Supporting Information

Mitochondrial-targeted tetrahedral DNA nanostructures for doxorubicin delivery and enhancement of apoptosis

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EXPERIMENTAL SECTION

Materials

All DNA oligonucleotides (S1, S2, S3, S4, Table S1) and DNA oligonucleotides modified with Cy5 or azide (S2-Cy5, S1-N_3, S3-N_3, S4-N_3, Table S1) were synthesized by Sangon Biotech (Shanghai) Co. Ltd. (Shanghai, China). KLA peptide with a terminal alkynyl ($_D$-(KLAKLAK)$_2$-alkynyl, MW=1,619.09, KLA-Pra) was purchased from GL Biochem (Shanghai) Co. Ltd. (Shanghai, China). GelRed DNA gel stain solution was purchased from Biotium (USA). DOX hydrochloride (DOX·HCl) were purchased from Zhejiang Hisun Pharmaceutical (Zhejiang, China). NIH/3T3 fibroblast cells and mice breast cancer cells (4T1) were purchased from the Chinese Academy of Science Cell Bank for Type Culture Collection (Shanghai, China). Dulbecco's modified Eagle's medium (DMEM), Roswell Park Memorial Institute 1640 medium (RPMI 1640), fetal bovine serum (FBS), penicillin–streptomycin and click reaction kit were

purchased from Life Technologies Co. (Gibco, USA). Lyso-tracker® Green, Mito-tracker® Green, antibody anti-cytochrome c and cell mitochondria isolation kit were purchased from Beyotime Institute of Biotechnology (Nanjing, China). Caspase 3 or 9 colorimetric assay kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

Synthesis and preparation of KLA-TDNs and 3KLA-TDNs

The N_3-TDNs (S1-N_3, S2 or S2-Cy5, S3, S4, 2 μM) and $3N_3$-TDNs (S1-N_3, S2 or S2-Cy5, S3-N_3, S4-N_3, 2 μM) were synthesized by the same method. The KLA-TDNs and 3KLA-TDNs were synthesized with the help of a click reaction kit. TDNs modified with azide (2 μM) and KLA-Pra (2 μM) were mixed in click kit buffer at a ratio of 1:4. The mixture were then put into a constant-temperature shaker at 37 °C and 150 rpm overnight. Afterward, the reaction mixture was transferred to a centrifuge tube (MW > 10 kDa). The unreacted peptide and the dissociative ions were removed by centrifugation, and the yielding DNA nanoparticles were resuspended in TM buffer.

Cell culture

NIH 3T3 cells were cultured in Dulbecco's Modified Eagel's Medium (DMEM) supplemented with 10% FBS and 1% penicillin–streptomycin in an incubator under 5% CO_2 at 37 °C. 4T1 cells were cultured in Roswell Park Memorial Institute 1640 (RPMI-1640) supplemented with 10% FBS and 1% penicillin–streptomycin in an incubator under 5% CO_2 at 37 °C.

Western blot and PCR analysis

For western blot assay, the 4T1 cells were collected, rinsed with PBS, and suspended in EBC 250 lysis buffer. Protein concentration of cells was determined by Bio-Rad protein assay reagent (Bio-Rad, USA). An equal amount of protein was separated on SDS-PAGE gel, transferred and hybridized to primary antibody and secondary antibody for subsequent detection.

For PCR assay, total RNA was collected after isolation using Trizol reagent (Invitrogen, USA) according to the instructions. cDNA was synthesized by total RNA as template in the cDNA synthesis reaction mixture (SuperScript II; Invitrogen) at 42 °C, denaturation at 70 °C and then quick cooling. qPCR was performed in a CFX96 Real-time PCR system (Bio-Rad, USA). The reactions were incubated in 96-well plates, and then by 40 cycles at 95 °C for 30 s, 60 °C for 30 s. β-Actin was used as an endogenous control for normalization. Primer sequences are follows:

Primer	**Primer sequences （5’ - 3’）**
p21 Fwd	5’-CGAGAACGGTGGAACTTTGACTTC-3’
p21 Rev	5’-AGAGTGCAAGACAGCGACAAGG-3’
p53 Fwd	5’-CGGCTCTGAGTATACCACCATCCA-3’
p53 Rev	5’-TTCTTCTTCTGTACGGCGGTCTCT-3’
Bax Fwd	5’-CCAGGATGCGTCCACCAAGAAG-3’
Bax Rev	5’-GCTCACGGAGGAAGTCCAGTGT-3’
Bcl-2 Fwd	5’-CCTGTGGATGACTGAGTACCTG-3’
Bcl-2 Rev	5’-AGCCAGGAGAAATCAAACAGAGG-3’
β-Actin Fwd	5’-TCACTATTGGCAACGAGCGGTTC-3’
β-Actin Rev	5’-GCACTGTGTTGGCATAGAGGTCTT-3’

