Supporting Information

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SI Materials and Methods

Materials. NHS, 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride, 1,2-epoxytetradecane, ethylenediamine core- poly (amidoamine) (PAMAM) generation 0 dendrimer, decanoic anhydride, and *cis,cis,trans*-[$Pt(NH_3)_2Cl_2(OH)_2$] were purchased from Aldrich. Poly(lactide-coglycolide) (PLGA) with acid end groups was purchased from Adsorbable Polymers International. A PEG polymer of molecular weight 3,400 with a terminal amine and carboxylic group (NH_2-PEG-COOH) was purchased from Jen-Kem Technology USA. ^{1}H, ^{13}C, and ^{195}Pt NMR spectra were recorded on a Bruker AVANCE-400 spectrometer with a Spectro Spin superconducting magnet at the Massachusetts Institute of Technology Department of Chemistry Instrumentation Facility. Electrospray ionization (ESI)-MS analyses were performed on an Agilent 1100 series autosampler instrument. Atomic absorption spectroscopic measurements were taken on a PerkinElmer AAnalyst 300 spectrometer. Lipofectamine 2000 (Lipo2000) and Quant-iT RiboGreen RNA quantitation reagent were purchased from Invitrogen. A Dual-GloTM Luciferase Assay System was purchased from Promega.

siRNA. siRNAs targeting luciferase (siLuc), Rev1 (si*REV1*), and Rev3L (si*REV3L*) were synthesized by and purchased from Dharmacon. Cy3-labeled siRNA was purchased from IDT. The siRNA sequences are as follows:

siLuc sense: 5′-CUUACGCUGAGUACUUCGA-3′

Antisense: 5′-UCGAAGUACUCAGCGUAAG-3′

si*REV1* sense: 5′-GCUGUAUAAUGCAUGUUGAUA-3′

Antisense: 5′-UAUCAACAUGCAUUAUACAGC-3′

si*REV3L* sense: 5′-UCUUCUUGCUGGUUUGGAAAG-3′

Antisense: 5′-CUUUCCAAACCAGCAAGAAGA-3′

General Cell Culture. A HeLa cell line stably expressing firefly and *Renilla reniformis* luciferase-expressing HeLa-derived cells (Dual-Luc HeLa) was obtained from Alnylam Pharmaceuticals, Inc. The cells were maintained in DMEM (Invitrogen) with high glucose, 10% (vol/vol) FBS, 500 μg/mL zeocin, and 0.5 μg/mL puromycin. Human Lymph Node Carcinoma of the Prostate and MDA-MB-231 cell lines (both bioluminescent and nonbioluminescent) were purchased from the American Type Culture Collection. Cell lines were cultured in RPMI-1640 media (Invitrogen) supplemented with 10% (vol/vol) FBS and 1% penicillin/streptomycin. Cells and biological experiments were conducted at 37 °C in 5% CO_2.

Animals. All animals were obtained from the Charles River Laboratories International, Inc. The animals were allowed free access to sterile food pellets and water. The animal use protocol was approved by the Institutional Animal Care and Use (MIT and Harvard Medical School) Committees on animal care. All in vivo studies were performed in accordance with National Institutes of Health animal care guidelines.

Synthesis of G0-C14. G0-C14 was synthesized by reacting 1,2-epoxytetradecane with generation 0 of ethylenediamine core-PAMAM dendrimer according to a previously described procedure (1). To increase the proportion of products with one less tail than the total possible for a given amine monomer, a substoichiometric amount of 1,2-epoxytetradecane was added to PAMAM dendrimer at a molar ratio of 7:1. The mixture was reacted under vigorous stirring at 90 °C for 2 d. The crude reaction mixture was separated by chromatography on silica with a gradient elution from CH_2Cl_2 to 75:22:3 CH_2Cl_2/MeOH/NH_4OH. The separated product was characterized by ^{1}H NMR (Fig. S7).

