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Supplementary Information

Tumor-penetrating peptide modified and pH-sensitive polyplexes for tumor targeted siRNA delivery

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Synthesis of poly(ethylene glycol)methyl ether methacrylate (PEGMA-CTP, PMC) via RAFT polymerization

AIBN (8.80 mg, Mn 164.21, 0.053 mmol), PEGMA (4.00 g, average Mw 500, 8 mmol), and 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid (CTP) (149.00 mg, Mn 279.38, 0.53 mmol) were taken in a 25 mL flask, and then 10 mL dioxane was then added to dissolve the mixture. After purging the reaction mixture with argon for 30 min (for complete removal of oxygen), the flask was sealed and stirred for 8 h at 80 °C, followed by rapid cooling to 25 °C. Finally, the reaction mixture was dialyzed (MWCO 3, 500 Da) against dioxane and purified water for 48 h, respectively, to obtain (PEGMA-CTP)

(PMC).

Synthesis of poly(ethylene glycol) methyl ether methacrylate block 2-(di- isopropyl amino)ethyl methacrylate (PEGMA-*b*-PDPA-CTP, PMDC) and poly-(ethylene glycol) methyl ether methacrylate block 2-(dimethylamino)ethyl methacrylate (PEGMA -*b*-PDMA-CTP, PMMC) via RAFT polymerization

AIBN (8.80 mg, Mn 164.21, 0.053 mmol), PEGMA-CTP (2.00 g, Mw 5, 000, 0.4 mmol), and DPA (2.13 g, Mn 213.32, 10 mmol) were taken in a 25 mL flask, and 10 mL dioxane was then added to dissolve the mixture. After purging the reaction mixture with argon for 30 min, the flask was sealed and stirred for 8 h at 80 °C, [1] followed by rapid cooling to 25 °C. The reaction mixture was finally dialyzed (MWCO 3, 500 Da) against dioxane and purified water for 48 h, respectively, to obtain (PEGMA-*b*-PDPA-CTP, PMDC).

The synthesis procedure of PMMC is similar to that of PMDC.

Synthesis of poly-(ethylene glycol) methyl ether methacrylate block 2-(di-isopropyl amino)ethyl methacrylate block 2-(dimethylamino)ethyl methacrylate (PEGMA-*b*-PDPA-*b*-PDMA-CTP, PMDMC) via RAFT polymerization

AIBN (8.80 mg, Mn 164.21, 0.053 mmol), PEGMA-*b*-PDPA-CTP (1.60 g, Mw 8, 200, 0.195 mmol), and DMA (0.983 g, Mn 157.21, 6.25 mmol) were taken in a 25 mL flask, and 10 mL dioxane was then added to dissolve the mixture. The flask was sealed after purging with argon for 30 min. The reaction mixture was stirred for 8 h at 80 °C, followed by rapid cooling to 25 °C. Finally, the reaction mixture was dialyzed (MWCO 7, 000 Da) against dioxane and purified water for 48 h, respectively, to obtain (PEGMA-*b*-PDPA-*b*-PDMA-CTP, PMDMC).

Synthesis of PEGMA-*b*-PDPA-*b*-DMA, PEGMA-*b*-PDPA (PMD) and PEGMA-*b*-PDMA (PMM)

The PEGMA-*b*-PDPA-*b*-DMA-CTP (PMDMC) (2.20 g, Mw 11, 000, 0.20 mmol) and AIBN (0.657 g, Mn 164.21, 4 mmol) were dissolved in dioxane (10 mL). After purging the reaction mixture with high-purity argon for 30 min, the flask was sealed and stirred for 8 h at 80 °C, [2, 3] followed by rapid cooling to 25 °C. The reaction mixture was finally dialyzed (MWCO 7, 000 Da) against dioxane and purified water for 48 h, respectively to obtain PEGMA-*b*-PDPA-*b*-DMA.

The synthesis method of PEGMA-b-PDPA and PEGMA-b-PDMA is similar to that of PEGMA-*b*-PDPA-*b*-DMA.

