Supplementary Information

Novel aromatic moieties-modified poly(glycidyl amine)s with potent siRNA delivery and cancer treatment effect

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1. Experimental Section

1.1. Synthesis of Polyepichlorohydrin1 (PECH1)

The epichlorohydrin and toluene were purified by vacuum distillation. Tetraoctylammonium bromide ($NOct_4Br$) (144 mg, 0.26 mmol) and epichlorohydrin (2.78 g, 30 mmol) were dissolved in 10 mL toluene under N_2. After cooling the mixture to -30 ℃ for 1 h, the reaction was started by adding 1 M triisobutyl aluminum (iBu_3Al) (0.7 mL, 0.7 mmol) to mixture. The mixture was stirred overnight at room temperature. After reaction, the mixture was washed with methanol multiple times and the precipitate was dried under vacuum to afford PECH1 as colorless oil. PECHs with different molecular weights was synthesized by similar method. ^{1}H NMR (500 MHz, $CDCl_3$). Repeat unit: δ 3.49 – 3.82 (CH_2Cl; CH_2CHO, 5H).

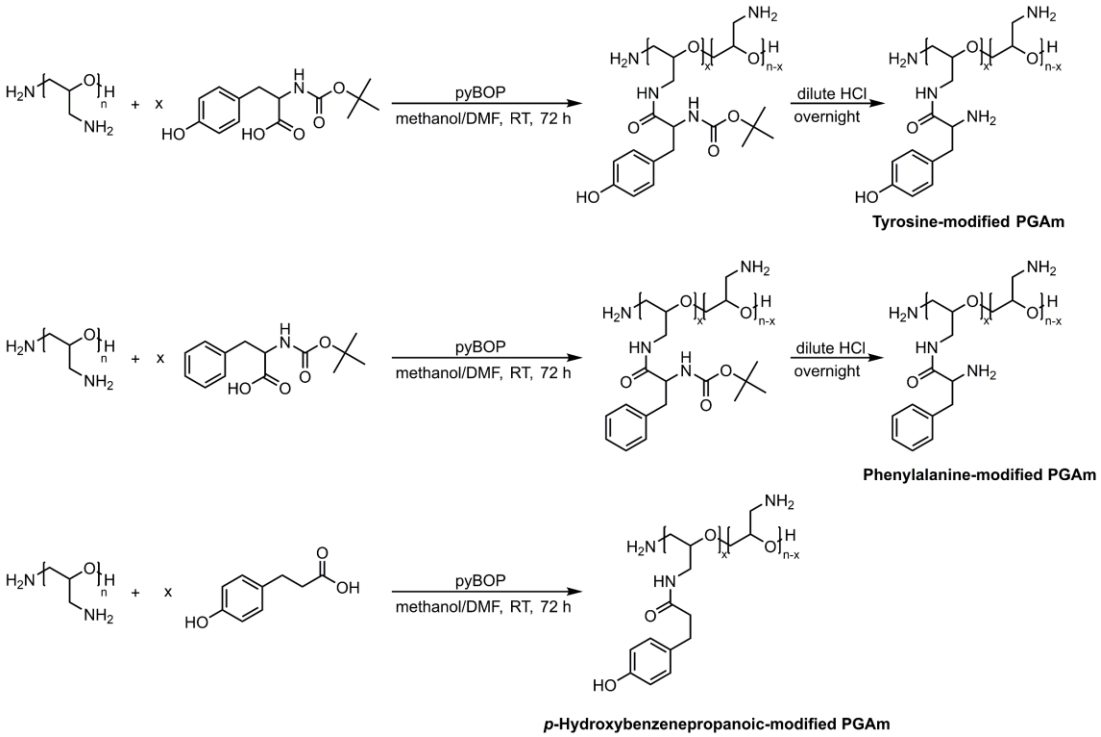
1.2. Synthesis of Poly(glycidyl azide)1 (PGA1)

PECH1 (2.5 g, 27 mmol in epichlorohydrin units) and sodium azide (1.3 g, 32 mmol) were dissolved in 30 mL dimethylformamide (DMF) and then stirred overnight at 110 ℃. After reaction, the salt was removed by filtration and PGA1 was precipitated in water as yellow oil. PGAs with different molecular weights were synthesized by corresponding PECHs in the same way. ^{1}H NMR (500 MHz, $CDCl_3$). Repeat unit: δ 3.79 – 3.49 (CH_2CHO, 3H), 3.45 – 3.21 (CH_2N_3, 2H). ^{13}C NMR (126 MHz, $CDCl_3$). Repeat unit: δ 78.72 (CHO), 77.66 – 76.20 (CH_2O), 51.75 (CH_2N_3).

