Supplementary Materials

A Novel Therapeutic Strategy Utilizing EpCAM Aptamer-Conjugated Gemcitabine for Targeting Bladder Cancer and Cancer Stem Cells

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Experimental

General reagents

The anti-EpCAM rabbit monoclonal antibody was purchased from Abcam (Cambridge, MA, USA). The anti-OCT4 rabbit monoclonal antibody, the anti-SOX2 rabbit monoclonal antibody, and the anti-NANOG rabbit monoclonal antibody were purchased from ZENBIO (Chengdu, China). Super GelRed was purchased from US Everbright® Inc (Sayreville, NJ, USA). Beijing Labor Technology Co, Ltd. (Beijing, China) provided the Cell Counting Kit-8 (CCK-8). Alexa Fluor 488-labeled goat anti-mouse secondary antibody, Radioimmunoprecipitation assay (RIPA) lysis buffer, Hoechst 33342, the phosphatase inhibitor cocktail, protease inhibitors, and Lysotracker were all purchased from Beyotime Biotech Inc. (Jiangsu, China). All tubes and plates for cell culture were purchased from NEST Biotechnology (Jiangsu, China). Unless otherwise stated, all other reagents were purchased from Sigma (St. Louis, MO, USA).

Oligonucleotide synthesis and purification

According to the reported method and synthesis procedure shown in **Fig. S1, Supporting Information**, Li_2CO_3 (900 mg) and Lutidine (1350 mg) were first added to compound 1 (gemcitabine, 269 mg, ~1.0 mmol, purity 97%) suspended in anhydrous CH_2Cl_2 (100 mL). 4,4'-dimethoxytrityl tetrafluoroborate salt (DMTrBF4) (~460 mg, 1.12 mmol) was added, followed by 150 mL of CH_2Cl_2, and the diluted solution was washed with saturated NaCl solution and dried over anhydrous Na_2SO_4. After removal of the solvent, the residue was purified using a flash column, giving compound 2 (~400

mg, yield 73%; $M+H^+$ = 543.3). Compound 2 (264 mg, ~0.5 mmol) was resuspended in 40 mL of CH_2Cl_2, added with N, N-Diisopropylethylamine (DIEA) (650 mg, 5.0 mmol) and cooled at 0 °C. N N-diisopropylchlorophosphoramidite (596 mg, 2.44 mmol) was then added and the reaction was monitored using thin-layer chromatography (TLC). When the starting material had disappeared, the reaction solution was diluted using CH_2Cl_2 (~100 mL) and rinsed using saturated $NaHCO_3$ and saturated NaCl. Then, the obtained products were dried over anhydrous Na_2SO_4. The dried solution was concentrated, and the residue was purified using a flash column, giving GEM phosphoramidite 3 (~380 mg, 80% yield; MW calculated for 950.03; $M+Na^+$ = 973.09) as a white powder. Compared with the calculated structure, the MS, ^{1}H-NMR, and ^{13}C-NMR were correct (**Fig. S2-S4**). Using the EpCAM-GEMs sequence, oligonucleotide synthesis was run automatically according to the requirements of the DNA synthesizer (PolyGen GmbH, Langen, Germany). After auto-synthesis, the crude products were treated with reference to the reported standard. Briefly, the DNA products were treated with about 400 μL of 28% ammonium hydroxide at 65 °C for 30 min to cleave the CpG of the oligonucleotides. The cleaved DNA was mixed with 1 mL of ice-cold ethanol and 40 μL of 3 M NaCl, and the oligonucleotides were precipitated at for 1 h at –20 °C, followed by centrifugation for 20 min at 4 °C and 12, 000 rpm; the pellet was retained. The precipitate was dissolved in 400 μL of 0.1 M triethylamine acetate (TEAA) for subsequent HPLC purification using a C18 column. The DNA product was lyophilized, resuspended in sterilized ultrapure water, and then desalted using a desalt mini column. The fluorescein isothiocyanate (FITC)- and cyanine 5 (Cy5)-labeled strands were

synthesized and purified. All DNA sequences were quantified and stored in sterilized water for further experiments.

Cell cultures

Bladder cancer cell lines (BIU-87, RT4, 5637, and HT-1376), the teratoma cell line (PA-1), and the normal cell line (HEK 293T) were purchased from ATCC (Manassas, VA, USA). RPMI-1640 Medium (Promocell, Heidelberg, Germany) was used to culture BIU-87 and 5637 cells. McCoy's 5A Medium (Promocell) was used to culture RT4 cells. Minimal Eagle's Medium (MEM, Promocell) was used to culture HT-1376 cells. PA-1 cells were cultured in Eagle's Minimum Essential Medium (EMEM, Promocell). HEK 293T cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Promocell). The above media were supplemented with 1% penicillin–streptomycin, 1% glutamine, and 10% fetal bovine serum (FBS). All cells were grown in a humidified atmosphere at 37 ℃ with 5% CO_2.

Immunofluorescence

Medium (200 μL) was pipetted in to into each well of two chamber slides and left for 10–15 min. We then aliquoted BCa cells into the wells and incubated them for 1 h to form a confluent monolayer. Ice cold ethanol was then used to fix the cells, which were rehydrated using buffer, subjected to 0.1% Triton X-10 permeabilization for 1 min, and washed in buffer. The first slide was washed in buffer, mounted using FluorSave™ (Calbiochem, San Diego, CA, USA), and stored under foil at 4 ℃. The other slide was

subjected to 10% serum in TBS blockade for 40 min and then reacted with primary antibodies against EpCAM (Abcam; 1:1000 dilution) for 1 h. The cells were then washed and the secondary antibody was added to each well and left for 1 h. Cells were then washed, mounted using FluorSave™, and stored under foil before being imaged under an Olympus BX51 Fluorescence microscope (Olympus, Tokyo, Japan) equipped with a Hamamatsu Orca ER digital camera (Hamamatsu City, Japan).

Western blotting

SDS lysis buffer was used to prepare cell and tissue lysates, the protein concentration of which was assessed using the Bradford assay (Bio-Rad Laboratories, Hercules, CA, USA). Electrophoresis through 10.5% sodium dodecyl sulfate polyacrylamide gels was used to separate equal amounts of protein, which were then electrotransferred onto a nitrocellulose membrane. The membrane was blocked for 1 h using 5% skim milk in Tris-buffered saline with Tween-20 (TBST), and then incubated with primary antibodies recognizing EpCAM (1:500 dilution), OCT4 (1:1000 dilution), SOX2 (1:1000 dilution), NANOG (1:1000 dilution), and β-actin (Sigma-Aldrich; 1:2000). After washing with TBST, secondary antibodies recognizing immunoglobulin G were incubated with the membrane. The enhanced chemiluminescence detection system (Amersham Biosciences Europe, Freiberg, Germany) was then used to visualize the immunoreactive proteins on the membrane, following the supplier's protocol.

Polyacrylamide gel electrophoresis

EpCAM-GEMs (2 μM) were incubated in 10% FBS-supplemented DMEM medium at 37 °C for 0, 1, 2, 4, 8, 12, 24, and 36 h, respectively. The samples were then denatured in for 5 min at 95 °C and subjected to 12% polyacrylamide gel electrophoresis at 110 V for 30 min. According to the supplier's instructions, Super GelRed (US Everbright® Inc.) was used to stain the gel for 10 minutes for imaging.

CCK-8 assay

Cells were inoculated in 96-well plates (4000 cells/well) in advance and cultured for 24 hours to allow the cells to adhere to the wall. Then, 100 μL of a concentration gradient (0, 15, 30, 60, 180, 360, 900, 2100 nM) of samples (GEM, Lib-GEMs, and EpCAM-GEMs) were added to each well and incubated for 12 h, followed by washing with phosphate-buffered saline (PBS). Fresh complete DMEM medium was added and the cells were incubated for 72 h. Then, 10 μL of CCK-8 reagent was added to each well and incubated for a certain time. The absorbance of the wells was read at 450 nm using a Synergy 2 Multi-Mode Microplate Reader (Bio-Tek, Winooski, VT, USA) to determine cell viability.

Flow cytometry determination of the cell cycle

BIU-87 cells treated with EpCAM-GEMs, LIB-GEMs, and GEM. Cells (~ 2×10^6) were washed once using PBS, centrifuged for 5 min at 1000 rpm, fixed in 75% pre-cooled ethanol, and incubated at 4 °C overnight. Next day, the 75% ethanol was discarded, the cells were washed with PBS once, suspended in 800 μL of PBS

containing 1% BSA, and 100 μL of propidium iodide (PI) dye solution (in 3.8×10^{-2} M sodium citrate, pH 7.0) was added. We then added 100 μL of Rnase A (10 mg/mL) and incubated the samples for 30 min at 37 ℃ in the dark. The cells were then tested using flow cytometry (Beckman Instruments, Inc, CA, USA).

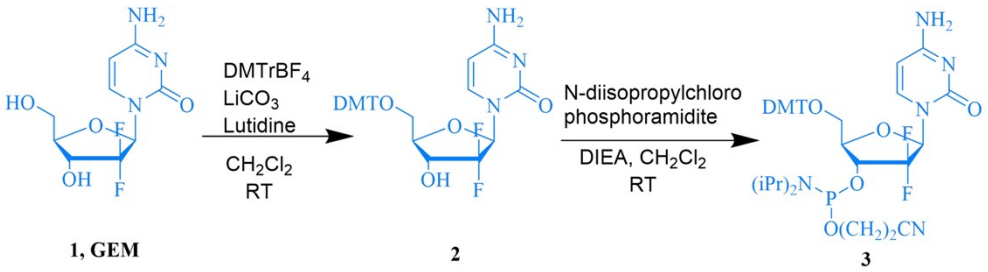

Fig. S1 The synthesis of GEM phosphoramidite 3.

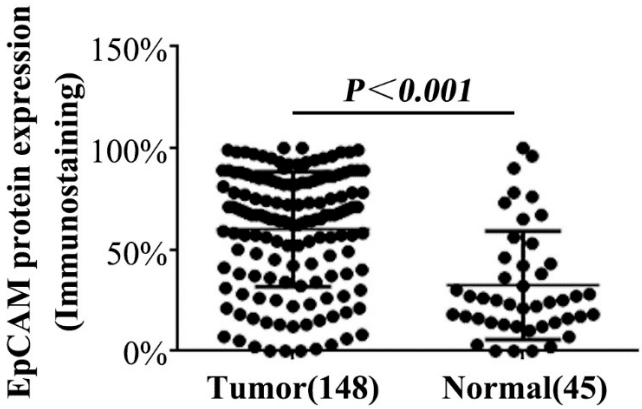

Fig. S2 EpCAM expression was higher in BCa tissues than in adjacent noncancerous tissues according to IHC analyses.