Synthesis of mal-PEGMA-*b*-PDPA-*b*-DMA (PMDM)

Mal-PEG-OH (Mw=5, 000) was synthesized following a published procedure with slight modification, [4] and PMDM was synthesized with dicyclohexyl- carbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) as coupling agent. PMDM (1.10 g, Mw 11, 000, 0.10 mmol), DMAP (0.018 g, Mn 122.17, 0.15 mmol), and DCC (0.031 g, Mn 206, 0.15 mmol) were dissolved in dichloromethane (25 mL). The reaction mixture was filtered after 30 min, and mal-PEG-OH (0.75 g, Mw 5, 000, 0.15 mmol) was then added to the filtrate. The resultant solution was kept at 25 °C for 72 h and later dialyzed (MWCO 14, 000 Da) against dioxane and purified water for 48 h, respectively, to obtain mal-PEGMA-*b*-PDPA-*b*-DMA (PMDM).

References

1 J. Liu, R. C. Li, G. J. Sand, V. Bulmus, T. P. Davis and H. D. Maynard, *Macromolecules*, 2012, **46**, 8-14.

2 A. B. Lowe and C. L. McCormick, *Prog. Polym. Sci.*, 2007, **32**, 283-351.

3 J. Zhou, L. Huang, W. Wang, J. Pang, Y. Zou, X. Shuai and X. Gao, *Chinese Sci.*

Bull., 2009, **54**, 3137-3146.
4 N. Nasongkla, X. Shuai, H. Ai, B. D. Weinberg, J. Pink, D. A. Boothman and J. Gao, *Angew. Chem., Int. Ed.*, 2004, **116**, 6483-6487.

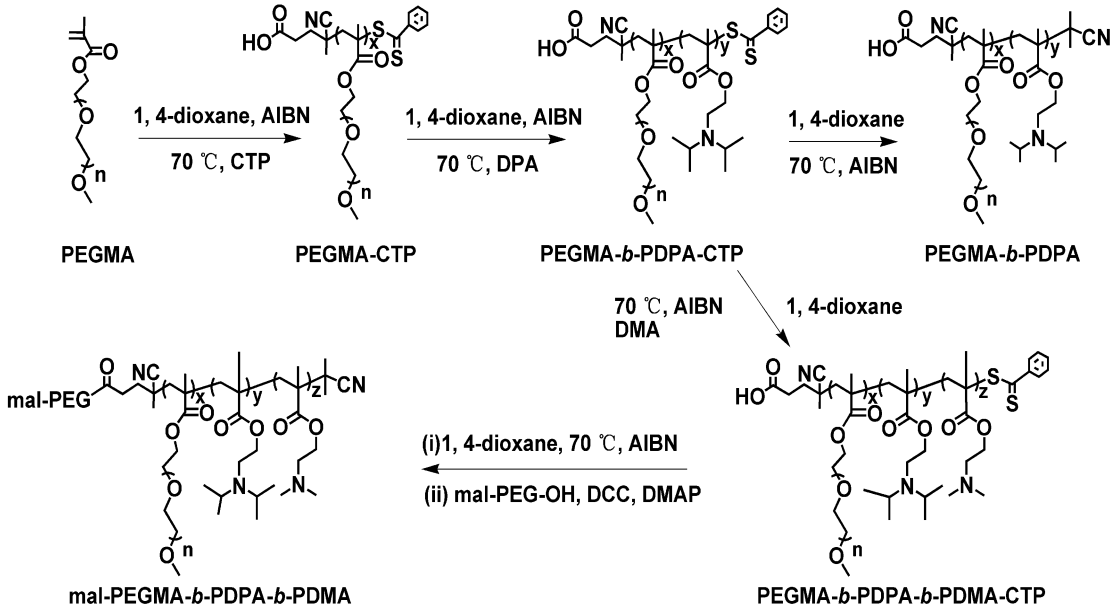

Fig. S1 Synthetic route of the copolymer mal-PEGMA-*b*-PDPA-*b*-PDMA (PMDM) and PEGMA-b-PDPA (PMD).

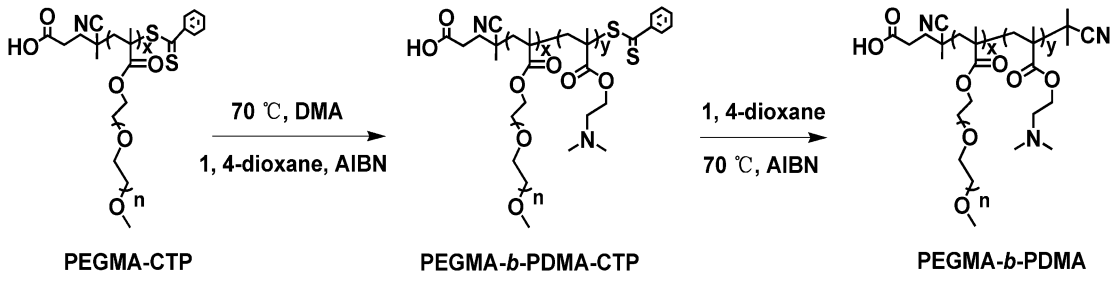

Fig. S2 Synthetic route of the copolymer PEGMA-b-PDMA (PMM).

