Electronic Supplementary Information (ESI) for:

1550 nm excitation-responsive upconversion nanoparticles to establish dual-photodynamic therapy against pancreatic tumor

Khang-Yen Pham,[a] Liu-Chun Wang,[a] Chia-Ching Hsieh,[a] Ya-Ping Hsu,[a] Li-Chan Chang,[b] Wen-Pin Su,[b,c] Yi-Hsin Chien*[d] and Chen-Sheng Yeh*[a,e,f]

[a] Department of Chemistry, National Cheng Kung University, Tainan, Taiwan
E-mail: csyeh@mail.ncku.edu.tw

[b] Institute of Clinical Medicine, College of Medicine, National Cheng Kung University, Tainan, Taiwan.

[c] Departments of Internal Medicine and Oncology, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University, Tainan, Taiwan.

[d] Department of Material Science and Engineering, Feng Chia University, Taichung, Taiwan
E-mail: yhchien@fcu.edu.tw

[e] Center of Applied Nanomedicine, National Cheng Kung University, Tainan, Taiwan

[f] Department of Medicinal and Applied Chemistry, Kaohsiung Medical University, Kaohsiung, Taiwan

Materials. Anhydrous $ErCl_3$ (99.9%), $GdCl_3$ (99.9%), NH_4F (99.9%), Rose bengal (RB, 95%), IGEPAL CO-520, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT, $C_{18}H_{16}BrN_5S$, 97.5%) were obtained from Sigma-Aldrich. Anhydrous $YbCl_3$ (99.9%), LiOH (99.9%), 1-octadecene (90%), oleic acid (90%), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC, 98%), *N*-hydroxysulfosuccinimide sodium salt (sulfo-NHS, 97%), and 1,3-Diphenylisobenzofuran (DPBF, 97%) were purchased from Alfa Aesar. Tetraethyl orthosilicate (TEOS, 98%) and (3-aminopropyl)triethoxysilane (APTES, 99%) were bought from Acros. Chlorin e6 (Ce6, 93-98%) was purchased from Frontier Scientific. Ammonia solution (NH_3 (aq), 28-30%), Ethanol (EtOH, 99.9%) and Dimetyl sulfoxide (DMSO, 99.9%) were obtained from J.T. Baker. H_2N-PEG-COOH (PEG, MW 3400) was acquired from Nanocs. Hydrochloric acid (HCl, 36%) and nitric acid (HNO_3, 70%) were bought from BASF. Dulbecco's modified eagle medium (DMEM, high glucose, pyruvate) and 0.25% trypsin-EDTA were obtained from Gibco. Fetal bovine serum (FBS) was used as purchased from HyClone. Calcein-AM ($C_{46}H_{46}N_2O_{23}$) was acquired from Invitrogen. Propidium iodide (PI, $C_{27}H_{34}I_2N_4$) was bought from BD Biosciences. 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) was obtained from Thermo Fisher Scientific. Water was obtained by using a Millipore direct-Q deionized water system throughout all studies.

Characterization. The size and shape of the nanoparticles (NPs) were characterized by a transmission electron microscope (TEM, Hitachi H-7500 at 200 kV). The high-resolution TEM was observed on a high-resolution TEM (HR-TEM, JEOL JEM-2100F at 200 kV). UV−vis spectra were recorded on the UV–vis absorption spectrometer (Hitachi U-2900). Zeta-potentials and hydrodynamic diameters of NPs were performed by a dynamic light scattering spectrometer (DLS, Malvern, U.K., Zetasizer Nano ZS90). An X-ray diffractometer (Shimadzu XRD-7000S, CuKα radiation λ = 1.54060 Å, 30 kV, 30 mA)) was used to collect the XRD data. An FTIR (JASCO 200E FTIR) was used to measure the FTIR spectrum of prepared NPs. The emission

spectra of UNCPs was obtained using a Fluoromax-4 spectrofluorometer (Horiba Scientific) equipped with a CW diode laser excitation 1550 nm. The *in vitro* cells images were captured using a confocal laser scanning microscope (ECLIPSE Ti series, NIKON). The Er^{3+}, Yb^{3+} concentration (μg/mL) of UCNPs were quantified by an inductively coupled plasma-atomic emission spectrometer (ICP-OES, Thermo iCAP 7000). The quantification of cell viability was done using an enzyme-linked immunosorbent assay reader (ELISA reader, Thermo Scientific Multiskan EX).

