Electronic Supplementary Information

Multifunctional PEI-entrapped gold nanoparticles enable efficient delivery of therapeutic siRNA into glioblastoma cells

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Part of Experimental Details:

Materials: Branched PEI (Mw = 25 000), EDC, and N-hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich (St Louis, MO). *m*PEG-COOH (PEG monomethyl ether with the end of carboxyl group, Mw = 2 000) and NH_2-PEG-COOH (PEG with one end of carboxyl group and the other end of amine group, Mw = 2 000) were purchased from Shanghai Yanyi Biotechnology Corporation (Shanghai, China). N-Succinmidyl 6-maleimidohexanoate (6-MAL) was from J&K Chemical Ltd. (Shanghai, China). Primary Amino Nitrogen (PANOPA) Assay Kit was obtained from Megazyme (Wicklow, Ireland). Cell Counting Kit 8 (CCK 8) was from Beyotime Institute of Biotechnology (Shanghai, China). Dulbecco Modified Eagle Medium (DMEM), penicillin and streptomycin were from Gino Biomedical Technology Co., Ltd. (Hangzhou, China). Fetal bovine serum (FBS) was purchased from Gibco (Carlsbad, CA). Bcl-2 siRNA (sense, 5'-GUA CAU CCA UUA UAA GCU G dtdt-3'; antisense, 5'-CAG CUU AUA AUG GAU GUA C dtdt-3') were supplied from Shanghai GenePharma Co., Ltd. (Shanghai, China). U87MG cells were obtained from Institute of Biochemistry and Cell Biology, the Chinese Academy of Sciences (Shanghai, China). Regenerated cellulose dialysis membranes with a molecular weight cutoff (MWCO) of either 1 000 or 14 000 were acquired from Fisher (Pittsburgh, PA). Water used in all experiments was purified using a Milli-Q Plus 185 water purification system (Millipore, Bedford, MA) with a resistivity higher than 18.2 MΩ·cm.

Preparation and activation of COOH-PEG-RGD: NH_2-PEG-COOH (16 mg, 8 mmol) dissolved in DMSO (5 mL) was added into a solution of 6-MAL (2.46 mg, 8 mmol) under vigorous stirring to get the raw product of MAL-PEG-COOH after 8 h. RGD peptide (5.53 mg, 8 mmol) in DMSO (2 mL) was dropwise added to a solution of COOH-PEG-MAL while stirring. After 12 h, the raw product of the COOH-PEG-RGD conjugate was obtained. EDC (4.0 mg, 20.88 mmol) and NHS

(2.4 mg, 20.88 mmol) were added to the COOH-PEG-RGD solution to obtain the activated COOH-PEG-RGD after stirring for 3 h.

Templated synthesis of Au NPs within the functional PEI templates: In brief, an aqueous solution of $HAuCl_4$ (8.24 mg, 2 mmol, 2 mL) was dropwise added to a water solution of PEI-(PEG-RGD)$_{10}$-*m*PEG$_{10}$ (10 mL) at the molar ratio of Au salt/PEI of 25:1 under vigorous magnetic stirring for 30 min. $NaBH_4$ (3.78 mg, 10 mmol, 2 mL) was then rapidly added into the Au salt/PEI-(PEG-RGD)$_{10}$-*m*PEG$_{10}$ mixture solution while stirring for 2 h, and the mixture was dialyzed against water for 3 days using a dialysis bag with an MWCO of 14000 to give rise to the formation of the {$(Au^0)_{25}$- PEI-(PEG-RGD)$_{10}$-*m*PEG$_{10}$} PENPs.

