Supporting Information

S1. Identification of Dox sensitivity in MCF-7 and MCF-7/MDR cells.

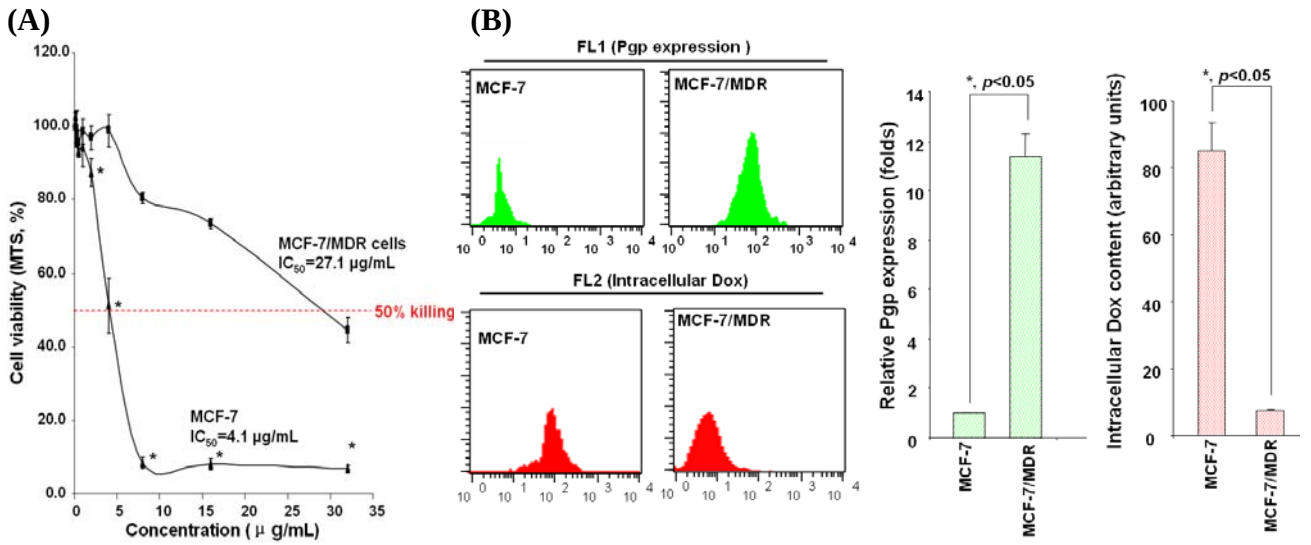
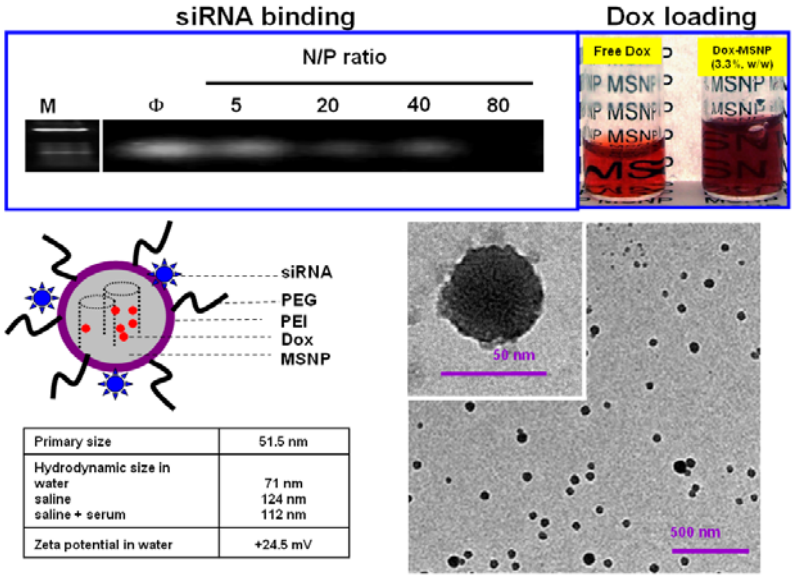


Fig. S1 (A) MCF-7 (Dox-sensitive) and MCF-7/MDR (Dox-resistant) cells were plated at a concentration of 1×10^4 cells per well in 96 well plates. The cells were treated with free Dox in doses ranging from 0.25-32 μg/mL for 72 h. The cell viability was determined by the MTS assay. After treatment, the cells were incubated with the MTS solution for 1 h before measurement. Non-treated cells served as reference for calculating 100% cellular viability. **(B)** In order to further characterize the MCF-7/MDR cells, we confirmed through flow cytometry that Pgp expression in MCF-7/MDR wells was ~11.5 times higher than MCF-7 cells. This leads to a significantly lower drug level in MCF-7/MDR when treated by free Dox compared to drug sensitive MCF-7 cells receiving the same amount of free Dox. In order to measure Pgp expression, 1×10^6 of cells were collected, washed in PBS, and fixed using 2% paraformaldehyde in PBS for 1 h at 4 ºC. The cells were then washed once in 1% FCS in PBS and incubated with anti-Pgp primary antibody for 2 hours at room temperature. After PBS washing, the cells were further incubated with the FITC-labeled secondary for 1 hour at room temperature. The cells were washed twice in PBS, and green signals (FL-1) were acquired using FACScan flow cytometer (Becton Dickinson, San Jose, CA) equipped with argon laser. In order to determine intracellular Dox retention, MCF-7 and MCF-7/MDR cells were incubated with 2 μg/mL free Dox for 3 hours at 37 ºC. After PBS washing, the red fluorescence was recorded in FL-2 channel using the same flow cytometer. Please note that Dox and FITC-labeled Pgp expression were measured separately due to the minor overlap of Dox with FITC fluorescence. Further data analysis showed that Pgp expression in MCF-7/MDR wells was ~11.5 times higher than MCF-7 cells. The intracellular Dox content in MCF-7/MDR cells is ~9 times lower than MCF-7 cells at the same Dox concentration.

S2. Design and characterization of drug and siRNA loading, cellular uptake, subcellular localization, and cargo release from MSNP in MCF-7/MDR cells.

S2.1 Demonstration of drug and siRNA loading of the particles in water and biological media: To demonstrate siRNA loading capability, an agarose gel retardation assay was used to determine the optimal particle/nuclei acid (N/P) ratio. Since this showed an optimal N/P ratio >40 (Fig. S2A), we used an N/P ratio of 100 in subsequent experiments. siRNA attachment leaves the pores assessable for Dox loading by



Primary size	51.5 nm
Hydrodynamic size in water saline saline + serum	 71 nm 124 nm 112 nm
Zeta potential in water	+24.5 mV

Fig. S2 (A) An agarose gel retardation assay was used to determine the siRNA binding to Dox-loaded MSNP. 0.1 µg of Pgp siRNA was mixed with 0.5-8 µg MSNP to obtain particle/nuclei acid (N/P) ratios of 5-80. 10 µL of the polyplex solution was mixed with 2 µL of 6× loading buffer and electrophoresed in a 1.5% agarose gel containing 0.5 µg/mL ethidium bromide at 100 V for 30 minutes in Tris-boric acid (TBE) running buffer. Nucleic acid bands were detected by UV light and the photos taken with a Bio-Rad imaging system (Hercules, CA). The N/P threshold ratio is defined as the lowest value that prevents free siRNA from entering the gel. The N/P ratio was determined to be 40. Through the measurement of Dox fluorescence intensity, the drug loading capacity was determined to be 3.3% (w/w). The scheme depicts a 50 nm MSNP coated with a 1.8 kD PEI polymer in which some of the amines were reacted with 5 kD PEG. The negatively charged, phosphonate coated particle provides an electrostatic surface for binding the cationic PEI-PEG copolymer, which, in turn, allows complexing of siRNA. Since the copolymer attachment leaves the porous interior free for Dox binding and delivery, also *via* an electrostatic binding mechanism, it allows simultaneous drug and nucleic acid delivery. TEM imaging demonstrates the copolymer coated morphology of the particles in saline. Size and zeta potential of dual drug/siRNA loaded particles in pure water, saline, and saline supplemented with mouse serum were assessed with a ZetaSizer Nano (Malvern) at a particle concentration of 100 µg/mL.

