Supporting Information for

Anti-EGFR antibody conjugation of fucoidan-coated gold nanorods as novel photothermal ablation agents for cancer therapy

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Materials

Fucoidan, cetyltrimethylammonium bromide (CTAB), gold (III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$), sodium borohydride ($NaBH_4$), L-ascorbic acid, silver nitrate ($AgNO_3$), anti-EGFR monoclonal antibody, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), acridine orange (AO), and propidium iodide (PI) were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). All other chemical reagents were of analytical grade and they were purchased from Sigma–Aldrich Co.

Preparation of anti-EGFR conjugated fucoidan-coated gold nanorods

The anti-EGFR conjugated Fu-coated gold nanorods (anti-EGFR Fu-AuNRs) were conjugated according to the previous literature.[1] The Fu-coated gold nanorods (Fu-AuNRs) were added to an anti-EGFR monoclonal antibody solution (5 μL/mL) that was diluted in PBS and allowed to interact for 30 min. The antibodies are presumed to be bound to the Fu-AuNRs surface by an electrostatic physisorption interaction.[2] The Fu-AuNRs conjugated with antibodies were centrifuged and dispersed into PBS to from a stock solution with an optical density of ~ 0.9 at 842 nm. The anti-EGFR Fu-AuNRs were stable at 4 °C for several days. The binding efficiency was estimated by bicinchoninic acid (BCA) protein assay kit (Pierce).

Stability of anti-EGFR conjugated Fu-AuNRs

The Fu-AuNRs and anti-EGFR Fu-AuNRs were suspended in 300 μL PBS in a 1.5 mL microcentrifuge tube and they were incubated for 24 h at 37 °C under shaking condition (100 rpm). After incubation, the solutions were centrifuged and the resulting supernatant was used to quantify the protein using a BCA assay kit. Protein concentrations were calculated against a standard curve, values for Fu-AuNRs were subtracted from the values for anti-EGFR Fu-AuNRs

to substract the background from the AuNRs since AuNRs alone also absorbs light at the wavelength used for the protein estimation (560 nm). After calculation, it was found that ~ 85% of EGFR antibodies remain attached to the Fu-AuNRs probe after 24 h at 37 °C under shaking conditions (100 rpm).

BCA protein assay

A BCA protein assay kit measured protein concentrations in the anti-EGFR Fu-AuNRs solutions. Antibodies alone (5 μL of anti-EGFR antibody + 200 μL PBS to represent total antibodies), anti-EGFR Fu-AuNRs (5 μL of anti-EGFR antibody + 200 μL Fu-AuNRs in PBS, wherein the supernatant contains an unbound antibody), and supernatant from the AuNRs solution (200 μL AuNRs in PBS) were measured and calculated against a standard curve composed of a serial dilution of a known concentration of bovine serum albumin (BSA) diluted in PBS. Aside from subtracting a reagent blank, the AuNRs supernatant was also subtracted from the anti-EGFR Fu-AuNRs supernatant measurements to reduce the background from unreacted AuNRs. The binding efficiency was then calculated based on this formula

$$\text{Binding efficiency} = \mu g \text{ protein of total antibody} - (\mu g \text{ protein of unbound antibody} - \mu g \text{ protein AuNRs only})/\mu g \text{ protein of total antibody} \times 100$$

The assay was preformed more than three times. The binding efficiency was found to be 79.52% by the BCA protein assay kit.

Characterization

The UV spectrum was measured with a DU-640 spectrophotometer from Beckman Coulter (Fullerton, CA, USA). The powder X-ray diffraction (XRD) patterns of the samples were measured using a X'Pert-MPD PW 3050 diffractometer (Phillips, The Netherlands). Fourier

transform infrared (FTIR) measurements were observed on a spectrum on a Spectrum 100 FTIR spectrometer (Perkin Elmer Inc., USA). The morphology, particle size, and composition were examined by field emission transmission electron microscopy (FETEM; JEM-2100F, JEOL, Japan), selected area electron diffraction pattern (SAED), and energy dispersive X-ray spectroscopy (EDX). The particle size and the zeta potential of the nanoparticles were measured using dynamic light scattering (DLS) and zeta potential (ZP) and performed with an ELS-8000 electrophoretic light scattering spectrophotometer (OTSUKA Electronics Co. Ltd., Japan) at a fixed angle of 90° at room temperature.

