Supporting Information

Receptor-targeting Drug and Drug Carrier for Enhanced Killing Efficacy against Non-muscle-invasive Bladder Cancer

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Experimental Section

Uptake of FITC-mBSA or FITC-txCD47-BSA-14 by UMUC3 cells after pretreatment with B6H12 and IgG1 antibodies: Anti-CD47 monoclonal antibody, B6H12 and mouse IgG1 kappa isotype control antibody were purchased from Thermo Fisher Scientific, USA. UMUC3 cells ($3x10^5$ cells per well) were placed in a 24-well plate and cultured overnight. The culture medium was aspirated and fresh medium containing the antibodies at 0.01 μg/μL per well (as per supplier recommended concentration) was added to the cells and incubated for 1.5 h at 37 ^oC.[1] After incubation, the cells were gently washed 3 times with 1x PBS and further incubated with FITC-mBSA or FITC-txCD47-BSA-14 at a concentration of 0.12 μM for 2 h at 37 ^oC. The cells were then trypsinized, washed again with 1x PBS and resuspended in 0.5 mL of 1x PBS supplemented with 2% FBS. The cellular uptake was then analyzed using a flow cytometer (CytoFLEX LX, Beckman Coulter, USA).

Results

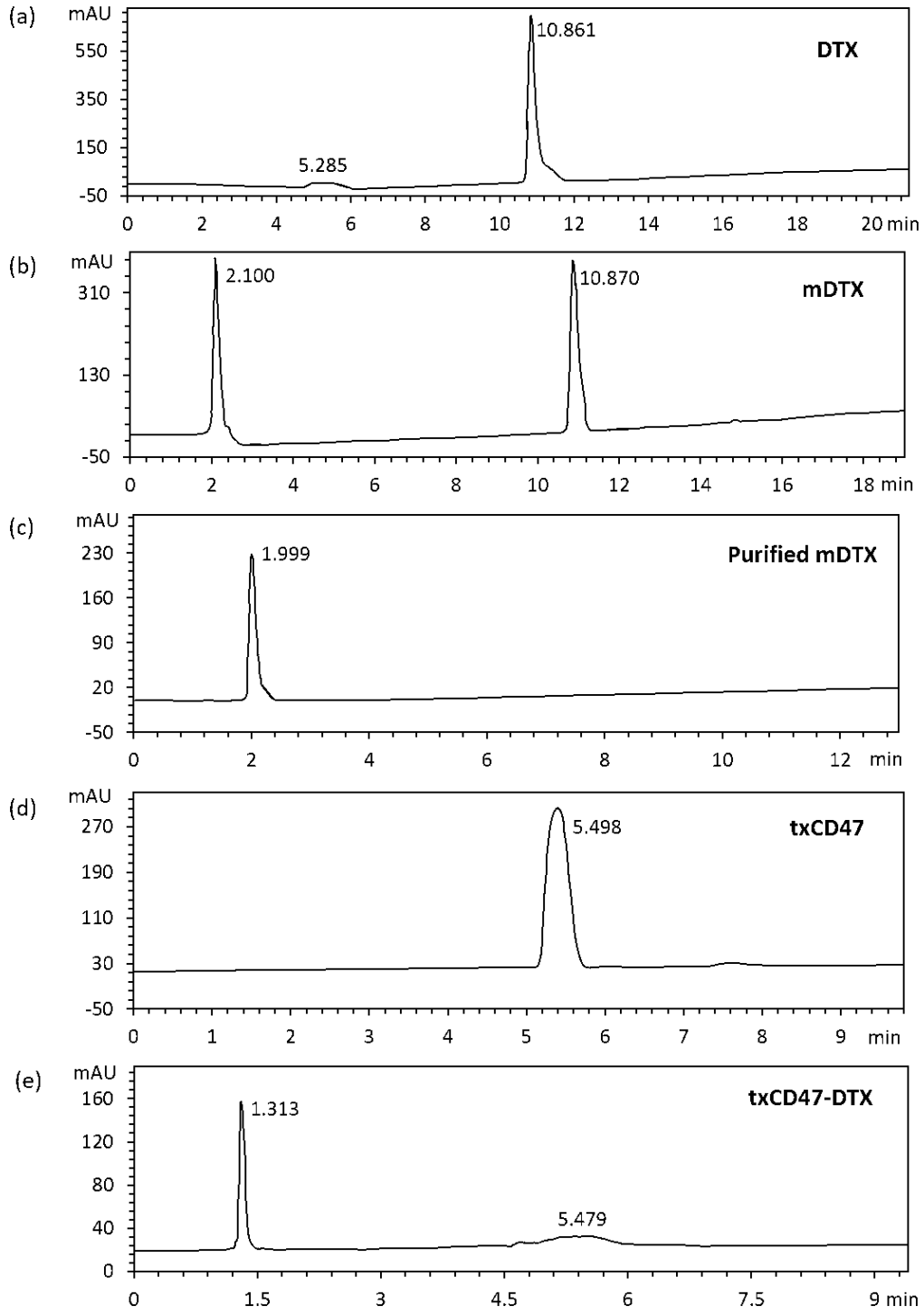

Figure S1. HPLC chromatograms of (a) DTX, (b) mDTX, (c) purified mDTX, (d) txCD47 and (e) txCD47-DTX

