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Supplementary Materials for

Near-infrared upconversion–activated CRISPR-Cas9 system: A remote-controlled gene editing platform

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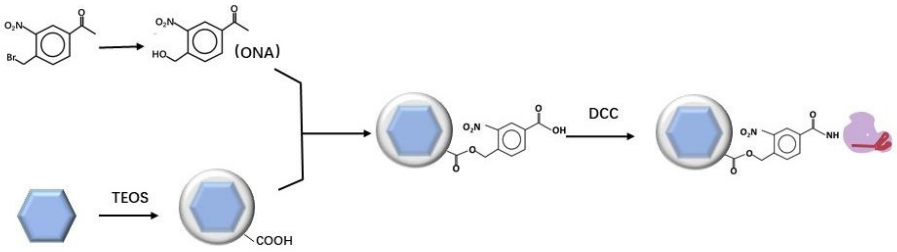

Fig. S1. Schematic for the synthesis of UCNPs-Cas9.

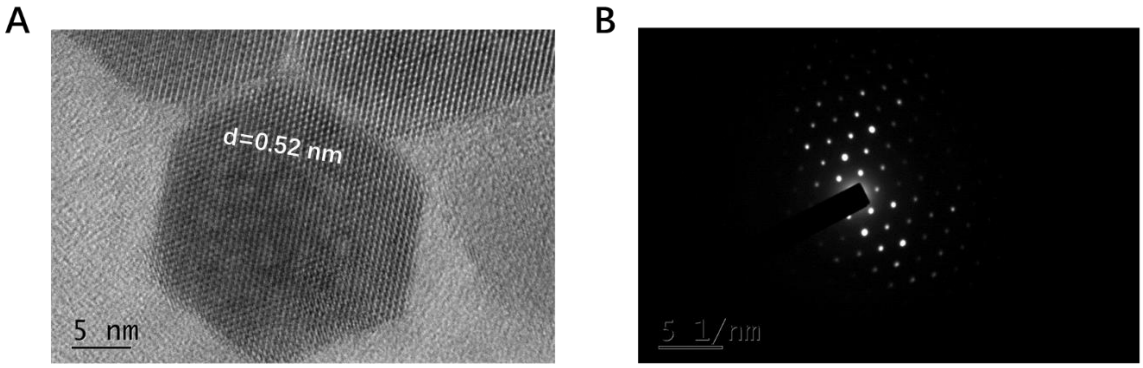

Fig. S2. The structure of UCNPs confirmed by HRTEM and SAED pattern. (A) HRTEM image and **(B)** SAED pattern of UCNPs. The interplanar spacing of 0.52 nm was corresponding to the typical (100) plane of the hexagonal structure.