Stability of gold nanorods

The stability of Fu-AuNRs at various situations was observed by UV-Vis spectra collected within a range of 300–1100 nm. To determine the Fu-AuNRs stability with respect to different conditions, the Fu-AuNRs dispersion was observed for up to 6 months using UV-Vis spectroscopy. The pH of Fu-AuNRs was adjusted (pH 2.0–12.0). The Fu-AuNRs dispersion was observed at different concentrations of NaCl (10^1 to 10^5 M). To check the dispersion stability of anti-EGFR Fu-AuNRs in the various aqueous solutions, anti-EGFR Fu-AuNRs were dispersed in each of deionized water, PBS, and a cell culture medium (DMEM without phenol red) containing 10% fetal bovine serum (FBS). The solutions were maintained at 25 °C and observed for 7 days and UV-Vis spectra of the samples solutions were taken periodically.

Animals

Female BALB/c nude mice (18–21 g body weight) were purchased from Orient Bio Inc. (Gyeonggi-Do, Korea). All the experiments were approved by the animal care and use committee of Pukyong National University and carried out ethically and humanely. MDA-MB-231 cells (4×10^6) were subcutaneously injected into the right leg of each mice.

Histology

For hematoxylin and eosin (H&E) staining, tumor tissues and major organs (the heart, kidney, spleen, lung, and liver) were collected, fixed in 10% neutral buffered formalin, processed routinely into paraffin, sectioned into thin slices, and stained with H&E for histological analysis. The stained samples were examined by a light microscope in a bright field.

Statistical analysis

Data were expressed as mean ± standard deviation (SD). All statistical analyses were performed using the SPSS software version 14.0 (SPSS Inc., Chicago, IL, USA) and significant differences between the groups were determined using one-way ANOVA analysis.

