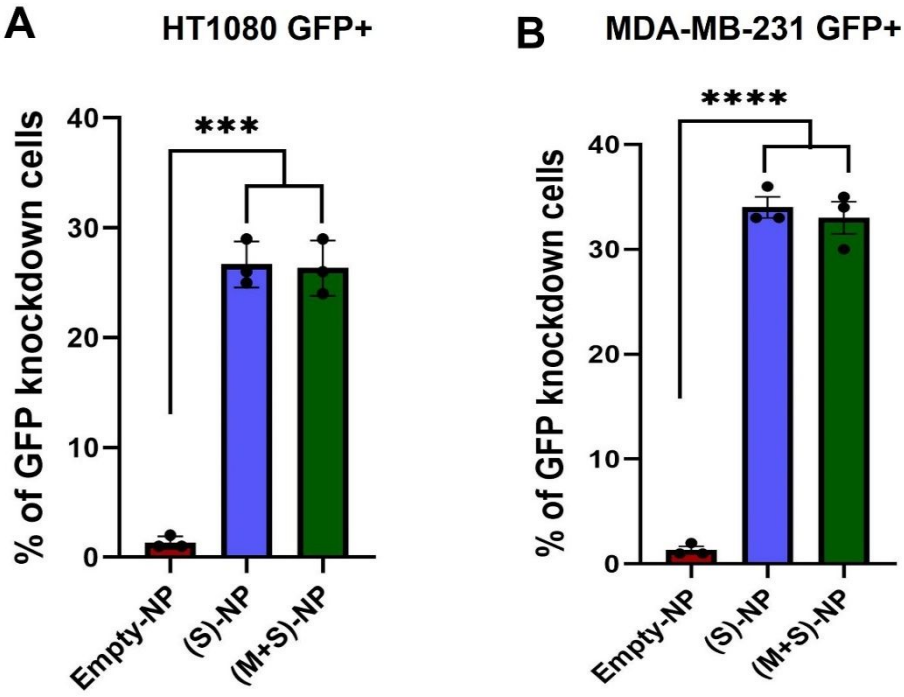

Figure S10. Percentage of GFP knockdown in (A) HT1080 GFP+ (B) MDA-MB-231 GFP+ was quantified with flow cytometry analysis; n=3 (Data represent means ± SD, ****p < 0.0001 ***p < 0.001).

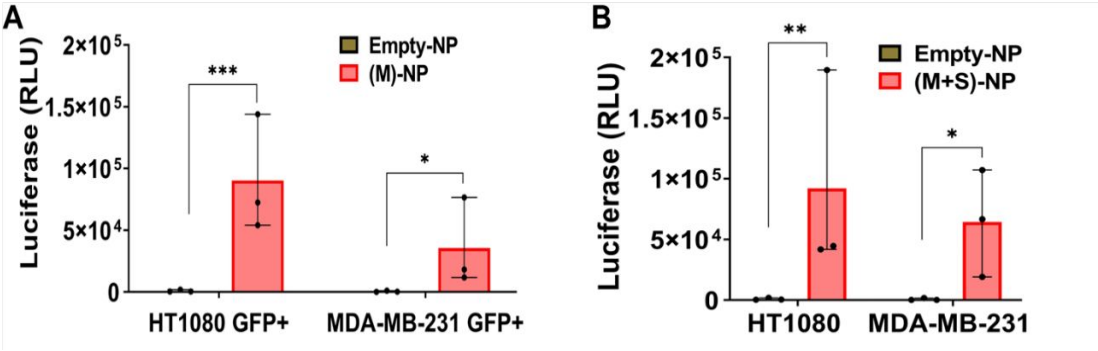

Figure S11. (A) Single drug (M)-NPs (0.016 nmol) and Empty-NPs (10 μM) treated to HT1080 GFP+ and MDA-MB-231 GFP+ cells, and 48 h post-treatment luciferase (RLU) measured by plate reader n=3 (Data represent means ± SD, *p < 0.05 ***p < 0.001). (B) HT1080 and MDA-MB-231 cells treated with (M+S)-NPs co-loaded with siRNA-GFP (1 nmol) and Luc mRNA (0.016 nmol) for 48 h, luciferase (RLU) measured by plate reader n=3 (Data represent means ± SD, *p < 0.05 **p < 0.01).

References

1. Billingsley, M. M.; Singh, N.; Ravikumar, P.; Zhang, R.; June, C. H.; Mitchell, M. J. Ionizable Lipid Nanoparacle-Mediated mRNA Delivery for Human CAR T Cell Engineering. Nano LeM 2020, 20 (3), 1578-1589. DOI: 10.1021/acs.nanole`.9b04246 From NLM Medline