Supplementary Materials

Selective Intratumoral Drug Release and Simultaneous Inhibition of Oxidative Stress by a Highly Reductive Nanosystem and Its Application as an Anti-tumor Agent

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Table S1. Size, Polydispersity Index (PDI), Zeta Potential and the content of DTX of the DTX-VNS.

Days	4 ℃				25 ℃			
	d_n (nm)	PDI	ζ (mV)	C (mg/g)	d_n (nm)	PDI	ζ (mV)	C (mg/g)
1	108.5 ± 3.4	0.199	-37.6 ± 2.3	2.10	108.5 ± 3.4	0.199	-37.6 ± 2.3	2.10
7	109.5 ± 2.6	0.172	-37.3 ± 1.2	2.06	114.0 ± 2.7	0.134	-39.7 ± 1.4	2.05
14	109.5 ± 1.3	0.161	-32.9 ± 2.6	2.04	115.4 ± 0.7	0.108	-34.5 ± 1.4	2.03
30	119.9 ± 1.4	0.147	-34.1± 1.3	2.02	118.9 ± 2.0	0.130	-36.3 ± 1.2	2.00
60	113.5 ± 2.3	0.161	-40.5± 1.5	2.05	124.4 ± 4.3	0.142	-39.2 ± 0.9	2.03

The dn, PDI, ζ and C values represent the diameter, PDI, zeta potential and the content of DTX in DTX-VNS, respectively.

Table S2 The mean and standard deviation (SD) values for each of the hematologic variables.

Groups	WBC (10^9/L)	%NEUT (%)	%LYMPH (%)	%MONO (%)	%EO (%)	%BASO (%)	RBC (10^{12}/L)	Hb (g/dL)	Hct (%)	MCV (fL)	MCH (Pg)	MCHC (g/dL)	PLT (10^9/L)	RET (10^9/L)
Saline	3.87±1.06	13.8±5.1	83.0±3.5	2.2±1.3	1.0±0.4	0.0±0.0	6.83±0.31	13.2±0.4	37.3±1.2	54.6±0.9	19.3±0.4	35.4±0.3	859±51	232.2±51.2
Blank VNS	7.22±2.77	26.9±22.1	68.1±19.4	3.9±2.6	1.1±0.3	0.0±0.0	6.67±0.28	12.8±0.4	37.1±1.1	55.6±1.9	19.2±0.4	34.6±0.4	866±112	365.1±63.6
DTX-VNS N	1.90±1.87	23.2±1.0	70.7±2.0	5.2±1.6	0.0±0.0	0.9±0.6	5.16±0.67	10.7±0.9	32.3±2.1	63.0±3.9	20.8±1.0	33.1±0.6	683±135	934.7±33.5
DTX-VNS H	2.61±0.88	23.3±14.0	72.3±14.7	4.2±0.8	0.0±0.0	0.2±0.3	5.36±0.12	10.9±0.3	32.8±1.4	61.1±2.1	20.3±0.5	33.2±0.5	732±150	1,092.7±306.7
Taxotere N	3.49±0.54	8.6±4.3	83.1±2.4	7.8±3.0	0.0±0.0	0.5±0.3	4.79±0.67	9.8±1.3	29.7±3.4	62.2±2.5	20.5±0.7	33.0±0.7	554±143	729.7±236.4
Taxotere H	1.92±0.55	10.4±3.5	80.7±6.8	8.3±3.3	0.0±0.0	0.6±0.2	5.16±0.74	10.0±1.4	29.5±4.2	57.1±0.9	19.4±0.2	34.0±0.4	358±141	467.4±145.3

Table S3 The mean and standard deviation (SD) values for each of the blood biochemical variables.

Groups	TP g/L	Alb g/L	ALT U/L	AST U/L	TB μmol/L	ALP U/L	BUN mmol/L	Cr μmol/L	Glu mmol/L	K mmol/L	Na mmol/L	Cl umol/L	TG mmol/L	TC mmol/L	CK U/L
Saline	59.5±3.2	46.5±2.2	21.9±2.2	77.3±6.4	1.0±0.2	59±17	4.6±0.4	29±4	6.39±0.53	3.66±0.12	140±1	101.1±0.8	0.25±0.03	1.59±0.22	389±55
Blank VNS	61.3±2.5	45.0±3.2	75.9±76.2	147.7±62.1	1.3±0.1	98±9	5.8±1.6	35±8	6.57±0.17	3.65±0.26	140±1	100.3±1.3	0.22±0.11	0.83±0.07	390±161
DTX-VNS N	48.0±3.0	38.4±3.9	25.7±4.3	109.5±4.0	1.1±0.4	46±18	6.5±0.3	22±1	6.21±0.25	4.13±0.17	140±2	100.0±0.5	1.00±0.55	1.73±0.41	332±83
DTX-VNS H	49.7±2.4	36.8±0.5	50.6±11.6	146.7±27.6	1.2±0.2	41±4	8.3±1.9	24±3	7.00±1.45	4.09±0.15	140±1	97.8±2.2	0.29±0.12	2.48±0.57	341±43
Taxotere N	45.7±5.0	35.5±4.0	29.5±1.2	99.4±14.9	1.9±0.2	37±7	8.8±0.7	27±4	6.91±0.43	4.19±0.20	141±1	100.6±1.0	0.71±0.26	2.16±0.26	222±34
Taxotere H	50.2±3.9	35.5±3.8	28.6±3.0	134.0±31.2	2.0±0.6	59±28	10.0±1.9	23±6	6.81±1.05	4.30±0.36	140±2	101.4±1.4	0.33±0.11	2.23±0.30	289±66