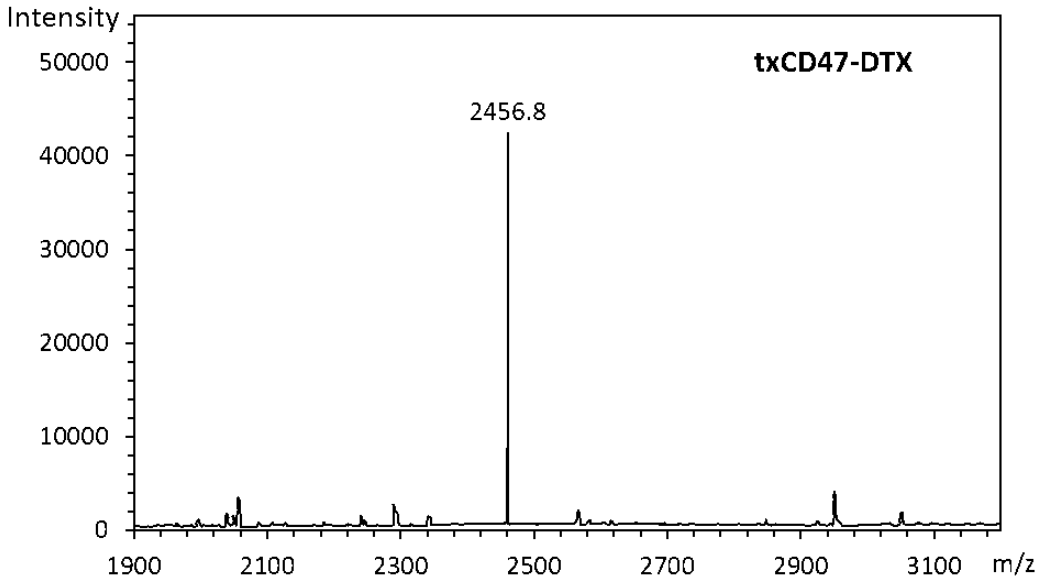

Figure S2. Mass spectrum of txCD47-DTX

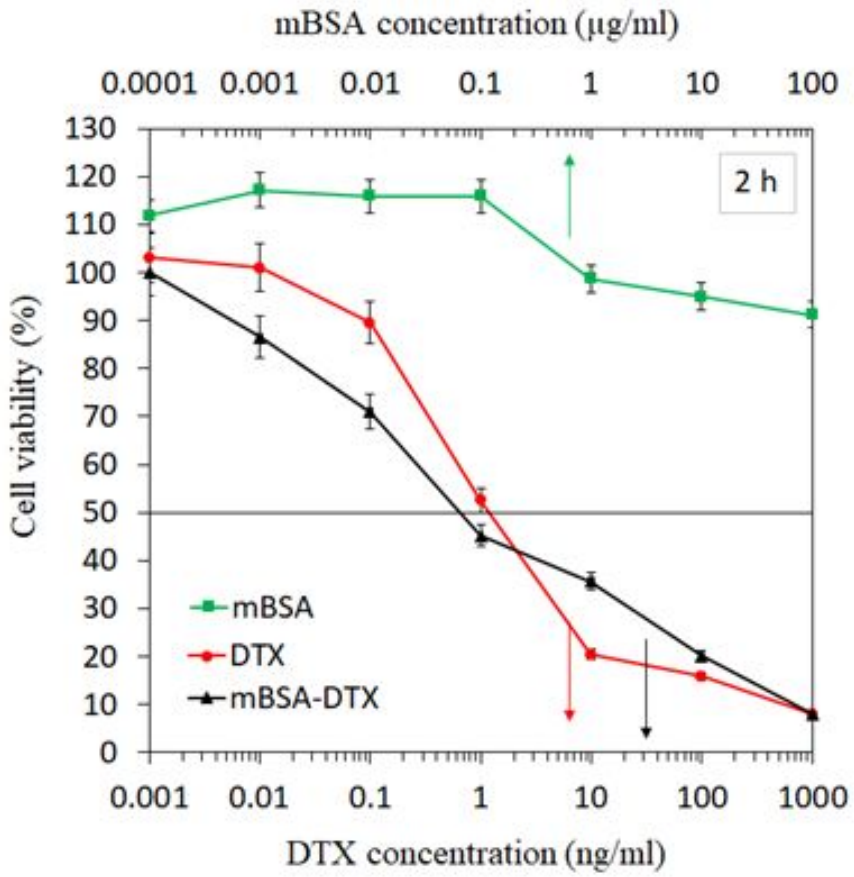

Figure S3. In vitro viability of UMUC3 cells after incubation with mBSA, free DTX and mBSA-DTX, for 2 h followed by 72 h in fresh medium. Control experiments were carried out without carrier or DTX.

