Supporting Information

Se-modified gold nanorods for enhancing the photothermal therapy efficiency--avoiding the off-target problem induced by biothiols

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Experimental section

Materials and Instruments.

Hydrogen tetrachloroaurate(III) ($HAuCl_4 \cdot 4H_2O$, 99.99%), cetyltrimethyl ammonium bromide (CTAB), sodium borohydride ($NaBH_4$), silver nitrate ($AgNO_3$), hydrochloric acid (HCl), ascorbic acid (AA), sodium dodecylsulfate (SDS) and mercapto-ethanol (ME) were obtained from China National Pharmaceutical (Shanghai); 3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyltetra zolium bromide (MTT), Hoechst 33342, N-ethylmaleimide (NEM), paraformaldehyde and human Caspase-9 recombinant proteins were purchased from Sigma. Caspase-9 inhibitor (Z-LEHD-FMK) was obtained from R&D systems (Minneapolis). All chemicals were analytical grade and used without further purification. Ultrapure water was purified to a resistivity of 18.2 MΩ·cm. FITC-Leu-Glu-His-Asp-Ser-Gly-(Se-Cys), FITC-Leu-Glu-His- Asp-Ser-Gly-Cys, RGD-PEG8-(Se-Cys) and RGD-PEG8-Cys were synthesized and purified by Karebay Biochem (Ningbo, China). The human hepatoma cells (B16-F10) and normal hepatic cells (7702) were purchased from KeyGEN biotechnology (Nanjing, China).

High resolution transmission electron microscopy (HRTEM) was carried out on a JEM-2100 electron. Centrifugation was performed on an Eppendorf 5417R Centrifuge. Zeta potential and dynamic light scattering measurements were performed on a Malvern Zeta Sizer Nano (Malvern Instruments). Fluorescence spectra were obtained with an FLS-920 Edinburgh Fluorescence Spectrometer with a xenon lamp. Absorbance was measured in a microplate reader (Synergy 2, Biotek, USA) in the

MTT assay. Fluorescence imaging was performed with the ImageStreamX multispectral imaging flow cytometer (Amnis Corporation). All pH values were measured by a pH-3c digital pH meter (LeiCi, China) with a combined glass-calomel electrode. Confocal fluorescence imaging experiments were performed with TCS SP8 confocal laser scanning microscopy (Leica Co., Ltd. Germany). PA imaging was accomplished using an Endra Nexus 128 (Ann Arbor, Michigan).

Evaluation of the amount of peptides loaded on AuNRs

The FITC-labeled peptides loaded on the AuNRs were quantified by fluorescence measurement of the labeled dye. 10 mM selenolcysteine was added into the 1 nM Casp-RGD-Se-AuNRs solution, and 10 mM ME was added into the 1 nM Casp-RGD-S-AuNRs solution. Selenolcysteine was in situ generated by mixing Cys and $(Cys\text{-}Se)_2$ at an equivalence ratio of 2:1. The mixture was stirred for 24 h at room temperature to almost completely release the fluorochrome-labeled peptides. Then the released peptides were collected *via* centrifugation, and their fluorescence intensity was measured using a fluorescence spectrometer. The fluorescence of the FITC-labeled peptides was excited at 488 nm and measured at 520 nm. The fluorescence was converted to the molar concentrations of the peptides *via* interpolation from a standard linear calibration curve prepared with known concentrations of the fluorochrome-labeled peptides with an identical buffer pH, ionic strength and peptide concentrations. By dividing the molar concentrations of the peptides by the AuNR concentration, the peptide loading amount per AuNR was obtained.

Cell culture

Dulbecco's modified Eagle's medium (DMEM) was used for the human hepatoma cells (B16-F10) culture, RPMI 1640 medium was used for normal hepatic cells (7702) culture, which supplemented with 10% fetal bovine serum and 100 U/mL 1% antibiotics (penicillin/streptomycin), and incubated in a humidified atmosphere of 5% CO_2 and 95% air at 37°C.