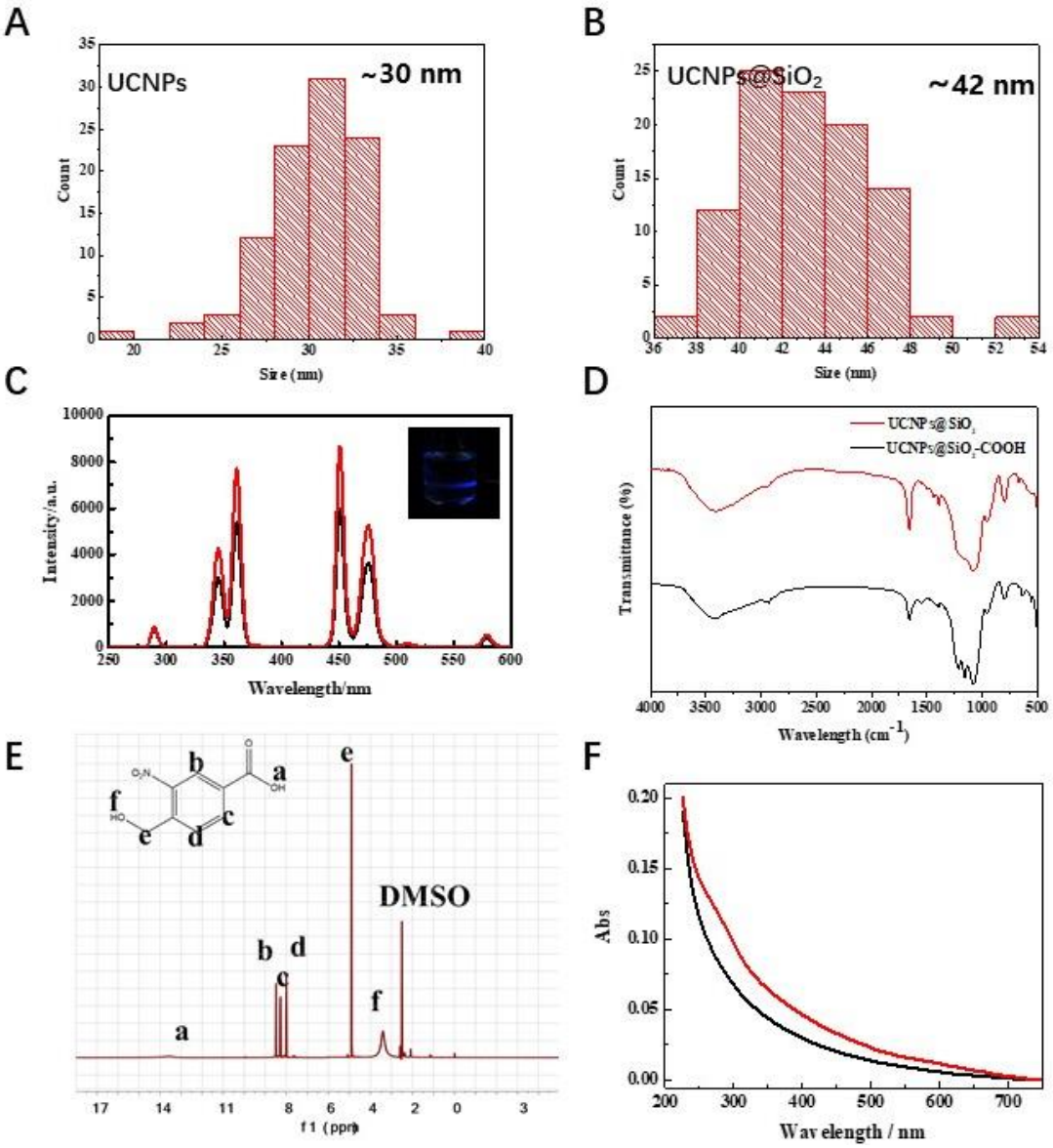

Fig. S3. The surface modification process of UCNPs. Size histograms for the UCNPs **(A)** and UCNPs@SiO_2 **(B). (C)** Upconversion luminescence spectra of the UCNPs (red line) and UCNPs@SiO_2 (black line) under 980 nm laser illumination. Inset photograph shows a blue light of UCNPs@SiO_2 excitated by 980 nm laser. **(D)** FITR spectra of UCNPs@SiO_2 (red line) and the UCNPs@SiO_2-COOH (black line). For UCNPs@SiO_2-COOH, new band at about 1600 cm-1 emerged, which is consistent with the stretching vibration of carboxyl group. These suggested the successful connection of –COOH and the nanoparticles. **(E)** 1H NMR of 4-(hydroxymethyl)-3-nitrobenzoic acid (ONA). δ 8.49 (d, 1H), 8.27 (dd, 1H), 8.00 (d, 1H), 4.93 (s, 2H). **(F)** UV−vis absorption spectra of UCNPs@SiO_2-COOH (black line) and after immobilization of ONA (red line). The absorption bands in 300−450 nm were obtained, corresponding to the absorption of photocleaved nitro-sobenzaldehydes (*34*).

