ADVANCED MATERIALS

Supporting Information

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Nanoparticle-Laden Macrophages for Tumor-Tropic Drug Delivery

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Methods

Chemicals. Tetraethyl orthosilicate (TEOS, ≥99.0%, Sigma-Aldrich), ammonium hydroxide (28.0-30.0%, Sigma-Aldrich), ethanol (200 proof, Decon Labs, Inc.), doxorubicin hydrochloride salt (Dox·HCl, LC Labs), Doxoves® - stealth liposomal Dox·HCl (2.0 mg mL^{-1}, FormuMax), hexadecyltrimethylammonium bromide (CTAB, ≥99%, Sigma-Aldrich), ethyl acetate (Fisher Scientific, HPLC Grade), HCl (J.T. Baker, 36.5-38%).

DSN synthesis. Dox-encapsulated silica nanocomplex (DSN) was synthesized by a modified procedure based on a previous study[1]. Different volumes of 4.2-4.5% ammonium hydroxide aqueous solutions (e.g., 0.15 mL, 0.3 mL, 0.6 mL) and TEOS (e.g., 2.5, 5, and 10 μL) were added into ethanol to form a 4.0 mL Dox solution of varied concentrations (e.g., 0.10, 0.25, and 0.50 mg mL^{-1}). The mixture was magnetically stirred at room temperature for 24 hours. The drug-loaded NPs were collected by repeated wash with ethanol and centrifugation (12,000 rpm, 5 min). The as-synthesized particles were lyophilized and stored at -80 ˚C in the dark. Specifically, DSN-0 NP was synthesized using 0.3 mL ammonium hydroxide, 5 μL TEOS, and 0.25 mg Dox mL^{-1}. For silica coating onto DSN-0, the as-synthesized DSN-0 was

re-dispersed in 4.0 mL 200 proof ethanol with brief sonication. Then, 0.3 mL ammonium hydroxide solution (4.2-4.5%) and different volumes of TEOS (e.g., 2.5, 5.0, and 10.0 μL) were dropwisely added to the colloidal solution. The mixture was magnetically stirred at room temperature for 24 hours. The coated DSN nanoparticles were washed, collected, lyophilized, and stored at -80 ^{o}C following the same protocol. According to the coating thicknesses measured by TEM, the coated DSNs were designated as DSN-12, DSN-22, and DSN-52, respectively.

Mesoporous silica nanoparticle synthesis. Mesoporous silica nanoparticles were synthesized following a published protocol[2]. Briefly, nanoparticles were prepared by mixing 3 mL TEOS with CTAB (5.5 mM) in a 300 mL, 70 ^{o}C aqueous solution containing 4.2 mmol NaOH, followed with the addition of 18 mL ethyl acetate. Free CTAB was removed by stirring nanoparticles in 100 mL ethanol containing 1 mL 37% HCl at 60 ^{o}C for 3 hours. The as-synthesized nanoparticles were dried at 60 ^{o}C overnight. To load Dox, mesoporous silica nanoparticles were stirred in a Dox ethanol solution (2.5 mg mL^{-1}) overnight at room temperature in the dark. The resulting Dox-encapsulated NPs were washed with water twice, lyophilized, and stored at -80 ˚C.

Characterization of nanoparticles. The morphology, size distributions, zeta potential, and EDS of nanoparticles were characterized by transmission electron microscope (TEM, H-9500), scanning electron microscope (SEM, FEI Teneo), and dynamic light scattering (DLS, Malvern Zetasizer Nano S90). The temporal degradation of nanoparticles at pH 5.0 and 7.4 was examined using TEM. Briefly, nanoparticles dispersed in PBS (pH 5.0 and 7.4) were incubated at 37 ^{o}C under constant shaking for 2, 6, 24, and 72 h. The remaining nanoparticles

were collected and examined under TEM for morphology and size changes. Absorbance at 470, 480, 490 nm was used to quantify Dox content. The loading capacity (%LC) was calculated by the following equation: %LC = (Drug loaded)/(nanoparticle weight) × 100%, where the amount of drug loaded was determined by absorbance, and the nanoparticle weight determined either by directly weighing lyophilized nanoparticles or calculating the silica weight based on inductively coupled plasma-optical emission spectrometry (ICP-OES) results. In the latter case, it was assumed that silica dioxide (SiO_2) was the major silica component and that nanoparticle weight = Dox weight + SiO_2 weight. Drug release of different nanoparticle formulations was determined using a Slide-A-Lyzer 10K MWCO mini dialysis device (Thermo Scientific). Briefly, nanoparticles containing the same Dox content were dispersed in 0.5 mL PBS and dialyzed against 14 mL PBS (pH 5.0 and 7.4) at 37 ^{o}C under constant shaking. At different time points (i.e., 0, 0.5, 1, 2, 4, 8, 12. 24, 48, 72, 96, 120 hour), a 0.5 mL PBS exmple from the bottom chamber was collected, which was supplanted with 0.5 mL fresh PBS. Cumulative Dox release over 5 days was quantified by subtracting the remaining Dox in the cassette from the initial loading amount. Dox concentration in the sample solutions were measured by fluorescence spectroscopy analysis (ex/em: 470/590 nm).

