Supporting Information

SI Materials and Methods

Materials. Cationic amino molecules including ethylenediamine core-poly (amidoamine) (PAMAM) generation 0 dendrimer (G0); polyethylenimine, ethylenediamine branched (M_w ~ 800) (PEI); polypropylenimine tetramine dendrimer, generation 1 (DAB); and diethylene triamine (114) were purchased from Sigma-Aldrich. Ester-terminated poly($_{D,L}$-lactide-*co*-glycolide) (PLGA, viscosity of 0.26-0.54 dL/g) was purchased from Durect Corporation. DSPE-PEGs (1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)]) with PEG molecular weight 3000 (DSPE-PEG3K) and 5000 (DSPE-PEG5K), C16 PEG5000 ceramide (ceramide-PEG5K), 1,2-dioleoyl-3-trimethylammonium-propane (chloride salt) (DOTAP), and 1,2-di-O-octadecenyl-3-trimethylammonium propane (chloride salt) (DOTMA) were obtained from Avanti Polar Lipids, and soybean lecithin from Alfa Aesar. Lipofectamine 2000 (Lipo2K) was purchased from Invitrogen. Steady-Glo luciferase assay system was purchased from Promega. siRNAs targeting luciferase (siLuc) and PHB1 (siPHB1), and fluorescent dye-labeled siRNAs (DY547-siRNA, DY647-siRNA, and DY677-siRNA) were acquired from Dharmacon. The siRNA sequences are as follows: siLuc, 5'-CUU ACG CUG AGU ACU UCG AdTdT-3' (sense) and 5'-UCG AAG UAC UCA GCG UAA GdTdT-3' (antisense); and siPHB1, 5'-GCG ACG ACC UUA CAG AGC GUU-3' (sense) and 5'-CGC UCU GUA AGG UCG UCG CUU-3' (antisense). DY547 and DY647 were labeled at the 5'-end of the sense strand of siLuc. DY677 was labeled at the 5'-end of both the sense and antisense strand of siLuc. MitoTracker Green (Life Technologies) was resuspended in dimethyl sulfoxide (DMSO; Sigma) and used at a final concentration of 200 nM. Alexa Fluor 488 phalloidin was purchased from Life Technologies. Antibodies used in this work included the following: anti-caspase 3 (9665), anti-caspase 9 (9504), anti-cleaved caspase 3 (9664), anti-cleaved caspase 9 (9509), anti-PARP (9532) (Cell Signaling); Alexa Fluor® 568 Goat-anti Rabbit IgG (Life Technologies, A-11011); anti-PHB1 (Abcam, ab70672); and anti-β-actin (Sigma, clone AC-15, A5441).

Synthesis of cationic lipid-like compounds. Cationic lipid-like compounds were synthesized by reacting alkyl epoxides with a selection of amines (Supplementary Fig. 2) according to a previously described ring-opening procedure[15,22]. Substoichiometric amounts of epoxide were added to increase the proportion of products with one less tail than the total possible for a given amine monomer. The amine (1 equiv) and epoxide (1/2N - 1 equiv, where N is the number of secondary amines plus 2× number of primary amines in the amine starting material) were reacted under vigorous stirring at 90 °C for 2-2.5 days. For example, G0-C14 compound was synthesized by mixing PAMAM dendrimer G0 with 1,2-epoxytetradecane at a molar ratio of 1:7. The crude reaction mixture was separated by chromatography on silica with gradient elution from CH_2Cl_2 to 75:22:3 CH_2Cl_2/MeOH/NH_4OH.

Preparation and characterization of lipid-polymer hybrid NPs. The siRNA-encapsulated lipid-polymer hybrid NPs were prepared by an innovative and robust self-assembly method. In brief, 5 mg PLGA and a quantity (0.3-1.1 mg) of cationic lipid or lipid-like compound (e.g., 0.5mg G0-C14) were dissolved in 1 mL acetone solvent. A 50 μL siRNA (4 nmol) solution is mixed with the acetone solution to form siRNA/cationic lipid nanocomplexes. Next, the polymer

solution with the nanocomplexes was added dropwisely into a 20 mL aqueous solution containing lipid-PEG (e.g., DSPE-PEG5K) and lecithin. The NPs formed instantly upon mixing. The residual acetone in the suspension was evaporated by continuously stirring the suspension at room temperature for 2 hours. NPs were washed three times in Amicon tubes (MWCO 100kDa; Millipore) to remove remaining organic solvent and free compounds with ice-cold water, and concentrated in 1 mL phosphate buffered saline (PBS) solution. The size and surface charge (zeta potential) were determined by Dynamic Light Scattering or DLS (15-mW laser, incident beam of 676 nm; Brookhaven Instruments Corporation). NP samples for transmission electron microscopy (TEM) were stained with 1% uranyl acetate and imaged using Tecnai G^2 Spirit BioTWIN microscope (FEI Company) operating at 80 kV.

Serum stability study. For the siRNA stability test, naked siRNA, NP(siRNA) or lipid-siRNA complexes were added to 100% murine serum (1:1 v/v) and incubated at 37ºC for the indicated times (0, 6, 12, and 24 hours). After incubation, siRNA NPs and siRNA-lipid complexes were ultra-centrifuged for 15 min. The pellet was dissolved in chloroform and siRNA was extracted with 0.1% SDS/0.5 M NaCl aqueous solution. Then the siRNA extracts or naked siRNA were loaded and separated on E-Gel precast agarose gels (4%) containing ethidium bromide (Life Technologies) and visualized under UV light.

siRNA release kinetics. To determine release kinetics, DY547-labeled siRNA was first encapsulated into the hybrid NPs. A suspension of NPs in PBS was added into a Float-a-lyzer G2 dialysis device (MWCO 100 kDa; Spectrum) and dialyzed against frequently renewed PBS (pH 7.4) at 37ºC with gentle stirring. At a predetermined time, a small volume of the NP solution was withdrawn and the NPs were disintegrated with DMSO. The fluorescence intensity of DY547-siRNA (ex/em: 530/590 nm) was measured using Synergy HT multi-mode microplate reader (BioTek Instruments).

Lipid-PEG dissociation kinetics. The dissociation of lipid-PEG molecules from NPs in simulated serum (4% serum albumin in PBS) was studied using a modified method based on spectrophotometric measurement of a complex formed between PEG and barium iodide[23]. NPs were first incubated in albumin solution at 37ºC. At a predetermined time point, NP suspension was ultra-centrifuged, washed with de-ionized water, and re-suspended in 100 µL water. Then the NP solution (1.25 mg/ml at PLGA polymer weight) was mixed with 100 µl DMSO, 20 µl $BaCl_2$ (5%) and 20 µl I_2 solution (0.1 N, Alfa Aesar). Calibration curve was prepared with a corresponding lipid-PEG solution containing the same concentration of PLGA (1.25 mg/ml) and G0-C14 (0.125 mg/ml). After incubation at room temperature for 15 minutes, absorbance at 535 nm was measured on the microplate reader.

NP surface charge measurement. To measure the change of surface charge associated with lipid-PEG dissociation, NPs (5 mg/ml) were first incubated with 4% serum albumin at 37ºC. At a predetermined time point, the NP suspension was ultra-centrifuged, and washed with nuclease-free water. The NP pellet was then re-suspended for zeta potential measurement using dynamic light scattering (DLS).

Cell culture. Luciferase-expressing HeLa (Luc-HeLa) cells and RAW264.7 macrophage cells (ATCC) were maintained in Dulbecco's Modified Eagle's Medium (DMEM; Invitrogen) with

high glucose, 10% (v/v) fetal bovine serum (FBS; Sigma). Non-small cell lung cancer A549 cells (ATCC) were maintained in F-12K medium (Invitrogen) supplemented with 10% (v/v) FBS. NCI-H460 cells (ATCC) were maintained in RPMI-1640 medium (Invitrogen) supplemented with 10% (v/v) FBS. All cells were incubated at 37ºC in 5% CO_2.

Luciferase Silencing. Luc-HeLa cells were seeded into 96-well plates (5,000 cells per well) and allowed to attach in growth medium at 37ºC in a 5% CO2 incubator overnight. Cells were then transfected with NP(siLu) or Lipo2K-siLuc complexes at siRNA concentration of 50 nM, 10 nM, 5 nM, and 1 nM for 24 hours unless otherwise specified. Lipo2K-siLuc complexes were prepared according to the manufacturer's protocol. Luc-HeLa cells were then washed with fresh medium and further cultured in medium for another 48 hours unless otherwise specified. The expression of firefly luciferase in HeLa cells was determined using Steady-Glo luciferase assay kits. Cell number or cytotoxicity was measured using AlamarBlue assay. The luminescence or fluorescence intensity was measured using microplate reader. All the *in vitro* transfection experiments were performed in triplicate.

