Supplementary Information (SI) for Journal of Materials Chemistry B.

Supporting Information

MicroRNA Nanocapsules for Modulating Macrophage Polarization to Promote Bone Repair

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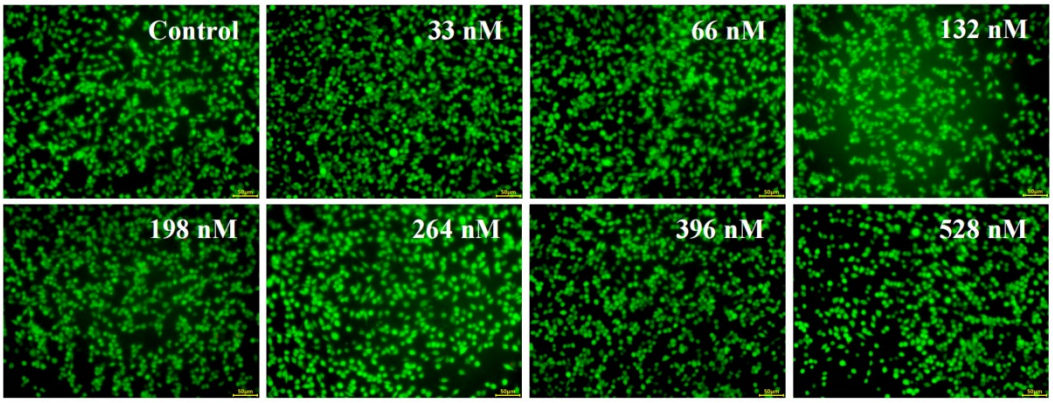

Figure S1. Live/dead cell viability assay. The L929 cells were coincubated with different concentrations of n(miR-21) for 24 h.

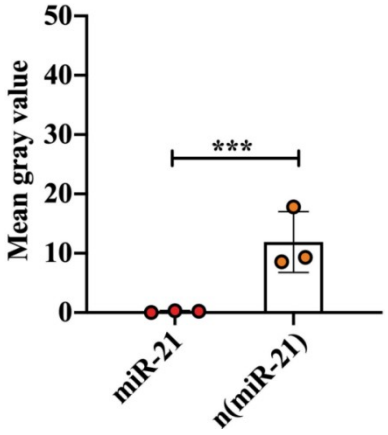

Figure S2. Mean gray value by image J. Semiquantitative analysis of the pictures of uptake efficiency.

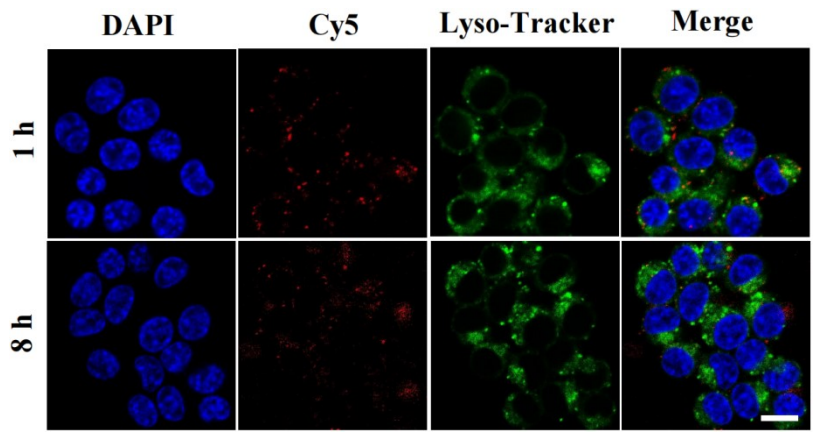

Figure S3. Confocal images of RAW264.7 cells incubated with n(miR21) for different times (1 h, 8 h). The fluorescence spots of n(miR21) (red) were observed. The nucleus and lysosome were stained with Hoechst 33342 (blue) and Lysotracker Green (green), respectively. The scale bar was 10 μm.

