Supplementary methods

Preparation of aptamer-siRNA chimera. The longer and shorter strands of the PEGylated chimeras were synthesized by TriLink BioTechnologies, Inc, followed HPLC purification. The molecular weight for the longer strand and shorter strand are 14,692 g/mol and 28,432 g/mol respectively. Both of the strands were dissolved in pH 7.4 buffer containing 300 mM KCl, 30 mM HEPES and 10 mM $MgCl_2$. For chimera folding, the two strands were mixed with equal molar amount and denatured at 95°C for 5 min, following cooling down to room temperature. The creation of chimeras was confirmed using a 4% MetaPhor® agarose gel.

Cell culture. MCF-7, T47D, MDA-MB-231, KATO III, HEK293T, U118MG cell lines were purchased from the American Type Culture Collection while MCF-7/Adr cells were generated by continued culturing of MCF-7 cells in 300 nmol/L Dox for 20 passages and maintained in 300 nmol/L Dox (Sigma, 44583) every other passage. All the above cells were cultured in DMEM (Invitrogen, 12800-017), supplemented with 10% FBS (Hyclone, A50111) and 1× Glutamax (Life Technologies, 35050-061) at 5% CO_2 and 37°C. MCF-7/Adr and MCF-7 cells were authenticated by the CellBank Australia using short tandem repeat profiling technology. In all in vitro studies, MCF-7/Adr were cultured in the presence of 300 nM of Dox, unless specifically noted. Primary breast cancer cells and single tumour cells were dissociated from clinical samples or xenograft tumours using collagenase II (Sigma, C6885) and cultured in DMEM supplemented with 15% FBS and 1× Glutamax at 5% CO_2 and 37°C.

Cell viability assay. MTT assay was conducted as previously reported[1]. Briefly, cells were seeded into 96-well plates (1200 cells/well). After 24 h, the culture medium was replaced by medium containing Dox at indicated concentrations. After 72 h of incubation, cells were treated with MTT (Sigma, M5655) for 4 h and the absorbance was measured at the wavelength of 570 nm using a VICTOR^{TM} X5 Multilabel Plate Reader (PerkinElmer Life and Analytical Sciences).

Immunoblotting analysis. Cells from cell culture or tumours were lysed with lysis buffer containing 1% Triton-X100 and 1 × protease inhibitor cocktails (Roche, 11697498001). Lysates (50 μg/lane) were separated on SDS-PAGE gels and blotted onto nitrocellulose membranes (Whatman, 10401196). Mouse anti-human antibodies to survivin (Santa Cruz, SC-17779), MDR1 (Santa Cruz, SC-55510), Bcl-2 (Cell Signaling, AB117115), Nanog (Abcam, ab62734), SOX2 (AbD Serotec, MCA 5659T), Notch1 (AbD Serotec, MCA 4804Z), ABCG2 (Cell Signaling, OBT 1177), Nestin (AbD Serotec, OBT 1610), BIM1 (AbD Serotec, MCA 3993Z), OCT4 (AbD Serotec, MCA5684T) and β-actin (Sigma, Cat No: A5441) were detected using goat anti-mouse antibody (Sigma, 31430) and visualized through a Super Signal West Dura substrate (Thermo Fisher Scientific, 34075). Quantification of ECL signals was conducted using a LAS-4000 Imaging System (GE Healthcare Life Sciences) with β-actin as a loading control.

Table S1. Summary of clinic-pathological features of primary breast tumour used in this project

				Immunohistochemistry				
ID	*Age*	*Tumour pathology*	*Grade*	*ER*	*PR*	*HER2*	*Ki-67*	*Survivin*
#1	46	IDC	3	Negative	Negative	Negative	> 15%	Positive
#2	52	Adenocarcinoma	2	Negative	Negative	Negative	< 15%	Positive
#3	76	IDC	2	Negative	Negative	Negative	< 15%	Positive
#4	45	IDC	2	Negative	Negative	Positive	> 15%	Positive
#5	42	IDC	3	Negative	Negative	Positive	> 15%	Negative
#6	72	IDC	3	Negative	Negative	Negative	> 15%	Positive
#7	54	Adenocarcinoma	2	Negative	Negative	Positive	< 15%	Negative
#8	61	IDC	2	Negative	Positive	Negative	< 15%	Negative

IDC, Invasive ductal carcinoma; ER, estrogen receptor; PR, progesterone receptor; HER2, human growth factor receptor 2.

5'RACE PCR. Total RNA was isolated from chimera treated cells or tumours with Trizol. Subsequently, total RNA (3.5 μg) was ligated to a GeneRacer RNA adaptor (Invitrogen, 46-0243) without prior treatment. Ligated RNA was reverse-transcribed with High Capacity cDNA Reverse Transcription Kit. To detect cleavage products, a nested PCR using Platinum *Taq* DNA Polymerase High Fidelity reagent (Invitrogen, 11304-011) was carried out with primers as detailed below. The specific PCR products were recovered with S.N.A.P. columns (Invitrogen, 46-0261) and then cloned into TOPO TA cloning vector (pCR 4-TOPO vector, Invitrogen, K4575-02). Individual clones were identified through DNA sequencing using a M13 reverse primer (5'-CAGGAAACAGCTATGAC-3').

Table S2. Primers used for 5'RACE PCR

GeneRacer adaptor	5'-CGACUGGAGCACGAGGACACUGACAUGGACUG AAGGAGUAGAAA-3'
5' RACE primer	5'-CGACTGGAGCACGAGGACACTGA-3'
Survivin specific primer	5'-CGCACTTTCTCCGCAGTTTCCTCAA-3'
Nested 5' RACE primer	5'-GGACACTGACATGGACTGAAGGAGTA-3'
Nested survivin primer	5'-CCTAGCGAGTGGTTCTTC-3'

qRT- PCR. Total RNA was extracted using Trizol (Invitrogen, 15596-026) and reverse transcribed using High Capacity cDNA Reverse Transcription Kits (Applied Biosystems, 4368814). Real-time PCR reactions were carried out on the Stratagene Mx3000P system (Agilent Technologies) with Fast SYBR Green Master Mix (Invitrogen, 4385612). The PCR was conducted according to the manufacturer's instruction with 400 nM of each primer per reaction. Relative mRNA levels were analyzed using the comparative $\Delta\Delta C_T$ method and normalized to the housekeeping gene GAPDH. Data processing was performed using the MxPro software (Agilent Technologies).