NP cellular uptake. For cellular uptake kinetics study, HeLa cells or RAW264.7 cells were seeded into 96-well plates and allowed to attach for 24 hours. Then the cells were incubated with NP(DY547-siRNA) at the siRNA concentration of 50 nM for different periods of time (1, 3, 6, 12, 18, and 24 hours), and then washed with PBS, fixed with 4% paraformaldehyde, and stained with Hoechst 33342 (2 μg/ml) for nuclei identification. For the uptake mechanism study with small molecule inhibitors, HeLa cells were first pre-incubated for 30 minutes with the small molecules, and then treated with NP(DY547-siRNA) for 6 hours in the presence of endocytic inhibitors. 5-(N-ethyl-N-isopropyl) amiloride (EIPA) was used as the macropinocytosis inhibitor, chlorpromazine (CPZ) as the clathrin-mediated endocytosis inhibitor, and filipin as the caveolae-mediated endocytosis inhibitor (Cayman Chemical). Images were acquired on a laser scanning confocal fluorescence microscope (Leica SP5 X, Leica Microsystems) or an inverted fluorescence microscope (Zeiss Axiovert 200) and analyzed using Fiji/Image-J software.

Immunofluorescent staining. For immunofluorescent staining, cells were first washed with ice-cold PBS, fixed in 4% paraformaldehyde at room temperature for 15 minutes, followed by three washes with PBS (5 minutes each). Subsequently, cells were permeabilized by incubation in 0.2% Triton X-100-PBS for 8 minutes on ice and blocked with PBS blocking buffer containing 2% normal goat serum, 2% BSA and 0.2% gelatin for 1 hour at room temperature. Cells were incubated in appropriately diluted primary antibody for 1 hour at 4 ^{o}C overnight. After rinsing three times with PBS, fluorescent dye-linked secondary antibodies were applied for 1 hour at room temperature. The cells were washed again with PBS, counterstained with Hoechst 33342 or DAPI and mounted on slides with Prolong Gold antifade mounting media (Life Technologies). For mitochondrial staining, MitoTracker Green was added into cells and incubated for 1 hour before fixation according to the manufacturer's instructions (Life Technologies). For actin staining, cells were incubated with Alexa Fluor 488 phalloidin (Life Technologies). PHB1 and mitochondria staining were visualized using a Leica DM-IRE2 inverted fluorescence microscope.

***In vitro* cell proliferation and NP cytotoxicity.** A549 or NCI-H460 cells were plated on 12-well plates (20,000 cells/well) and allowed to attach overnight. The cells were transfected with NP(siPHB1) or NP(siControl) for 24 hours, and then washed with fresh medium for further

incubation. At different time points, cell number or cytotoxicity was measured by AlamarBlue assay according to the manufacturer's protocol. The AlamarBlue assay agent is non-toxic, which allows us to continuously monitor cell proliferation in real time. After each measurement, the AlamarBlue agents were washed away and replaced with fresh growth medium for further cell growth. To examine the cytotoxicity of combination treatment by NP(siPHB1) and cisplatin, A549 or NCI-H460 cells were seeded in 96-well plates and allowed to adhere overnight. Then the cells were transfected with NP(siPHB1) or NP(siControl) with an siRNA concentration of 10 nM for 24 hours. One or two days after transfection, cells were incubated with cisplatin at varying concentrations for another 72 hours. The cytotoxicity was then measured with AlamarBlue assay.

Caspase-3/7 activity measurement. Forty-eight hours after NP treatment, A549 or NCI-H460 cells were seeded in 96-well plates at a density of 5,000 cells/well in triplicate. Twenty four hours post seeding, caspase-3/7 activity was measured using the Caspase-Glo 3/7 assay kits (Promega) according to the manufacturer's instructions.

Soft agar colony formation assay. Anchorage-independent growth of tumor cells (A549 and NCI-H460) was investigated by soft agar colony formation assay. Cells were cultured overnight in a 6-well plate and treated with NP(siPHB1) or NP(siControl) at 70% confluence. 48 hours later, the NP-treated cells (5,000 cells/well) were suspended in 0.36% agarose (Invitrogen) diluted in complete medium and were poured on a 0.75% preformed layer of agarose in triplicate 6-well plates. Cells were incubated at 37°C for 21 days. The colonies were stained with 0.005% crystal violet, and visible colonies > 20 μm in diameter were counted.

Flow cytometry analysis. Forty eight hours post-NP treatment, both NP(siPHB1)- and NP(siControl)-treated cells were seeded in 6-well plates and grown overnight. The supernatant and the cell monolayer were collected, washed with PBS, and processed for detection of apoptotic cells using an Annexin V-PE/7-AAD apoptosis detection kit (BD Biosciences) according to manufacturer's instructions.

Western blot analysis. Protein extracts were prepared using modified radioimmunoprecipitation assay lysis buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1% NP-40 substitute, 0.25% sodium deoxycholate, 1mM sodium fluoride, 1mM Na_3VO_4, 1mM EDTA), supplemented with protease inhibitor cocktail (Cell Signaling) and 1 mM phenylmethanesulfonyl fluoride or complete Mini protease inhibitor tablets (Roche). Equal amounts of protein, as determined with a bicinchoninic acid (BCA) protein assay kit (Pierce/Thermo Scientific) according to the manufacturer's instructions, were resolved on SDS-PAGE gels and transferred to nitrocellulose or polyvinylidene difluoride membrane. The blots were blocked with 5% nonfat dry milk or 3% BSA in TBST (50 mM Tris-HCl, pH 7.4 and 150 mM NaCl, and 0.1% Tween 20) and then incubated with appropriate primary antibodies. Signals were detected with horseradish peroxidase (HRP)-conjugated secondary antibodies and an enhanced chemiluminescence (ECL) detection system (Pierce). When indicated, membranes were subsequently stripped for reblotting.

Animals. Animals were obtained from Charles River Laboratories. All *in vivo* studies were performed in accordance with National Institutes of Health animal care guidelines and in strict pathogen-free conditions in the animal facility of Brigham and Women's Hospital. Animal

protocol was approved by the Institutional Animal Care and Use Committees on animal care (Harvard Medical School). The animals were allowed free access to sterile food pellets and water.

NSCLC xenograft tumor model. For the NCI-H460 tumor xenograft model, 3×10^6 cells in 100 μL of culture medium were implanted in the subcutaneous tissue on the bilateral flanks of 4-5-week-old female athymic nude mice. For A549 tumor xenograft model, 3×10^6 cells were suspended in 1:1 (v/v%) media and Matrigel (BD Biosciences) and implanted subcutaneously into the flanks of 4-5-week-old female athymic nude mice.

Pharmacokinetic study. For *in vivo* pharmacokinetic study, normal BALB/c mice (5-6 weeks old, n = 3 per group) were intravenously injected with fluorophore (DY647)-labeled siRNA NPs or naked DY647-siRNA through the tail vein. At different time points, blood was drawn retroorbitally and siRNA fluorescence was measured using the BioTek microplate reader. Standard curve was generated by measuring the fluorescence intensity of different amounts of NP(DY647-siRNA) or free DY647-siRNA added in blood from untreated mice. Total blood volume was estimated as 58.5 ml blood per kg of body weight.

Biodistribution. To study biodistribution *in vivo*, female athymic nude mice (n = 4 per group) bearing NCI-H460 xenograft were injected intravenously with NIR fluorophore DY677-labeled siRNA NPs or naked DY677-siRNA via the tail vein. Twenty-four hours after administration, mice were imaged using the Syngene PXi imaging system (Synoptics Ltd). Organs and tumors were also harvested and imaged. To quantify the siRNA accumulation, organs and tumors harvested from mice were weighed and homogenized. Fluorescence of the homogenate was measured using the Syngene PXi imaging system and quantified by Image-J.

