Supplementary

for

Circulating Tumour Cells-Targeting Au-Nanocages-Mediated Bimodal Phototherapeutic Properties Enriched by Magnetic Nanocore

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Table S1. Characterization of TPVA modified with different TGA volume concentrations. The initial PVA concentration was fixed at 2 wt % for all samples.

Sample	TGA (vol %)	Thiol content (mmol g^{-1})	Size[a] (nm)
$TPVA_{0.5}$	0.5	0.179	223.8 ± 18.1
$TPVA_{1}$	1	0.34	262.8 ± 34.8
$TPVA_{2.5}$	2.5	1.23	358.2 ± 50.5

[a] the size of the magnetic core synthesized from TPVA with different thiol content.

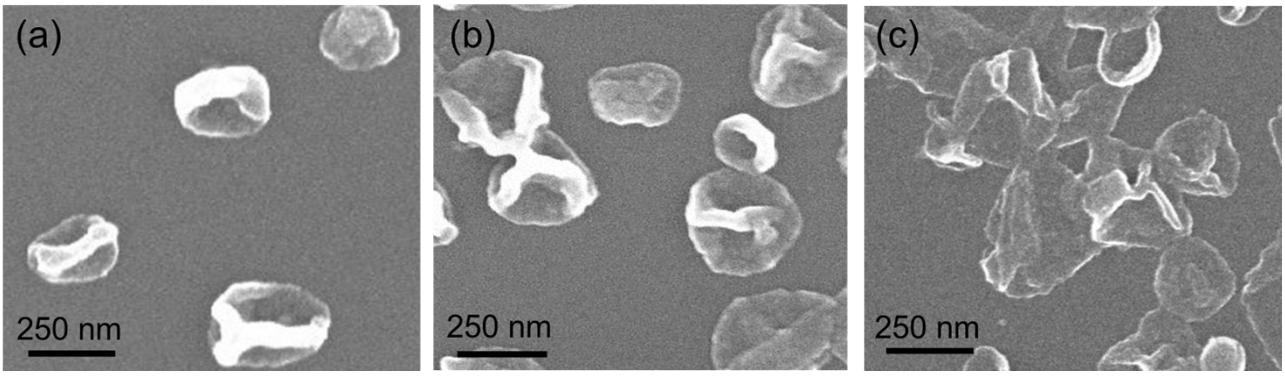

Fig. S1 The morphology of the magnetic core was related to the thiol content of TPVA. SEM images of MN synthesized from TPVA modified with (a) 0.5, (b) 1, and (c) 2.5 % (v/v) TGA after an esterification reaction, respectively.