Table S3. Primers used for qRT-PCR

Gene	*Primer sequence*	*Amplicon (bp)*
GAPDH (Human)	F 5'-GAAATCCCATCACCATCTTCCAGG-3'	120
	R 5'-GAGCCCCAGCCTTCTCCATG-3'	
MDR1 (Human)	F 5'-ATATCAGCAGCCCACATCAT-3'	154
	R 5'-GAAGCACTGGGATGTCCGGT-3'	
Survivin (Human)	F 5'-GAACTGGCCCTTCTTGGAG-3'	93
	R 5'-AAGTCTGGCTCGTTCTCAGT-3'	
GAPDH (Mouse)	F 5'- GGAAGCCCATCACCATCTTCCAGG-3'	120
	R 5'- GGGCCCCGGCCTTCTCCATG -3'	
IFIT1 (Mouse)	F 5'-CTGAGGAGTTCTGCTCTGCT-3'	86
	R 5'-ACCTGGTCACCATCAGCATT-3'	

Flow cytometry. The binding assays were conducted as previously reported[2]. The equilibrium dissociation constant was calculated after subtracting the mean fluorescence intensity obtained from target cells to that of negative control cells according to a method described by Ellington and colleagues[3]. For cell surface marker analysis and cell sorting, single cell suspensions were washed with PBS containing 0.1% BSA and stained with following human-specific antibodies: PE-conjugated CD24 (BD Biosciences, 555428), PerCP-Cy5.5-conjugated CD44 (BD Biosciences, 560531) and FITC-conjugated EpCAM (BD Biosciences, 347197) using the concentration recommended by the manufacturer for 30 min at 4°C. After thorough washing with PBS, the population of CSCs (defined as $EpCAM^{+}/CD44^{+}/CD24^{-}$) were read using FACS Canto II (Becton Dickinson) or sorted using the FACSAria flow cytometer (BD Biosciences). Side and forward scatter were used to eliminate debris and cell doublets. Cells were routinely sorted twice, and the cells were reanalyzed for purity, which was typically >95%.

Confocal microscopy of endocytosis. The EpCAM aptamer-medicated cell binding and the inhibition of receptor-mediated endocytosis assays were carried out as previously reported (Shigdar et al, 2011). The reversal of endocytosis blockade was conducted by changing potassium-depleted buffer to PBS. To study the co-localization of chimera with late endosome/lysosome, cells were incubated with 100 nM chimera for 30 min at 37°C, followed by thorough washing. The surface chimera was quenched with 0.04% Trypan Blue (Sigma, T8154) for 3 min followed by thorough washing. Cells were then incubated in DMEM medium for a further 2 h with 50 nM Lysotraker (Life technologies, L-7526) applied in the last 30 min. After washing, cells were imaged using a FluoView FV10i laser scanning confocal microscope (Olympus). Quantification of cytoplasmic chimera was conducted via Image-Pro Premier 9.0 software (Media Cybernetics).

In vivo imaging. Dy647-labelled chimera was injected i.v. to MCF-7/Adr tumour-bearing NOD/SCID mice (n=5) with a dose of 2 nmol/mouse. The mice were imaged at 10 min, 30 min, 1 h, 2 h, 3 h, 4 h, 6 h, 8 h, 10 h, 14 h, or 24 h with a Xenogen IVIS Lumina II imaging system (Caliper Life Sciences). A circular region of interest (ROI) around the tumour site of each mouse was made and the total flux in the region was quantified using Living Image Software V2.50 (Xenogen) with the units of photons/s/cm2/sr. The data were used for semi-quantification of chimera signal in tumours.

Control for specificity of RNAi via overexpression of target protein. For plasmid rescue, a pEX-3 expression vector driven by a CMV promoter with a survivin cDNA which contains eight silent mutations (RNAi-resistant) in the region corresponding to survivin siRNA was obtained from GenePharma and transfected to cells using Lipofectamine 2000. Specifically, the survivin siRNA guide strand matching region 5'-GGACCACCGCATCTCTACA-3', corresponding to nucleotide positions 166 - 184 of survivin cDNA, was mutated to 5'-AGATCATCGTATTTCCACT-3'.

RNA-ELISA. Mouse anti-FITC antibody (Sigma, F5636, 10 μg/mL) was prepared in washing buffer (PBS containing 0.1 mg/ml tRNA and 1mg/ml BSA) and added to a 96-well plate that had been pre-coated with goat anti-mouse lgG (Sapphire Bioscience, 600-11050). After incubation at room temperature for 1 h, the wells were washed and blocked with the addition of 50 μL 1 × SuperBlock Blocking buffer (Thermo Scientific, 37537) at room temperature for 1 h. Samples containing FITC- and biotin-labelled chimera were added to the washed wells and incubated for 1 h at room temperature. After extensive washing, 50 μL of Pierce High Sensitivity Streptavidin HRP conjugates (1:5000 dilution, Thermo Scientific, 37537) was added to each well to bind biotin which is conjugated on chimera. After 1 h incubation at room temperature, absolute fluorescence intensities (ex = 325 nm, em = 420 nm) for the chimera were measured using a using a VICTORTM X5 Multilabel Plate Reader (PerkinElmer).

Mammosphere formation assay. Mammosphere assay was conducted according to previously reported protocols for the quantification of breast CSC frequency (Shaw et al, 2012). Briefly, 2000 viable single cells from different treatment groups were cultured in DMEM/F-12 media (Invitrogen, 12500-096) supplemented with 1 × B27 (Gibco, 10889-038), 20 ng/mL EGF (Sapphire Bioscience, 701-02360), 10 ng/mL FGF (Sapphire Bioscience, 701-23300) and 4 ng/mL insulin (Sigma, 19278) in ultralow attachment surface 6-well plates (Corning) at 5% CO2 and 37°C. Seven days later, first-generation spheres were counted. The spheres were incubated with trypsin for 3 minutes at 37°C and dispersed by pipetting with a 23-gauge needle. The cells were pelleted and replated under the same conditions as above. The sphere numbers were counted seven days after the incubation. Only spheres with size more than 50 μm in diameter were counted.