electrostatic attachment. This binding is disrupted by protons in the acidifying endosomal compartments in the cells. The loading capacity of Dox-laden MSNP, which showed optical transparency in saline supplemented with serum, was ~3.3% (w/w). We also determined the morphology, size, and surface charge of the dual loaded particles in different biological media. TEM demonstrated that the primary particles are mono-dispersed and exhibit a 51.5 nm diameter in saline. DLS analysis showed an average hydrodynamic size of a ~71 nm, ~124 nm and ~112 nm in pure water, saline, and saline supplemented with 10% mouse serum, respectively. Copolymer coating resulted in particles with a positive zeta-potential of +24.5 mV in water (Fig. S2A).

S2.2 Demonstration of pH dependent Dox release under abiotic conditions and in acidifying subcellular compartments in MCF-7/MDR cells: Dox remains stable encapsulated in MSNP that is introduced to cell culture medium or saline supplemented with serum at pH=7.4 for at least 96 h. However, Dox is released in a time-dependent manner from the particle in artificial lysosomal fluid (pH=4.5) or by lowering the saline pH to 4.5 (Fig. S2, B1). We further demonstrated that the particles are taken up and localize in LAMP-1 positive endosomal compartments. This objective was achieved by co-incubating the FITC-labeled MSNP with MCF-7/MDR cells, followed by confocal microscopy and staining the late endosomal and lysosomal compartments with a TRITC-labeled anti-LAMP-1 antibody as previously described by us (J Am Chem Soc. 2010, 132, 12690-12697). Image analysis with Image J software demonstrated an 83% co-localization ratio (CR) of the green-labeled nanoparticles with the red-labeled lysosomes. Dox is released from the porous interior as a result of the interference in electrostatic binding of the drug by the protons being pumped into the endosomal compartment. This was confirmed by the finding that NH_4Cl, which neutralizes the acidification, keeps the Dox confined in the particles (discrete spots) instead of being released to the nucleus.

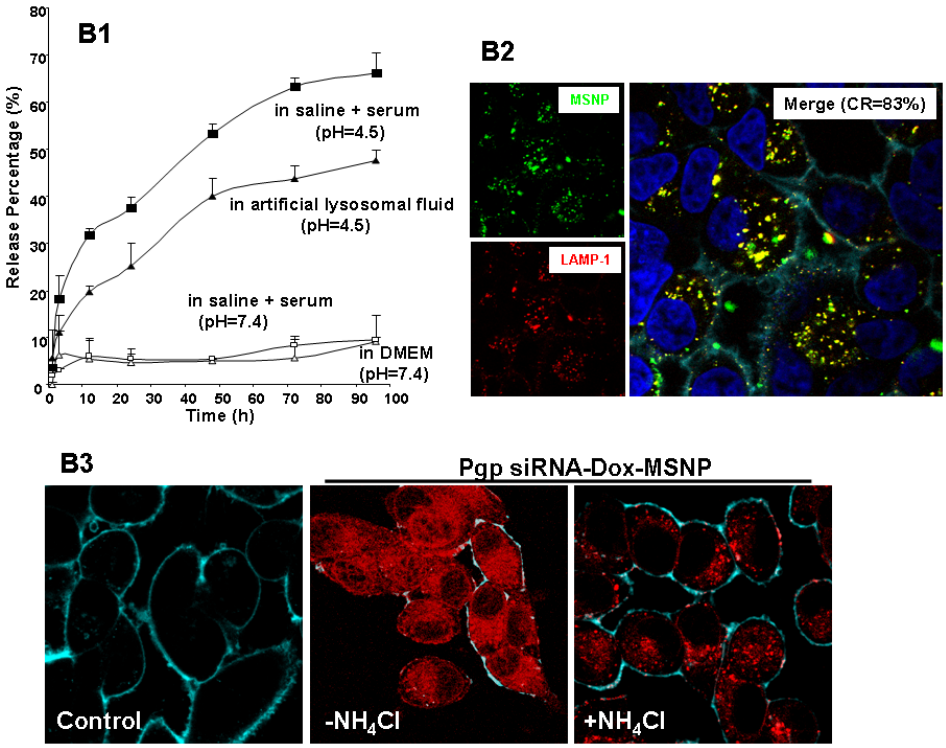


Fig. S2 (B) B1. Dox release from MSNP by a proton-sensitive mechanism. 1 mg MSNP containing Dox and siRNA was re-suspended in 3 mL phenol-red free complete DMEM (pH=7.4), saline supplemented with mouse serum (pH=7.4), artificial lysosomal fluid (pH=4.5), or acidified mouse serum at a pH=4.5. Following incubation for 96 h, 40 µL of each particle suspension was collected and centrifuged. At this point the pH in the collected supernatant was readjusted to ~7.0 by 0.1 M NaOH and the fluorescence spectrum of Dox measured at excitation and emission wavelength of 485/550 nm in a microplate reader (M5e, Molecular Device, USA). **B2.** MCF-7/MDR cancer cells were grown on 8-well chamber slides, and were exposed to FITC-labeled 50 nm PEI-PEG-MSNP (20 µg/mL) for 6 h, before being fixed, permeabilized, and labeled with a standard immunocytochemistry protocol. LAMP-1 staining was performed by using a 1:500 dilution of mouse-anti-human mAb (H4A3) for 16 h at 4 °C. This procedure was followed by a TRITC-conjugated 1:500 dilution of goat-anti-mouse secondary antibody for 1 h at room temperature. Cell membranes were co-stained with Alexa Fluor 633 conjugated wheat germ agglutinin (WGA, 5 µg/mL) in PBS for 30 min. Slides were mounted with Hoechst 33342 and visualized under a confocal microscope (Leica Confocal 1P/FCS). Image analysis by Image J software demonstrated 83% co-localization of the green-labeled nanoparticles with the red-labeled acidic lysosomal compartments. **B3.** Since Dox release is proton-sensitive, the effect of neutralizing the lysosomal pH with NH_4Cl was investigated in MCF-7/MDR cells. These cells were treated with 35 µg/mL Pgp-siRNA-Dox-MSNP with or without the addition of 20 mM NH_4Cl. The cell membrane was stained by WGA 633, a cyan-fluorescent dye. Confocal microscopy showed intracellular Dox release from the MSNP to the cytoplasm and nucleus in MCF-7/MDR cells 24 hours after the particle treatment. However, the lysosomal pH neutralizer, NH_4Cl, significantly interferes in drug release in MCF-7/MDR cells.

S2.3 Demonstration of MSNP-mediated siRNA uptake and intracellular release of siRNA in MCF-7/MDR cells: Texas red labeled siRNA was used to demonstrate intracellular delivery in association with FITC labeled MSNP in MCF-7/MDR cells. Additional images taken at a late time point provided evidence for siRNA release from the particle.

