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Supporting Information

Photocontrolled chondrogenic differentiation and long-term tracking of mesenchymal stem cells *in vivo* by upconversion nanoparticles

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Keywords: photocontrolled chondrogenic differentiation, long-term tracking, mesenchymal stem cells, upconversion nanoparticles, *in vivo*

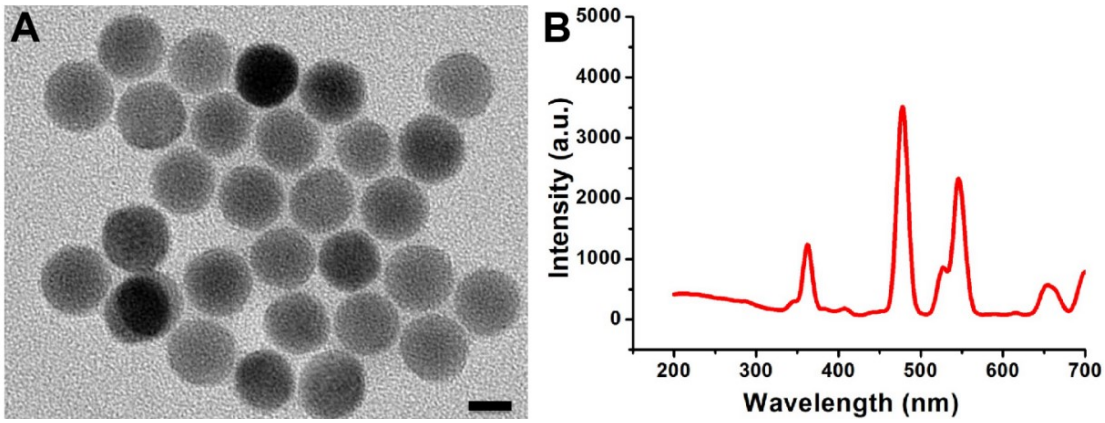

Figure S1. Characterization of the Tm/Er doped naked UCNPs ($NaYF_4$: Tm/Er,Yb). A) TEM imaging of the Tm/Er doped UCNPs. The size of UCNPs was about 30 nm. Scale bar: 20 nm. B) Fluorescent emission spectrum of the Tm/Er doped UCNPs. The UCNPs showed a strong fluorescent emission with 365/475/545/647 nm under the 980 nm NIR irradiation (1 W/cm^2).

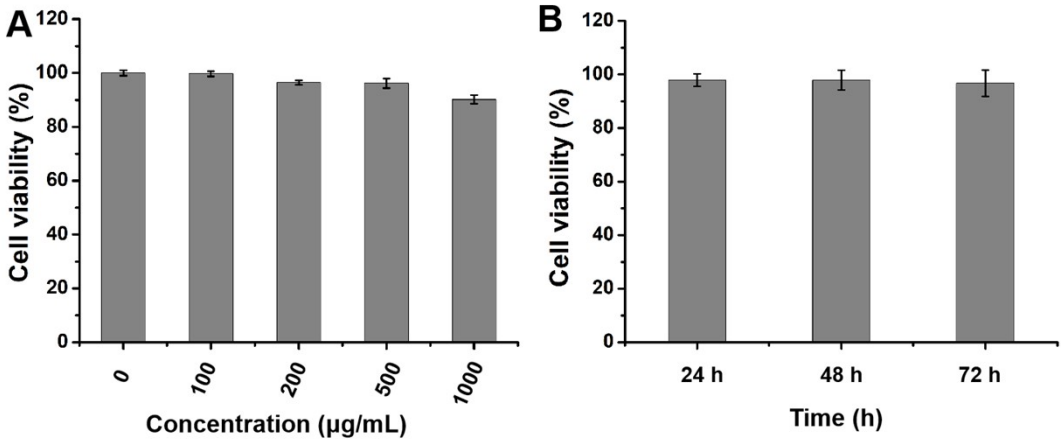

Figure S2. Investigate the cytotoxicity of UCNP@$mSiO_2$-azo-RGD in MSCs by Almar blue. A) The cell viability assay of UCNP@$mSiO_2$-azo-RGD in MSCs after 24 h culture with different concentration (0/100/200/500/1000 μg/mL). The cell viability of MSCs remained above 90% after 24 h incubation under the concentration of 1000 μg/mL UCNP@$mSiO_2$-azo-RGD. B) The cell viability assay of UCNP@$mSiO_2$-azo-RGD in MSCs with different incubation time (24/48/72 h) under 1000 μg/mL. The cell viability of MSCs maintained above 90% after 72 h incubation with 1000 μg/mL UCNP@$mSiO_2$-azo-RGD, indicating a low cytotoxicity of UCNP@$mSiO_2$-azo-RGD in MSCs.