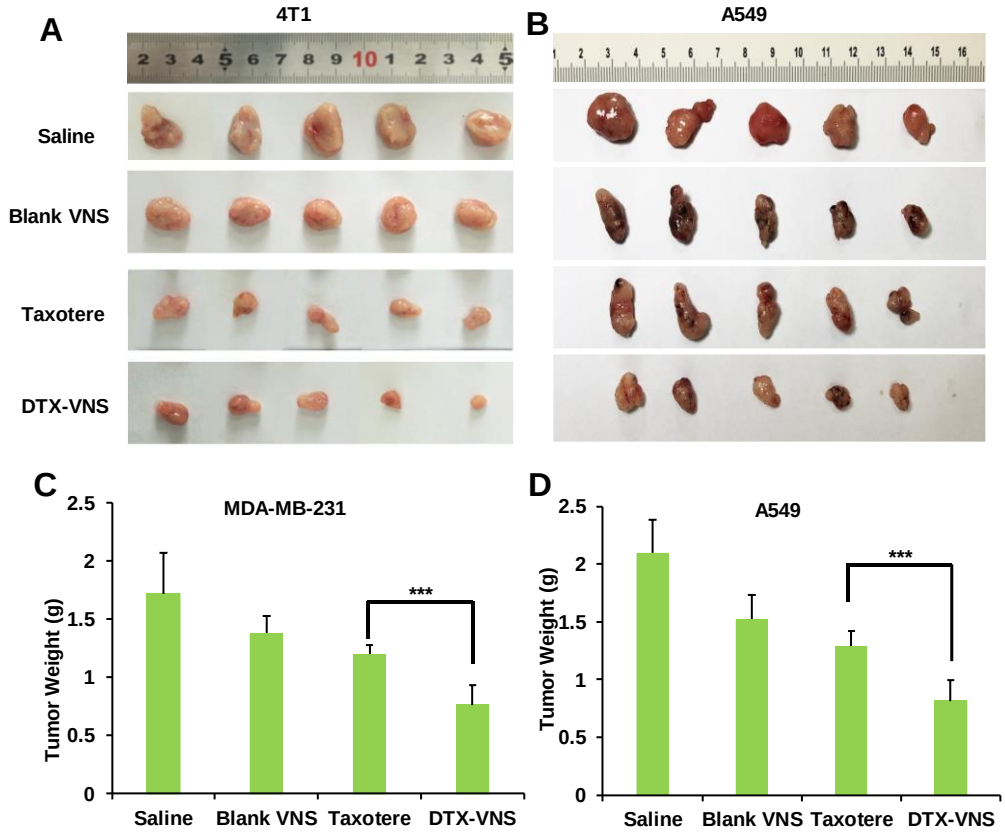

Figure S1 Representative tumor imaging of each group of 4T1 (**A**) and A549 (**B**) tumor models at the end of the study. Average tumor weight of each group of MDA-MB-231 (**C**) and A549 (**D**) tumor models at the end of the study. *p < 0.05, **p < 0.01, ***p <0.005.