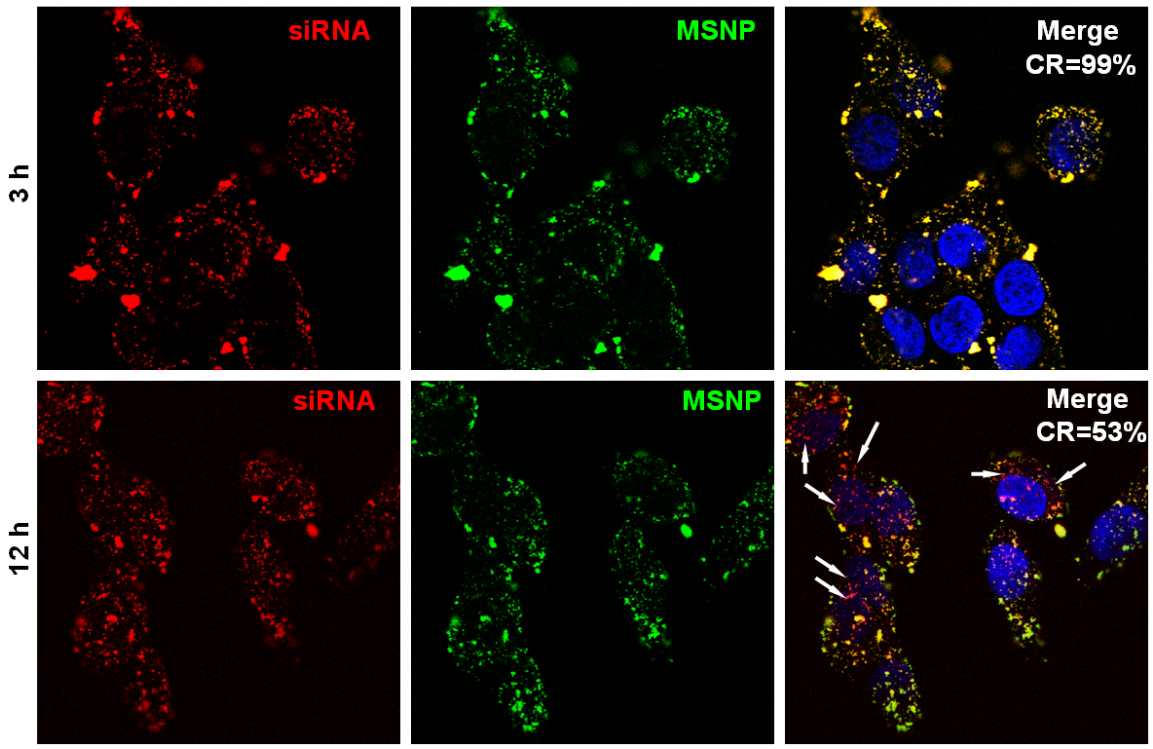


Fig. S2 (C) Confocal microscopy to demonstrate Texas red-labeled siRNA (another siRNA species which targets GFP protein of molecular weight of ~15 kD) uptake in MCF-7/MDR cells. 200 µg copolymer-coated MSNPs were incubated with 2 µg Texas red-labeled siRNA for 30 minutes. Cellular uptake of siRNA-loaded MSNP was performed by adding 30 µg/mL MSNP to 8-well chamber slides for 3 or 12 hours, followed by PBS washing. Each well contained 5×10^4 cells in 0.4 mL culture medium. Slides were mounted with Hoechst 33342 and visualized under a confocal microscope (Leica Confocal 1P/FCS) using 100× objective. Image J software was used to determine the co-localization ratio (CR) of red-labeled siRNA versus FITC-labeled particle. The merged image at 3 hours showed a high rate of particle-associated cellular uptake of siRNA. However, the merged images at 12 hours showed a lower CR value, providing evidence of intracellular siRNA release (arrows showing non-particle associated nucleic acid) from the endosomal compartment into which the particles were taken up.

S3. Comparison of the cytotoxic effects of different delivery modalities of Dox in MCF-7/MDR cells.

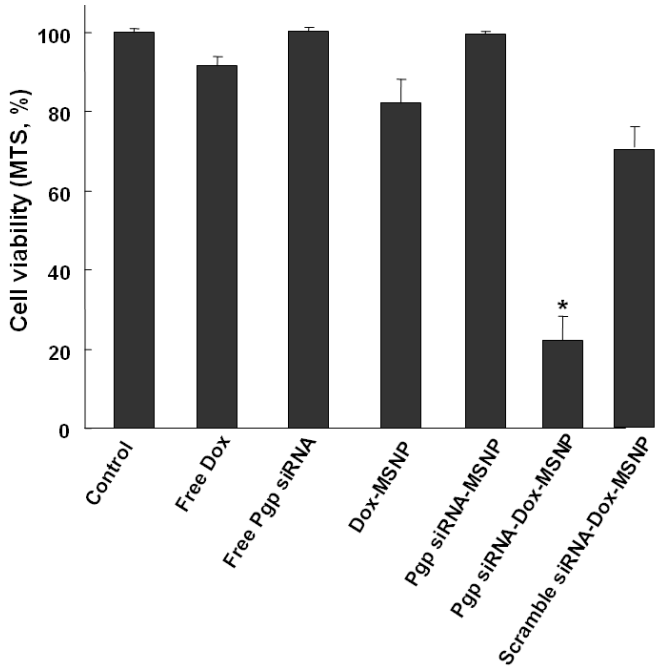


Fig. S3 Comparison of the cytotoxic effects of different delivery modalities of Dox in MCF-7/MDR cells. The MCF-7/MDR cells were treated by Pgp siRNA-Dox-MSNP at a particle dose of 40 μg/mL (Dox: 1.3 μg/mL; siRNA: 400 ng/mL) for 72 hours and compared to a list of controls at equivalent drug or siRNA doses. Consistent with the HTS assay, the MTS viability assay showed that MSNP delivery of Dox plus Pgp siRNA is capable of restoring Dox sensitivity as compared to free Dox, particle-associated Dox or MSNP attached to siRNA only. Since no cytotoxicity was obtained in cells treated with Pgp siRNA-MSNP only, we did not include this control in Fig. 2 for logistical reasons. However, we did demonstrate that there is no effect on MDR tumor growth in mice. *, $p<0.05$.

S4. Identification of Dox sensitivity and Pgp expression in MDR xenograft.