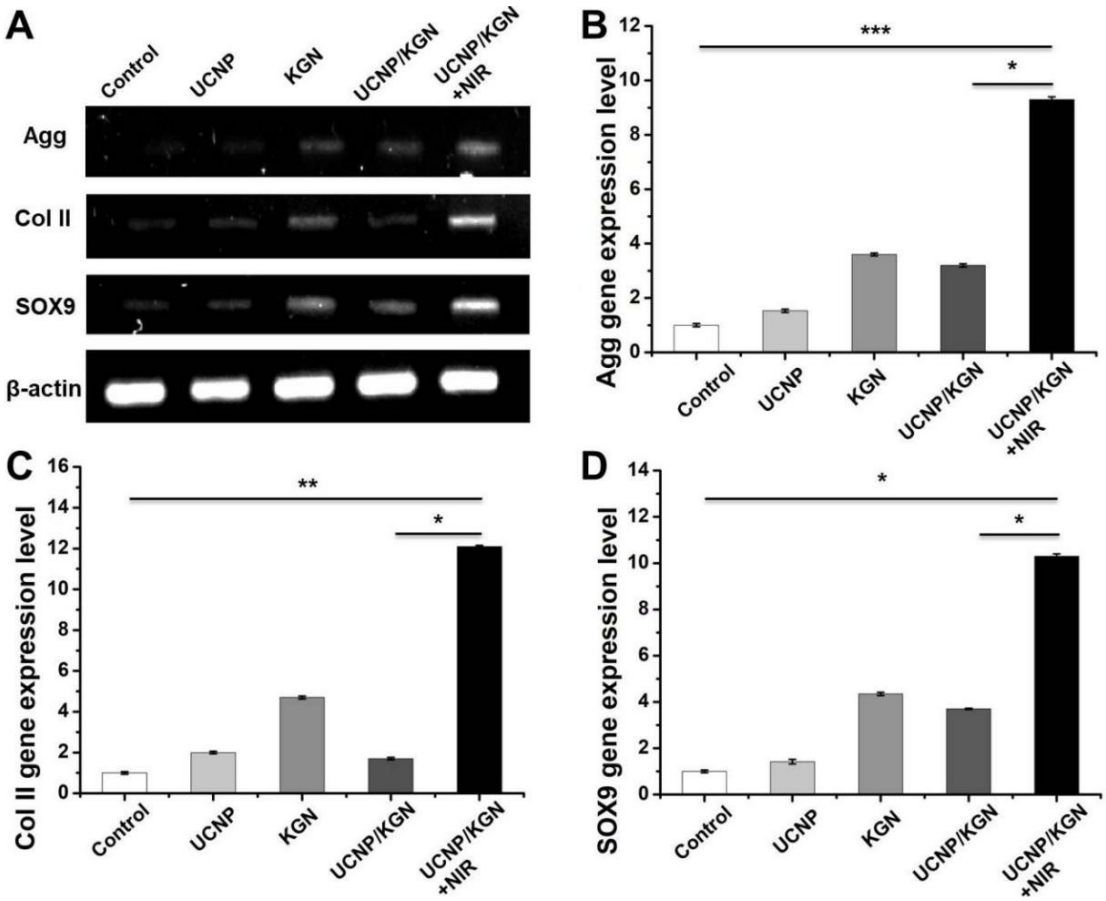

Figure S3. RT-PCR analysis of chondrogenic differentiation-related genes: Agg, Col II and SOX9 in differentiated MSCs with different treatments after 14 days consecutive culture. A) Agarose gel electrophoresis result of chondrogenic differentiation-related genes: Agg ,Col II and SOX9 expression in differentiated MSCs after different treatments by RT-PCR, the UCNP/KGN+NIR group showed the highest gene expression in all groups. B) Measuring the related gene expression of Agg with different treatments in chondrogenic-differentiated MSCs from agarose gel electrophoresis result by Image J. C) Measuring the related gene expression of Col II with different treatments in chondrogenic-differentiated MSCs from agarose gel electrophoresis result by Image J. D) Measuring the related gene expression of SOX9 with different treatments in chondrogenic-differentiated MSCs from agarose gel electrophoresis result by Image J. UCNP/KGN nanocomplexes: 200 μg/mL; KGN: 100 nM; NIR: 1.5 W/cm^2, 30 min; n=3, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