***In vivo* PHB1 silencing and tumor cell apoptosis.** To evaluate *in vivo* silencing efficacy and tumor cell apoptosis, athymic nude mice bearing NCI-H460 xenograft were administered daily by intravenous injection of siRNA NPs at 600 μg siRNA per kg of animal weight, for three consecutive days. Two days after the final injection, mice were sacrificed and tumors were extracted and cut into two pieces. One was lysed for Western blot analysis as described above. The other was fixed in formalin, embedded in paraffin and sectioned at a thickness of 5 mm. Tumor cell apoptosis was determined by the TdT-mediated dUTP Nick-End Labeling (TUNEL) assay (Promega). The TUNEL staining was performed according to the manufacturer's protocol. Tumor sections were imaged with an inverted fluorescence microscope (Zeiss Axiovert 200).

***In vivo* therapeutic efficacy.** For NCI-H460 xenograft model, when the tumor size reached ~ 100 mm^3, mice were randomly divided into the following groups (n = 4 per group): (i) saline, (ii) naked siPHB1 (iii) NP(siControl) and (iv) NP(siPHB1), and treated intravenously at a dose of 600 μg siRNA per kg of animal weight at day 8, 10 and 12.

A549 xenografts were grown to ~ 100 mm^3. The mice were then randomly divided into five groups (n = 6 per group). Animals were treated with the following regimens according to the timeline shown in Fig. 4d: (i) saline, (ii) NP(siControl), (iii) NP(siControl) + cisplatin, (iv) NP(siPHB1) and (v) NP(siPHB1) + cisplatin. NP(siPHB1) and NP(siControl) were administrated intravenously at a dose of 600 μg of siRNA per kg of animal weight for each injection. For cisplatin treatment, mice were injected intraperitoneally with cisplatin (3 mg/kg, one injection

per week) prepared in 0.9% USP saline. The tumor size was measured by caliper, and tumor volume was calculated as $volume = length \times (width)^2 / 2$.

Hematologic examination and histology. For *in vivo* toxicity studies, normal female BALB/c mice (5-6 weeks old, n = 4 per group) were administered daily by intravenous injection of (i) saline, (ii) naked siPHB1, (iii) NP(siControl) or (iv) NP(siPHB1) at 600 μg siRNA/kg animal, for three consecutive days. Blood was drawn retroorbitally and serum was separated for hematologic examination. The serum level of aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urine nitrogen (BUN), creatinine and troponin-1 was measured using assay kits for AST (BioVision), ALT and Creatitine (Cayman Chemical), BUN (Arbor Assays), and troponin-1 (Life Diagnostics) according to the manufacturer's protocol. For histology, organs were collected 2 days post the final injection, fixed with 4% paraformaldehyde, and embedded in paraffin. Tissue sections were stained with H&E.

Immune response. Female BALB/c immunocompetent mice (5-6 weeks old, n = 4 per group) were injected intravenously with saline, naked siPHB1 (600 μg siRNA/kg), blank NPs or siPHB1 NPs. Serum samples obtained 6 hours or 24 hours post injection were processed for measurement of representative cytokines (TNF-α, IL-6 and IL-12) by enzyme-linked immunosorbent assay or ELISA (PBL Biomedical Laboratories and BD Biosciences) in accordance with the manufacturer's instructions.

Analysis of PHB1 tumor expression in NSCLC patients. Archived formalin-fixed paraffin embedded material was obtained from the Lung Cancer specialized Program of Research Excellence Tissue Bank at The University of Texas MD Anderson Cancer Center. Utilization of these tissues has been approved by the MD Anderson Cancer Center Institutional Review Board.

In total, 522 NSCLC tumor tissue samples were collected from surgical resected lung cancer specimens (344 adenocarcinoma and 178 squamous cells carcinoma). Tumor tissues were histologically analyzed and classified according to the 2004 World Health Organization Classification of Tumors. The tumor tissues were placed in tissue microarray using three 1-mm-diameter cores, including tissue from the center, intermediate, and periphery zone of the tumor. Detailed clinicopathologic information including demographics, smoking status (never, former, and current smokers), clinicopathologic TNM stage, overall survival, duration and time to recurrence were available for most cases. Note that patients who died from diseases other than lung cancer or from unexpected events were excluded from the case collection. After excluding 57 cases with incomplete clinicopathologic information or losing tissue during TMA preparing and staining, 465 patients were finally analyzed.

Immunohistochemical (IHC) staining was performed using automated immunostainer (Dako Inc, Carpinteria, CA). 5-μm-thick sections from formalin-fixed and paraffin-embedded tissues were deparaffinized and hydrated. Antigen retrieval was performed in pH 6.0 citrate buffer in a decloaking chamber (Biocare, Concord, CA) for 30 minutes at 121ºC, followed by 10 minutes at 90ºC, and Tris buffer washing. Peroxide blocking was performed with 3% H_2O_2 in methanol at room temperature for 30 minutes. The slides then were incubated with anti-PHB1 antibody (dilution 1:200, rabbit polyclonal, ab1836, Abcam, Cambridge, MA) at room temperature for 65 min and washed with Tris buffer, followed by incubation with Envision Dual-Link system-HRP (Dako Inc) for 30 min. Staining was developed with 0.5% 3,3'-diaminobenzidine freshly prepared with Imidazole-HCl buffer pH 7.5 containing H_2O_2 and an anti-microbial agent (Dako

Inc) for 5 minutes, and then counter stained with hematoxilyn, dehydrated, and mounted. Positive tumor tissues were used as control. As the negative control, we used the same tissue used in positive control replacing the primary antibody with universal anti-rabbit negative control antibody (Dako Inc). We analyzed PHB1 cytoplasmic expression in tumor cells using a score (range 0 - 300) calculated using staining intensity (0 - 3) multiplied by extension of expression (i.e., percentage of tumor cells with positive staining) (0 - 100%). For the survival analysis, we considered it as high PHB1 expression when the score was higher than 50. Survival curves were estimated based on the Kaplan-Meier method and assessed with log-rank test.

Statistics. Statistical analyses of the data were performed using the IBM SPSS program 16.0 to perform the two tailed student's *t*-test. All experiments, unless otherwise stated, were performed in triplicate. Error bars used in this work are S.D., unless otherwise noted. A *p value* <0.05 is considered statistically significant. Statistical values are indicated in figures according to the following scale: * = $p<0.05$, ** = $p<0.01$ and *** = $p<0.001$.

SI Appendix

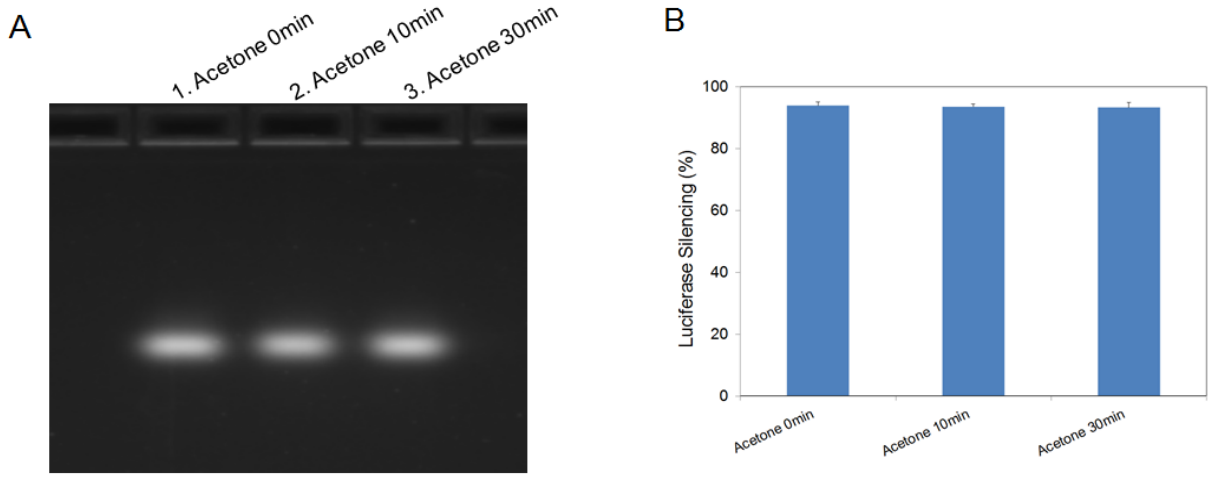

Figure S1. siRNA stability in acetone. (**A**) Gel electrophoresis and (**B**) silencing activity of luciferase siRNA incubated with acetone for 0, 10 and 20 min. Lipo2K was used as the transfection agent in testing the siRNA silencing efficacy.