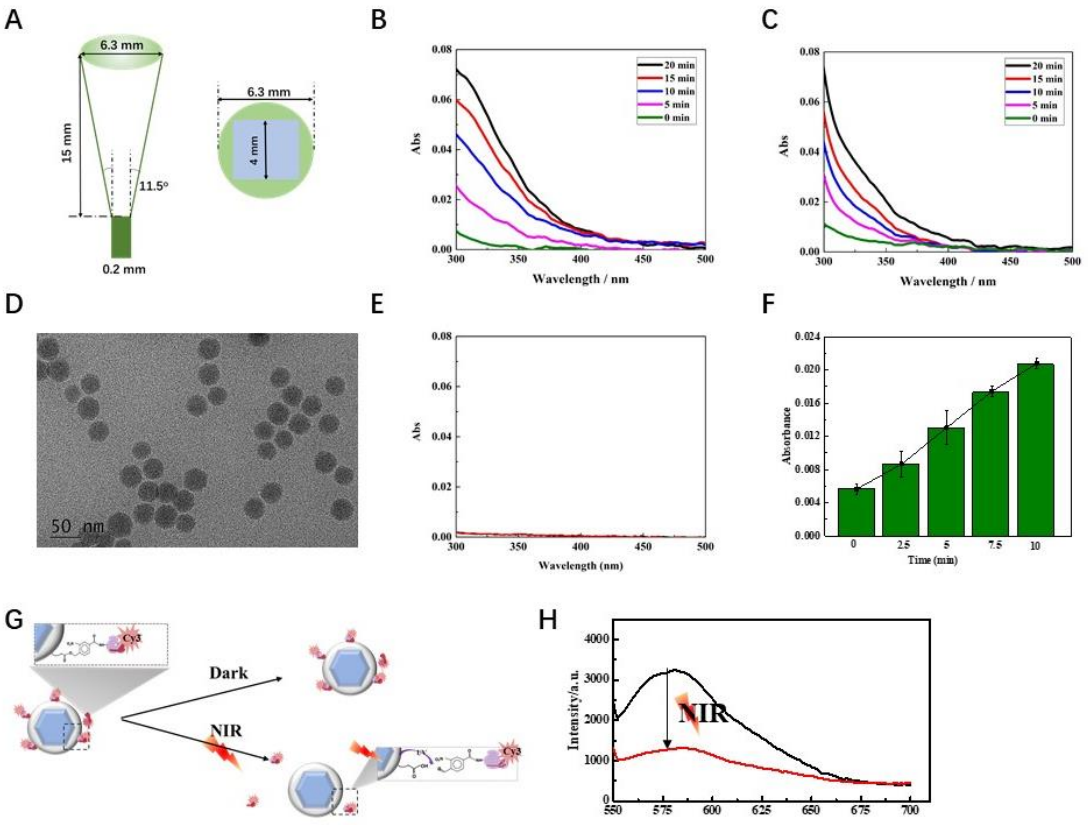

Fig. S4. Photocleavage reaction triggered by NIR light on the surface of UCNPs. (A) Scheme of the optical fiber placement (left) and the NIR irradiation area of substrate (right). The blue denotes substrate and the green circle denotes NIR light. UV-vis absorption spectra of the supernatant solution of UCNPs@SiO_2-ONA upon direct **(B)** UV irradiation and **(C)** NIR irradiation with different time. **(D)** TEM image of SiO_2-ONA. **(E)** UV-vis absorption spectra of the supernatant solution of SiO_2-ONA upon NIR irradiation (0 min: black line; 20 min: red line). **(F)** UV-vis absorption at 280 nm of the supernatant solution of UCNPs-Cas9 irradiated by UV with different irradiation time. **(G)** Schematic illustration of the release of Cas9 triggered by NIR. **(H)** Fluorescence of UCNPs-Cas9 irradiated by NIR with different irradiation time.

