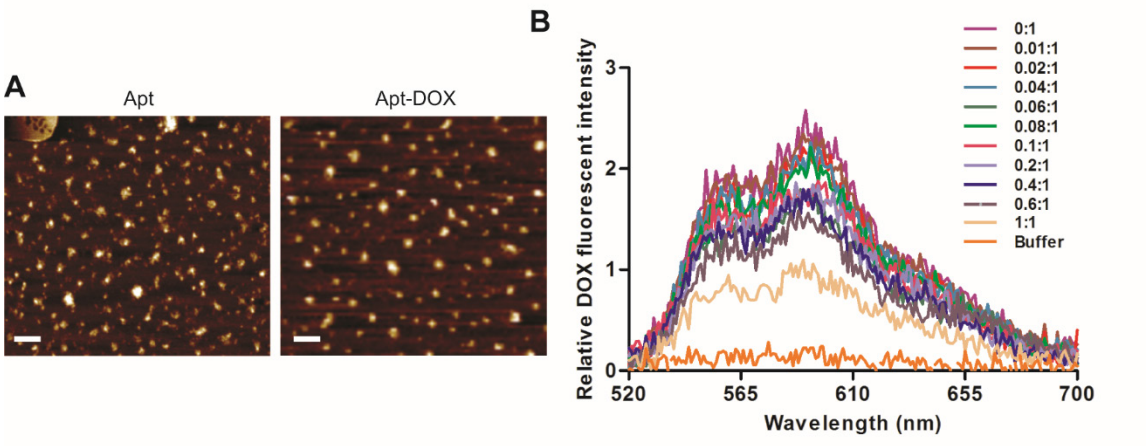
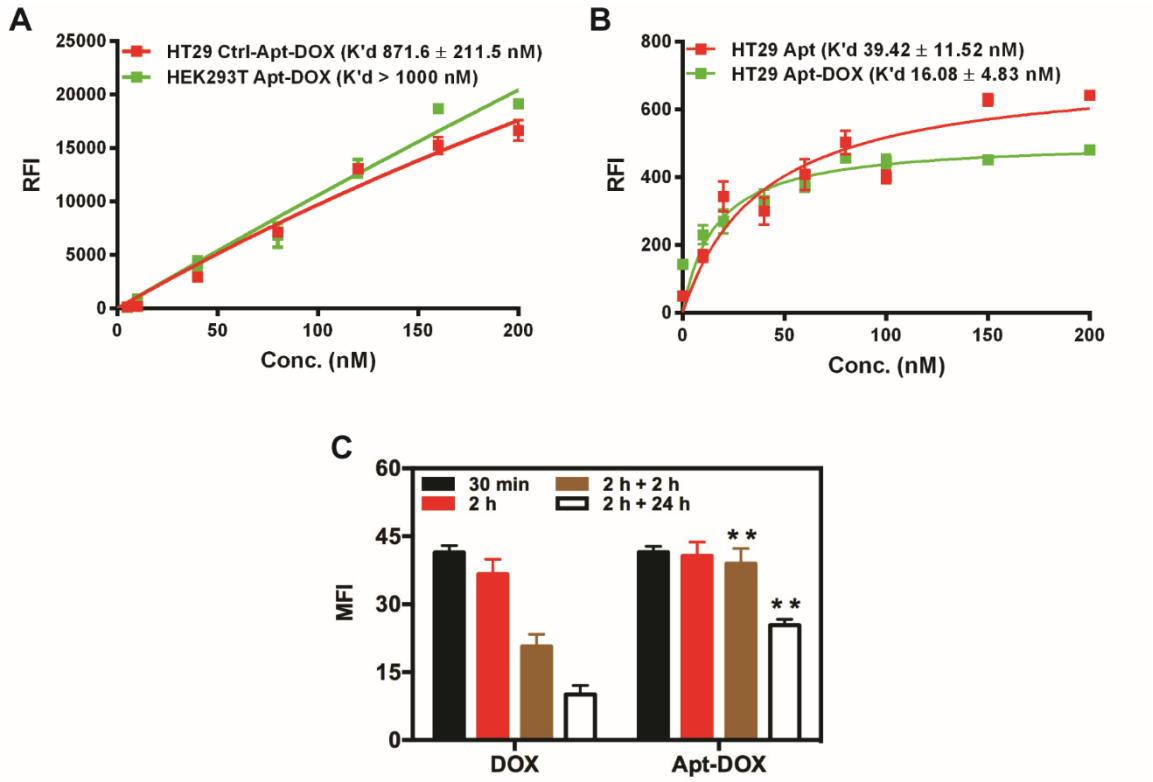
Supplementary Figure Legends
Supple. Fig. 1



