Supporting Information

Near-infrared control and real-time detection of osteogenic differentiation in mesenchymal stem cells by multifunctional upconversion nanoparticles

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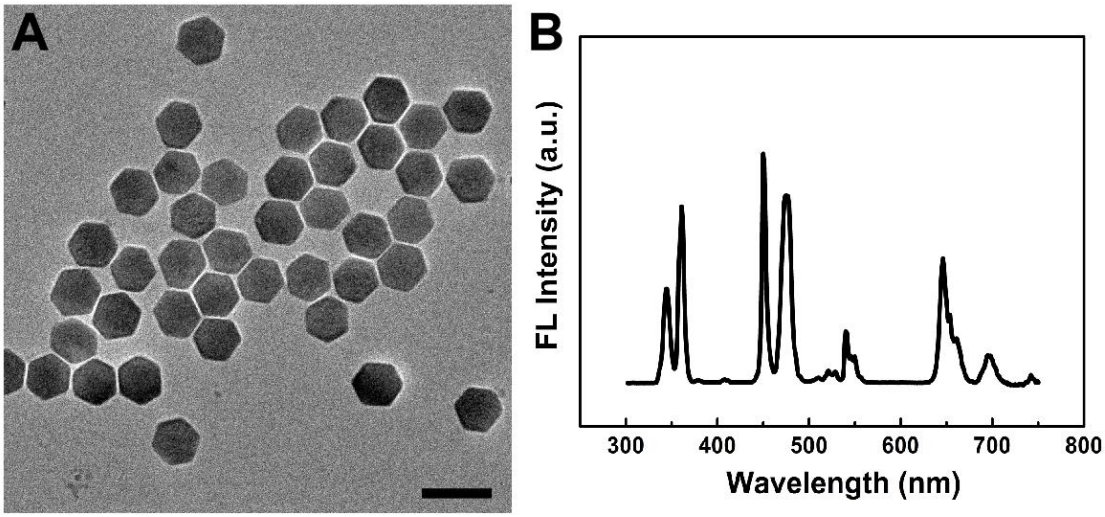

Fig. S1 Characterization of the Tm/Er doped core-shell UCNPs ($NaYF_4$: Tm/ErYb@$NaYF_4$). A) TEM imaging of the Tm/Er doped core-shell UCNPs. The size of UCNPs was about 30 nm. Scale bar: 50 nm. B) Fluorescent emission of the Tm/Er doped core-shell UCNPs. The UCNPs showed a strong fluorescent emission with 365/475/540/650 nm under the 980 nm NIR irradiation (1 W/cm^2).

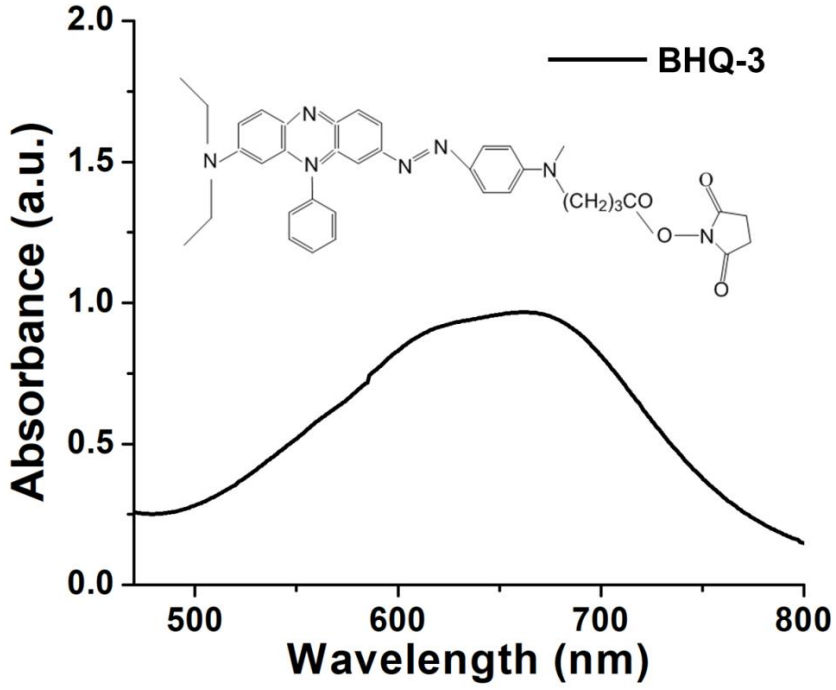

Fig. S2 The structural formula and the UV-vis absorption of black hole quencher-3 (BHQ-3).