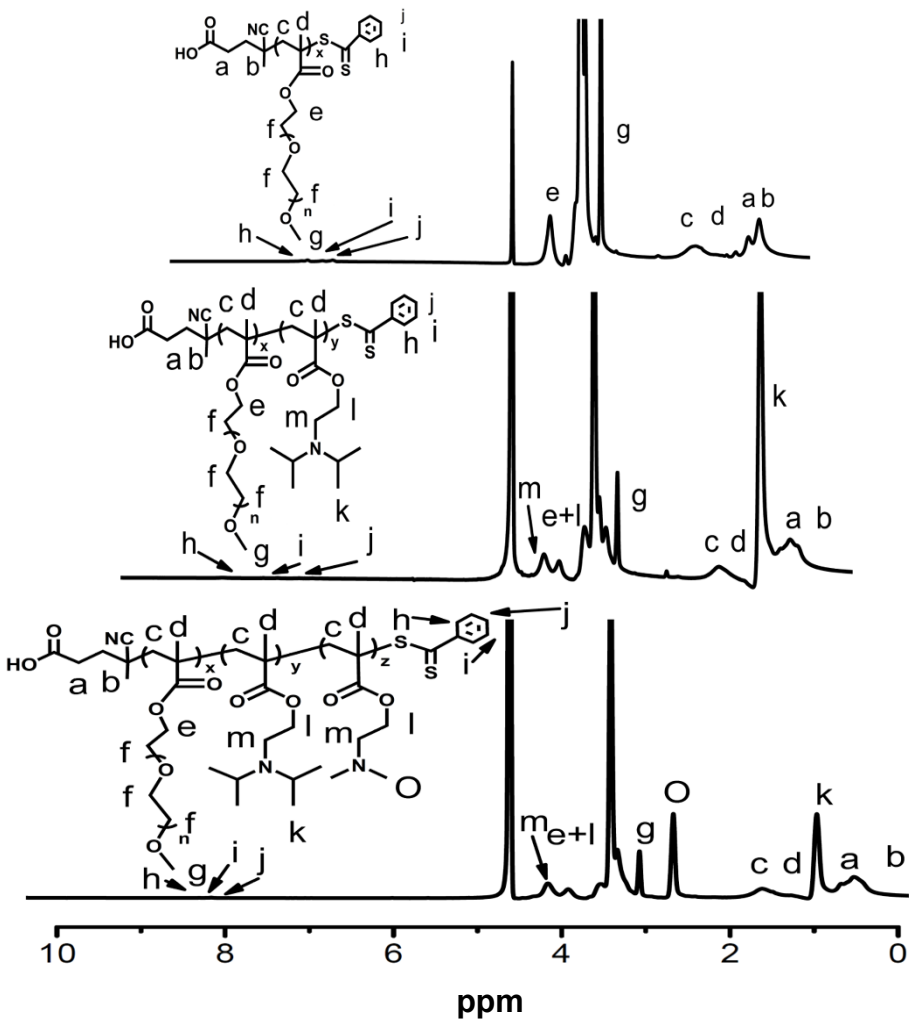

Fig. S3 ^{1}H NMR spectra of PEGMA-CTP, PEGMA-*b*-PDPA-CTP, PMDM-CTP in D_2O (pH 5.0).