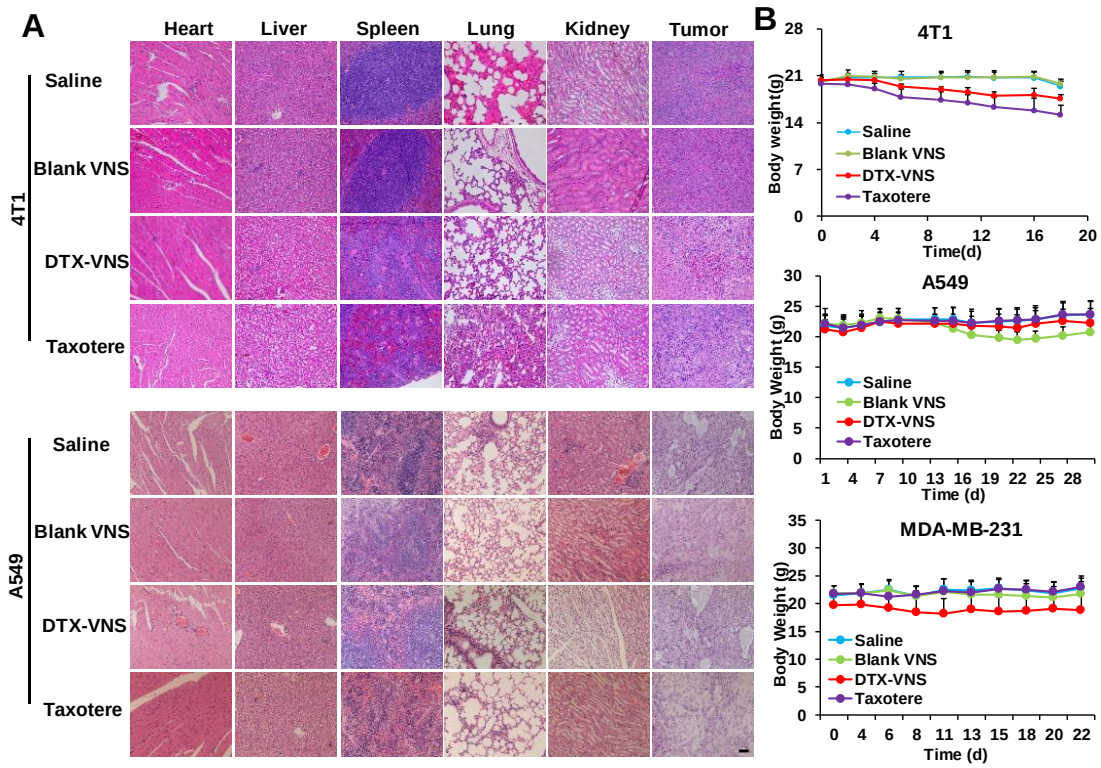

Figure S2 *In vivo* anticancer effect for the mice bearing different tumors. (**A**) Representative H&E staining images of various tissues (heart, liver, spleen, lung, kidney, and tumor) for four groups; the scale bar is 100 μm. (**B**) The mean body weight in the whole experiment process (n=5).

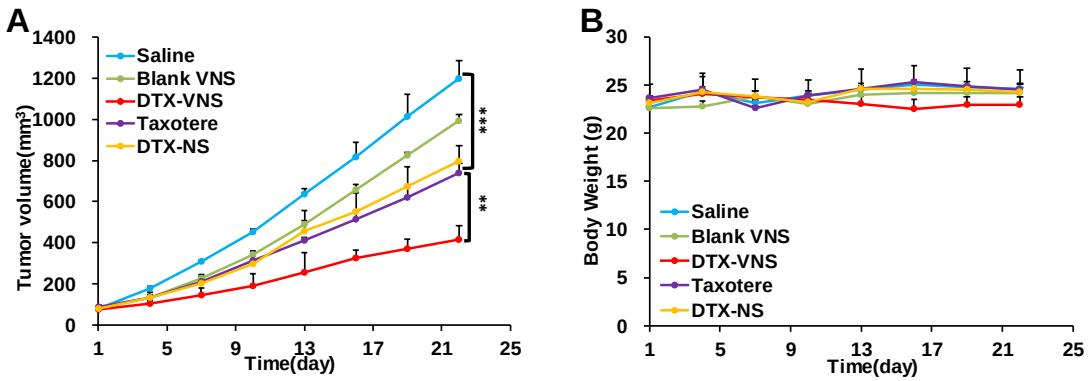

Figure S3 *In vivo* anticancer effect for the mice bearing 4T1 tumors. (**A**) Tumors growth curves. (**B**) The mean body weight in the whole experiment process. *p < 0.05, **p < 0.01, ***p <0.005.

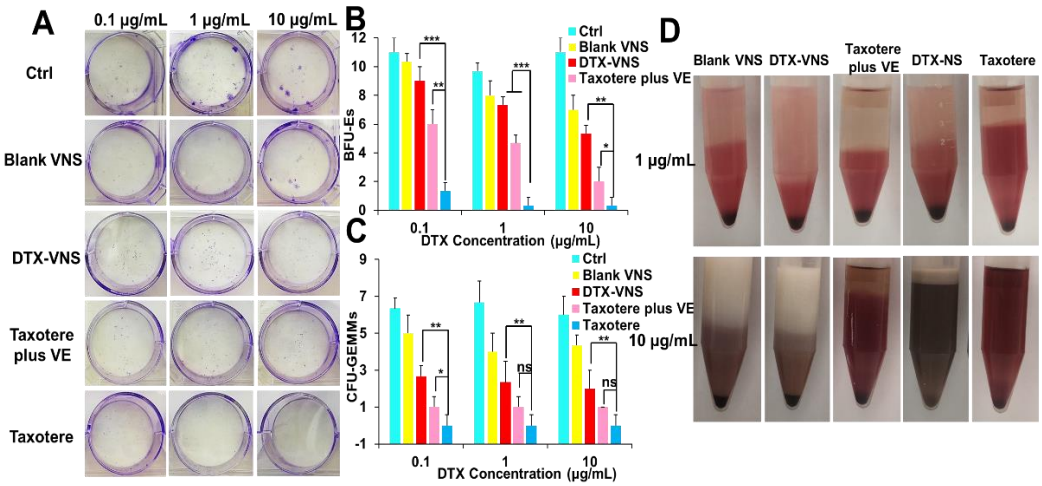

Figure S4 The detoxification of DTX-VNS. (**A**) Bone marrow cells from mice were incubated with Blank VNS, DTX-VNS, Taxotere, and Taxotere plus VE at the dose of 0.1 μg/mL, 1 μg/mL or 10 μg/mL for 14 days in methylcellulose-based media. Burst-forming units that generate erythroids (**B**) and colony forming units that generate granulocytes, erythroids, macrophages, and megakaryocytes (**C**) were counted using an inverted microscope. The data are presented as mean ± standard error from three experiments. *p < 0.05, **p < 0.01, ***p <0.005. (**D**) Photographs of defibrinated blood mixed with with Blank VNS, DTX-VNS, Taxotere plus VE, DTX-NS, and Taxotere in different concentration (high: 10 μg/mL; low: 1 μg/mL) for 3 hours.