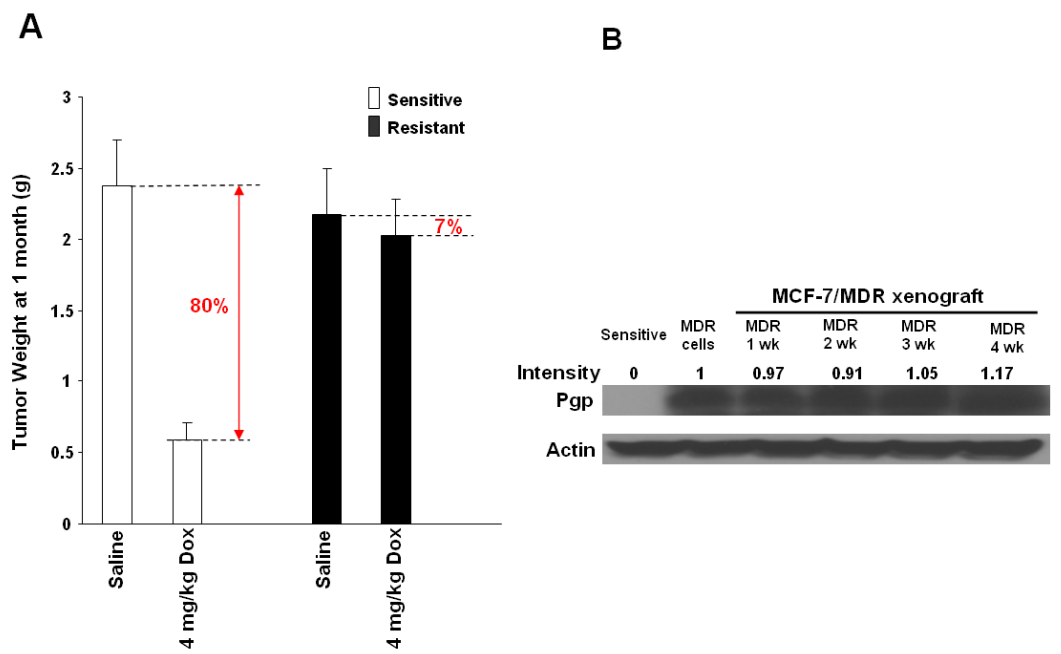


Fig. S4 (A) To ensure that the MDR cancer cells retain resistance to Dox under xenograft conditions, an animal experiment was conducted. Briefly, MCF-7 and MCF-7/MDR cells were used to grow tumor xenografts. One week after tumor implantation, free Dox was intravenously injected into these tumor-bearing animals at a Dox dose of 4 mg/kg weekly for 4 weeks. At the end of experiment, the tumor tissues were harvested to calculate tumor inhibition rate compared to the treatment using saline. **(B)** In a separate experiment, one animal was sacrificed for tumor harvesting every week post MCF-7/MDR tumor cells implantation in nude mice. Pgp expressions in the xenografts at each time points were compared through the use of immunoblotting at each time point.

S5. Biodistribution of i.v. injected MSNP in MCF-7/MDR xenografts grown in nude mice.

In order to optimize MSNP biodistribution in MDR tumor-bearing mice, imaging studies were performed in a nude mice. Tumor visualization was done by using luciferase expression in MCF-7/MDR cells and detecting bioluminescent images at the xenograft site following intraperitoneal (i.p.) injection of D-Luciferin. To simultaneously visualize the particles, the MSNP were labeled with near-infrared (NIR) dye that exhibits high photon penetration in the animal tissue. Reference images, which were captured before particle injection, showed little NIR background in the mice. Xenograft bearing animals were i.v. injected with 50 mg/kg NIR-labeled MSNP and the fluorescence images were captured 3, 24, and 72 h post particle injection. Prominent MSNP uptake was found in the tumor tissue after 24 h and imaging was sustained for at least 72 h, suggesting abundant particle retention at the xenograft site (Fig. S5A). The mice were sacrificed at 72 h and *ex vivo* images were obtained of the tumor tissue as well as major organs (liver, spleen, lung, kidney, brain, and heart) (Fig. S5B). This demonstrated strong fluorescence at the tumor site using *ex vivo* imaging. The data also showed that a considerable amount of particles were captured in the reticuloendothelial system (RES) system such as liver and spleen. These organs were accurately weighed and used to quantitatively express particle distribution by by using fluorescence intensity per unit mass tissue. When further expressed as a percentage of the total mass of the particles administered, ~8% of MSNP were retained at the tumor site at 72 h (Fig. S5C). We have previously demonstrated that the quantification of NIR fluorescence is in good agreement with calculation of the EPR effect by according to the elemental Si content (ICP-MS analysis) of the same tissues (ACS Nano, 2011, 5, 4131-4144). An 8% EPR effect is exceptionally good compared to other particle types (Curr. Opin. Mol. Ther. 2001, 3, 153– 158, Int. J. Pharm. 2007, 331, 167– 175, J. Drug Target 2004, 12, 585–591), demonstrating the novelty of our design and quantitative assessment of particle delivery at the tumor site.

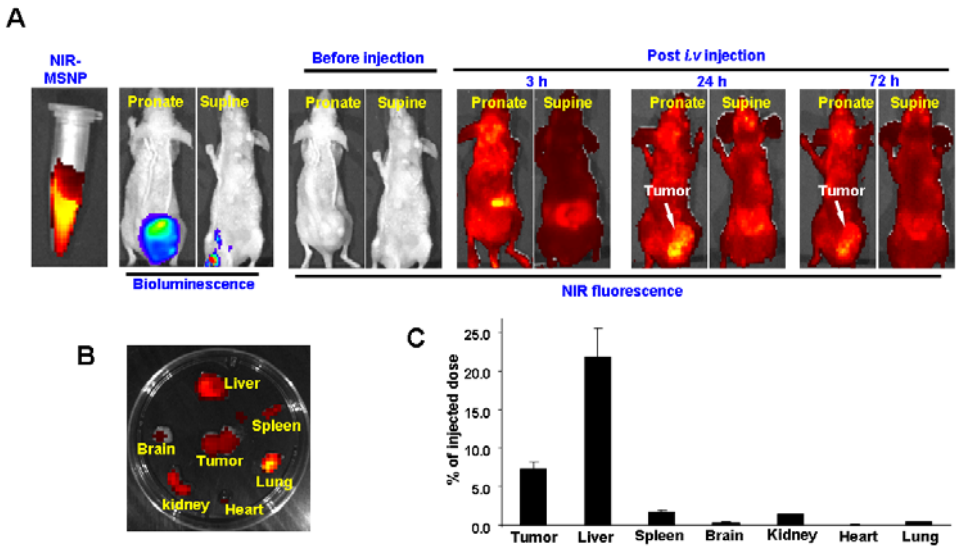


Fig. S5 Biodistribution of NIR-labeled MSNP delivered *i.v.* in a MCF-7/MDR tumor xenograft model in nude mice. (A) An IVIS optical imaging system (Xenogen) was used to study the biodistribution of NIR dye labeled-MSNP in the tumor-bearing mice. To visualize the luciferase expression in the cancer cells, the anesthetized mice received D-Luciferin were used for bioluminescence images. Reference fluorescence images were captured before *i.v.* injection of 50 mg/kg NIR-labeled particles into the tumor-bearing mice. Pronate and supine images were obtained at 3 h, 24 h, and 72 h following the particle injection. (B) 72 h after injection, the animals were sacrificed and tumor tissues as well as major organs were collected for *ex vivo* imaging. (C) Measurement of the fluorescence intensities of individual organs from mice treated with NIR-labeled MSNP. The tissue for each organ was accurately weighted, homogenized and the fluorescence intensity obtained at excitation and emission wavelength of 680/715 nm in a microplate reader (SpectraMax M5e, Molecular Device, USA). The biodistribution of MSNP was expressed as % of total particle load distributing to the individual organs.

S6. Morphological observation of tumor xenografts after six *i.v.* injections at day 30.

