**ELECTRONIC SUPPLEMENTARY INFORMATION FOR**

# Mn-DNA Coordination Nanoparticles for an Efficient Chemodynamic Therapy

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## Experimental Section

**Chemicals:** DNA sequences listed in Table S1 were purchased from Shanghai Sangon Biotech, China. $T_4$ DNA ligase was purchased from New England Biolabs, USA. φ29 DNA polymerase was purchased from Thermo Fisher, USA. dNTPs and 15000 bp DNA ladder were purchased from Takara, China. $H_2O_2$ was purchased from Shanghai Sangon Biotech. GelRed (×10,000) was obtained from Biotium, USA. Ethylene diamine tetra-acetic acid (EDTA), $MnCl_2$, $FeCl_2$, $MgCl_2$, $Na_2HPO_4$ and other common reagents were purchased from Aladdin, China. Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), penicillin–streptomycin solution (Anti), and phosphate-buffered saline (PBS) were supplied by Hyclone, USA. 5-tert-Butoxycarbonyl-5-methyl-1-pyrroline-N-oxide (BMPO) from Dojindo, Shanghai; Ultrapure water used throughout the experiments was prepared using ELGA water purification system (PURELAB Classic).

### Synthesis of metal ions condensed DNA particles

***RCA process for preparing lssDNA:***

(1) Annealing: DNA template (0.33 μM) and ligation primer (0.67 μM) were mixed in $T_4$ ligase buffer (50 mM Tris-HCl, 10 mM $MgCl_2$, 10 mM Dithiothreitol, 1 mM ATP, pH = 7.5) at 90 °C for 10 min, followed by cooling down to room temperature gradually.

(2) Ligation: $T_4$ DNA ligase (10.4 $U•\mu L^{-1}$) was added into the annealing product and cultured at 16 °C with continuous shaking (500 rpm) for 16 h. Subsequently, the ligation product was heated to 65 °C and kept for 10 min to stop the ligation.

(3) Amplification: The ligation product, φ29 DNA polymerase buffer (50 mM Tris-HCl, 10 mM $(NH_4)_2SO_4$, 10 mM $MgCl_2$, 4 mM Dithiothreitol), dNTPs (0.2 mM each) and φ29 DNA polymerase (0.2 $U•\mu L^{-1}$) were mixed at 30 °C with continuous shaking (500 rpm) for 24 h, followed by heating to 90 °C and keeping for 10 min to stop the RCA reaction.

(4) Purification: After diluting the RCA product 3 times with nuclease-free water, an appropriate amount of EDTA (0.5 M) was slowly added until the solution changed from viscous to clear, which was performed under 300 rpm shaking and 90 °C incubation. The clear solution was centrifuged at 3000 × g for 10 min in a clean and humid 100 k ultrafiltration centrifuge tube at 4 °C. The centrifugal washing operation was repeated 5 times with ultrapure water, and finally the products were diluted in an appropriate amount of ultrapure water to obtain pure lssDNA.

(5) Quantification: Pure lssDNA was diluted 10-fold with nuclease-free water, and the absorption at 260 nm was measured with a microplate reader (BioTek Cytaion3; USA) to determine the concentration and yield.

***Condensation process for MDNs:***

The $MnCl_2$ and $MgCl_2$ stock solution (50 mM and 200 mM respectively) were added into the lssDNA solutions (0.27 mM = 81 ng•μ$L^{-1}$, according to phosphate groups) to achieve molar ratio (phosphate : Mn : Mg = 1 : 20 : 200), followed by gentle mixing for 2 h at room temperature. To remove extra metal ions and purify nanoparticles, the filtrate was eliminated by centrifuging at 8000 × g for 15 min by the 3k ultrafiltration centrifuge tube at 4 °C. Finally, the nanoparticles were resuspended in ultrapure water for storage and further experiments.

***Interaction forces in MDNs:***

Solution of the short template ssDNA (4 μM) (T68-c) (shown in Table S2) mixed with and without $Mn^{2+}$ (5.4 mM) solution in D.I. water for 2 hours, followed by the gel electrophoresis in 15% polyacrylamide gel (PAGE) with one-hour constant voltage running of 80 V (Bio-Rad MINI-PROTEAN Tetra; USA). Then the gel block was imaged by the gel imaging system (Tannon 3500; Shanghai, China).

**Characterization of metal ions condensed DNA particles**

***Morphological and component analyses***

Transmission electronic microscopy (TEM; Hitachi HT7700; Tokyo, Japan) was used to obtain high resolution digital images of MDNs for morphological characterization at an accelerating voltage of 100 kV. For TEM observation, MDNs were dried on a plasma-cleaned copper wire in a vacuum drying oven at 30 °C for overnight. The map of composition was detected by high-angle annular dark-field scanning transmission electron microscopy (HAADF-STEM; FEI Talos F200X; USA) with a voltage of 200kV. The real concentrations of $Mn^{2+}$ and $Mg^{2+}$ in MDNs were detected by the inductively coupled plasma mass spectrometry (ICP-MS) (Agilent 7700X; USA). The chemical states of manganese were determined by the X-ray photoelectron spectroscopy (XPS) analysis with a model ESCALAB 250Xi instrument (Thermo Fisher Scientific Co., Waltham, MA, USA). The XPS spectra were recorded at a constant pass energy of 100 eV and 1.000 eV/step with Al Kα X-ray source. The high resolution XPS spectra of Mn 2p were recorded at a constant pass energy of

30 eV and 0.050 eV/step with Al Kα X-ray source. The Thermo Avantage 4.51 software (Thermo Fisher Scientific Co.) was used to fit the obtained XPS spectra, binding energies were referred to the C 1s level at 284.8 eV, the background of spectra was achieved by using the Shirley method. The Mn 2p spectra were fitted using a 2:1 peak area ratio and 12.3 eV splitting for Mn $2p_{3/2}$ and Mn $2p_{1/2}$.