Characterization Techniques: ^{1}H NMR spectra were collected *via* a Bruker DRX 400 nuclear magnetic resonance spectrometer. Au DENPs were dispersed in D_2O before measurements. UV-vis spectra were recorded using a Lambda 25 UV-vis spectrophotometer (PerkinElmer, Boston, MA). Au DENPs were dissolved in water before analysis. TEM was performed using a JEOL 2010F analytical electron microscope (JEOL, Tokyo, Japan) operating at 200 kV. A typical TEM sample was prepared by depositing of a diluted Au DENP suspension (1 mg/mL, 5 μL) onto a carbon-coated copper grid and air-dried before observation. The number of the primary amines on the surface of Au PENPs were measured on the basis of the manufacturer's instruction of the PANOPA Kit. The obtained Au PENPs were dispersed in PBS with a concentration of 2 mg/mL before assays.

Gel Retardation Assay: Au PENP/siRNA polyplexes were formed by incubating 1 μg Bcl-2 siRNA and appropriate amount of Au PENPs under different N/P ratios in PBS and incubated for 30 min at room temperature. The final volume of the polyplexes was 20 μL. Gel The gel was prepared by dissolving 1% (w/v) agarose gel and 0.1 μg/mL ethidium bromide (EB) in Tris-acetate-EDTA buffer, and melted using a microwave. Au PENP/siRNA polyplexes were added into the wells of the

gel and electrophoresis was carried out at 80 V for 30 min. The retardation of the siRNA was visualized using a UV transilluminator (Shanghai FURI Science & Technology, Shanghai, China).

Statistical Analysis: All the data was assessed using one way ANOVA method in order to determine the statistical differences. A value of $p < 0.05$ was considered as a significance level. All the data was labeled with (*) for $p < 0.05$, (**) for $p < 0.01$, and (***) for $p < 0.001$, respectively.

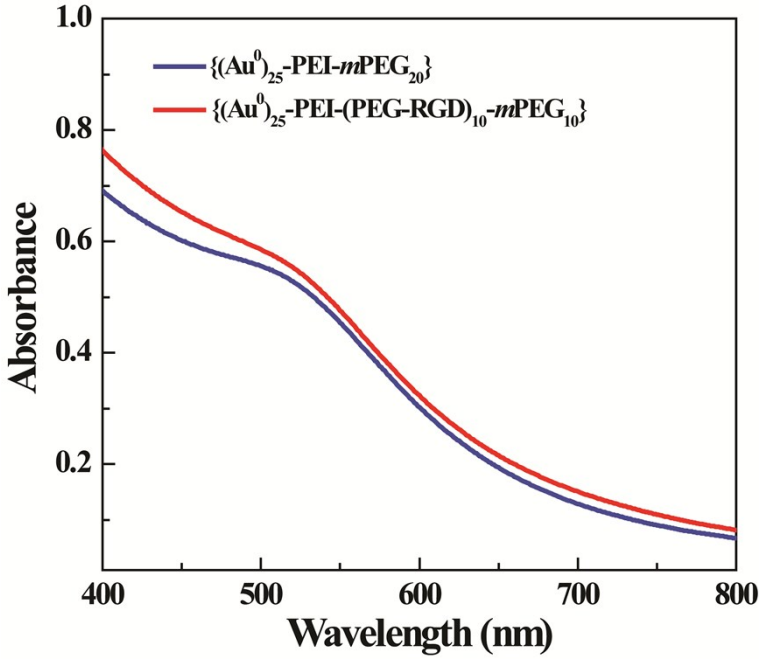

Figure S1. UV-vis spectra of $\{(Au^0)_{25}$-PEI-mPEG$_{20}\}$ and $(Au^0)_{25}$-PEI-(PEG-RGD)$_{10}$-mPEG$_{10}\}$ PENPs.