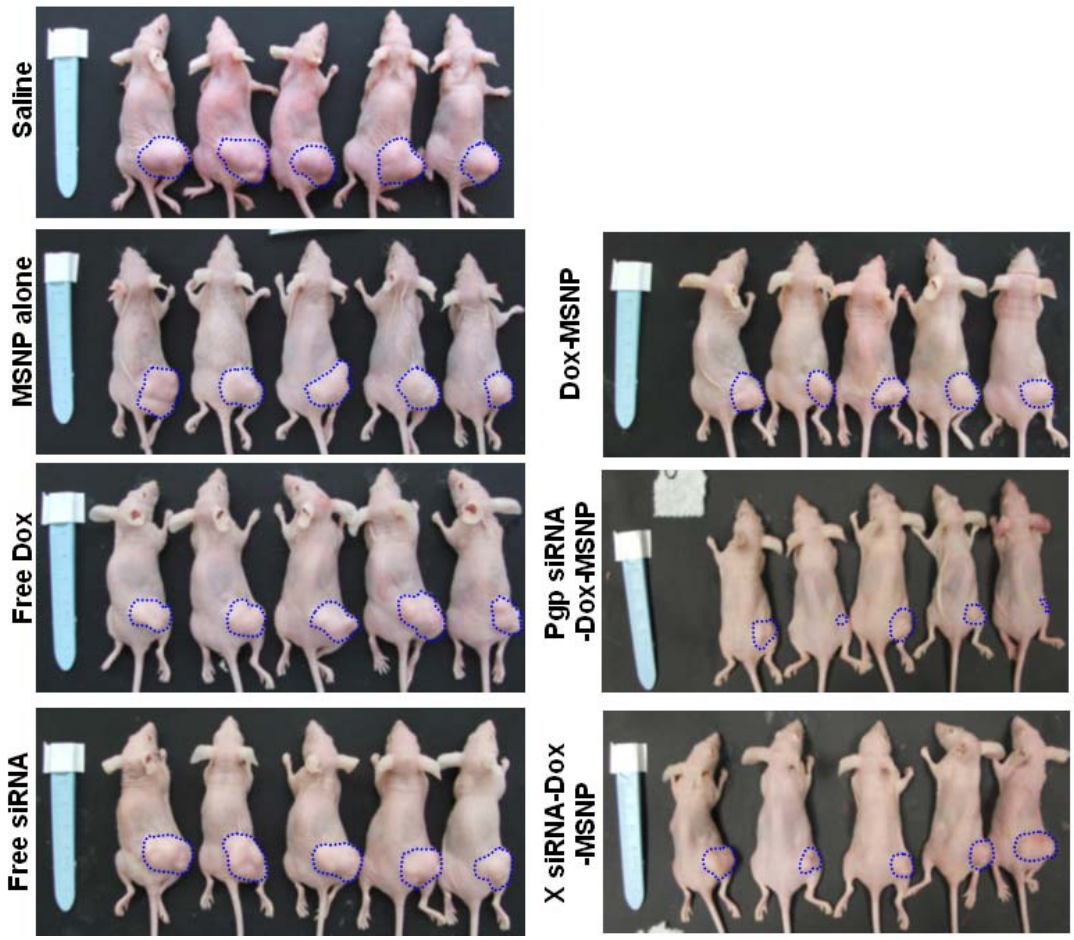


Fig. S6 Tumor-bearing animals were treated with saline, MSNP alone, free Dox, free siRNA, Dox-laden MSNP, Dox-laden MSNP with Pgp siRNA, and Dox-laden MSNP with surface attached scramble (X) siRNA as described in material and methods. The localization of the tumor is outlined by a broken line. No difference in tumor shrinking was observed in animals receiving Pgp siRNA-MSNP without Dox-co-delivery.

S7. Analysis of Pgp expression in saline-treated MCF-7/MDR tumor using IHC staining and western blotting.

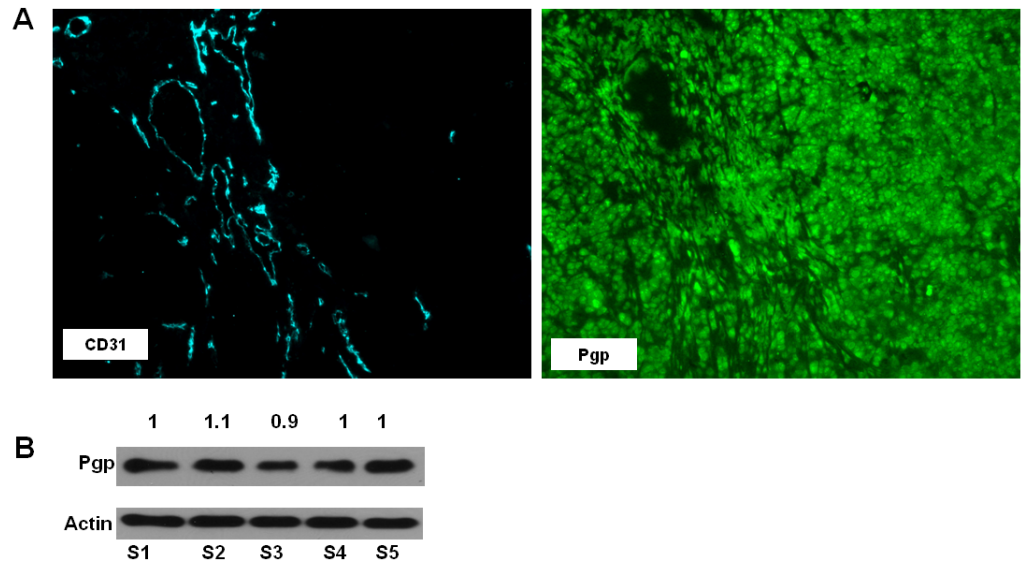


Fig. S7. Analysis of Pgp expression in tumor tissue of animal treated by saline only, using IHC staining and western blotting. **(A)** In order to visualize the Pgp expression in the MDR tumors, tissue sections were used for CD31 (cyan) and Pgp (FITC) staining. The experimental details were described in the supporting information S10. **(B)** The immunoblotting analysis using randomly selected multiple biopsies (labeled as S1→S5) in the same tumor xenograft demonstrated a stable and homogenous Pgp expression pattern.

S8 Serum biochemistry profiles duing safety analysis of our MSNP platform.

Treatment/ Parameters	Saline	MSNP alone	free Dox	Free siRNA	Dox-MSNP	Pgp siRNA-Dox-MSNP	X siRNA -Dox-MSNP
CHOL (mg/dL)	80.4±26.1	86.4±13.0	81.2±13.0	102.8±25.7	83.0±14.6	75.6±1.6	82.5±30.2
TRG (mg/dL)	63.2±11.6	73.0±4.6	60.2±15.5	67.4±20.9	62.8±5.2	84.8±9.5	57.8±25.1
ALT (u/L)	34.8±7.4	41.5±4.9	41.2±12.4	36.8±18.6	39.5±4.9	40.0±11.0	34.8±11.1
AST (u/L)	121.8±69.0	156.3±48.0	326.0±74.6*	137.0±57.0#	170.0±48.0	123.0±3.9#	156.3±68.7
TBIL (mg/dL)	0.2±0.1	0.3±0.1	0.3±0.1	0.2±0.1	0.3±0.2	0.3±0.1	0.2±0.1
GLU (mg/dL)	6.1±1.3	5.8±0.5	5.4±1.7	5.6±3.1	4.6±0.6	4.7±1.3	2.0±1.9
PHOS (mg/dL)	37.4±70.8	5.6±0.6	5.4±0.6	4.8±1.2	5.2±0.6	5.1±1.3	4.2±2.8
TPR (g/dL)	10.5±0.6	10.3±0.5	9.9±0.4	11.4±2.5	9.5±0.6	9.8±0.4	9.7±0.5
CAL (mg/dL)	34.2±6.5	38.4±5.0	31.6±3.4	24.2±11.1	31.2±5.3	38.0±0.2	39.0±11.5
BUN (mg/dL)	0.2±0.1	0.2±0.1	0.2±0.1	1.2±2.3	0.3±0.1	0.2±0.0	0.2±0.0
CRE (mg/dL)	2.6±0.1	2.7±0.1	2.7±0.2	4.4±3.6	2.8±0.1	3.0±0.0	2.8±0.5
ALB (g/dL)	80.4±26.1	86.4±13.0	81.2±13.0	102.8±25.7	83.0±14.6	75.6±1.6	82.5±30.2

Table S8 Serum biochemistry profiles. Blood was collected from the sacrificed animals and the serum obtained by centrifuging the whole blood at 5,000 rpm for 15 min. The biochemical parameters were assayed by UCLA Division of Laboratory Animal Medicine (DLAM) diagnostic laboratory services. These parameters include cholesterol (CHOL), triglycerides (TRG), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBILI), glucose (GLU), inorganic phosphorus (PHOS), total protein (TPR), calcium (CAL), blood urea nitrogen (BUN), creatinine (CRE), and albumin (ALB). *, $p<0.05$, compared to saline; #, $p<0.05$, compared to free Dox.