MTT assay

The toxicity of the AuNRs and the two nanoprobes were respectively measured *via* the following MTT assay to the B16-F10 and 7702 cells respectively. The cells were dispersed in replicated 96-well microtiter plates and incubated at 37 °C in 5% CO_2 and 95% air for 24 h. After the original medium was removed, 1 nM of the AuNRs, Casp-RGD-Se-AuNRs and Casp-RGD-S-AuNRs was respectively added to each well, and then incubated for 2, 4, 8, 12 and 24 h respectively. The original medium was removed and replaced with another medium containing MTT (0.5 mg/mL). The cells were incubated at 37 °C for another 4 h, and then the medium was removed. DMSO (100 μL) was added to the cells to dissolve the produced formazan. The absorbance at 490 nm was recorded for each well. The viability was calculated based on the recorded data. All the experiments were repeated at least three times.

The degree of apoptosis was also investigated by MTT assay. B16-F10 cells were cultured in a confocal dish for 24 h at 37 °C. After 80% coverage, the cells were washed with PBS buffer for three times. Casp-RGD-Se-AuNRs (1 nM) was added to the dishes and incubated for 4 h at 37 °C and then exposed to NIR irradiation at 2 W/cm^2 for 0, 2, 4, 6, 8, 10 min, respectively. The medium was removed, and the cells

were washed with PBS for three times. Then, 100 μL MTT solutions (0.5 mg/mL) were added to each well and further incubated for 4 h. Afterwards, the remaining MTT solution was discarded and 100 μL of DMSO was added to the cells to dissolve the purple formazan. The absorbance at 490 nm was recorded for each well. The viability was calculated based on the recorded data. All the experiments were repeated at least three times.

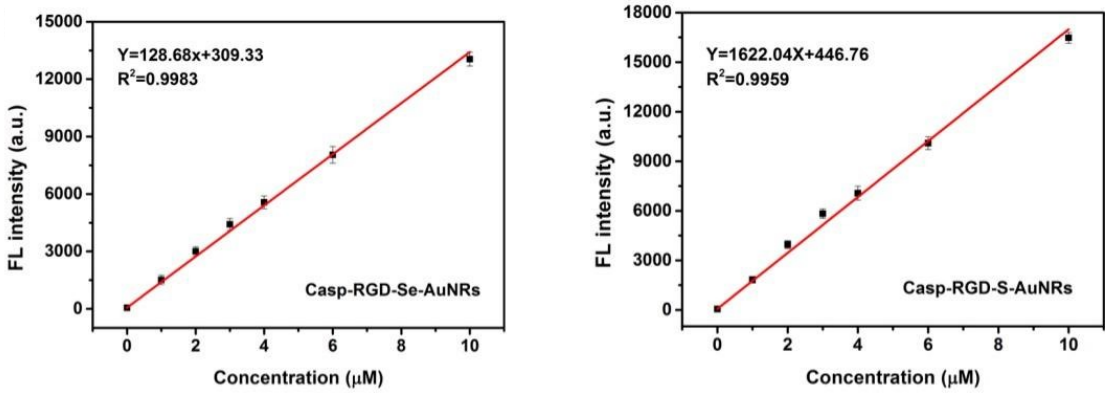

Figure S1. Standard linear calibration curves of the FITC labeled peptides. Error bars were estimated from three replicate measurements.

Table S1. Quantification of peptide chains loaded on each AuNR.

Reaction Concentration AuNRs / Peptides		1:1000	1:2000	1:4000	1:6000	1:8000	1:10000
Number of Peptides On each AuNR	Au-Se	725 ± 20	1025 ± 22	1830 ± 25	2750 ± 30	3020 ± 32	3955 ± 45
	Au-S	640 ± 16	968 ± 18	1735 ± 20	2320 ± 25	2755 ± 28	3530 ±35

