Exploring the Translational Potential of PLGA Nanoparticles for Intra-articular Rapamycin Delivery in Osteoarthritis Therapy

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Table S1. List of primer sequences.

Gene	Forward primer (5'to3')	Reverse primer (5'to3')
Col2a1	CTGGTGGAGCAGCAAGAGCAA	CAGTGGACAGTAGACGGAGGAAAG
Sox9	AGGAGAGCGAGGAAGATAAG	ACGTGTGGCTTGTTCTTG
Mmp13	CAGTTGACAGGCTCCGAGAA	CGTGTGCCAGAAGACCAGAA
β-actin	GGGACCTGACTGACTACCTC	TCATACTCCTGCTTGCTGAT

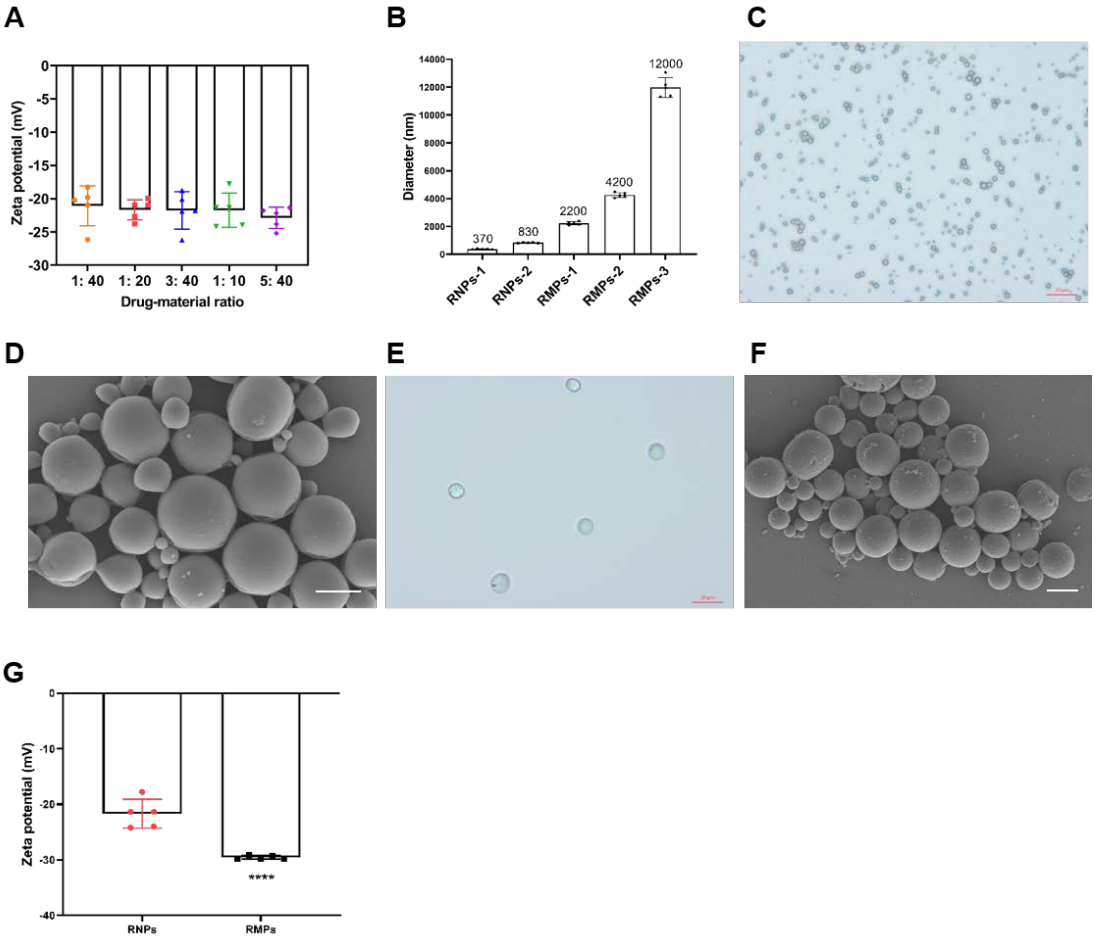

Figure S1. Preparation and characterization of RNPs and RMPs. (A) Zeta potential of RNPs with different drug-to-material ratios. (B) Average diameter of RNPs and RMPs (0.5% PVA concentration, drug-to-material ratio of 1:10). Average diameter of RNPs-1, RNPs-2, RMPs-1, RMPs-2 