Supplementary information

Detailed methods and materials

1, synthesis of DSPE-PEG5000- 4-Arm-(PEG-Amine)

One molar equivalent (eq.) DSPE-PEG5000-Amine (SUNBRIGHT® DSPE-050PA, NOF cooperation) was reacted with 5 eq. succinic anhydride in dichloromethylene (CH_2Cl_2, Aldrich) overnight at room temperature. After evaporating the solvent, the product was dissolved in water. The solution was dialyzed against water with a 3.5 kDa molecular weight cut off (MWCO) membrane for 2 days and then lyophilized into powder. The resulting DSPE-PEG5000-COOH was activated by 1.5 eq. dicyclohexylcarbodiimide (DCC, Aldrich) and 2 eq. hydroxybenzotriazole (HOBt, Aldrich) in CH_2Cl_2 at for 1 hour. 4 eq. 4-Arm-(PEG-Amine) (10kDa, P4AM-10, Sunbio) was added and the reaction solution was stirred for 2 days. After evaporating the solvent, water was added into the container and stirred for 1 hour. Solid precipitate (leftover DCC and HOBt) was removed by filtration via a 0.22 μm filter, yielding clear water solution of DSPE-PEG5000-4-Arm-(PEG-Amine) (see Fig.1a). The product was confirmed by MALDI (matrix-assisted laser desorption/ionization) mass spectrometry in Stanford PAN facility, showing no existence of starting DSPE-PEG5000 material. Excess 4-Arm-(PEG-Amine) was removed by dialysis with a 14 kDa MWCO membrane in the synthesis of DSPE-PEG-PTX.

2, Tumor slice staining

Tumors were placed into Optimal Cutting Temperature (OCT) medium immediately after being taken out from the mice, frozen by dry ice and stored at -80^{o}C. 5 μm thick tumor slices were cut by a Heidelburg microtome. The slices were stored at -80^{o}C until use.

TUNEL staining. Frozen tumor tissue slices from treated mice in storage were taken out from freezer and warmed for 20min at room temperature, then fluorescent TUNEL staining were conducted following manual instruction of In Situ Cell Death Detection kit (Roche, Indianapolis, IN).

Fluorescent staining of Ki67. Frozen tissue slices from treated mice in storage were fixed with ice-cold acetone for 10 min and then dried for 30 min at room temperature, After 3x 5min rinse with PBS, slides were blocked with 10% goat serum in PBS for 15 min at room temperature. The slices were then incubated with rabbit anti-mouse Ki67 antibody (Abcam, Cambridge, MA) for 1 h at room temperature. After 3x 5 min washing with PBS, slides were incubated with Cy3-conjugated goat anti-rabbit secondary antibody (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) for 1 hour at room temperature. After staining, slides were mounted with VECTASHIELD mounting medium (Vector Laboratories, Burlingame, CA).

CD31 Staining. The tumor tissue slices were obtained from tumor bearing mice injected with free AF488 dye and AF488 labeled SWNT (SWNT-AF488) solutions at 4h p.i. Frozen tumor tissue slices were taken out from freezer and warmed for 20min at room temperature. Slides were blocked with 10% goat serum in PBS for 15 min at

room temperature and then incubated with rat anti-mouse CD31 antibody (BD bioscience, San Jose, CA) for 1 hour at room temperature. After 3x 5 min washing with PBS, slides were incubated with FITC-conjugated goat anti-rat secondary antibody (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA). After staining, slides were mounted with VECTASHIELD mounting medium (Vector Laboratories, Burlingame, CA).

3, Fluorescence imaging of tumor slices

PEGylated SWNTs were labeled by NHS-Alexa Fluor 488 (Invitrogen) at pH 7.5 for 4 h. Excess dye molecules were removed by filtration. SWNT-AF488 and free AF488 with the same fluorescence intensity normalized by a fluorimeter were injected into 4T1 tumor bearing mice, which were sacrificed at 4 h p.i. Tumor slices were stained with Cy3-anti CD31 antibody to visualize the vasculature and imaged by a Zeiss LSM 510 confocal microscope.