Pharmacokinetics and biodistribution studies. To investigate the plasma drug concentration-time curve of chimera, healthy male Sprague-Dawley (SD) rats (200 to 250 g) were injected intravenously with chimera at a single dose of 100 nmol/kg. Blood was serially collected from animals in heparinized tubes from the tail vein at time points of 10 min, 0.5 h, 1 h, 2 h, 3 h, 4 h, 6 h, 8 h, 10 h, 12 h, 14 h and 24 h. Chimera concentration in plasma was measured via an RNA-ELISA method. For biodistribution assay, MCF-7/Adr tumour-bearing NOD/SCID mice were randomly divided into 4 groups (5 mice per group, termed 0.5 h, 3 h, 6 h and 10 h time point groups) once tumours reached the volume of 150 mm3. Chimera was delivered via tail vein injection with a dose of 2 nmol/mouse. Organs including stomach, eye, brain, skin, heart, liver, spleen, kidney, lung, intestine, tumour and excrement were collected 0.5 h, 3 h, 6 h or 10 h after injection. Tissues were then thoroughly homogenized in PBS (tissue: PBS weight ratio 1:3). After centrifugation at 10,000 × g at 4°C for 10 min, the supernatants were collected and the chimera concentration was quantified by RNA-ELISA. The pharmacokinetic parameters were analyzed using the DAS 2.0 software (Mathematical Pharmacology Professional Committee of China).

Innate immune response assay. For in vivo assay, 8-week old Balb/c mice were randomly divided into 4 groups (n=6). Mice in chimera and negative control chimera groups were intravenously injected with 2 nmol of the respective chimera. Poly I:C (200 ng /mouse, InvivoGen, PIC-34-08) and PBS were used in the positive and negative control groups, respectively. Blood (100 μL) was collected from each mouse 4 h and 24 h after injection. The blood was allowed to coagulate at 25°C for 30 min before centrifuging at

17,000 × g for 10 min to collect serum. Levels of the IFN-α and TNF-α in the serum were determined using mouse IFN-α (PBL Biomedical Laboratories, 42120-1) and mouse TNF-α (BD, 560478) ELISA kits. For IFIT1 expression assay, total RNA was processed from mouse livers and lungs 24 h after treatments. IFIT1 expression was determined by qRT-PCR with GAPDH as internal control. For human peripheral blood mononuclear cells (PBMCs) assay, PBMCs were treated with 750 nM chimera alone or duplexed with DOTAP, or with a known immunostimulatory ssRNA control (B-406-AS) (Gantier et al, 2008). The cells were incubated overnight and supernatants were assayed for human IFN-α and TNF-α, as previously described [4]

Apoptosis assay. Apoptosis was assessed using ApopTag Red In Situ Apoptosis Detection Kit (Millipore, S-7165) while cell proliferation was studied using a mouse anti-human Ki-67 antibody (DAKO, M7240) and a DAB peroxidase substrate solution kit (Vector Laboratories, SK-4105) in accordance with the manufacturers' instructions.

Survival analysis. MCF-7/Adr tumour bearing mice were grouped and treated as described in "Tumour implantation and treatment". The mice were evaluated daily for disease-free survival and disease-related events. The endpoint was the same as described above. The survival curves were derived using a log-rank test with a 95% confidence interval.

Supplementary results

Optimization of EpCAM aptamer-survivin siRNA chimera structure.

In order to engineer an optimal chimera with potent silencing activity, enhanced *in vivo* stability, and minimized immunogenicity, a total of 10 chimera structures were tested, which are presented in Figure S1A.

Chimera 1 consisted of a 23-mer survivin siRNA with its longer strand modified with 2'-fluoropyrimidine. Although it achieved an *in vitro* half-life of 9.17 h in 50% human serum, it was a poor Dicer substrate with low efficacy in silencing survivin (40% knockdown at the dose of 20 nM) (Figure S1B, C). In order to increase the efficiency of being processed by Dicer, the 23-mer siRNA portion was extended to 27-mer, with 8-bp at the 5'-end of the guide strand of the siRNA portion unmodified (Chimera 2), intending not to interfere with Dicer cleavage at the predicted site of Dicer processing [5]. Indeed, the potency for Chimera 2 as a Dicer substrate increased significantly and achieved approximately 80% knockdown even at the concentration of 5 nM. However, its serum half-life decreased dramatically to 0.47 h. To improve the serum stability of Chimera 2, the unmodified AA linker was changed to 2'-*O*-methyl modified AA linker or a hexaethylenglycol (HEGL) linker to derive Chimera 3 and Chimera 4, respectively. However, no improvement in serum stability was observed for either of them. Further engineering entailed alternative modification of the shorter strand with 2'-*O*-methyl, except the 8 nucleotides from 3'-end that gave rise to Chimera 5. Again, no improvement of the serum stability was achieved. At this point in time, it became clear that it was the 8-bp unmodified nucleotide at the 5'-end of the guide strand of the siRNA portion that contributed to the instability of the chimera. Therefore, for Chimera 6 and Chimera 7, 2'-*O*-methyl or 2'-fluoropyrimidine modification was introduced to the 8-nt at the 3'-end of short strand, respectively. Disappointedly, these modifications not only failed to increase the stability of the chimera but also compromized the silencing potency. Next, in Chimeras 8 and 9, the longer strand was fully modified with 2'- fluoropyrimidine and the 8-nt at the 3'-end of the shorter strand was modified with either 2'-*O*-methyl or 2'-fluoropyrimidine, respectively. Although these chimeras demonstrated an enhanced stability in 50% serum ($t_{1/2} \approx 11$ h), the gene knockdown potency lost completely. It was now apparent that the unmodified 8-nt sequence on the 5'-end of the guide strand was

critical for both the potency of gene silencing and the stability of the chimera. To achieve a balance between potency and stability, Chimera 10 was engineered in such a way that the longer strand was kept fully modified with 2'-fluoropyrimidine while the 8-nt at the 3'-end portion of the shorter strand remained unmodified. As shown in Figure S1, Chimera 10 demonstrated a prolonged serum stability ($t_{1/2}$ =8.34 h) with sufficient gene knockdown potency (> 80% silencing at the concentration of 20 nM).