***In vitro* cellular loading studies.** RAW264.7 (murine macrophages) and U87MG (human glioblastoma) were purchased from ATCC. RAW264.7 cells were cultured in RPMI1640 medium (Corning, USA) supplemented with 10% FBS (Corning, USA) and 1% penicillin-streptomycin (MediaTech, USA). During the nanoparticle loading stage, FBS-free RPMI1640 medium was used for culturing. U87MG cells were grown in DMEM medium (Corning, USA) supplemented with 10% FBS, 1% non-essential amino acids, and 1% penicillin-streptomycin. These two cell lines were incubated under 37 ^{o}C and 5% CO_2 in a humid chamber. For nanoparticle loading studies, nanoparticles (DSN-22 or DSN-52) of different concentrations

(i.e., 0, 10, 20, and 40 μg Dox mL^{-1}) were incubated with RAW264.7 cells for 1, 2, and 4 h, followed with gentle wash with PBS or complete medium. Depending on the purpose of each study, the DSN-laden macrophages were either collected using trypsin treatment or cultured further with complete growth medium. To determine the amount of Dox loaded into cells, DSN-laden cells were counted and then lysed by sonication in PBS (pH 5.0). The amounts of released Dox was measured by spectroscopic analysis. The Dox content on a per cell basis was calculated compared with macrophages without nanoparticle loading. To study whether DSN-52 nanoparticles were laden into cells via internalization, nanoparticles were incubated with RAW264.7 cells under the same condition described above except using 4℃ for incubation and adding 0.1 wt.% NaN_3 (to minimize energy-consuming internalization process). The Dox loading amount on a per cell basis was compared with control. To evaluate the impact of loaded DSNs on macrophages, the viability change was first assessed at different time points post nanoparticle loading by 3-(4,5-dimethythiazon-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay or Live/Dead Cytotoxicity assay and the results were compared with normal macrophages. In the end, DSN-52 NPs were selected for cell loading, and the loading process was accomplished by 2-h incubation at a concentration of 20 μg Dox mL^{-1}. All *in vitro* experiments were repeated at least twice.

Cell invasion/migration assay. Cell invasion/migration assay was used to examine whether DSN-laden RAW264.7 (DSN-MF) cells remained tumor-tropic. Unladen RAW264.7 (MF) cells served as controls. A transwell polycarbonate membrane cell culture insert set (Corning, 8.0 μm pore sized) was fitted into a 6-well cell culture plate for this study. Migration assay required coating the upper surface of the inserts with a layer of Matrigel beforehand. For experimental groups, U87MG cells (0.2 million cells) as a lure were seeded to the bottom of each well and cultured overnight. For the control group, only medium was added into each

bottom well. Then, 0.4 million DSN-MF cells or normal RAW264.7 cells (dispersed in 1.0 mL FBS-free RPMI1640 medium) were seeded onto the upper chamber of each insert. The transmigration process took 16 hours to accomplish under normal incubation conditions (37 ^{o}C, 5% CO_2). Afterwards, the transwell inserts were collected, washed twice with PBS, fixed with formaldehyde (3.7% in PBS) for 2 min, washed twice with PBS again, permeabilized with methanol for 20 min, and subjected to Giemsa staining. Those cells that failed to transmigrate (i.e., remained on top of the film) were scraped off with cotton swabs. Optical and fluorescence images of the transmigrated RAW264.7 cells were captured. Due to Giemsa staining, invaded/migrated cells were blue in bright-field images. In fluorescence images, DSN-MF cells were visualized due to the intrinsic fluorescent properties of Dox. For each sample, 25 images of different areas were acquired for cell counting to obtain a statistically significant result. The experiment was repeated twice.

#	Upper chamber	Assay Type	Lower chamber
1	MF	Invasion	U87MG cells
2			Empty
3		Migration (+ Matrigel)	U87MG cells
4			Empty
5	DSN-MF	Invasion	U87MG cells
6			Empty
7		Migration (+ Matrigel)	U87MG cells
8			Empty

Cell phenotype change study. Enzyme-linked immunosorbent assay (ELISA) were used to examine the cytokine released by macrophages after they were loaded with DSN-52 nanoparticles. Briefly, the medium supernatants from DSN-MF seeded plates were collected at different time points (i.e., 2 and 24 h post loading) to quantify the concentrations of different cytokines, including interleukin-1beta (IL-1β), IL-6, IL-10, IL-12p70, and tumor necrosis factor-α (TNF-α). The results were compared with normal macrophage control groups with the same cell numbers, the same culture medium volumes, and the same culturing

conditions. The experiments were conducted by following vendor-provided protocols (RayBiotech) and the concentrations of each cytokine were calculated by comparing to standard calibration curves. All ELISA tests were repeated at least twice.

***In vitro* therapy study.** DSN-MF as well as MF cells were cultured with complete growth medium for 12, 24, and 48 hours (2 million cells, 5 mL medium for each group). Supernatants from each group were collected. The amounts of Dox in the supernatants were assessed by fluorescence spectroscopy analysis with the help of standard calibration curves. To evaluate the cytotoxicity to cancer cells, U87MG cells that were pre-cultured in a separate 6 well plate overnight (confluence ~ 0.4 million cells per well). Supernatants taken DSN-MF cell cultures at 12, 24 h, and 48 h were added into the U87MG cell culture medium. For controls, 1.0 mL complete RPMI1640 medium was added. The viability of U87MG cells at 48 h was examined by MTT assay. For imaging studies to visualize the accumulation of Dox in cancer cells, U87MG cells were co-incubated with different supernatant medium for 6 h, washed with PBS, and then imaged under a fluorescence microscope.