4, Raman spectroscopy measurement

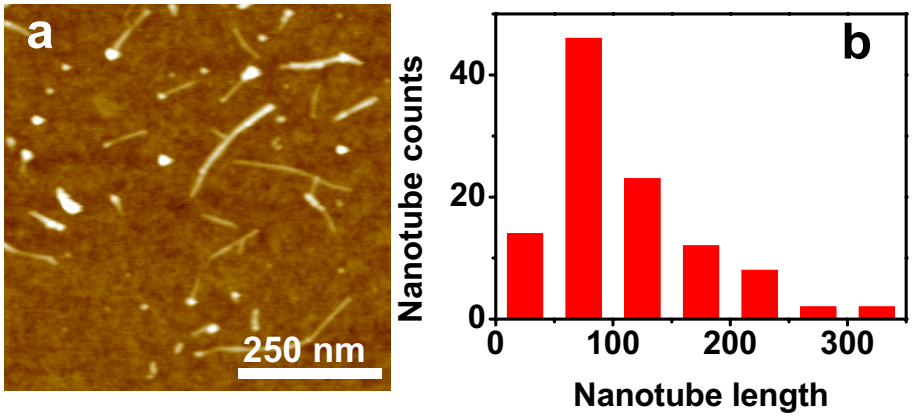
A glass capillary tube filled with tissue lysate solution was placed under the objective (20×) of the Raman microscope. As low as 2 μL of solution sample was needed for each measurement. After focusing at the center of the capillary, we recorded the Raman spectrum of the solution (100 mW power with laser spot size of ~ 25 μm^2, 10 second collection time). At least 4 spectra were taken for each sample for averaging. The Raman intensity was obtained by integrating the SWNT G-band peak

area from 1570 cm^{-1} to 1620 cm^{-1} and averaged over several spectra. SWNT concentration in blood samples or tissue lysate was determined by comparing the raman intensity with a standard calibration curve obtained for SWNTs in lysis buffer with various known concentrations. The linear dependence between Raman intensity of a SWNT solution and concentration allows for accurate measurement of nanotube concentration in aqueous phase.(18, 22) The concentrations of injected SWNT or SWNT-PTX solutions were also determined by the Raman method prior to injections into mice.

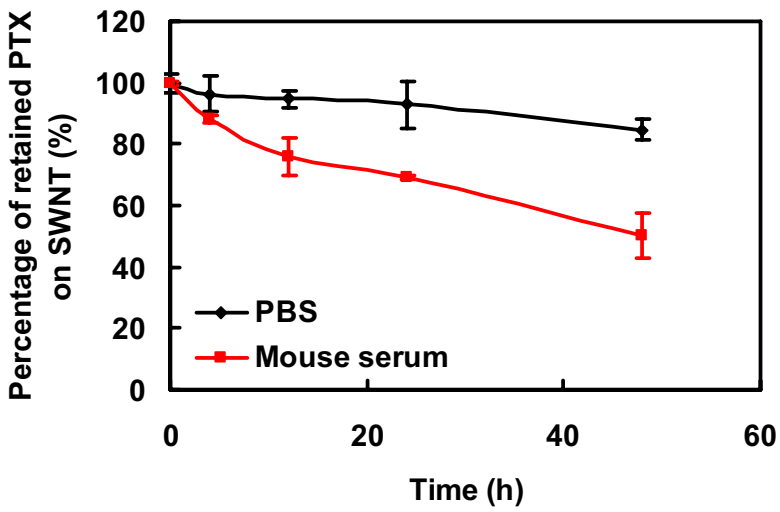
5, Necropsy, blood chemistry and histology study

24 days after initiation of treatment, 3 mice from each treatment group (SWNT-PTX and Taxol®) and 2 age-matched female Balb/c control mice were sacrificed by CO_2 asphyxiation. Blood was collected via cardiac puncture at time of sacrifice for analysis of serum chemistries by the Diagnostic Laboratory, Veterinary Service Center, Department of Comparative Medicine, Stanford University School of Medicine. Serum chemistries were run on an Express Plus Chemistry Analyzer (Chiron Diagnostics) and electrolytes were measured on a 644 Na/K/Cl Analyzer (CIBA-Corning). A full necropsy was performed and all internal organs were harvested, fixed in 10% neutral buffered formalin, processed routinely into paraffin, sectioned at 4 microns, stained with hematoxylin & eosin (H&E) and examined by light microscopy. Examined tissues included: liver, kidneys, spleen, heart, salivary gland, lung, trachea, esophagus, thymus, reproductive tract, urinary bladder, eyes,