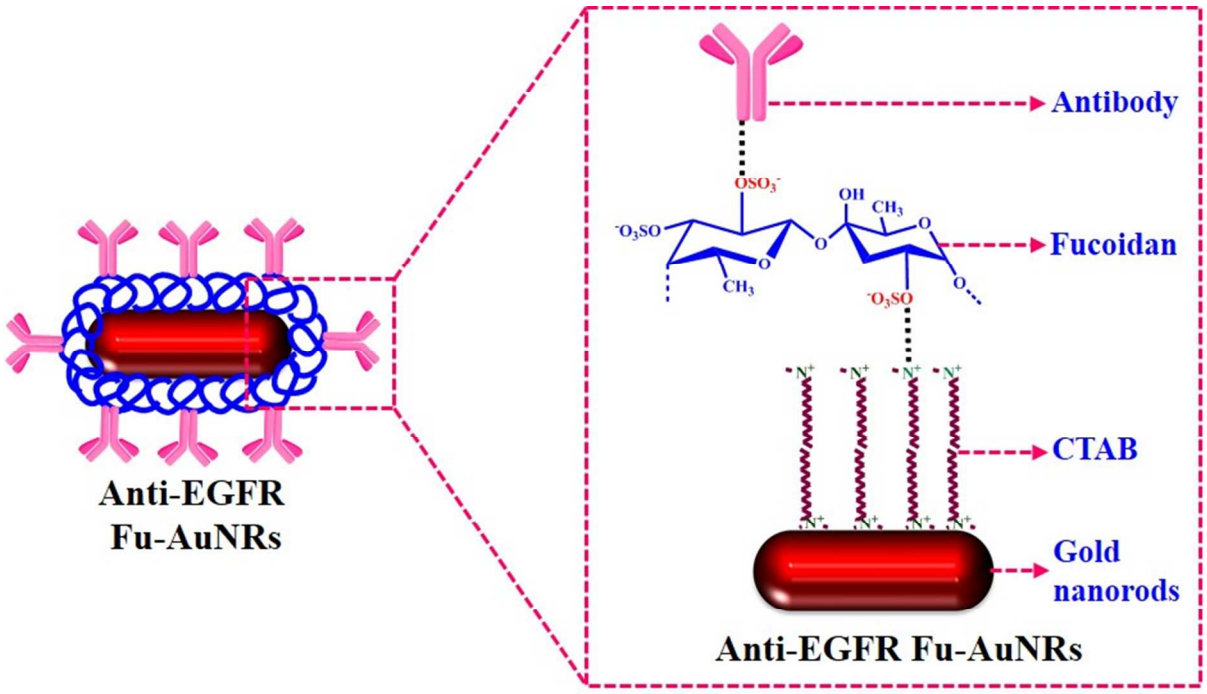

Fig. S1. Schematic diagram showing the fucoidan-coated gold nanorods (Fu-AuNRs) and subsequently conjugated with anti-EGFR antibody.

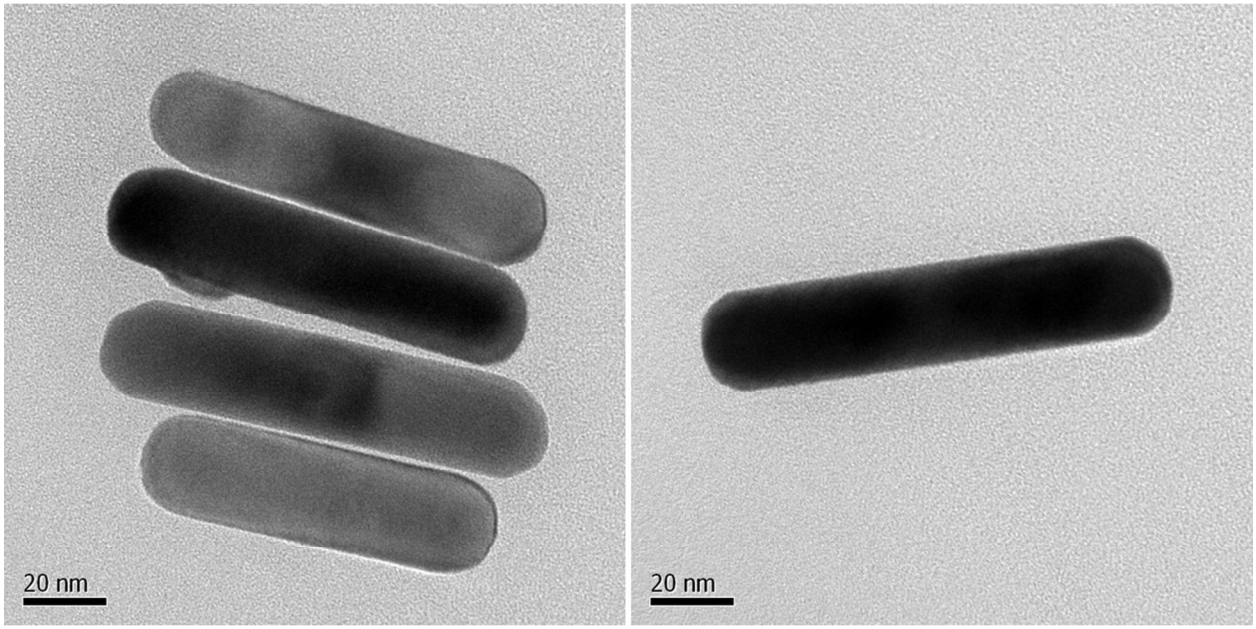

Fig. S2. FETEM image of gold nanorods (AuNRs).