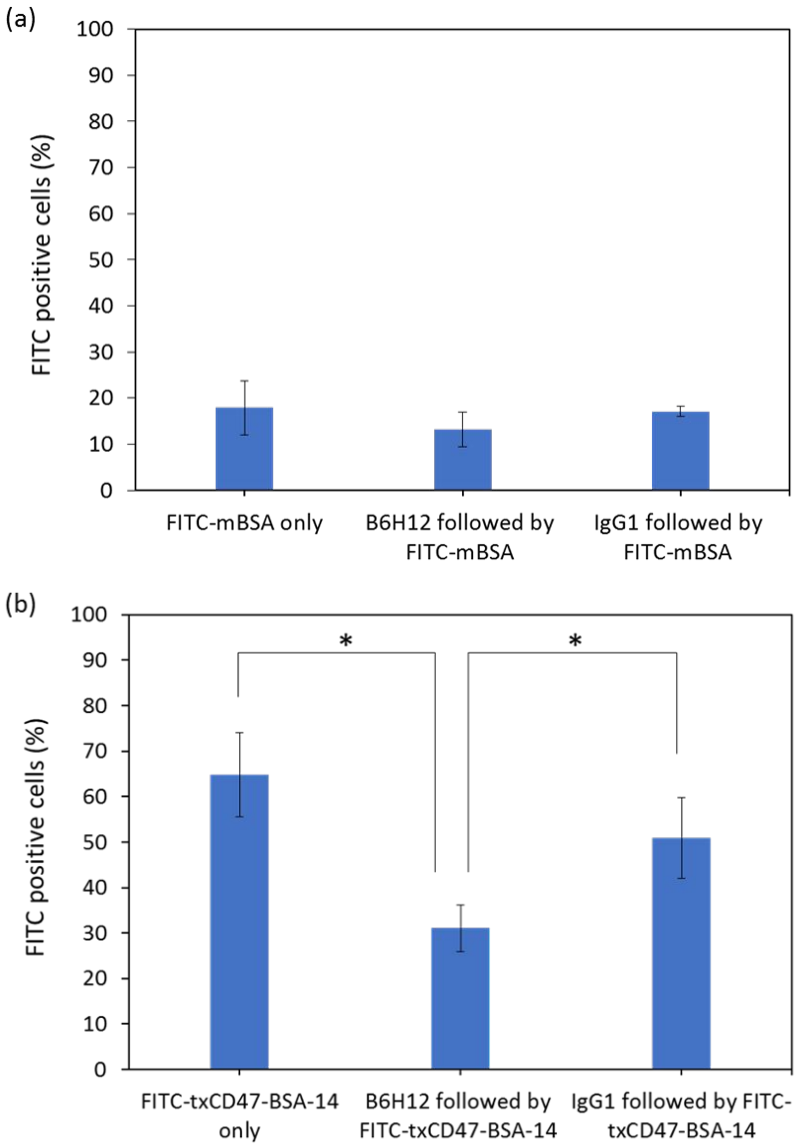

Figure S4. Cellular uptake of (a) FITC-mBSA with and without pretreatment with B6H12 or IgG1 antibodies, and (b) FITC-txCD47-BSA-14 with and without pretreatment with B6H12 or IgG1 antibodies. * Significant difference ($p < 0.05$) between samples indicated.