***Physicochemical properties of MDNs***

The size distribution and zeta potential of MDNs and DOX@MDNs were determined by a particle size and zeta potential analyzer (Nano DLS; BI-200SM; Brookhaven, USA). All samples were dispersed in D.I. water under 15 s ultrasound, and were measured at 25 °C. In the part of stability, the particle size and zeta potential of MDNs and DOX@MDNs were also tested in dibasic sodium phosphate-citric acid buffer ($Na_2HPO_4$-CA) with different pHs or after 7 days' incubation in D.I. water.

***Biostability of MDNs***

MDNs were dispersed in nuclease-free water for the control group and in 10% FBS for serum stability group for 2 h to 3 days' incubations. The samples were mixed with 1 × loading buffer, and thereafter were loaded into the 0.75% agarose gel which was stained by GelRed. After running under a constant voltage of 80 V for 1 h on gel electrophoresis system (Bio-Rad MINI-PROTEAN Tetra; USA), the gel block was imaged by the gel imaging system (Tannon 3500; Shanghai, China).

***Fenton-like reaction of MDNs in vitro***

The types of reactive oxygen species (ROS), such as hydroxyl radicals (•OH) and hydroperoxyl radical (•OOH) were determined in the system of MDNs/$H_2O_2$ (8 mM $H_2O_2$, 10 mM $Na_2HPO_4$-CA buffer with different pHs, 25 mM BMPO) by the electron paramagnetic resonance spectrum (EPR) (Bruker BioSpin EMXplus-10/12, Bruker; Germany). The level of ROS was also performed by using the classic colorimetric method based on the degradation of methylene blue (MB). The absorbance at $\lambda$= 660 nm of MB solution (25 mg•$L^{-1}$) with different concentrations of $H_2O_2$ (0, 0.5, 1, 2, 4, 8 mM) in $Na_2HPO_4$-CA buffer solution with different pHs (5.0, 5.5, 6.0, 6.5, 7.0, 7.4) were measured after the adding of MDNs (400 μM according to $Mn^{2+}$).

***DOX loading and releasing of MDNs in vitro***

Doxorubicin (DOX) is a kind of anti-cancer drug with high DNA-binding affinity. When DOX bound to DNAs, the fluorescence of DOX will decrease; on the contrary, the fluorescence will

recover when DOX is released. Hence the processes of drug loading and release were investigated by the fluorescence spectroscopy. The fluorescence of free DOX solution ($F_0$) with different concentrations from 0 to 10 μM were tested to achieve a standard curve (Fig. S11a). MDNs with different concentrations of lssDNA from 0.1 to 4 μM were mixed with 2 μM DOX in 1 × PBS (pH 7.4) for 1 h, and then which were washed with PBS through 3 k ultrafiltration tubes at 10000 × g for 10 min. We measured the fluorescence of the filtrate ($F_{s1}$) at 595 nm under 480-nm excitation by a microplate reader (BioTek Cytaion3; USA), and the drug loading efficiency (Fig. S11b) was calculated through the following equation:

$$Encapsulation\ Efficiency\ (\mathrm{EE\%}) = 1 - \frac{F_{S1}}{F_0}\% \quad (1)$$

$$Drug\ Loading\ (L\%) = \frac{\frac{EE\% * n_{DOX}}{n_{DNA}} * MW_{DOX}}{MW_{DOX} + MW_{DNA}} \quad (2)$$

$MW_{DOX} = 543.52\ g \cdot mol^{-1};\ MW_{DNA} = 20400\ g \cdot mol^{-1}$

$F_{s1}$: The fluorescence of filtrate at 595 nm.

$F_0$: The fluorescence of free DOX solution at 595 nm.

The releasing curve in different incubate conditions also be drawn by testing the fluorescence of the filtrate ($F_{s2}$) at different time (0, 5, 15, 30, 60, 90, 120, and 180 min).

$$Drug\ Releasing\ (\mathrm{R\%}) = \frac{F_{S2}}{F_0}\% \quad (3)$$

$F_{s2}$: The fluorescence of filtrate at 595 nm.

$F_0$: The fluorescence of free DOX solution at 595 nm.

***Functionalization of MDNs' backbone ---- long-single-strand DNA***

On the one hand, the high hybridization of cDNA-Cy5 and lssDNA indicated the potential of MDNs on the delivery and therapy with antisense oligonucleotide (ASO), microRNA, small interfering RNA (siRNA) and etc. On the other hand, the modification with fluorescence Cy5 would make it easy to track the MDNs during the cell uptake pathway assay. Solutions containing 10 μM of cDNA-Cy5 (54 ng•μL$^{-1}$) and 20 μM of lssDNA (408 ng•μL$^{-1}$, according to the number of repeat unit) in annealing buffer (pH 7.4, 10 mM PBS) were heated at 95 °C for 5 min and then naturally cooled to room temperature. The hybridized product contains 20 μM of lssDNA and 10 μM of cDNA-Cy5

was defined as 20 μM of lssDNA-Cy5. The successful hybridization of lssDNA-Cy5 was determined by gel electrophoresis in 1% agarose gel with a constant voltage of 100V and running time of 1 hour (Fig. S8b). Similar with the condensation process of MDNs, we mixed lssDNA-Cy5 (0.27 mM = 103 ng•μL$^{-1}$, according to phosphate groups), $MnCl_2$ and $MgCl_2$ with the molar ratio of phosphate / Mn / Mg = 1 / 20 / 200 to achieve the MDNs-Cy5. The condensation product contains 4 μM of lssDNA-Cy5 (103 ng•μL$^{-1}$, according to the number of repeat unit) and 1 mM of Mn( II ) was defined as 1 mM of MDNs-Cy5.