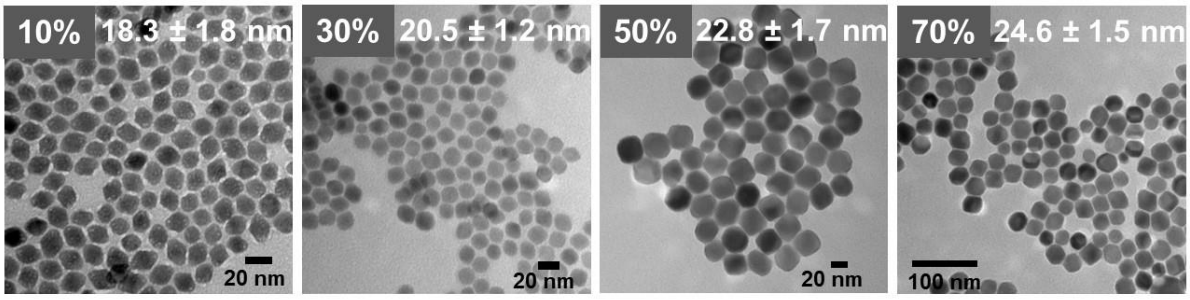

Fig. S1 TEM images of $LiYbF_4$:Er with different %Er (10, 30, 50, and 70%).

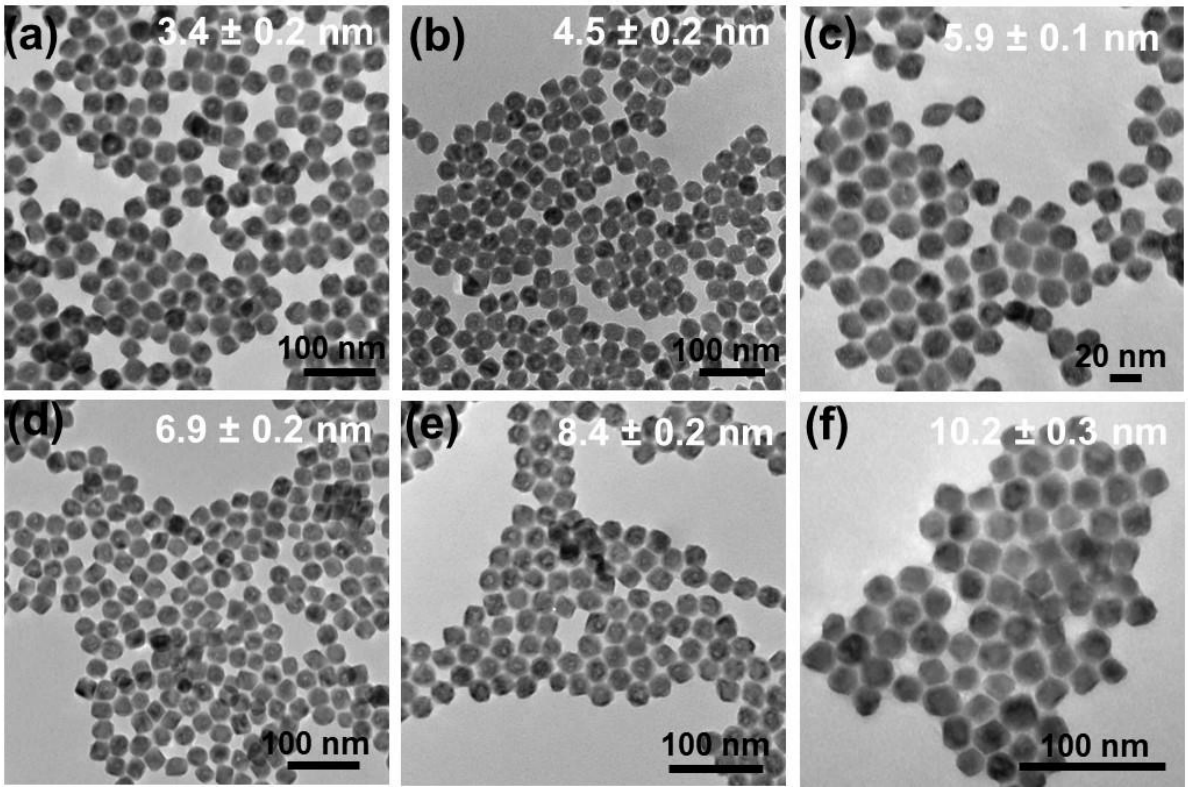

Fig. S2 TEM images of $LiYbF_4$:30%Er@$LiGdF_4$ with different shell thicknesses.