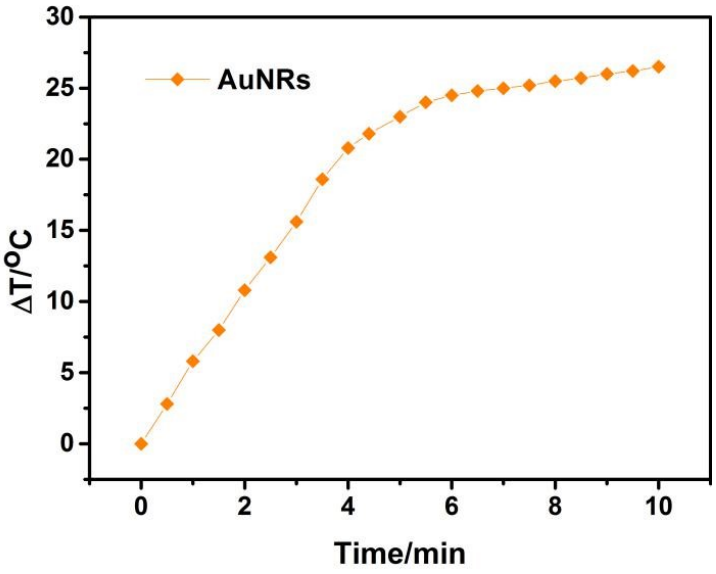

Figure S2. Temperature change curves of AuNRs upon 808 nm laser irradiation at 2 W/cm^2 for 10 min.

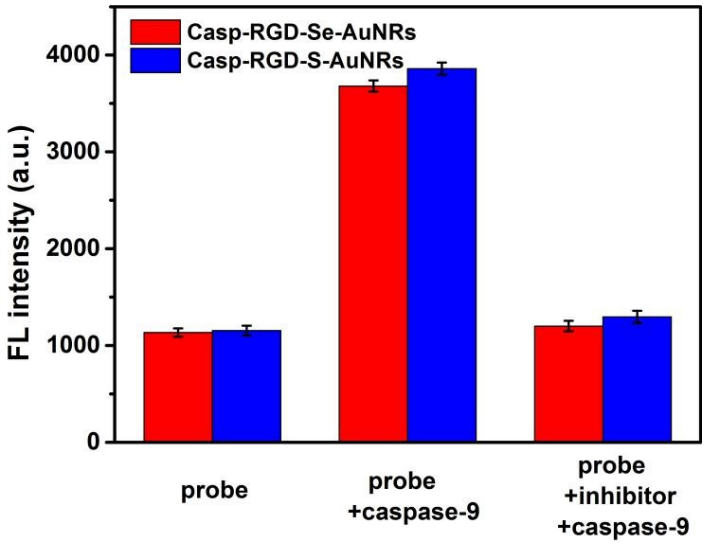

Figure S3. (a) Fluorescence response of Casp-RGD-Se-AuNRs (1 nM) and Casp-RGD-S-AuNRs (1 nM) after mixing with Caspase-9 (300 ng/mL) in the absence and presence of the Caspase-9 inhibitor (10 μM). Error bars were estimated from three replicate measurements.

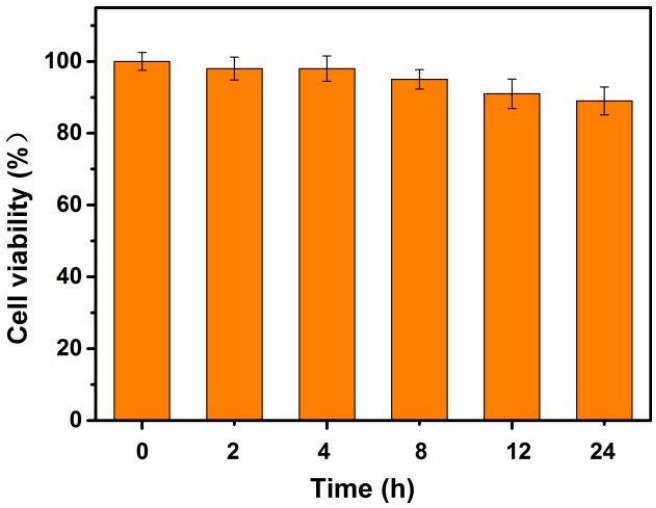

Figure S4. The MTT assay of B16-F10 cells incubated with AuNRs (1 nM) for different time respectively. The absorbance of MTT at 490 nm is dependent upon the degree of activation of the cells. Error bars were estimated from three replicate measurements.