***Cell* experiments of metal ions condensed DNA particles**

***Cell culture***

A549 cells and 293T cells were incubated at 37 °C with 5% $CO_2$ in DMEM medium mixed with 1% Anti and 10% FBS in a carbon dioxide incubator (INCO2/153C; Memmert, Germany;).

***Fenton-like reaction of MDNs in cells level***

A549 cells with a density of 4000 cells/well were planted into a 96-well plate with glass bottom, and MDNs or ions were added respectively after 24 hours of cells culture (the medium was changed 1 h in advance when the nanoparticles were added). After 4 hours of coculture, DCFH-DA (25 μM) solution was added and need another 30 min of coculture. The medium was replaced with fresh one and the cells were washed twice with 1×PBS buffer before the observation under a laser scanning confocal microscope (LSCM) (Leica TCS-SP8; Germany) with an excited laser of 488 nm and emission of 529 nm.

***Cell uptake assay***

Cell uptake pathway assay by tracking the fluorescence of cy5 on MDNs-Cy5. A549 cells with a density of 4000 cells/well were planted into a 96-well plate with the glass bottom, and 200 μM MDNs-Cy5 with or without a competitive inhibitor of SR, polyinosinic acid (Poly I, 40 μg•mL$^{-1}$) were added after 24 h of cells culture. After additional 1 h, 6 h or 24 h of coculture, the cells were washed twice with 1 × PBS buffer. Then, we marked the lysosome and nucleus by Lyso Tracker Green DND-26 (250 nM, 5 min) and Hoechst 33258 (25 μg•mL$^{-1}$, 15 min), respectively. Solution in the hells were replaced with fresh DMEM before the observation by a laser scanning confocal microscope (LSCM) (Leica TCS-SP8; Germany). The emission intensity was collected at 460 nm, 550 nm, 670 nm under the 405-nm, 488-nm, 638-nm excitation, respectively (Fig. S9).

Cell uptake assay by imaging the releasing of DOX. A549 cells with a density of 4000 cells/well were planted into a 96-well plate with the glass bottom, and free DOX or DOX@MDNs were added respectively after 24 h of cells culture. After additional 1 or 6 h of coculture, the cells were washed twice with 1 × PBS buffer before the observation by a laser scanning confocal microscope (LSCM) (Leica TCS-SP8; Germany). The emission intensity was collected at 590 nm under a 488-nm excitation (Fig. S12).

Cell uptake assay also determined by testing the concentrations of metal ions in cells. A549 cells with a density of 8000 cells/well were planted into a 96-well plate, and free ions or MDNs were added respectively after 24 h of cells culture. After additional 6 h of coculture, the cells were washed twice with 1 × PBS buffer and digested in 1% $HNO_3$ overnight before the measurement by the inductively coupled plasma mass spectrometry (ICP-MS) (Agilent 7700X; USA) (Fig. 3c).

***Cytotoxicity assay***

(1) Coculture: A549 cells with a density of 8000 cells/well were planted into a 96-well plate, and MDNs, DOX@MDNs, DOX@MgDNs, free ions, and free DOX were added respectively after 24 h of cells culture (the medium was changed 1 h in advance when the nanoparticles were added). Similarly, 293T cells with a density of 8000 cells/well were planted into a 96-well plate, followed by the adding of MDNs after 24 h of cells culture. After 24 h of coculture, the medium was removed and the cells were washed twice with 1 × PBS buffer.

(2) MTT assay: MTT solution (0.5 mg•$mL^{-1}$, 100 μL) was added to each well, and the mixtures were incubated at 37 °C for 4 h. MTT solution was replaced with 150 μL DMSO followed by shaking at 800 rpm at room temperature for 10 min. Finally, the cell viability was calculated from the change of absorbance at 570 nm (tested by microplate reader (BioTek Cytaion3; USA)).

***Statistical analysis***

Experimental data are expressed as the mean ± standard error (SE) for three samples per group. Differences between groups were analyzed using one-way ANOVA with a Fisher LSD test, a function of the SPSS software package version 21.0. The data were marked as *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

**Table S1.** Comparison of the apoptosis rate of MDNs toward cancer cells and previously published nanoparticles

| Nanoparticles | | Absence of additive | Presence of additive | | | | |
|---|---|---|---|---|---|---|---|
| **Type** | **Conc. ( g•mL$^{-1}$)** | **Cancer cell apoptosis (%)** | **Source** | **Conc. (μg•mL$^{-1}$)** | **Cancer cell apoptosis (%)** | **Cell lines** | **Ref.** |
| $Fe_3O_4$@carbon | 20 | 10 | ascorbic acid | 352 | 61 | PC-3 | 1 |
| GOx-MnCaP | 5 | 10 | glucose | 1000 | 80 | U87MG | 2 |
| $MnCl_2$ | 25 | 5 | endogenous $H_2O_2$ | - | 5 | U87MG | 2 |
| | 50 | 35 | | - | 35 | | |
| | 100 | 60 | | - | 60 | | |
| MnGdOP@PDA-PEG | 100 | 8 | endogenous $H_2O_2$ pH = 6.5 | - | 27 | U87MG | 3 |
| $MnO_2$@HMCuS | 100 | 30 | endogenous $H_2O_2$ | - | 30 | MCF-7 | 4 |
| rMOF(Fe)-FA | 100 | 80 | endogenous $H_2O_2$ | - | 80 | Hela | 5 |
| MDNs | 21.6 | 70 | endogenous $H_2O_2$ | - | 70 | A549 | This study |