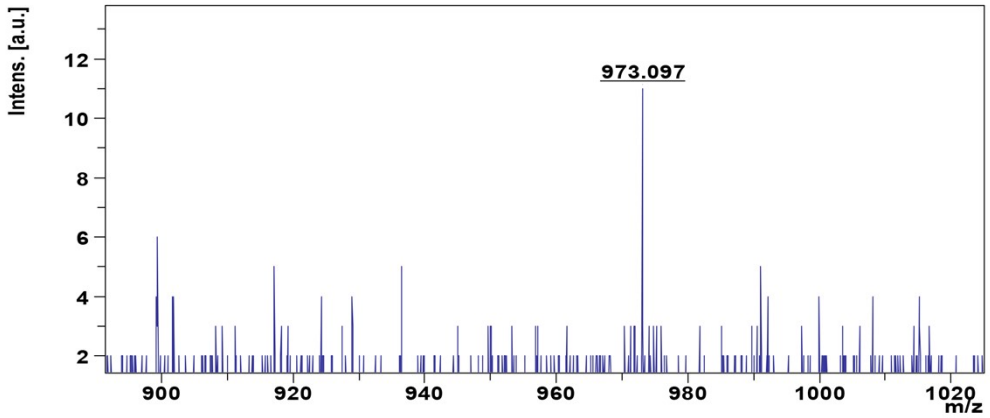

Fig. S3 The mass spectrum of GEM phosphoramidite 3. The calculated molecular weight was 950.03, the observed M/Z+ was 973.09 ($M+Na^+ = 973.09$).

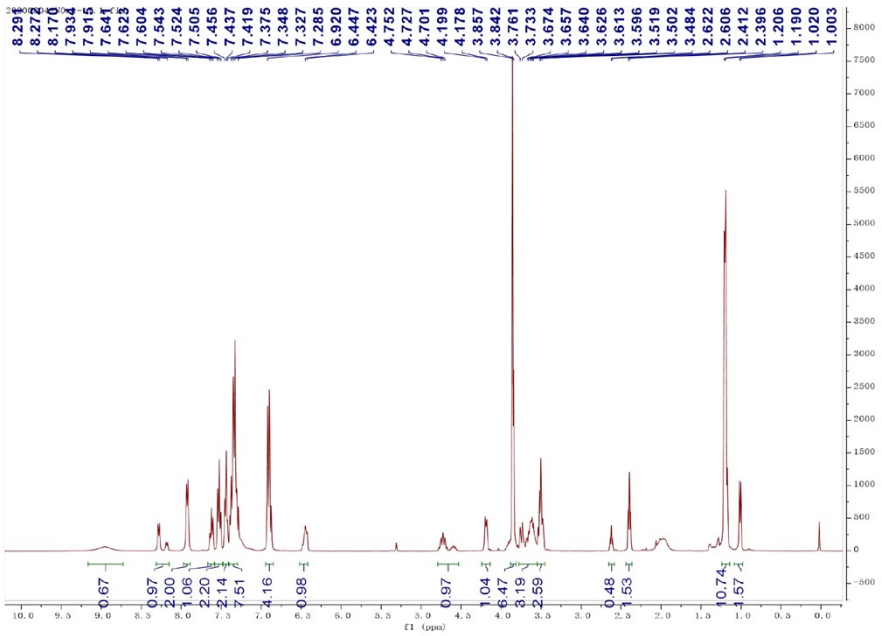

Fig. S4 The ^{1}H-NMR spectrum of GEM phosphoramidite 3.

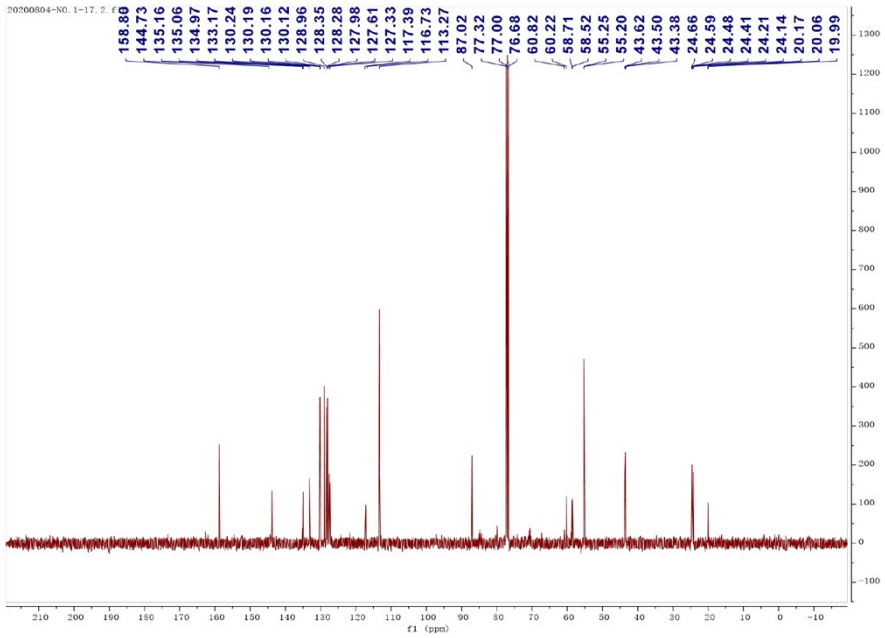

Fig. S5 The ^{13}C-NMR spectrum of GEM phosphoramidite 3.

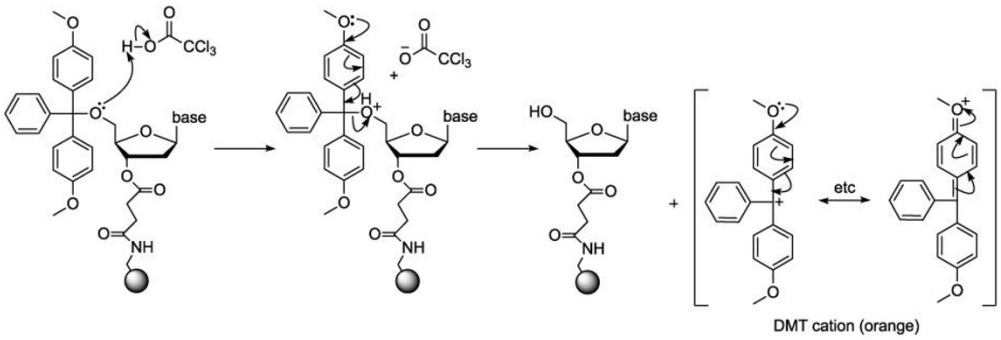

Fig. S6 Step one of synthesis. The CpG dinucleotide is linked with one of the bases A, G, C and T is selected as the solid phase carrier.

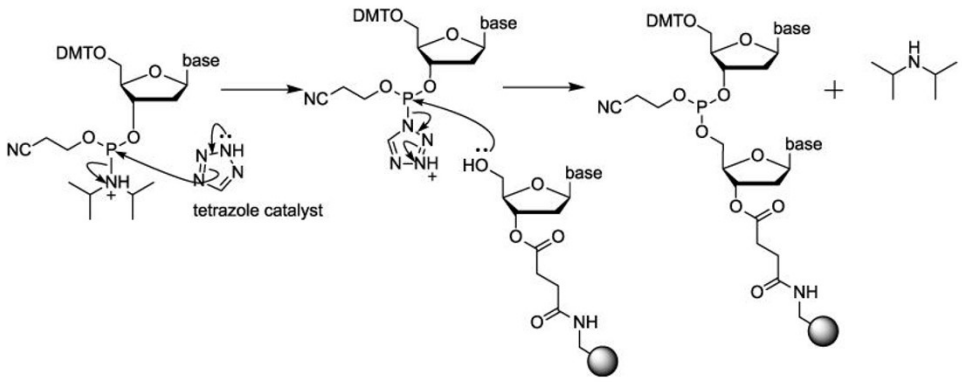

Fig. S7 Formation of a phosphite bond.

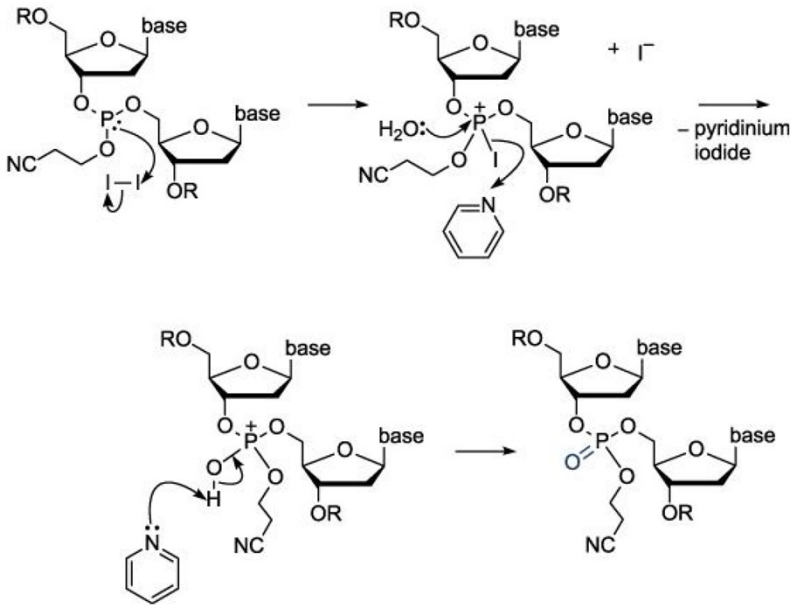

Fig. S8 Formation of a stable phosphodiester bond.

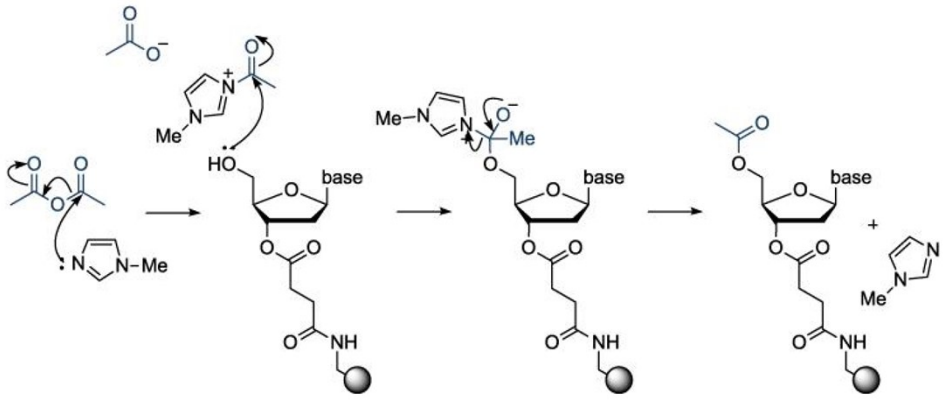

Fig. S9 Acetyl blocking 5' OH is not involved in the reaction.