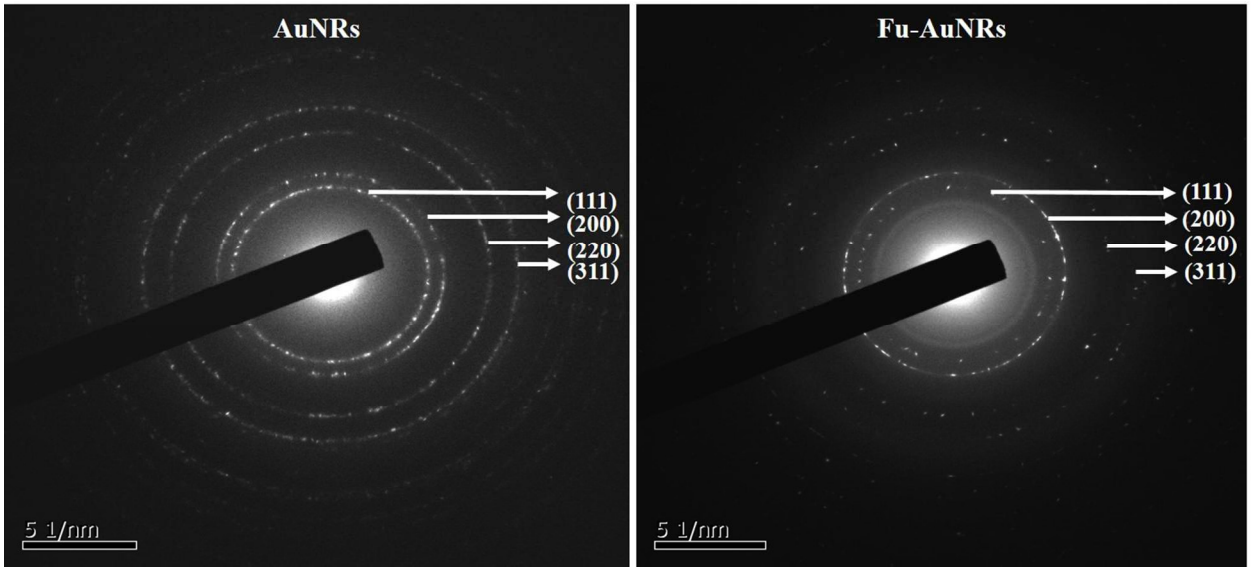

Fig. S3. Selected area electron diffraction pattern (SAED) of AuNRs and Fu-AuNRs.