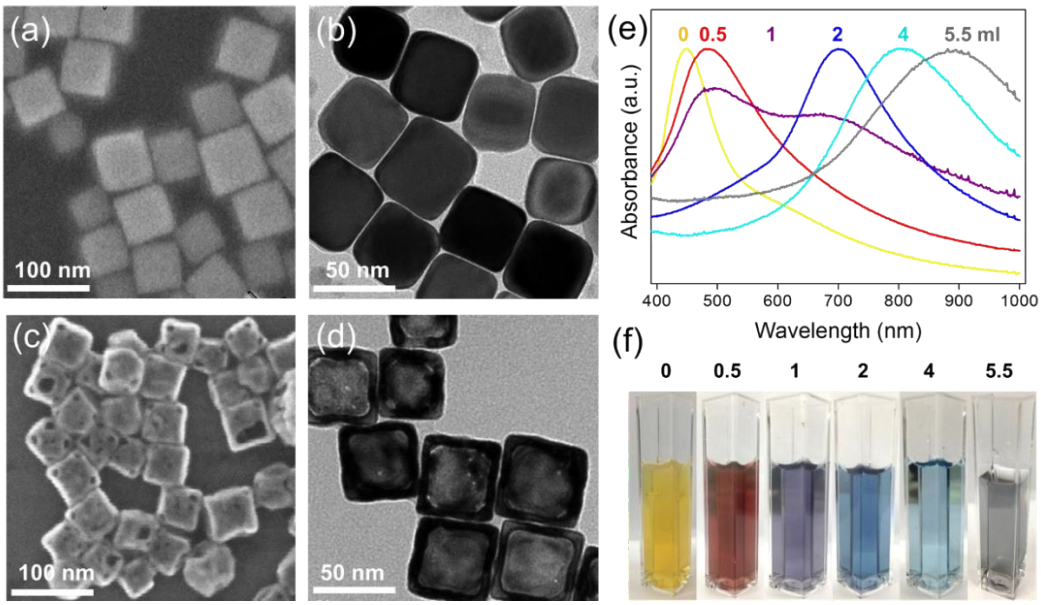

Fig. S2 Images and optical properties of Ag nanocubes and AuNCs. (a) SEM and (b) TEM image of the Ag nanocubes, which showed approximately 50 nm in edge length. (c) SEM image and (d) TEM image of AuNCs in a hollow and porous structure. (e) UV-visible-NIR absorbance spectra of Ag nanocubes and AuNCs. Different volumes of the $HAuCl_4$ solution (0, 0.5, 1, 2, 4, 5.5 ml) were used to titrate the Ag nanocubes suspension. (f) Brilliant color changes during the Ag-Au alloy formation.

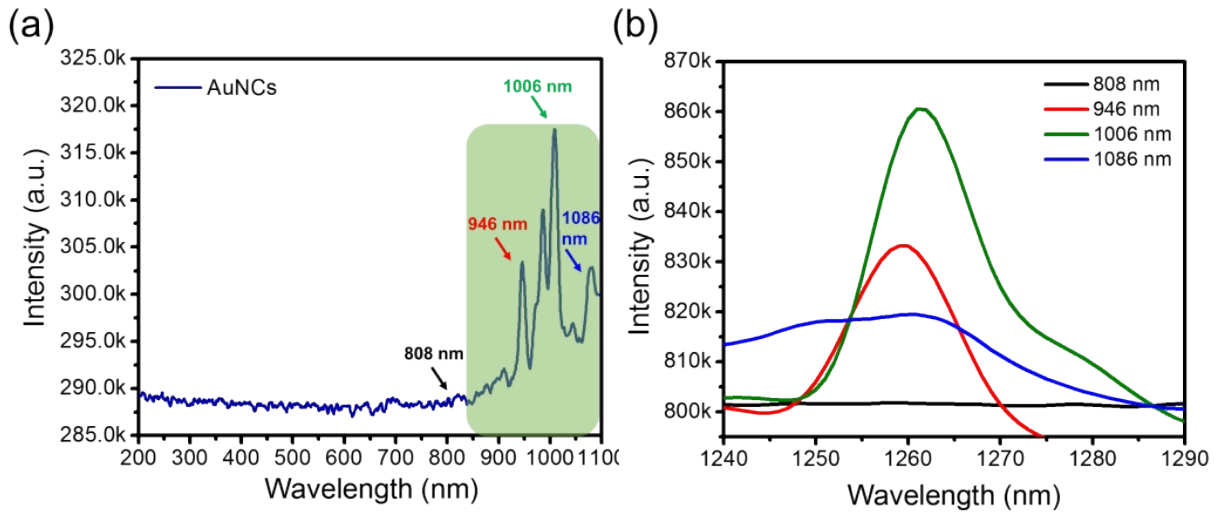

Fig. S3 Direct detection of singlet oxygen ($^1O_2$) phosphorescence from the photoexcitation of AuNCs in a D_2O solvent. (a) The excitation spectrum of AuNCs and strong spectral peaks that exist after 900 nm. (b) $^1O_2$ phosphorescence emission spectra by sensitization of AuNCs using different NIR wavelengths.