and RMPs-3 measured by dynamic light scattering. Average diameter of RMPs-4 obtained from Scanning Electron Micrograph measured by Image J (n = 3). (C) Upright light microscope image of RMPs-1, Scale Bar 20 μm. (D) Scanning Electron Micrograph of RMPs-1, Scale Bar 2 μm. (E) Upright light microscope image of RMPs-3, Scale Bar 20 μm. (F) Scanning Electron Micrograph of RMPs-3, Scale Bar 10 μm. (G) Zeta potential of RNPs and RMPs-1 (0.5% PVA concentration, drug-to-material ratio of 1:10).

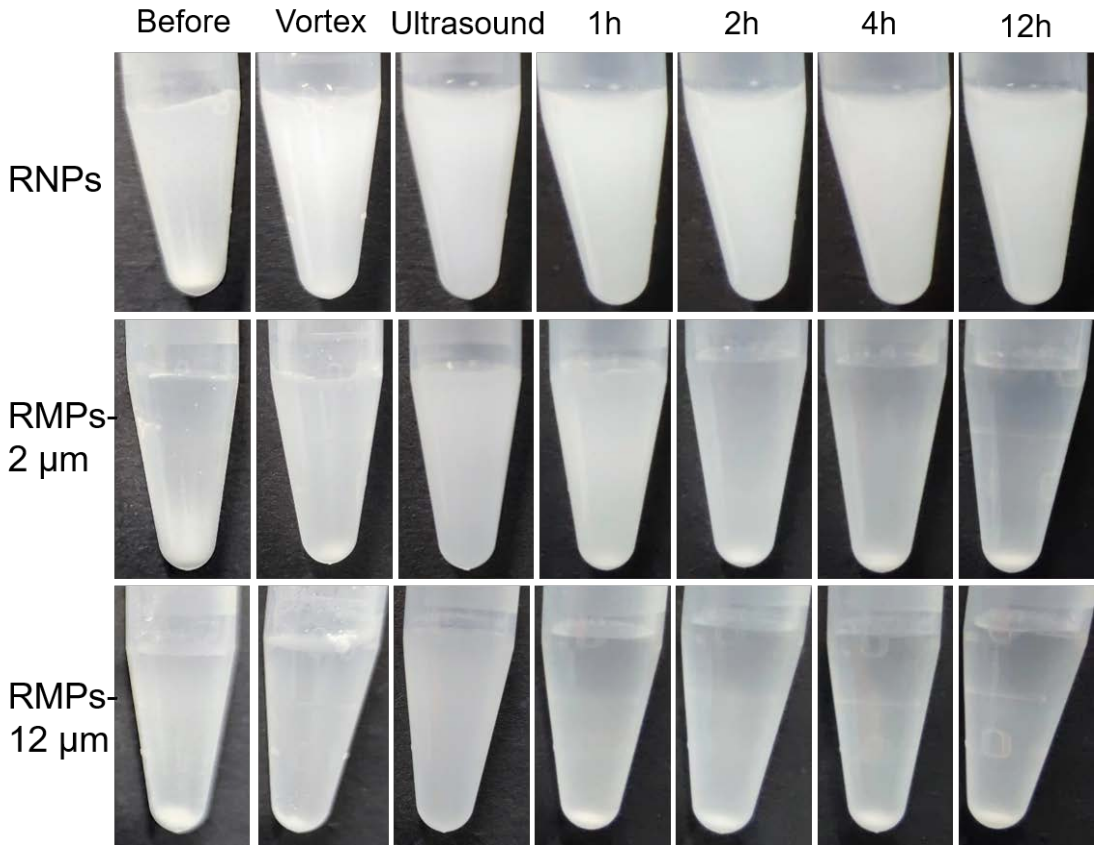

Figure S2. Stability of RNPs and RMPs suspended in physiological saline. Photographs in sequence showing the suspensions before vortex or ultrasound, after vortex, after ultrasound, 1, 2, 4, and 12 h after ultrasound, respectively.