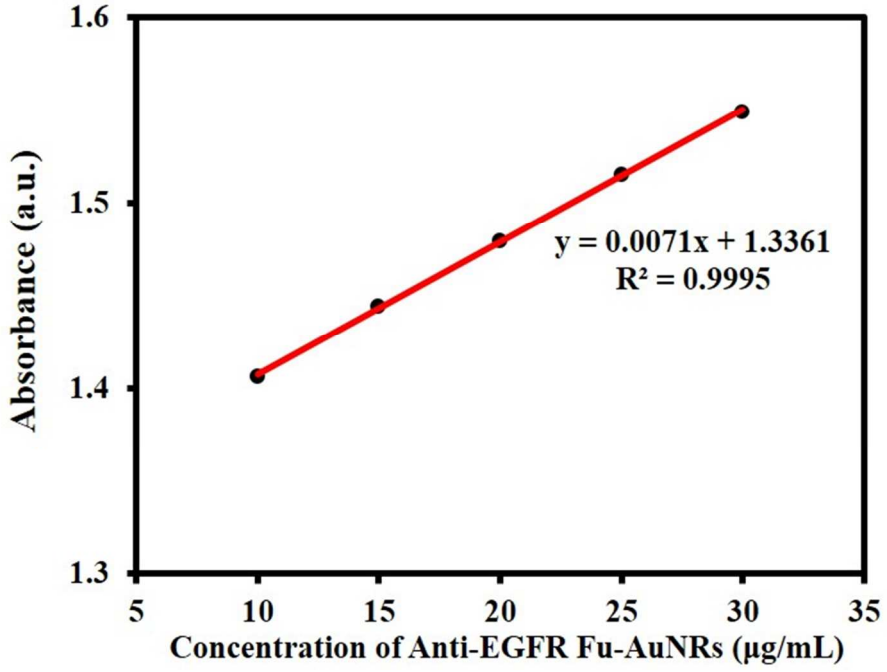

Fig. S4. A linear relationship for the absorbance at 808-nm wavelength as a function of the concentration.

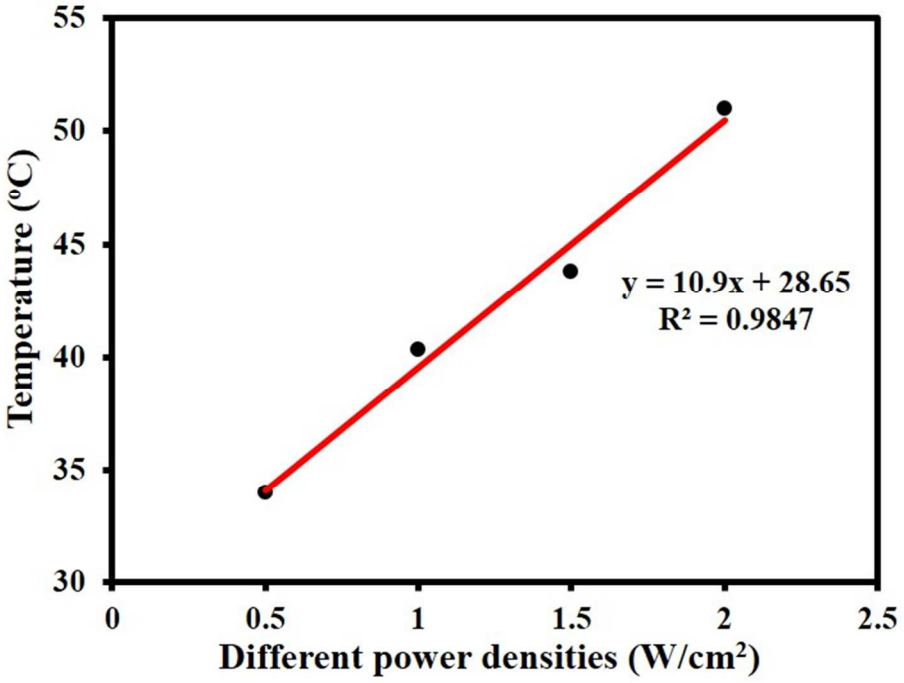

Fig. S5. Linear fitting of temperature elevation of 30 μg/mL anti-EGFR Fu-AuNRs solution under 808-nm laser irradiation at different power densities (0.5 W/cm^2, 1.0 W/cm^2, 1.5 W/cm^2, and 2.0 W/cm^2) for 5 min.