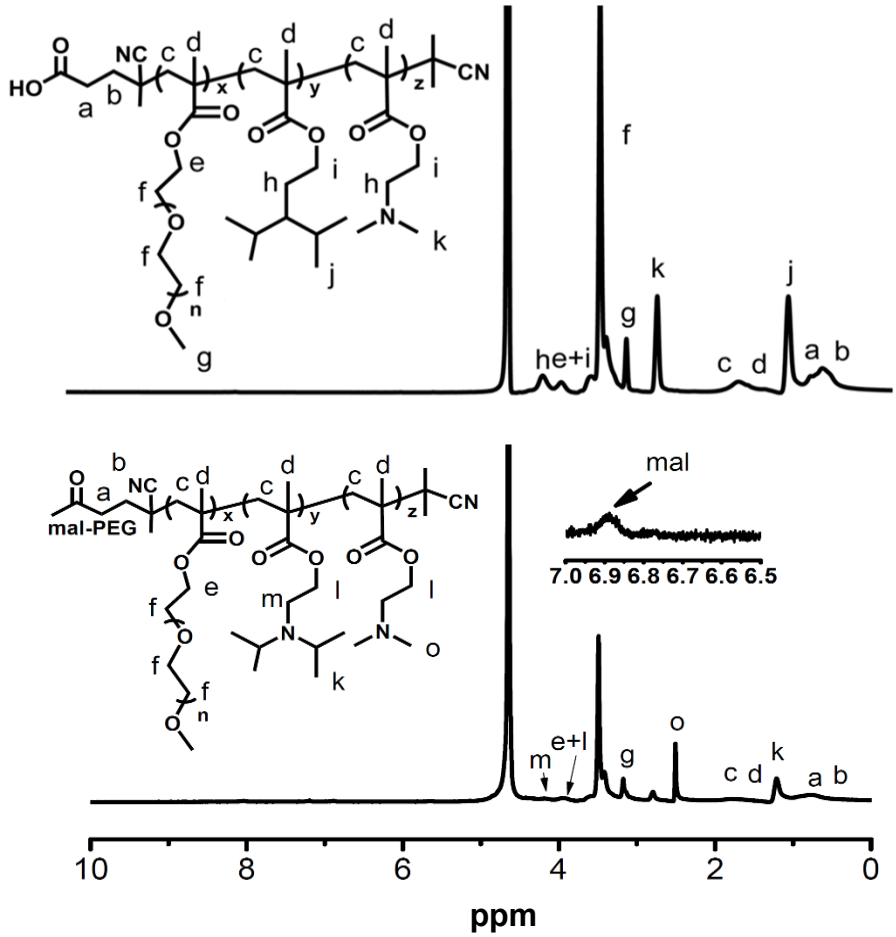

Fig. S4 ^{1}H NMR spectra of PMDM (unmodified with mal-PEG-OH) and PMDM (modified with mal-PEG-OH) in D_2O (pH 5.0).

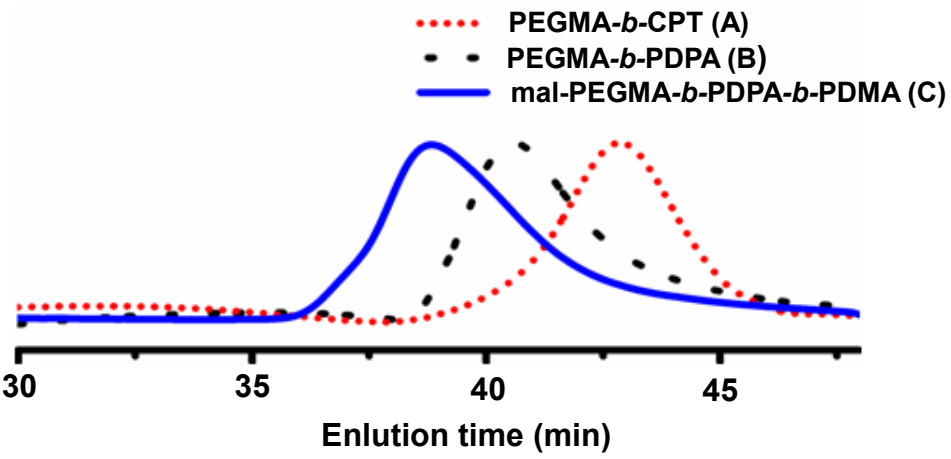

Fig. S5 Gel permeation chromatography (GPC) spectra of (A) PEGMA-CTP, (B) PEGMA-*b*-PDPA, and (C) mal-PEGMA-*b*-PDPA-*b*-PDMA (PMDM), 1/100M CH_3COOH-CH_3COONa buffer solution of pH 5.0.