Synthesis of *cis,cis,trans*-[$Pt(NH_3)_2Cl_2(OOC(CH_2)_8CH_3)_2$] (Compound 1). Decanoic anhydride (0.41 g, 1.26 mmol, 3.8 eq) was added to a solution of *cis,cis,trans*-[$Pt(NH_3)_2Cl_2(OH)_2$] (0.11 g, 0.33 mmol) in 10 mL of Dimethylformamide (DMF), and the mixture was then stirred at 55 °C. After 16 h, unreacted *cis,cis,trans*-[$Pt(NH_3)_2Cl_2(OH)_2$] was removed by the filtration and the resulting yellow solution was then reduced to 3 mL under reduced pressure (2). This concentrated solution was then added drop-wise to a rapidly stirred volume of diethyl ether (50 mL), forming a pale yellow precipitate. The buff-colored powder was filtered and washed twice with 30 mL of diethylether. The final product was dried in a desiccator. The yield of the pale yellow solid was 65% (0.138 g, 0.21 mmol). The ESI-MS *m/z* of calculated deprotonated molecular ion mass $[M-H]^-$ was 641.2, and that found was 641.1. ^{1}H NMR (DMSO-d_6) was as follows: δ 0.85 (t, 6H, 8.8 Hz), 1.22 (m, 24H), 1.44 (q, 4H, 8.2 Hz), 2.19 (t, 4H, 8 Hz), and 6.53 (s, 6H). ^{13}C NMR (DMSO-d_6) was as follows: δ 14.15, 22.11, 25.37, 28.66, 28.97, 31.47, 35.86, and 180.02. ^{195}Pt NMR (DMSO-d_6) was δ 1,217.76. Elemental analysis calculated for $C_{20}H_{44}Cl_2N_2O_4$ was as follows: C, 37.38; H, 6.90; N, 4.36, and that found was as follows: C, 37.38; H, 7.08; N, 4.71.

Preparation and Characterization of Nanoparticles Containing siRNAs and Compound 1. Copolymer PLGA-PEG was synthesized by the amide coupling of HOOC-PEG-NH_2 to PLGA-COOH in methylene chloride as previously described (2). The nanoparticles (NPs) encapsulated with a combination payload of siRNAs and compound **1** were formulated via the double-emulsion solvent evaporation technique. In brief, copolymer PLGA-PEG and G0-C14 were codissolved in dichloromethane (DCM) with or without compound **1**. siRNAs were reconstituted in HyPureTM molecular biology grade water (HyClone Laboratories, Inc.). The siRNA solution (0.4 mL) was added drop-wise into 1 mL of PLGA-PEG and G0-C14 solution and emulsified by probe sonification to form the first emulsion. Next, the emulsified mixture was added into 3 mL of aqueous solution containing 1% poly(vinyl alcohol) (PVA), followed by probe sonification to form the double emulsion. The final emulsion solution was poured into 15 mL of water and stirred for 3 h to allow the DCM solvent to evaporate and the particles to harden. The remaining organic solvent and free molecules were removed by washing the particle solution three times using an Amicon Ultra-4 centrifugal filter (MWCO 100kDa; Millipore). The NP size and zeta potential were determined by using a ZetaPALS dynamic light-scattering detector (15-mW laser, incident beam of 676 nm; Brookhaven Instruments Corporation). Samples for transmission EM were stained with 1% uranyl acetate and observed using a JEOL 2011 instrument at 200 kV. The Pt content in the NPs was measured by atomic absorption spectroscopy (AAS). The siRNA in the NPs was analyzed by using the Quant-iT RiboGreen assay according to the manufacturer's protocol. Drug loading is defined as the mass fraction of drug in the NPs, whereas entrapment efficiency is the fraction of initial drug that is encapsulated by the NPs.

Release of Compound 1 and siRNA from the NPs. To determine release kinetics, Cy3-labeled siRNA was first encapsulated into the NPs. A suspension of NPs in PBS was aliquoted (100 μL) into

several semipermeable minidialysis tubes (molecular mass cutoff of 100 kDa; Pierce) and dialyzed against frequently renewed PBS (pH 7.4) at 37 °C with gentle stirring. At a predetermined time, an aliquot of the NP suspension was removed and the platinum content was analyzed by AAS. For siRNA quantification, a standard curve correlating fluorescence and Cy3-siRNA concentration was used to determine the amount of siRNA encapsulated within the NPs. The fluorescence intensity was measured by a Synergy HT multimode microplate reader (excitation/emission 530/590 nm; BioTek Instruments, Inc.).