**Table S2.** DNA sequences for the rolling circle amplification reaction.

| Strand | DNA Sequences |
|---|---|
| **Template (68nt)** | 5'-ACT TCA CCC TAA CTC TGC CTC CAC TCA CTT TCA CTC TTG TTC ATC TTC CAC ACT CCA CTC CCT ACT CG-3' |
| **Template-c (68nt)** | 5'-CGA GTA GGG AGT GGA GTG TGG AAG ATG AAC AAG AGT GAA AGT GAG TGG AGG CAG AGT TAG GGT GAA GT-3' |
| **Primer (14nt)** | 5'-GTG AAG TCG AGT AG-3' |
| **cDNA (18nt)** | Cy5-5'-CCT AAC TCT GCC TCC ACT-3' |

**Table S3.** MDNs were purified by D.I. water in 3 k ultrafiltration tube at 10,000×g for 20 min every cycle. Then NPs were digested in 1% $HNO_3$ overnight before the ICP-MS test.

| Sample | Total Conc./ppb | Tested Conc./ppb | Conc. RSD/% | Loading Efficient |
|---|---|---|---|---|
| **Mn** | 1031 | 218.7743064 | 1.2328410 | 21.2% |
| **Mg** | 1800 | 211.7557534 | 1.1057063 | 11.8% |

**Table S4.** A549 cells were incubated with 400 μM MDNs or free ions for 6hrs followed by washed with 1x PBS and digested in 1% $HNO_3$ overnight. The concentration of $Mn^{2+}$ in cells were tested by ICP-MS.

| Sample | Total Conc./ppb | Tested Conc./ppb | Conc. RSD/% | Uptaking Efficient |
|---|---|---|---|---|
| **MDNs** | 54945 | 2028.92 | 2.98 | 3.7% |
| **Free ions** | 54945 | 51.48 | 1.88 | 0.1% |

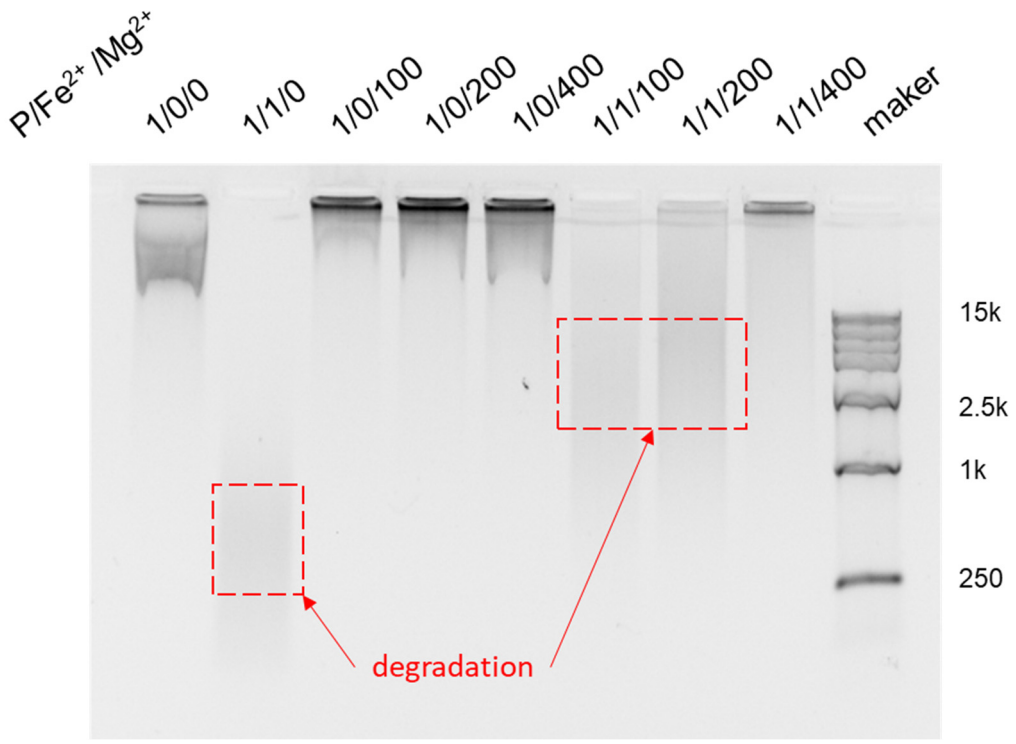


**Fig. S1** Agarose gel electrophoresis (AGE) images: The condensation effect of different metal ions on lssDNA (4 μM) with varied P/ $Fe^{2+}$/ $Mg^{2+}$ ratios.