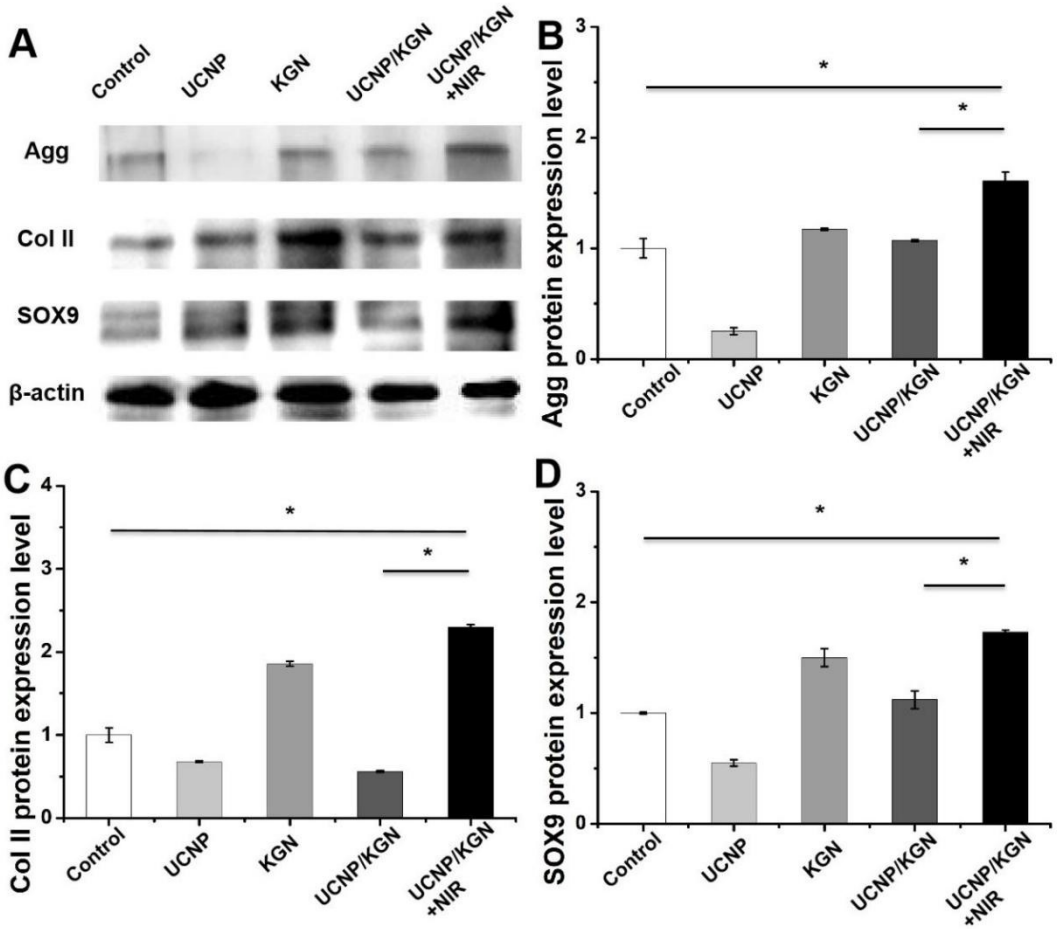

Figure S4. Western blot analysis of chondrogenic differentiation-related proteins: Agg, Col II and SOX9 in differentiated MSCs with different treatments after 14 days consecutive culture. A) Polyacrylamide gel electrophoresis result of chondrogenic differentiation-related proteins: Agg ,Col II and SOX9 expression in differentiated MSCs after different treatments by Western blot, the UCNP/KGN+NIR group showed the highest protein expression in all groups. B) Measuring the Agg protein expression in different treatments of chondrogenic-differentiated MSCs from Western blot result by Image J. C) Measuring the Col II protein expression in different treatments of chondrogenic-differentiated MSCs from Western blot result by Image J. D) Measuring the SOX9 protein expression in different treatments of chondrogenic-differentiated MSCs from Western blot result by Image J. UCNP/KGN nanocomplexes: 200 μg/mL; KGN: 100 nM; NIR: 1.5 W/cm^2, 30 min; n=3, $^*p < 0.05$.