Platinum Uptake Evaluation. The cellular uptake of compound **1**-encapsulated NP [NP(compound **1**)] and free compound **1** was studied using an LNCaP cell line. LnCaP cells were seeded into six-well plates (2.5×10^6 cells per well) and allowed to adhere overnight in growth medium. NP(compound **1**) and free compound **1** were added to the growth media at a final platinum concentration of 0.1 mM. Six hours after NP incubation, the cells were washed with PBS buffer, followed by treatment with nitric acid for 3 h. The cell lysate was collected, and the cellular platinum concentration was quantitatively determined by AAS. The platinum content was normalized against protein concentration in the cell extracts, measured by a bicinchoninic acid (BCA) protein kit. Data were reported as mean ± SD for triplicate samples, and the experiment was repeated twice.

In Vitro siRNA Transfection. Dual-Luc HeLa cells were seeded into 96-well plates (15,000 cells per well) and allowed to attach in growth medium at 37 °C in a 5% CO_2 incubator overnight. Cells were then transfected with naked siLuc, NPs encapsulating siLuc [NP(siLuc)], blank NP, and Lipo2000-complexed siLuc. Lipo2000 and siRNA were mixed to form lipoplex according to the manufacturer's protocol as a positive control. After 24 h, the cells were washed with fresh medium and further incubated in the medium for 1 d. The expression of firefly and *Renilla* luciferase in HeLa cells was determined by Dual-GloTM Luciferase assay kits. The luminescence intensity was measured using a microplate reader (Tecan). All the in vitro transfection experiments were performed in quadruplicate.

REV1/*REV3L* siRNA transfection of LNCaP and MDA-MB-231 cells was conducted in six-well plates (400,000 cells per well). The cells were allowed to adhere overnight in growth medium and were then transfected with NP(si*REV1*, si*REV3L*). Twenty-four hours posttransfection, the cells were washed with fresh medium, and the cellular levels of *REV1* and *REV3L* mRNAs were assessed on 3 consecutive days using quantitative real-time PCR (qRT-PCR). In brief, the total RNA from transfected cells was extracted using RNeasy minikits (Qiagen) according to the manufacturer's protocol. The qRT-PCR assays were performed using SYBR green on a BioRad thermal cycler. The primer sequences used in qPCR assays are human housekeeping gene GAPDH: CAATGACCCCTTCATTGACC and GACAAGCTTCCCGTTCTCAG, human *REV1*: TTGTGATGAAGCGCTGGTAG and AGAGGCAGCACATTTCGTCT, and human *REV3L*: TTTGTGCCAGCAACAGAAAG and CTGGGATCCATCGCTGTAGT. Relative gene expression values were determined using StepOne Software (Applied Biosystems). Data are presented as the fold difference in target gene expression normalized to GAPDH as the endogenous reference and relative to the untreated control cells.

In Vitro Viability Assays. To examine the cytotoxicity of the siRNA-containing NPs, Dual-Luc cells were seeded in quadruplicate in 96-well plates (15,000 cells per well). The cells were allowed to adhere overnight in growth medium and then treated with formulations containing an escalating dose of NP(siLuc). Cells in media containing 10% (vol/vol) FBS with nothing added were used as controls. Twenty-four hours after treatment, cell viability was measured using an XTT [2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide] assay (Roche Applied Science) following the manufacturer's protocol. To investigate the dose response of the siRNA-containing formulations, the LNCaP cells were first transfected with NP(si*REV1*, si*REV3L*) for 24 h and the medium was replaced with fresh medium thereafter. We incubated the cells for another 24 h, after which the transfected cells were seeded in quadruplicate in 96-well plates (15,000 cells per well) and treated with two different formulations with an escalating dose of compound **1**. The first formulation consists of NP(si*REV1*, si*REV3L*) with compound **1** in solution form, and the second formulation contains compound **1** within the NP(si*REV1*, si*REV3L*) [NP(si*REV1*, si*REV3L*, compound **1**)]. Meanwhile, untransfected cells were seeded and treated with an escalating dose of compound **1** in either solution or NP form. Cell viability was then measured using the XTT assay 24 h posttreatment.