Since both unmodified 21-mer siRNA and 27-mer siRNA can remain stable in 50% serum for more than one hours[5], it was surprising that the unmodified 8-bp at the 5'-end of the siRNA portion on Chimera 2 contributed to the serum instability. This was probably due to that the 47-nt longer strand in the chimera was much longer than 21- or 27-mer siRNA and therefore facilitated RNases entry or recognition. As a result, this longer chimera structure had to be modified more extensively.

Supplementary references

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3. Li N, Ebright JN, Stovall GM, Chen X, Nguyen HH, Singh A *et al.* Technical and Biological Issues Relevant to Cell Typing with Aptamers. *J Proteome Res.* 2009; **8:** 2438-2448.
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Supplementary figures

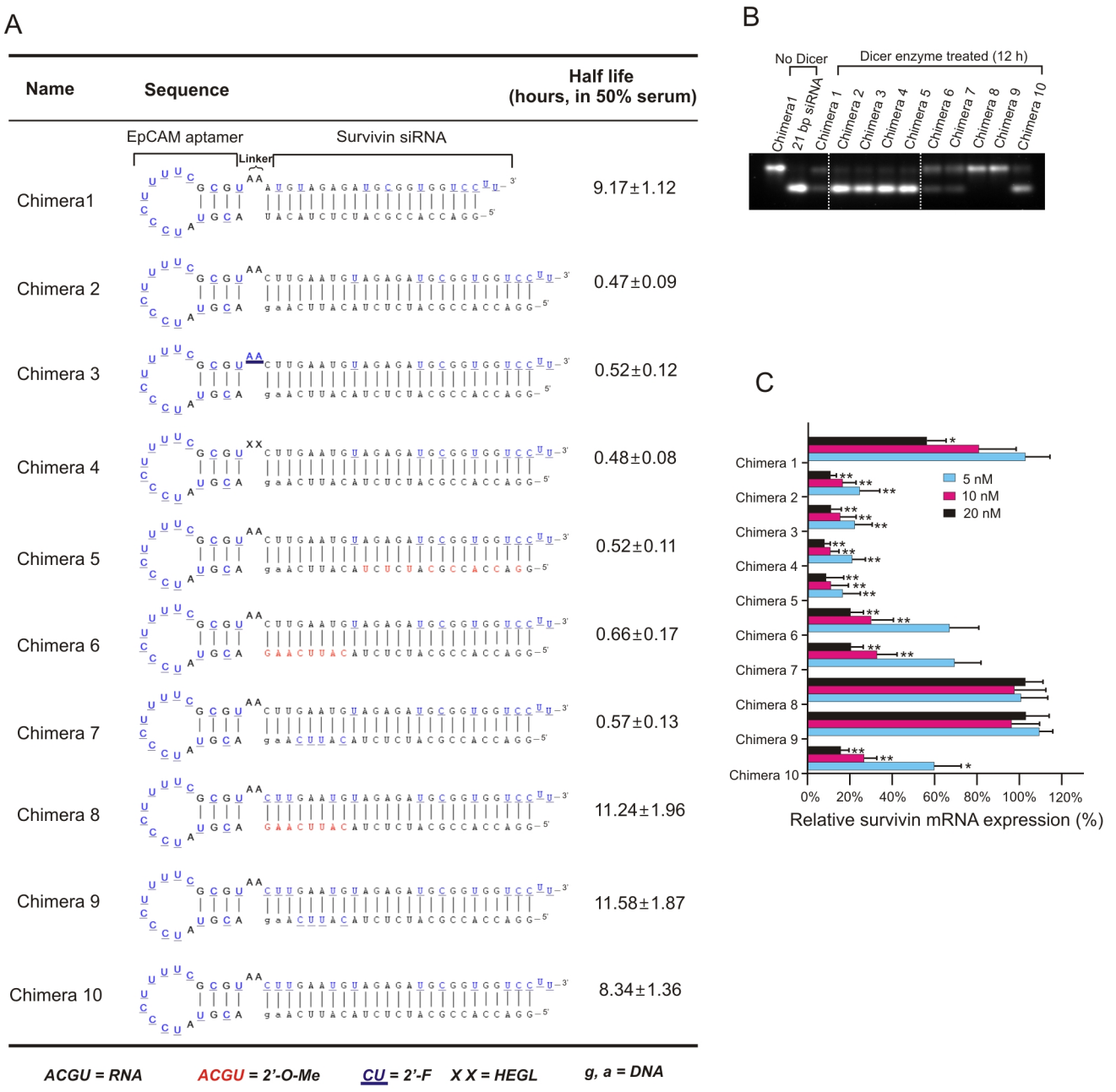


Figure S1. Optimization of EpCAM aptamer-survivin siRNA chimera. (A) Schematic representations of serially engineered chimeras and their *in vitro* stability in 50% human serum. Black letters, RNA; red letters, 2' *O*-methyl RNA; blue letters, 2'-fluoropyrimidine RNA; XX, 18-atom hexaethylenglycol spacer and, lowercase letters, DNA bases. (**B)** Analysis of chimera processing by the RNAi machinery. Chimeras were incubated *in vitro* with recombinant human Dicer enzyme for 12 hours. The Dicer cleavage or uncleaved (No Dicer) products were visualized after electrophoresis through a 4% MetaPhor® agarose gel. (**C)** Dose-dependent knockdown of survivin mRNA by chimeras. MCF-7/Adr cells were incubated with various concentrations of different versions of the chimera. The knockdown of survivin mRNA was analyzed 24 h after the treatment using qRT-PCR. The levels of survivin mRNA in samples treated with negative control chimera were designated as 100%. Data are means ± SEM, n=3. *, $P < 0.01$; **, $P < 0.001$; compared with saline treated.