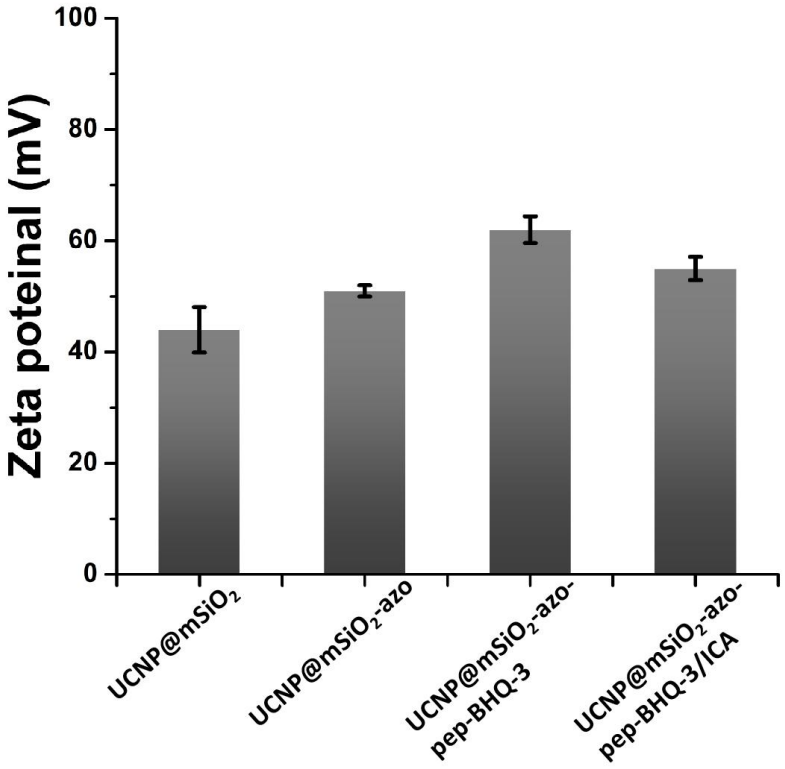

Fig. S3 The zeta poteinal of UCNP@mSiO$_2$, UCNP@mSiO$_2$-azo, UCNP@mSiO$_2$-azo-peptide-BHQ-3 and UCNP@mSiO$_2$-azo-peptide-BHQ-3/ICA.

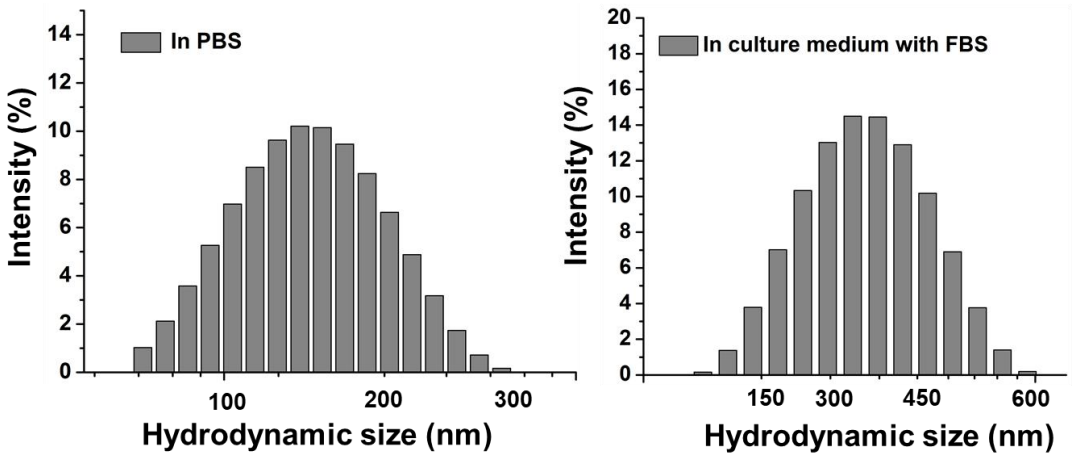

Fig. S4 The dynamic light scattering (DLS) of UCNP@mSiO$_2$-azo-peptide-BHQ-3 in PBS and in culture medium.