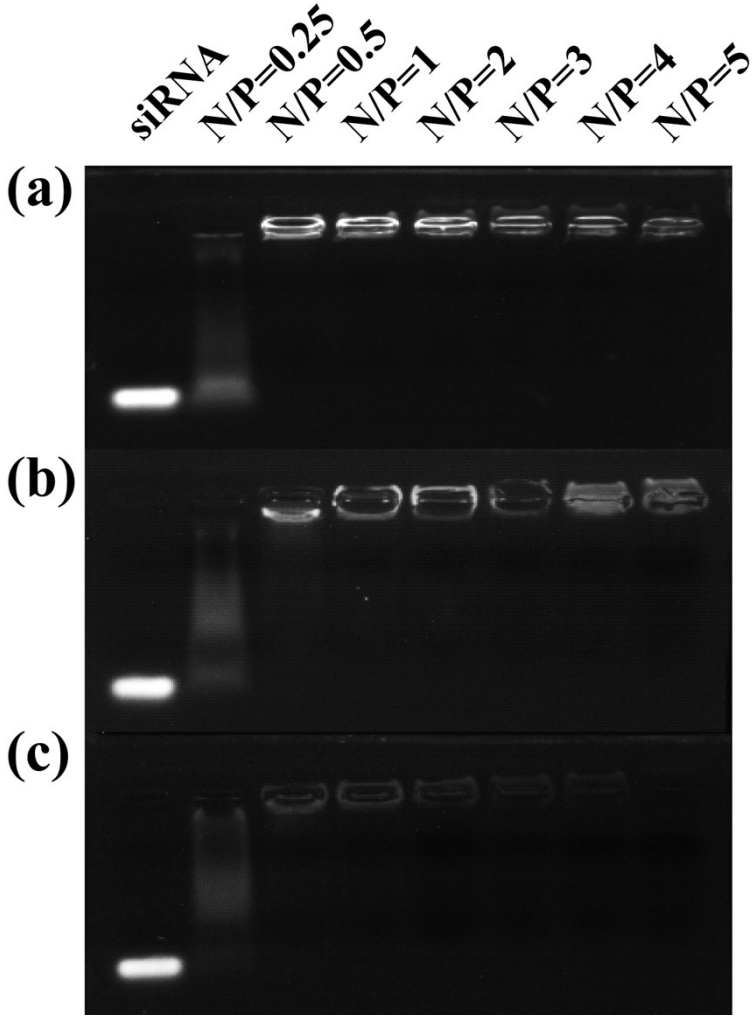

Figure S2. Gel retardation assay of PEI/Bcl-2 siRNA (a), $\{(Au^0)_{25}$-PEI-*m*$PEG_{20}\}$PENP/Bcl-2 siRNA (b), $\{(Au^0)_{25}$-PEI-(PEG-RGD)$_{10}$-*m*$PEG_{10}\}$PENP/Bcl-2 siRNA (c) polyplexes at different N/P ratios.