S9 Histological analysis to demonstrate lack of adverse tissue impact of the MSNP

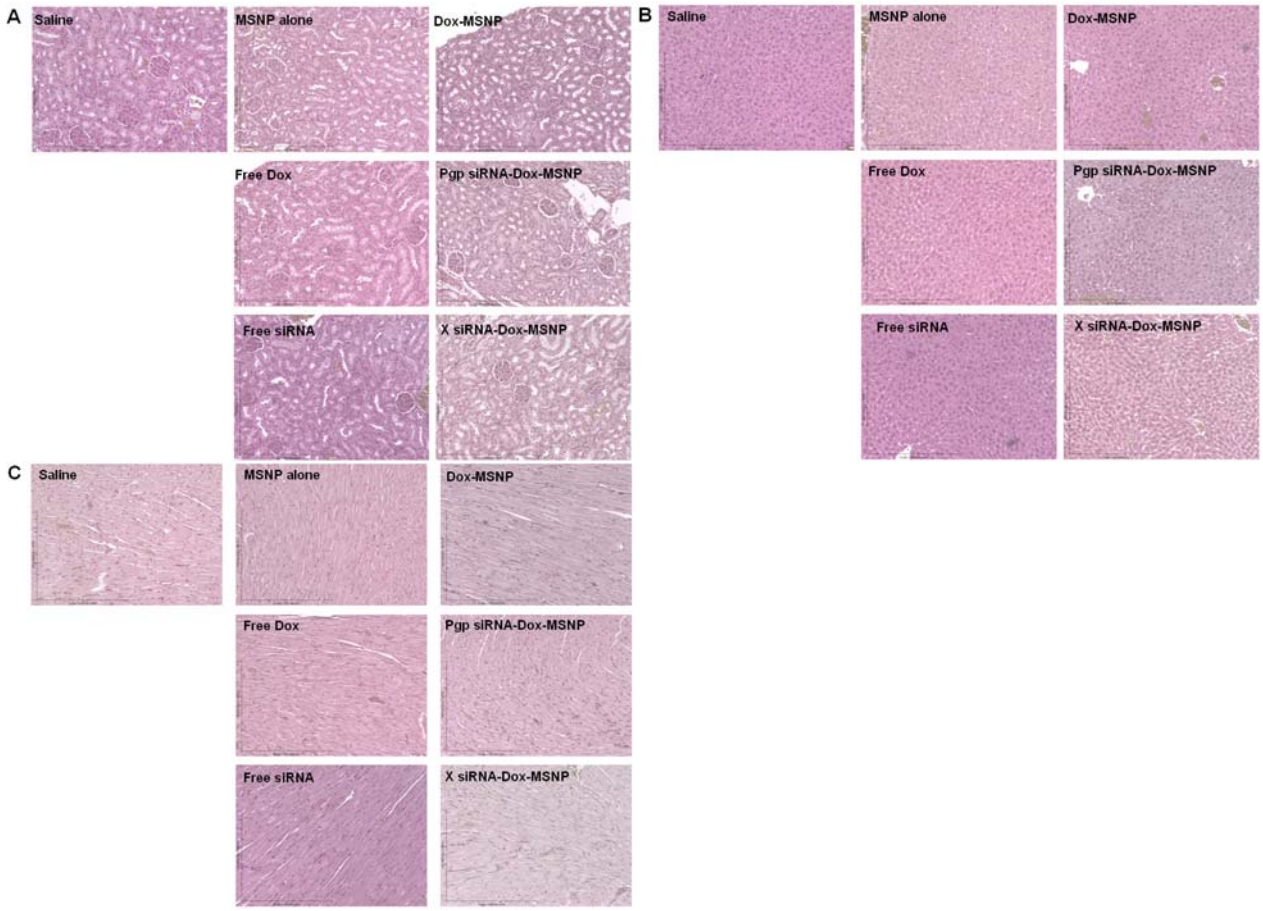


Fig. S9 Comparison of kidney (A), liver (B), and heart (C) histology in animals receiving various treatments in the tumor inhibition experiment. The animal experiment is described in material and methods. The animals were sacrificed 30 days after the first treatment. Histological analysis of these tissue sections were performed by the UCLA DLAM diagnostic laboratory services. The sections were stained with hematoxylin/eosin (H&E) and examined by light microscopy. Representative images are shown. No histological abnormalities were observed in any of the organs.

S10. Materials and methods

Materials

Cetyl trimethylammonium bromide (CTAB, 95%), Pluronic F127, tetraorthoethylsilicate (TEOS, 98%) 3-(trihydroxysilyl)propyl methylphosphonate (42% in H_2O), polyethyleneimine (PEI, 1.8 kD), 4-(dimethylamino)pyridine (99%), *N*,*N*'-disuccinimidyl carbonate (95%), poly(ethylene glycol) methyl ether (m-PEG, MW 5 kD), phthalic anhydride (99%) and polybrene were purchased from Sigma (St. Louis, MO). *N*-(2-aminoethyl)-3-aminopropyltrimethoxysilane (NAPTS) was purchased from Gelest (Morrisville, PA). Amine-reactive near infrared fluor DyLight 680 NHS ester was purchased from Thermo Scientific (Rockford, IL). D-Luciferin was purchased from Xenogen (Alameda, CA). Apoptosis TUNEL detection kit (Click-iT TUNEL kit), bovine serum albumin (BSA), DPBS solution, L-glutamine, penicillin, streptomycin, and DMEM medium were obtained from Invitrogen. Fetal bovine serum (FBS) was purchased from Atlanta Biologicals. The siRNAs used are either custom-synthesized (Pgp siRNA 5'-CGGAAGGCCUAAUGCCGAAtt-3'; Bcl-2 5'-GUGAAGUCAACAUGCCUGCTT-3') or purchased from the commercially available siRNA products (MRP1: sc-35962, ABCG2: sc-41151, c-Myc: sc-29226, and PXR: sc-44057) from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-CD31 antibody was purchased from BD Bioscience. Anti-Pgp antibody was purchased from Abcam (Cambridge, MA). All reagents were used without further purification.

Synthesis of MSNP

In order to synthesize 50 nm MSNP, 250 mg of CTAB and 200 mg of Pluronic F127 was mixed with 120 mL of H_2O and 875 μL of 2M NaOH aqueous solution. The solution was kept at 80 °C before 1 mL of TEOS was added. 20 min later, 300 μL of trihydroxysiylpropyl methylphosphonate was added. The solution was then stirred for 2 h and the particles were collected by centrifugation. The particles were then resuspended in a solution of 60 mL methanol with 60 mL of H_2O and mixed with 0.8 g of NH_4NO_3. After stirring at 50 °C for 30 min, the particles were centrifuged and washed with methanol. In order to perform copolymer modification, 100 mg of particles were suspended in 10 mL of 2.5 mg/mL PEI ethanolic solution and the solution was

stirred at room temperature for 30 min. The particles were collected by centrifugation and washed with ethanol. In order to perform biodistribution study, 20 mL of anhydrous DMF was used to resuspend part of PEI-treated particles, and 1 mg of DyLight 680 NHS ester was added into the solution. 500 mg of activated m-PEG was added and these solutions and stirred for another 12 h. The resulting particles were centrifuged and washed with DMF, methanol, and H_2O respectively.