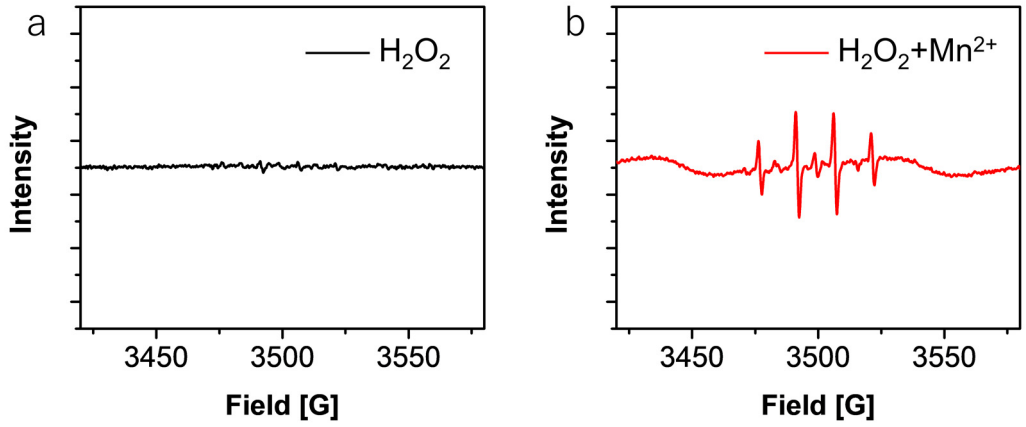


**Fig. S2** Electron paramagnetic resonance (EPR) spectra of 100 mM DMPO with 8 mM $H_2O_2$ in the a) present and b) absent of 100 μM $Mn^{2+}$ in 25 mM $NaHCO_3$, 5% $CO_2$ system.

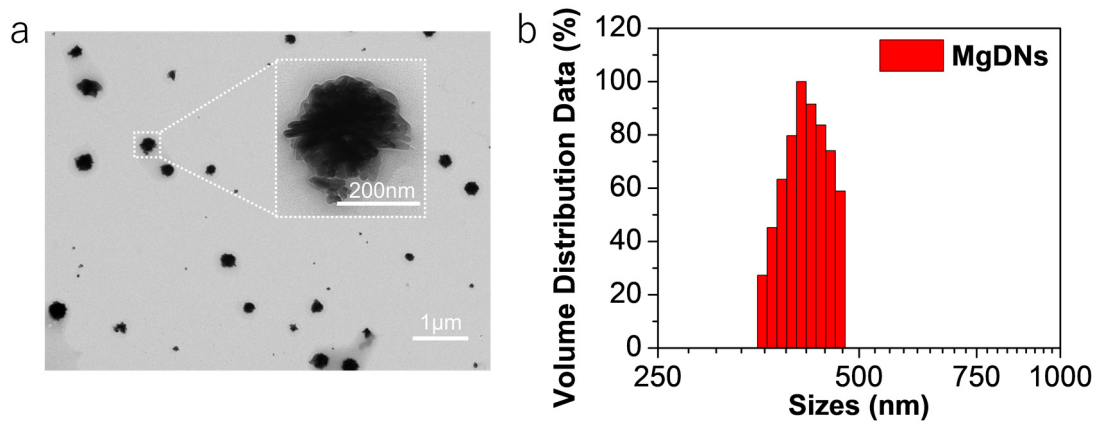


**Fig. S3** TEM image of lssDNA NPs condensed by pure $Mg^{2+}$. Morphological and particle size distribution of lssDNA NPs condensed by pure $Mg^{2+}$. (a) TEM images; (b) DLS data: particle size is approximately 400 nm.