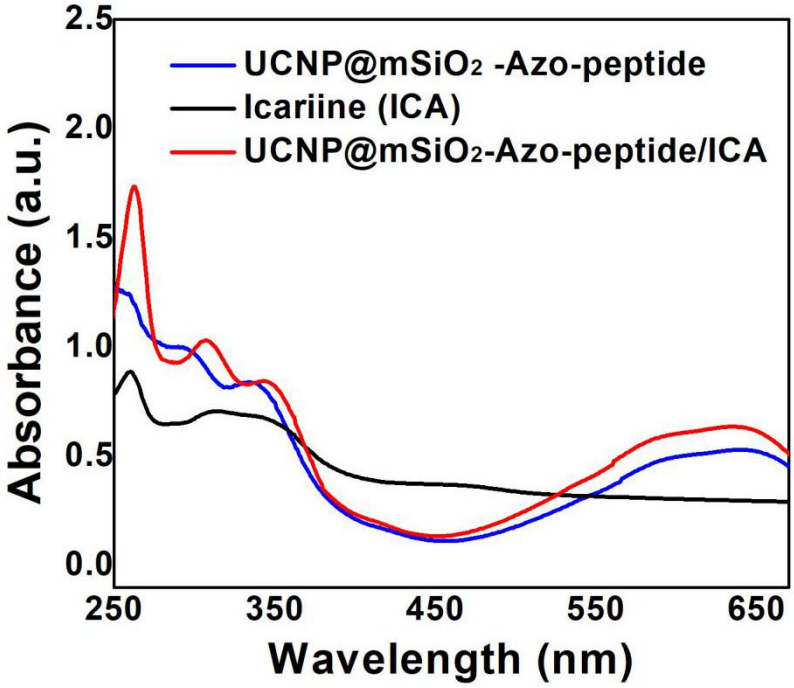

Fig. S5 The characteristic of ICA, UCNP@mSiO$_2$-azo-peptide-BHQ-3 and UCNP@mSiO$_2$-azo-peptide-BHQ-3/ICA nanocomplexes via UV-vis.

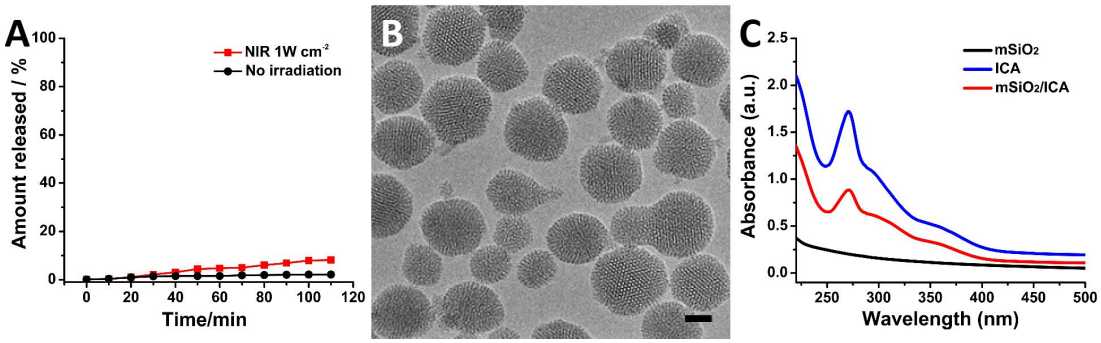

Fig. S6 The heating effect experiment that used mesoporous silica nanoparticles (mSiO$_2$) without doping ions as the negative control to testify whether the ICA can be released under the 980 nm laser with the heating effect. A) The release of ICA from mSiO$_2$/ICA nanocomplexes with or without 980 nm NIR irradiation (1 W/cm^2, irradiation time: 110 min). B) The TEM imaging of mSiO$_2$, Scale bar: 50 nm. C) The UV-vis absorbance of mSiO$_2$, ICA and mSiO$_2$/ICA nanocomplexes.