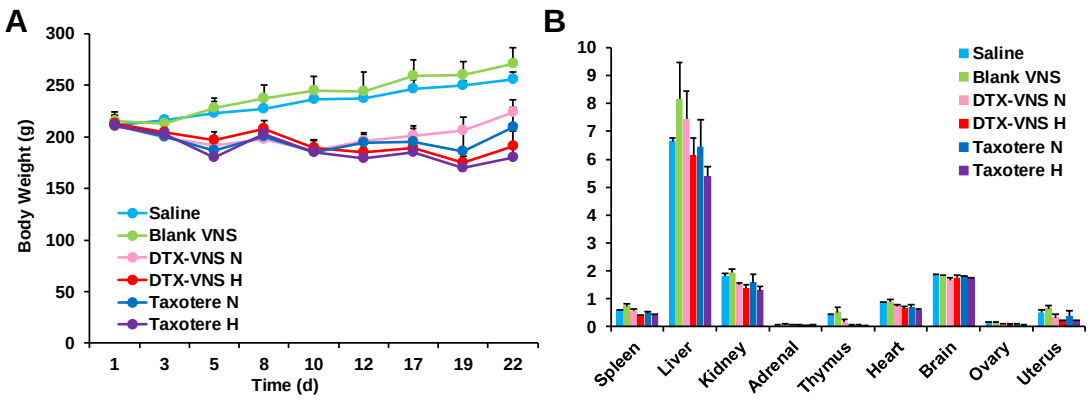

Figure S5 Toxicity evaluation. (**A**) Rats body weight changes under different treatment in the whole experiment process. (**B**) Relative organ weights of rats receiving different treatments for 22 days.

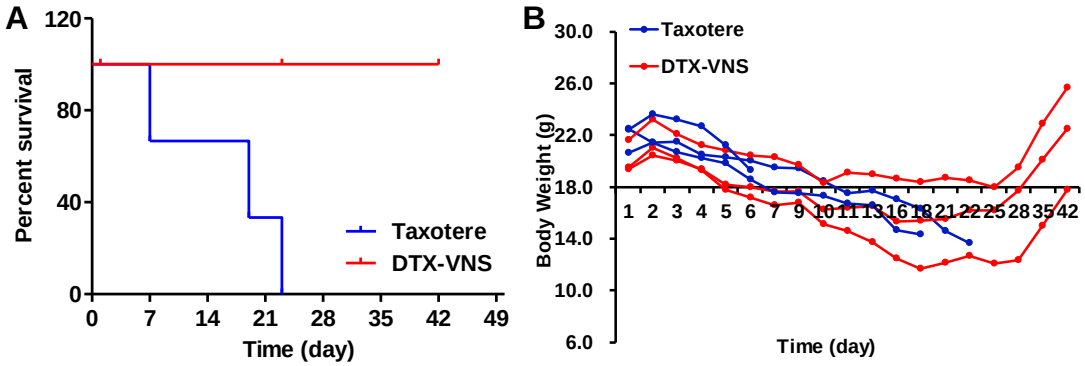

Figure S6 The tolerance dose study. (**A**) The percent survival of the mice after treated with Taxotere and DTX-VNS. (**B**) Individual body weight of the mice in the whole experiment process (n=3).

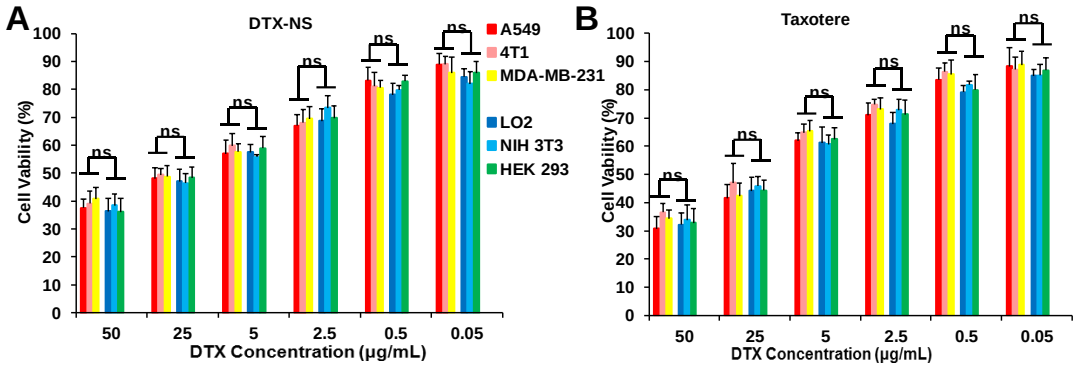

Figure S7 MTT assay of three tumor cells (A549, 4T1, MDA-MB-231) and three normal cells (LO2, NIH 3T3, HEK 293) treated with DTX-NS (**A**) or Taxotere (**B**) for 48 hours (n=5). All error bars are expressed as ± SD, n=5; *p < 0.05, **p < 0.01, ***p <0.005.