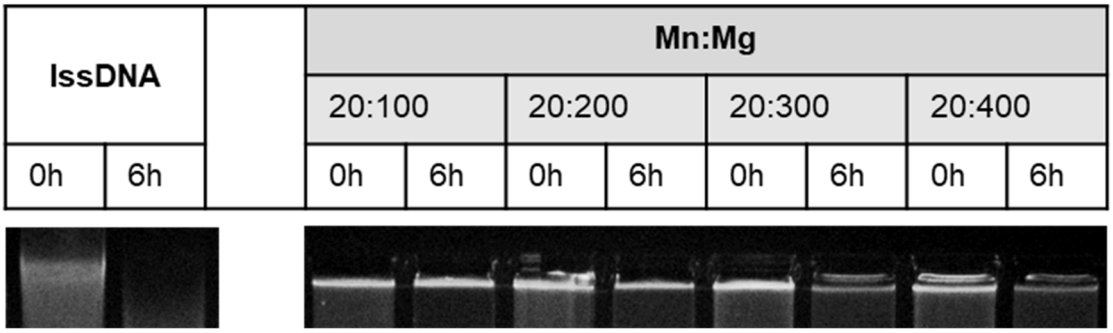


**Fig. S4** AGE image for the stability of the MDNs and lssDNA during the 6-h incubation in 10% FBS.

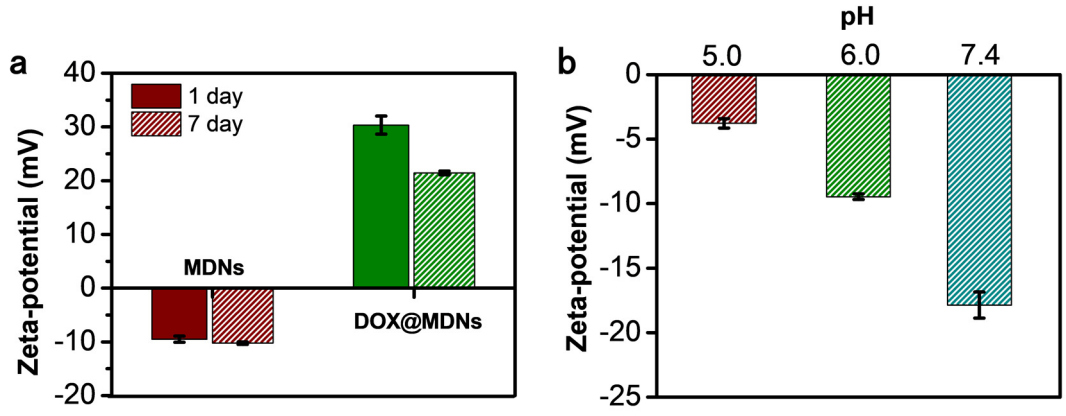


**Fig. S5** a) The zeta-potential of MDNs and DOX@MDNs were tested immediately or after 7-day

incubation in D.I. water; b) The zeta-potential of MDNs in dibasic sodium phosphate-citric acid buffer with pH = 5.0, 6.0, and 7.4. Considering the negatively charged DNAs shell, MDNs would be readily ionized by cations in the solution and became less negatively charged when lowering the buffer pH.

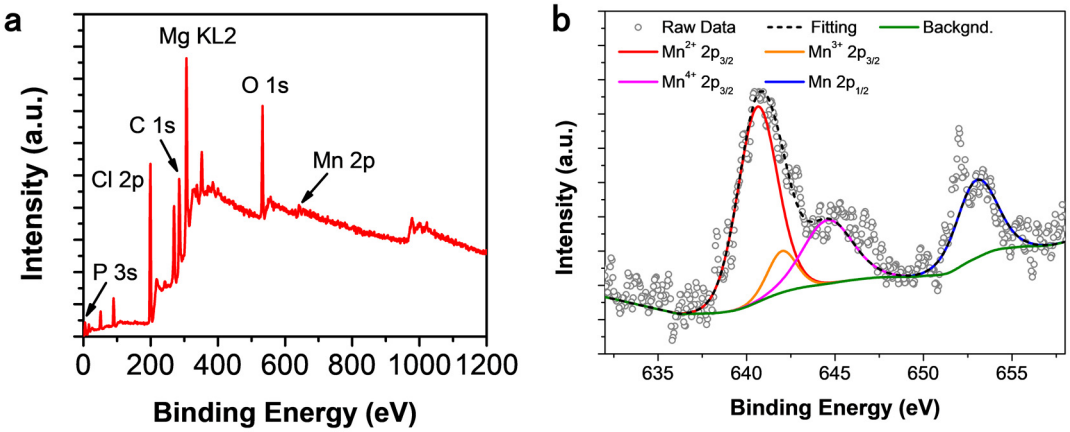


**Fig. S6** a) The successful assembly of MDNs was validated by the X-ray photo-electron spectroscopy (XPS) analysis; b) The high-resolution XPS of Mn 2p showed that the Mn 2p3/2 mainly consisted of 64.21% $Mn^{2+}$ (640.5 eV), 9.86% $Mn^{3+}$ (641.5 eV), and 25.92% $Mn^{4+}$ (644 eV)

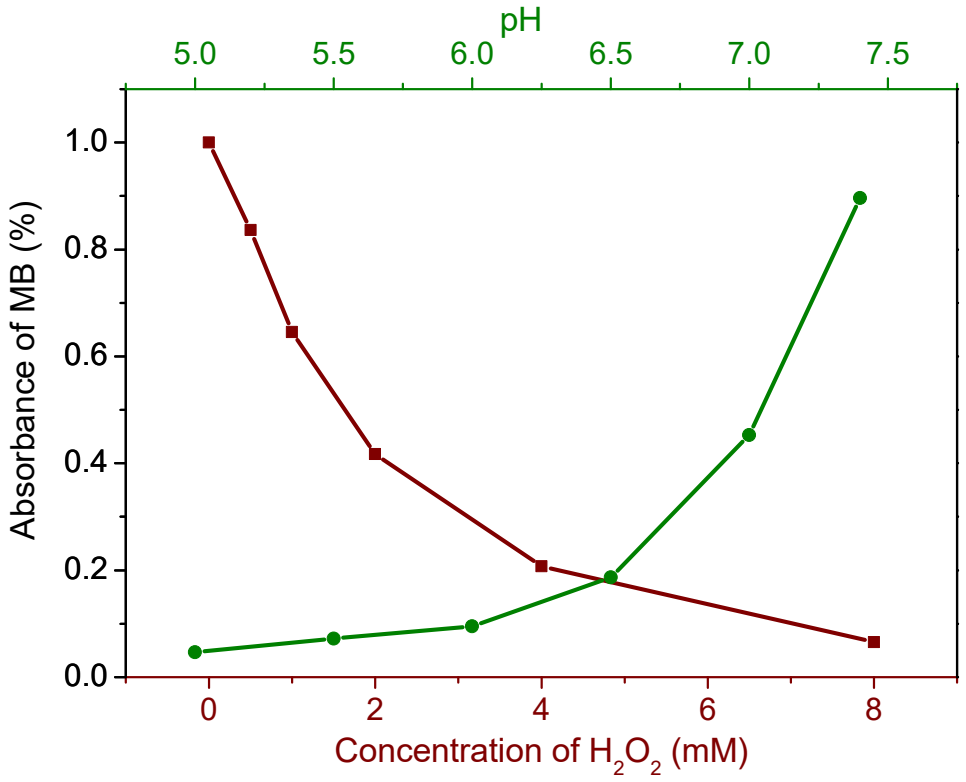


**Fig. S7** Absorbance of MB with 8mM $H_2O_2$, 0.4mM MDNs and 25mM $NaHCO_3$ in different conditions: In $Na_2HPO_4$-citric acid buffer from 5.0 to 7.4 (green line); In $Na_2HPO_4$-citric acid buffer pH 5.0 with different concentration of $H_2O_2$ (brown line).