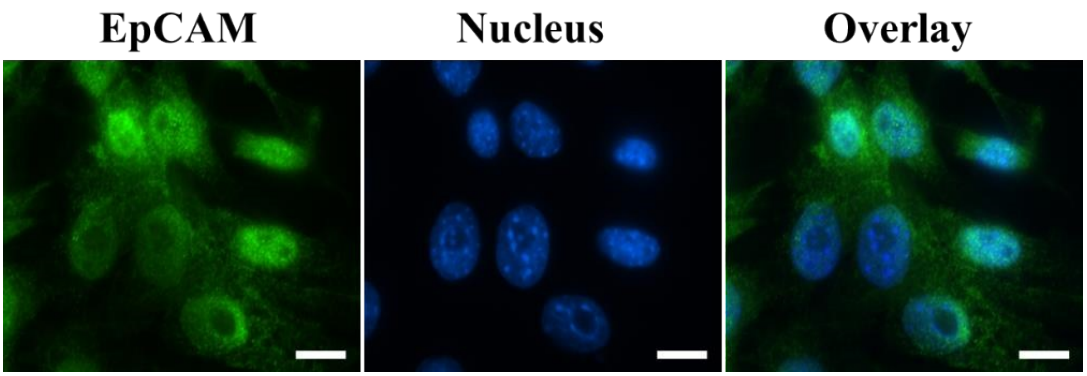

Fig. S4 EpCAM is a surface antigen used for the targeted delivery of QD-labeled TPMN to 4T1 cells. High expression of EpCAM on 4T1 cells was confirmed using immunofluorescence staining on 4T1 cells with a secondary FITC-labeled anti-rabbit antibody. Scale bars = 10 μm.

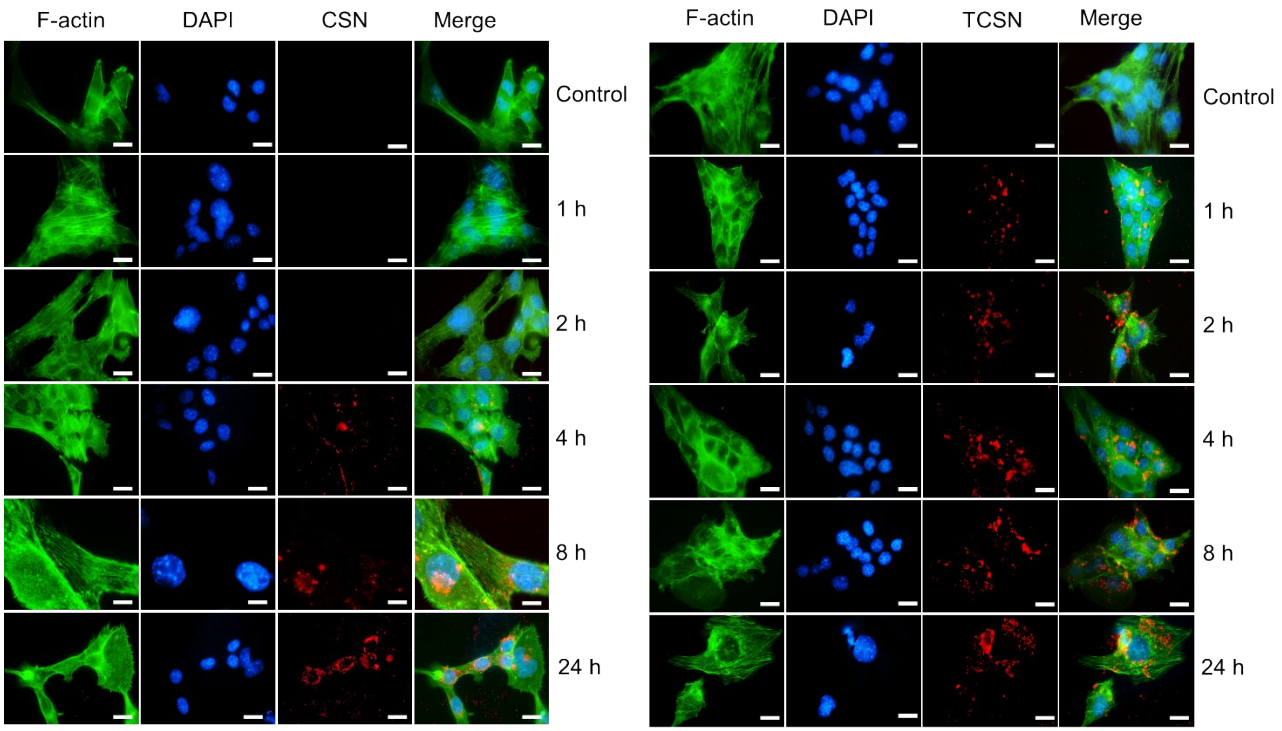

Fig. S5 Cell association of non-targeted QD-labeled CSN and TCSN (red) incubated for 1, 2, 4, 8, and 24 h with 4T1 cells. F-actin and nuclei of 4T1 cells were stained with Alexa-488 (green) and DAPI (blue). Scale bars = 10 μm.