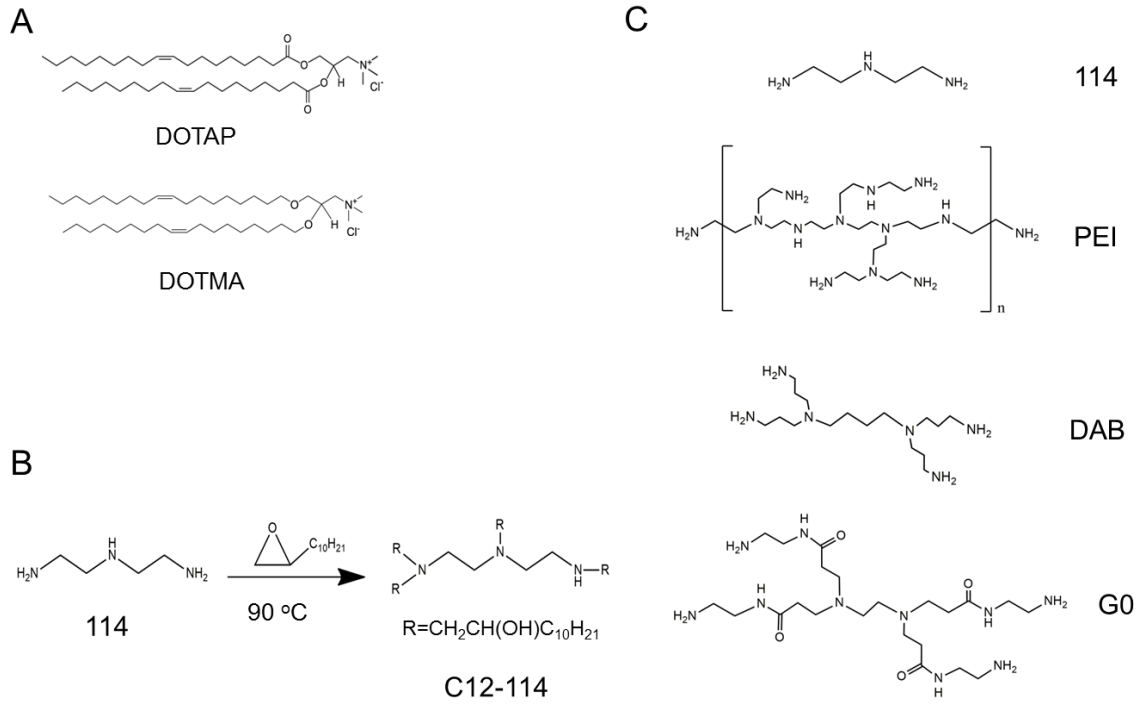

Figure S2. Chemical structure of cationic lipids or lipid-like compounds. (**A**) Cationic lipid DOTAP and DOTMA. (**B**) Synthesis of cationic lipid-like compounds through ring opening reaction. (**C**) Amino moieties of the cationic lipid-like compounds.

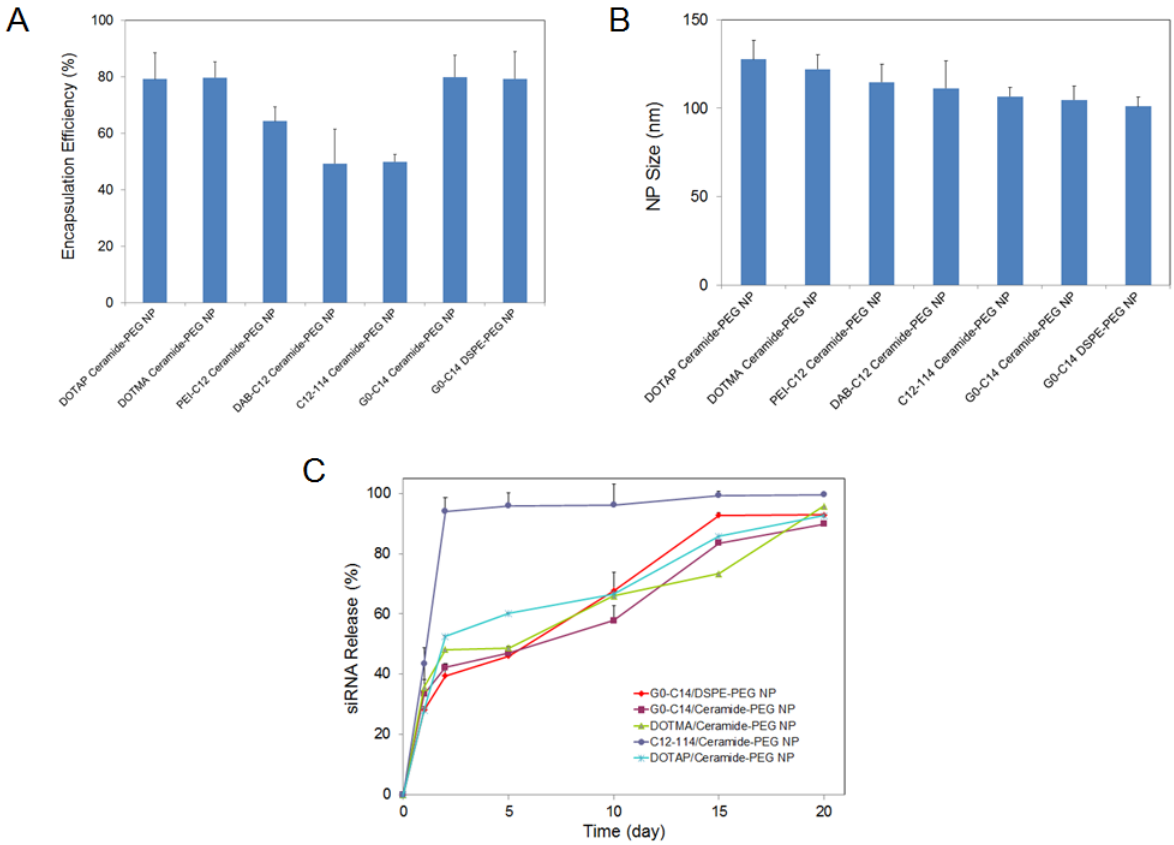

Figure S3. Effect of cationic lipid and lipid-PEG on (**A**) siRNA encapsulation, (**B**) NP size, and (**C**) siRNA release kinetics.

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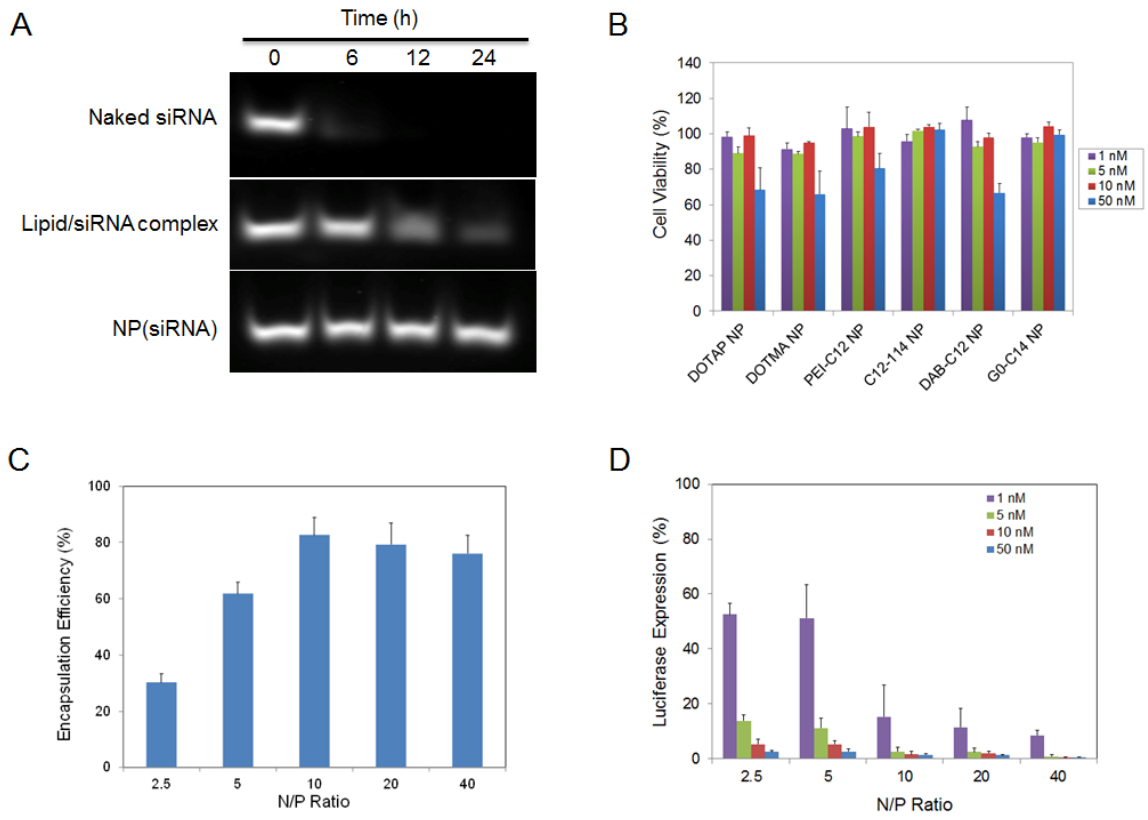