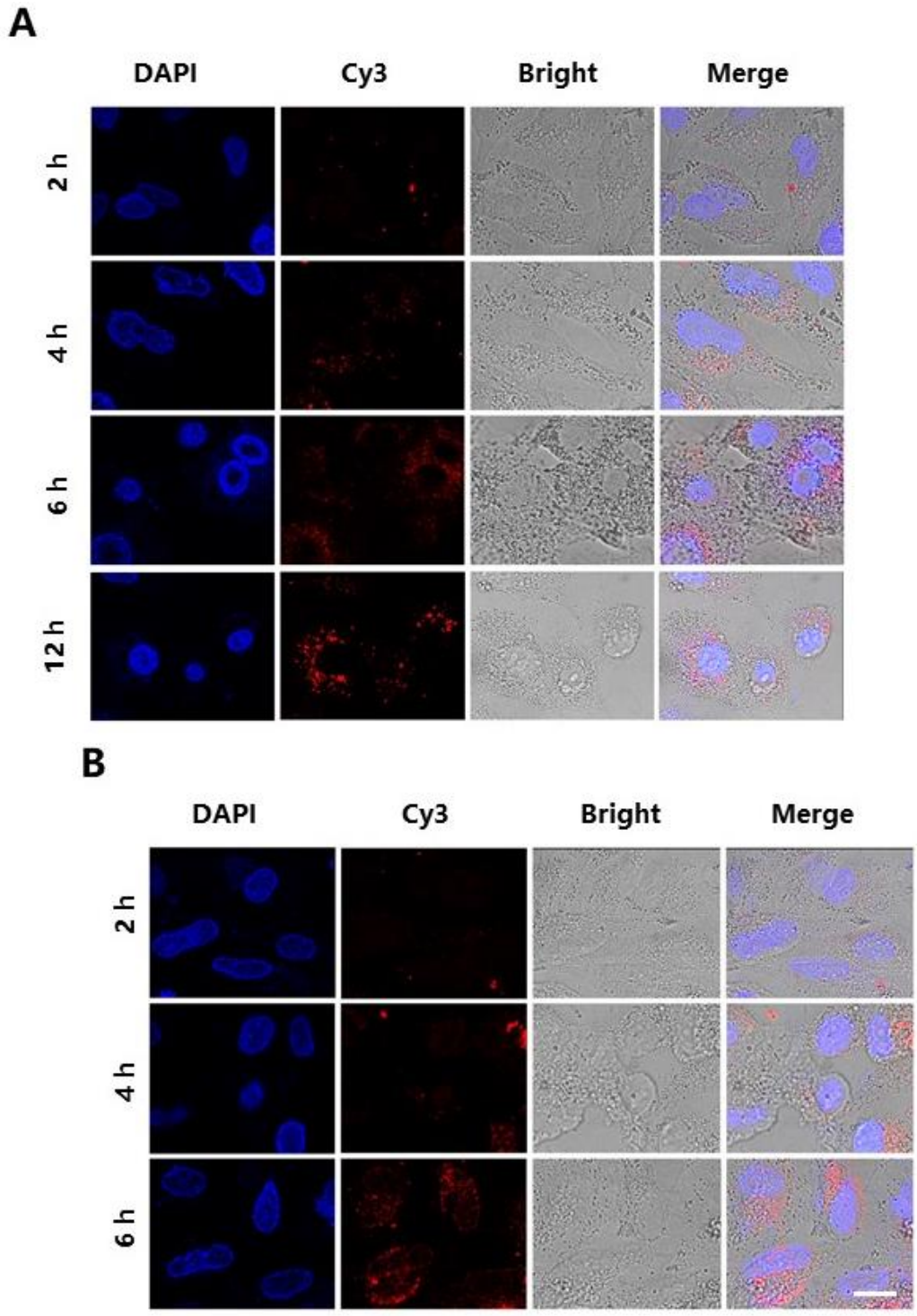

Fig. S5. Cellular internalization of UCNPs-Cas9@PEI monitored by confocal microscopy. (A) A549 cells were treated with UCNPs-Cas9@PEI in dark for 2, 4, 6 or 12 h, respectively, and no red was observed in cell nuclei. **(B)** Cells were incubated with UCNPs-Cas9@PEI under different irradiation time. 6 h later, Cas9 appeared in cell nuclei. Scale bar: 10 μm; blue: DAPI; red: Cy3-label UCNPs-Cas9@PEI.