1.3. Synthesis of Poly(glycidyl amine)1 (PGAm1)

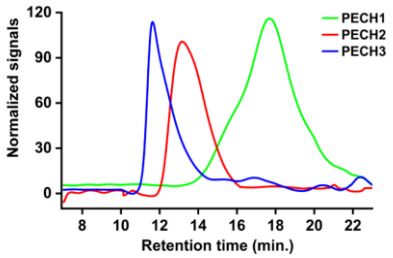
PGA1 (2.5 g, 25 mmol in 2-(azidomethyl)oxirane units) and the triphenylphosphine (PPh_3) (7.82 g, 30 mmol) were dissolved in 30 mL DMF. The reaction mixture was stirred at room temperature for 3 h and subsequently 5 mL H_2O was added. The solution was dialyzed against methanol (MWCO 1000 Da) for 3 days. Finally, PGAm1 was acquired as yellow oil after died in vacuum. ^{1}H NMR (400 MHz, D_2O). Repeat unit: δ 3.87 – 3.47 (CH_2CHO, 3H), 2.62 – 2.89 (CH_2NH_2, 2H).

1.4. The synthetic route of poly(glycidyl amine) derivatives



Scheme S1. Synthesis of poly(glycidyl amine) derivatives.

2. Supporting Data



Sample name	M_n	M_w	PDI
PECH1	10 000	15 000	1.5
PECH2	120 000	160 000	1.4
PECH3	400 000	540 000	1.3

Figure S1. The GPC curves and molecular weights of PECHs.

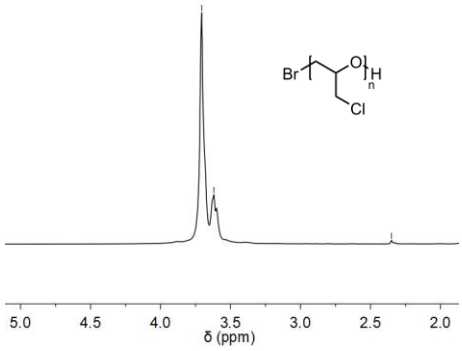


Figure S2. ^{1}H NMR spectra of PECH.

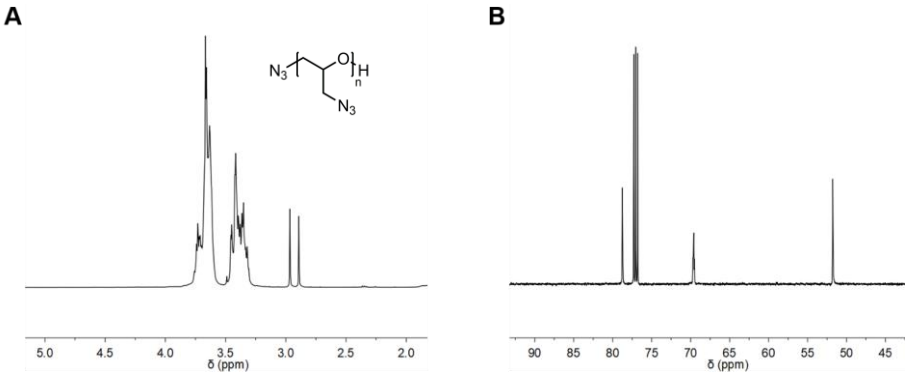


Figure S3. (A) ^{1}H NMR spectra and (B) ^{13}C NMR spectra of PGA.

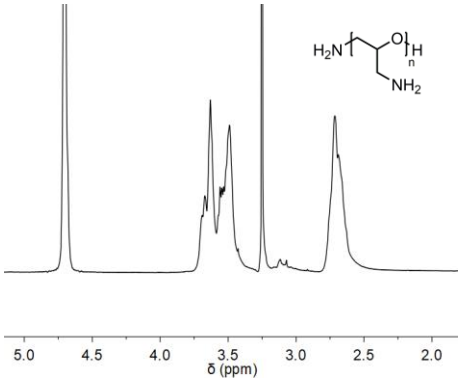


Figure S4. ^{1}H NMR spectra of PGAm.