In Vivo Bioluminescence Imaging. Luciferase-expressing xenograft tumors were induced in the mammary fat pad of 8-wk-old BALB/C nude mice (Charles River Laboratories International, Inc.) by s.c. injection of 1×10^6 luciferase-expressing MDA-MB-231 cells suspended in 1:1 media and Matrigel (BD Biosciences). After 2 wk, NP(siLuc) and NP(negative siRNA) were administered to the tumor-bearing nude mice (tumor size of ∼100–150 mm^3). Tumor length and width were measured with calipers, and tumor volume was calculated using the following equation: Tumor Volume (V) = Length × Width × Width/2. The NP solution was concentrated to 15 mg/mL with the total encapsulated siRNA concentration being ∼150 μg/mL The siRNA-containing NPs were directly injected into the tumor at an equivalent dose of ∼0.4 mg/kg of entrapped siRNA. Right before dosing, the mice were monitored using an IVIS Spectrum bioluminescent and fluorescent imaging system (Caliper Life Sciences). Tumor bioluminescence images were then taken daily for 3 d postinjection. The bioluminescence intensity was analyzed using LivingImage (Caliper Life Sciences, Inc.) acquisition and analysis software. All the in vivo imaging experiments were performed in quintuplicate.

In Vivo Gene Silencing of *REV1* and *REV3L*. LNCaP cells were first retrovirally infected to express GFP protein stably. The retroviral vector used is murine stem cell virus (MSCV)/LTRmiR30-SV40-GFP, which is under control of the SV40 promoter. MSCV vector was transfected into the 293T cell line by the calcium method to produce retrovirus. Following incubation with retrovirus, LNCaP cells were sorted on a MoFlo flow cytometer (BD Biosciences). The GFP-labeled LNCaP cells (10^6 cells, 0.1 mL) were injected s.c. into the flank of a male SCID-beige mouse using 50% (vol/vol) Matrigel. Tumor nodules were allowed to grow to a volume of ∼100 mm^3 before treatment. Tumor-bearing mice were randomly assigned to two groups ($n = 4$) to minimize weight and tumor size differences between the groups. Each group was administered either NP(negative siRNA) (control group) or NP(si*REV1*, si*REV3L*) at a dose of 0.4 mg of siRNA per kilogram of animal weight via intratumoral injection. After euthanasia, the tumor tissues were dissected from the mice at the designated time points and pure populations of live LNCaP cells were isolated by GFP sorting. The cellular expression levels of si*REV1* and si*REV3L* mRNAs from the sorted cells were assessed using qRT-PCR as described above.

In Vivo Anticancer Efficacy Evaluation. The mouse LNCaP xenograft tumor model was developed by injecting 0.1 mL of LNCaP cell suspension (10^6 cells) into the flank of a male SCID-beige mouse using 50% (vol/vol) Matrigel. Tumor nodules were allowed to grow to a volume of ∼100 mm^3 before initiating treatment. Tumor-bearing mice were randomly assigned to five groups before treatment, and their weight and the initial tumor volume

were measured and recorded, respectively. Starting from day 4, at intervals of 3 or 4 d, test animals received two intratumoral injections weekly of the following formulations: (*i*) saline, (*ii*) compound **1** in solution form, (*iii*) NP(compound **1**), (*iv*) NP (si*REV1*, si*REV3L*) with compound **1** in solution, and (*v*) NP (si*REV1*, si*REV3L*, compound **1**) for 34 d ($n = 5$ for groups *i*–*iv*, $n = 8$ for group *v*). It should be noted that before treatment with the two siRNA-containing NP formulations (regimens *iv* and *v*), the mice were injected with NP(si*REV1*, si*REV3L*) on day 0 and day 2 at an equivalent dose of ∼0.4 mg/kg of entrapped siRNA in order for *REV1*/*REV3L* depletion to occur at noticeable levels. On day 4, we started injections with the aforementioned five formulations at an equivalent dose of 4 mg/kg for compound **1** and 0.4 mg/kg for siRNA per injection. The weight and tumor volume of each mouse were measured twice weekly over a period of 37 d.