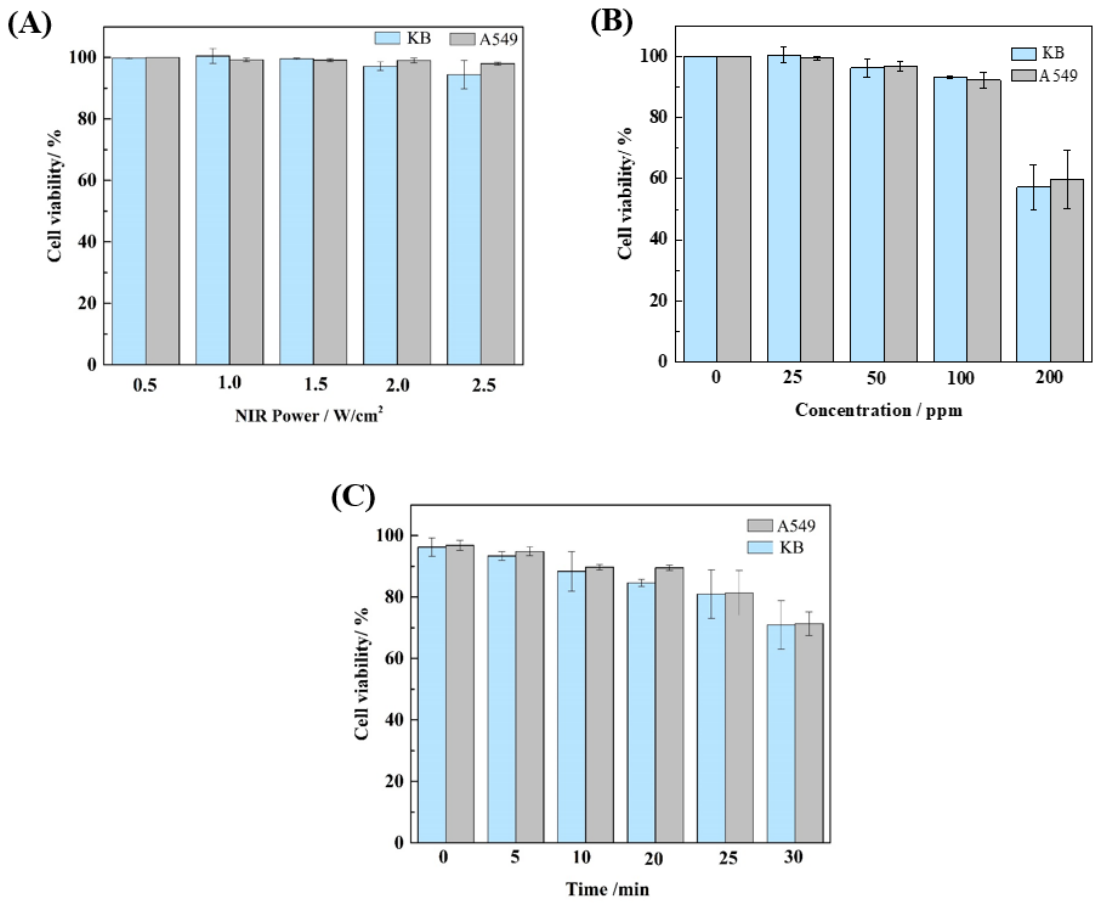

Fig. S6. The viability of cells treated under different conditions. (A) Different NIR power (no nanoparticles), **(B)** Different concentration of UCNPs-Cas9@PEI (NIR power: 2.0 W/cm^2) and **(C)** Different NIR irradiation time (concentration of UCNPs-Cas9@PEI: 50 ppm; NIR power: 2.0 W/cm^2). There existed few differences in viability between the two kinds of cells.