The examples for the calculation of modification ratio:

For 3T10: the total integrated area of the 9th and 10th hydrogen atoms were set as A, and 1-6, 8 were set as B

$$graft\ ratio = \frac{A/4}{(B - A/2)/5} * 100\% = 11.2\%$$

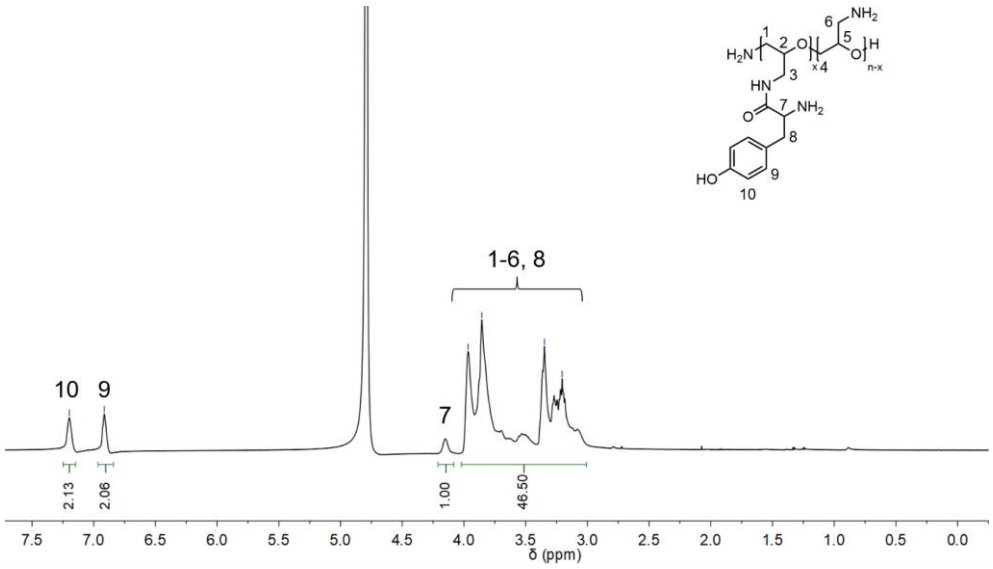


Figure S5. [1]H NMR spectra of 3T10.

For 3A10: the total integrated area of the 9th and 10th hydrogen atoms were set as A, and 1-6 were set as B

$$graft\ ratio = \frac{A/4}{B/5} * 100\% = 11.4\%$$

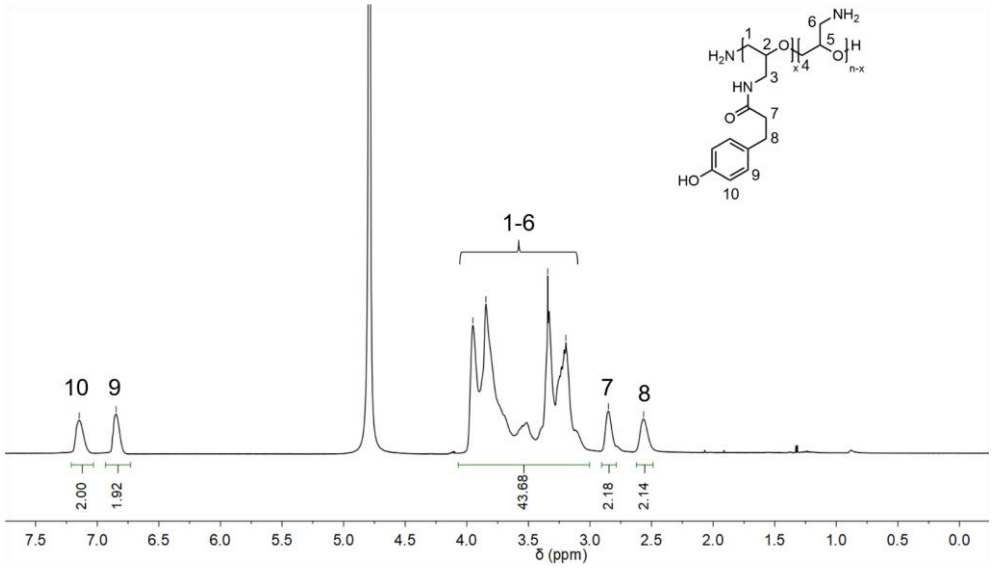


Figure S6. [1]H NMR spectra of 3A10.