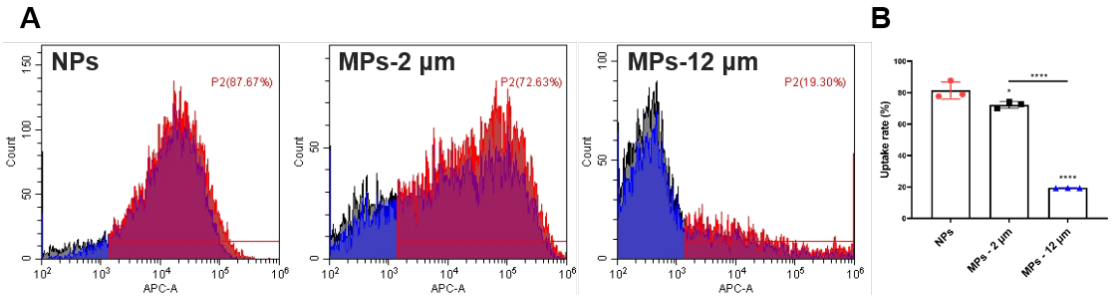

Figure S3. *In vitro* cellular uptake. Cellular uptake of RNPs and RMPs by flow cytometry in RAW 264.7 cells (n = 3). Statistical analysis was performed using one-way ANOVA with Tukey's *post hoc* analysis. Data are presented as means ± SD. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001.

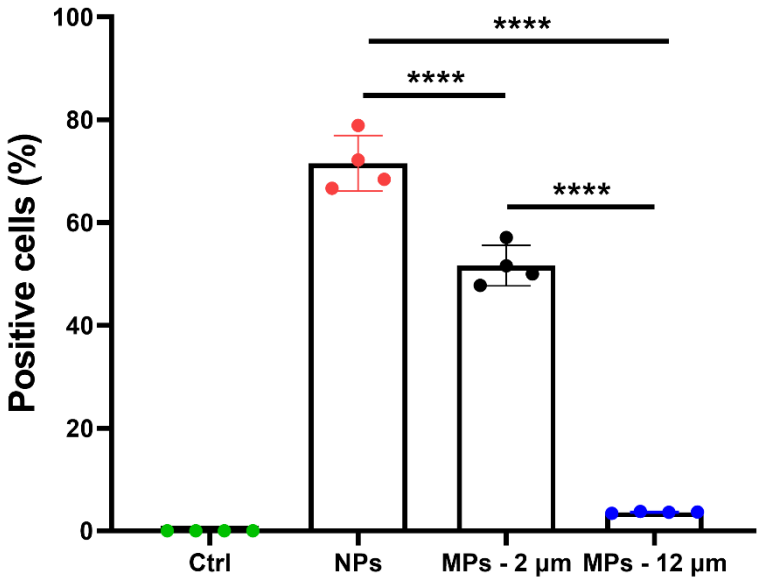

Figure S4. Quantification of RNPs and RMPs visualized by CLSM in mouse primary articular chondrocytes, data are presented as means ± SD. ****$P < 0.0001$, (n = 4).

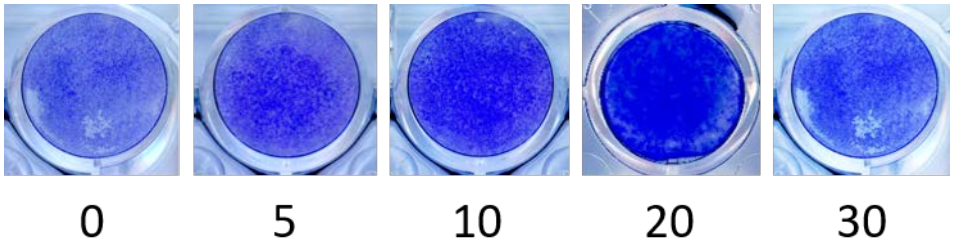

Figure S5. Toluidine blue staining of ATDC5 cells cultured in chondrogenic medium for 7 days treated with 0, 5, 10, 20 and 30 nM rapamycin, respectively.