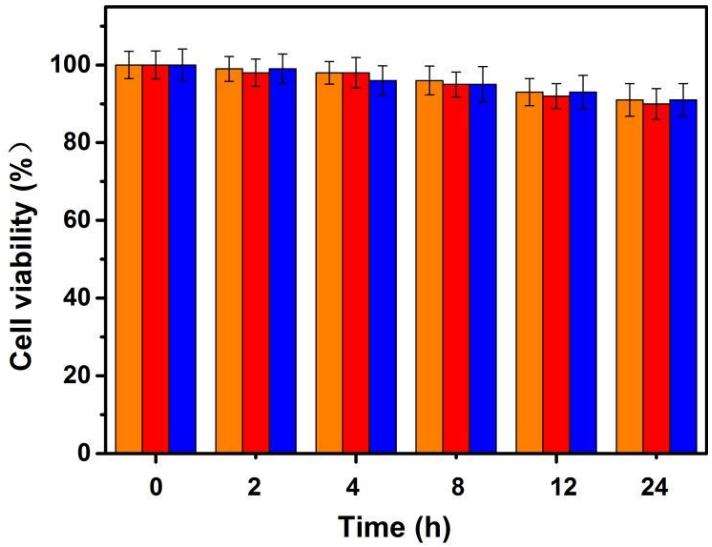

Figure S5. The MTT assay of 7702 cells incubated with AuNRs (1 nM, yellow), Casp-RGD-Se-AuNRs (1 nM, red) and Casp-RGD-S-AuNRs(1 nM, blue) for different time respectively. Error bars were estimated from three replicate measurements.

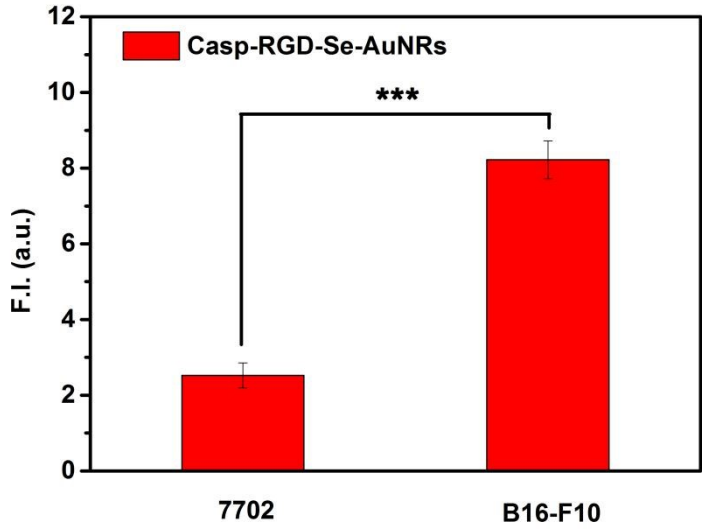

Figure S6. The fluorescence signal intensities of Casp-RGD-Se-AuNRs (1 nM) cultured with 7702 and B16-F10 cells for 4h (*p < 0.05, **p < 0.01, and ***p < 0.001; n = 3).

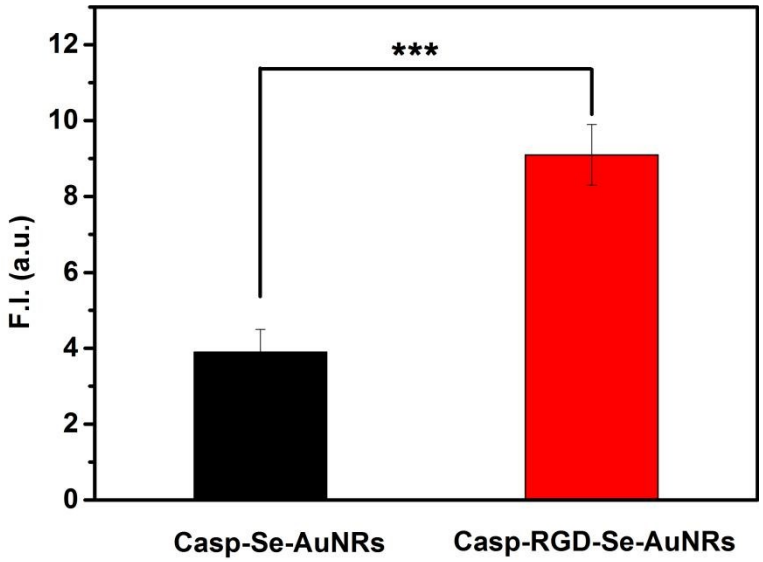

Figure S7. The fluorescence signal intensities of Casp-Se-AuNRs (1 nM) and Casp-RGD-Se-AuNRs (1 nM) cultured with B16-F10 cells for 4h (*p < 0.05, **p < 0.01, and ***p < 0.001; n = 3).