For 3E10: the total integrated area of the 9-11th hydrogen atoms were set as A, and 1-6, 8 were set as B

$$graft\ ratio = \frac{A/5}{(B - A * 2/5)/5} * 100\% = 10.4\%$$

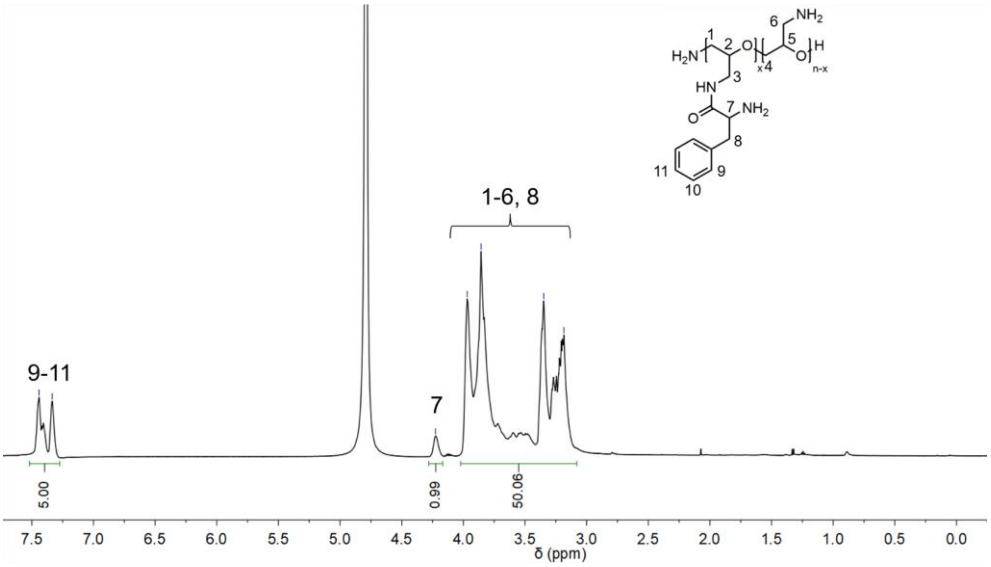


Figure S7. ^{1}H NMR spectra of 3E10.

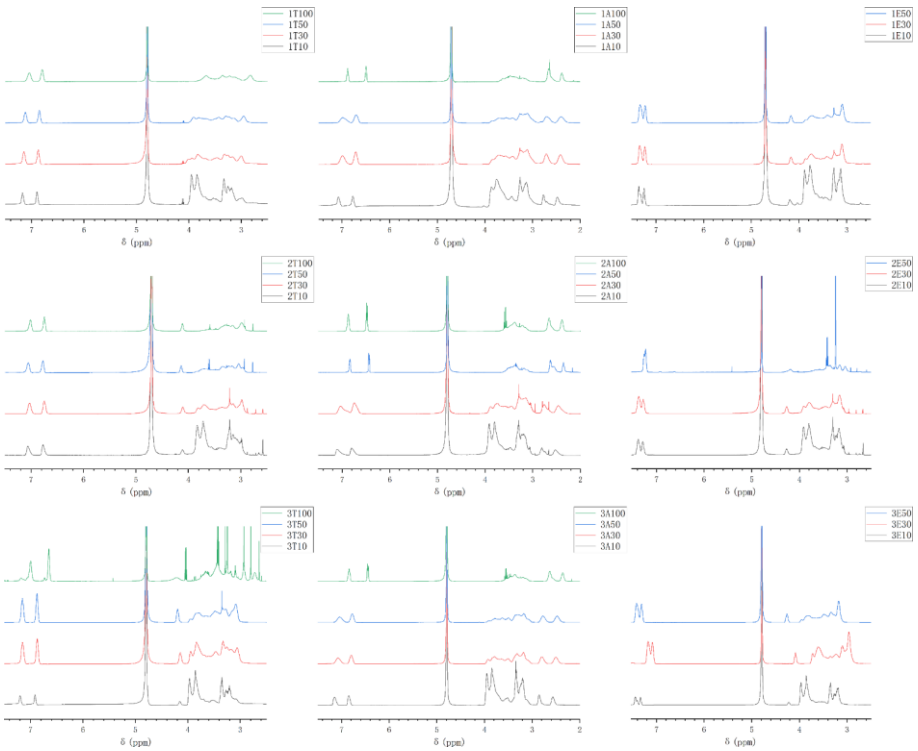


Figure S8. ^{1}H NMR spectra of tyrosine-, *p*-hydroxybenzenepropanoic acid- and phenylalanine-modified PGAms.