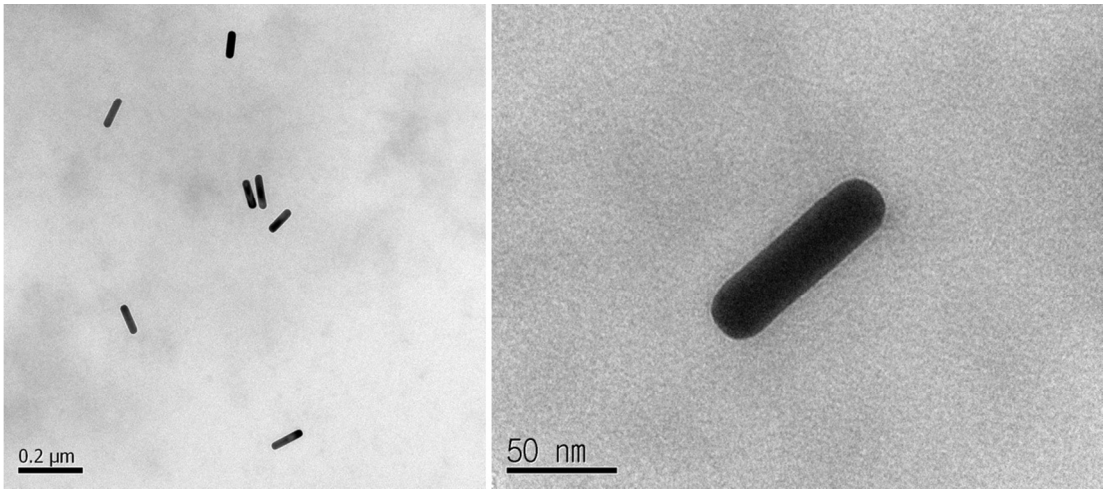

Fig. S6. FETEM image of anti-EGFR Fu-AuNRs after six cycles of laser on/off.

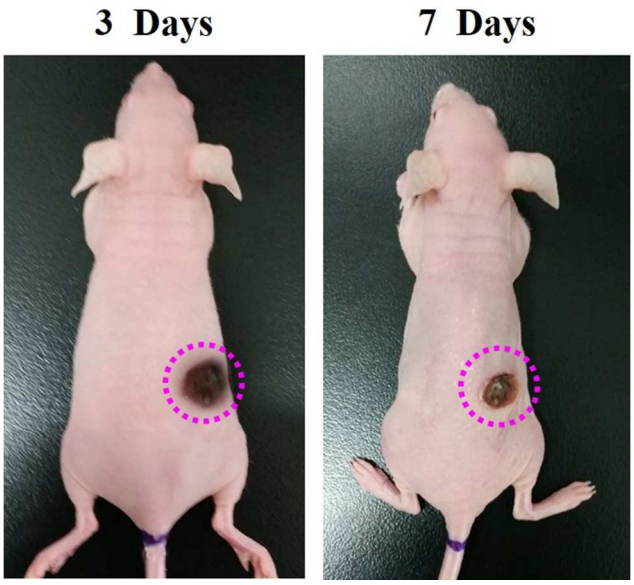

Fig. S7. Representative photographs of tumors in mice taken at 3 and 7 days after treatments of anti-EGFR Fu-AuNRs under 808-nm NIR laser irradiation at 2.0 W/cm^2 for 5 min.