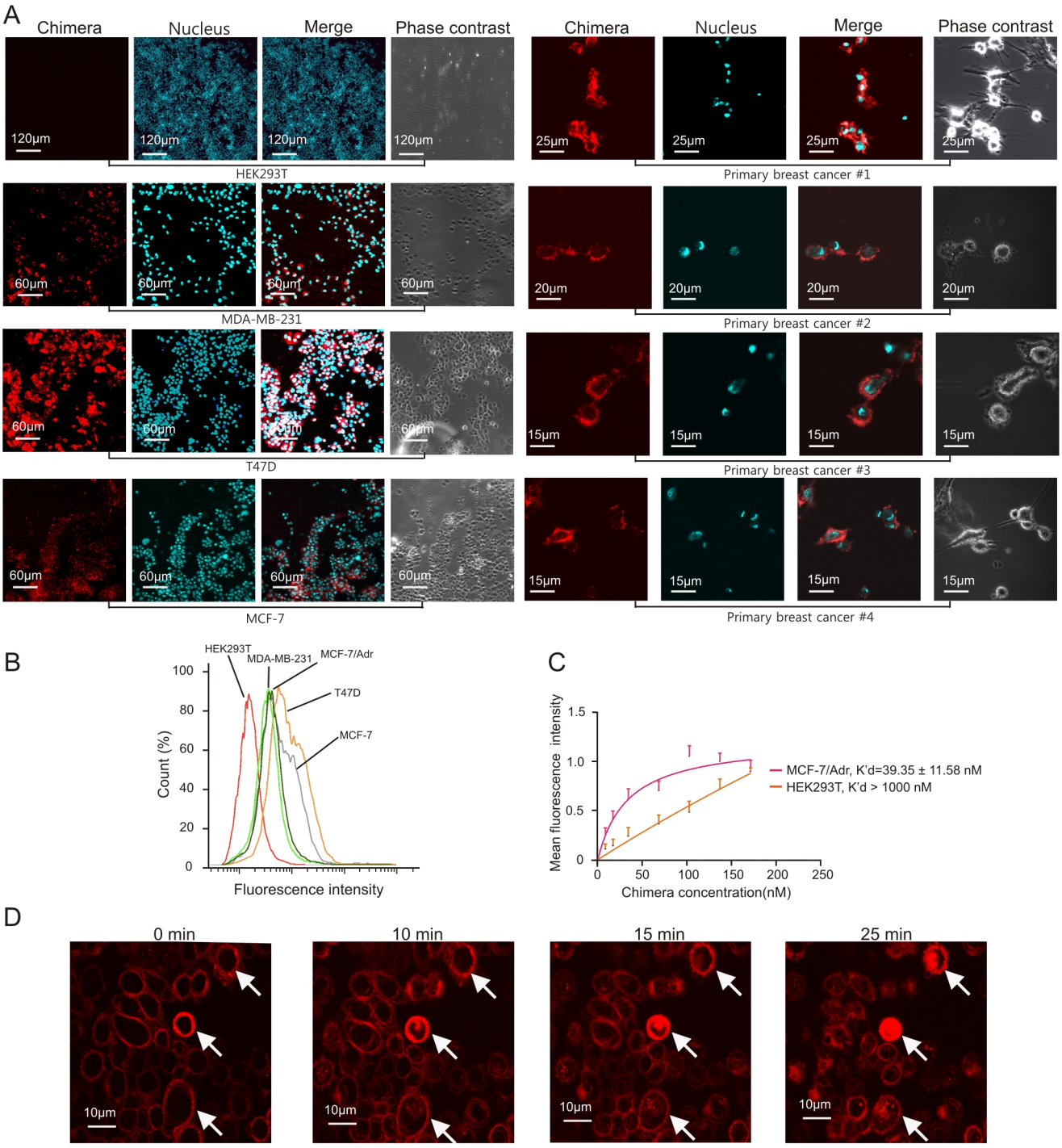
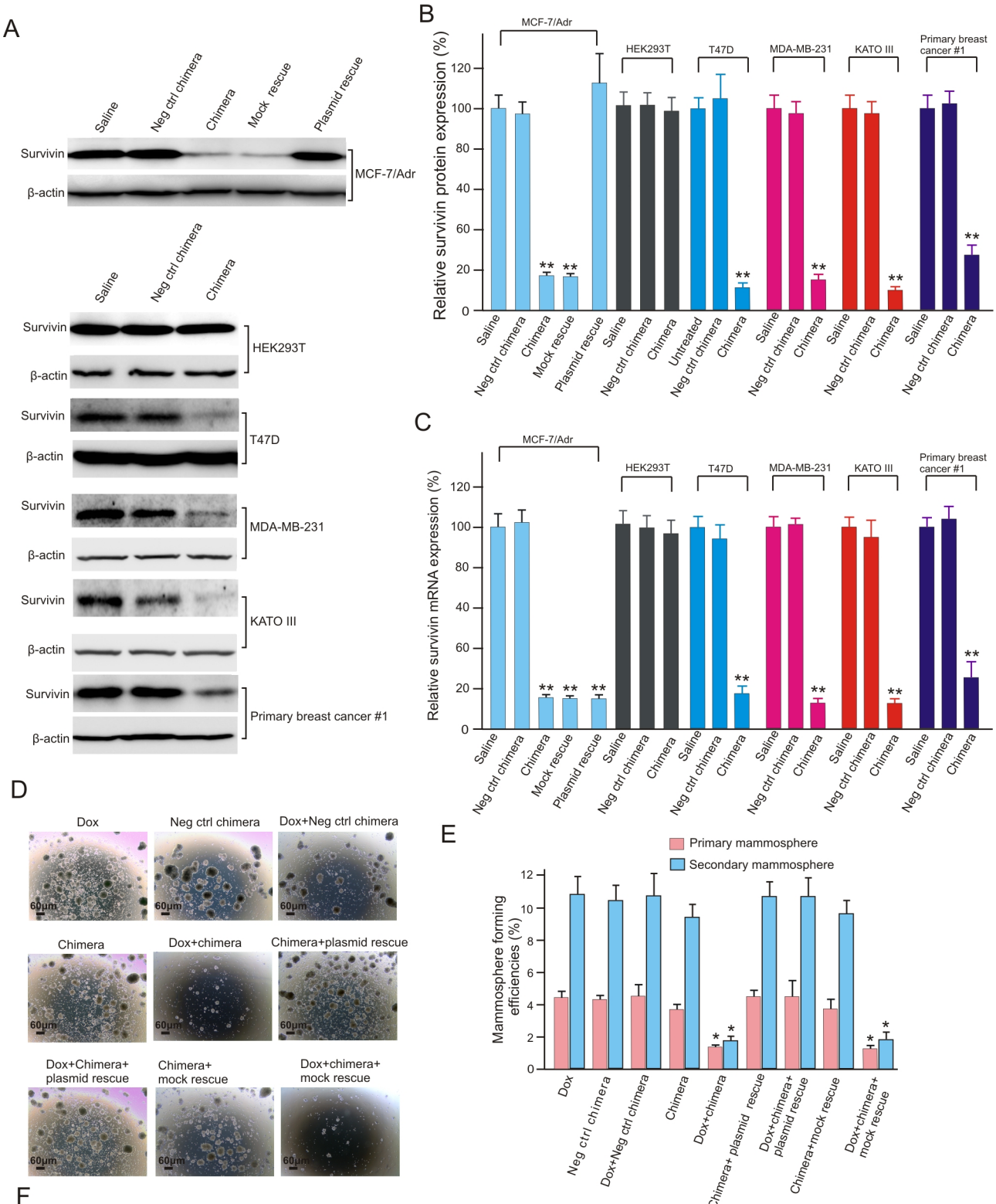