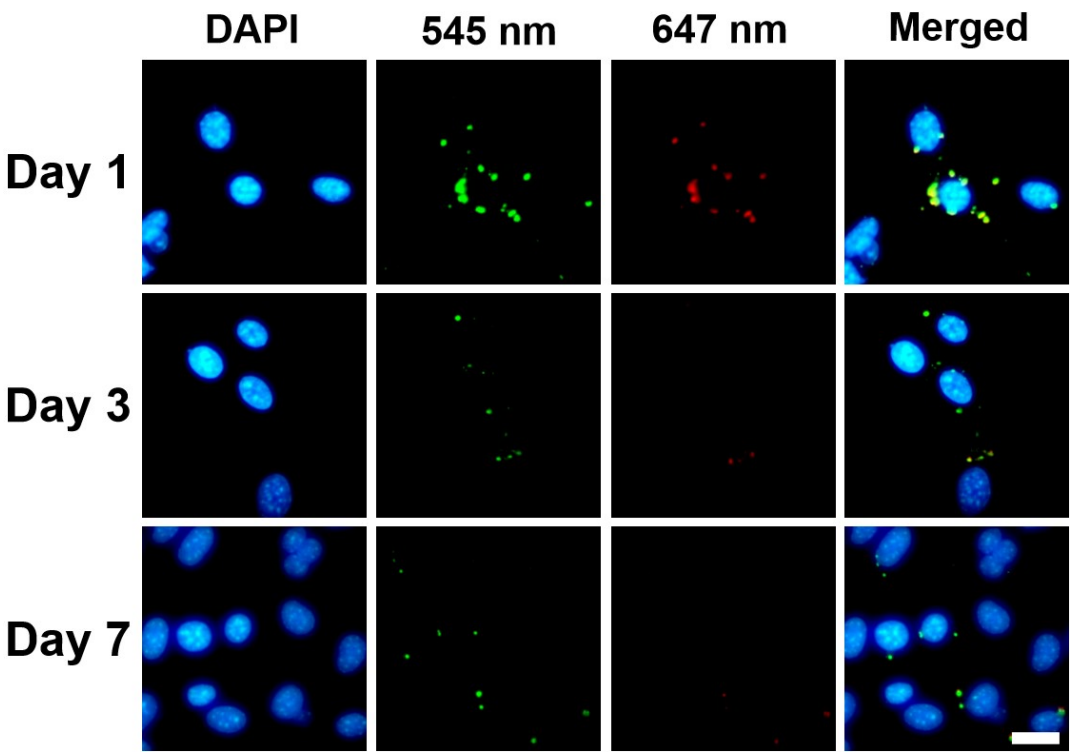

Figure S5. Long-term tracking of non-differentiated MSCs by UCNP nanoprobes with day 1, 3 and 7. The fluorescence images of MSCs were photographed by 980 nm near infrared fluorescence inverted microscope after MSCs incubated with UCNP nanoprobes and continued to culture in the grow medium. The UCNP nanoprobes could stay in the cytoplasm and track the MSCs for a long-term of 1, 3 and 7 days. UCNP nanoprobes: 200 μg/mL; Scale bar: 20 μm.