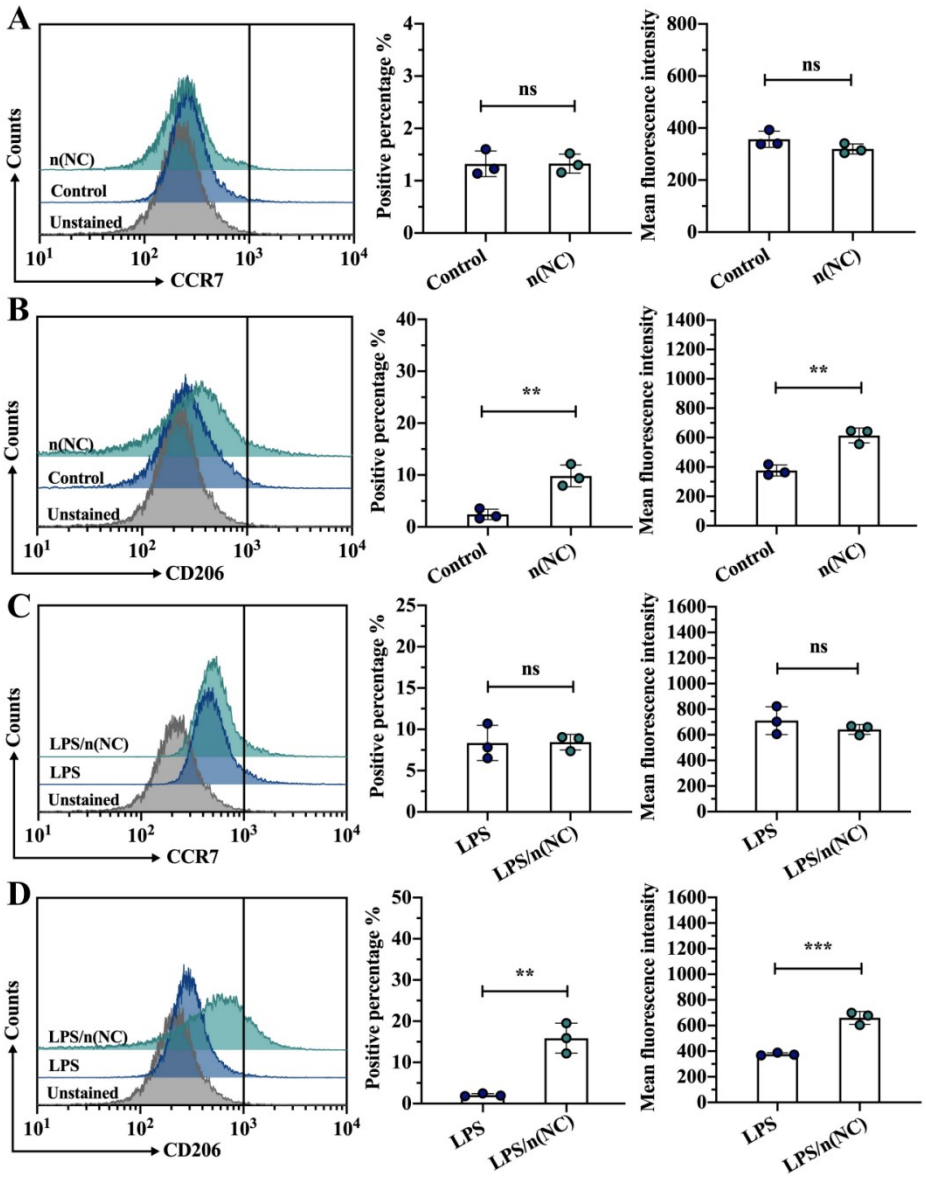

Figure S4. *In vitro* macrophages polarization treated with different treatment. A) The M1 macrophages marker CCR7 analyzed by flow cytometry under normal conditions. B) The M2 macrophages marker CD206 analyzed by flow cytometry under normal conditions. C) The M1 macrophages marker CCR7 analyzed by flow cytometry under inflammatory conditions. D) The M2 macrophages marker CD206 analyzed by flow cytometry under inflammatory conditions. **p<0.01; ***p<0.001; ns indicates that the groups are not significantly different from each other.