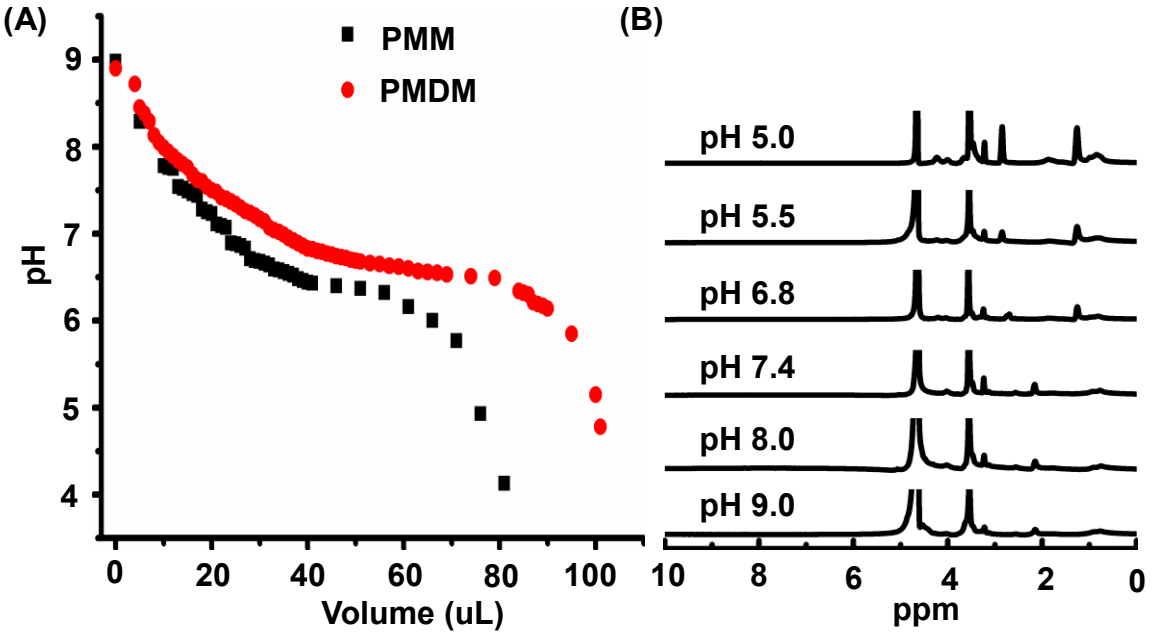

Fig. S6 (A) The buffering capacity of PMM and PMDM polymer. (B) pH sensitive ^1H NMR spectra of PMDM at pH 5.0, 5.5, 6.8, 7.4, 8.0, and 9.0, respectively.