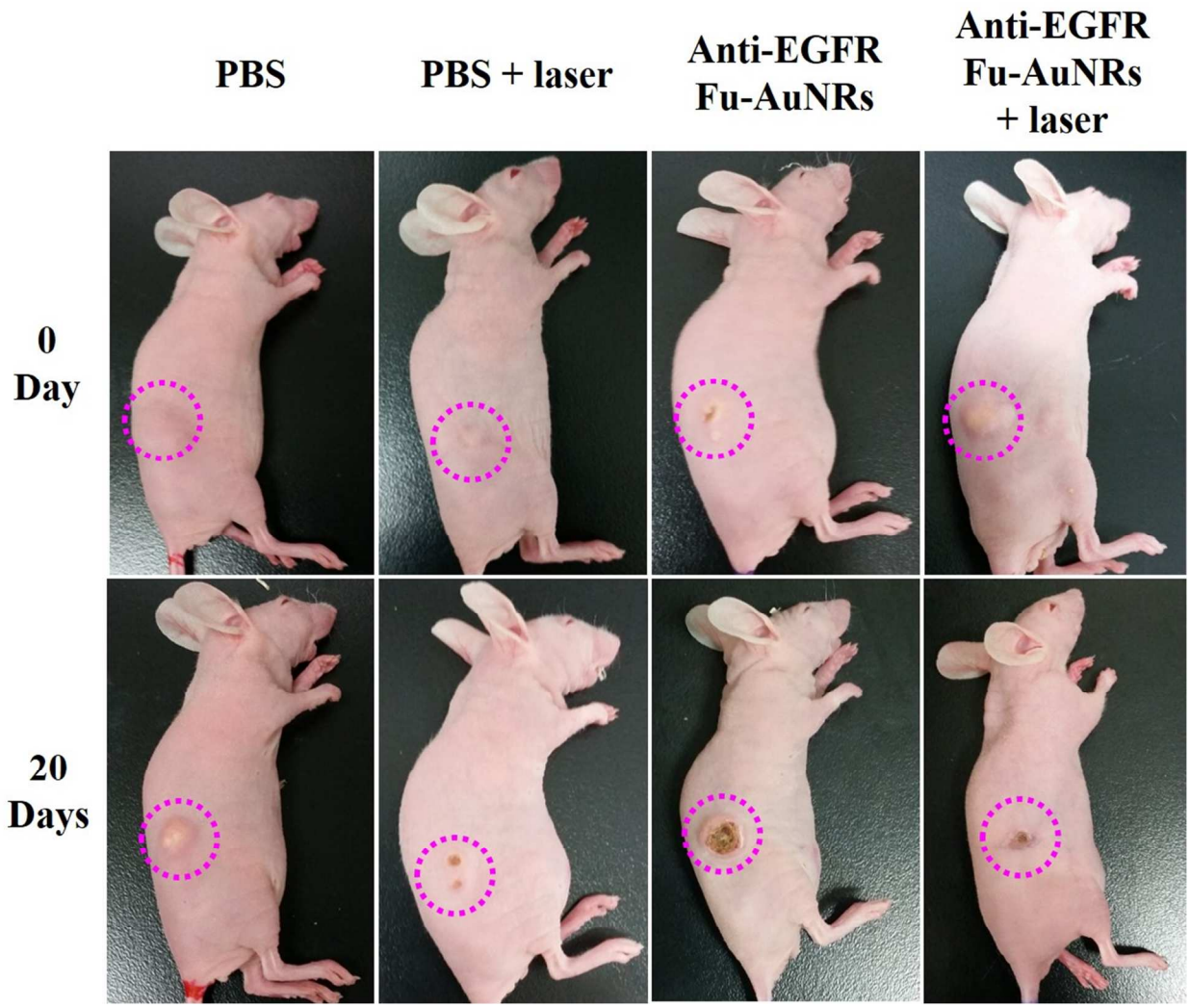

Fig. S8. Representative photographs of tumors in mice before and after various treatments for 0 and 20 days.