Exosome isolation and analysis. Ten T75 flasks were each seeded with 2 million RAW264.7 cells. After overnight culturing, cells were laden with DSN-52 nanoparticles following above mentioned protocol. The medium supernatant was collected at 45 h post loading. Exosomes in the supernatant were enriched via a series of centrifugation: (1) centrifugation at 300 ×g for 10 min at 4 ºC to remove the living cells, (2) 2000 ×g at 4 ºC for 10 min to remove dead cells, (3) 10,000 ×g at 4 ºC for 30 min to remove the cell debris, (4) a ultracentrifugation step at 100 000 ×g at 4ºC for 90 min, and (5) 100 000 ×g at 4ºC for 60 min after PBS wash. Collected exosomes were dispersed in (1) PBS, (2) DI water, and (3) radioimmunoprecipitation assay

(RIPA) buffer. For TEM imaging, 5 μL exosome dispersion in DI water was dropped onto a TEM grid and air-dried for 10 min, followed by addition of 5 μL of 1 wt.% uranyl acetate in DI water for negative staining. The hydrodynamic size of exosomes in PBS was analyzed by DLS after filtration twice through a 0.45 μm filter unit. The amount of Dox in exosome was quantified by measuring Abs at 470 nm and comparing to a calibration curve. Alternatively, Dox was quantified by measuring fluorescence with excitation at 470 nm and emission at 590 nm. The protein concentration of exosome lysates was determined by DC protein assay (Bio-Rad Laboratories). Standards and samples (5 μL) were added into a 96-well plate, followed by addition of 25 μL reagent A:S at a ratio of 50:1, and 200 μL of reagent C. The absorbance at 750 nm was measured after 15 min incubation at room temperature. The concentration of exosome lysates was calculated based on a standard calibration curve. For western blotting analysis, the collected exosomes were lysed in RIPA buffer containing protease inhibitor (1X). After denaturing at 95 ℃ for 5 min, the lysate was resolved in SDS-PAGE gel and transferred onto nitrocellulose membrane, followed by incubation with primary antibody (1:1000 dilution) at 4 ºC overnight and secondary antibody (1:5000 dilution) at room temperature for 1 h. The blot was imaged using enhanced chemiluminescence (ECL).

Small animal models. For imaging and therapy studies, a U87MG subcutaneous mouse model was used. The animal model was established by subcutaneously inoculating 1 million U87MG cells onto the right hind leg or the right flank (for PET imaging only) of a 5-6 week old athymic female nude mice (Harlan). For toxicity studies, normal 5-6 week old balb/c mice (Envigo laboratories). All the animal studies were performed according to a protocol approved by the Institutional Animal Care and Use Committee (IACUC) of University of Georgia.

***In vivo* MRI and Prussian blue staining.** Human serum albumin decorated iron oxide nanoparticles (HSA-IONPs) were prepared according to a previously published protocol[3]. HSA-IONPs (20 μg Fe mL^{-1}) and DSN-52 (20 μg Dox mL^{-1}) were co-incubated with RAW264.7 cells for 2 h for cell labeling. The resulting, IONP labeled cells at a dose of around 2 million cells per mouse were i.v. administrated to nude mice bearing U87MG tumors on the right hind leg (tumor size ≈ 200 mm^3). The mice were scanned on a 7.0 T Varian small animal MRI system before cell injection, as well as 1, 4 and 24 h after the administration. The scan parameters were the following: TR = 2500 ms, TE = 40 ms, field-of-view (FOV) = 40 mm × 80 mm, matrix size = 2562, thickness = 2 mm. After the 24-h scan, the mice were euthanized. The liver, spleen, lung, heart, kidney, brain, and tumor tissues were collected and frozen in optical cutting temperature (OCT) compound gel at -80 ^oC for Prussian Blue staining purpose. The tissue blocks were cryo-sectioned into 8 μm thick slices and fixed in formalin solutions for 10 min. The slides were carefully rinsed with PBS twice and then submerged in a solution containing 20% HCl and 10% $K_4[Fe(CN)_6]\cdot 3H_2O$ for 20 min (Prussian Blue Staining). Afterwards, the slices were washed twice with PBS and counter-stained with Fast Red for 5 min, followed by PBS wash.

Small-animal Positron Emission Tomography. Small-animal PET was performed on a micro-PET R4 scanner. U87MG tumors were inoculated to the right flanks of the nude mice instead of their hind legs to minimize the impact from tracer uptake in the abdomen. Imaging started once the tumor size reached 50-100 mm^3. DSN-MF and MF cells were co-incubated with ^{64}Cu-pyruvaldehyde-bis(N4-methylthiosemicarbazone) (^{64}Cu-PTSM) in 1 mL serum-free medium at 37 ^oC for 1.5 hours. After washing, 1 million of ^{64}Cu-labelded cells in 0.25

mL PBS (pH 7.4) were i.v. injected into each mouse under isofluorane anesthesia. Static scans were performed at various time points (i.e., 1, 8, and 23 h after the injection). The average radioactivities accumulated within the tumor and other major organs were quantified from decay-corrected coronal images and the results were converted to percentage injected dose per gram (%ID g^{-1}).