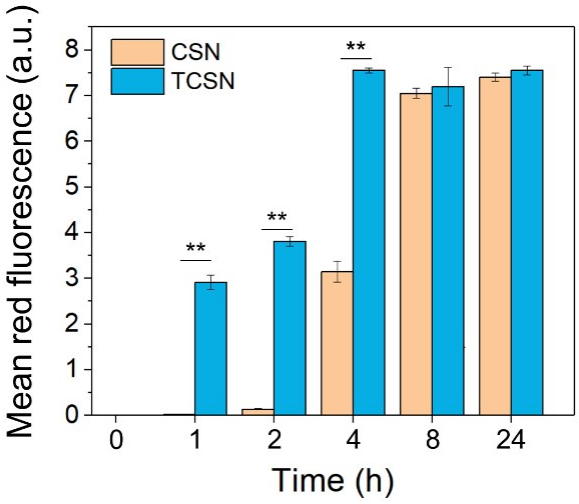

Fig. S6 Statistical analysis of cellular association at 0, 1, 2, 4, 8, and 24 incubation with CSN or TCSN. Images from fluorescent microscopy were analyzed using ImageJ. Cell association level between CSN and TCSN showed significant difference at short culture period including 1, 2, and 4h incubation, but no significant difference was found in longer culture time (i.e., 8 and 24h). Data are expressed as mean ± SD, n = 6 biologically independent samples. *$p < 0.05$ and ** $p < 0.01$ between groups using an independent sample t-test.

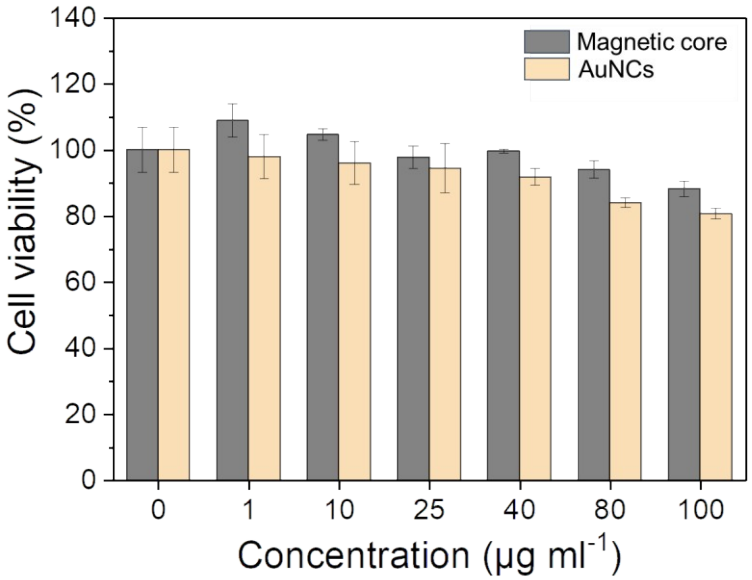

Fig. S7 Evaluation of cell viability of 4T1 cells after incubation with various concentrations of the magnetic core and AuNCs in the dark for 24 h. The result is expressed as the mean ± SD, n= 5.