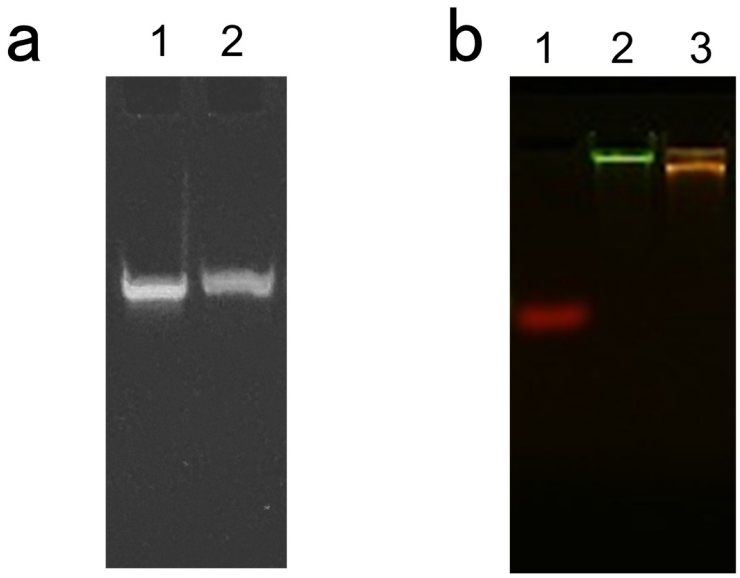


**Fig. S8** a) Native-polyacrylamide gel-electrophoresis (PAGE) images: Short template ssDNA (4 μM) (T68-c) *lane 1*: without and *lane 2*: with $Mn^{2+}$ (5.4 mM); b) Agarose gel electrophoresis (AGE)

images: *lane 1*: cDNA-Cy5; *lane 2*: lssDNA; *lane 3*: hybridization product of cDNA-Cy5 and lssDNA .

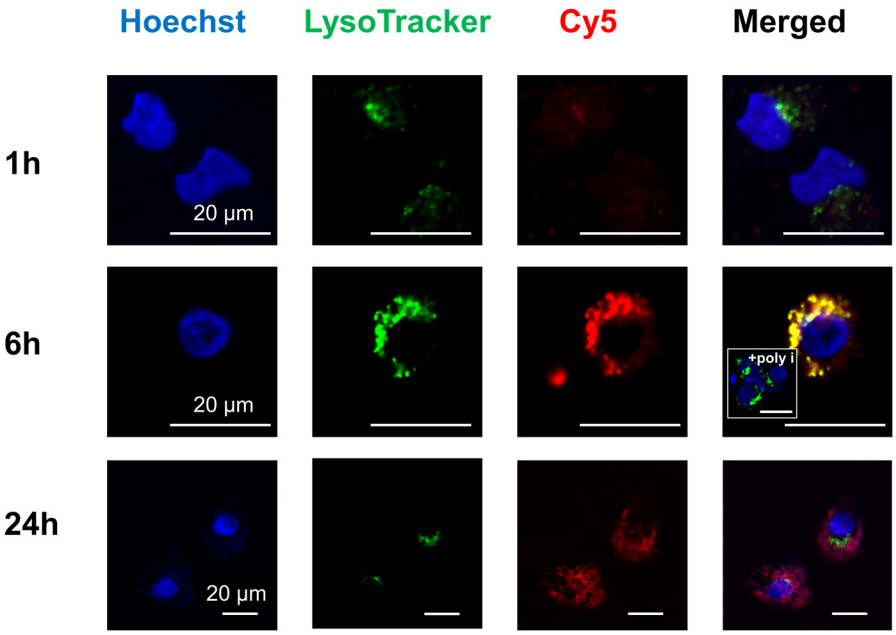


**Fig. S9** High-resolution LSCM image of the intracellular distribution of MDNs-Cy5 at different time points. Nucleus, lysosomes and MDNs were marked by Hoechst (blue), Lyso Tracker (green) and Cy5 (red), respectively. Scale bar: 20 μm.