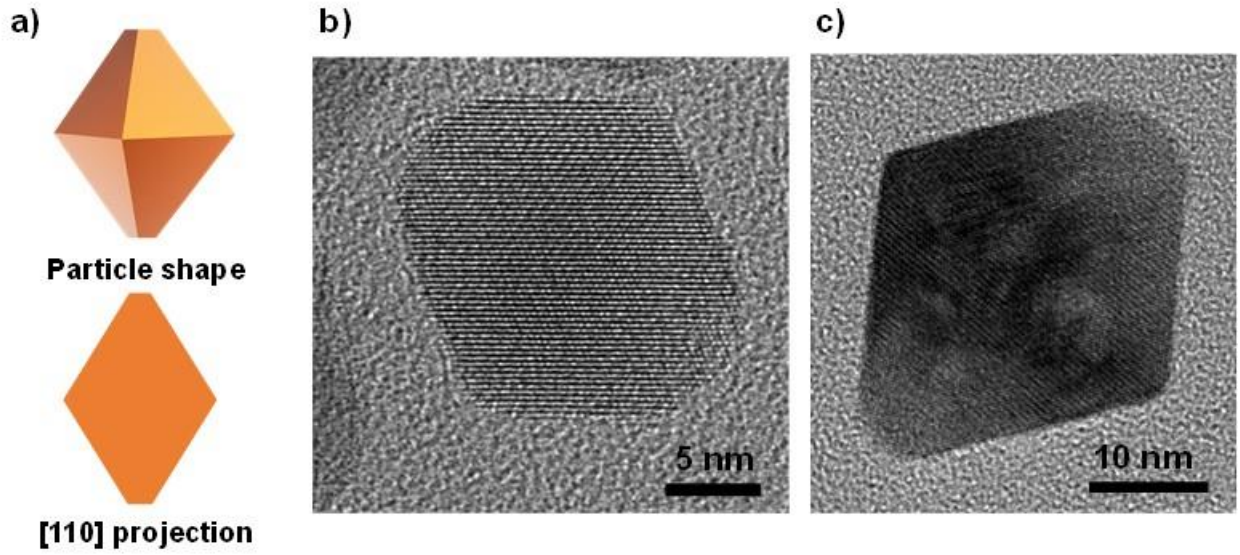

Fig. S3 (a) The schemes of the truncated bipyramidal shape and its [110] projection. HR-TEM images of (b) core $LiYbF_4$:30%Er and (c) core-shell $LiYbF_4$:30%Er@$LiGdF_4$ observed along the [110] direction.