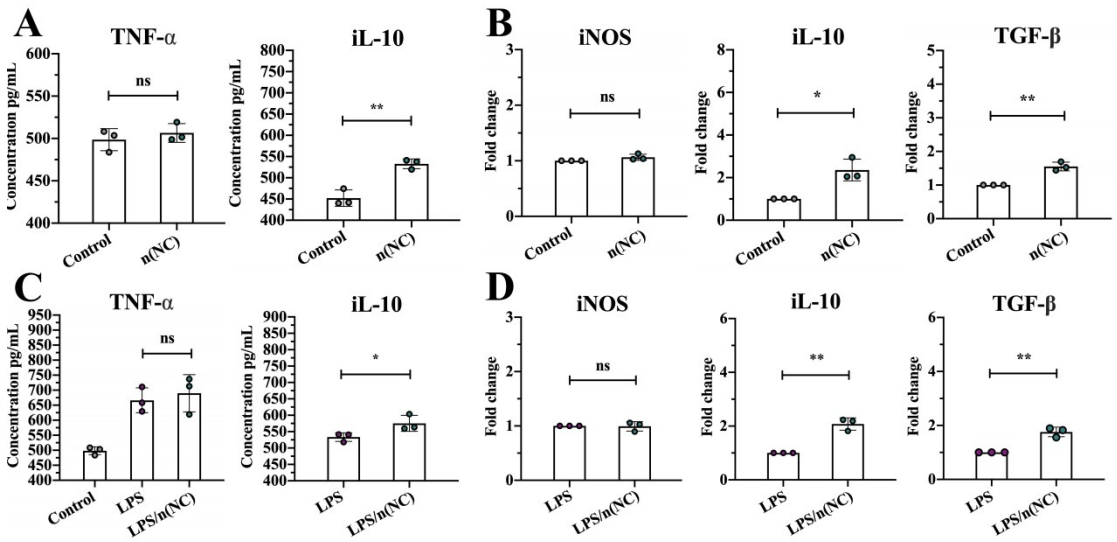

Figure S5. A) ELISA assay for TNF-α and iL-10 in the supernatant of RAW264.7 cells with different treatment under normal conditions. B) Relative gene expression of the M1-related iNOS and M2-related iL-10, TGF-β detected by RT-qPCR under normal conditions. C) ELISA assay for TNF-α and iL-10 in the supernatant of RAW264.7 cells with different treatment under inflammatory conditions. D) Relative gene expression of the M1-related iNOS and M2-related iL-10, TGF-β detected by RT-qPCR under inflammatory conditions. *$p<0.05$; **$p<0.01$; ns indicates that the groups are not significantly different from each other.

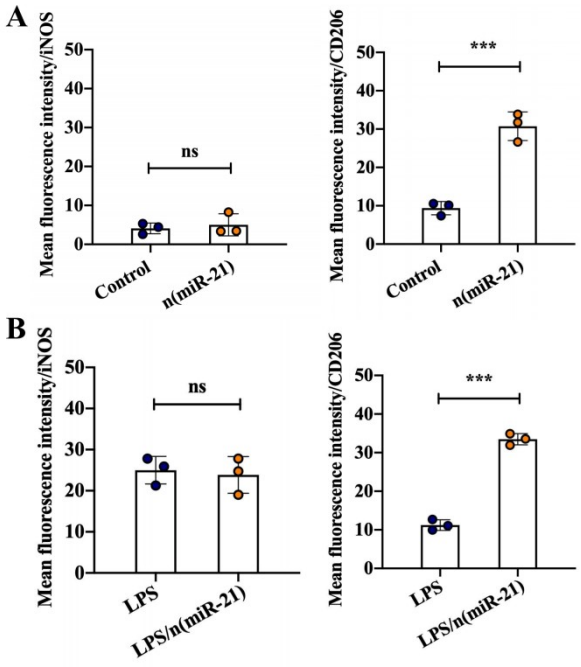

Figure S6. Semiquantitative analysis of immunofluorescence staining of RAW264.7 cells with different treatment under normal conditions (A) and under inflammatory conditions (B). ***p<0.001, ns indicates that the groups do not differ significantly from each other.