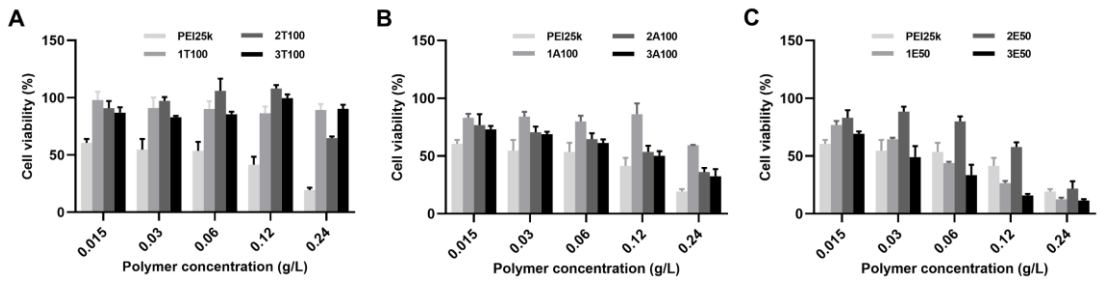


Figure S9. Cytotoxicity of parts of (A) tyrosine-; (B) *p*-hydroxybenzenepropanoic acid-; (C) phenylalanine-modified PGAms.

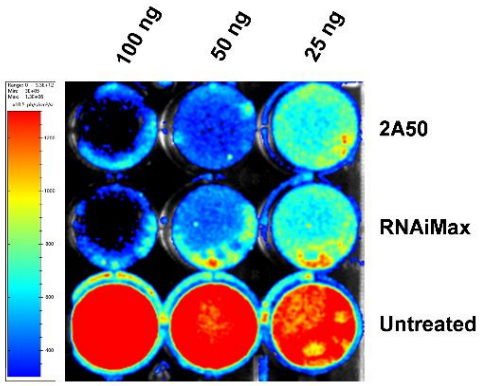


Figure S10. Luminescence images of HeLa-Luc cells after 24 h-treatment with 2A50/siLuc NPs at varying siLuc doses. RNAiMax was used as the positive control.

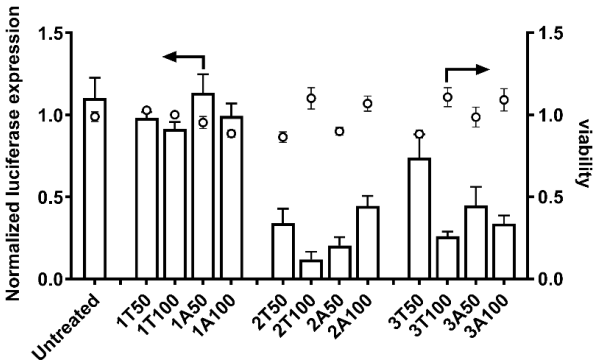


Figure S11. siRNA delivery efficiency of the selected NPs on A549-Luc cells.

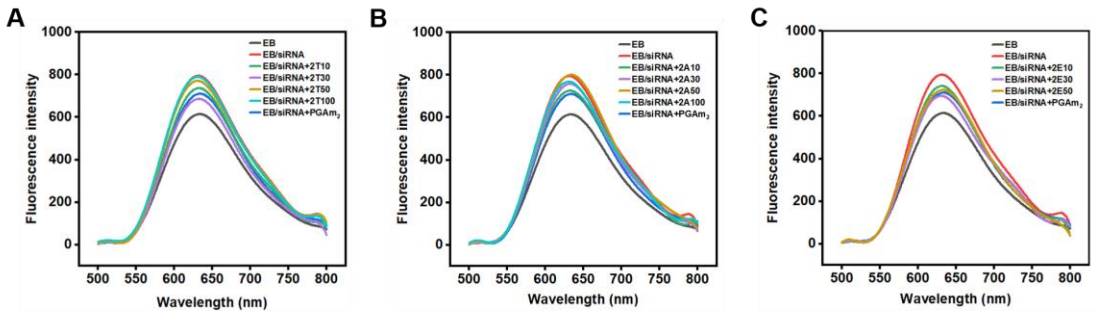


Figure S12. Fluorescence spectra of NPs based on (A) tyrosine-; (B) *p*-hydroxybenzenepropanoic acid-; (C) phenylalanine-modified PGAms.