***In vivo* therapy study.** Treatments started when tumor size reached 100~150 mm^3. 25 mice were randomly divided into 5 groups and were i.v. injected with PBS, free Dox, DSN-52 in PBS, MF cells, and DSN-MF cells on Day 0. Dox, DSN-52, and DSN-MF were injected at 3 mg Dox kg^{-1}, and 4 million cells were injected into the mice in MF and DSN-MF groups. The body weight and tumor volume of each mouse were measured every other day for 2 weeks. The tumor volume was calculated by the following equation: tumor volume = 0.5 × length × $(width)^2$, where length ≥ width. Mice were euthanized once the tumor volume was above 1,700 mm^3.

In separate studies, animals were euthanized 24 h after cell/drug injection, and the tumors were collected and frozen in OCT compound gel at -80 ^oC. The tissues were cryo-sectioned into 8 μm thick slices for *in situ* apoptosis detection staining (ab206386 from abcam) following the vendor's protocol. The apoptotic nuclei were stained as dark brown and the cytoplasm components were green.

Toxicity studies. Fifteen normal balb/c mice were randomly divided into 5 groups and received regimens specified in the *in vivo therapy study* section. The body weight and anal temperature of each mouse were measured daily at the same time (starting from 6 days before injection through Day 6). On Day 7, all mice were euthanized and the whole blood was

collected. Part of the blood samples were used for a complete blood count (CBC) test. The rest were centrifuged at 5000 rcf for 5 min and the resulting serum samples were stored at -80 ^{o}C and then subjected to ELISA or colorimetric assays to quantify C-reactive protein (CRP), TNF-α, alanine transaminase (ALT), aspartate aminotransferase (AST), and blood urea nitrogen (BUN) amounts. All tests were conducted by following the vendors' protocols. CRP and TNF-α kits were purchased from RayBiotech, ALT (MAK055) and AST (MAK052) kits from Sigma-Aldrich, and BUN from Arbor Assays. Each assay was repeated at least twice.

Statistical methods. Quantitative data were expressed as mean ± SEM. Two-tailed Student's t-test and Chi-squared test were used for statistical comparison between experimental groups and control groups for different studies. $P < 0.05$ was considered statistically significant.

Estimation of drug loading capacity via surface backpack strategy

The number of cells injected during one transfer procedure is calculated to evaluate the drug loading capacity of nanoparticle-laden cell system via surface backpack strategy. Three types of nanoparticles that are commonly used for drug loading are considered here: (1) poly(lactic-co-glycolic acid) (PLGA) nanoparticle; (2) lipid-type nanoparticle, such as liposome; (3) Stöber silica nanoparticle. For each particle, three parameters are considered for the estimation: (a) the amount drug in each particle, (b) the number of nanoparticles that are tethered on surface of each cell, and (c) the injection dose. The following equation is used to estimate the drug loading amount per nanoparticle: drug loading by weight = %Loading Capacity × nanoparticle weight. Except some rare examples[4], the %Loading Capacity by weight of PLGA nanoparticle, lipid nanoparticle, and Stöber silica nanoparticle is typically lower than or close to 20%[5–8]. The nanoparticle weight can be calculated by mass = density

× volume. The density of PLGA nanoparticle, lipid nanoparticle, and Stöber silica nanoparticle is around 1.3 g cm^{-3}, 1.06 g cm^{-3}, and 1.8-2.2 g cm^{-3} respectively. Assuming each nanoparticle is a perfect sphere, the volume of a single nanoparticle equals to $(4/3)\pi r^3$, where r is the radius of nanoparticles, typically ranging from 100 to 200 nm. According to Stephan et al.[9], attachment of up to 100-150 nanoparticles with a diameter of ~200 nm onto the plasma membrane is benign to T cells and hematopoietic stem cells. A clinically relevant treatment dose usually ranges from 1 to 10 mg kg^{-1}.

To simplify the calculation, we assume drug encapsulated nanoparticle as a homogeneous entity with a fixed density, the %Loading Capacity = 20%, and the number of nanoparticles attached onto each single cell = 300. At 1 mg kg^{-1}, which is at the low end of typical drug injection dose, and assuming a body weight of 25 g for mouse and 50 kg for human, the amount of drug injected is 25 μg per mouse and 50 mg per person. As shown in the following table, the drug loading capacity on a per cell basis is estimated around 0.03-0.50 pg drug $cell^{-1}$, which requires to inject tens to hundreds million cells per mouse to achieve the 1 mg kg^{-1} dose. In a recent report by Huang et al. about using nanoparticle-carrying T cells for drug delivery[10], the drug loading was 0.1~0.125 pg drug $cell^{-1}$, which coincides well with our estimation.

Table S1. Estimation of drug loading per cell via the surface backpack strategy

NP type	**density**	**radius**	**volume**	**NP mass**	**drug per NP**	**drug per cell**	**#cell per mouse**	**#cell per person**
	g cm^{-3}	nm	cm^3	pg	pg NP^{-1}	pg $cell^{-1}$	million	billion
PLGA	1.30	50	0.52×10^{-15}	0.68×10^{-3}	1.36×10^{-4}	0.04	612	1224
		100	4.19×10^{-15}	5.45×10^{-3}	10.89×10^{-4}	0.33	77	153
Lipid	1.06	50	0.52×10^{-15}	0.56×10^{-3}	1.11×10^{-4}	0.03	751	1501
		100	4.19×10^{-15}	4.44×10^{-3}	8.88×10^{-4}	0.27	94	188
Stöber Si	2.00	50	0.52×10^{-15}	1.05×10^{-3}	2.09×10^{-4}	0.06	398	796
		100	4.19×10^{-15}	8.38×10^{-3}	16.76×10^{-4}	0.50	50	99