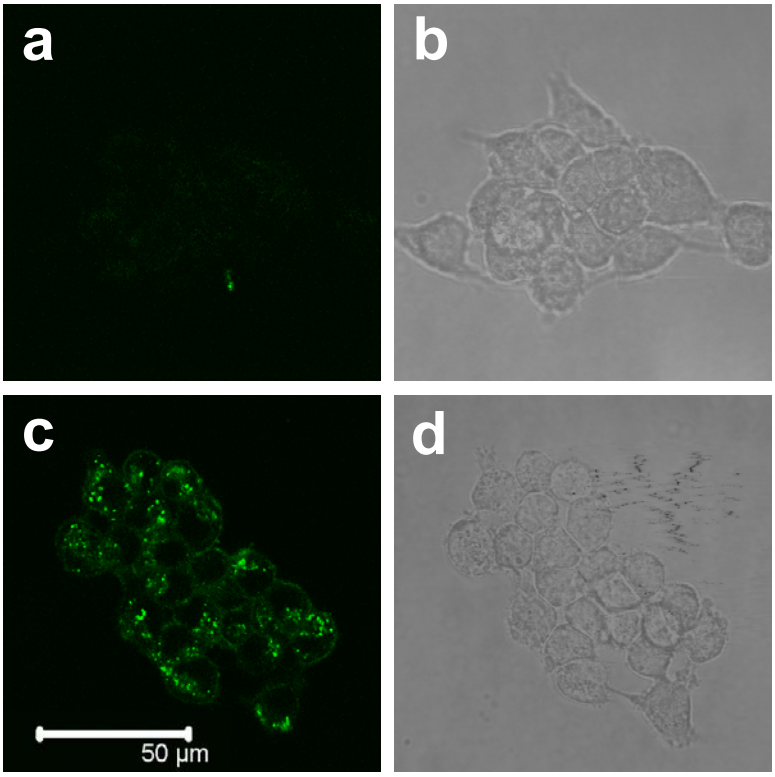
lymph nodes, brain, thyroid gland, adrenal gland, gastrointestinal tract, pancreas, bone marrow, skeletal muscle, nasal cavities, middle ear, vertebrae, spinal cord and peripheral nerves.



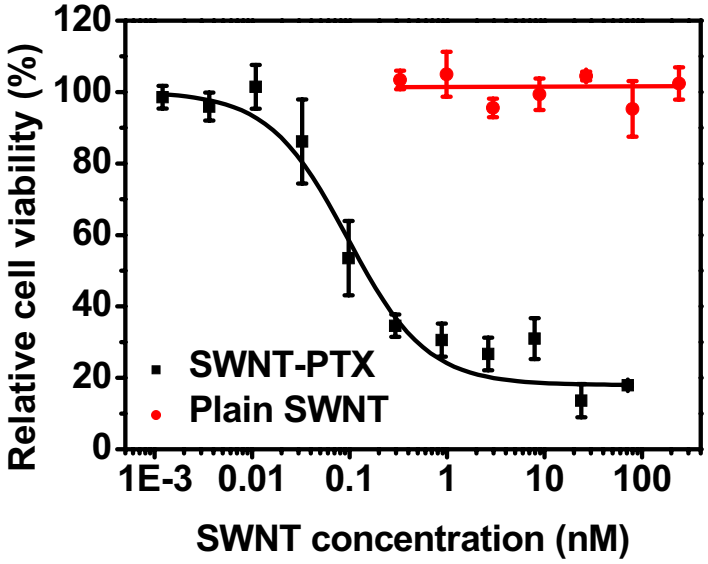
Supplementary Figure S1. Characterization of SWNT-PTX material by atomic force microscope (AFM). **a,** an representative AFM image of PEGylated SWNTs conjugated with PTX. The image was obtained after depositing SWNT-PTX from solution onto a SiO2 substrate. **b,** Nanotube length distribution histogram of SWNT-PTX measured by AFM images. Over 100 nanotubes were measured for the statistics. The average length of SWNT-PTX was 106 nm with a standard deviation of 64 nm.