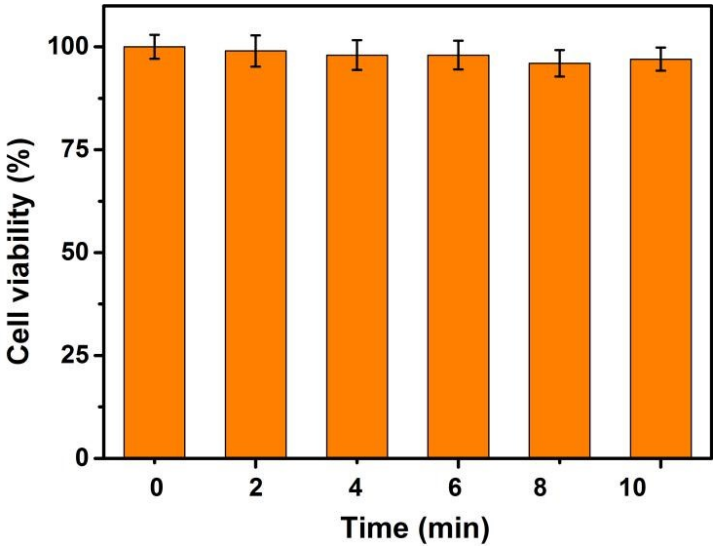

Figure S8. Viability test of 7702 cells after NIR irradiation at 2 W/cm^2 for different time intervals. Error bars were estimated through at least three experiments.

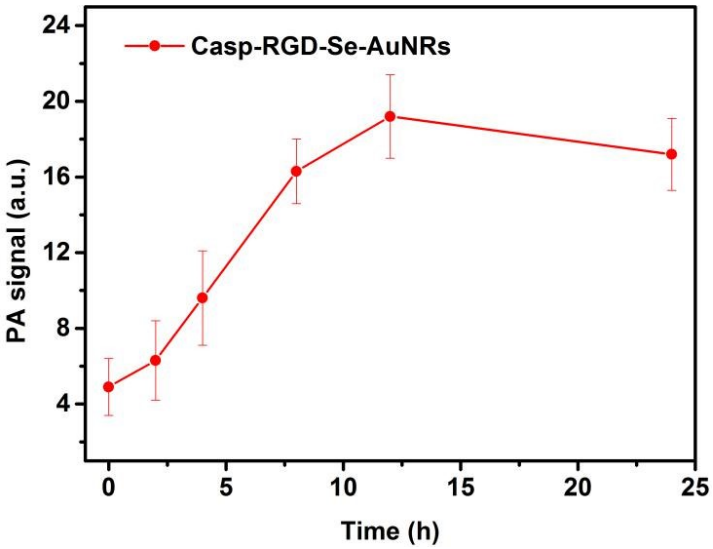

Figure S9. *In vivo* photoacoustic signals of B16-F10 tumor bearing mice treated with intravenous injection of Casp-RGD-Se-AuNRs (1 nM) for 0-24h. Error bars were estimated from three different measurements.

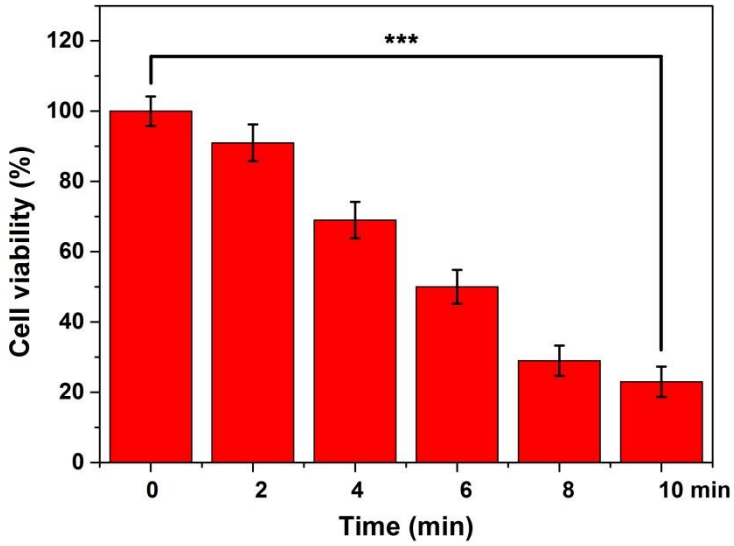

Figure S10. MTT assay of B16-F10 cells incubated with Casp-RGD-Se-AuNRs (1 nM) for 4 h and then treated with NIR irradiation at 2.0 W/cm^2 for different time intervals (*p < 0.05, **p < 0.01, and ***p < 0.001; n = 3).