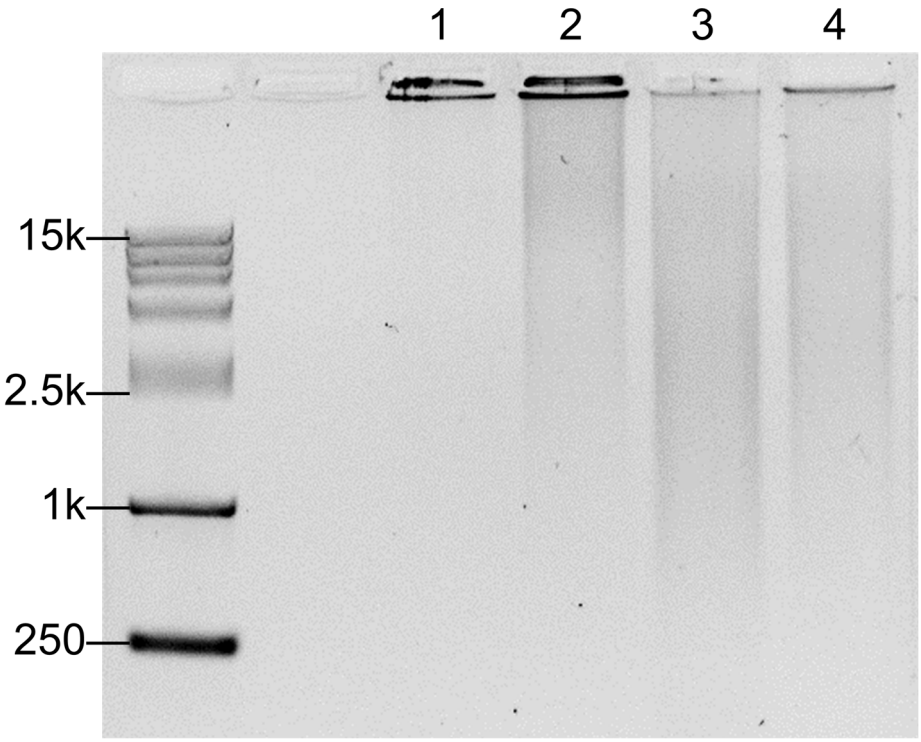


**Fig. S10** Agarose gel electrophoresis (AGE) image of DOX@MDNs to show the degree of degradation at different conditions: lane1 pH = 7.4, lane2 pH = 6.0, lane3 pH = 7.4 + 10 mM $H_2O_2$, lane4 pH = 6.0 + 10 mM $H_2O_2$.

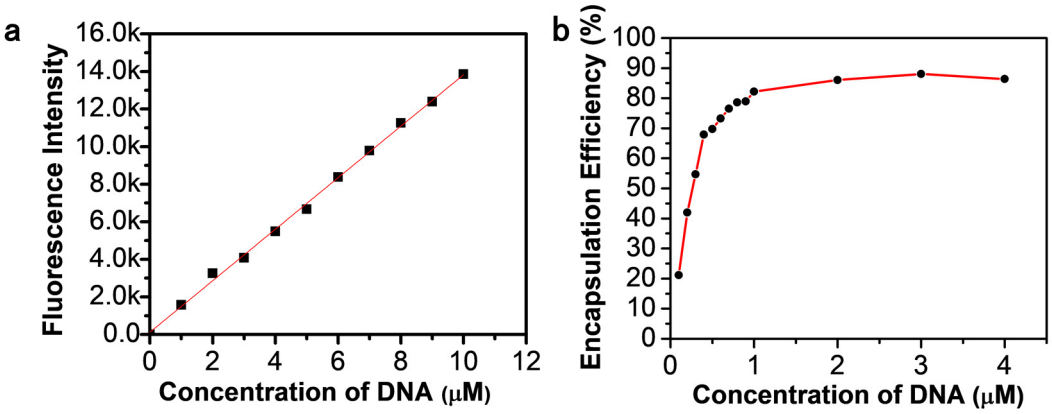


**Fig. S11** a) Standard curve of free DOX; b) DOX encapsulation curve of MDNs.

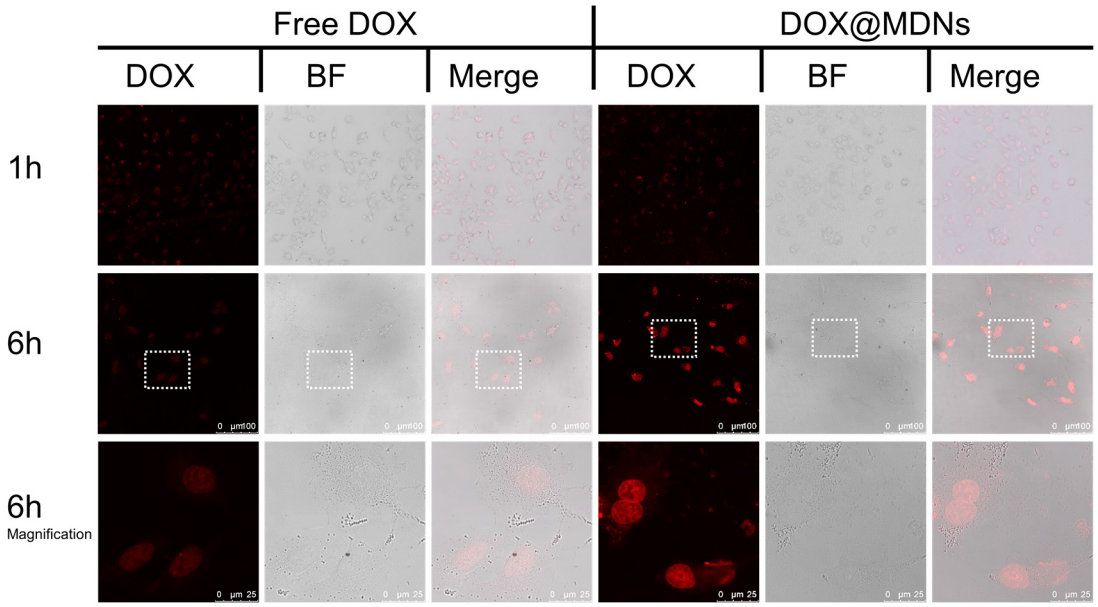


**Fig. S12** LSCM images of A549 cells after 1 and 6 h incubating with free DOX or DOX@MDNs. DOX delivered by MDNs completely entered the cell nucleus after a 6-h incubation, showing the high cell uptake efficiency.

**Notes and References**


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