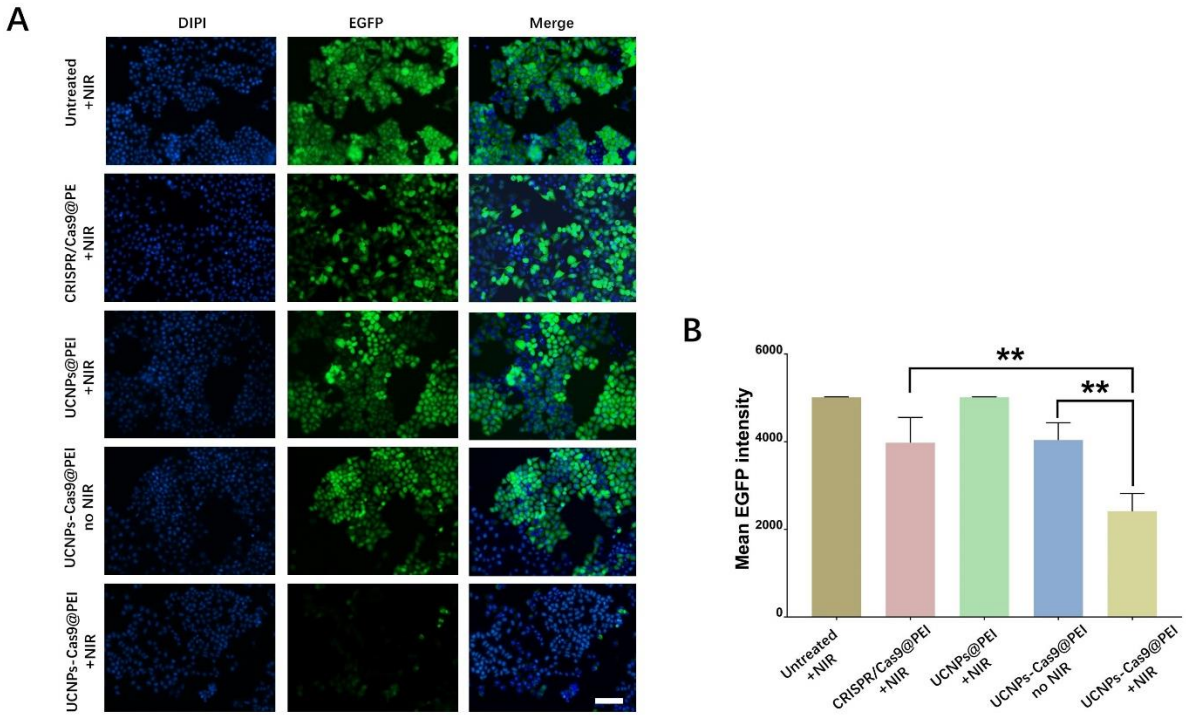

Fig. S7. The assay of EGFP expression in cells. (A) Fluorescence microscopy images of KB cells with different treatments as indicated. Green: EGFP; blue: nuclei stained with DAPI. Scale bar: 100 μm. **(B)** Quantitation of EGFP expression in cells treated with different formulations via flow cytometry analysis. UCNPs: 50 ppm.