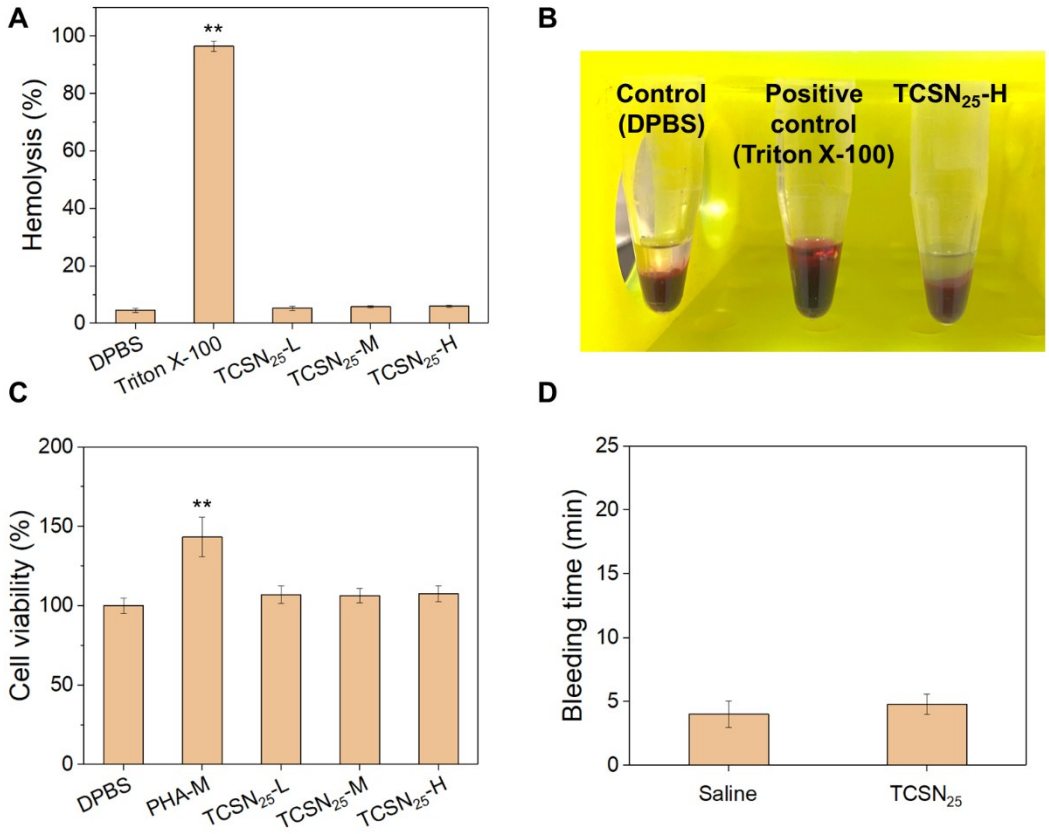

Fig. S8 Blood compatibility of TCSN. (A) Evaluation of hemolytic reaction of DPBS (negative control), Triton X-100 (positive control), low-concentration $TCSN_{25}$ ($TCSN_{25}$-L, [Au]= 10 μg mL^{-1}), medium-concentration $TCSN_{25}$ ($TCSN_{25}$-M, [Au]= 20 μg mL^{-1}), or high-concentration $TCSN_{25}$ ($TCSN_{25}$-H, [Au]= 40 μg mL^{-1}), n= 3. (B) Visual observation of the blood after incubation with DPBS, Triton X-100, and TCSN-H for 3h. (C) Cell viability of peripheral blood mononuclear cell (PBMC) after incubation with DPBS, PHA-M containing RPMI-1640 media, $TCSN_{25}$-L, $TCSN_{25}$-M, and $TCSN_{25}$-H for 24 h, n= 5. (D) Tail bleeding test for monitoring the coagulation time. After the mouse was treated with saline or $TCSN_{25}$, the tail was amputated and the blood were collected and measured to confirm the bleeding time, n= 3. All results are expressed as mean ± SD, n ≥ 3 biologically independent animals or samples. Comparison between two groups was performed using an unpaired two-tailed t-test. Symbol (**) represents a significant difference ($p < 0.01$) compared with control.

Table S2. Semi-quantification of Prussian blue stain

Group	**1 day after treatment**			**7 day after treatment**		
Animal ID	1	2	3	4	5	6
Lung	3	1	1	1	1	1
Liver, red pulp	3	1	2	1	1	1
Spleen, sinusoid	3	1	2	2	1	1
Kidney	0	0	0	0	0	0
Heart	0	0	0	0	0	0

The Perls' Prussian blue stained preparations were graded semi-quantitatively: 0= no iron; 1= minimal or very small amounts; 2= slight and patchy; 3= moderate and diffuse; 4= strong, extensive and diffuse content.

Table S3. Histopathology incidence table

Group	**1 day after treatment**			**7 days after treatment**		
Animal ID	1	2	3	4	5	6
Lung						
Foreign body inflammation	3	2	3	1	1	1
Liver						
Pigment deposition, sinusoid	3	1	2	1	X	1
Spleen						
Pigment deposition, red pulp	3	1	3	1	1	X

X: No significant lesions; degree of lesions was graded from one to five depending on severity: 1= minimal (< 1%); 2= slight (1-25%); 3= moderate (26-50%); 4= moderate/severe (51-75%); 5= severe/high (76-100%).