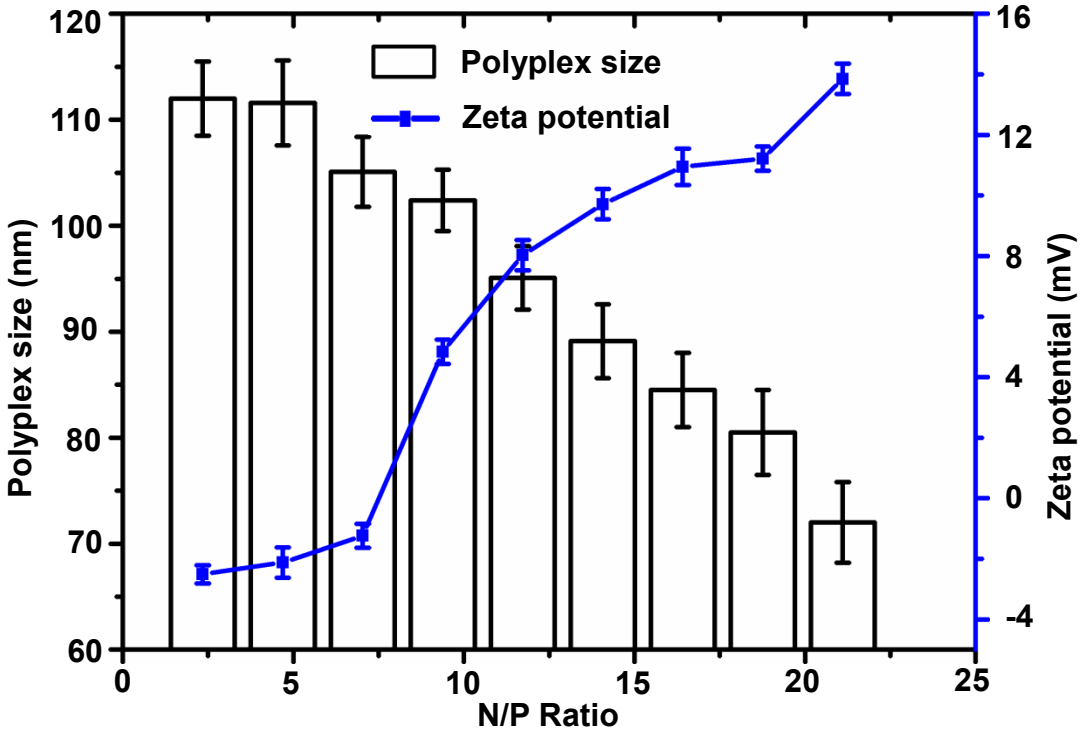

Fig. S7 Particle size and zeta potential of the PMDM polyplexes at various N/P ratio when complexing with siRNA at pH 5.0.

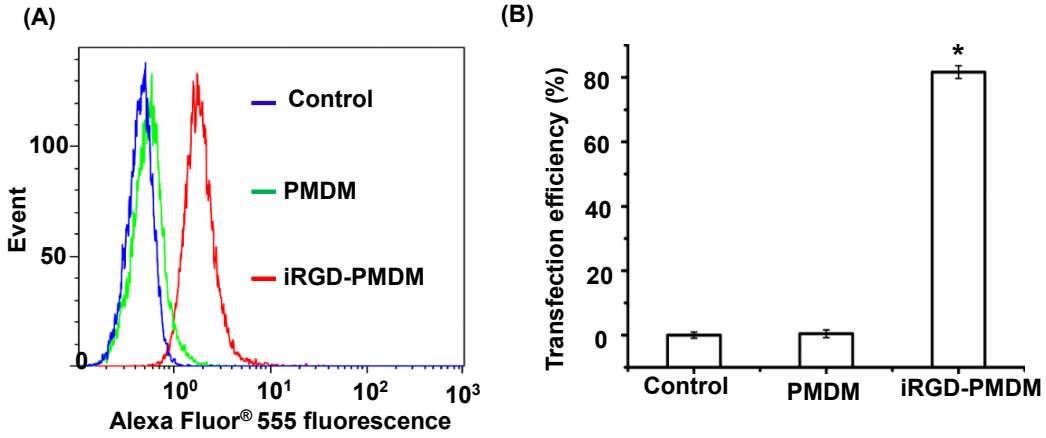

Fig. S8 The flow cytometric measurement of siRNA transfection in A549-Luc cells using PMDM polyplexes or iRGD-PMDM polyplexes. Incubation time: 2 h, dose: 100 nM. siRNA was labeled with Alexa Fluor®555 dye (N/P 13). *$P < 0.01$ *vs* PMDM polyplexes.