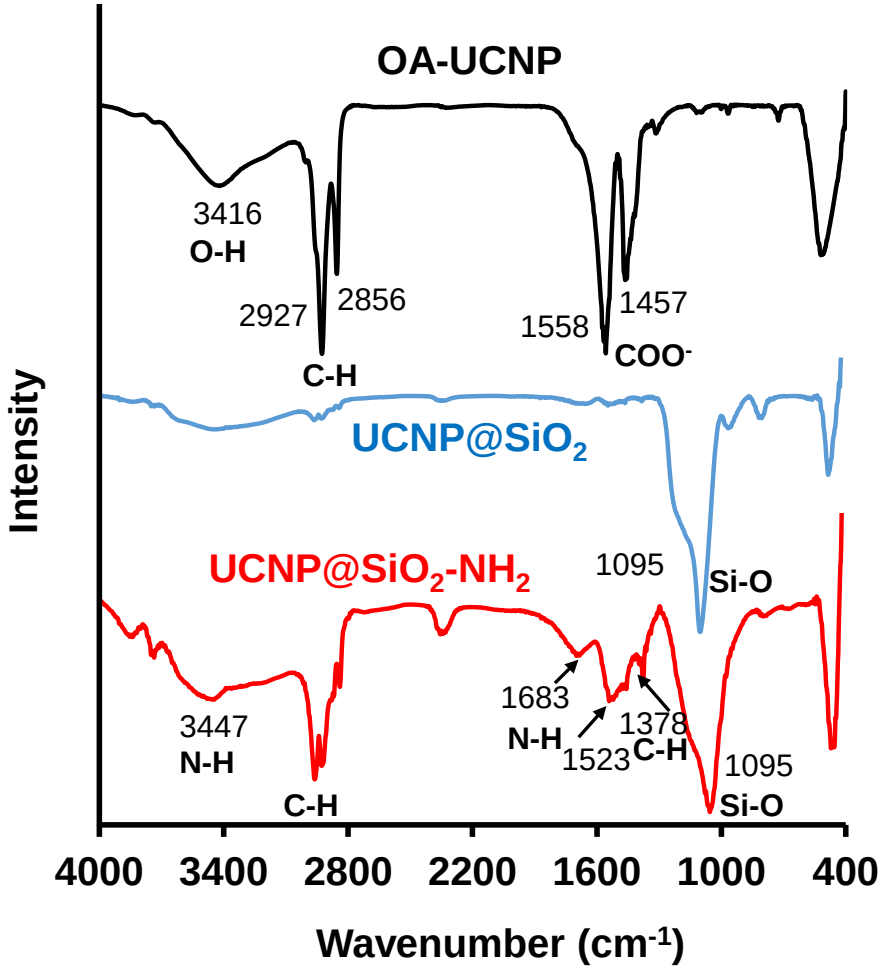

Fig. S4 FT-IR spectra of oleic acid (OA)-UCNP, UCNP@SiO_2, and UCNP@SiO_2-NH_2 (APTES modification). The 1095 cm^{-1} of Si-O showed the silica shell formation. The characteristic peak at ~3447 cm^{-1} indicated N-H bond in NH_2. The new peaks at 1683, 1523, and 1378 cm^{-1} assigned to N-H, and C-H bond, respectively.

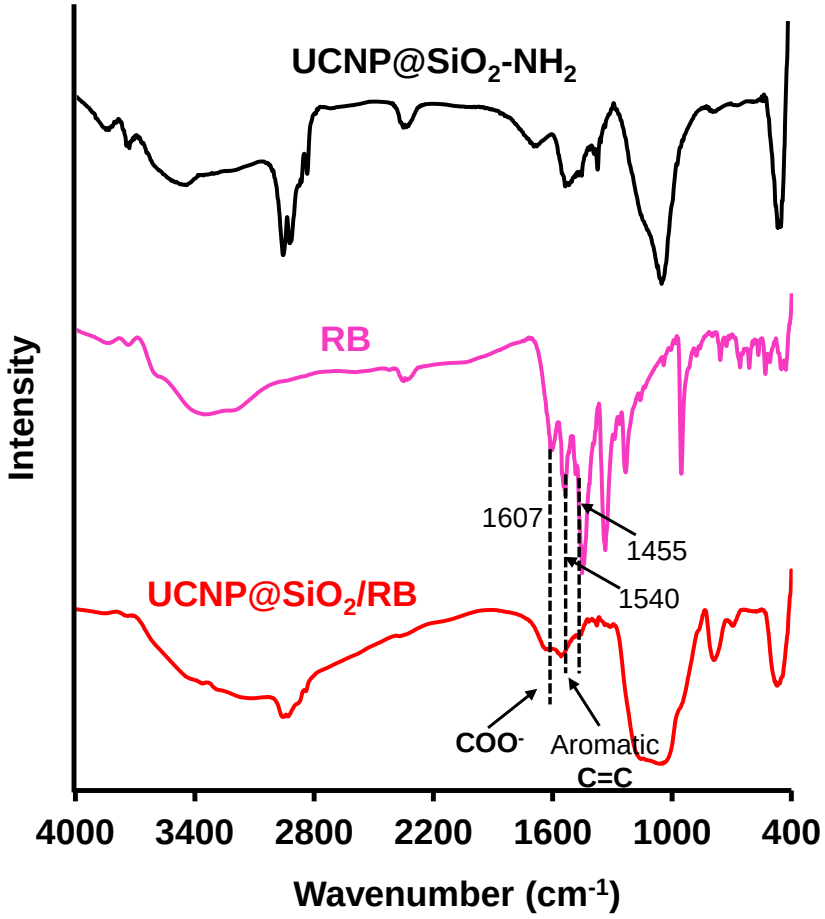

Fig. S5 FT-IR spectra of UCNP@SiO_2-NH_2, pure RB, and UCNP@SiO_2/RB. The signal at ~1607 cm^{-1} indicated COO^- group in RB. The peaks at 1540 and 1455 cm^{-1} assigned to the aromatic C=C bond in RB.