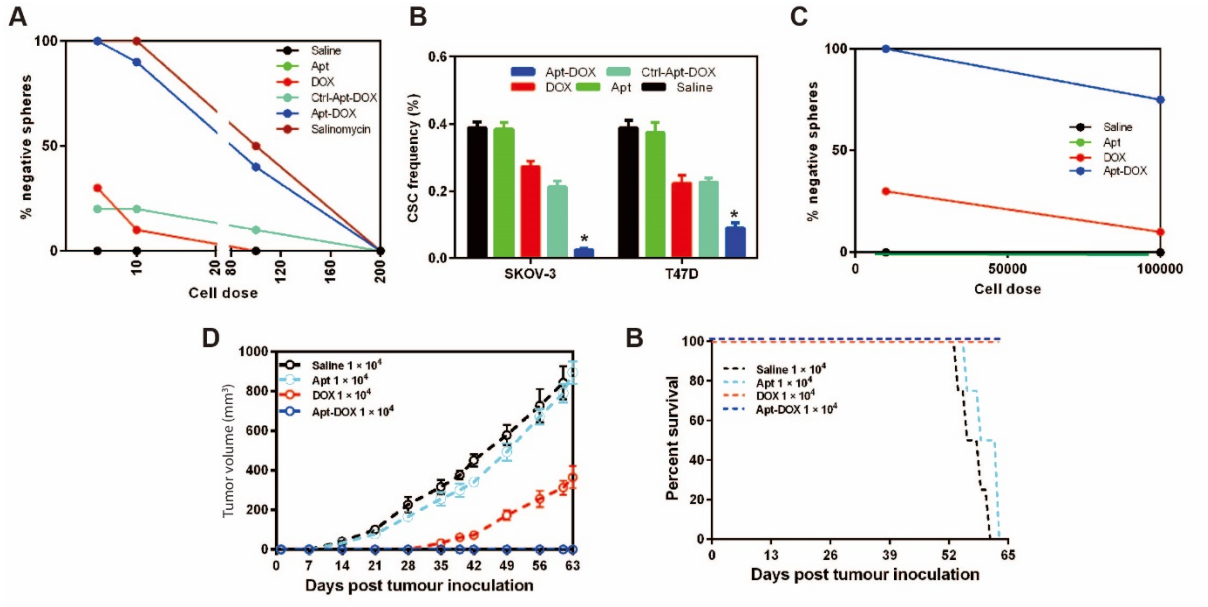
Supplementary Figure 1. Successful intercalation of DOX to aptamers. **(A)** AFM images of aptamer and the conjugation of aptamer and DOX. Scale bar is 10 nm. **(B)** Fluorescence quenching of DOX solution (10 μM) with increasing molar ratios of the aptamer: DOX (from top to bottom: 0, 0.01, 0.02, 0.04, 0.06, 0.08, 0.1, 0.2, 0.4, 0.6, 1 and buffer) as determined by scanning fluorescence spectroscopy. Data shown are means ± SEM. (n=3).

Supple. Fig. 2



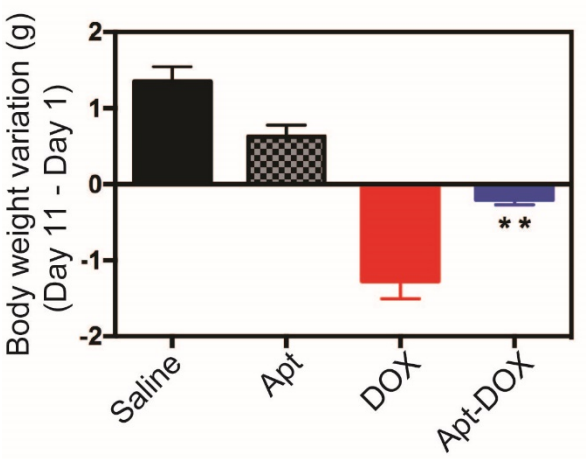
Supple. Figure 2. Specific and enhanced delivery of DOX to target cells by EpCAM Apt-DOX conjugate. (**A-B**) Uncompromised binding affinity and specificity of the EpCAM aptamer after DOX intercalation. The *Kd* of aptamer-DOX was evaluated using flow cytometry with fluorescently labelled Apt-DOX conjugates with concentrations ranging from 0 to 200 nM. (**A**) The binding of Apt-DOX or Ctrl-Apt-DOX to EpCAM-negative cell line HEK293T and HT29 cells, respectively. (**B**) The binding of the free aptamer and Apt-DOX to HT29 cells. (**C**) Quantitative analysis of aptamer-guided delivery of DOX to the nuclei of HT29 cells after incubating cells with Apt-DOX conjugates (1.5 μM of DOX equivalent) at 37 °C. Scale bar is 5 μm. Data shown are means ± SEM. (n=3). $^{**}P < 0.01$ compared with free DOX administration groups (two-tailed Student's *t-test*).