Figure S2. Robust and specific binding and internalization of EpCAM aptamer-survivin siRNA chimera. (A) EpCAM aptamer-survivin siRNA chimera specifically binds to EpCAM-positive primary breast cancer cells and breast cancer cell lines but not to EpCAM-negative HEK293T cells. Cells were imaged via confocal microscopy after incubation with 100 nmol/L of chimera at 37°C for 30 min. Red, Dy647 (chimera), and blue, Hoechst 33342 (nuclei). (**B)** Quantification of binding of chimera to EpCAM-positive breast cell lines versus that to the EpCAM-negative HEK-293T via flow cytometric analysis. **(C)** Determination of the equilibrium dissociation constants of chimera to MCF-7/Adr cells using flow cytometry by incubating cells at varying concentrations of chimera (1–200 nmol/L) using HEK-293T as a negative control. K_d was derived using GraphPad Prism program 3.03. (**D)** Chimera internalized via receptor-mediated endocytosis. MCF-7/Adr cells were treated in potassium-depleted medium for 30 min followed by the addition of 100 nmol/L chimera for an additional 30 min. The potassium-depleted medium was then replaced with phosphate buffered saline (0 min), and cells were imaged using a confocal

microscope at indicated time points to follow the entry of the chimera. Red, Dy647 (chimera).



F

Percentage of EpCAM^{+}/CD44^{+}/CD24^{-} population in MCF-7/Adr cells

	Dox alone	Neg ctrl chimera alone	Dox+neg ctrl chimera	Chimera alone	Dox+ chimera	Dox+chimera + mock rescue	Dox+chimera+plasmid rescue
% of EpCAM+/ CD44+/CD24- cells	22.10 ± 3.62	21.97 ± 2.95	21.68 ± 3.42	17.9 ± 2.34	9.202 ± 1.63**	10.26 ± 1.29**	19.96±2.55

In *in vitro* assay, cells were maintained in 300 nM Dox except in chimera only and negative chimera only groups.

Figure S3. Specific and effective knockdown of survivin by chimera *in vitro* leads to sensitization of CSCs to Dox. (A)

Representative immunoblots demonstrate specific knockdown of survivin by chimera after 48 h treatment using 20 nmol/L chimera and/or other indicated reagents with RNAi rescue performed as in *Figure 2A*. EpCAM-negative and survivin-positive HEK293T cells were used as a control for the specificity of aptamer targeting. (**B)** Quantification of survivin protein knockdown. (**C)** Quantification of survivin mRNA knockdown using qRT-PCR. As the forward primer for survivin corresponds to the 5'-UTR region, and thus the transcripts from the rescue plasmid were not amplified in qRT-PCR. (**D**, **E)** Effect of treatment of chimera or controls plus 300 nmol/L Dox on the self-renewal efficacy of MCF-7/Adr cells with representative images of secondary mammosphere assays (***D***) and quantification of primary and secondary mammosphere formation (***E***). (**F)** Efficacy and specificity of targeting tumorigenic EpCAM^{+}/CD44^{+}/CD24^{-} cells *in vitro* by 20 nmol/L chimera or controls with or without combined treatment of 300 nmol/L Dox as indicated. Data are means ± SEM, n=4. *, $P < 0.01$; compared with cells treated with 300 nM Dox alone.

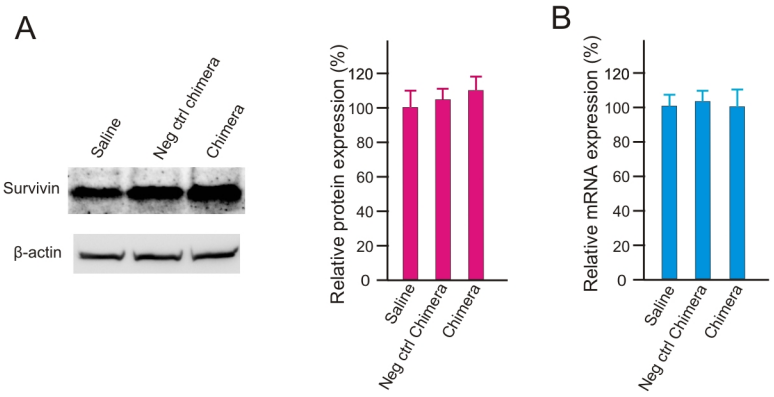


Figure S4. Non-PEGylated chimera is inefficient in silencing survivin *in vivo*. NOD/SCID mice bearing orthotopic MCF-7/Adr tumour with a tumour volume of approximately 60 mm^3 received a bolus intravenous injection of 2 nmol/mouse non-PEGylated chimera every other day over 5 days. Two days after the third injection, the tumours were removed and total protein and RNA were prepared. (**A)** Representative immunoblots for *in vivo* survivin silencing and quantification. (**B)** Quantification of survivin mRNA in treated tumours via qRT-PCR.