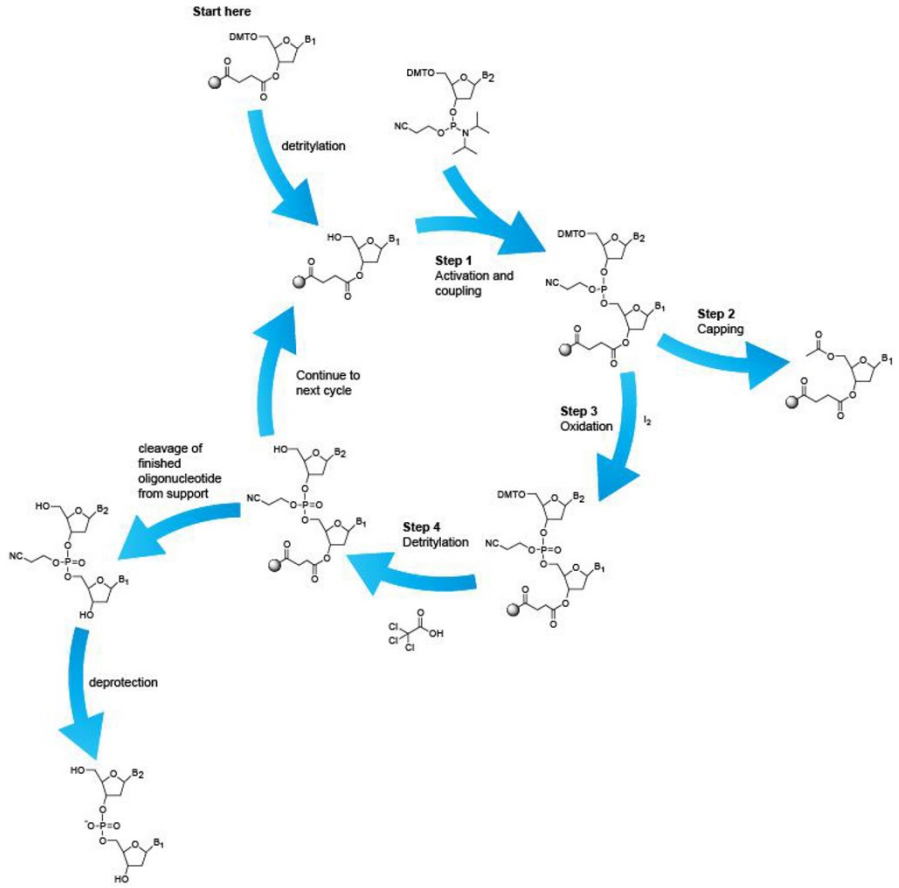

Fig. S10 The synthesis of EpCAM-GEMs. Synthesis proceeded from the 3' to the 5' end of the oligonucleotide, adding one base in each cycle.

Name :	**EpCAM (SYL3C)**		
Sequence(5'to3'):	TTCACTACAGAGGTTGCGTCTGTCCCACGTTGTCATGGGGGGTTGGCCTGTTTTT		
Lot No. :	AX109284774	**Length :**	55
Purification :	HPLC	**Modification(5'to3'):**	
nmoles:	3.98	**Add water to 100uM:**	39.8
TM(°C) :	74.8	**GC(%) :**	52.7
MW (target):	16967.95	**MW(observed):**	16965.6
Conclusion:		**Qualified:**	
Inspector:		**Auditor:**	

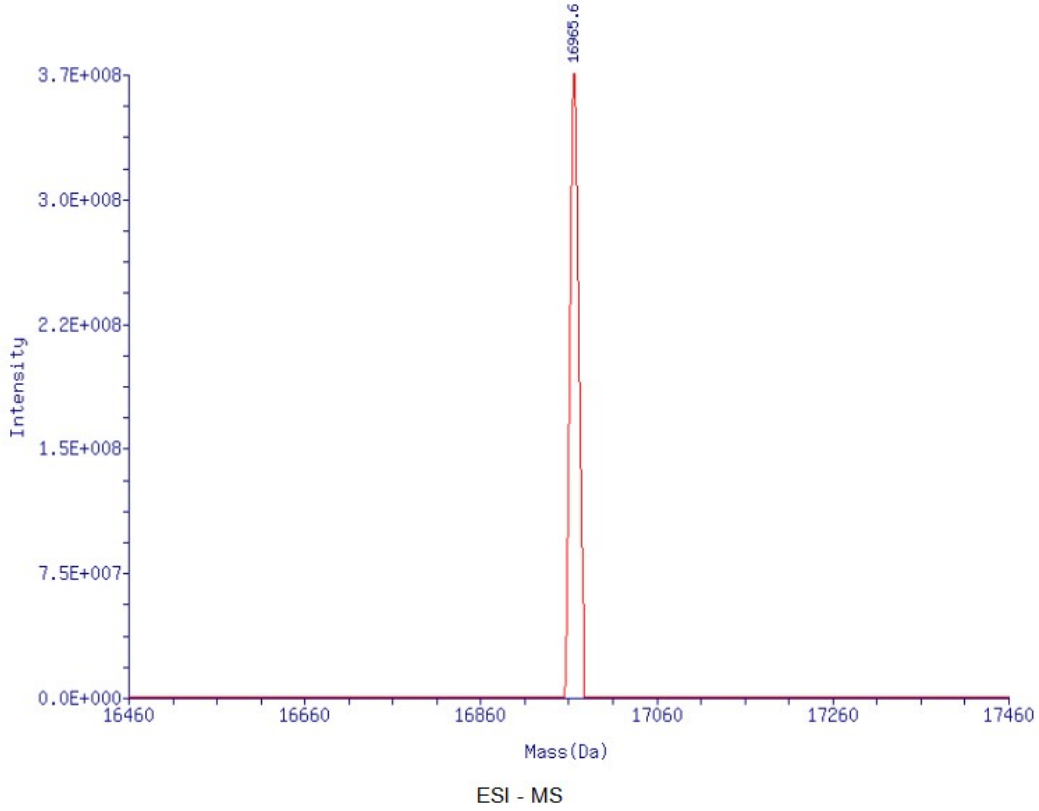

Fig. S11 The ESI-MS spectrum of EpCAM.

Name :	**Control-TDO5**		
Sequence(5'to3'):	TTCACCGGGAGGATAGTTCGGTGGCTGTTCAGGGTCTCCTCCCGGTGTT		
Lot No. :	AX109284769	**Length :**	49
Purification :	HPLC	**Modification(5'to3'):**	
nmoles:	4.44	**Add water to 100uM:**	44.4
TM(°C) :	76.4	**GC(%) :**	59.2
MW (target):	15133.77	**MW(observed):**	15131.3
Conclusion:		**Qualified:**	
Inspector:		**Auditor:**	

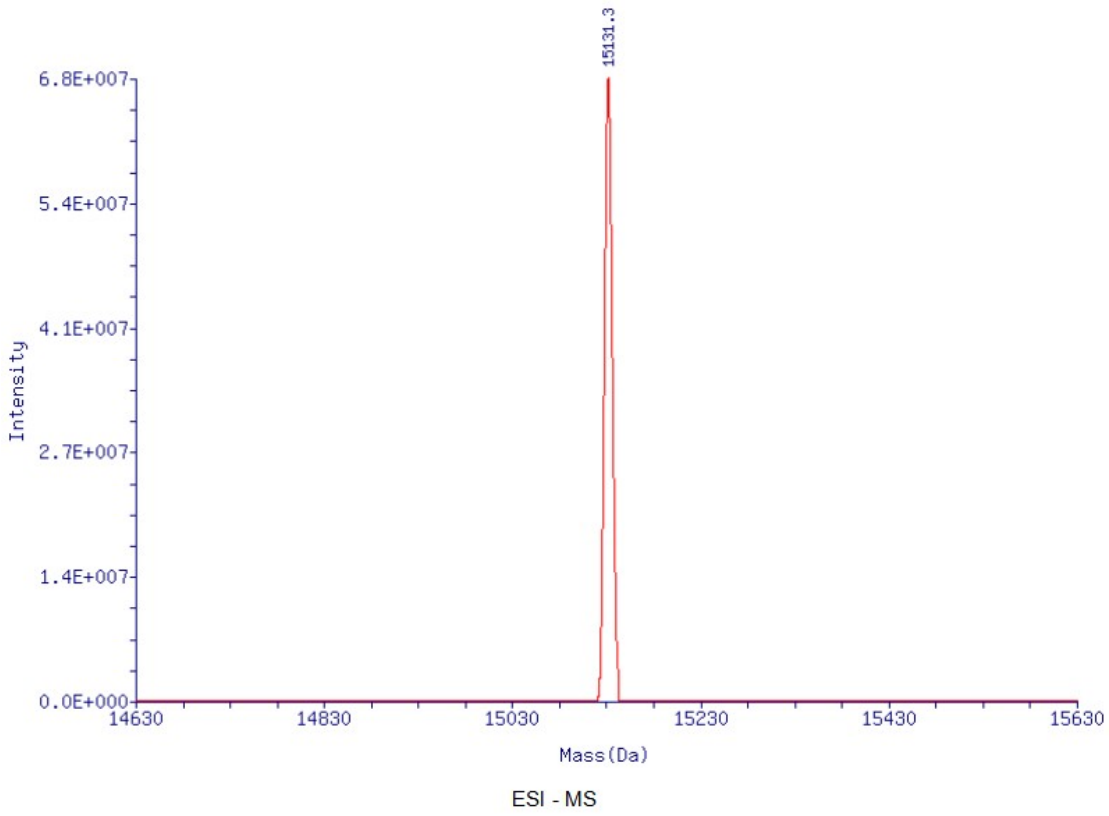

Fig. S12 The ESI-MS spectrum of LIB.