NP: nanoparticle

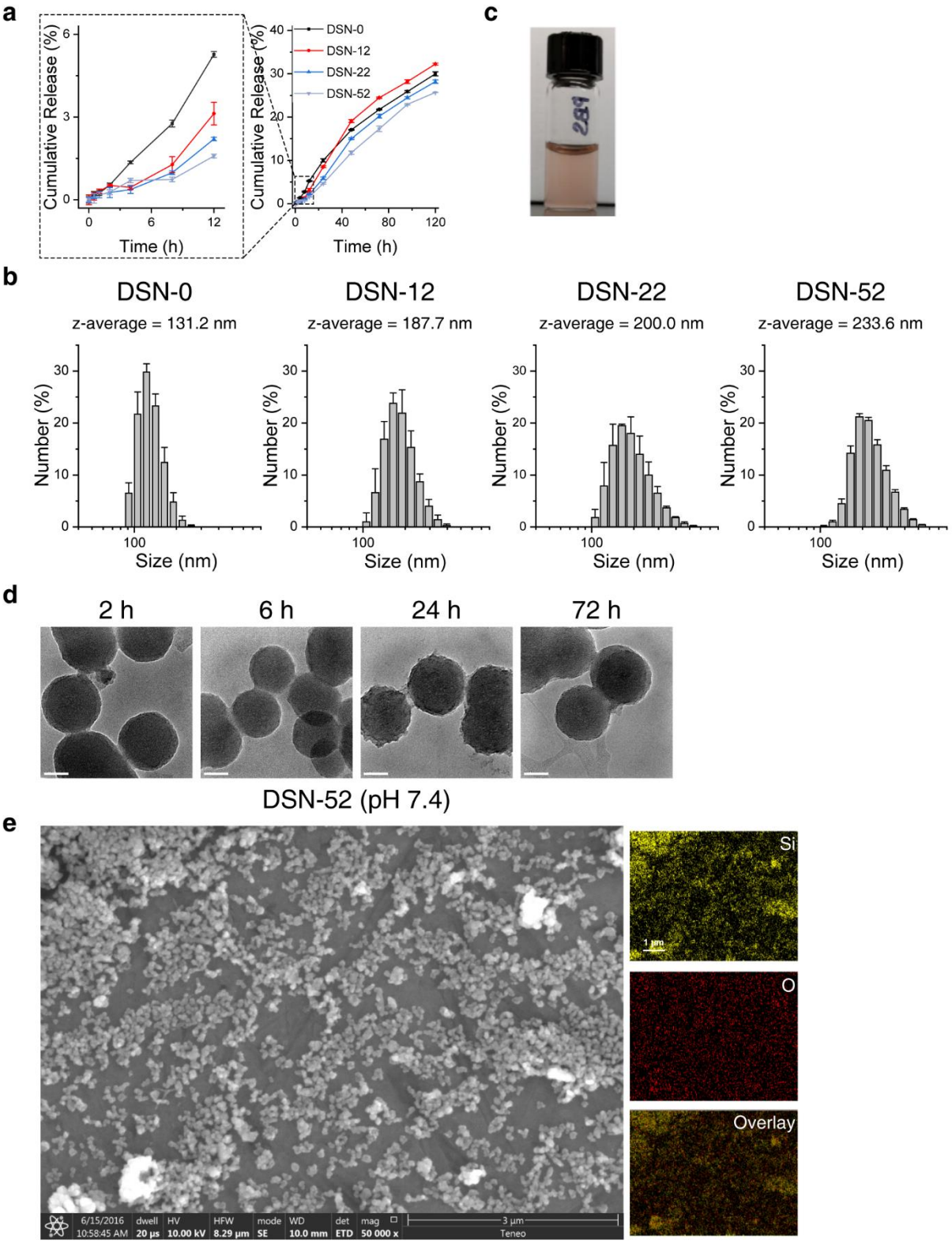


Figure S1. a) Drug release profiles at pH 7.4 and **b)** hydrodynamic sizes of DSN-0, DSN-12, DSN-22, and DSN-52 nanoparticles. **c)** Digital photograph of DSN-52 nanoparticle dispersed

in PBS. **d)** TEM images showing DSN-52 nanoparticles' morphology changes over time in a pH 7.4 PBS solution. Scale bar, 50 nm. **e)** SEM and elemental mapping by EDS with DSN-52 nanoparticles.

Table S2. Loading capacity (%LC) of DSNs

	Total Wt.	**Dox**	**SiO_2**	**Dox Ratio**
	mg	mg	mg	wt%
DSN-0	5.0	0.83	4.17	16.70%
DSN-12	2.6	0.29	2.39	11.15%
DSN-22	4.7	0.42	8.34	8.94%
DSN-52	6.7	0.34	6.36	5.13%

Table S3. Loading capacity (%LC) of DSN-52 by ICP-OES.