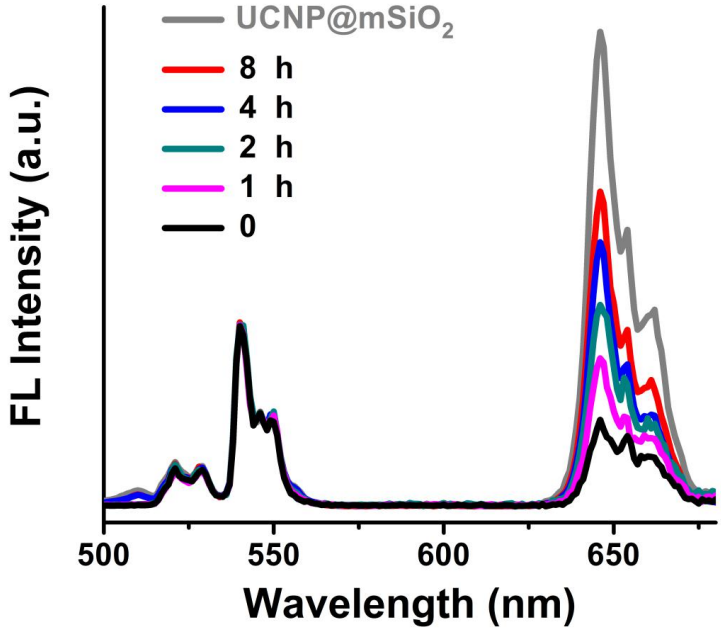

Fig. S7 Detecting MMP13 enzyme by UCNP@mSiO$_2$-azo-pep-BHQ-3 nanoprobes in enzyme digestion buffer. Similar with concentration dependent experiment, the fluorescent emission of UCNPs in 650 nm was gradual recovery after the longer incubation time with MMP13 enzyme at 980 nm NIR excitation. Incubation time: 0, 1, 2, 4 and 8 h; MMP13: 20 nM; NIR: 1 W/cm^2.

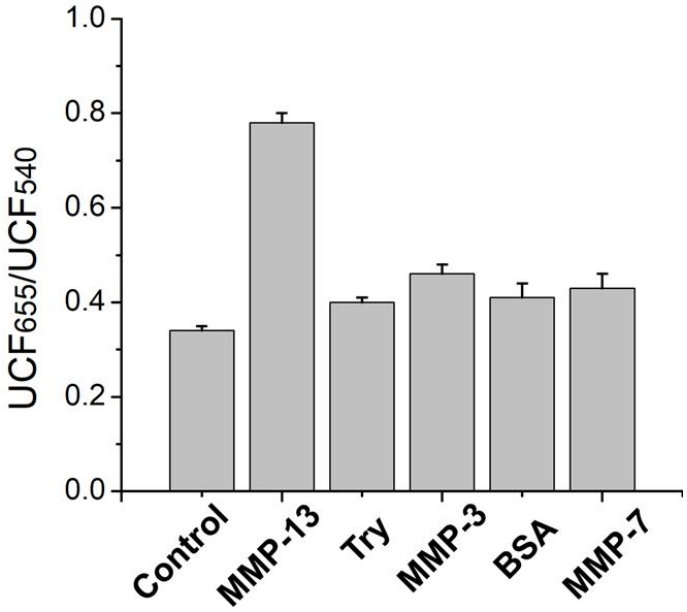

Fig. S8 Fluorescence emission kinetic spectra of the UCNPs nanoprobes in the presence of different enzymes (MMP-3, 7 and 13, BSA, Trypsin, 20 nM) with 2 h incubation at 37 ℃. The UCNP@mSiO$_2$-azo-peptide-BHQ-3 nanoprobes showed special fluorescence recovery reaction by the special MMP13 enzyme sensitive peptide digestion on the surface of UCNPs. Control: only UCNPs, no enzyme. UCNP@mSiO$_2$-azo-peptide-BHQ-3: 1 mg/ml. NIR: 1 W/cm^2.