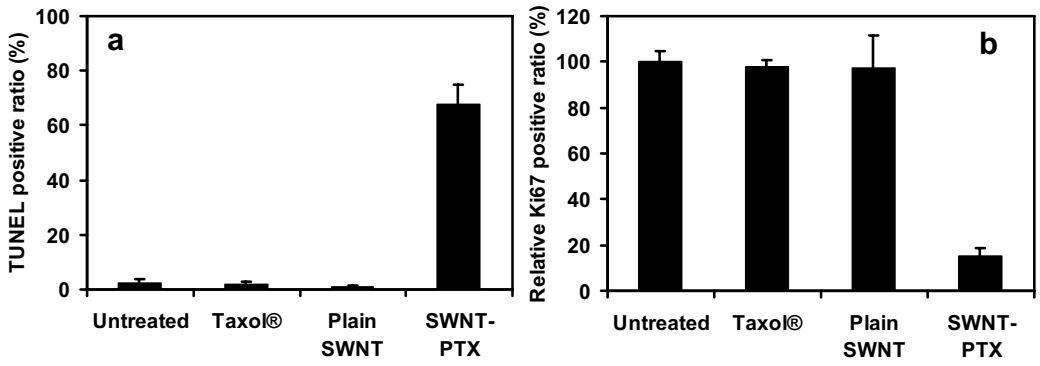
Supplementary Figure S2. PTX releasing curves for SWNT-PTX in phosphate buffered saline (PBS) and balb/c mouse serum (Innovative Research) respectively. SWNT-^{3}H-PTX was incubated in PBS or serum at 37^{o}C over 48 hours. Released PTX was removed by filtration through 100kDa MWCO filters at different time points (4h, 12h, 24h, 48h). The released and retained ^{3}H-PTX were measured by scintillation counting. SWNT-PTX exhibited high stability in the physiological buffer and increased releasing rate in the mouse serum, which is likely due to the carboxylase in the serum.



Supplementary Figure S3. Confocal fluorescence images of 4T1 cells incubated with ~5nM of fluorescein labeled SWNT-PTX at 4°C (a) and 37°C (c) for 2 hours. (b) and (d) are white field images. SWNT-PTX uptake was observed at 37°C but not 4°C, suggested that nanotubes enter cells by energy dependent endocytosis mechanism. The bright dots inside cells (c) indicated the accumulation of nanotubes in cell endosomes. To make the labeled nanotube conjugate, 0.1 mM of NHS-fluorecein (perice) was added during the PTX conjugation reaction. Excess labels and PTX were removed by repeated filtration and washing. Cells were washed by PBS after incubation and imaged under a Leica SP2 AOBS Confocal Laser Scanning Microscope.



Supplementary Figure S4. In vitro toxicity tests of SWNT-PTX and plain SWNT. 4T1 cells were incubated with SWNT-PTX or plain SWNT at series of concentrations over 72 hours. Cell viability was tested by MTS assay using a Celltiter 96 kit (Promega). Note that the absorbance contribution from SWNT at high concentrations was subtracted from the absorbance values measured by the microplate reader. While SWNT-PTX showed very strong cell killing ability at extremely low SWNT concentration (~150 PTX per SWNT), plain SWNT had no noticeable effect to the cell viability and cell growth even at very high concentrations.



Supplementary Figure S5. Quantitative data of tumor staining experiments in Fig. 3. **a,** percentage of TUNEL positive cells in the untreated, Taxol® treated, plain SWNT treated and SWNT-PTX treated tumors. The ratio was calculated by the number of TUNEL positive cells vs. the total number of cells counted by DAPI (nucleus) staining in each fluorescent microscope field. **b,** relative Ki67 positive cell ratio in Taxol®, plain SWNT and SWNT-PTX treated tumors compared with untreated tumor. The numbers of Ki67 positive cells were counted in fluorescent microscope fields with the same size and magnification. The error bars were based on 6 microscope fields for each tumor slice.

	Taxol®	PEG-PTX	SWNT-PTX
Volume of distribution (L / kg)	2.02 ± 0.49	0.55 ± 0.05	0.44 ± 0.02
$AUC_{0-\infty}$ (mg · min / L)	67.6 ± 11.8	298.8 ± 25.9	1328 ± 47
Circulation half-life (min)	18.8 ± 1.5	22.8 ± 1.0	81.4 ± 7.4

Supplementary table S1. Pharmacokinetic data of three PTX formulations. One compartment first order decay fitting is applied in the data analysis. The decreased volumes of distribution in the PEG-PTX and SWNT-PTX cases were consistent to the increased hydrophilicity (to reduce rapid non-specific binding/uptake of hydrophobic PTX by tissues) of these two formulations. In the mean time, SWNT-PTX showed significantly increased circulation half-life and AUC compared with Taxol® and PEG-PTX.