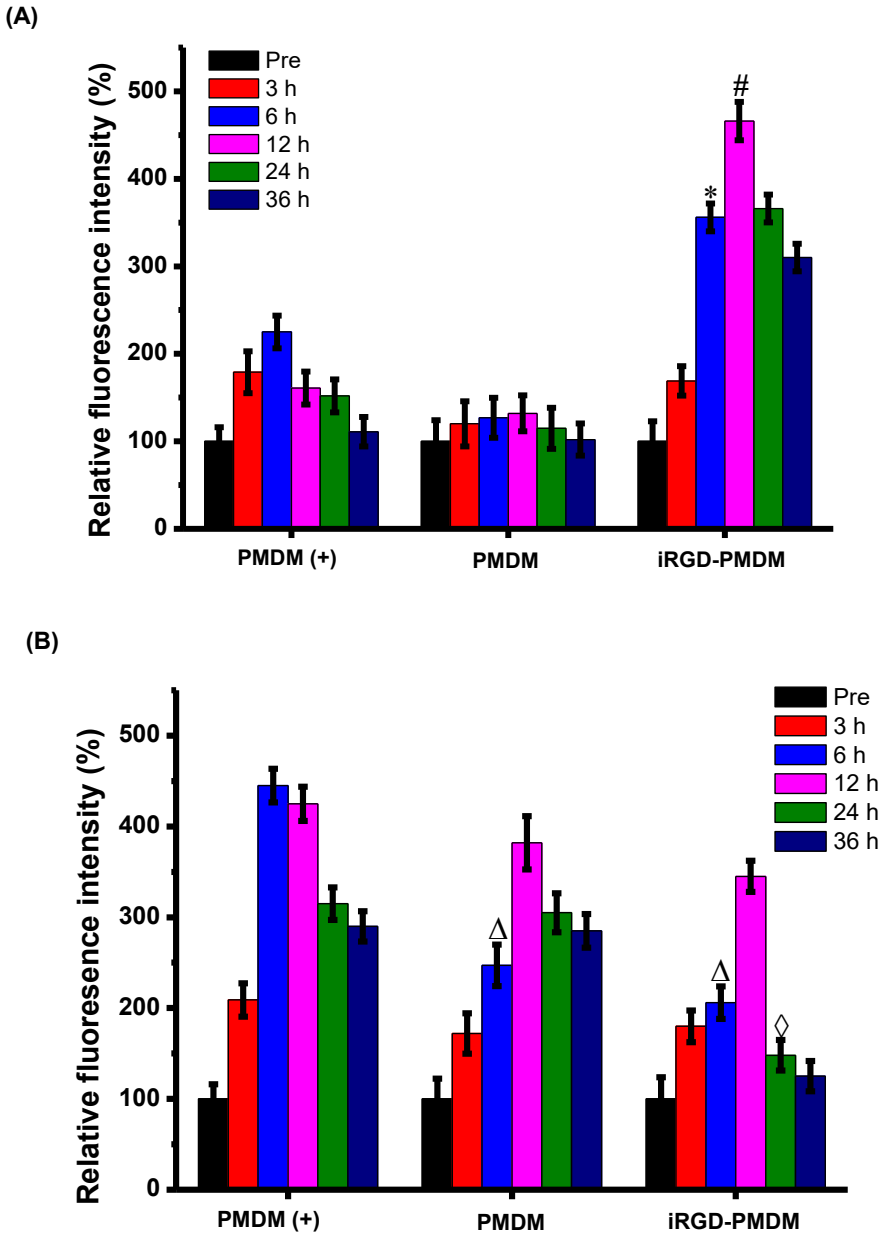

Fig. S9 The fluorescence intensities (n = 3) in the tumor site (A) and liver site (B) of nude mice receiving different polyplexes *via* intravenously injection. The polyplexes was labeled with Alexa Fluor®750 for *in vivo* imaging. The fluorescence intensities were normalized using the "Pre" group as a standard (i.e. 100% luminescence intensity) for each polyplex group. $^{*}P < 0.01$ *vs* PMDM (+) and PMDM polyplexes group at 6 h time point after injection, $^{\#}P < 0.01$ *vs* iRGD-PMDM polyplexes at 6 h time point after injection, $^{\Delta}P < 0.01$ *vs* PMDM (+) polyplexes at 6 h time point after injection, $^{\lozenge}P < 0.01$ *vs* PMDM (+) and PMDM polyplexes group at 24 h time point after injection.