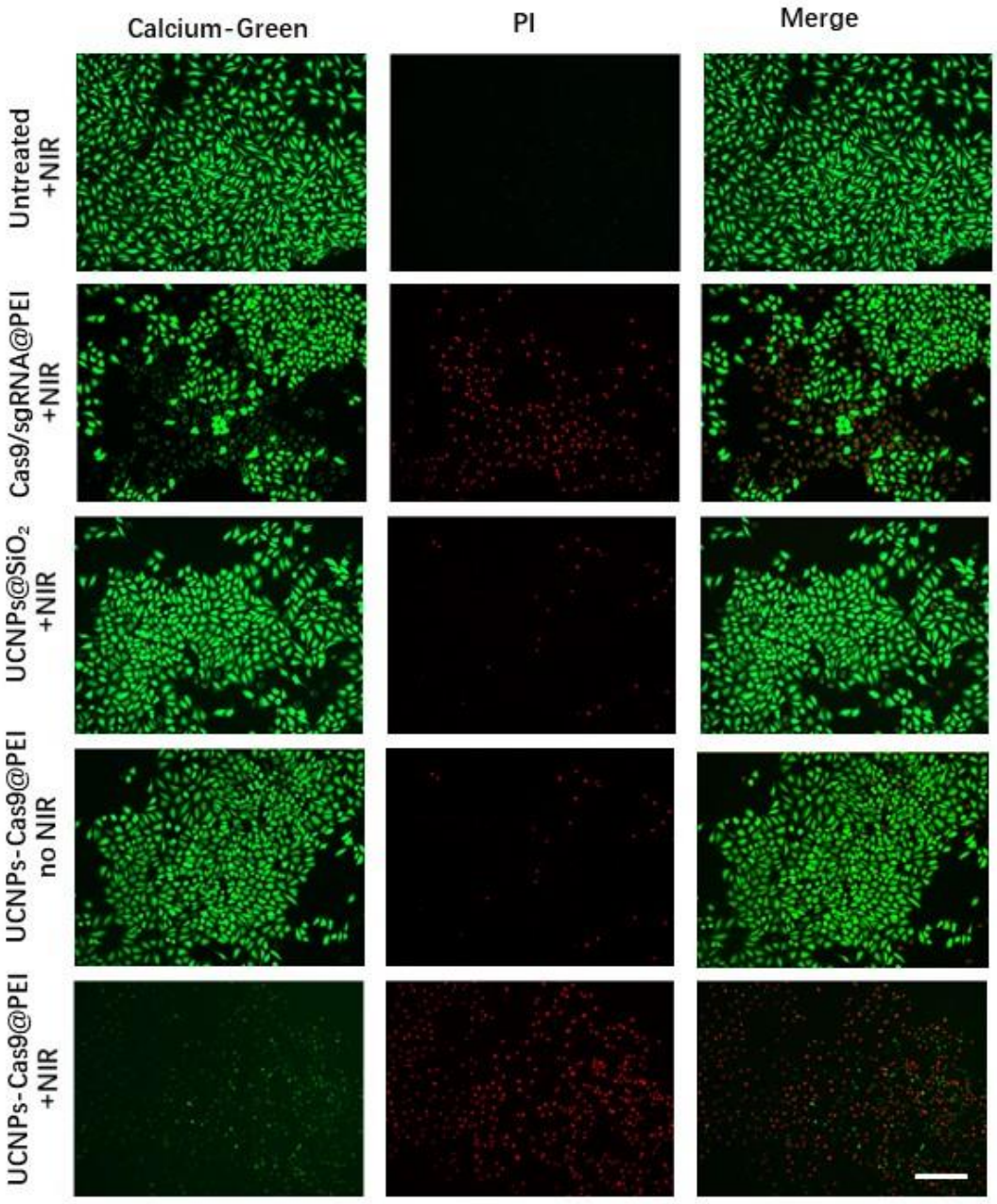

Fig. S8. Cell viabilities observed by CLSM images. Green: lived cells stained with calcium-green; red: dead cells stained with PI. Scale bar: 200 μm; UCNPs: 50 ppm.