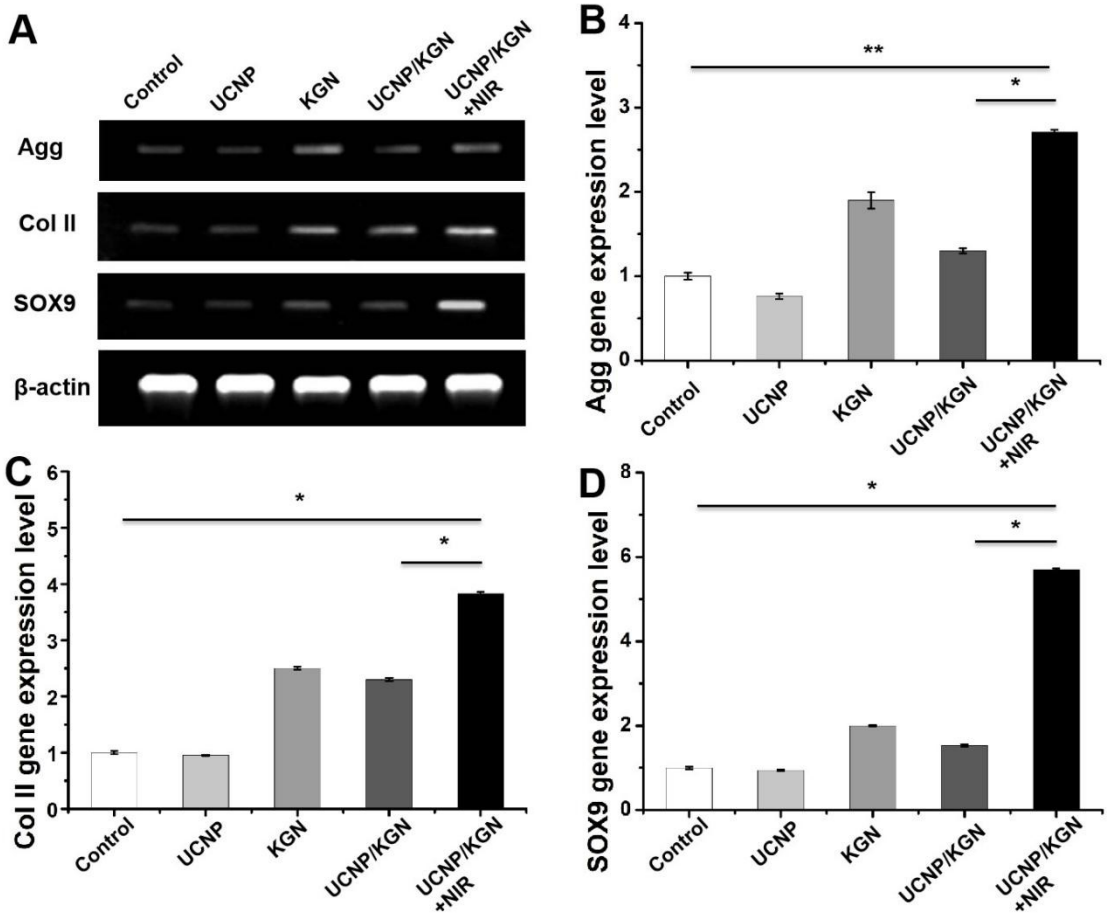

Figure S6. RT-PCR analysis of chondrogenic differentiation-related genes: Agg, Col II and SOX9 in differentiated MSCs with different treatments in the 3D hydrogel. The MSCs were treated with KGN or particles and then were implanted into the hydrogel for induced differentiation culture of 7 days. A) Agarose gel electrophoresis result of chondrogenic differentiation-related genes: Agg, Col II and SOX9 expression in differentiated MSCs after different treatments in the 3D hydrogel by RT-PCR, the UCNP/KGN+NIR group showed the highest gene expression in all groups. B) Measuring the related gene expression of Agg with different treatments in chondrogenic-differentiated MSCs from agarose gel electrophoresis result by ImageJ. C) Measuring the related gene expression of Col II with different treatments in chondrogenic-differentiated MSCs from agarose gel electrophoresis result by ImageJ. D) Measuring the related gene expression of SOX9 with different treatments in chondrogenic-differentiated MSCs from agarose gel electrophoresis result by ImageJ. UCNP/KGN nanocomplexes: 200 μg/mL; KGN: 100 nM; NIR: 1.5 W/cm^2, 30 min; n=3, $^{*}p < 0.05$, $^{**}p < 0.01$.

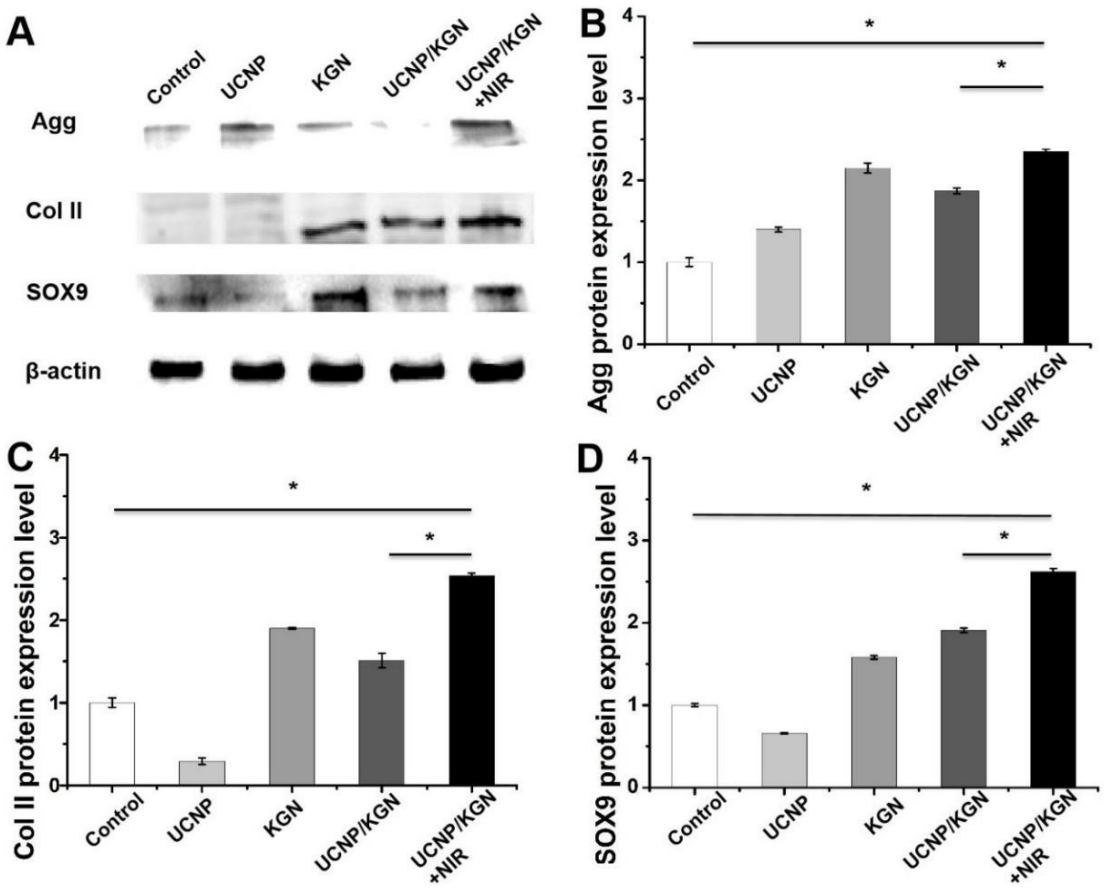

Figure S7. Western blot analysis of chondrogenic differentiation-related proteins: Agg, Col II and SOX9 in differentiated MSCs with different treatments in the 3D hydrogel. The MSCs were treated with KGN or particles and then were implanted into the hydrogel for induced differentiation culture of 7 days. A) Polyacrylamide gel electrophoresis result of chondrogenic differentiation-related proteins: Agg ,Col II and SOX9 expression in differentiated MSCs after different treatments in the 3D hydrogel by Western blot, the UCNP/KGN+NIR group showed the highest gene expression in all groups. B) Measuring the related protein expression of Agg with different treatments in chondrogenic-differentiated MSCs from Western blot result by Image J. C) Measuring the related protein expression of Col II with different treatments in chondrogenic-differentiated MSCs from Western blot result by Image J. D) Measuring the related protein expression of SOX9 with different treatments in chondrogenic-differentiated MSCs from Western blot result by Image J. UCNP/KGN nanocomplexes: 200 μg/mL; KGN: 100 nM; NIR: 1.5 W/cm^2, 30 min; n=3, $^*p < 0.05$.