1. Love KT, et al. (2010) Lipid-like materials for low-dose, in vivo gene silencing. *Proc Natl Acad Sci* USA 107(5):1864–1869.
2. Johnstone TC, Lippard SJ (2013) The Effect of Ligand Lipophilicity on the Nanoparticle Encapsulation of Pt(IV) Prodrugs. *Inorg Chem* 52(17):9915–9920.
3. Farokhzad OC, et al. (2006) Targeted nanoparticle-aptamer bioconjugates for cancer chemotherapy in vivo. *Proc Natl Acad Sci USA* 103(16):6315–6320.

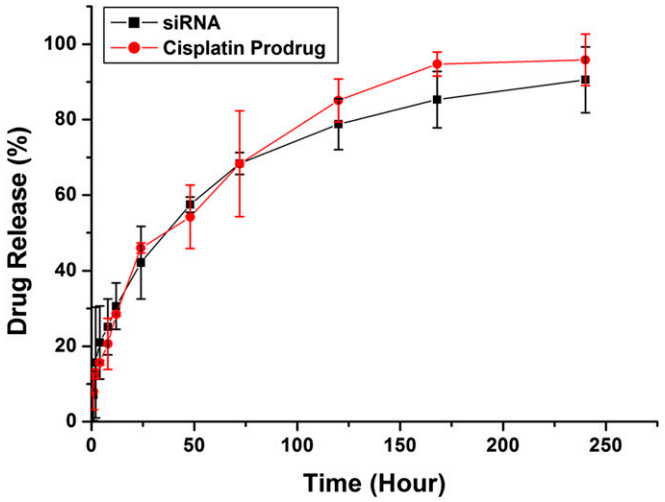

Fig. S1. In vitro release profile of the siRNA and compound **1** from PLGA-PEG/G0-C14 NPs.

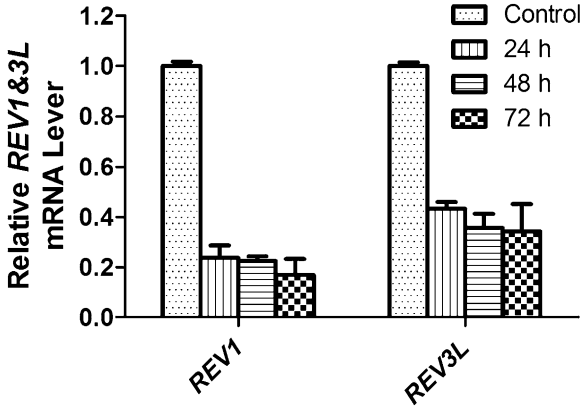

Fig. S2. qRT-PCR confirmation of *REV1* and *REV3L* gene suppression using NP(si*REV1*, si*REV3L*) in MDA-MB-231 human breast carcinoma cells.