Name ：	**EpCAM (SYL3C)**		
Sequence(5'to3'):	/invdT/TCACTACAGAGGTTGCGTCTGTCCCACGTTGTCATGGGGGGTTGGCCTGTTTTT		
Lot No. :	AX108081533	**Length :**	55
Purification :	HPLC	**Modification(5'to3'):**	3`FITC /invdT/
nmoles:	3.82	**Add water to 100uM:**	38.2
TM(°C) :	74.8	**GC(%) :**	52.7
MW (target):	17479.02	**MW(observed):**	17480.5
Conclusion:		**Qualified:**	
Inspector:		**Auditor:**	

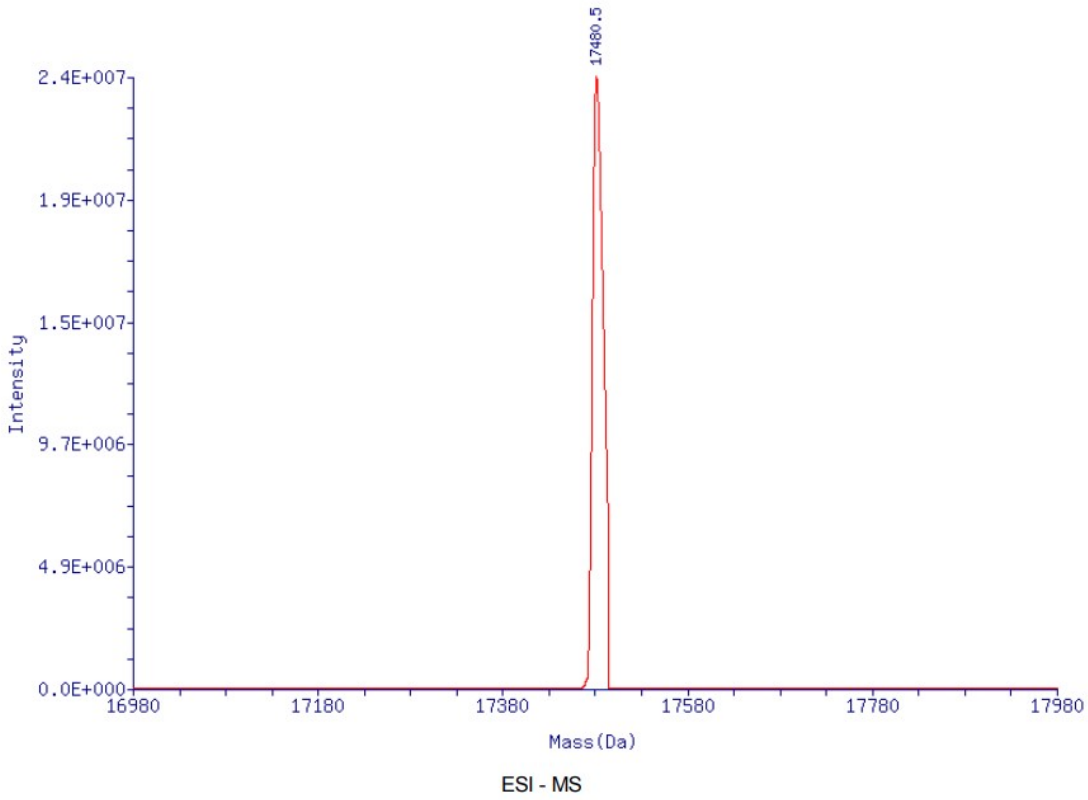

Fig. S13 The ESI-MS spectrum of EpCAM-FITC.

Name ：	**Control-TDO5-1**		
Sequence(5'to3'):	TTCACCGGGAGGATAGTTCGGTGGCTGTTCAGGGTCTCCTCCCGGTGTT		
Lot No. :	AX108081527	**Length :**	49
Purification :	HPLC	**Modification(5'to3'):**	3`FITC
nmoles:	4.24	**Add water to 100uM:**	42.4
TM(°C) :	76.4	**GC(%) :**	59.2
MW (target):	15644.84	**MW(observed):**	15647.0
Conclusion:		**Qualified:**	
Inspector:		**Auditor:**	

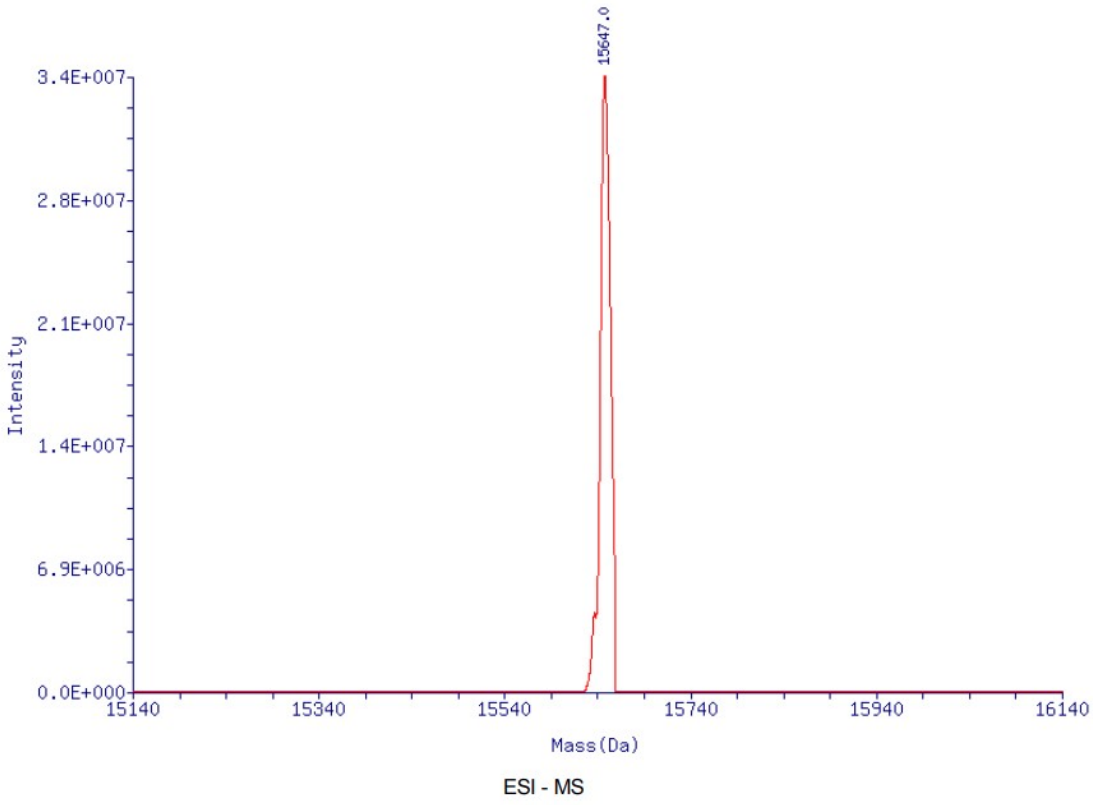

Fig. S14 The ESI-MS spectrum of LIB-FITC.

Name ：	**EpCAM (SYL3C)-Cy5**		
Sequence(5'to3'):	/invdT/TCACTACAGAGGTTGCGTCTGTCCCACGTTGTCATGGGGGGTTGGCCTGTTTTT		
Lot No. :	AX109284773	**Length :**	55
Purification :	HPLC	**Modification(5'to3'):**	3`Cy5 /invdT/
nmoles:	3.92	**Add water to 100uM:**	39.2
TM(°C) :	74.8	**GC(%) :**	52.7
MW (target):	17500.95	**MW(observed):**	17498.0
Conclusion:		**Qualified:**	
Inspector:		**Auditor:**	

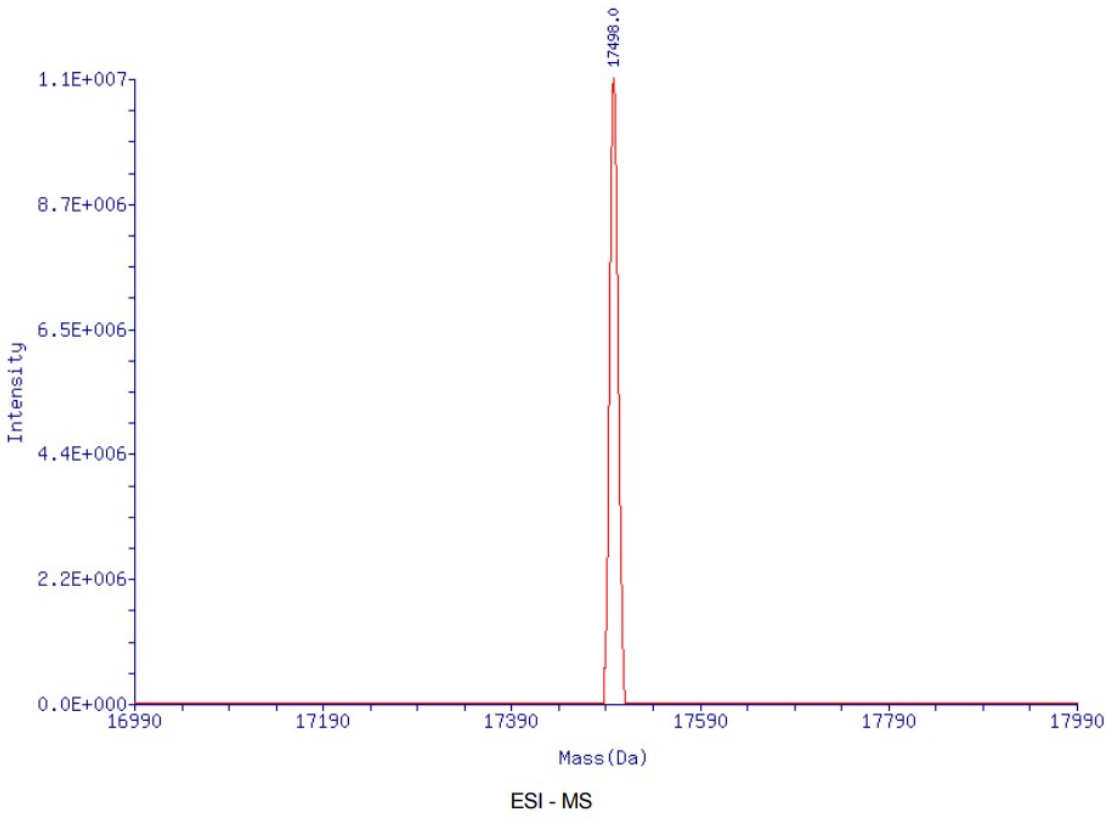

Fig. S15 The ESI-MS spectrum of EpCAM-Cy5.

Name ：	**Control-TDO5-Cy5**		
Sequence(5'to3'):	TTCACCGGGAGGATAGTTCGGTGGCTGTTCAGGGTCTCCTCCCGGTGTT		
Lot No. :	AX109284765	**Length :**	49
Purification :	HPLC	**Modification(5'to3'):**	3`Cy5
nmoles:	4.34	**Add water to 100uM:**	43.4
TM(°C) :	76.4	**GC(%) :**	59.2
MW (target):	15666.77	**MW(observed):**	15664.1
Conclusion:		**Qualified:**	
Inspector:		**Auditor:**	