Dox loading and release

10 mg of various particles were suspended in 0.5 mL of 5 mg/mL Dox aqueous suspension. The solution was stirred for 24 h and the nanoparticles were collected through centrifugation and carefully washed with H_2O. To measure the loading yields of the Dox-laden MSNP, 1 mg of doxorubicin-loaded MSNP was resuspended and sonicated in 1 mL heated 0.1 M HCl for 15 min. After centrifugation, another 1 mL of fresh HCl aqueous solution was added. This process was repeated at least for 5 times until the particles became white color. The pH of supernatant was readjusted to 7.0 by 0.1 M NaOH and the fluorescence spectrum of doxorubicin was measured at excitation and emission wavelength of 485/550 nm in a microplate reader (SpectraMax M5Microplate Reader, Molecular Device, USA).

To study the Dox release profile in various conditions, 1 mg MSNP containing Dox and siRNA was respectively resuspended in 3 mL of phenol-red free complete DMEM (pH 7.4), saline supplemented mouse serum (pH 7.4), artificial lysosomal fluid (pH 4.5), as well as acidified mouse serum containing saline (pH 4.5) and followed by an incubation for 96 h. 40 μL particle suspensions were collected and centrifuged. At this point the pH in the collected supernatant was readjusted to ~7.0 by 0.1 M NaOH and the fluorescence spectrum of Dox measured at excitation and emission wavelength of 485/550 nm in a microplate reader (SpectraMax M5e Microplate Reader, Molecular Device, USA).

Characterization of Dox and siRNA loaded MSNP

The drug and siRNA loaded MSNP were characterized for size distribution and shape as well as for charge in water, saline, and saline supplemented with 10% mouse serum. The shape and morphology were characterized using a transmission electron microscope (JEOL JEM 2010, JEOL USA, Inc., Peabody, MA). TEM samples were prepared by using a drop of MSNP suspension in saline at 100 μg/mL onto copper grids and then dried at room temperature overnight. After Dox loading and siRNA attachment, the particle size and zeta potential in solutions were measured by ZetaSizer Nano (Malvern Instruments Ltd., Worcestershire, UK). The measurements were conducted with MSNP suspended at 100 μg/mL particle concentration.

Luciferase transfection and establishment of MCF-7/MDR cells

MCF-7 cells were cultured in DMEM medium (Carlsbad, CA) containing 10% FBS, 100 U/mL penicillin, 100 μg/mL streptomycin, and 2 mM L-glutamine (CDMEM). To generate a stable cell line constitutively expressing luciferase, 1.5×10^4 MCF-7 cells immersed in 40 μL complete DMEM were transduced with 10 μL of a lentivirus (Cignal Finder Lenti Pathway Reporter Qiagen/SA Biosciences; 1.4×10^7 TU/mL) using 96 well tissue culture plates. Centrifugal inoculation was performed at 1,200 g for 60 minutes. The viral containing media was removed after 16 h and the cultures replenished with fresh DMEM media. Cells were allowed to proliferate to a population size of 1.2×10^6 cells. The transduced cells were selected by using 1 μg/mL puromycin for 5 days before induction of drug resistance. MCF-7/MDR cells were developed from the luciferase containing MCF-7 cells by continuous treatment of a combination of cancer drugs for approximately 9 weeks. Week 0-3: CDMEM containing doxorubicin at 1 μg/mL, daunorubicin at 1 μg/mL, and vinblastine at 1 μg/mL; Week 4-6: CDMEM containing doxorubicin at 2 μg/mL, daunorubicin 1 μg/mL, and vinblastine at 1 μg/mL; Week 7-9: CDMEM containing doxorubicin at 4 μg/mL, daunorubicin 1 μg/mL, and vinblastine at 1 μg/mL. The MCF-7/MDR cells were established from a single clonal selection by performing limiting dilution methods. The resulting MCF-7/MDR cells were maintained in 1 μg/mL of vinblastine in CDMEM.

Determination of the optimal drug/siRNA combination using a cellular HTS assay

MCF-7/MDR cells in 50 μL of fresh phenol red free complete DMEM (containing 10% FBS) were plated into 384-well plate followed by overnight growth at 37 °C in a humidified 5% CO_2 incubator. Dox-loaded MSNP, which was sonicated for 15 s before use, were mixed with various siRNA at N/P of 100. The resulting complexes were freshly dispersed in CDMEM. The medium in the 384-well plates was aspirated and 25 μL CDMEM containing free drug, empty MSNP, or MSNP with various siRNA(s) were added into cells. After incubation with the indicated dose of MSNP for various lengths of time at 37 °C, another 25 μL CDMEM containing Hoechst 33342 nuclear dye at 1 μM was added into each well. Each 384-well plate received additional 25 μL of Hoechst containing CDMEM was incubated for 30 min in the dark. No washing step is involved before HTS screening. Epifluorescence readings were obtained at indicated time point using an Image-Xpressmicro (Molecular Devices, Sunnyvale, CA) equipped with a laser autofocus. DAPI and TRITC filters were used to image nuclear (blue fluorescence) and intracellular Dox (red fluorescence). After imaging experiment, the same 384-well plate was used to determine cellular viability using MTS assay. The old medium was replenished with phenol red free CDMEM containing MTS reagent. Following 1 h incubation, the absorbance was measured at 490 nm. The mean absorbance of non-exposed cells served as the reference for calculating 100% cellular viability.

Establishment of the MCF-7/MDR tumor xenograft model

Athymic BALB/c nu/nu female mice (8 weeks) were purchased from the Charles River Laboratory, and maintained under pathogen-free conditions. All animal experiments were performed using protocols approved by the UCLA Animal Research Committee. The tumor cell suspension (0.1 mL, 8×10^6 cells/mL) was injected subcutaneously into the mice. To perform biodistribution study, the tumor-bearing animals were used 3 week after tumor implantation. In the tumor growth inhibition experiments, the nude mice received different treatments 1 week after tumor implantation.

Biodistribution studies

Three tumor-bearing mice were used to study the biodistribution of MSNP in tumor-bearing animals after i.v administration. To visualize the tumor, anesthetized mice were i.p. injected with 75 mg/kg D-Luciferin. Eight minutes after injection, bioluminescence images were acquired using an IVIS Imaging System (Xenogen). Acquisition time was 30 s. Subsequently, the mice were i.v. injected with NIR dye-MSNP at 50 mg/kg, and the fluorescence images were taken at 3, 24, and 72 h post injection. The tumor tissue together with major organs (lung, spleen, liver, kidney, brain and cardiac muscle) were collected and used for *ex vivo* imaging. Around 100-200 mg tissue for each organ was accurately weighed out, washed, homogenated, and the fluorescence intensities per unitary amount of each organ were measured by a microplate reader (Molecular Device, M5e).