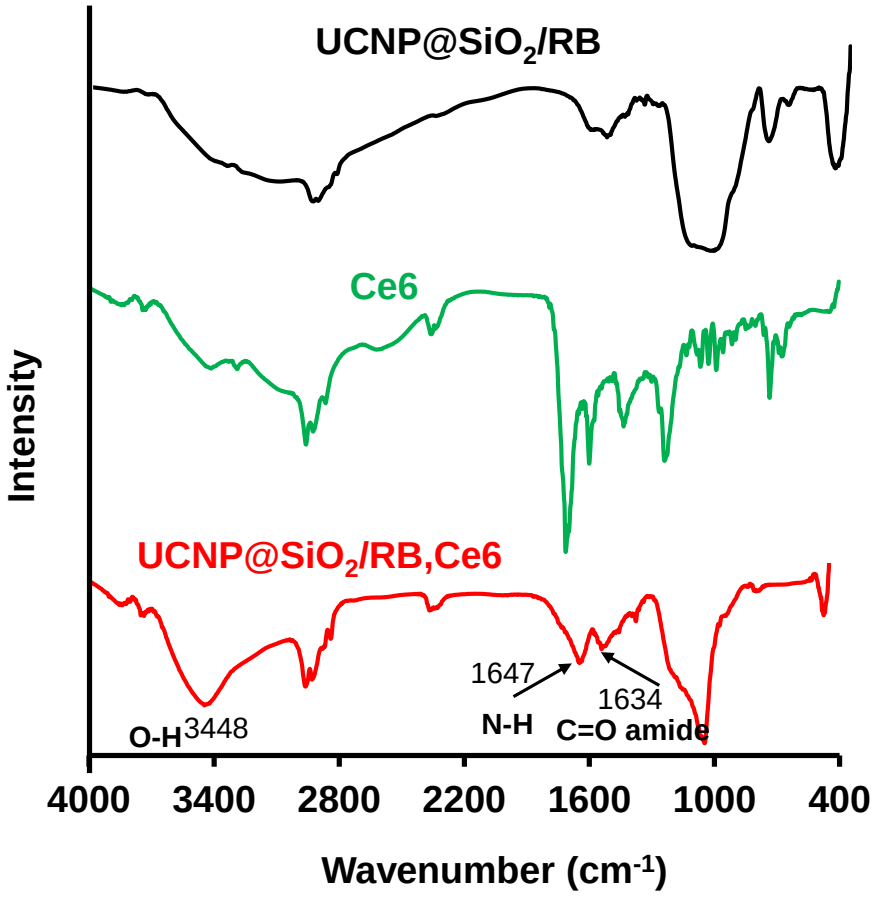

Fig. S6 FT-IR spectra of UCNP@SiO_2/RB, pure Ce6, and UCNP/RB,Ce6. The peak at ~3448 cm^{-1} (O-H) from the carboxylic group in Ce6 which contains three –COOH groups. The signal at 1634 cm^{-1} was assigned to C=O bond of amide group and the peak at 1647 cm^{-1} was from N-H group.

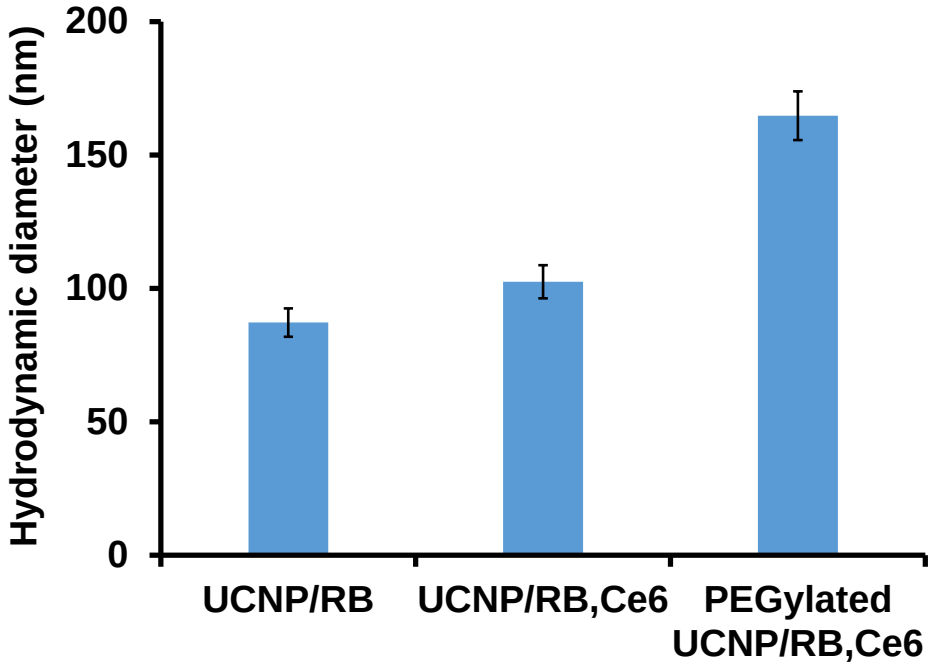

Fig. S7 The hydrodynamic diameters of UCNP/RB, UCNP/RB,Ce6, and PEGylated UCNP/RB,Ce6 observed through DLS measurements.