	Dox	**Si**	**Soln.**	**Si**	**Si**	**SiO2**	**Total**	**Dox Ratio**
	µg	ppm	g	ppm	µg	µg	µg	wt%
BKG	0	0.97	5.03	0	0	0	0	
#1	6	10.51	(5mL)	9.54	47.986	102.8271	108.8271	5.51%
#2	12	22.4		21.43	107.793	230.985	242.985	4.94%
							average	5.23%

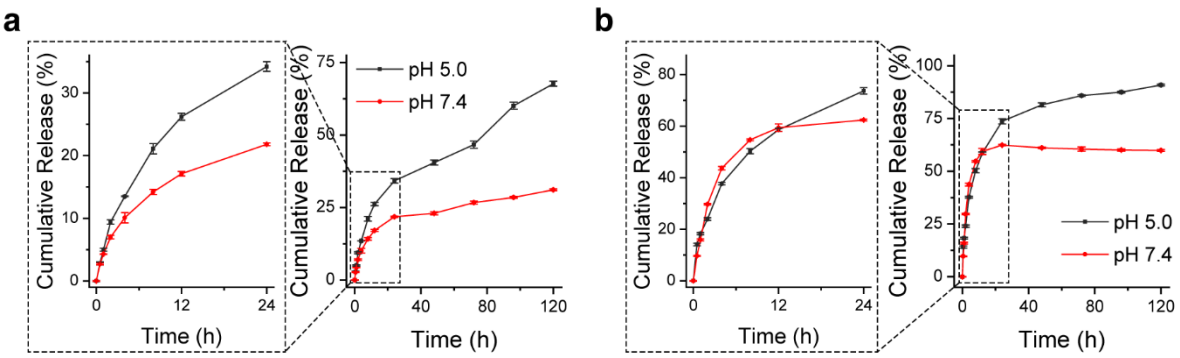


Figure S2. Drug release profiles measured at pH 5.0 and 7.4 with **a)** Doxove and **b)** Dox-encapsulated mesoporous silica nanoparticles.

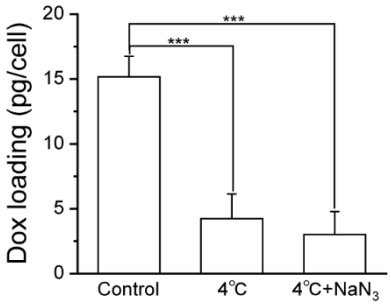


Figure S3. Drug loading assay showing the internalization of DSN-52 nanoparticles into macrophage. Control, DSN-52 nanoparticles were laden into RAW264.7 cells via incubation under normal condition; 4℃, loading was conducted at 4℃; 4℃+NaN_3, loading was conducted at 4℃ with the presence of 0.1 wt.% NaN_3. ***, $p<0.001$.

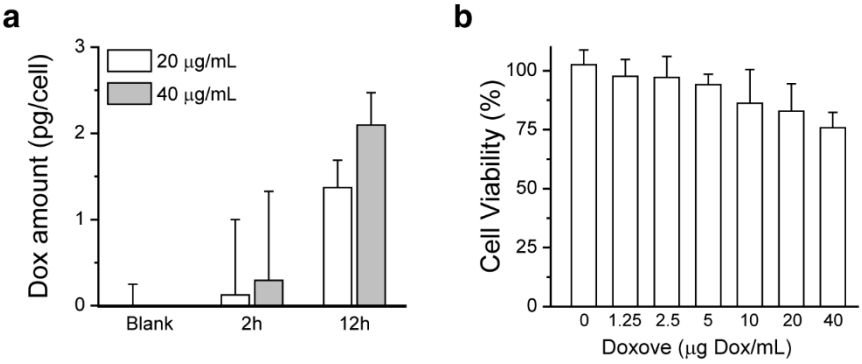


Figure S4. a) Intracellular Dox contents. RAW264.7 cells were incubated with Doxove at 20 and 40 μg Dox/mL for 2 or 12 h. **b)** Cell viability. RAW264.7 cells were incubated with Doxove at different concentrations for 2 h. After replenished with fresh media, the cells were cultured for 24 h, and the viability measured by MTT.