Figure S4. *In vitro* characterization of the hybrid siRNA NPs. (**A**) Serum stability of NP(siRNA). Agarose gel electrophoresis of naked siRNA (top), lipid-siRNA complexes using G0-C14/lipid-PEG (middle), and NP(siRNA) (bottom) in serum at 37 ºC at the indicated times. Bands were shown in the expected position of intact siRNA (~ 13 kDa). (**B**) Cytotoxicity of the siRNA NPs prepared with different cationic lipids on HeLa cells. Untreated cells were used as a control. Effect of N/P ratio on (**C**) siRNA encapsulation and (**D**) silencing activity of G0-C14 ceramide-PEG NPs (n = 3).

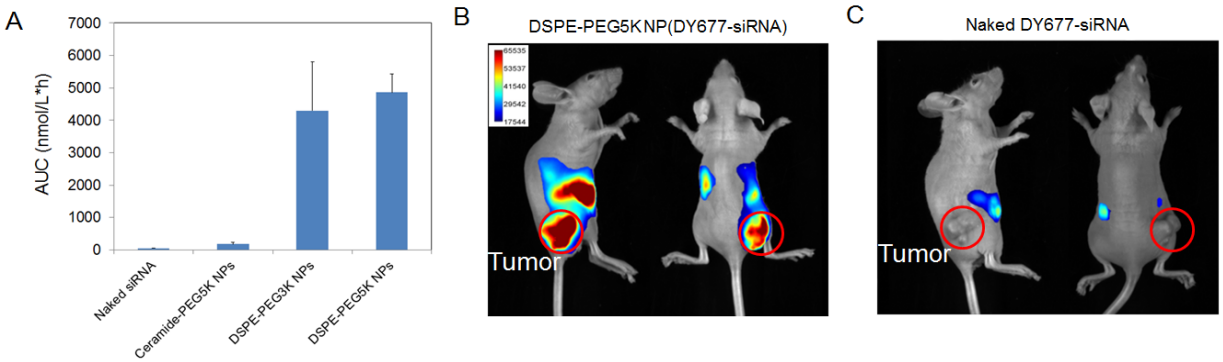

Figure S5. *In vivo* PK and BioD of the hybrid siRNA NPs. (**A**) The area under the curve (AUC) of naked siRNA and three different siRNA NPs, calculated from the circulation profiles shown in Fig. 2A. Whole animal fluorescence imaging 24 h post IV injection of (**B**) NP(DY677-siRNA) composed of DSPE-PEG5K and (**C**) naked DY677-siRNA in mice bearing NCI-H460 tumors.

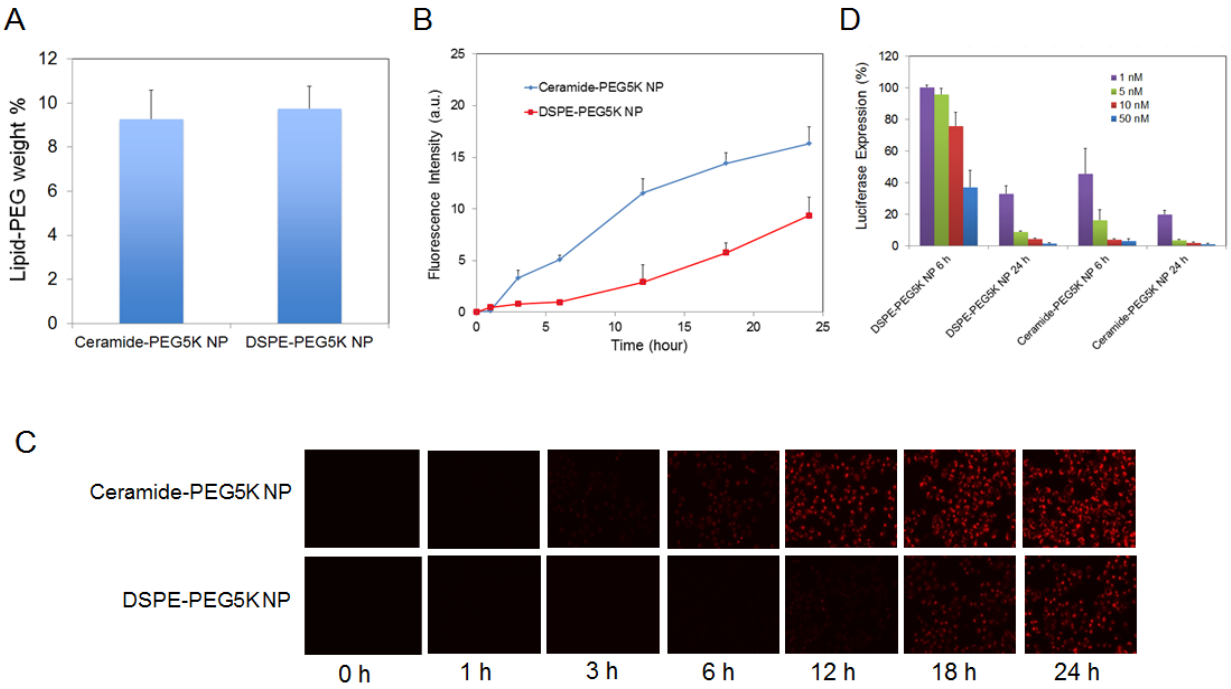

Figure S6. Effect of lipid-PEG on the NP properties. (**A**) The amount of lipid-PEG (ceramide-PEG5K *vs*. DSPE-PEG5K) on NP surface. *Y*-axis is the weight % of lipid-PEG relative to the PLGA polymer. (**B**) Uptake kinetics of NPs prepared with two different lipid-PEGs (ceramide-PEG5K *vs*. DSPE-PEG5K) in Luc-HeLa cells. (**C**) Representative fluorescence images for the cellular uptake of the two NPs at different time points. (**D**) Effect of NP incubation time with Luc-HeLa cells on *in vitro* luciferase silencing of DSPE-PEG5K *vs*. ceramide-PEG5K NPs.