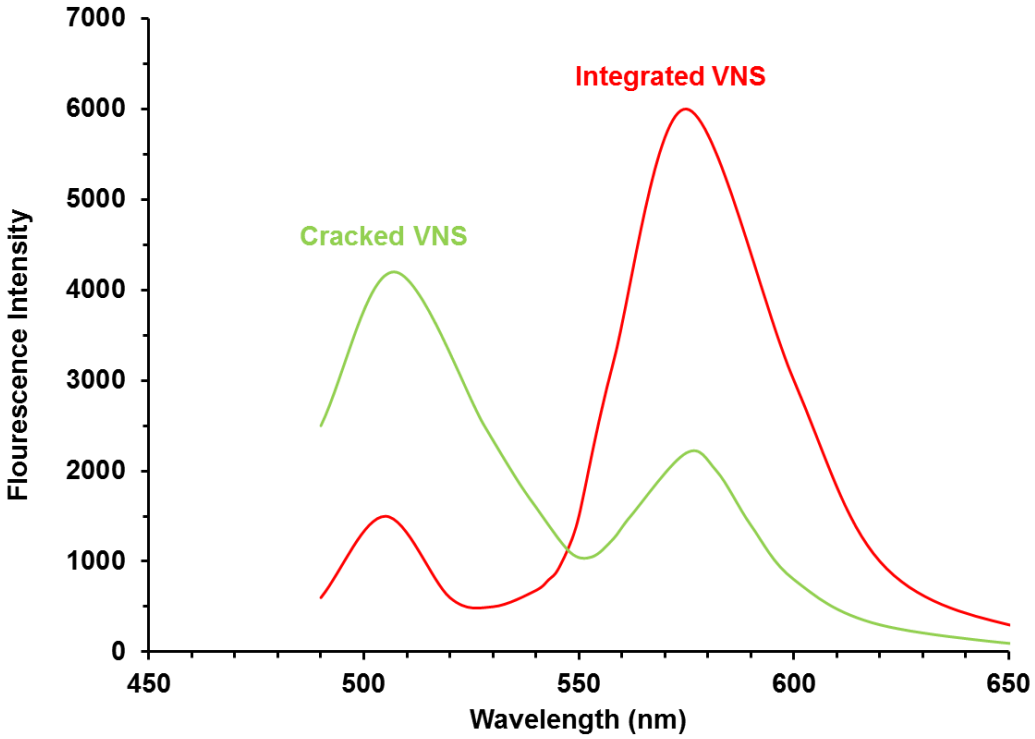

Figure S8 Spectra of DiO-, DiI-loaded DTX-VNS diluted by 100 X water (red curve, represented Integrated VNS) and 100 X alcohol (green curve, represented Cracked VNS), respectively.