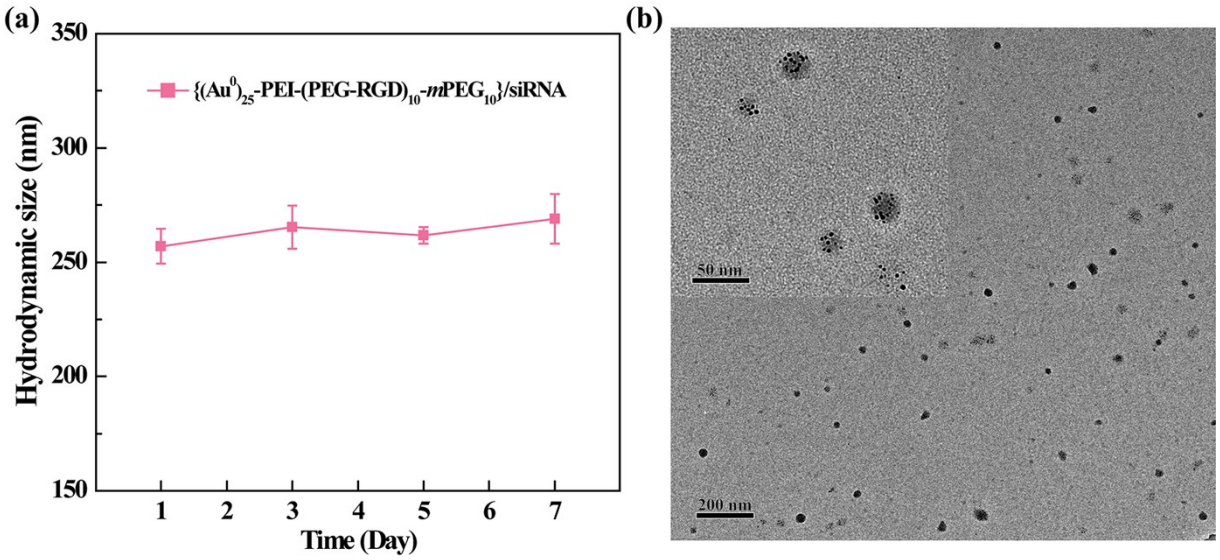

Figure S3. (a) Mean hydrodynamic size of $\{(Au^0)_{25}$-PEI-$(PEG\text{-}RGD)_{10}$-$m PEG_{10}\}$ PENP/Bcl-2 siRNA polyplex at an N/P ratio of 10 at different time points. (b) TEM image of the $\{(Au^0)_{25}$-PEI-$(PEG\text{-}RGD)_{10}$-$m PEG_{10}\}$PENP/Bcl-2 siRNA polyplex at an N/P ratio of 10.

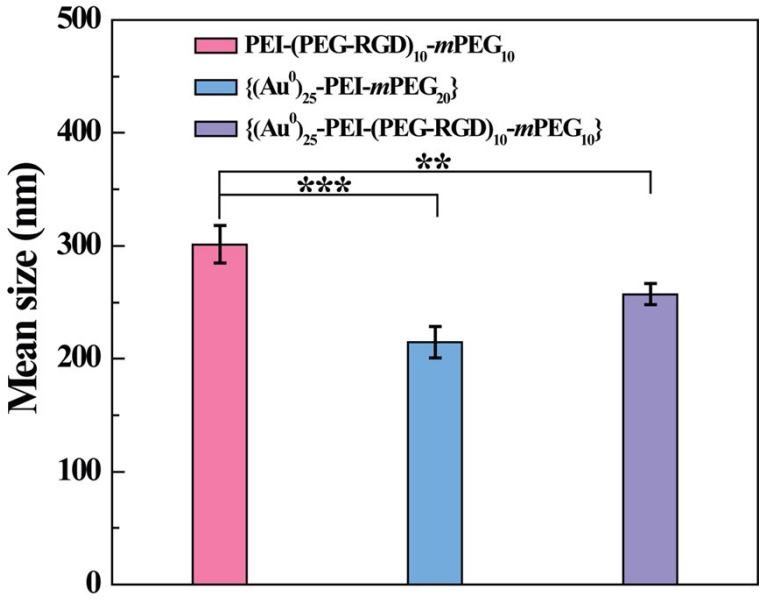

Figure S4. (a) Mean hydrodynamic size of PEI-$(PEG\text{-}RGD)_{10}$-$m PEG_{10}$/Bcl-2 siRNA, $\{(Au^0)_{25}$-PEI-$m PEG_{20}\}$PENP/Bcl-2 siRNA, and $\{(Au^0)_{25}$-PEI-$(PEG\text{-}RGD)_{10}$-$m PEG_{10}\}$ PENP/Bcl-2 siRNA polyplexes at an N/P ratio of 10.