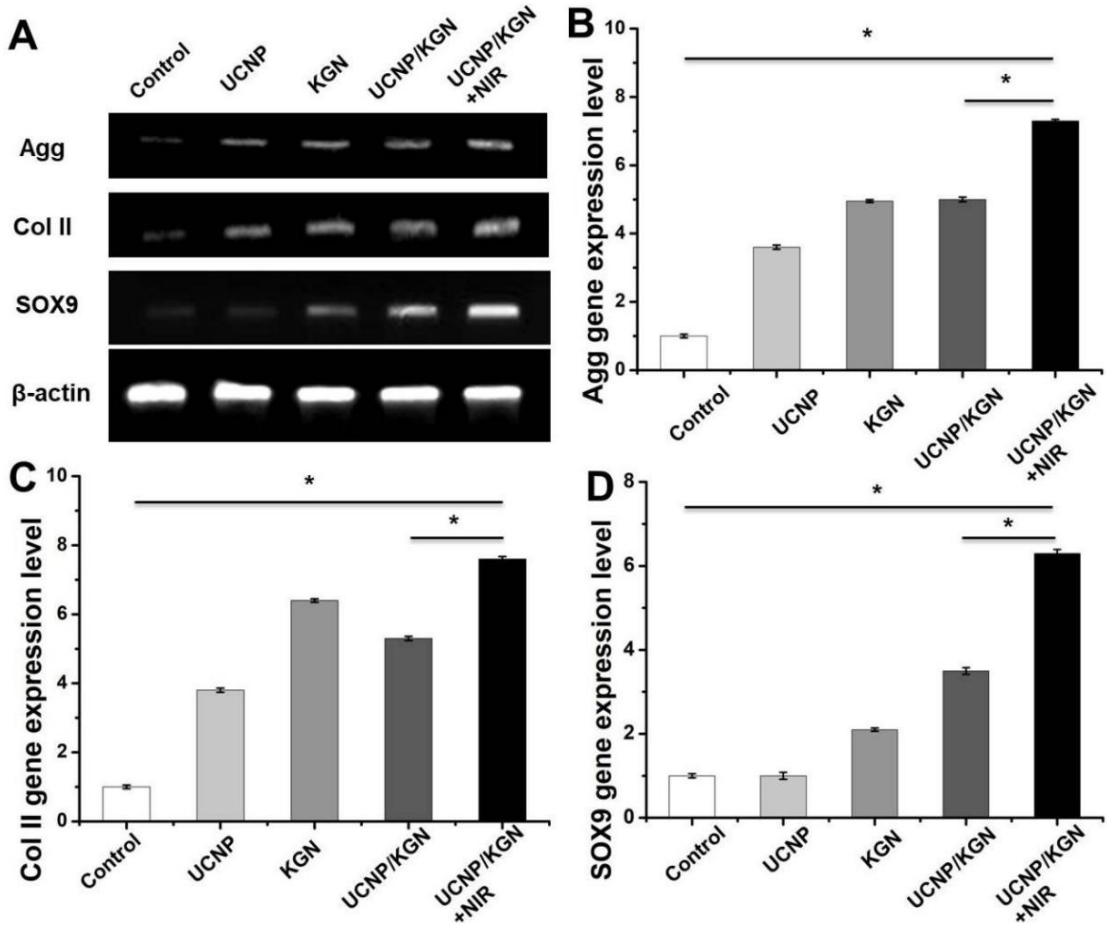

Figure S8. RT-PCR analysis of chondrogenic differentiation-related genes: Agg, Col II and SOX9 in differentiated MSCs with different treatments in the 3D hydrogel. The MSCs were treated with KGN or particles and then were implanted into the hydrogel for induced differentiation culture of 14 days. A) Agarose gel electrophoresis result of chondrogenic differentiation-related genes: Agg ,Col II and SOX9 expression in differentiated MSCs after different treatments in the 3D hydrogel by RT-PCR, the UCNP/KGN+NIR group showed the highest gene expression in all groups. B) Measuring the related gene expression of Agg with different treatments in chondrogenic-differentiated MSCs from agarose gel electrophoresis result by Image J. C) Measuring the related gene expression of Col II with different treatments in chondrogenic-differentiated MSCs from agarose gel electrophoresis result by Image J. D) Measuring the related gene expression of SOX9 with different treatments in chondrogenic-differentiated MSCs from agarose gel electrophoresis result by Image J. UCNPs: 200 μg/mL; KGN: 100 nM; NIR: 1.5 W/cm^2, 30 min; n=3, *$p < 0.05$.