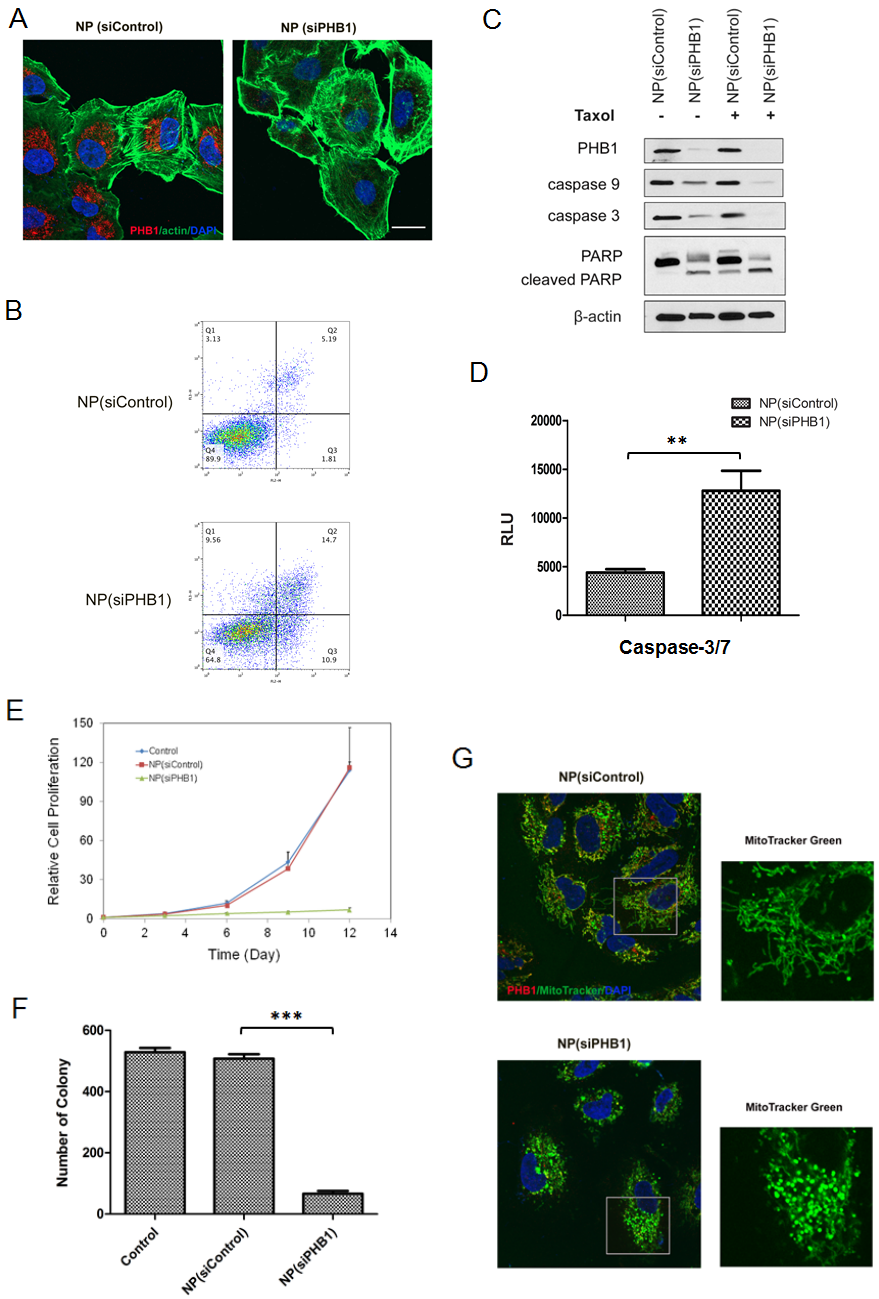

Figure S7. NP-mediated PHB1 silencing in A549 cells. (**A**) Immunofluorescence images of A549 cells after treatment with NP(siControl) or NP (siPHB1). PHB1: red; actin: green; and nucleus: blue. (**B**) Flow cytometry analysis of cell apoptosis 72 h post NP treatment. (**C**) Western blot analysis of PHB1, caspase-9, caspase-3, and PARP cleavage after NP treatment alone or in combination with taxol. β-actin was used as a control. (**D**) Caspase 3/7 activities measured by Caspase-Glo 3/7 assay, after NP treatment (** p<0.01). (**E**) Inhibition of cell proliferation of A549 cells upon PHB1 silencing. (**F**) Quantitative analysis of colony numbers in soft agar colony formation assay 3 weeks post NP treatment (*** p<0.001). (**G**) Mitochondrial staining of A549 cells after NP treatment. MitoTracker: green; PHB1: red; and nucleus: blue. Enlarged MitoTracker pictures depict (top) long tubular mitochondrial network for NP(siControl) and (bottom) punctuated form of mitochondria for NP(siPHB1). Scale bar: 20 μm.

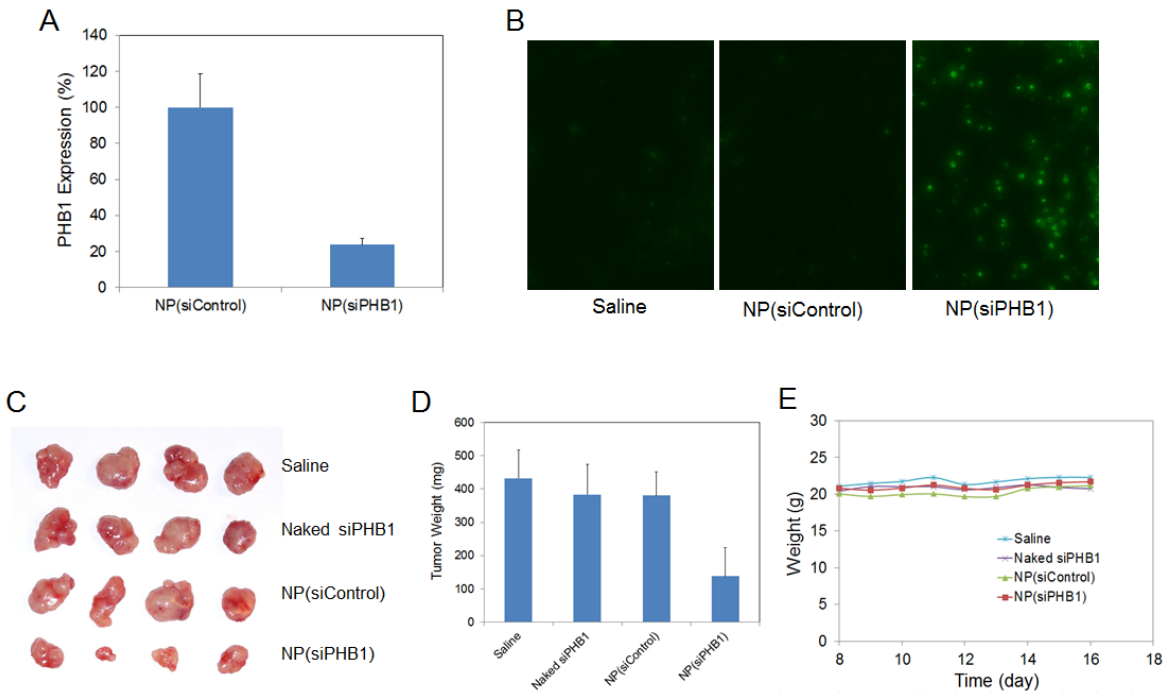

Figure S8. *In vivo* therapeutic efficacy of NP(siPHB1) in NCI-H460 xenograft. (**A**) Quantification of PHB1 silencing in NCI-H460 tumor tissue. (**B**) Representative image of apoptotic cell staining by TUNEL in the tumor tissue. (**C**) Image and (**D**) weight of the NCI-H460 tumors at day 16. (**E**) Total body weight of the NCI-H460 tumor-bearing mice over the course of therapy.