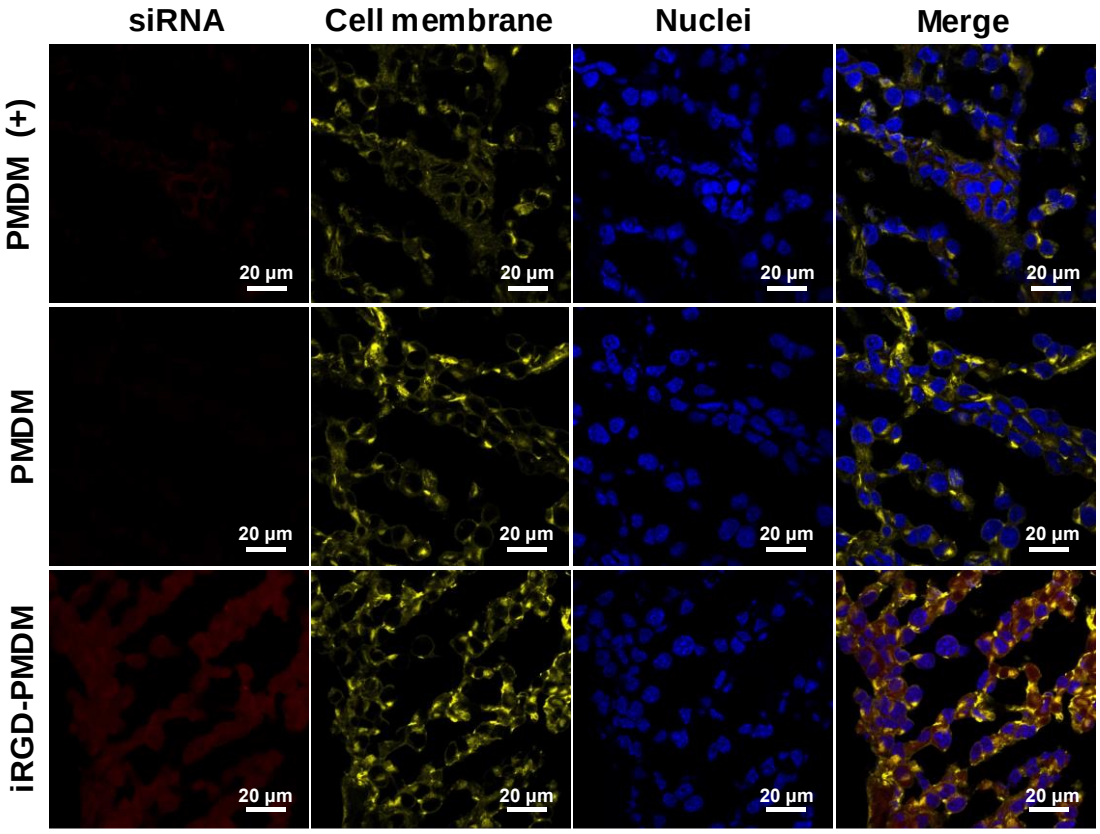

Fig. S10 The locations of siRNA in the tumor tissue of nude mice receiving various polyplexes were observed by confocal laser scanning microscopy assay (CLSM). The cell membranes and nuclei were stained by Alexa Fluor® 647 labeled WGA (wheat germ agglutinin) and Hoechst 33342. The siRNAs were labeled with Cy3.

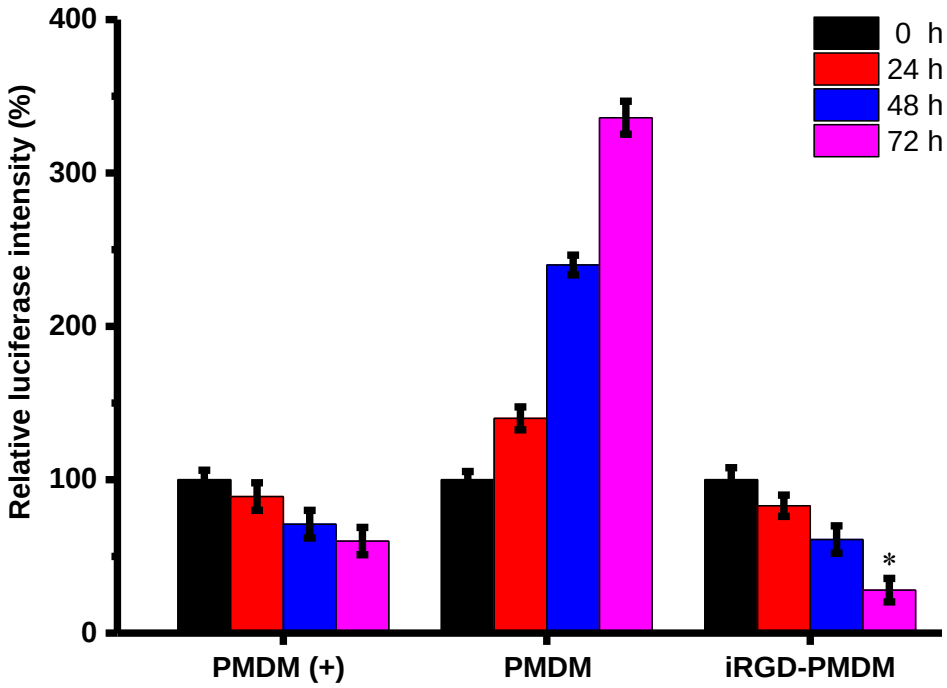

Fig. S11 *In vivo* firefly luciferase emission intensities (n = 3) showing the gene silencing activities of different polyplexes via intravenously injection. The luciferase emission fluorescence intensities were normalized using the "0 h" group as a standard (i.e. 100% luminescence intensity) for each polyplexes group. *P < 0.05 *vs* the PMDM and PMDM (+) polyplexes group.