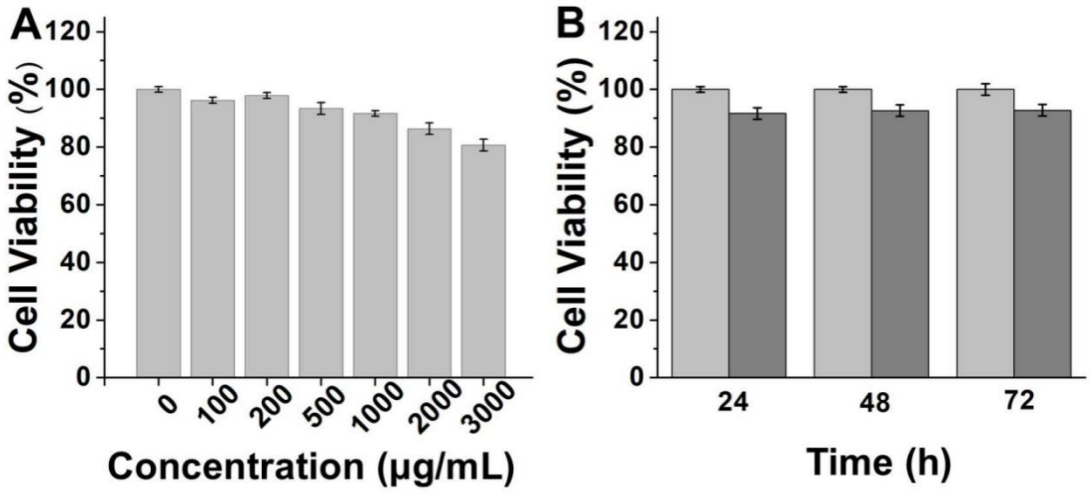

Fig. S9 Testing the cytotoxicity of UCNP@mSiO$_2$-azo-peptide-BHQ-3 in MSCs by Almar blue. A) The cell viability assay of UCNP@mSiO$_2$-azo-peptide-BHQ-3 in MSCs after 24 h incubation with different concentration (0/100/200/500/1000/2000/3000 μg/mL). The cell viability of MSCs maintained above 90% after 24 h incubation with 1000 μg/mL UCNP@mSiO$_2$-azo-peptide-BHQ-3. (B) The cell viability assay of UCNP@mSiO$_2$-azo-peptide-BHQ-3 in MSCs after different incubation time (24/48/72 h) with 1000 μg/mL. The cell viability of MSCs maintained above 90% after 72 h incubation with 1000 μg/mL UCNP@mSiO$_2$-azo-peptide-BHQ-3, showing a low cytotoxicity of UCNP@mSiO$_2$-azo-peptide-BHQ-3.

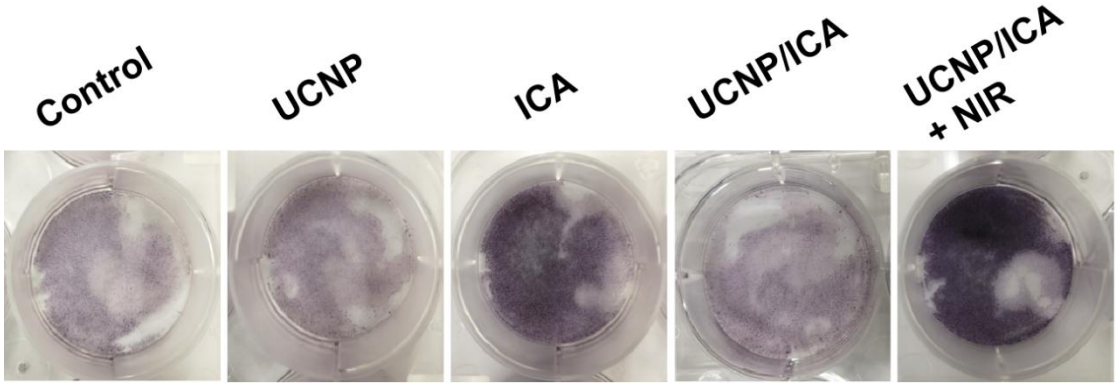

Fig. S10 ALP stain of MSCs with different treatments at 14 days inducing differentiation by UCNP@mSiO$_2$-azo-peptide-BHQ-3. Compared to others treatment, the ICA group and the UCNP/ICA+NIR group showed the obvious ALP activity by the ALP stain, which displayed the high osteogenic differentiation of MSCs through the free ICA and the NIR-triggered release ICA from the UCNPs nanocomplexes. UCNPs or UCNPs nanocomplexes: 100 μg/mL; ICA: 10 μM; ARS: 1%; NIR: 1 W/cm^2, 60 min (interval, for inducing differentiation); n=3.