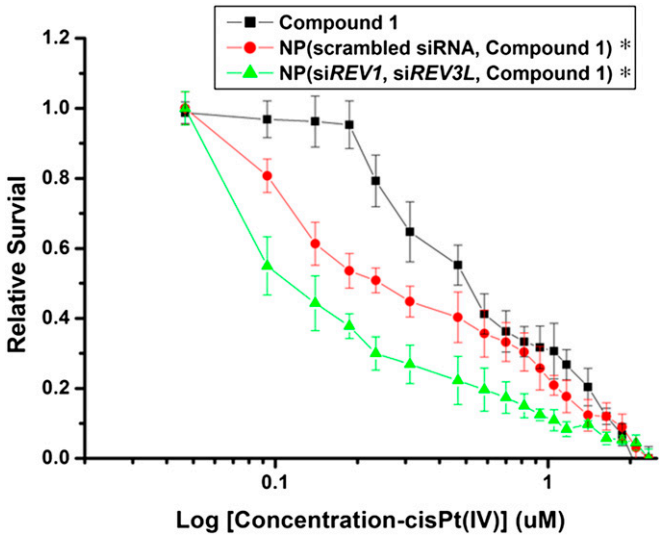

Fig. S3. Platinum dose–response curves of LNCaP cells treated with free compound **1** (black), NP(scrambled siRNA, compound **1**) (red) and NP(si*REV1*, si*REV3L*, compound **1**) (green). The experiment was conducted in quadruplicate ($n = 4$). *Before treatment with the two siRNA-containing NP formulations, the cells were transfected with NP(si*REV1*, si*REV3L*) or NP(scrambled siRNA) for 48 h.

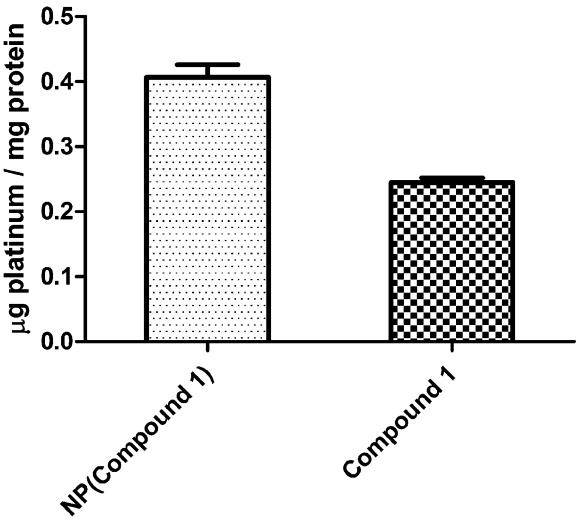

Fig. S4. AAS analysis of platinum content within LNCaP cells treated with an equivalent dose of compound **1** either in solution or NP form.

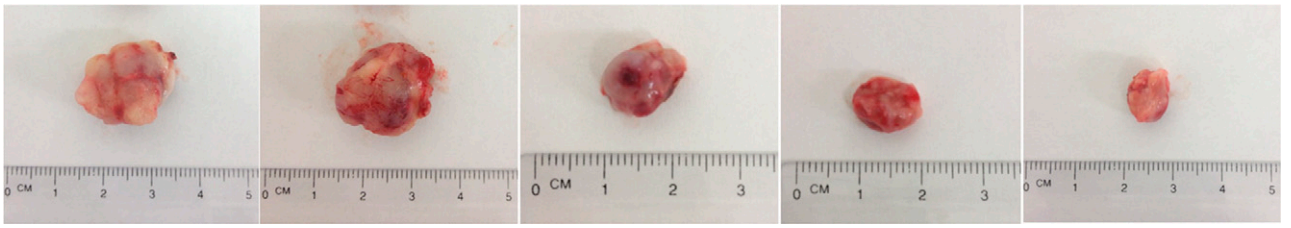

Fig. S5. Representative images of excised tumors at the end point for each group. From left to right, tumors were treated with the following regimens: (*i*) saline, (*ii*) compound **1** in solution form, (*iii*) NP(compound **1**), (*iv*) NP(si*REV1*, si*REV3L*) with compound **1** in solution, and (*v*) NP(si*REV1*, si*REV3L*, compound **1**).