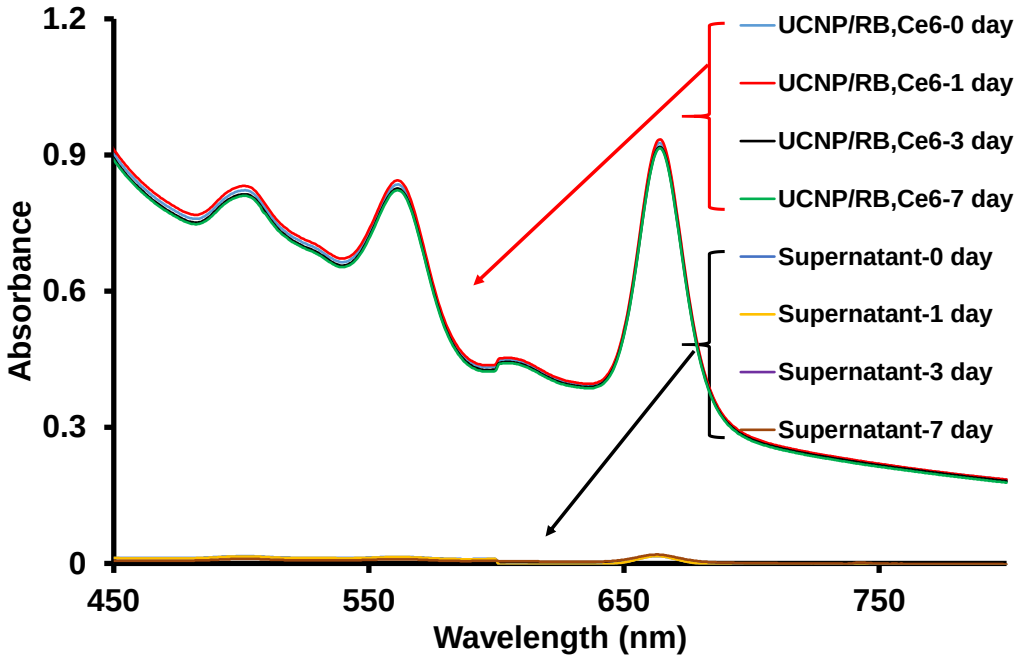

Fig. S8 A 200 ppm of UCNP/RB,Ce6 was dispersed in PBS (pH 7), stored in dark condition at 4 °C, and observed during 7 days using UV-vis spectra. The absorption of supernatant also was recorded at different time points after centrifugation. If RB and/or Ce6 are liberated from UCNPs, the characteristic peaks of RB and/or Ce6 would appear in the supernatant.