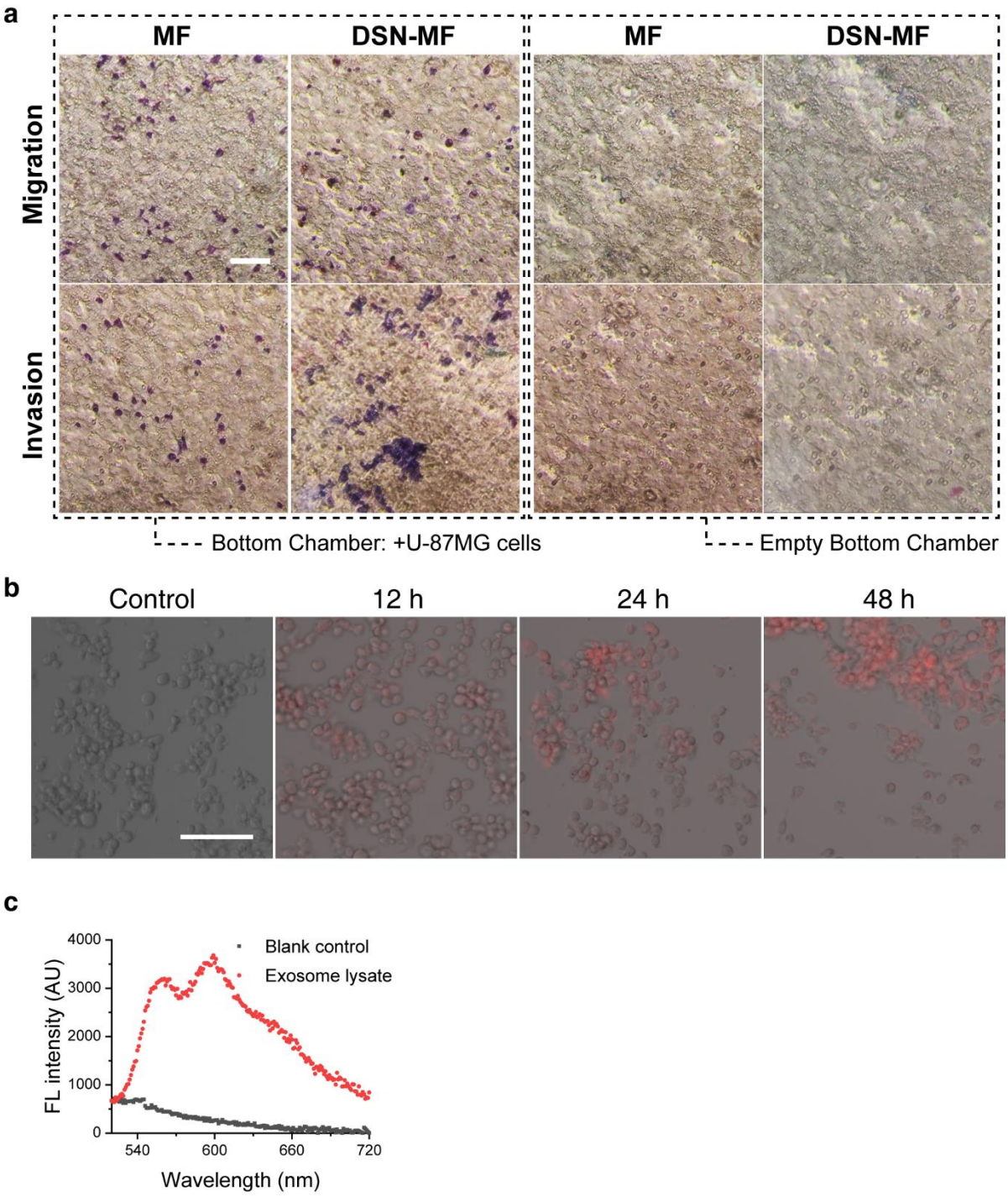


Figure S5. a) Transwell invasion/migration assay results. **b)** FL images of U87MG cells after incubating with different supernatants from DSN-MF cell cultures for 6 h. **c)** FL spectroscopy analysis of exosome lysates, which confirms the presence of Dox in the exosomes. Excitation was set at 470 nm.

Table S4. Dox release from DSN-MF

# cells	Dox_{load} (μg)	Dox_{exo} (μg)	Dox_{sup} (μg)	%Dox release	%Dox(exosome)
$5.03 \pm 0.13 \times 10^6$	80.03 ± 2.00	7.51 ± 0.47	37.93 ± 1.07	56.8%	16.5%

- %Dox release = $(Dox_{exo} + Dox_{sup})/Dox_{load} \times 100\%$
- %Dox(exosome) = $Dox_{exo}/(Dox_{exo} + Dox_{sup}) \times 100\%$

Dox_{load}: the amount of Dox loaded in $5.03 \pm 0.13 \times 10^6$ RAW264.7 cells; **Dox_{exo}**: the amount of Dox in secreted exosomes; **Dox_{sup}**: the amount of Dox present in the supernatant.

Note: a certain amount of Dox is retained in the cell debris, which was removed during centrifugation and excluded from calculation.