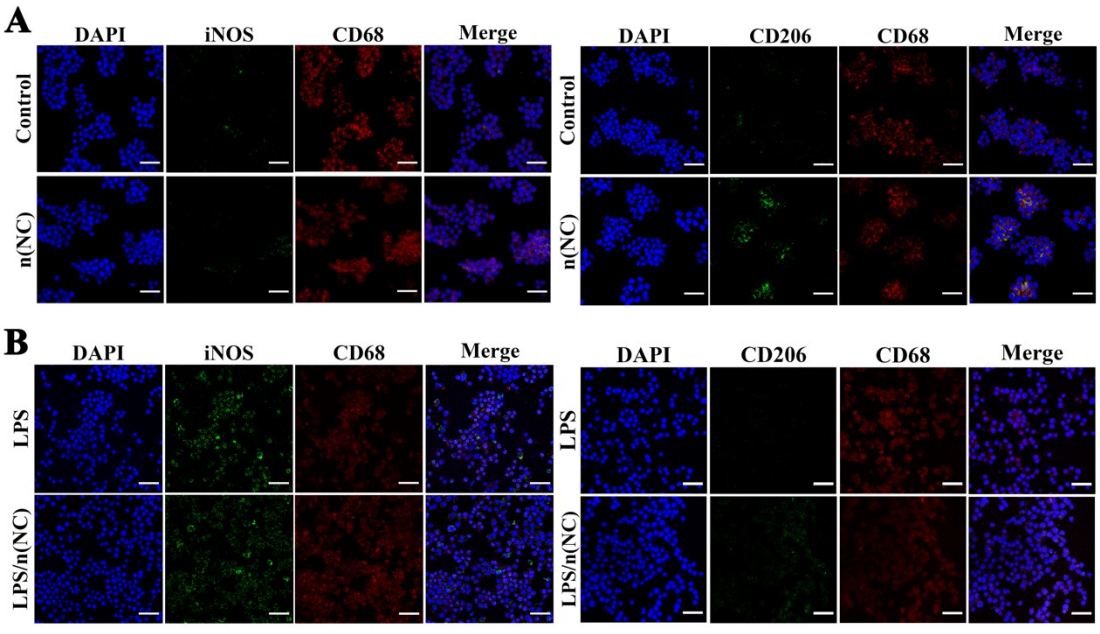

Figure S7. Immunofluorescence staining of RAW264.7 cells with different treatment

under normal conditions (A) and under inflammatory conditions (B). Cells were stained for iNOS/CD206 (green), CD68 (red), and nuclei (blue), respectively. The scale bars were 50 μm.

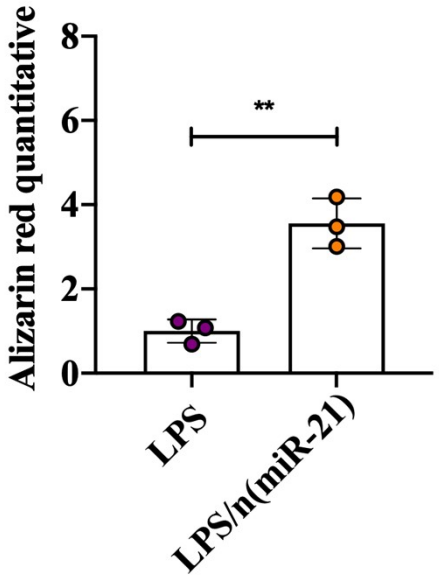

Figure S8. Semiquantitative analysis of alizarin red staining (**p<0.01).