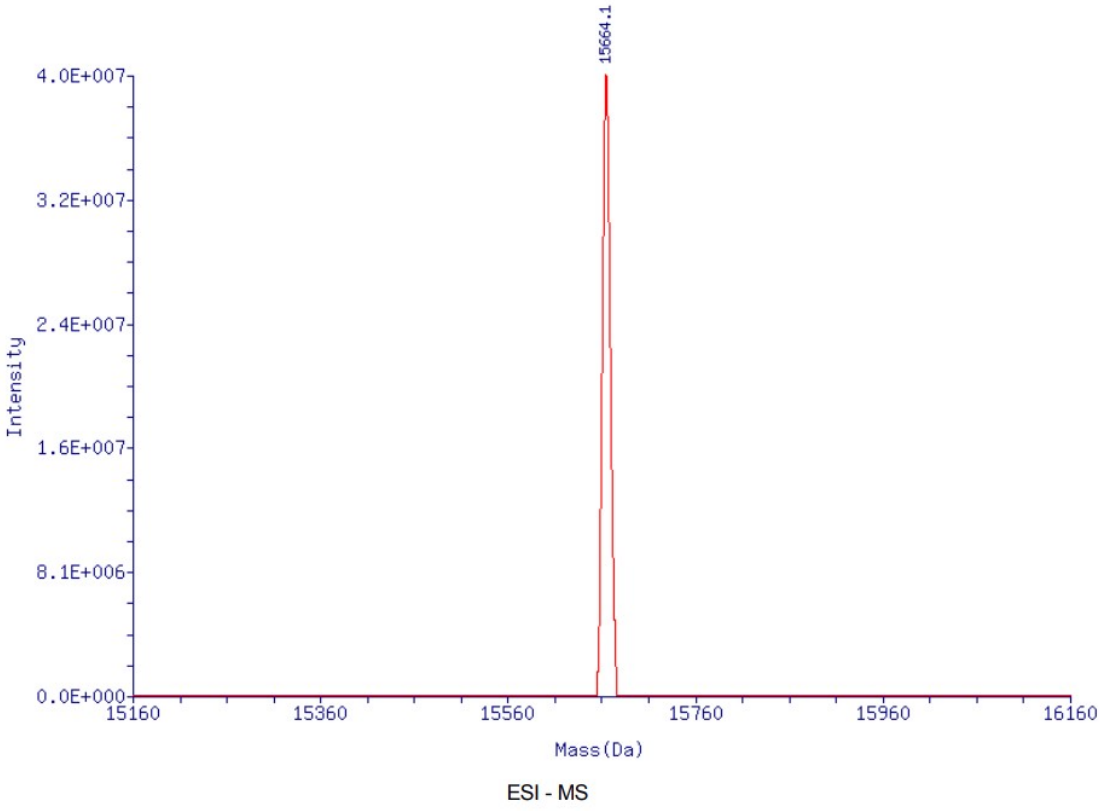

Fig. S16 The ESI-MS spectrum of LIB-Cy5.

Name :	**EpCAM (SYL3C)-3GEM**		
Sequence(5'to3'):	TTCACTACAGAGGTTGCGTCTGTCCCACGTTGTCATGGGGGGTTGGCCTG/iGem//iGem//iGem/TT		
Lot No. :	AX110120811	**Length :**	55
Purification :	HPLC	**Modification(5'to3):**	3Gem
nmoles:	50	**Add water to 100uM:**	500
TM(°C) :	77.1	**GC(%) :**	58.2
MW (target):	17030.5	**MW(observed):**	17028.0
Conclusion:		**Qualified:**	
Inspector:		**Auditor:**	

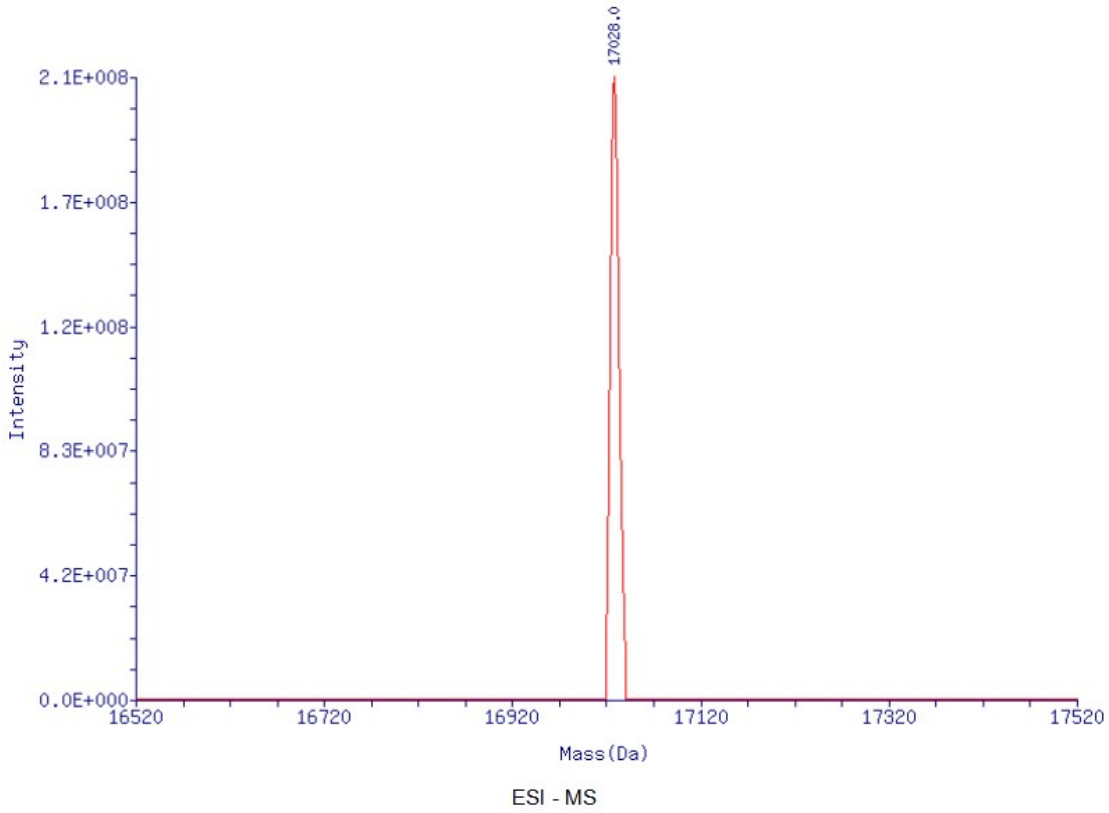

Fig. S17 The ESI-MS spectrum of EpCAM-GEMs.

Name :	**Control-TDO5-3GEM**		
Sequence(5'to3'):	TTCACCGGGAGGATAGTTCGGTGGCTGTTCAGGGTCTCCTCCCGGTG/iGem//iGem//iGem/TT		
Lot No. :	AX110120806	**Length :**	52
Purification :	HPLC	**Modification(5'to3'):**	3Gem
nmoles:	50	**Add water to 100uM:**	500
TM(°C) :	77.9	**GC(%) :**	61.5
MW (target):	16108.9	**MW(observed):**	16106.0
Conclusion:		**Qualified:**	
Inspector:		**Auditor:**	

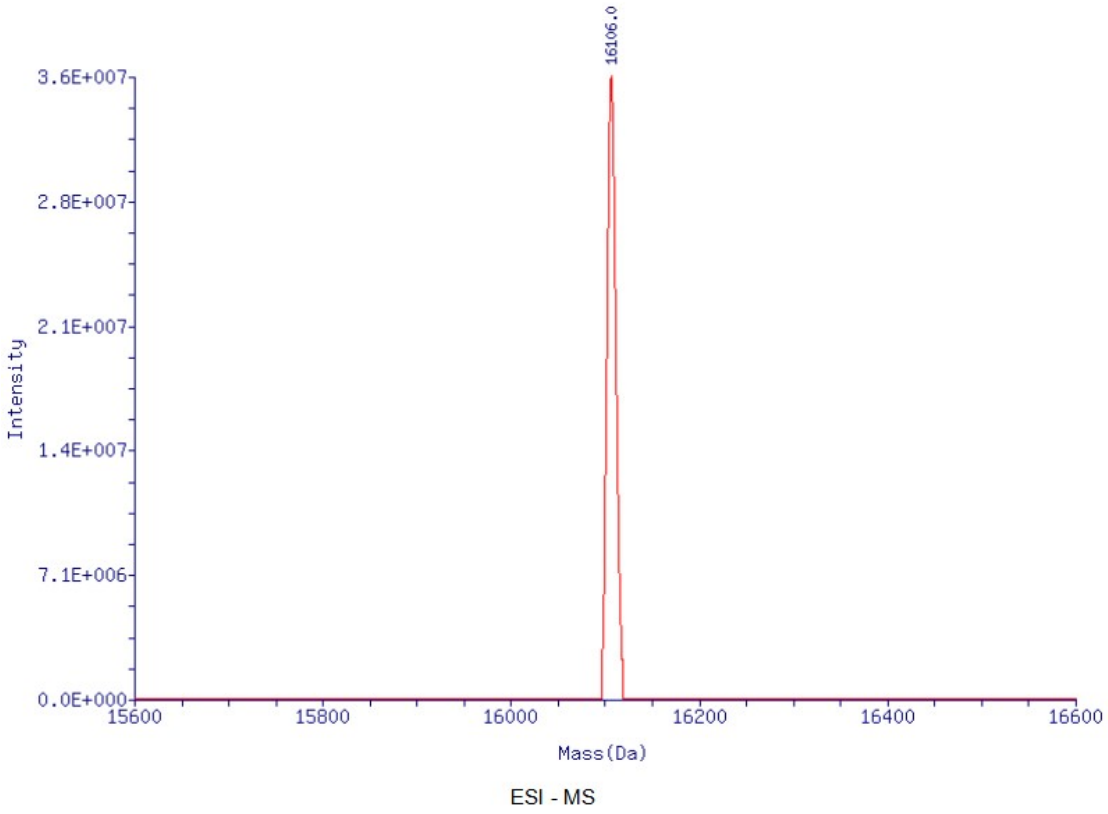

Fig. S18 The ESI-MS spectrum of LIB-GEMs.

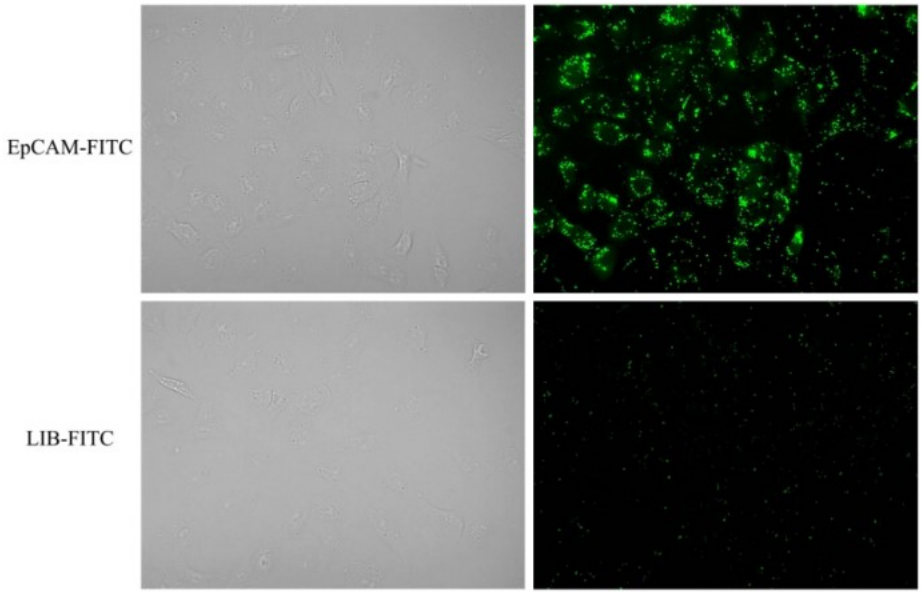

Fig. S19 Surface binding ability of FITC-labeled EpCAM-GEMs and LIB-GEMs to BIU-87 cells.