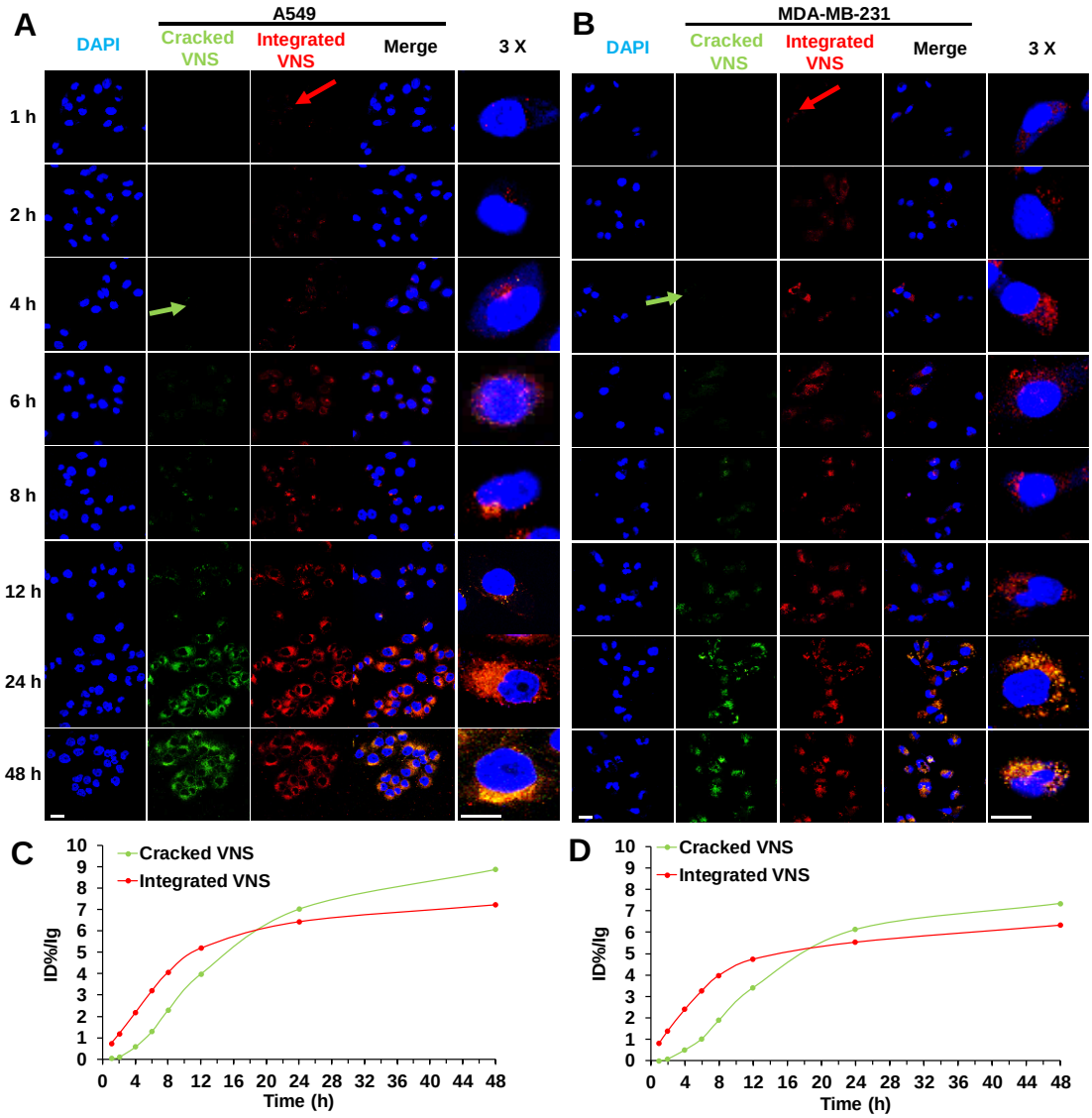

Figure S9 Selective release of DTX-VNS in tumor cells. Cellular uptake of DiO-, DiI-loaded DTX-VNS were incubated with A549 (**A**) and MDA-MB-231 (**B**) tumor cells at 37 °C for predetermined times (blue: cell nuclei; green: cracked VNS; red: integrated VNS), and the fluorescence images inspected by confocal fluorescence microscopy. The fluorescence intensity of cracked VNS and integrated VNS in A549 (**C**) or MDA-MB-231 tumor cells (**D**) was calculated based on (**A**) or (**B**) using ImageJ; the scale bar is 20 μm.

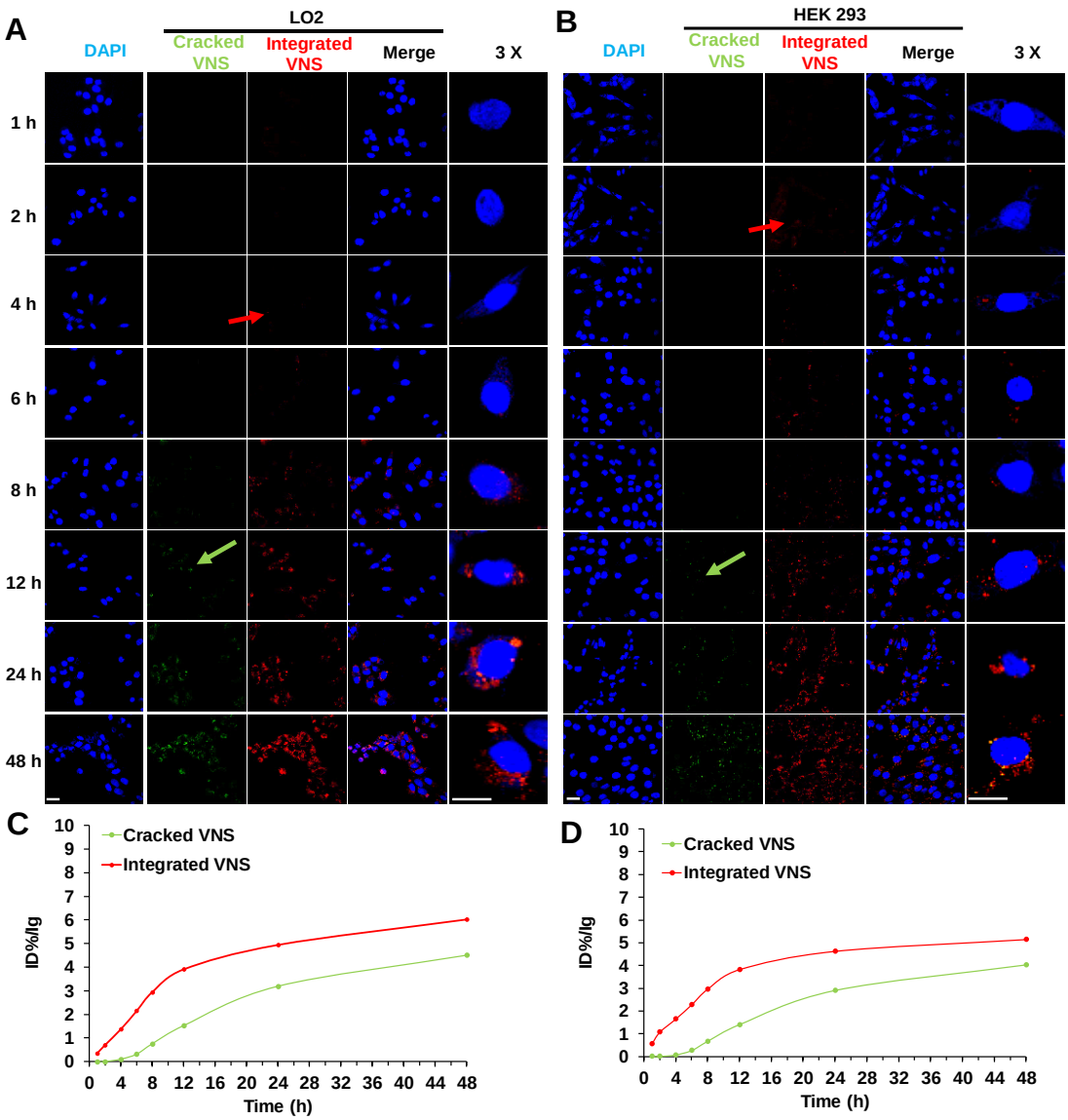

Figure S10 Selective release of DTX-VNS in normal cells. Celluar uptake of DiO-, DiI-loaded DTX-VNS were incubated with LO2 (**A**) and HEK 293 normal cells (**B**) at 37 °C for predetermined times (blue: cell nuclei; green: Cracked VNS; red: Integrated VNS), and the fluorescence images inspected by confocal fluorescence microscopy. The fluorescence intensity of Cracked VNS and Integrated VNS in LO2 (**C**) or HEK 293 normal cells (**D**) was calculated based on (**A**) or (**B**) using ImageJ; the scale bar is 20 μm.