Table S1. DNA oligonucleotides used in TDNs.

ssDNA	Sequence (5' – 3')
S1	ACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAGAGCCGCCATAGTA
S2	TATCACCAGGCAGTTGACAGTGTAGCAAGCTGTAATAGATGCGAGGGTCCAATAC
S3	TCAACTGCCTGGTGATAAAACGACACTACGTGGGAATCTACTATGGCGGCTCTTC
S4	TTCAGACTTAGGAATGTGCTTCCCACGTAGTGTCGTTTGTATTGG ACCCTCGCAT
S1-N_3	N3-ACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAGAGCCGCCATAGTA
S2-Cy5	Cy5-TATCACCAGGCAGTTGACAGTGTAGCAAGCTGTAATAGATGCGAGGGTCCAATAC
S3-N_3	N3-TCAACTGCCTGGTGATAAAACGACACTACGTGGGAATCTACTATGGCGGCTCTTC
S4-N_3	N3-TTCAGACTTAGGAATGTGCTTCCCACGTAGTGTCGTTTGTATTGG ACCCTCGCAT

Results

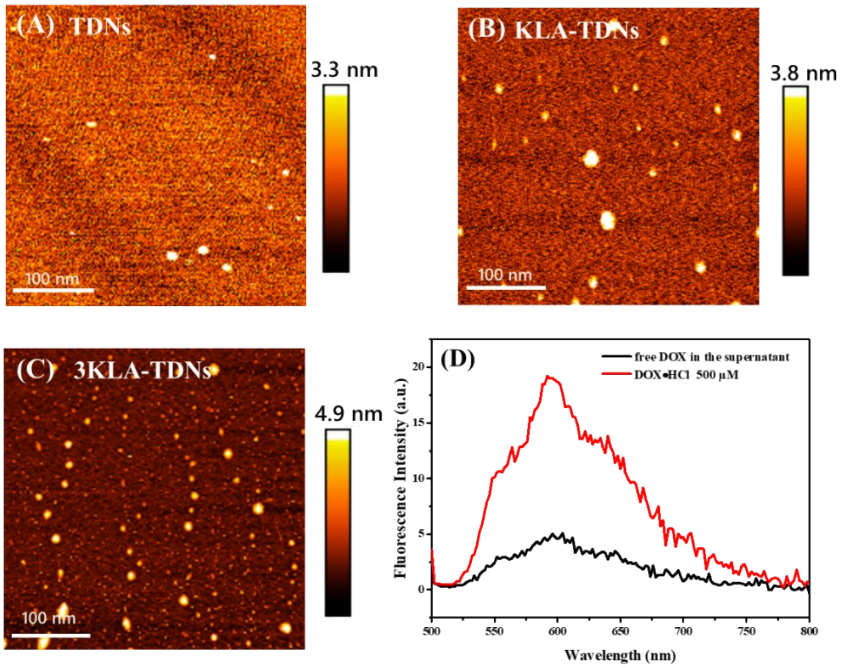

Figure S1. Atomic force microscope images of (A) TDNs, (B) KLA-TDNs, (C) 3KLA-TDNs. (D) Fluorescence spectra of DOX·HCl (500 μM) and the free DOX in the supernatant. DOX·HCl (500 μM) was mixed with TDNs (1 μM) at 37 °C and stirring 150 rpm for 3 h. Centrifugation (10,000 rpm, 10 min) gave the yielded DOX-encapsulating DNA nanostructures (TDNs/DOX) as a red sediment in the bottom of centrifuge tube and free DOX·HCl was dissolved in the supernatant.