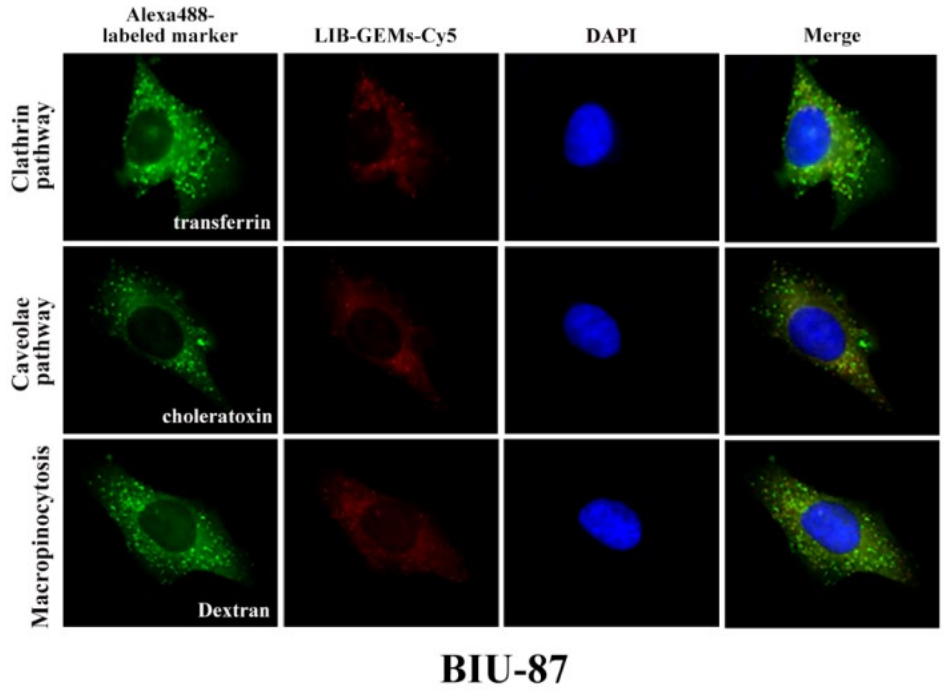

Fig. S20 Internalization and trafficking of LIB-GEMs in BIU-87 cells. Photographs show EpCAM-GEMs-cy5 (red) co-localized with endocytic markers transferrin,

cholera toxin, and dextran labeled with Alexa Fluor 488 (green), respectively. The nuclei were counterstained with DAPI (blue).

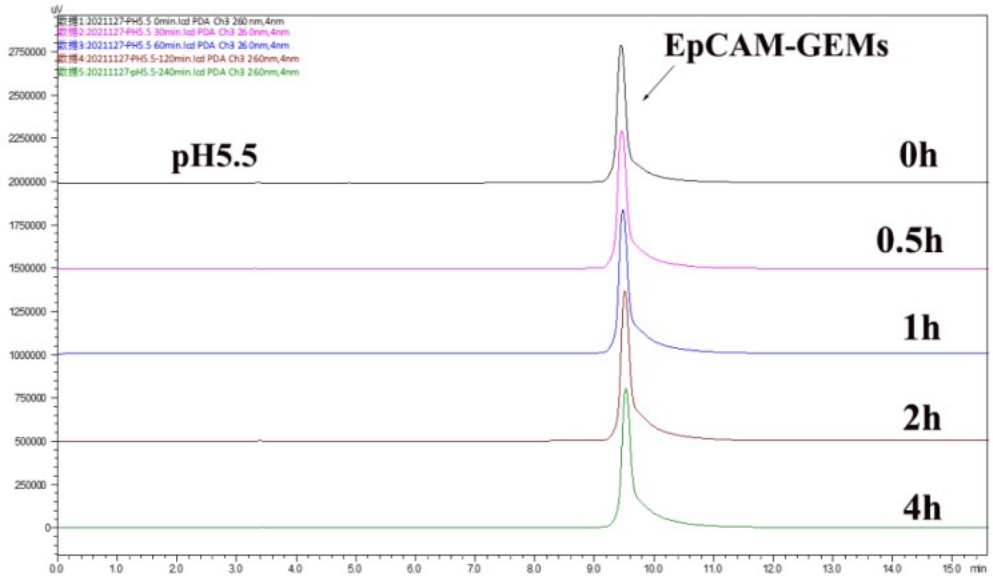

Fig. S21 The release of GEM from EpCAM-GEMs in pH5.5 buffer.

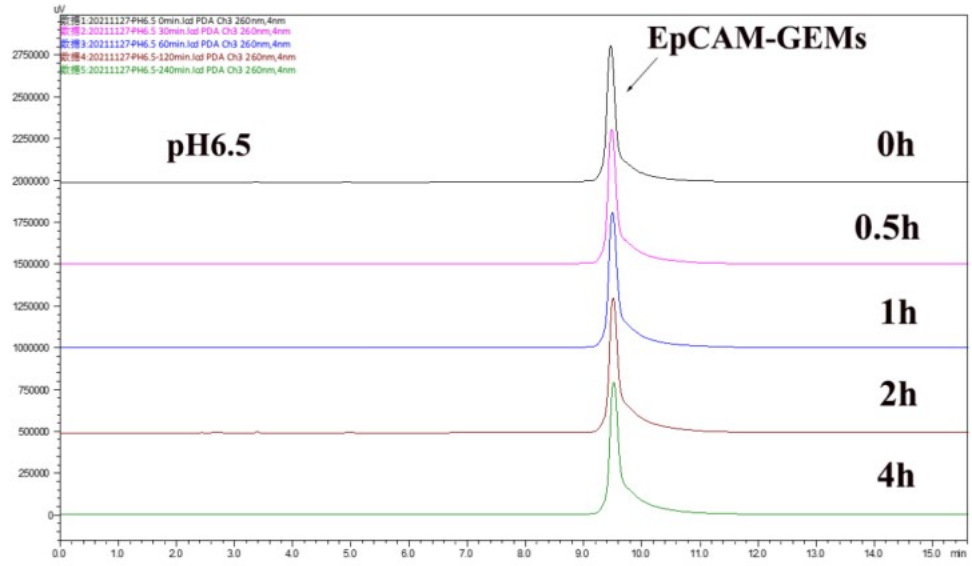

Fig. S22 The release of GEM from EpCAM-GEMs in pH6.5 buffer.

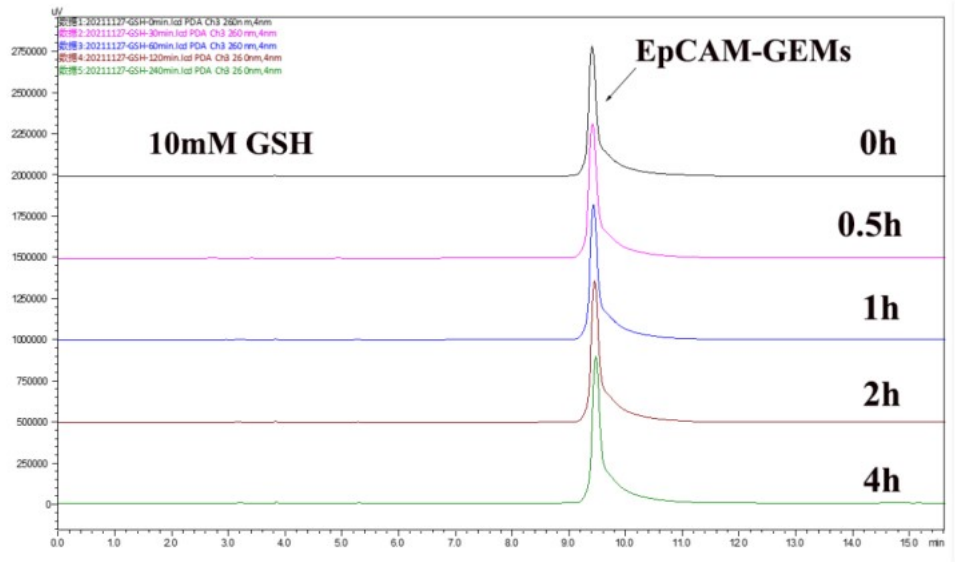

Fig. S23 The release of GEM from EpCAM-GEMs in 10 mM GSH.

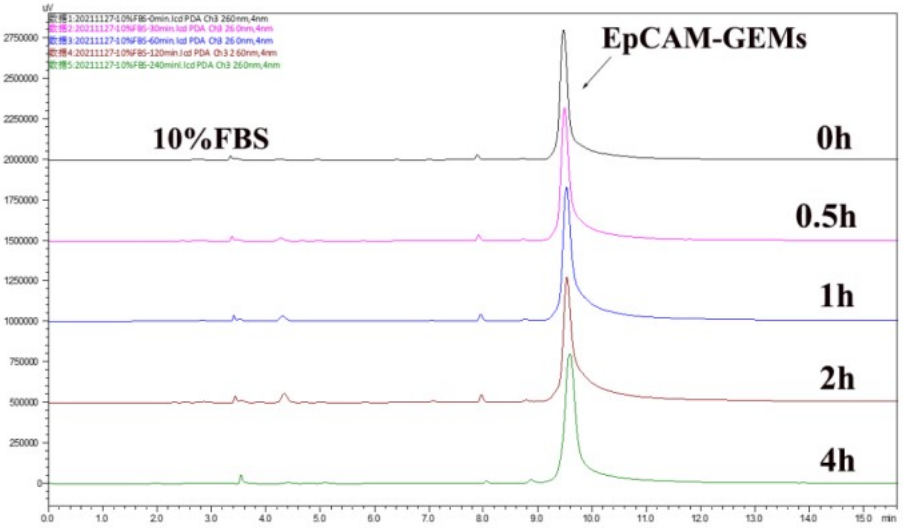

Fig. S24 The release of GEM from EpCAM-GEMs in 10% FBS.