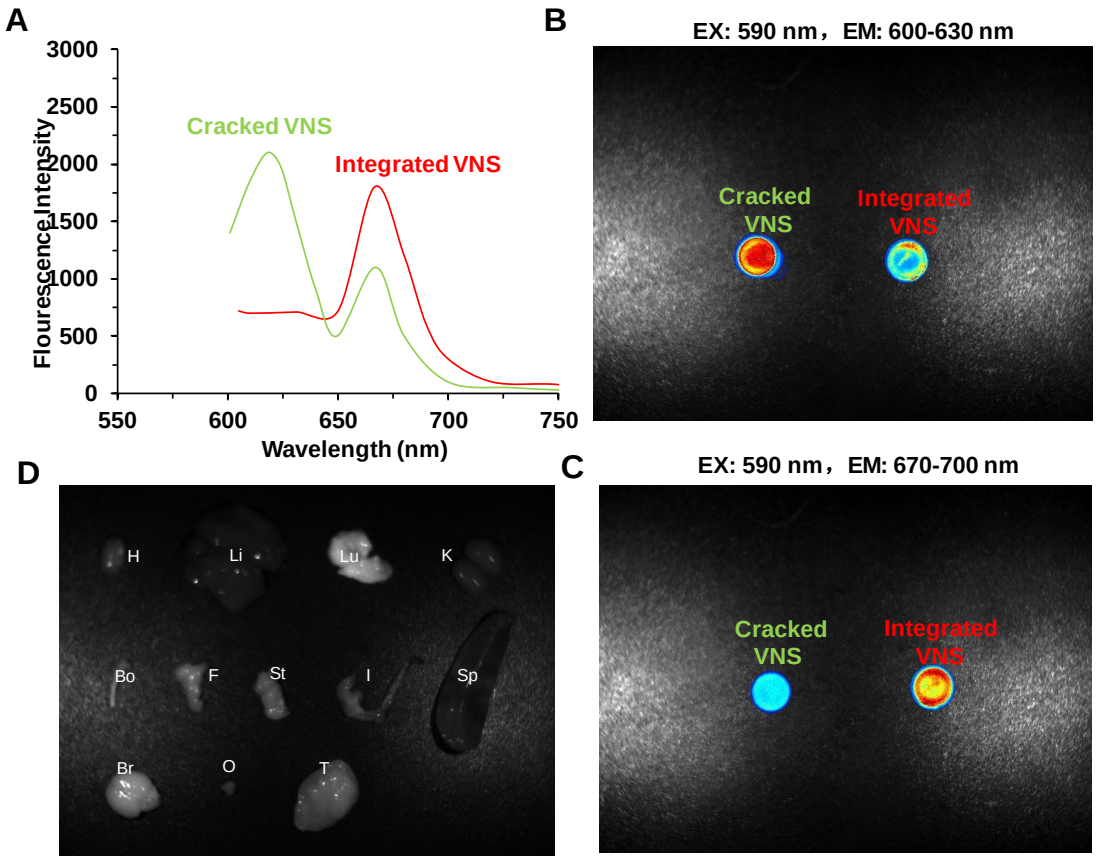

Figure S11 *In vitro* selective release of DTX-VNS. (**A**) Spectra of NR-, DiD-loaded DTX-VNS diluted by 100 X water (red curve, represented Integrated VNS) and 100 X alcohol (green curve, represented Cracked VNS), respectively. Fluorescence imaging of NR-, DiD-loaded DTX-VNS diluted by and 100 X alcohol (left, represented Cracked VNS) and 100 X water (right, represented Integrated VNS), respectively. The excitation was 590 nm, and the emission were 600-630 nm (**B**) or 670-700 nm (**C**). (**D**) Optical photos of various tissues (H: heart, Li: liver, Lu: lung, K: kidney, Bo: bone, F: fat, St: stomach, I: intestines, Sp: spleen, Br: brain, O: ovary, and T: tumor).