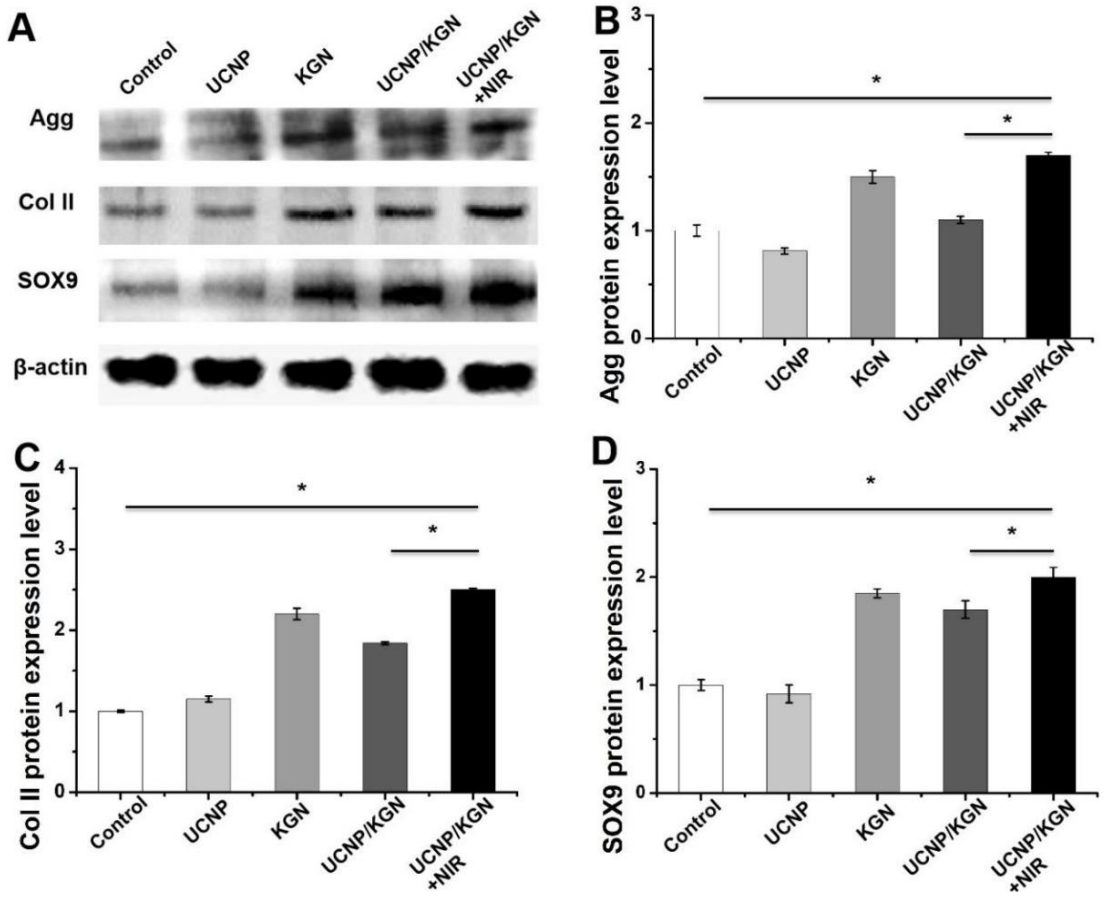

Figure S9. Western blot analysis of chondrogenic differentiation-related proteins: Agg, Col II and SOX9 in differentiated MSCs with different treatments in the 3D hydrogel. The MSCs were treated with KGN or particles and then were implanted into the hydrogel for induced differentiation culture of 14 days. A) Polyacrylamide gel electrophoresis result of chondrogenic differentiation-related proteins: Agg ,Col II and SOX9 expression in differentiated MSCs after different treatments in the 3D hydrogel by Western blot, the UCNP/KGN+NIR group showed the highest gene expression in all groups. B) Measuring the related protein expression of Agg with different treatments in chondrogenic-differentiated MSCs from Western blot result by Image J. C) Measuring the related protein expression of Col II with different treatments in chondrogenic-differentiated MSCs from Western blot result by Image J. D) Measuring the related protein expression of SOX9 with different treatments in chondrogenic-differentiated MSCs from Western blot result by Image J. UCNP/KGN nanocomplexes: 200 μg/mL; KGN: 100 nM; NIR: 1.5 W/cm^2, 30 min; n=3, $^*p < 0.05$.