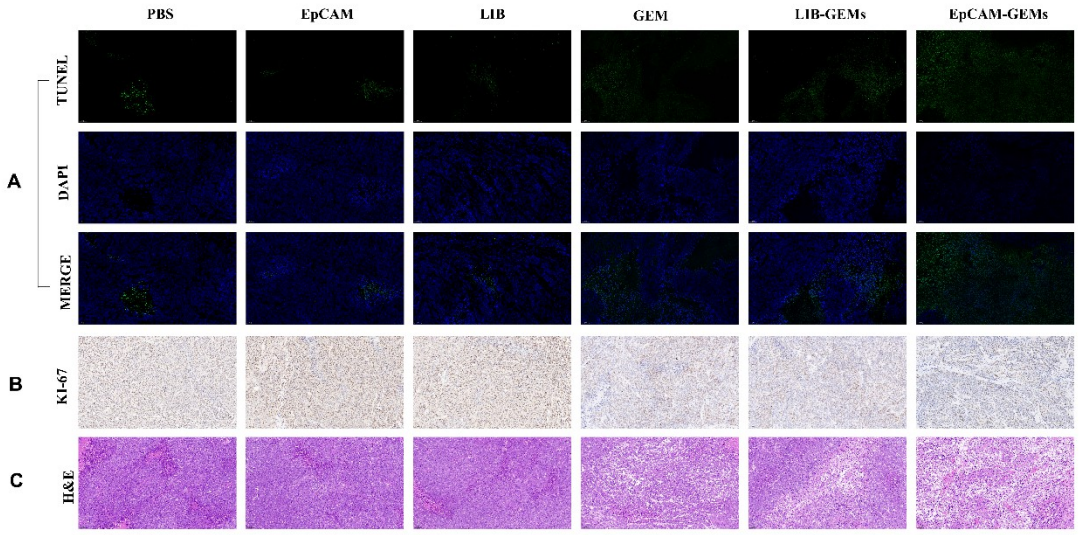

Fig. S25 Biosafety assessment of EpCAM-GEMs in stained tumor sections. Tumor sections of different groups with (A) TUNEL staining (green fluorescence, combined with blue nuclei), (B) Ki67 staining (brown signal), (C) H&E staining.

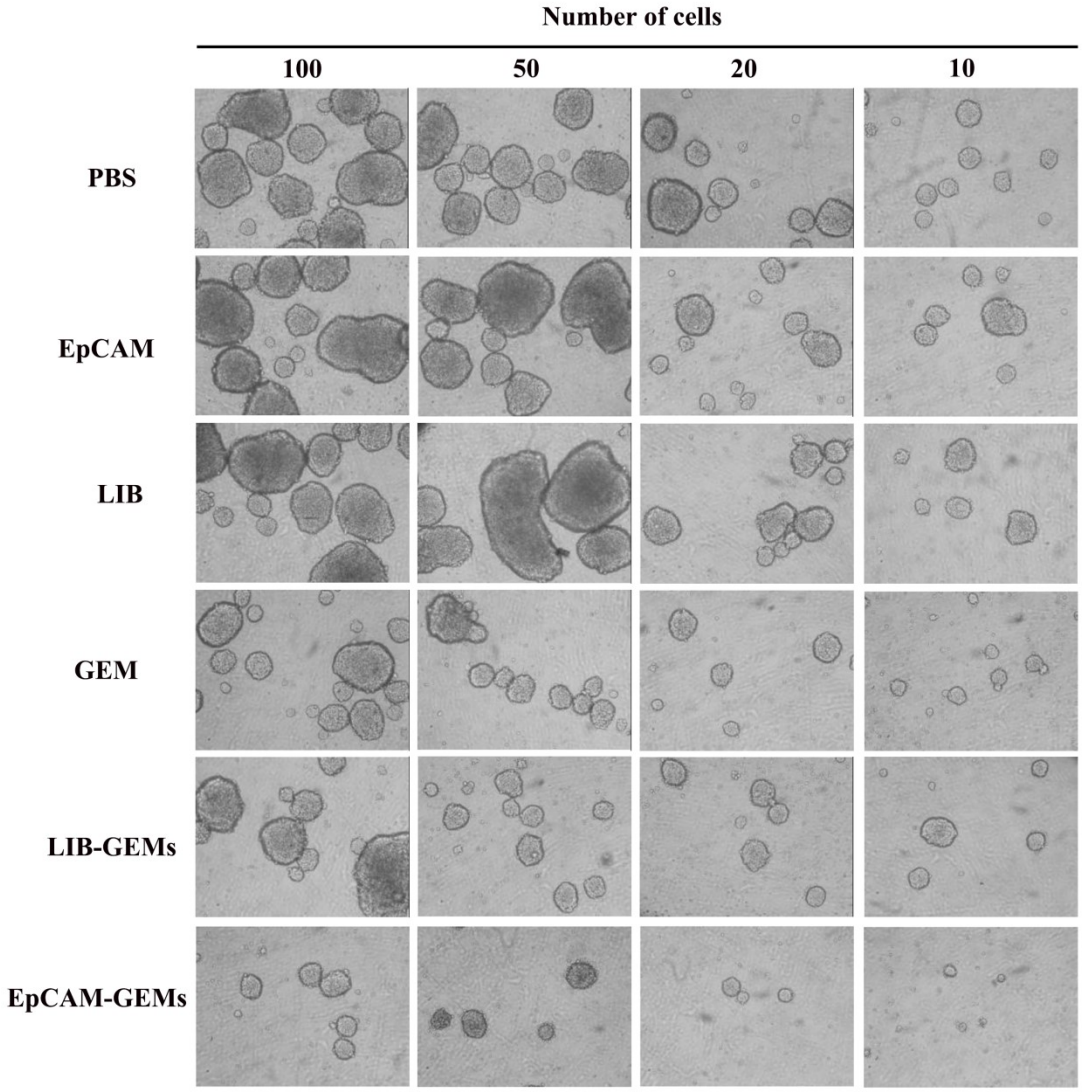

Fig. S26 Representative images of tumor spheres taken at two weeks after cell seeding.

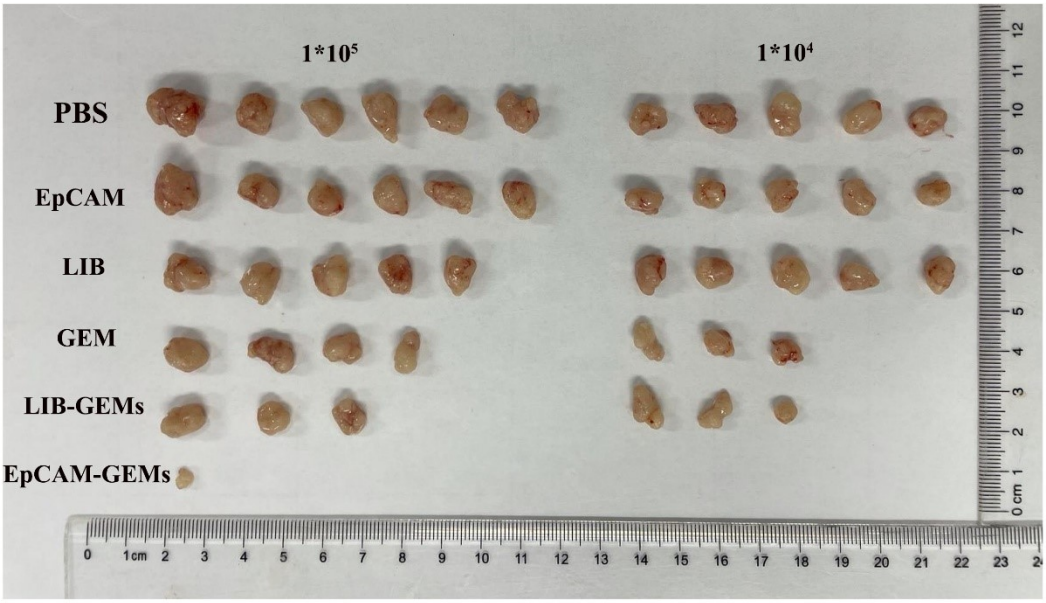

Fig. S27 Tumor growth images after transplantation of 1×10^4 and 1×10^5 BIU-87 cells after treatment with various drugs as indicated.

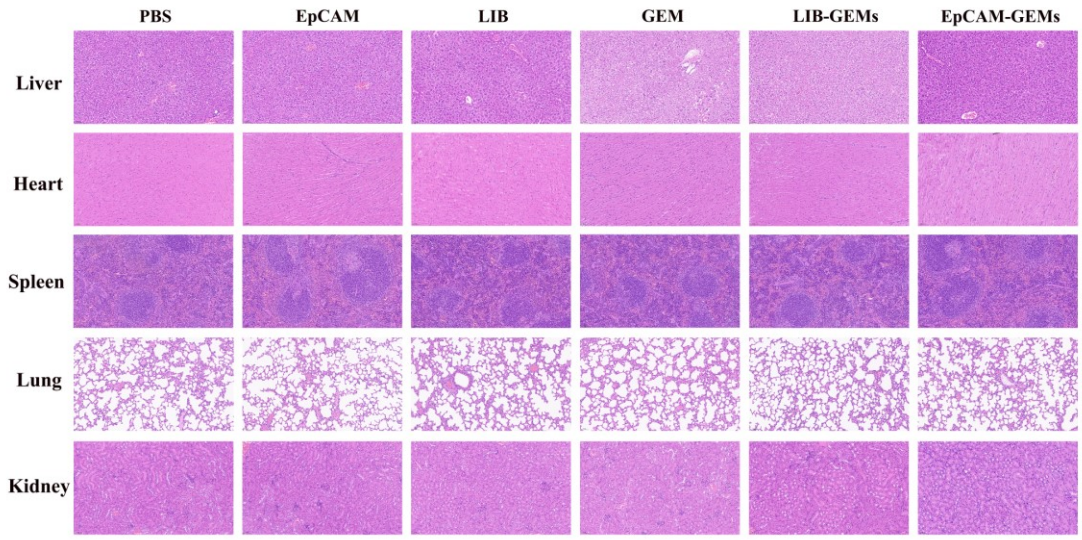

Fig. S28 The biosafety assessment of EpCAM-GEMs in the staining of tissue sections. H&E staining analysis of heart, liver, spleen, kidney, and lung tissues in the different groups.

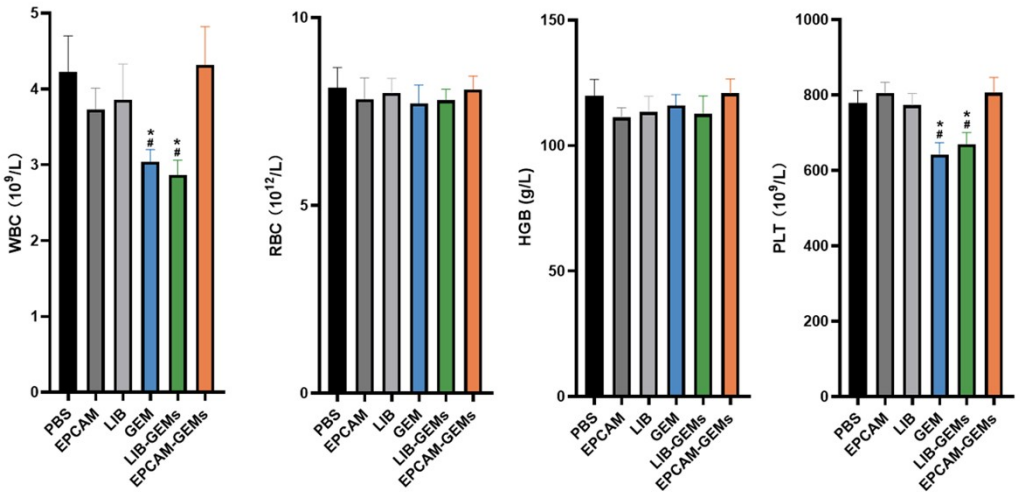

Fig. S29 The biosafety assessment of EpCAM-GEMs in biochemical assays. PLTs: platelets, HGB: hemoglobin, RBCs: red blood cells, WBCs: white blood cells. Data are the mean ± SEM, n = 5. **P* < 0.05 *vs*. the PBS group, $^{\#}$ *P* < 0.05 *vs*. the EpCAM-GEMs group.