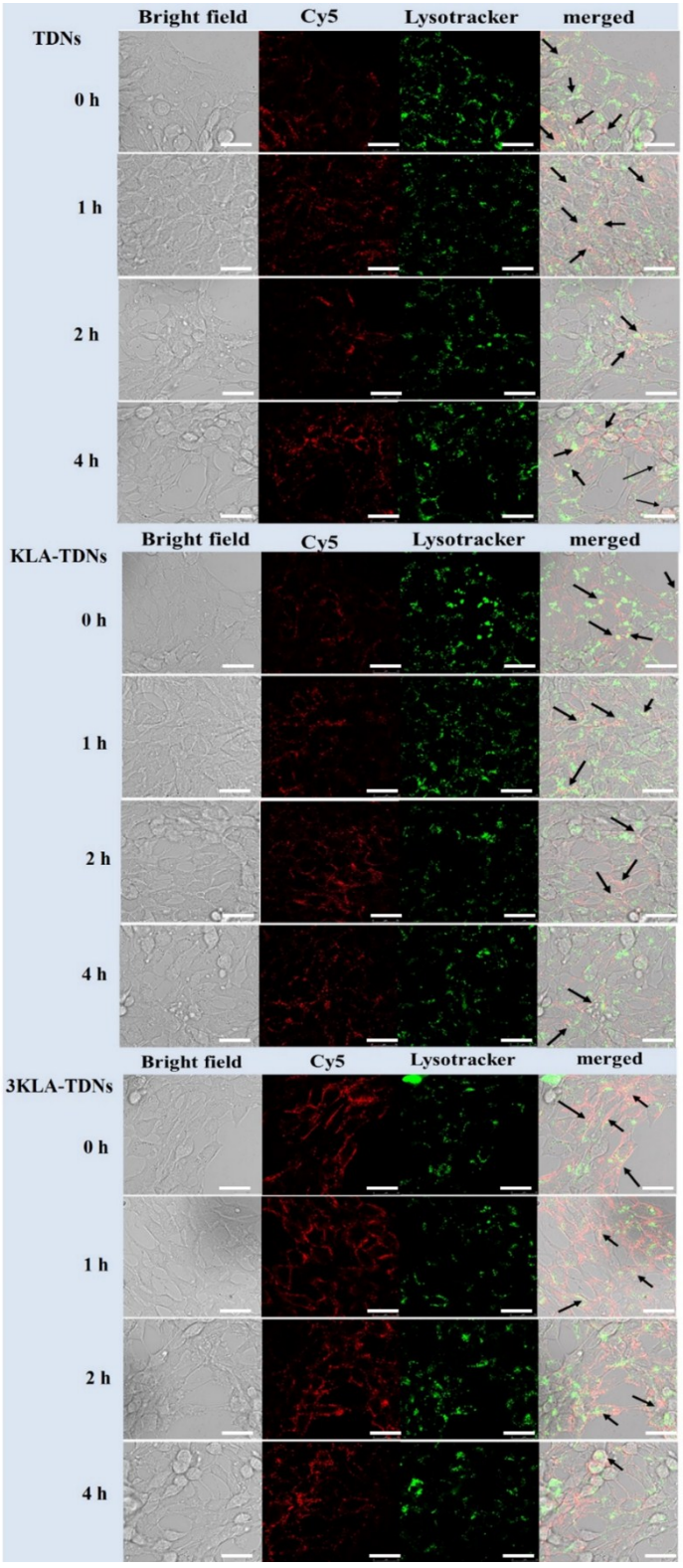

Figure S2. The CLSM images of endosomal escape capacity in 4T1 cells. Yellow spots in the merged pictures denote the colocalization of the TDNs, KLA-TDNs and 3KLA- TDNs within endosomal compartments. The arrow indicated the yellow overlapping area. The scale bar was 25 μm.

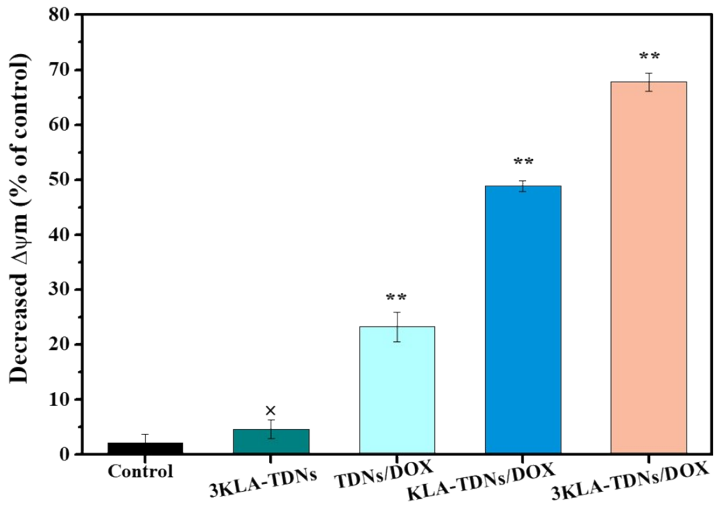

Figure S3. The change in mitochondrial membrane potential Δψm of 4T1 cells by flow cytometry. (means ± SD (n =3) , $^{\times}P > 0.05$ and $^{**}P < 0.01$ versus the control group)