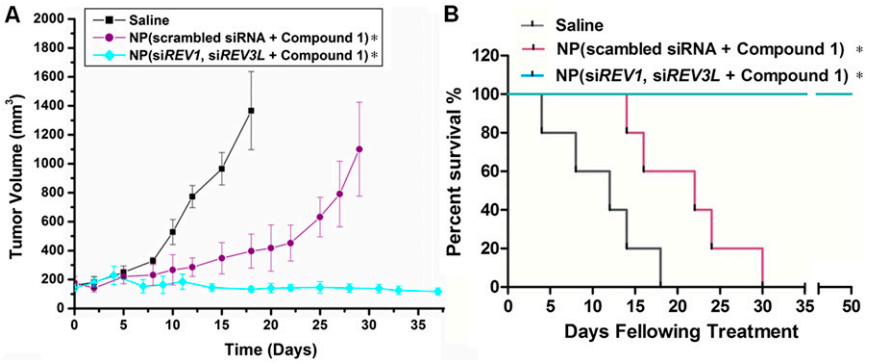

Fig. S6. Enhanced in vivo therapeutic efficacy mediated by NP(si*REV1*, si*REV3L*, compound **1**). (*A*) Inhibition of LNCaP xenograft tumor growth by regimen *iii* [NP(si*REV1*, si*REV3L*, compound **1**)] in comparison to regimens *i* (saline) and *ii* [NP(scrambled siRNA, compound **1**)]. The doses of compound **1** and siRNA per injection were 4 mg/kg and 0.4 mg/kg, respectively. (*B*) Survival curves of tumor-bearing mice treated with the aforementioned three formulations. Day 0 represents the first day of NP(si*REV1*, si*REV3L*) or NP(scrambled siRNA) administration (n = 5 for groups *i* and *ii*, *n* = 8 for group *iii*): $P < 0.003$ NP(scrambled siRNA, compound **1**) vs. NP(si*REV1*, si*REV3L*, compound **1**). *P* values for all survival studies were determined using log-rank curve comparison tests. *Before treatment with the two siRNA-containing NP formulations, the tumor-bearing mice were injected on day 0 and day 2 with NP(si*REV1*, si*REV3L*) or NP(scrambled siRNA). Starting from the fourth day, the mice received intratumoral injections of the aforementioned three formulations twice weekly.

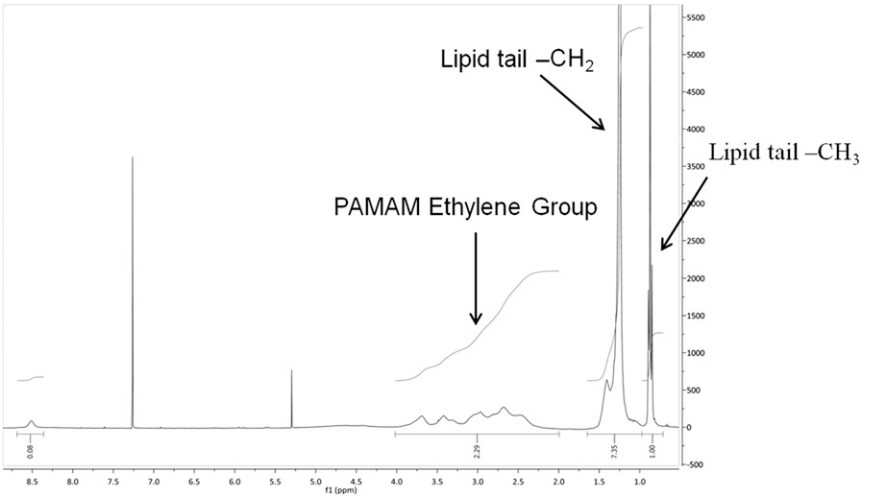

Fig. S7. NMR analysis of G0-C14. The product was dissolved in deuterated chloroform and characterized by ^{1}H NMR.

Table S1. PLGA-PEG/G0-C14 NP characterization

G0-C14/siRNA weight ratio*	Encapsulation efficacy, %	Size, nm	Polydispersity	Zeta potential, mV
10:1	87.0 ± 5.0	210 ± 8.5	0.23 ± 0.07	15.7 ± 4.5
20:1	95.0 ± 4.0	220 ± 12.5	0.18 ± 0.04	17.6 ± 3.1
30:1	99.0 ± 3.0	185 ± 10	0.10 ± 0.06	25.0 ± 2.6

*The initial amount of siRNA used for all weight ratios remains fixed.