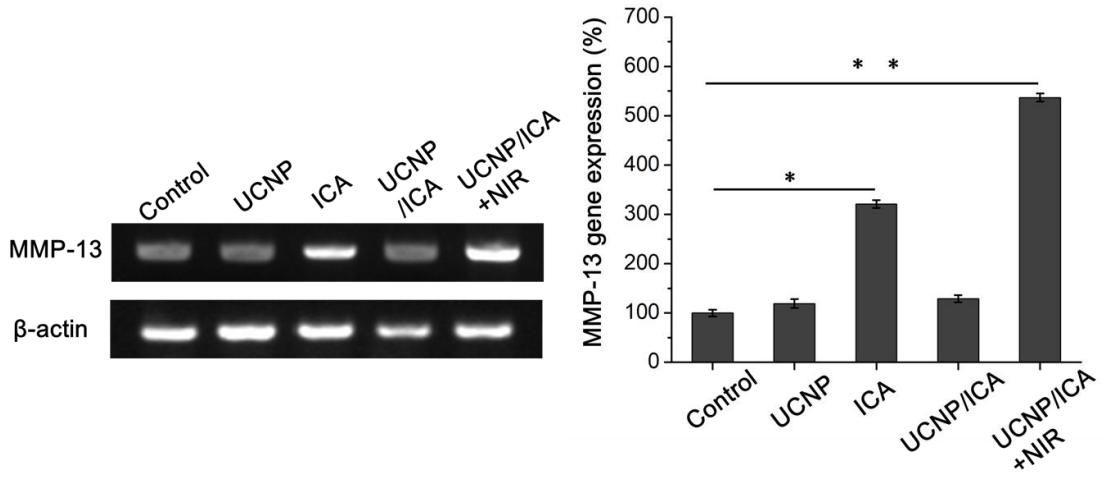

Fig. S11 The RT-PCR analysis of MMP13 gene expression after different treatments. The MMP13 gene showed a high expression with the ICA group and UCNP/ICA+NIR group, indicating that the osteogenic differentiation of MSCs and the expression of MMP13 can be used as a bio-marker for real-time detecting cell differentiation by UCNPs nanoprobes. UCNPs nanoprobes: 100 μg/mL; ICA: 10 μM; NIR: 1 W/cm^2, with 1 h; osteogenic differentiation: 7 days; n=3, $^{*}p<0.05$, $^{**}p<0.01$.