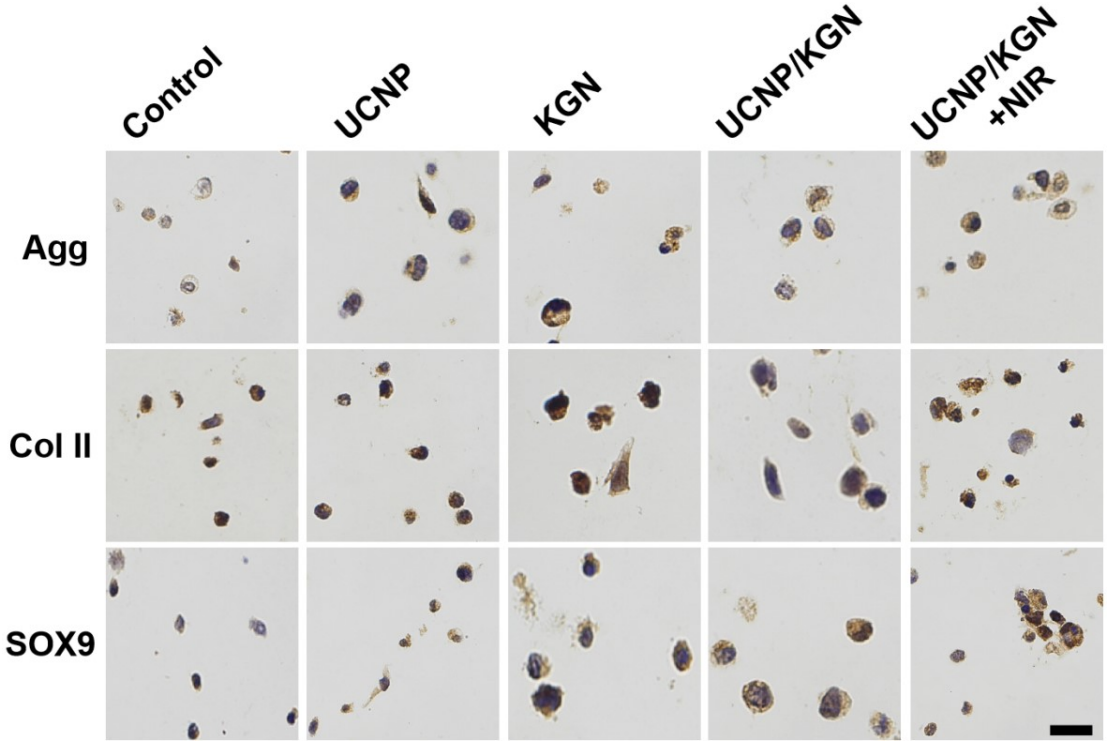

Figure S10. Immunohistochemical staining of MSC-laden 3D hydrogel after 21 days implantation against the chondrogenic differentiated-related proteins: Agg, Col II and SOX9 in different treatment groups (Control, KGN, UCNP/KGN and UCNP/KGN+NIR). The UCNP/KGN+NIR treatment group showed the darkest color of immunohistochemical staining with the highest protein expression of Agg, Col II and SOX9, indicating the NIR treatment induced significant chondrogenic differentiation of MSCs in the 3D Vitragel via the NIR-triggered release of KGN. UCNP/KGN nanocomplexes: 200 μg/mL; KGN: 100 nM; NIR: 1.5 W/cm^2, 30 min; Scale bar: 20 μm.

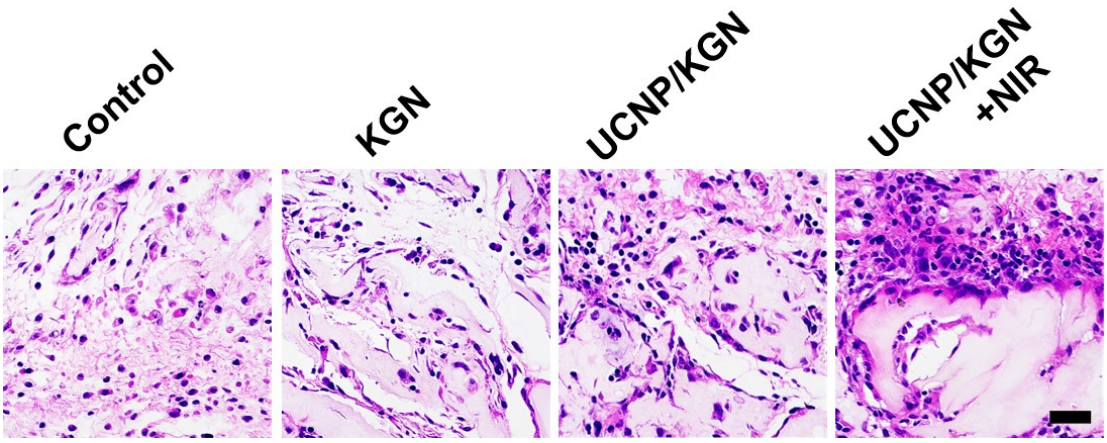

Figure S11. H&E staining of MSC-laden hydrogels after 28 days subcutaneous implantation in the back of Balb/c mice with different treatment groups (Control, KGN, UCNP/KGN and UCNP/KGN+NIR). The MSCs were treated with KGN or particles and then were implanted into the hydrogel for induced chondrogenic differentiation culture. The MSC-laden hydrogels were implanted in the back of Balb/c mice for continuous differentiation with 28 days and then the hydrogels were taken out for fixation and sectioning. The UCNP/KGN+NIR treatment group showed the darkest color of H&E staining with the most significant chondrocyte differentiation, indicating the NIR treatment induced significant chondrogenic differentiation of MSCs *in vivo* via the NIR-triggered release of KGN. UCNP/KGN: 200 μg/mL; KGN: 100 nM; NIR: 1.5 W/cm^2, 30 min; Scale bar: 50 μm.