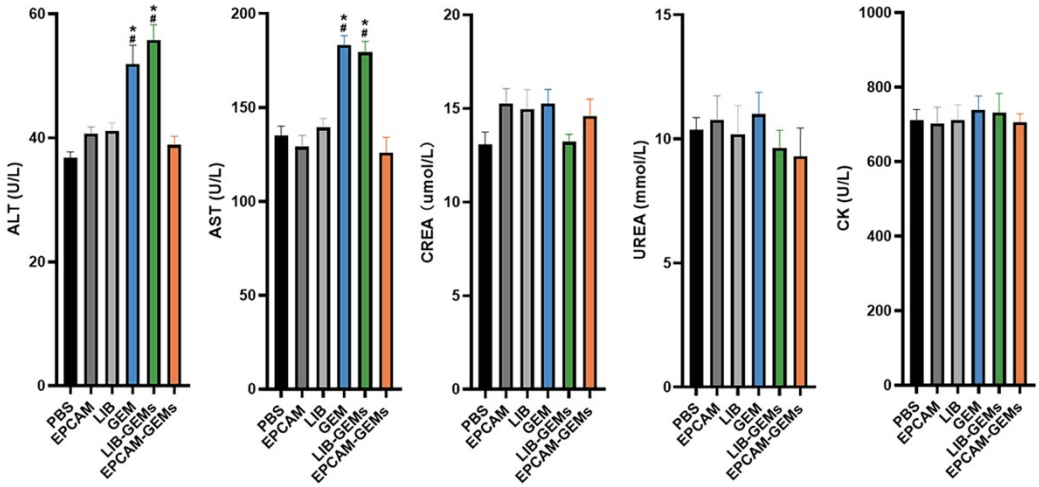

Fig. S30 Biosafety assessment of EpCAM-GEMs in enzymes assays. ALT: alanine aminotransferase, AST: aspartate aminotransferase, CREA: creatinine, CK: creatine phosphokinase. Data are the mean ± SEM, n = 5; **P* < 0.05 *vs*. the PBS group, $^{\#}$ *P* < 0.05 *vs*. the EpCAM-GEMs group.

Table S1. EpCAM expression and clinicopathological variables in BCa.

Clinicopathological variables	Number of cases (n = 148)	EpCAM expression level		*P* value[a]
		Low (%) (n = 46)	High (%) (n = 102)	
Age (years)				0.039
≤ 60[b]	62	25 (40.3)	37 (59.7)	
> 60	86	21 (24.4)	65 (75.6)	
Sex				0.573
Male	125	40 (32.0)	85 (68.0)	
Female	23	6 (26.1)	17 (73.9)	
Tumor size (cm)				0.45
≤ 3.8[c]	84	24 (28.6)	60 (71.4)	
> 3.8	64	22 (34.4)	42 (65.6)	
Tumor multiplicity				0.449
Unifocal	39	14 (35.9)	25 (64.1)	
Multifocal	109	32 (29.4)	77 (70.6)	
Tumor grade				0.81
Low	59	19 (32.2)	40 (67.8)	
High	89	27 (30.3)	62 (69.7)	
pT status				**0.032**
pT1	44	12 (27.3)	32 (72.7)	
pT2	52	23 (44.2)	29 (55.8)	
pT3/pT4	52	11 (21.2)	41 (78.8)	
pN status				**0.006**
pN-	123	44 (35.8)	79 (64.2)	
pN+	25	2 (8.0)	23 (92.0)	

[a]Chi-squared test. [b]mean age. [c]mean size.

Table S2. Univariate Cox proportional regression analysis for survival in BCa.

Variables	All cases	HR (95% CI)	*P* value[a]
Age (years)			0.39
≤ 60[b]	62	1	
> 60	86	1.288 (0.723–2.296)	
Sex			0.934
Male	125	1	
Female	23	0.967 (0.435–2.150)	
Tumor size (cm)			0.596
≤ 3.8[c]	84	1	
> 3.8	64	1.166 (0.661–2.055)	
Tumor multiplicity			0.998
Unifocal	39	1	
Multifocal	109	1.001 (0.545–1.837)	
Tumor grade			**< 0.001**
Low	59	1	
High	89	4.092 (1.978–8.466)	
pT status			**0.013**
pT1	44	1	
pT2	52	2.029 (0.847–4.860)	
pT3/pT4	52	3.365 (1.454–7.789)	
pN status			**< 0.001**
pN-	123	1	
pN+	25	3.878 (2.110–7.127)	
EpCAM			**0.001**
Low	46	1	
High	102	4.222 (1.796–9.926)	

[a]Chi-squared test. [b]mean age. [c]mean size. HR: hazard ratio. CI: confidence interval.

Table S3. Multivariate Cox proportional regression analysis for survival in BCa.

Variables	Hazards ratio	95% CI[a]	*P* value
Tumor grade (low *vs*. high)	3.805	1.762–8.219	**0.001**
pT status (pT1 *vs*. pT2 *vs*. pT3/pT4)	1.457	0.964–2.201	**0.012**
pN status (pN- *vs*. pN+)	1.621	0.799–3.285	**0.017**
EpCAM (low *vs*. high)	4.393	1.831–10.542	**0.001**

[a]CI: confidence interval.

Table S4. Aptamer sequences used in this study.

Name	Sequences and modification (5' to 3')
EpCAM (SYL3C)	TT CAC TAC AGA GGT TGC GTC TGT CCC ACG TTG TCA TGG GGG GTT GGC CTG TTT TT
LIB	TT CAC CGG GAG GAT AGT TCG GTG GCT GTT CAG GGT CTC CCG GTG TT
EpCAM (SYL3C)-FITC	dTT CAC TAC AGA GGT TGC GTC TGT CCC ACG TTG TCA TGG GGG GTT GGC CTG TTT TT(FITC)
LIB-FITC	TT CAC CGG GAG GAT AGT TCG GTG GCT GTT CAG GGT CTC CCG GTG TT(FITC)
EpCAM (SYL3C)-Cy5	dTT CAC TAC AGA GGT TGC GTC TGT CCC ACG TTG TCA TGG GGG GTT GGC CTG TTT TT(Cy5)
LIB-Cy5	TT CAC CGG GAG GAT AGT TCG GTG GCT GTT CAG GGT CTC CCG GTG TT(Cy5)
EpCAM (SYL3C)-GEMs	TT CAC TAC AGA GGT TGC GTC TGT CCC ACG TTG TCA TGG GGG GTT GGC CTG MMM TT
LIB-GEMs	TT CAC CGG GAG GAT AGT TCG GTG GCT GTT CAG GGT CTC CCG GTG MMM TT

FITC-labeled and Cy5-labeled DNA are modified on the corresponding 3' terminus. MMM stands for the three gemcitabine moieties.

Table S5. Aptamer-guided GEMs delivery reduced CSC frequency in BIU-87 xenograft tumours.

Groups	Cell numbers injected	Tumour incidence[a]	CSC frequency(95% CI)
PBS	1×10^5	6/6	1 in 42.5
	1×10^4	5/6	(89.9-20.2)
EpCAM	1×10^5	6/6	1 in 42.5
	1×10^4	5/6	(89.9-20.2)
LIB	1×10^5	5/6	1 in 58.8
	1×10^4	5/6	(118.7-29.3)
GEM	1×10^5	4/6	1 in 119.8
	1×10^4	3/6	(256.8-56.0)
LIB-GEMs	1×10^5	3/6	1 in 151.3
	1×10^4	3/6	(341.5-67.1)
EpCAM-GEMs	1×10^5	1/6	1 in 1201.2
	1×10^4	0/6	(8528.2-169.6)

[a]The number of tumours detected/number of mice received xenotransplantation.

Table S6. Tumor suppression effect of different treatments on bladder weight and histopathological changes in SD rat bladders of the different groups.

No. of rats sacrificed	Normal (T0)	Cancer *in situ* or Noninvasive papillary carcinoma (Tis/Ta)	Subepithelial connective tissue invasive BCa (T1)	Muscle invasive BCa (≥ T2)
12	1	0	2	9
12	0	1	3	8
12	1	2	1	8
12	2	4	1	5
12	2	3	2	5
12	6	4	1	1

Table S7. Hematological and biochemical assays of the sacrificed mice.

Group	PBS	EpCAM	LIB	GEM	LIB-GEMs	EpCAM-GEMs
WBCs (10^9/L)	4.2 ± 0.5	3.7 ± 0.3	3.9 ± 0.5	3.0 ± 0.2 $^{*\#}$	2.8 ± 0.2 $^{*\#}$	4.3 ± 0.5
RBCs (10^{12}/L)	8.1 ± 0.5	7.8 ± 0.6	8.0 ± 0.4	7.7 ± 0.5	7.8 ± 0.3	8.1 ± 0.4
HGB (g/L)	119.8 ± 6.6	111.2 ± 3.9	113.4 ± 6.3	116.0 ± 4.3	112.6 ± 7.2	120.8 ± 5.8
PLTs (10^9/L)	779.4 ± 32.3	804.8 ± 28.9	773.0 ± 31.0	642.4 ± 31.4 $^{*\#}$	668.8 ± 31.7 $^{*\#}$	806.2 ± 40.2
ALT (U/L)	36.8 ± 1.0	40.7 ± 1.1	41.1 ± 1.3	51.9 ± 3.0 $^{*\#}$	55.8 ± 2.5 $^{*\#}$	38.8 ± 1.4
AST (U/L)	135.1 ± 4.9	129.1 ± 6.0	139.4 ± 4.6	183.2 ± 5.1 $^{*\#}$	179.4 ± 5.9 $^{*\#}$	125.9 ± 8.3
CREA (μmol/L)	13.1 ± 0.7	15.3 ± 0.8	15.0 ± 1.0	15.3 ± 0.8	13.2 ± 0.4	14.6 ± 0.9
UREA (mmol/L)	10.4 ± 0.5	10.8 ± 1.0	10.2 ± 1.2	11.0 ± 0.9	9.6 ± 0.7	9.3 ± 1.1
CK (U/L)	711.5 ± 28.7	701.5 ± 44.8	711.8 ± 41.0	738.5 ± 37.7	731.2 ± 51.3	705.9 ± 22.5

WBCs: white blood cells, RBCs: red blood cells, HGB: hemoglobin, PLTs: platelets, ALT: alanine aminotransferase, AST: aspartate aminotransferase, CREA: creatinine, and CK: creatine phosphokinase. Data are mean ± SEM, n = 5， $^{*}P < 0.05$ *vs.* the phosphate buffered saline (PBS) group, $^{\#}P < 0.05$ *vs.* the EpCAM-GEMs group.