Supple. Fig. 3



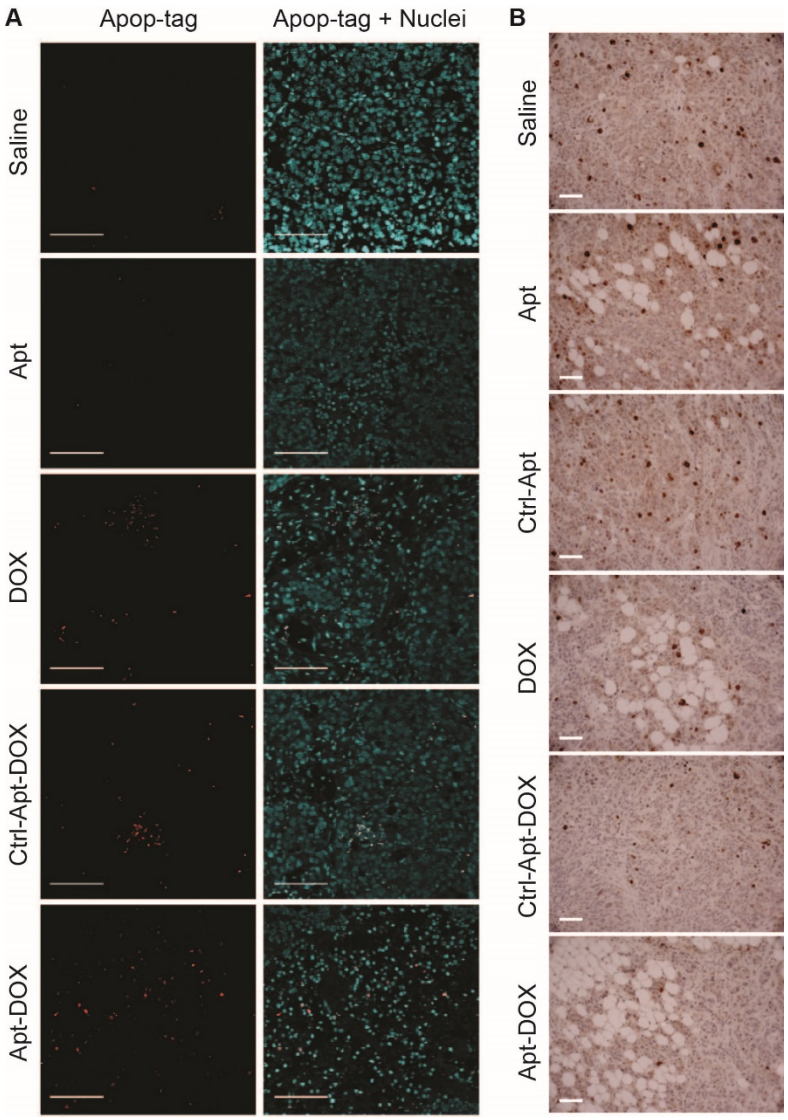
Supple. Figure 3. Quantification of the elimination of CSCs *in vitro* and *ex vivo*. **(A)** Limiting dilution assay of HT29 cells after *in vitro* tratment. **(B)** The percent of CSC frequency of SKOV-3 and T47D breast cancer cells based on *in vitro* limiting dilution assay. **(C)** Limiting dilution assay of HT29 cells after *ex vivo* tratment. Data shown are means ± SEM. (n=3, unless indicated otherwise). **(D)** Tumour growth of colorectal tumours in mice inoculated with 1×10^4 cells/mouse following treatment with various agents as indicated. **(E)** Survival curves of NOD/SCID mice-bearing xenograft tumours treated as described. Data shown are means ± SEM. (n=3, unless indicated otherwise).