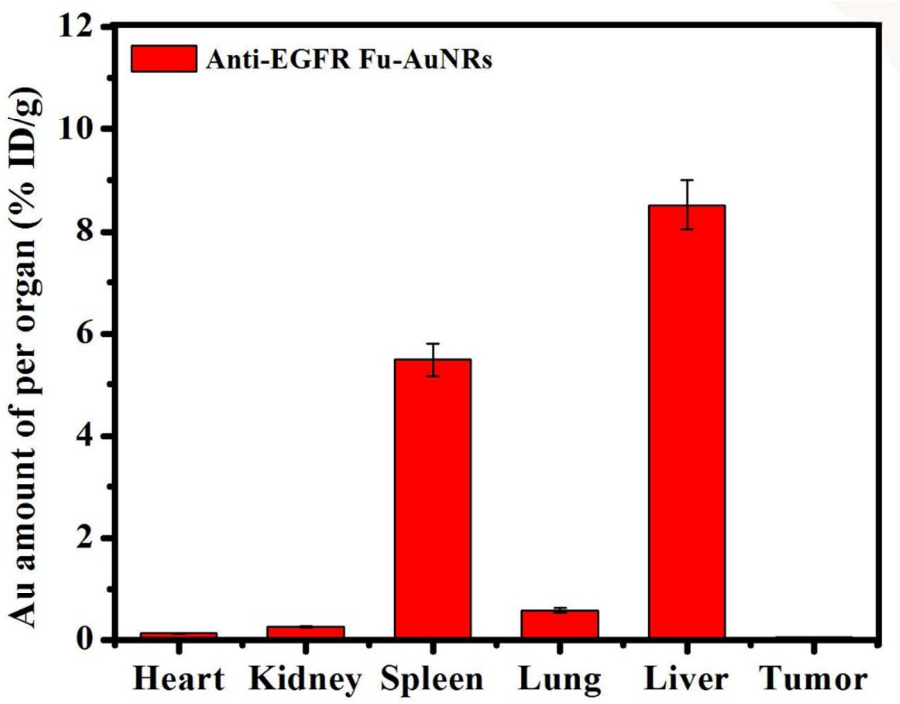

Fig. S9. Biodistribution of nanoparticles (Au concentration %ID/g) in tumor tissues and organs at 20 days after the intravenous injection. Data is expressed as mean ± SD of the three experiments.

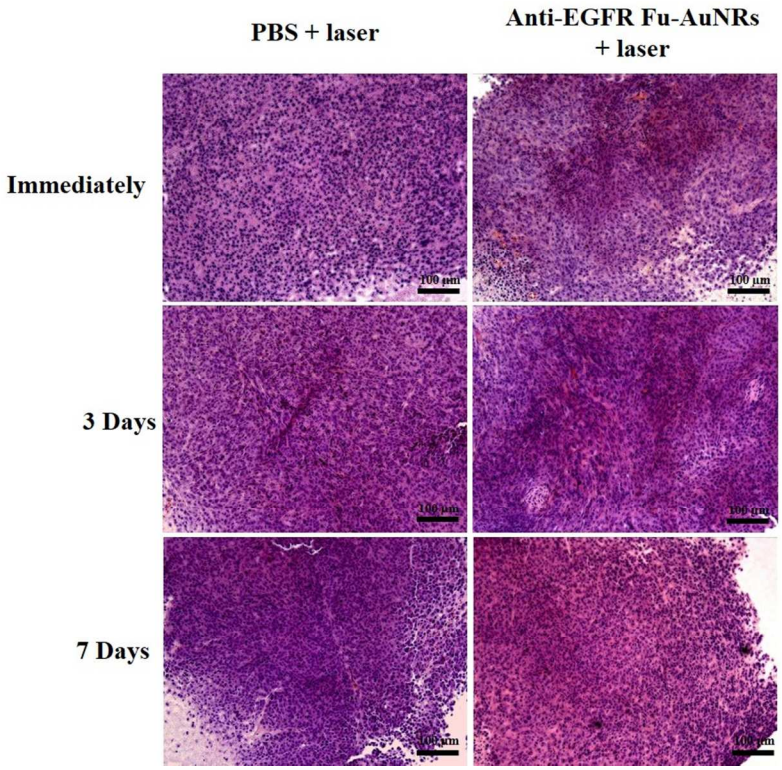

Fig. S10. Histology staining of tumor tissues slices collected from different groups of mice after laser irradiation.

References

1. Charan, S.; Sanjiv, K.; Singh, N.; Chien, F.-C.; Chen, Y.-F.; Nergui, N. N.; Huang, S.-H.; Kuo, C. W.; Lee, T.-C.; Chen, P., Development of chitosan oligosaccharide-modified gold nanorods for *in vivo* targeted delivery and noninvasive imaging by NIR irradiation. *Bioconjugate Chem.* **2012,** *23* (11), 2173-2182.

2. Sokolov, K.; Follen, M.; Aaron, J.; Pavlova, I.; Malpica, A.; Lotan, R.; Richards-Kortum, R., Real-time vital optical imaging of precancer using anti-epidermal growth factor receptor antibodies conjugated to gold nanoparticles. *Cancer Res.* **2003,** *63* (9), 1999-2004.