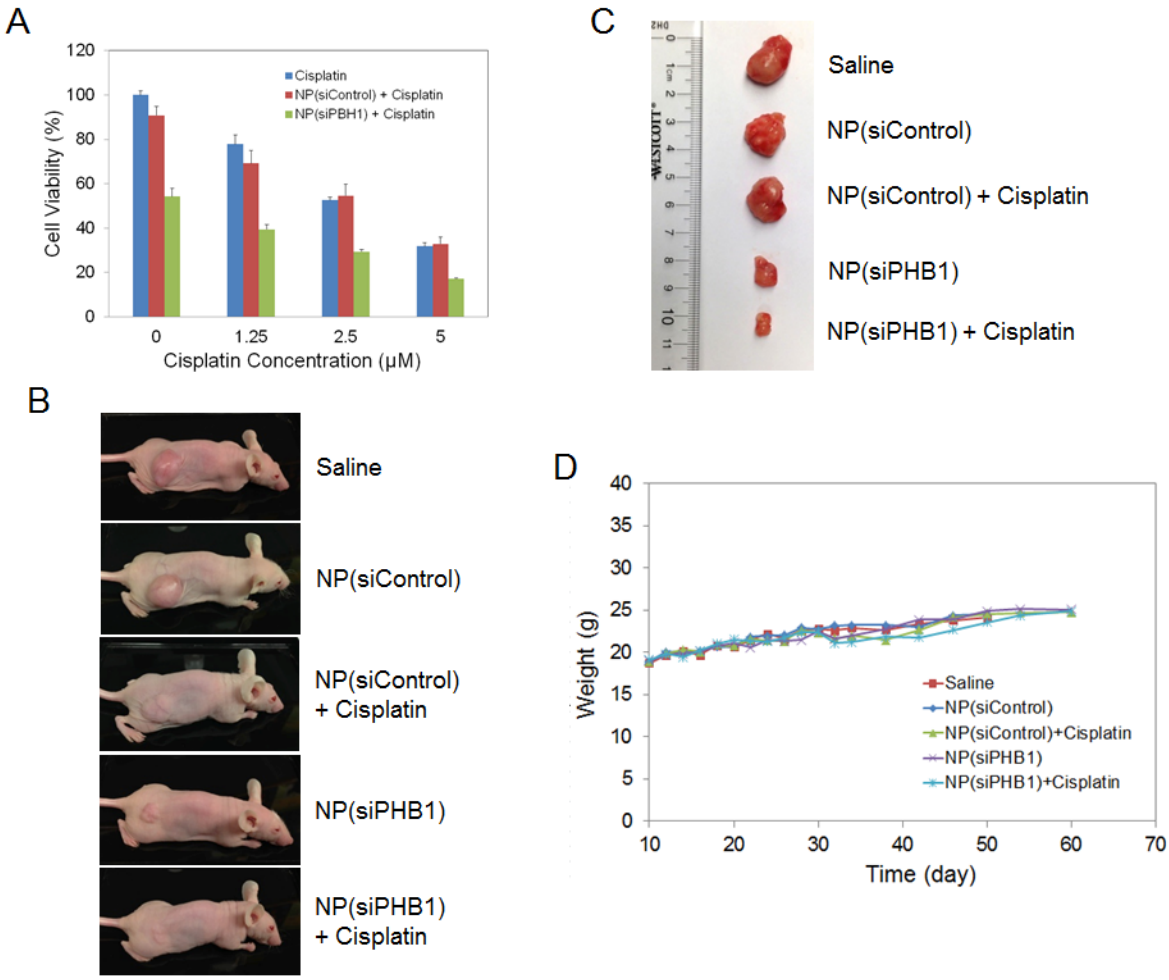

Figure S9. Therapeutic efficacy of NP(siPHB1) in combination with cisplatin. (**A**) Cytotoxicity of NCI-H460 cells incubated with cisplatin at different concentrations for 72 h with or without NP(siPHB1) treatment. (**B**) Representative image of mouse-bearing A549 tumor at day 34 for each group. (**C**) Representative image of the tumors from each group at the day of sacrifice. (**D**) Total body weight of the A549 tumor-bearing mice over the course of therapy.

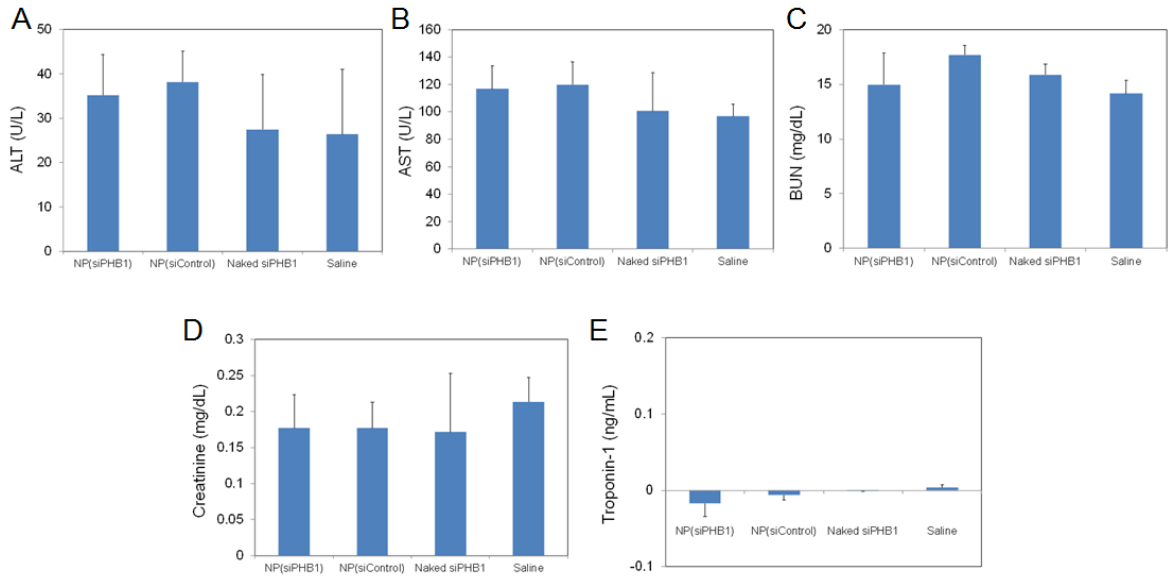

Figure S10. Evaluation of NP toxicity *in vivo*. Serum levels of (**A**) alanine aminotransferase (ALT), (**B**) aspartate aminotransferase (AST), (**C**) blood urine nitrogen (BUN), (**D**) creatinine and (**E**) troponin-1 were measured after three daily IV injections of NP(siPHB1) or control groups.

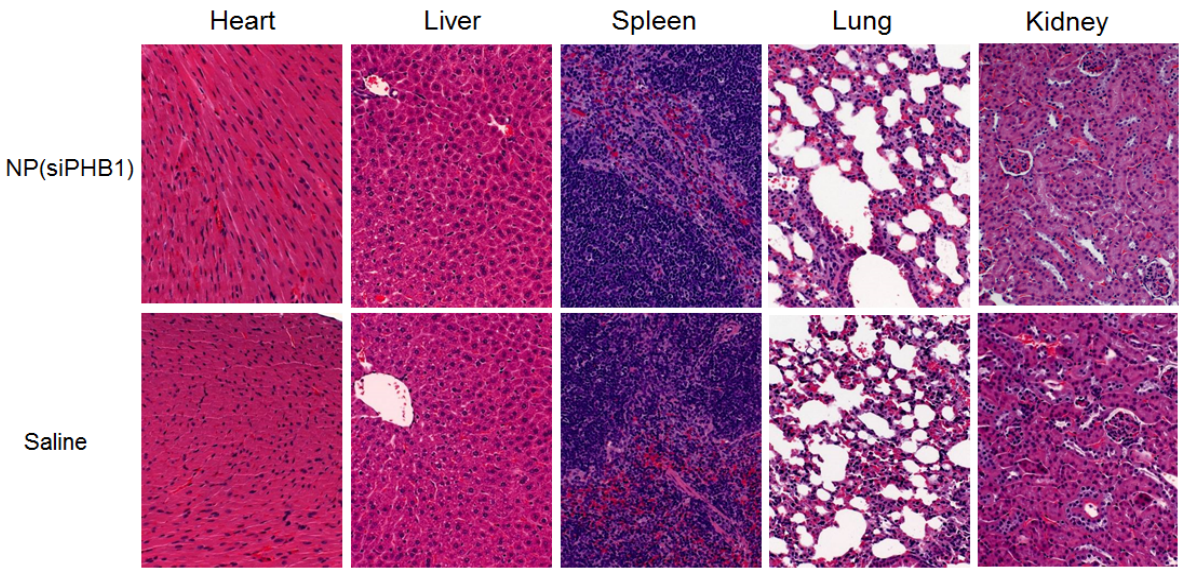

Figure S11. H&E staining of tissue sections of major organs after treatment of NP(siPHB1) *vs*. saline. Images were taken under 20× objective.

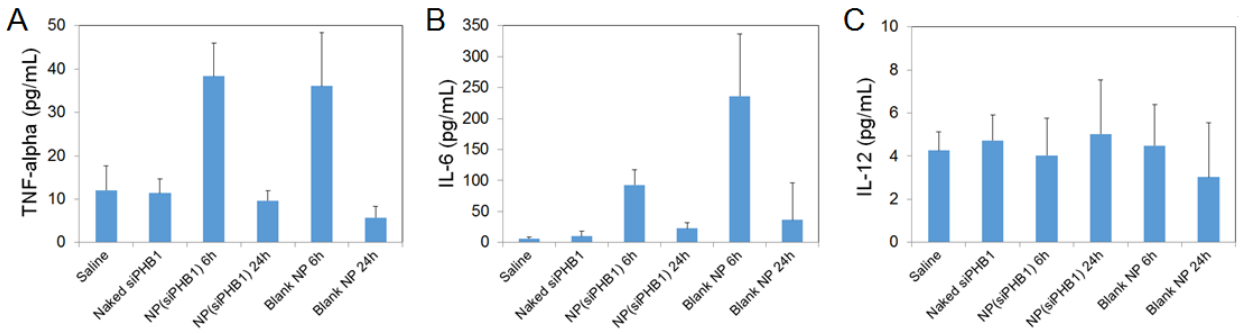

Figure S12. Immune response after NP treatment. Serum levels of (**A**) TNF-α, (**B**) IL-6 and (**C**) IL-12 at 6 and 24 h after IV injection of saline, naked siPHB1, blank NP, or NP(siPHB1).