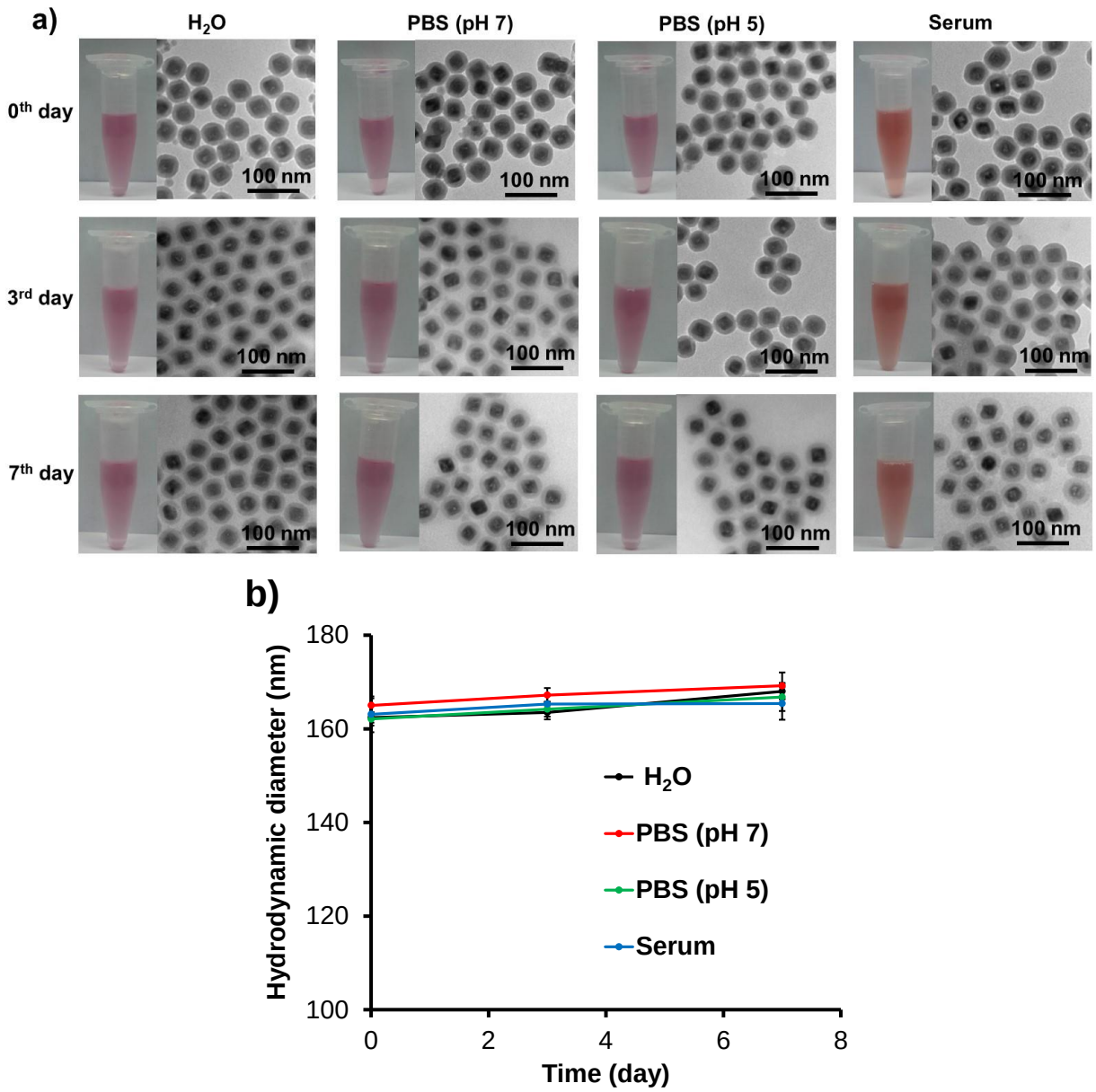

Fig. S9 The stability of PEGylated UCNP/RB,Ce6 under different conditions including H_2O, PBS (pH 7), PBS (pH 5), and serum at 37 °C for a period of a week. (a) TEM images and photographs showing the structures of PEGylated UCNP/RB,Ce6 remained intact and no precipitation was observed for colloidal solutions after 7-day incubation. (b) The hydrodynamic diameter as a function of day indicating no obvious change in size during 7-day incubation from all of the solutions.

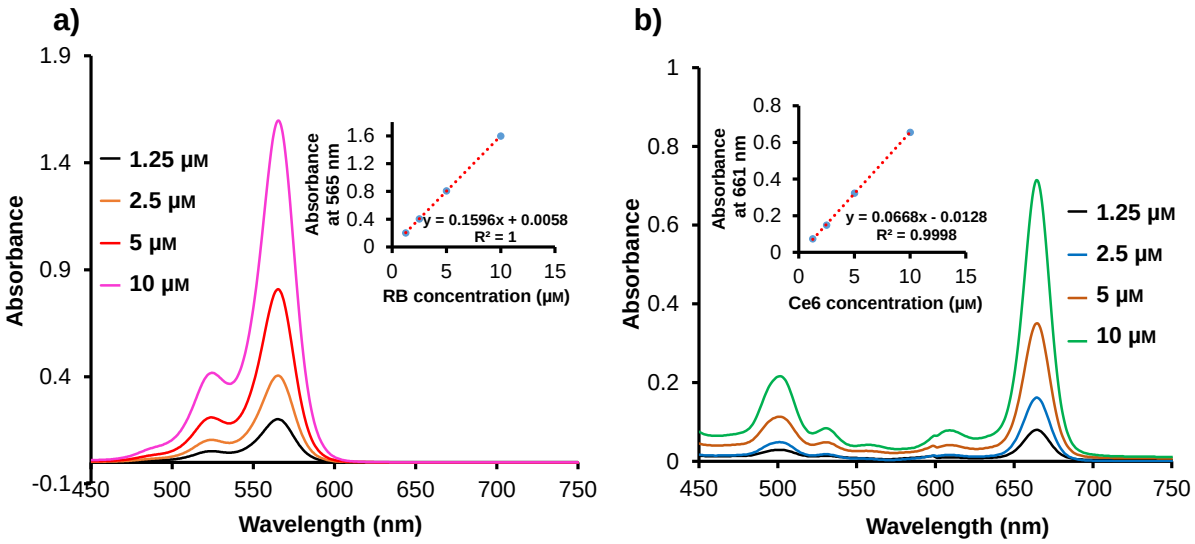

Fig. S10 The absorbance of (a) RB and (b) Ce6 and the corresponding standard curves.