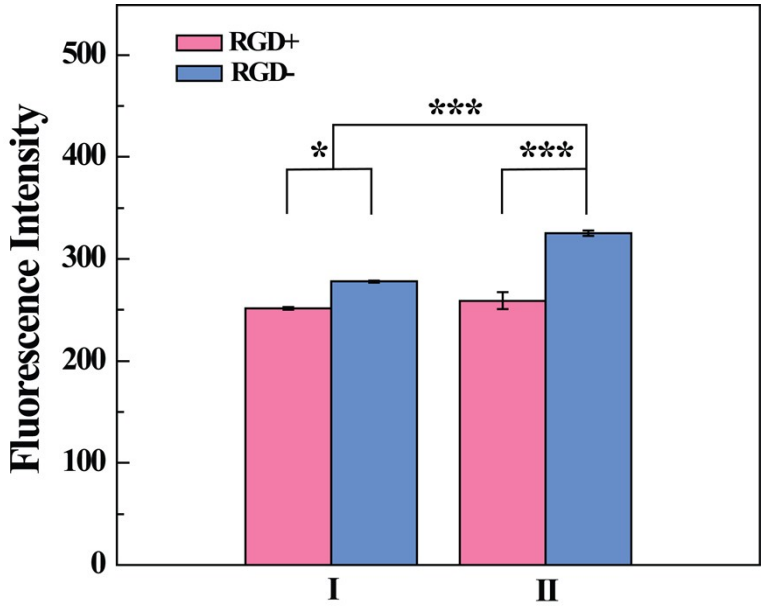

Figure S5. Fluorescence intensity of U87MG cells pre-treated with free RGD ((5 μg/well, RGD+) or normal U87MG cells (RGD-) after being transfected with $\{(Au^0)_{25}$-PEI-*m*$PEG_{20}\}$PENP/Cy3-labeled Bcl-2 siRNA (I) or $\{(Au^0)_{25}$-PEI-$(PEG\text{-}RGD)_{10}$-*m*$PEG_{10}\}$PENP/Cy3-labeled Bcl-2 siRNA (II) polyplexes. Data are presented as mean ± SD (n = 3).

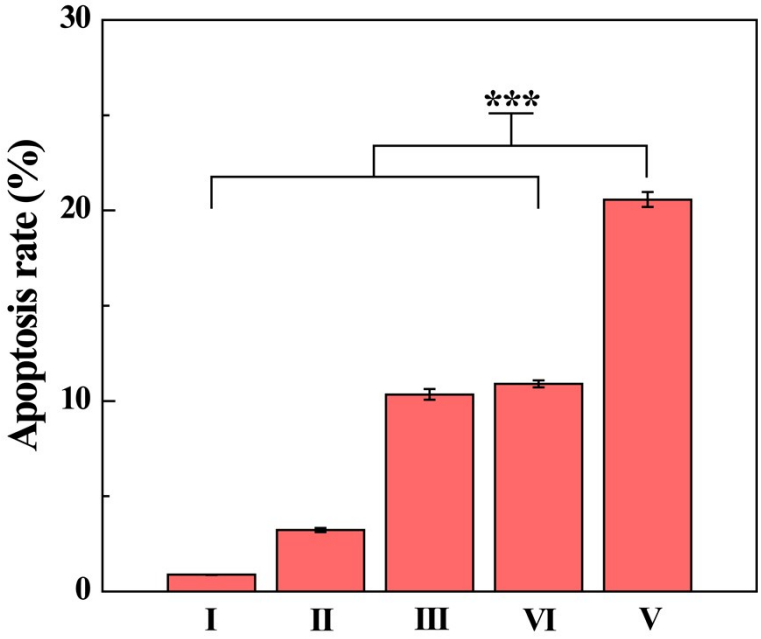

Figure S6. Cell apoptosis rate (TUNEL positive cells) of U87MG xenografted tumors after intratumorally injection with 0.9% normal saline (I), free siRNA (II), PEI/siRNA polyplexes (III), $\{(Au^0)_{25}$-PEI-*m*$PEG_{20}\}$ PENP/siRNA polyplex (VI), and $\{(Au^0)_{25}$-PEI-$(PEG\text{-}RGD)_{10}$-*m*$PEG_{10}\}$ PENP/siRNA polyplex (V), respectively.