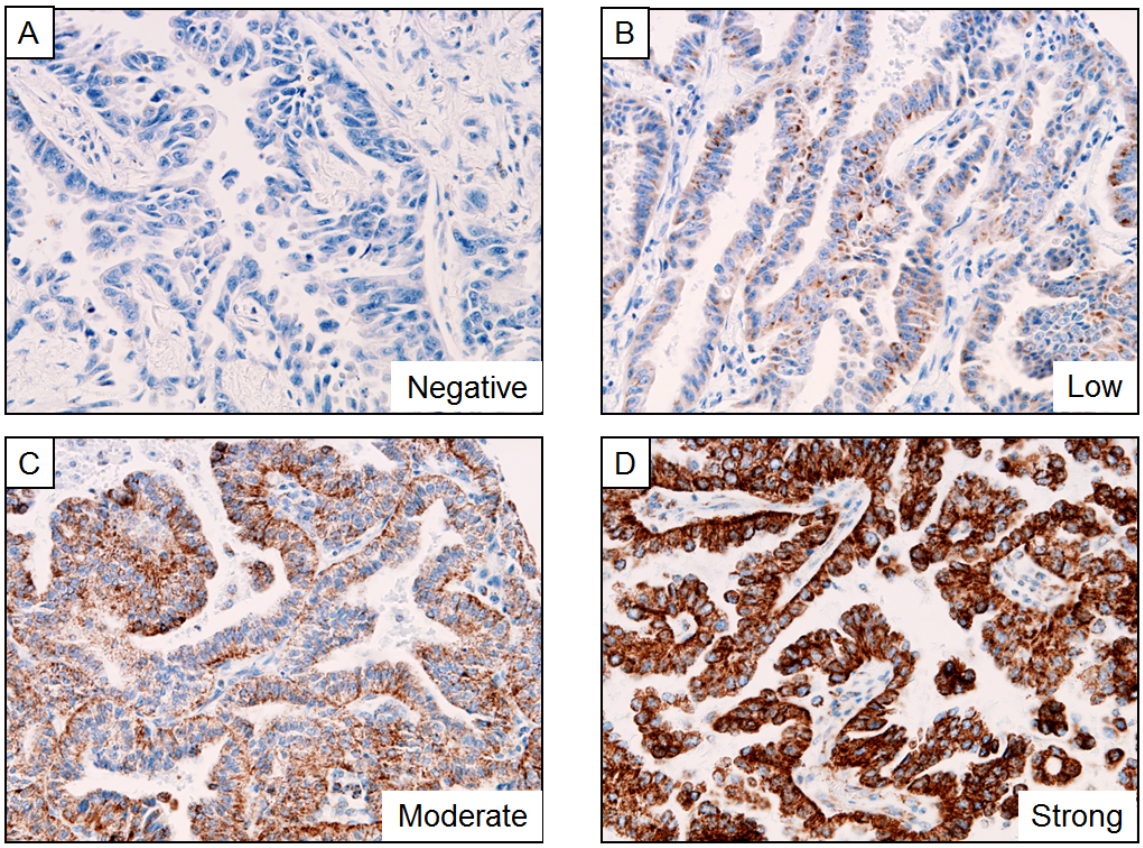

Figure S13. IHC microphotographs of different levels of PHB1 tumor expression in NSCLC patients with adenocarcinoma: (**A**) negative, (**B**) low, (**C**) moderate and (**D**) strong granular cytoplasmic expression in tumor cells.

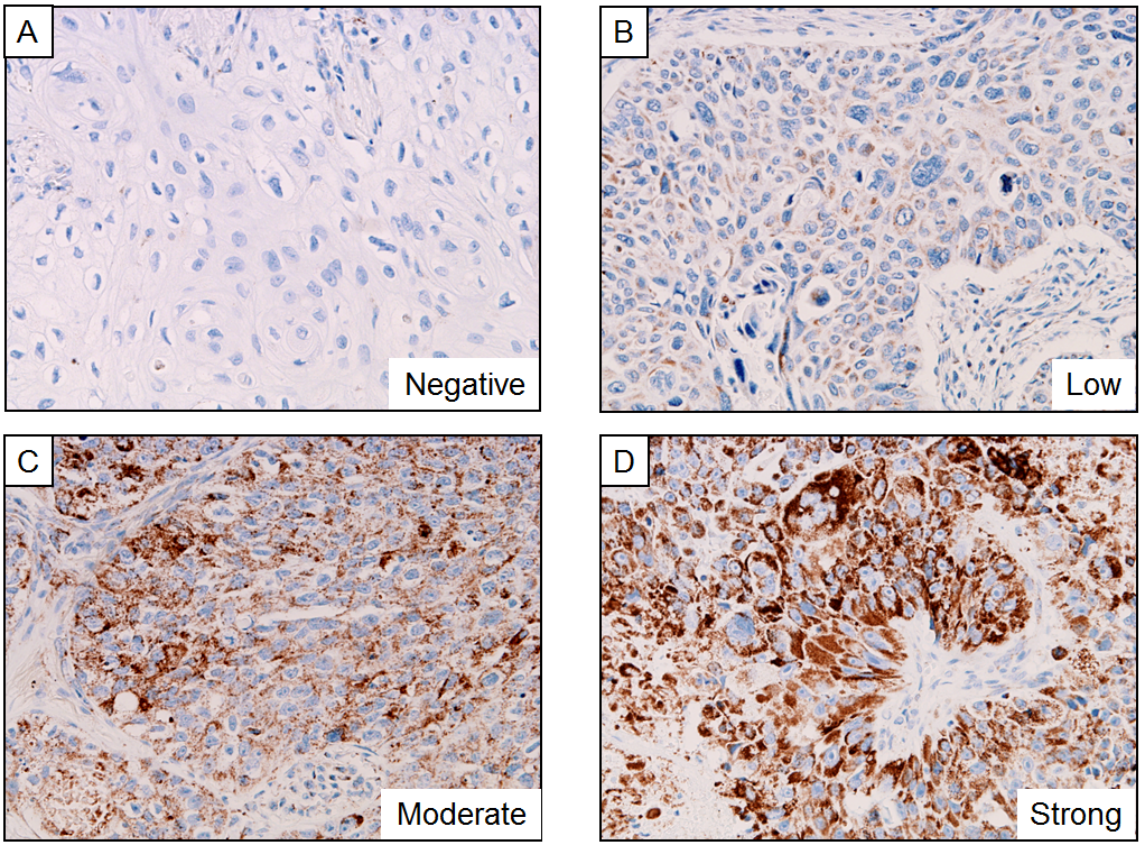

Figure S14. IHC microphotographs of different levels of PHB1 tumor expression in NSCLC patients with squamous cell carcinoma: (**A**) negative, (**B**) low, (**C**) moderate and (**D**) strong granular cytoplasmic expression in tumor cells.

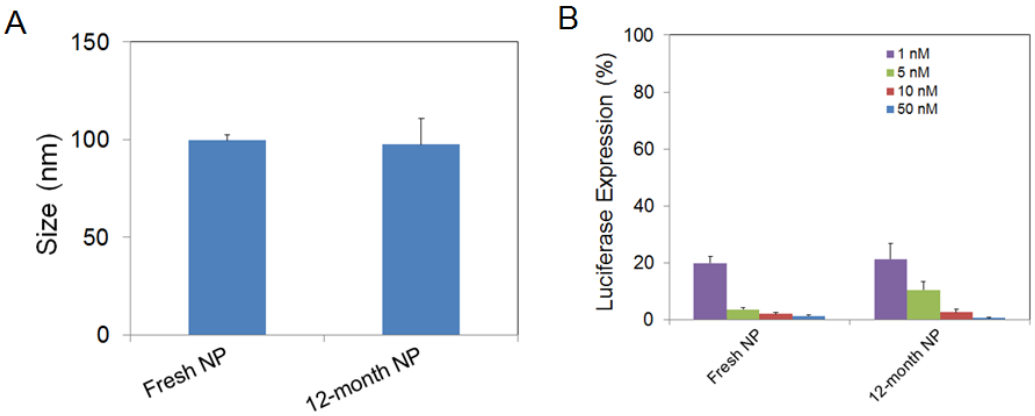

Figure S15. NP stability and silencing activity after storage at -80 ºC for 12 months. The comparison of (**A**) particle size and (**B**) silencing activity before and after storage.

Table S1. Mean and median PHB1 tumor expression scores according to histologic type

Characteristic	N	Positive N (%)	Mean Score	Median Score	Standard Deviation
Adenocarcinoma	307	235 (76%)	70.1	67.0	65.2
Squamous cell carcinoma	158	131 (83%)	67.8	70.0	52.1
TOTAL	465	366 (79%)	69.3	67.0	61.0

[a]