Supple. Fig. 4



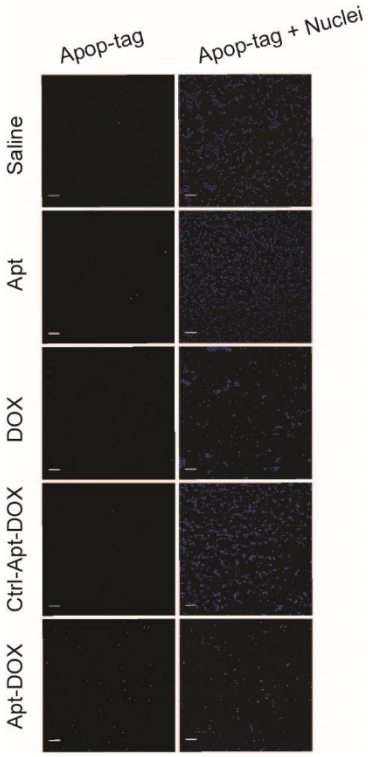
Supple. Fig. 4 Aptamer-guided DOX delivery reduced gross adverse effects. Body weight variation of tumour-bearing mice between day 1 and day 11 after treatment as indicated were recorded. Mice were treated with 1.5 μM of Apt-DOX/mouse or an equivalent dose of free DOX. Data shown are means ± SEM. (n=3, unless indicated otherwise). ** $P < 0.01$ compared with mice receiving free DOX.

Supple. Fig. 5



Supple. Figure 5. Apt-DOX treatment enhanced apoptosis and inhibited proliferation of HT29 xenograft tumour. NOD/SCID mice bearing HT29 xenograft tumours with a volume of 50 mm^3 were treated as indicated. **(A)** Representative of confocal micrographs of TUNEL assay of cells with illustrated treatments. **(B)** Representative images of Ki67 assay on HT29 xenograft tumour sections with illustrated treatments. Scale bar is 100 μm**.**

Supple. Fig. 6



Supple. Fig. 6 Representative of confocal micrographs of TUNEL assay of cells with illustrated treatments. Scale bar is 100 μm.

Supple. Tables

Supple. Table 1. *In vitro* limiting dilution assay of HT29 colorectal tumour cells.

Groups	Cell numbers seeded/well	Tumour sphere incidence[†]	CSC frequency (95% CI)
Saline	200	10/10	
	100	10/10	1 in 2.53
	10	10/10	(4.40-1.46)
	5	10/10	
	1	0/10	
Apt	200	10/10	
	100	10/10	1 in 2.53
	10	10/10	(4.40-1.46)
	5	10/10	
	1	0/10	
DOX	200	10/10	
	100	10/10	1 in 5.04
	10	9/10	(8.53-2.98)
	5	7/10	
	1	0/10	
Ctrl-Apt-DOX	200	10/10	
	100	9/10	1 in 12.06
	10	8/10	(22.67-6.41)
	5	8/10	
	1	0/10	
Apt-DOX	200	10/10	
	100	6/10	1 in 84.39
	10	1/10	(143.07-49.78)
	5	0/10	
	1	0/10	
Salinomycin	200	10/10	
	100	5/10	1 in 104.68
	10	0/10	(178.39-61.43)
	5	0/10	
	1	0/10	

Supple. Table 2. CSC frequency of ovarian and breast tumour cells treated by DOX and Apt-DOX and other controls *in vitro* with limiting dilution assay.

	Groups	Saline	Apt	DOX	Ctrl-Apt-DOX	Apt-DOX
CSC frequency (95% CI)	SKOV-3	1 in 2.53 (5.53-1.16)	1 in 2.53 (5.53-1.16)	1 in 3.61 (7.58-1.72)	1 in 4.75 (9.98-2.26)	1 in 57.54 (124.50-26.60)
	T47D	1 in 2.52 (5.48-1.16)	1 in 2.52 (5.48-1.16)	1 in 4.55 (9.01-2.30)	1 in 4.55 (9.01-2.30)	1 in 11.87 (23.72-5.94)

Supple. Table 3. *Ex vivo* limiting dilution assay of single suspension cells after *in vitro* treatment.

Groups	Cell numbers injected	Tumour incidence†	Latency (days)‡	CSC frequency (95% CI)
Saline	1×10^5	4/4	8	1 in 1
	1×10^4	4/4	16 – 18	(15582-1)
Apt	1×10^5	4/4	10	1 in 1
	1×10^4	4/4	18 – 19	(15582-1)
DOX	1×10^5	4/4	13	1 in 1
	1×10^4	4/4	31 – 32	(15582-1)
Apt-DOX	1×10^5	1/4	40	1 in 387857
	1×10^4	0/4	--	(2737697-54949)

†The number of tumours detected/number of mice received xenotransplantation.

‡Approximate number of days from tumour cell injection to the appearance of a tumour.