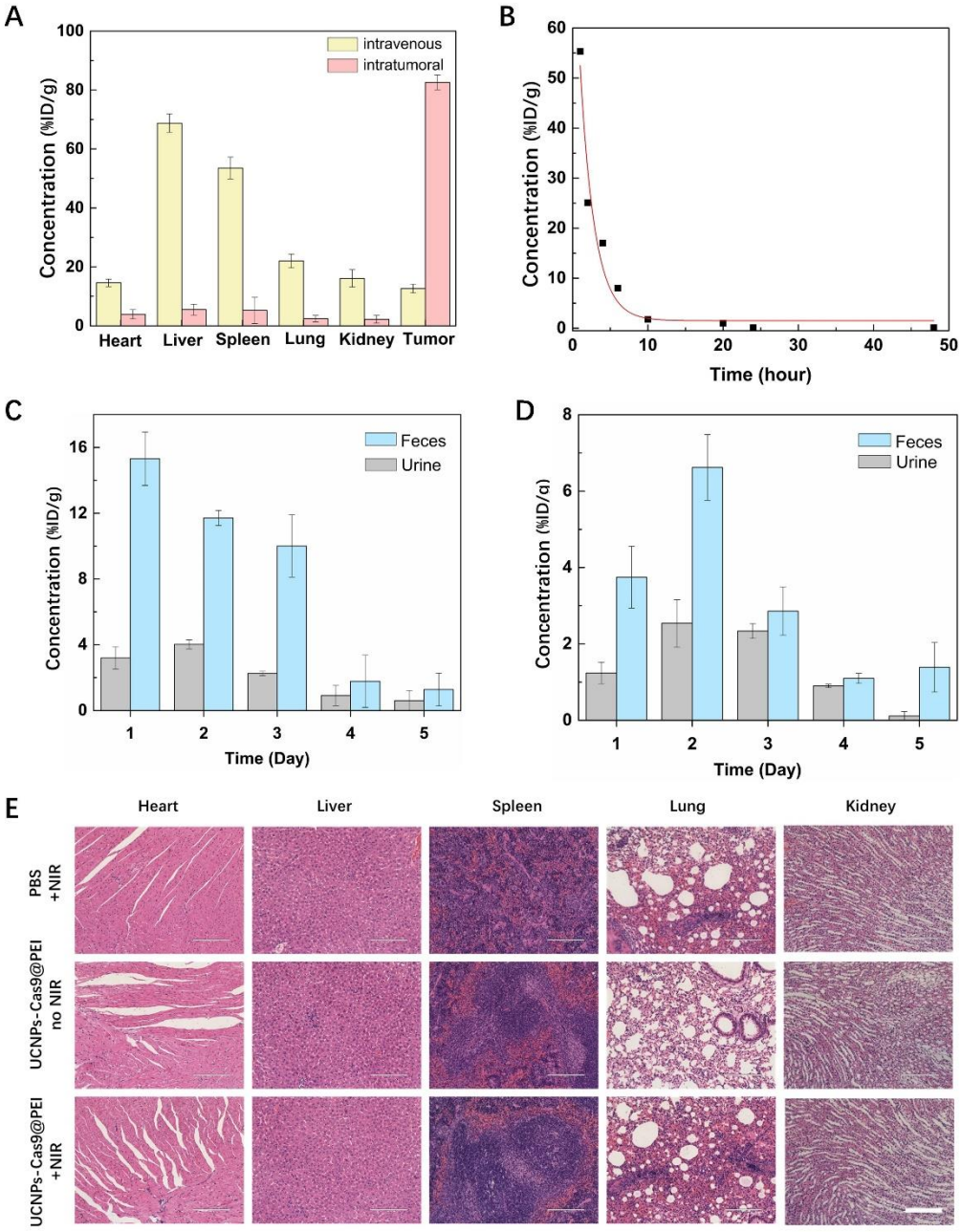

Fig. S9. The biocompatibility analysis of UCNPs-Cas9@PEI. (A) The biodistribution of the nanoparticles via intravenous (i.v.) administration (50 mg/kg) and intratumoral administration (100 μL, 3.5 mg/ml) after 24 h. **(B)** The pharmacokinetic of UCNPs-Cas9@PEI via i.v. administration. UCNPs levels in feces and urine determined by lanthanide ion (Y^{3+}) in 5 days after **(C)** i.v. and **(D)** intratumoral administration. The concentration of lanthanide ion (Y^{3+}) was detected by ICP-MS (NexION 1000, USA). **(E)** Histology analysis of major organs from nude mice with different 20-days treatments as indicated. Scale bar: 200 μm.