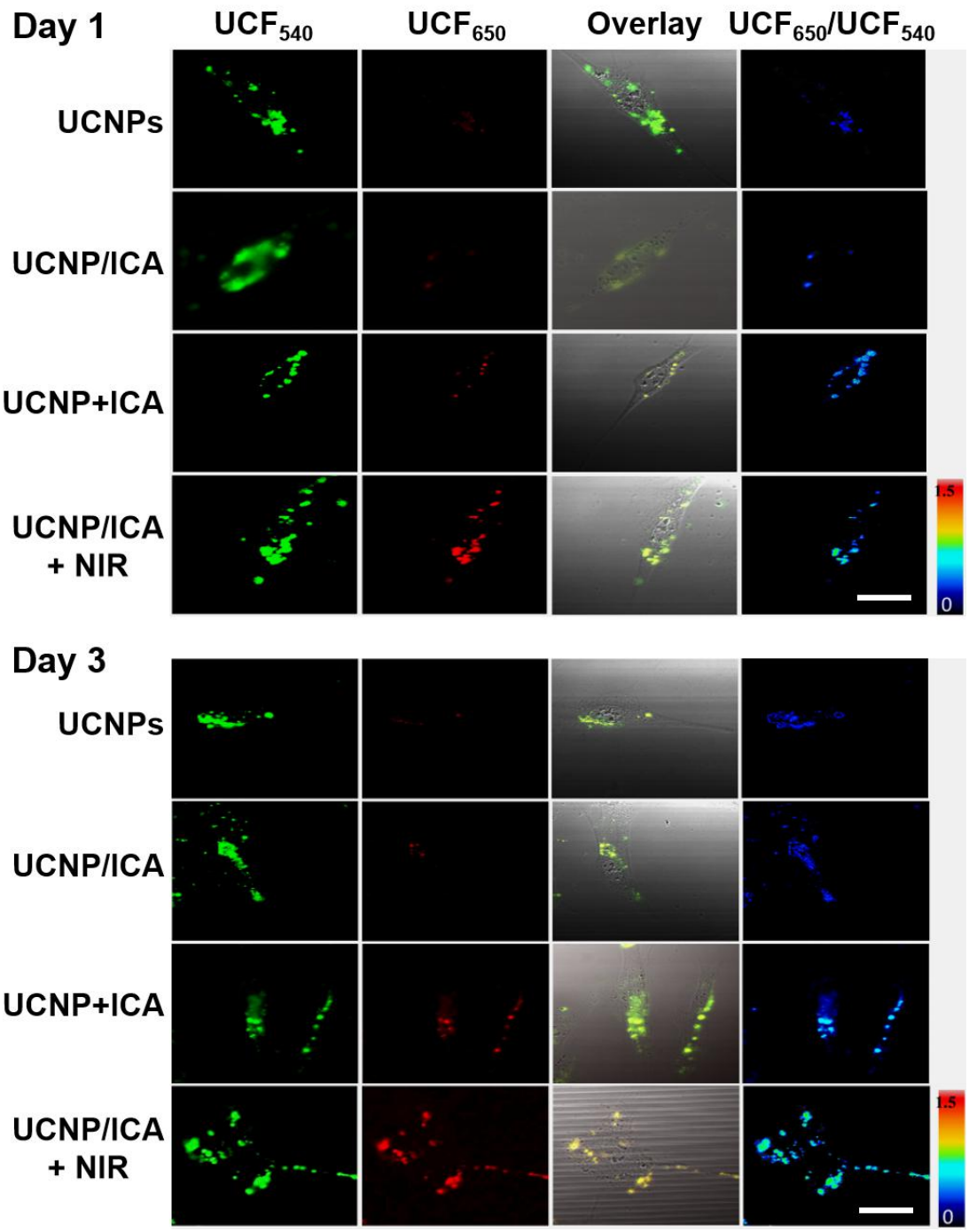

Fig. S12 980 nm confocal images of real-time detecting osteogenic differentiation of MSCs by UCNP@mSiO$_2$-azo-peptide-BHQ-3 nanoprobes. After controlled inducing osteogenic differentiation of MSCs, the UCNP@mSiO$_2$-azo-peptide-BHQ-3 became the nanoprobes to detect the activity of MMP13 enzyme that produced by osteogenic differentiation by enzyme digestion and UCNPs 650 nm fluorescence recovery for real-time detecting cell differentiation in living differentiated MSCs. UCNPs: 100 μg/mL; ICA: 10 μM; NIR: 1 W/cm^2 with 1 h; Scale bar: 20 μm.

Table 1. Primer sequences and conditions

Gene	NCBI number	Forward (5′-3′)	Reverse (5′-3′)	Annealing temperature
BMP-2	NM_001200	gtatcgcaggcactcaggtc	cacttccaccacgaatccat	Touch down
Runx2	NM_001024630	acttcctgtgctcggtgct	gacggttatggtcaaggtgaa	Touch down
OPN	NM_00582	ctaggcatcacctgtgccatacc	cagtgaccagttcatcagattcatc	55℃
B-actin	NM_001200	gctcgtcgtcgacaacggctc	caaacatgatctgggtcatcttctc	52℃
MMP13	NM_002427.3	tggtggtgatgaagatgatttgtct	agttacatcggaccaaactttgaag	52℃

Touch down： The annealing temperature was continuously reduced from 62 ℃ to 52 ℃, 0.5 ℃ per cycle, and the next 20 cycles were run at 52 ℃.

Table S1 The sequence and annealing temperature of primers used in RT-PCR.