Tumor inhibition experiment

MCF-7/MDR tumor-bearing mice were randomly divided into 7 groups, 5 animals per group. These groups were used for comparing the effects of saline, empty MSNP, free Dox, free siRNA, Dox-loaded MSNP, Dox-loaded MSNP together with Pgp siRNA, and Dox-loaded MSNP with scrambled siRNA, respectively. The Pgp siRNA-Dox-MSNP group received i.v. administration of MSNP at particle dose of 120 mg/kg (~2.4 mg per animal), which is equivalent to a Dox dose of 4 mg/kg (~0.08 mg per animal) and siRNA dose of 1.2 mg/kg (~0.024 mg per animal), for 6 times in a 30 days-period. The empty MSNP, free Dox, free siRNA groups received i.v. injections at same particle, drug and siRNA dose for 30 days. We also included a group using Dox-loaded MSNP with surface attached scramble siRNA to rule out any possible effects that may associate with gene knockdown. Saline was used as control. We also performed a separate experiment, for logistical reasons, in which the effect of Pgp siRNA-MSNP in the absence of doxorubicin was compared to the saline control and demonstrated no interference. The body weights and tumor sizes were accurately recorded. At the end of experiment, tumor tissues were harvested and precisely weighed for the calculation of tumor inhibition rates for each treatment.

Immunoblotting

To view Pgp expression, the cells or tumor tissue were washed in cold PBS and the samples were lysed using a lysis buffer containing protease inhibitors. The protein content of the supernatants was determined by the Bradford method. The protein samples were electrophoresed by 4-12% gradient SDS-PAGE (Invitrogen, Grand Island, NY) and transferred to a PVDF membrane. After blocking, the membranes were incubated with 1:1000 dilution of primary monoclonal antibody to Pgp. The membranes were overlayed with appropriate secondary antibody (1:1000 dilution) before the addition of the HRP-conjugated streptavidin-biotin complex. The proteins were detected using ECL reagent according to the manufacturer's instructions.

qPCR analysis

Pgp mRNA was analyzed by real-time PCR in tumor samples. First, tumor tissues were harvested, randomly spliced into 2-3 sections and separately placed in 1mL of TRIzol reagent (Invitrogen). RNA isolation was then proceeded using manufacturer's protocol. RNA isolation was then followed by RNA quantification, DNase treatment, and cDNA synthesis successively. The cDNA product was then added to a mixture of iQ SYBR Green Supermix (Bio-Rad Laboratories) along with Pgp forward and reverse primers (5'-AGG AAG CCA ATG CCT ATG ACT TTA-3' and 5'-CAA CTG GGC CCC TCT CTC TC-3' respectively). The PCR parameters consisted of a 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. The relative gene expressions were analyzed using Ct method and normalized using Beta-actin as an endogenous control.

Study of intratumoral Dox content and distribution and Pgp expression

Part of tumor tissues in saline, free Dox, Dox-MSNP, and Pgp siRNA-Dox-MSNP groups were frozen and OCT embedded before sectioning to provide 4 μm thick slices. The slices were washed three times in PBS, fixed in cold acetone for 15 min and the slide subsequently blocked using 1% normal goat serum at room temperature for 10 min. The sections were respectively overlayed with rat-anti-mouse CD31 monoclonal antibody (1:500) at 4 °C overnight. After removal of the primary antibody and washing in PBS for 3 times,

corresponding fluorescent labeled second antibody (1:500) was added and incubated for 1 h at room temperature. The slides were visualized under a fluorescence microscope (Zeiss, Germany). In order to visualize Dox, Pgp, and CD31 at the same time in Pgp siRNA-Dox-MSNP treated tumor, two closely adjacent tumor sections were cut. The 1st section was used for CD31 IHC staining. The 2rd section was used for Pgp IHC staining and Dox analysis (section 2). This arrangement allowed us to compare the abundance of the drug fluorescence as well as Pgp expression *in situ* in relation to tumor blood vessel at the same place.

Blood biochemistry and tumor and major organ histology

Following the animal experiments described above, the mice were sacrificed and serum, appropriate size of tumor, liver, kidney, spleen, lung, heart, and brain were collected for blood biochemistry analysis and histology examination performed by DLAM at UCLA.

cTnT assay

The mouse serum samples were also used for cTnT assay using a commercially available ELISA kit according to the manufacturer's instruction (MyBioSource, San Diego, CA). Briefly, 50 μL of each serum sample was diluted with 50 μL of 1X PBS for a total volume of 100 μL. The resulting 100 μL samples were then placed in the pre-coated 96 well plate. In addition, 100 μL of the provided standards were added to the plate in duplicates. The plate was left to incubate at 37 °C for 60 minutes and then was washed five times. 50 μL of substrate A and 50 μL of substrate B in the kit was then added to each well and incubated in dark for 15 minutes. The stop solution was added and the final product was then placed in microplate reader at 450 nm for optical density measurement.

S11. Table S11: Summary of *in vivo* publications using dual drug plus siRNA delivery to overcome MDR in cancer. We attempted to provide a comparison of the different types of nanocarriers, administration routes, tumor model, and major findings. We also highlight the significant differences between published data and our study **red**. Through such a comparison, we believe that our research is novel.

Literature data				
Nanocarrier type/Cargoes	**Administration route**	**Animal tumor model**	**Major findings**	**Reference**
Poly(D,L-lactide-co-glycolide) nanoparticles for codelivery of paclitaxel and P-gp targeted siRNA	i.v. administration	JC **mammary MDR murine cancer** xenograft	1. Dual agent nanoparticles results in enhanced anticancer effectiveness *in vivo*. 2. Enhanced paclitaxel accumulation in the presence of Pgp siRNA in tumor. **3. No attempt was made to demonstrate the distribution of *in vivo* Pgp knockdown**.	Patil et al., Biomaterials 31 (2010) 358–365
Mesoporous silica nanoparticles for simultaneous delivery of **doxorubicin, cisplatin, MRP1 siRNA and BCL2 siRNA**	**Lung inhalation study**	non-small cell **orthotopic lung carcinoma** model	1. Delivery of siRNAs plus drugs enhanced the cytotoxicity of anticancer drugs in tumor bearing mice. 2. *In vivo* gene knockdown was achieved **through inhalation of nanoparticles** **3. No demonstration of the distribution of *in vivo* gene knockdown**.	Taratula O, et al., J Drug Target. 2011 Dec;19(10):900-14. Epub 2011 Oct 10.
Cationic liposome-polycation-DNA and anionic **liposome**-polycation-DNA for **cMyc siRNA** and Doxorubicin delivery	i.v. administration	NCI/ADR-RES **multidrug-resistant ovarian cancer** xenograft	1. cMyc siRNA and Dox co-formulated in liposome nanoparticles showed a significant improvement in tumor growth inhibition and induction of apoptosis in NCI/ADR-RES xenograft tumor. **3. No demonstration of the distribution of *in vivo* gene knockdown**.	Chen Y., et al., J Biol Chem. 2010 July 16; 285(29): 22639–22650.
MDR1b, MDR1a, Bcl-2 siRNA-**lipofectamine 2000** complex followed by cyclophosphamide treatment	i.p. administration	RLS40 MDR **Ascites lymphosarcoma** -bearing CBA mice	1. MDR1b/1a siRNA followed by treatment with cyclophosphamide results in 3 folds decrease in tumor size compared to drug alone. 2. Silencing of mdr1b and mdr1a **genes in lymphosarcoma in ascites** **3. No demonstration of the distribution of *in vivo* gene knockdown effect**.	Patutina, et al., BMC Cancer 2010, 10:204
Novelty of the findings in our study				
PEI-PEG copolymer coated size controlled (50nm), monodispersed mesoporous silica nanoparticles	i.v administration	MCF-7/MDR human breast cancer xenograft model in nude mice	1. Multifunctional MSNP carrier for Dox/Pgp siRNA delivery 2. Comparison with other drug-resistant siRNAs using HTS to rationally select the most appropriate target, which happened to be Pgp 3. Synergistic tumor inhibition at sites that are assessable to the dual delivery carrier 4. The heterogeneity of gene knockdown due to heterogeneity in the tumor environment has never been reported	n/a