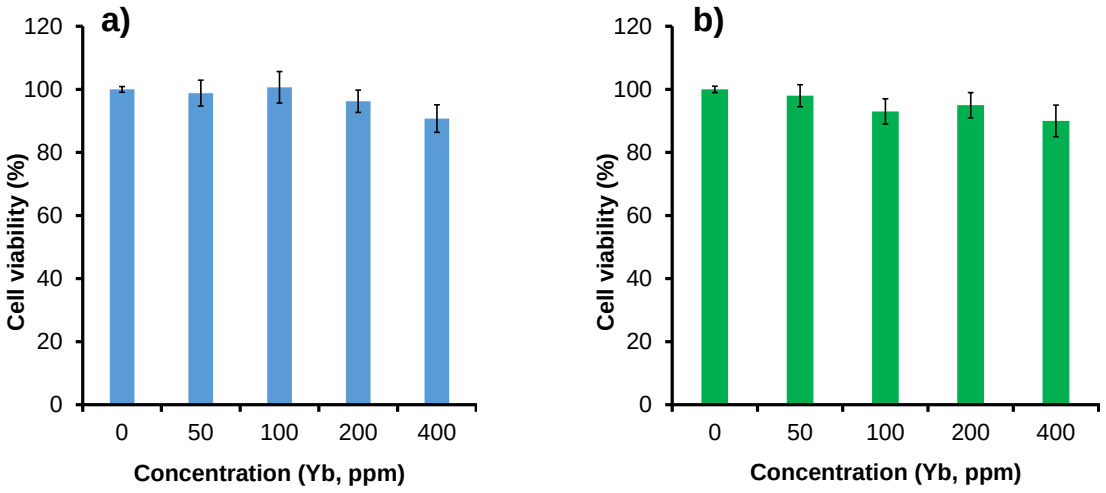

Fig. S11 (a) Panc-1 and (b) MRC-5 cell viability after incubation with PEGylated UCNP/RB,Ce6 for 24 h and quantitative assays by standard MTT method.

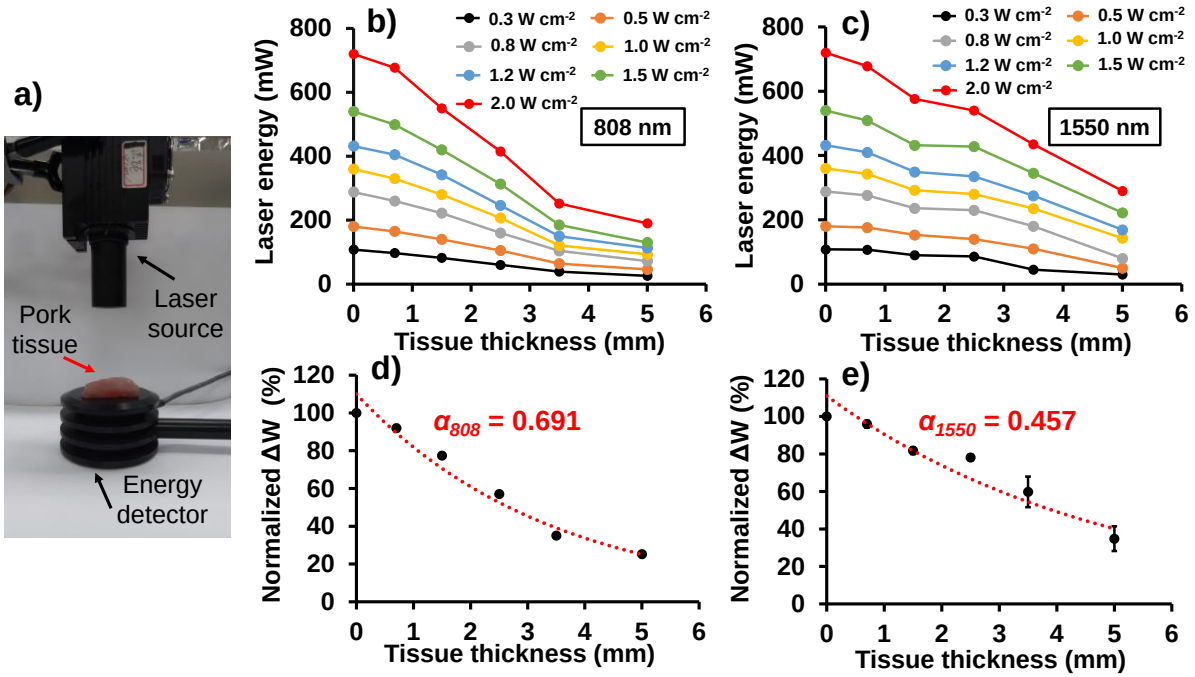

Fig. S12 Comparison of tissue penetration depth for different NIR laser wavelengths. (a) Photograph of the experimental setup for measuring tissue penetration ability of laser at 808 and 1550 nm. Energy intensities of (b) 808 nm and (c) 1550 nm laser after penetration through the various tissue thicknesses. Normalized energy penetrating through different tissue thicknesses for the attenuation coefficients (α) of (d) 808 nm and (e) 1550 nm laser.