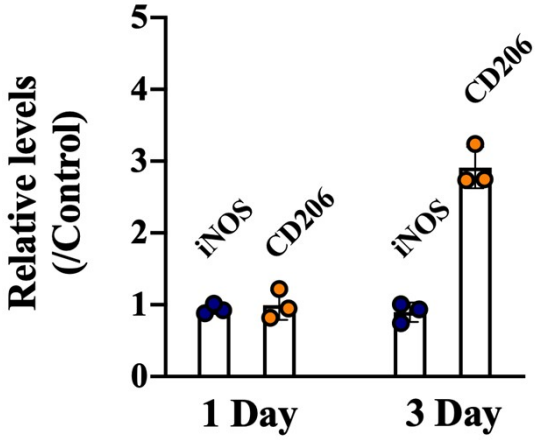

Figure S9. Semiquantitative analysis of immunofluorescence analysis of macrophage phenotype after n(miR-21) treatment *in vivo*.

Table S1. Primers used in the qRT-PCR.

Gene	Primer sequence
GAPDH	Forward 5'-ATCACTGCCACCCAGAAG-3' Reverse 5'-TCCACGACGGACACATTG-3'
iNOS	Forward 5'-CAGCTGGGCTGTACAAACCTT-3' Reverse 5'-CATTGGAAGTGAAGCGTTTCG-3'
iL-10	Forward 5'-ACTCTTCACCTGCTCCACTG-3' Reverse 5'-GCTATGCTGCCTGCTCTTAC-3'
TGF-β	Forward 5'- CTTCAGCCTCCACAGAGAAGAACT-3' Reverse 5'-TGTGTCCAGGCTCCAAATATAG-3'

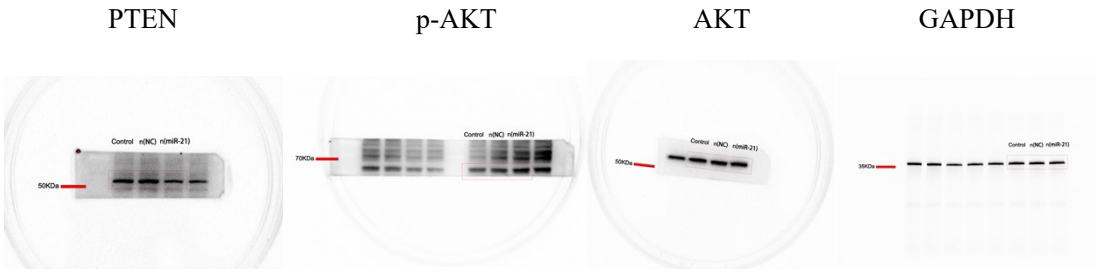

Figure S10. The raw data in the Western blot experiment.

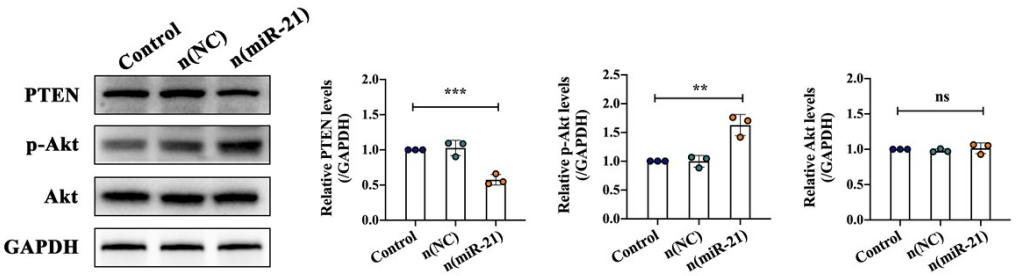

Figure S11. Western blot analysis of PTEN, p-Akt and Akt levels in RAW264.7 cells with different treatment. **p<0.01; ***p<0.001; ns indicates that the groups do not differ significantly from each other.