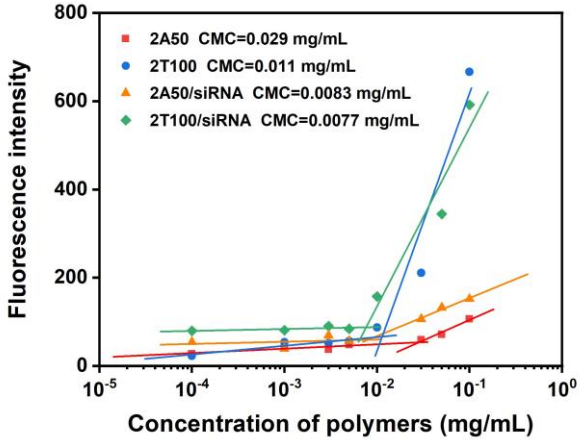


Figure S13. The CMC of selected polymers and corresponding polymer/siRNA NPs (the mass ratio of polymer to siRNA remained constant at 30:1).

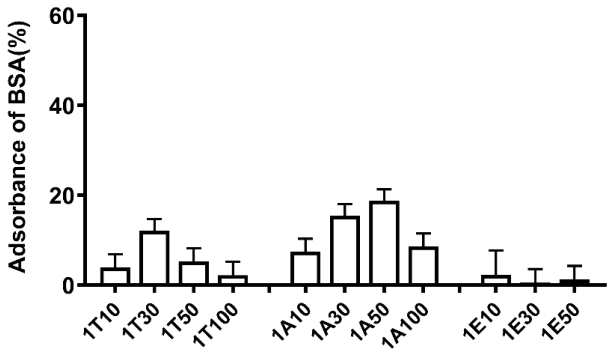


Figure S14. Protein adsorption of NPs based on PGAm1 derivatives.

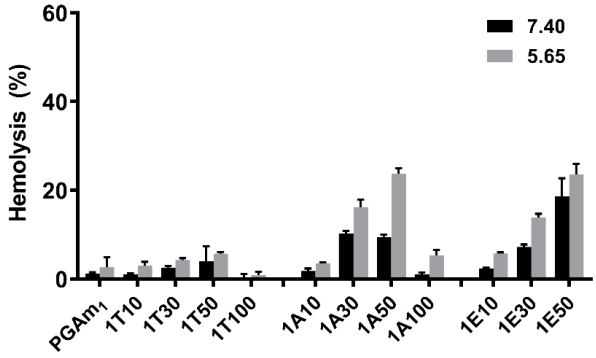


Figure S15. Hemolysis analysis of NPs based on PGAm1 derivatives.

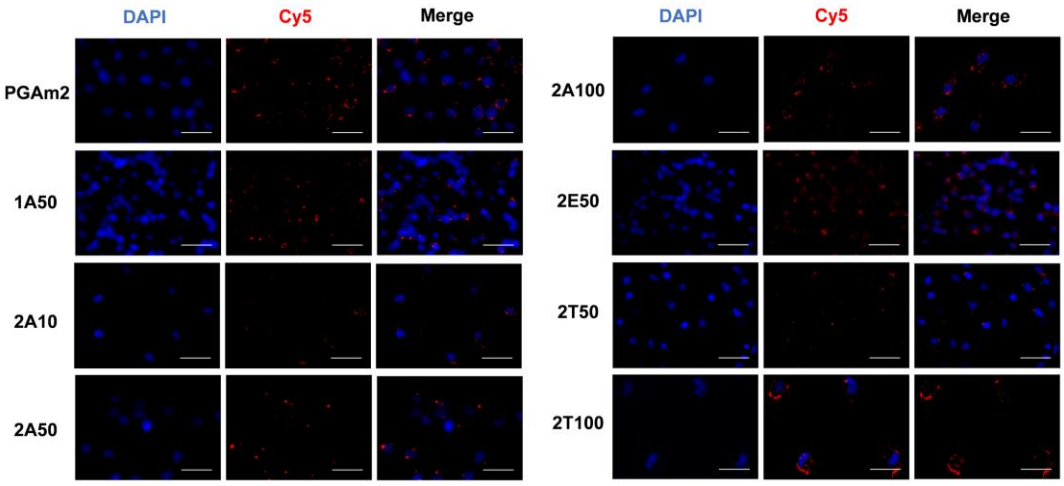


Figure S16. Internalization of NPs into HeLa-Luc cells observed by fluorescence microscopy at 6 h. Blue: DAPI, Red: Cy5-siRNA (scale bar: 50 μm).