Supple. Table 4. Pharmacokinetic parameters of free DOX, PEGylated Apt-DOX and control PEGylated Apt-DOX after *i.v.* administration at a dose of equivalent to 5 mg/kg DOX.

Pharmacokinetic paramete	Free DOX	Ctrl-Apt-DOX	Apt-DOX
C_{max} (μg/mL)	2.53 ± 0.78	23.02 ± 3.16	25.67 ± 4.58
$t_{1/2}\alpha$ (h)	0.07 ± 0.02	0.71 ± 0.15*	0.87 ± 0.11*
$t_{1/2}\beta$ (h)	0.87 ± 0.25	7.13 ± 3.16*	7.73 ± 2.35*
MRT (h)	2.48 ± 1.39	15.49 ± 2.55*	16.25 ± 2.42*
AUC (h*h*μg/mL)	428.358 ± 156.32	2836.54 ± 1032.41**	3118.64 ±1537.72**
CL (mL/h/kg)	331.33 ± 87.54	37.07 ± 3.65*	30.36 ± 3.44*
Vss (mL/kg)	9404.75 ± 1578.32	470.31 ± 112.38**	372.92 ± 98.65**

Cmax, maximum plasma concentration; $t_{1/2}\beta$, elimination half-life; MRT, mean retention time; AUC, area under the plasma concentration-time curves; CL, total body clearance; Vss, steady state volume of distribution. *, $P < 0.01$; **, $P < 0.001$ compared with rats received free DOX (two-tailed Student's t-test).

Supple. Table 5. *In vitro* limiting dilution assay of colorectal tumour cells prepared from xenograft tumours after *in vivo* treatment.

Groups	Cell numbers seeded/well	Tumour sphere incidence[†]	CSC frequency (95% CI)
Saline	200	10/10	
	100	10/10	1 in 2.53
	10	10/10	(4.40-1.46)
	5	10/10	
	1	0/10	
Ctrl-Apt	200	10/10	
	100	10/10	1 in 2.53
	10	10/10	(4.40-1.46)
	5	10/10	
	1	0/10	
Apt	200	10/10	
	100	10/10	1 in 2.53
	10	10/10	(4.40-1.46)
	5	10/10	
	1	0/10	
DOX	200	10/10	
	100	8/10	1 in 28.98
	10	6/10	(55.30-15.18)
	5	4/10	
	1	0/10	
Ctrl-Apt-DOX	200	10/10	
	100	8/10	1 in 29.18
	10	5/10	(55.60-15.30)
	5	5/10	
	1	0/10	
Apt-DOX	200	5/10	
	100	0/10	1 in 525.67
	10	0/10	(1246.2-221.74)
	5	0/10	
	1	0/10	

[†]The number of tumour sphere detected/number of cell seeded.

Supple. Table 6. Aptamer-guided DOX delivery increased tumour latency and reduced CSC frequency in HT29 xenograft tumours.

Groups	Cell numbers injected	Tumour incidence†	Latency (days)‡	CSC frequency (95% CI)
Saline	1 x 10^5	4/4	7 – 9	
	1 x 10^4	4/4	18 – 23	1 in 417
	1 x 10^3	4/4	36 – 42	(1259-138)
	1 x 10^2	0/4	–	
Ctrl-Apt	1 x 10^5	4/4	7 - 10	
	1 x 10^4	4/4	20 – 25	1 in 417
	1 x 10^3	4/4	38 – 45	(1259-138)
	1 x 10^2	0/4	–	
Apt	1 x 10^5	4/4	8 – 10	
	1 x 10^4	4/4	21 – 24	1 in 417
	1 x 10^3	4/4	37 – 48	(1259-138)
	1 x 10^2	0/4	–	
DOX	1 x 10^5	4/4	13 – 16	
	1 x 10^4	3/4	27 – 34	1 in 3609
	1 x 10^3	3/4	50 –	(10942-1190)
	1 x 10^2	0/4	–	
Ctrl-Apt-DOX	1 x 10^5	4/4	12 – 17	
	1 x 10^4	3/4	26 – 30	1 in 4677
	1 x 10^3	2/4	50 –	(13769-1589)
	1 x 10^2	0/4	–	
Apt-DOX	1 x 10^5	2/4	30 – 35	
	1 x 10^4	1/4	47 –	1 in 108037
	1 x 10^3	0/4	–	(360667-32362)
	1 x 10^2	0/4	–	

†The number of tumours detected/number of cell injected.
‡Approximate number of days from tumour cell injection to the appearance of a tumour.