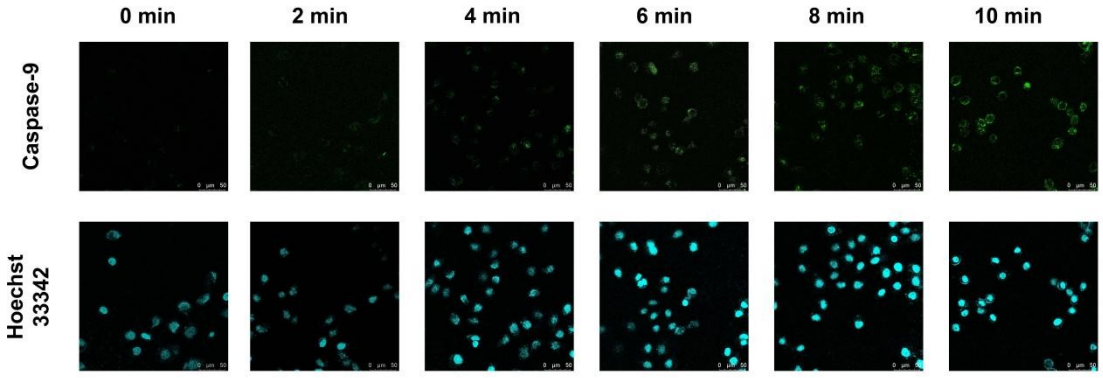

Figure S11. Real-time fluorescence imaging of the cell apoptosis process in B16-F10 cells incubated with Casp-RGD-Se-AuNRs (1 nM) for 4 h and then treated with NIR irradiation at 2.0 W/cm^2 for different time intervals: green fluorescence (Caspase-9) at $\lambda_{ex}/\lambda_{em}$ of 488/500−600 nm, blue fluorescence (Hoechst 33342) at $\lambda_{ex}/\lambda_{em}$ of 405/430−480 nm. Scale bar = 100 μm.

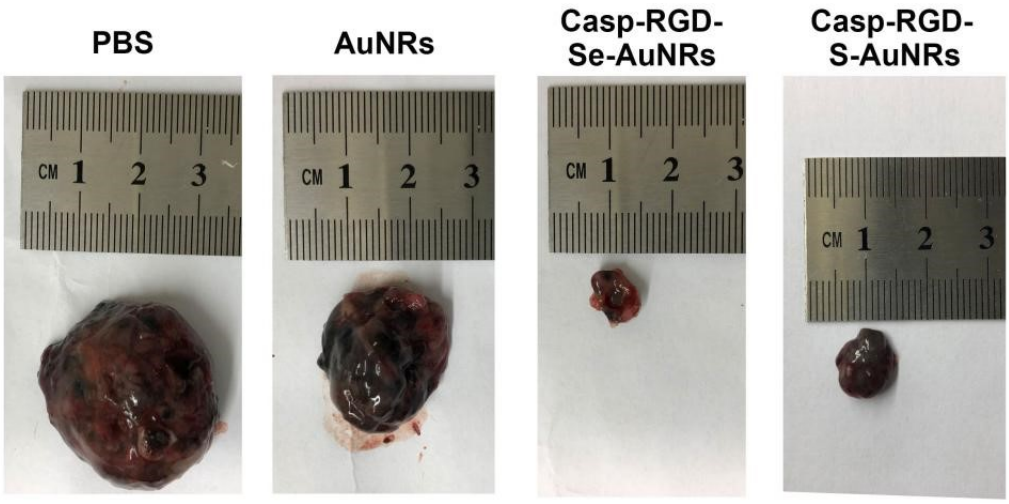

Figure S12. Images of the tumors in the different treatment groups at days 14.