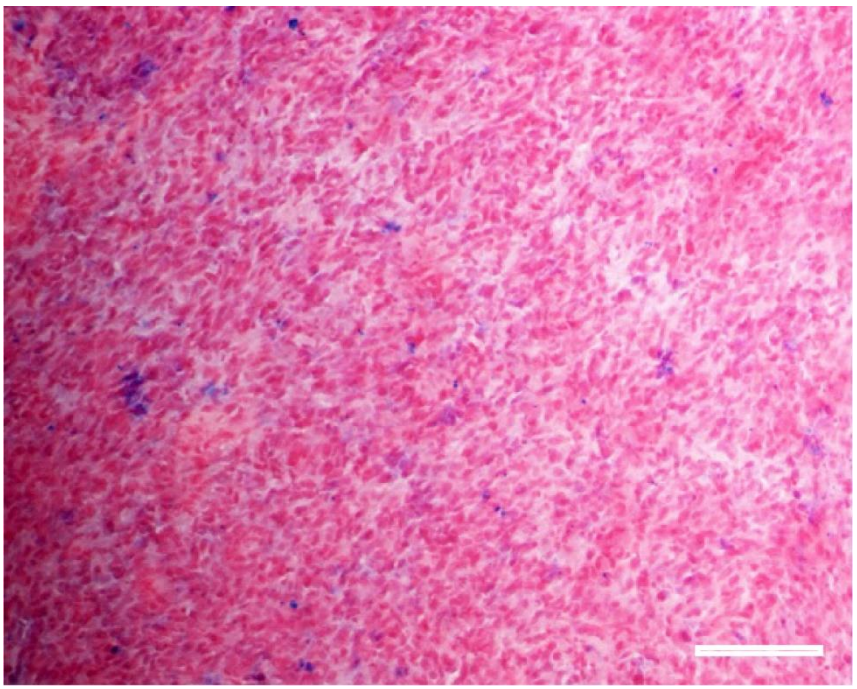
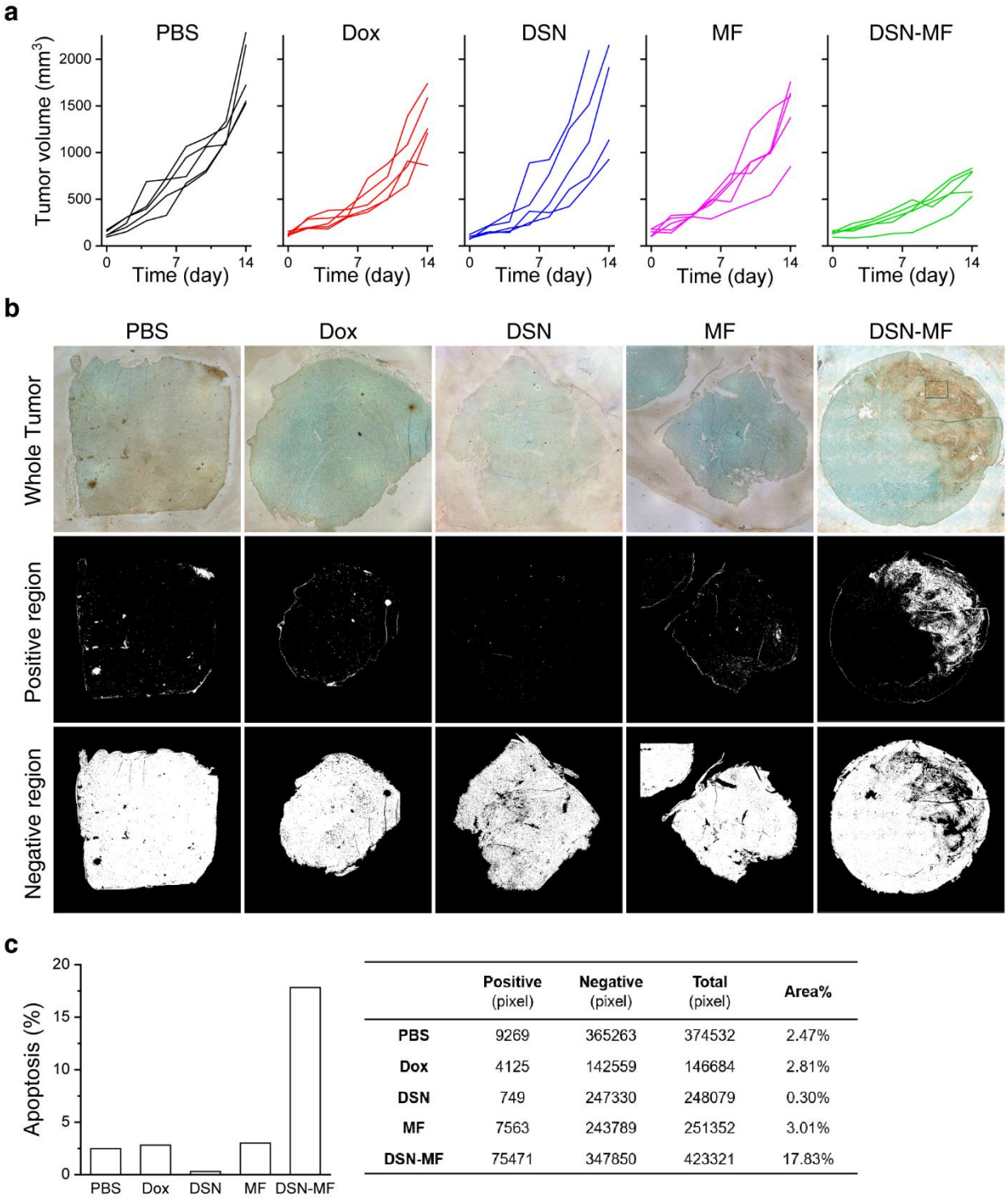


Figure S6. Prussian blue staining on tumor cryo-section. Tumors were collected at 24 h after i.v. injection with IONP-labeled DSN-MF cells.



	Positive (pixel)	Negative (pixel)	Total (pixel)	Area%
PBS	9269	365263	374532	2.47%
Dox	4125	142559	146684	2.81%
DSN	749	247330	248079	0.30%
MF	7563	243789	251352	3.01%
DSN-MF	75471	347850	423321	17.83%

Figure S7. a) Tumor growth curves for individual animals. **b)** *In situ* Apoptosis staining analysis of cryo-sectioned tumor tissues. The tumors were dissected 24 h post treatments. The whole tumors were subjected to staining and the positively stained areas quantified by Photoshop. **c)** Quantitative analysis based on the staining results of **b**.

Table S5. Complete Blood Count Report

	PBS	**Dox**	**DSN**	**MF**	**DSN-MF**	**REF. RANGE**
WBC ($\times 10^3$ μl^{-1})	5.62	13.49	6.31	3.77	8.52	6-15
RBC ($\times 10^6$ μl^{-1})	9.26	13.9	9.34	8.81	8.39	7-11
HGB (g dl^{-1})	14.0	44.0	14.1	13.7	13.1	1.2-16.6
HCT (%)	44.7	48.9	44.4	43.4	41.4	39-49
MCV (fl)	48.3	15.4	47.6	49.2	49.4	41-49
MCH (pg)	15.1	31.6	15.1	15.5	15.6	15-18
MCHC (g dl^{-1})	31.3	62	31.8	31.6	31.7	30-38
Segs (%)	10	0	13	13	13	10-40
Bands (%)	0	12	0	0	0	No data
Lymphs (%)	84	82	79	80	78	55-95
Monos (%)	6	6	8	7	9	1-4

Reference

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