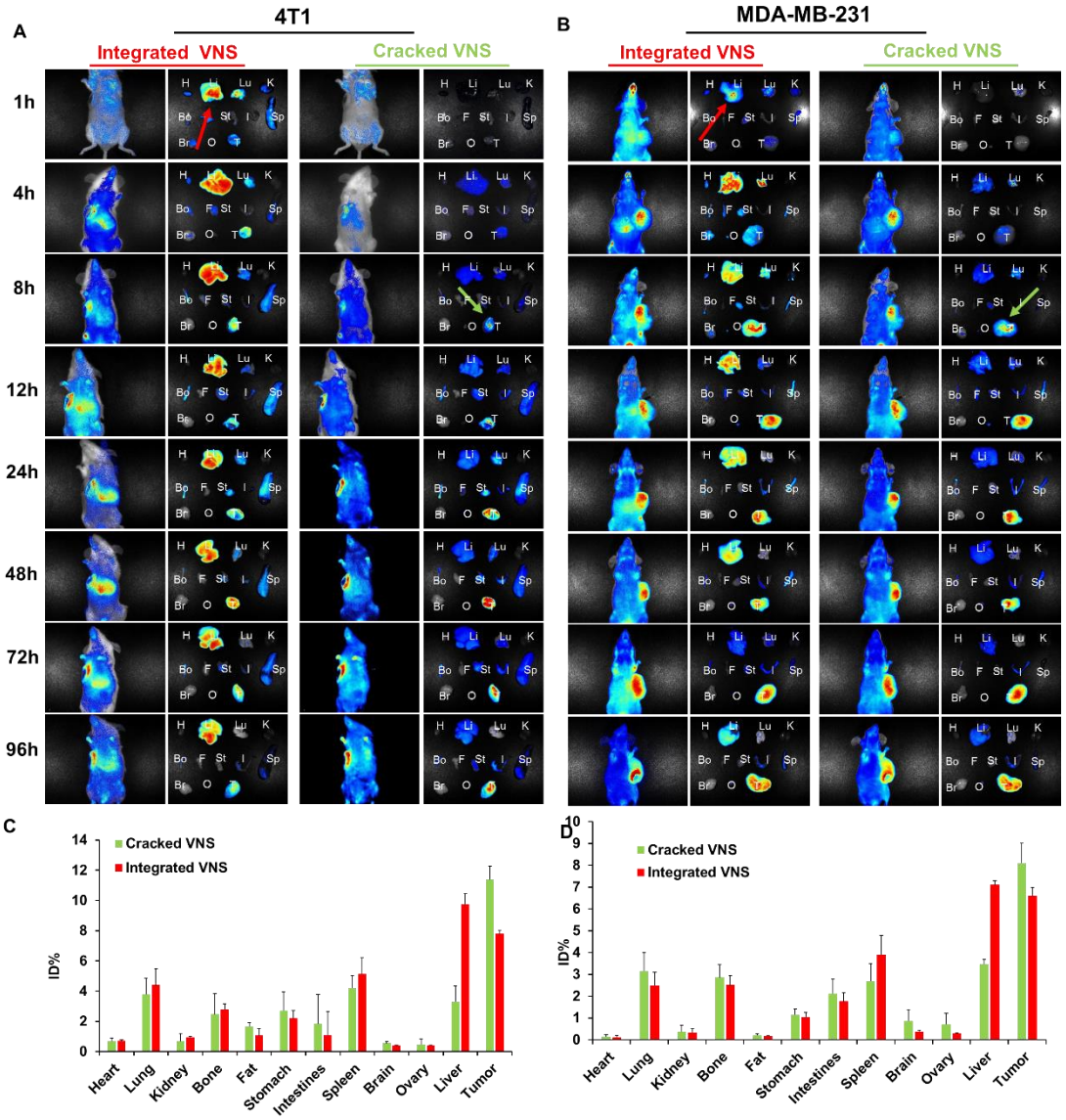

Figure S12 *In vivo* and ex *vivo* selective release of DTX-VNS. *In vivo* and *ex vivo* images of the mice bearing 4T1 tumors and various tissues (**A**) or MDA-MB-231 tumors and various tissues (**B**) were acquired at predetermined times post i.v. injection. %ID/g (percentage of the injected dose per gram of tissue) was analyzed to show the accumulation of cracked VNS and integrated VNS in 4T1 tumors and various tissues (**C**) or MDA-MB-231 tumors and various tissues (**D**) at 24 h after i.v. injection.

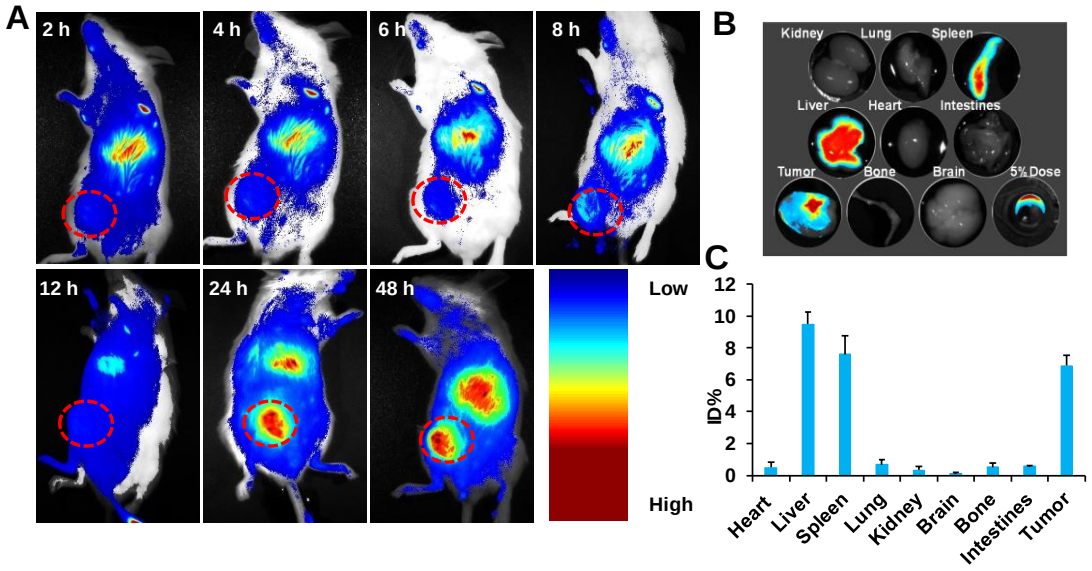

Figure S13 (**A**) *In vivo* fluorescence imaging of 4T1 tumor bearing mince i.v. injected with DiR-labeled DTX-VNS at different time points. (**B**) Fluorescent images of various tissues distribution of DTX-VeNP at 48 hours after injection. (**C**) Quantitative analysis of *ex vivo* fluorescence images in (**B**).