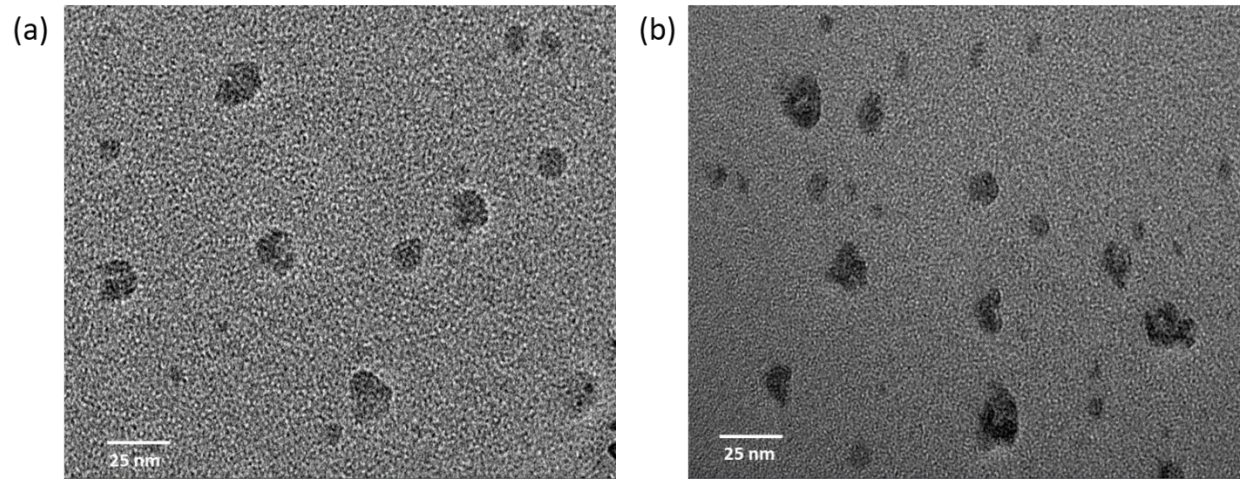

Figure S5. TEM images of (a) txCD47-BSA-14 and (b) txCD47-BSA-DTX-14.

Tables

Table S1. Fluorescence-positive cells (%) and mean fluorescence intensity of UMUC3 cells after incubation with APC x hu EGFR, FITC x hu CD47, and APC x hu FGFR3

	UMUC3 cells	
	Fluorescence-positive cells (%)	**Mean fluorescence intensity**
APC x hu EGFR	84.6 ± 4.6	3015 ± 556
FITC x hu CD47	80.2 ± 12.8	376 ± 172
APC x hu FGFR3	31.6 ± 15.2	1511 ± 881

Table S2. FITC-positive cells (%) and mean fluorescence intensity of UMUC3 cells after incubation with ~0.12 μM of FITC-mBSA, FITC-txCD47-BSA-4, FITC-txCD47-BSA-8, and FITC-txCD47-BSA-14 for 2 h

	UMUC3 cells	
	FITC-positive cells (%)	**Mean fluorescence intensity**
FITC-mBSA	29.1 ± 1.7	20.4 ± 1.9
FITC-txCD47-BSA-4	37.3 ± 1.3	20.8 ± 2.3
FITC-txCD47-BSA-8	52.1 ± 2.5	41.3 ± 9.4
FITC-txCD47-BSA-14	73.1 ± 5.7	149.1 ± 11.8

Reference

1. Kamerkar, S.; LeBleu, V.S.; Sugimoto, H.; Yang, S.; Ruivo, C.F.; Melo, S.A.; Lee, J.J.; Kalluri, R. Exosomes facilitate therapeutic targeting of oncogenic KRAS in pancreatic cancer. *Nature* **2017**, *546*, 498.