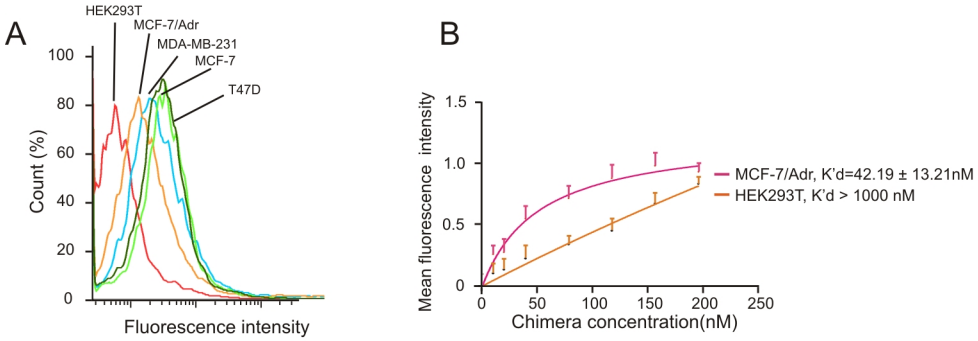


Figure S5. PEGylation does not compromize the affinity or specificity of the chimera. (A) Quantification of binding of chimera to EpCAM-positive breast cell lines versus that to the EpCAM-negative HEK-293T via flow cytometric analysis. (**B)** Determination of the equilibrium dissociation constants of chimera to MCF-7/Adr cells using flow cytometry by incubating cells at varying concentrations of the PEGylated chimera (1–200 nmol/L) using HEK-293T as a negative control. K_d was derived using GraphPad Prism program 3.03.

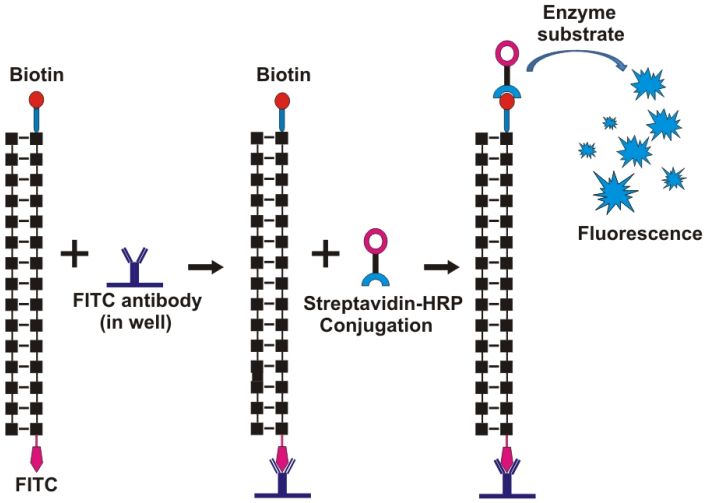


Figure S6. Schematic of RNA ELISA used to quantify aptamer-siRNA chimera. Mouse anti-FITC antibody was added to a 96-well plate that had been pre-coated with goat anti-mouse lgG. After 1 h incubation at room temperature, the wells were washed and blocked. Samples containing chimera were added to the washed wells and incubated for 1 hour. After extensive washing, Streptavidin HRP conjugates were added to each well to bind chimera-conjugated biotin. Absolute fluorescence intensities were then measured using a plate reader.