Tumor vs		Heart	Lung	Liver	Kidney	Spleen	Stomach	Intestine	Muscle
Taxol®	**Ave.**	**0.36**	**0.13**	**0.07**	**0.05**	**0.27**	**0.22**	**0.26**	**0.73**
2h p.i.	Std.	0.12	0.03	0.02	0.02	0.10	0.09	0.18	0.16
PEG-PTX	**Ave.**	**0.70**	**0.10**	**0.35**	**0.21**	**0.88**	**0.76**	**0.93**	**1.64**
2h p.i.	Std.	0.12	0.04	0.13	0.05	0.20	0.16	0.03	0.55
SWNT-PTX	**Ave.**	**1.22**	**0.95**	**0.46**	**0.69**	**0.46**	**1.12**	**0.38**	**5.54**
2h p.i.	Std.	0.18	0.07	0.15	0.06	0.12	0.04	0.05	2.09
Taxol®	**Ave.**	**0.18**	**0.20**	**0.07**	**0.14**	**0.17**	**0.09**	**0.73**	**1.16**
24h p.i.	Std.	0.08	0.01	0.01	0.07	0.10	0.04	0.22	0.35
PEG-PTX	**Ave.**	**0.28**	**0.20**	**0.18**	**0.33**	**0.19**	**0.46**	**0.45**	**3.41**
24h p.i.	Std.	0.13	0.08	0.11	0.15	0.13	0.11	0.31	0.28
SWNT-PTX	**Ave.**	**0.60**	**0.89**	**0.44**	**0.73**	**0.24**	**1.05**	**0.85**	**8.27**
24h p.i.	Std.	0.11	0.20	0.22	0.22	0.06	0.06	0.15	2.83

Supplementary Table S2. Tumor to normal organ PTX uptake ratios (T/N ratios). T/N ratio is the essential criteria to determine the efficiency of a drug delivery carrier. The T/N ratios of SWNT-PTX are higher than Taxol® in all cases and PEG-PTX expects tumor vs spleen and intestine at 2h p.i.

Chemistry Panel	Unit	Control		Taxol®		SWNT-PTX	
		Ave.	Std.	Ave.	Std.	Ave.	Std.
Glucose	mg/dL	187	12.7	177	45.6	183.7	21.0
AST	IU/L	134.5	7.8	123.6	59.4	181.0	27.9
ALT	IU/L	48.9	15.4	45.7	16.5	96.3	26.8
Alkaline Phosphatase	IU/L	126	14.1	97	18.2	150. 7	30.6
GGT	IU/L	12	2.8	4.3	4.5	18.3	17.0
Cholesterol	mg/dL	80	11.3	85	9.2	96	13.8
BUN	mg/dL	17.4	3. 7	17.6	3.1	17.1	2.2
Calcium	mg/dL	10.6	0.3	11.3	0.5	10	1.2
Phosphorus	mg/dL	7.9	0.1	7.5	1.7	7	2.9
Ca/P ratio	mg/dL	1.4	0. 1	1.6	0.4	1.8	1.2
Total Protein	mg/dL	4.6	0. 3	4.8	0.1	6.8	1
Albumin	mg/dL	2.9	0.4	3.2	0.2	3.4	0.5
Globulin	mg/dL	1.7	0.1	1.7	0.1	2.3	0.6

Supplementary Table S3. Serum chemistry data. Healthy Balb/c mice were treated with Taxol® and SWNT-PTX at the PTX dose of 5 mg/kg every 6 days. The mice will euthanized at 24^{th} day with blood collected for blood chemistry test. All the other enzyme, protein and metal ion levels for Taxol® and SWNT-PTX treated mice are in the normal fluctuation range. Although some blood chemistry levels in the SWNT-PTX treated mice are different from the control, the difference cannot be considered as physiologically significant. The standard deviation of SWNT treated mice were based on 3 mice per group.