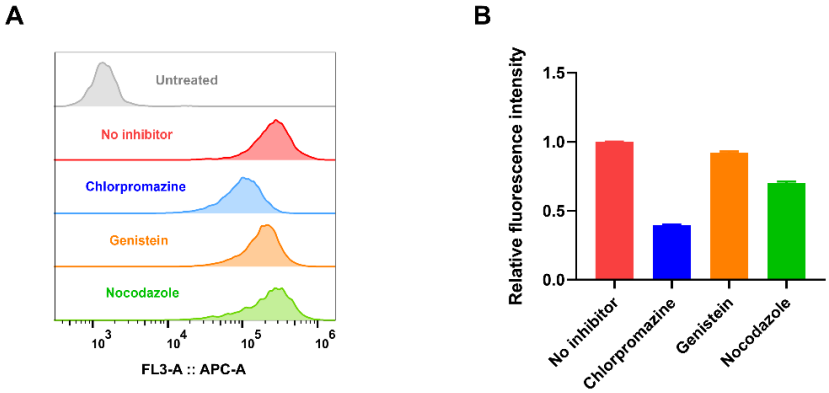


Figure S17. Quantitative analysis of cellular uptake of 2A50/Cy5-siRNA NPs into HeLa-Luc cells following pre-treatment with endocytosis inhibitors using flow cytometry.

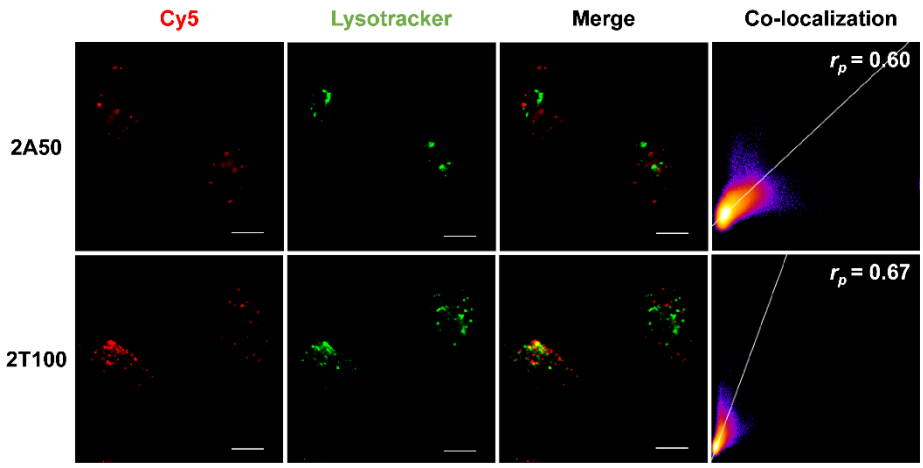


Figure S18. Endosome escape of 2A50/Cy5-siRNA NPs and 2T100/Cy5-siRNA NPs in HeLa-Luc cells observed by fluorescence microscopy at 6 h. Red: Cy5-siRNA; Green: Lysotracker (scale bar: 10 μm). Pearson's coefficient (r_p) is expressed.

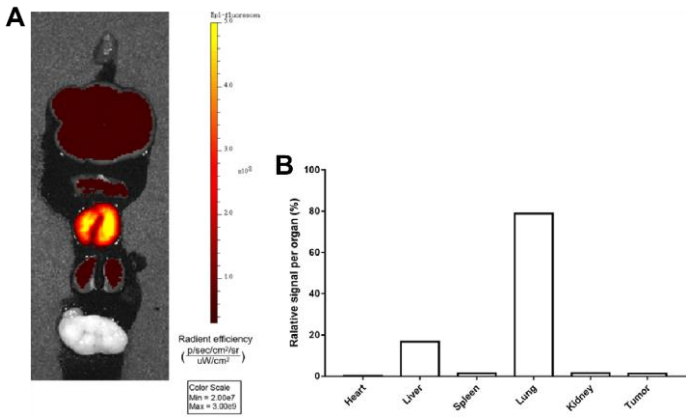


Figure S19. (A) *Ex vivo* fluorescence imaging of organs from mice dead after intravenous injection of 2A50/Cy5-siRNA NPs; (B) Quantified biodistribution in major organs.