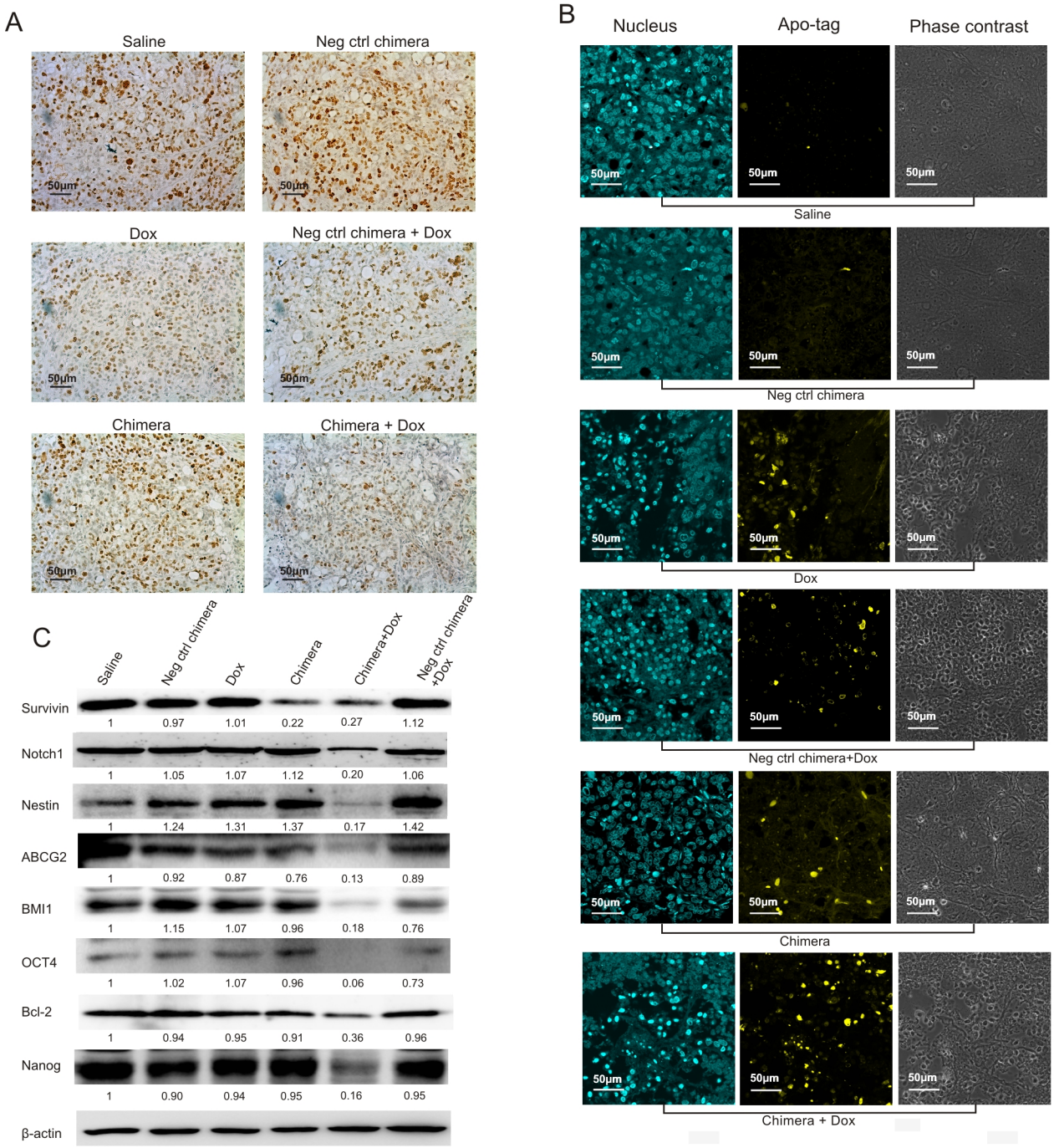


Figure S7. The PEGylated chimera reverses chemoresistance, inhibits cell proliferation and suppresses stemness *in vivo*. **(A)** Combined chimera and Dox treatment inhibited proliferation of MCF-7/Adr tumours. Tumours were treated as indicated. Representative images of Ki-67 staining on MCF-7/Adr xenograft tumour sections were shown. Brown, Ki-67-positive cells. (**B)** Representative micrograph of TUNEL assays on sections from MCF-7/Adr tumours treated as indicated. Blue, DAPI (nuclei), and yellow, Rhodamine for ApopTag-positive nuclei. (**C)** Representative immunoblots of anti-apoptotic and stemness proteins in tumours treated as indicated. Numbers under each band indicate the fold of change in each protein compared to the saline treated sample.

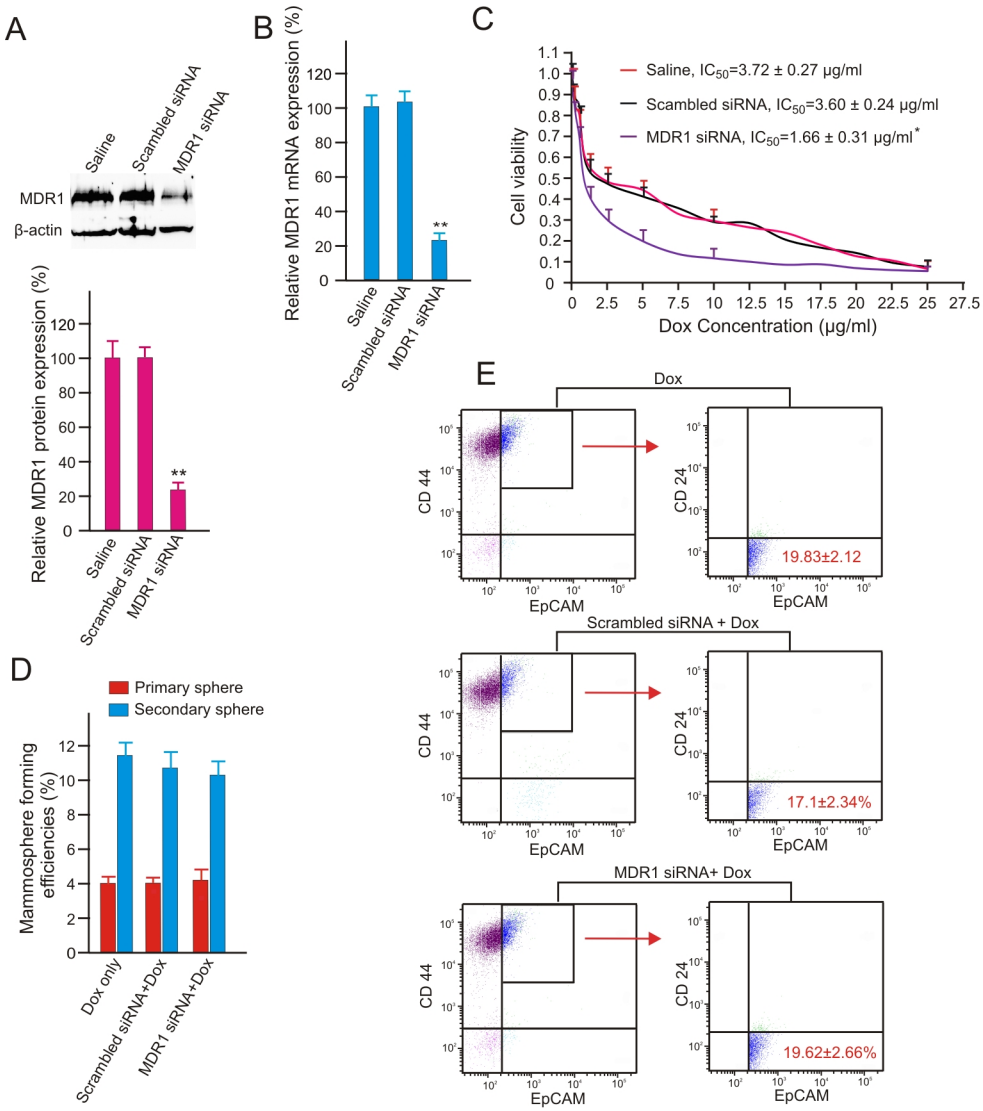


Figure S8. Silencing of MDR1 gene sensitizes bulk MCF-7/Adr cells to Dox but does not affect chemosensitivity and self-renewal efficacies of CSCs. (A) Representative immunoblots demonstrate the knockdown of MDR1 by siRNA transfection after 48 hours with indicated reagents. β-actin was used as a loading control. (**B)** The MDR1 mRNA levels after 24 hours siRNA treatment were determined by qRT-PCR and the expression was normalized to that of GAPDH mRNA. (**C)** Cell viability of MCF-7/Adr cells treated with 50 nM of indicated siRNA or controls and various concentrations of Dox for three days as determined using an MTT assay. (**D)** Self-renewal efficacy of cells after different treatments as determined by mammosphere formation assay. MCF-7/Adr cells were cultured in media containing 300 nM Dox throughout. (**E)** Changes in the abundance of the $EpCAM^{+}/CD44^{+}/CD24^{-}$ population in MCF-7/Adr cells after treatment with 300 nM Dox and indicated siRNA. Data are means ± SEM, n=3. *, $P< 0.05$; **, $P<0.001$; compared with those treated with saline.