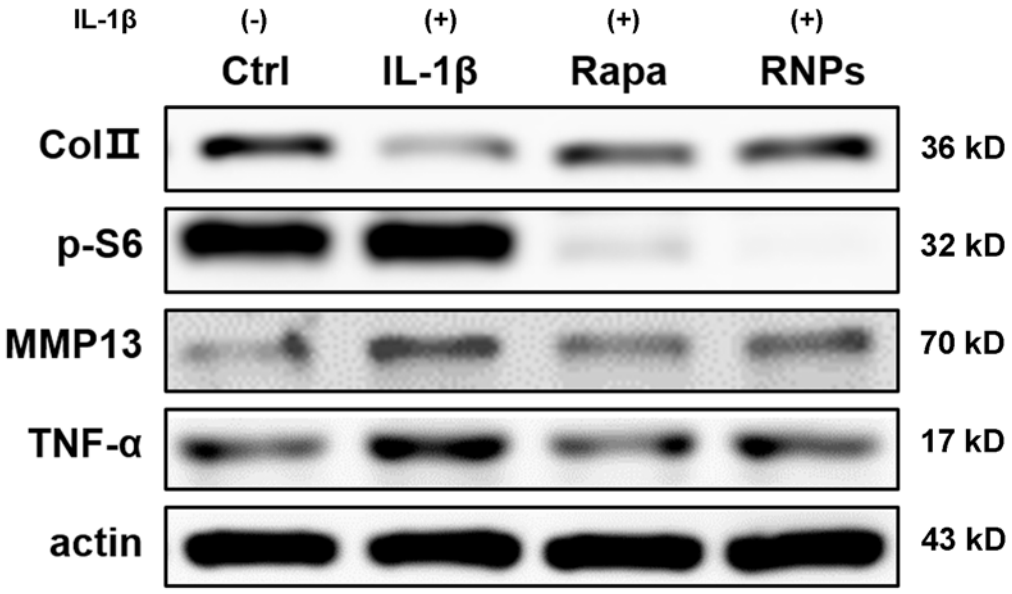

Figure S6. The expression of proteins (Col II, p-S6, MMP13 and TNF-α) in SW1353, which were induced with IL-1β for 24 h.

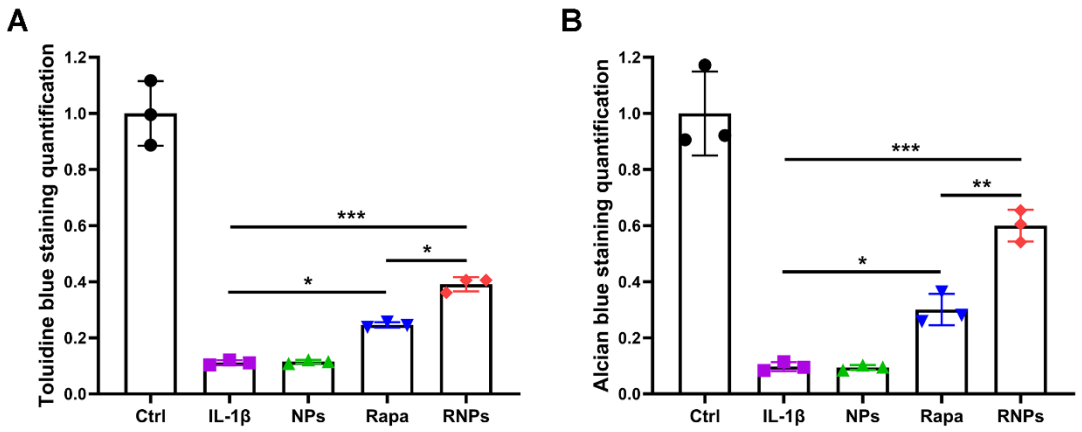

Figure S7. Quantification of Toluidine blue and Alcian blue staining, data are presented as means ± SD. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, (n = 3).

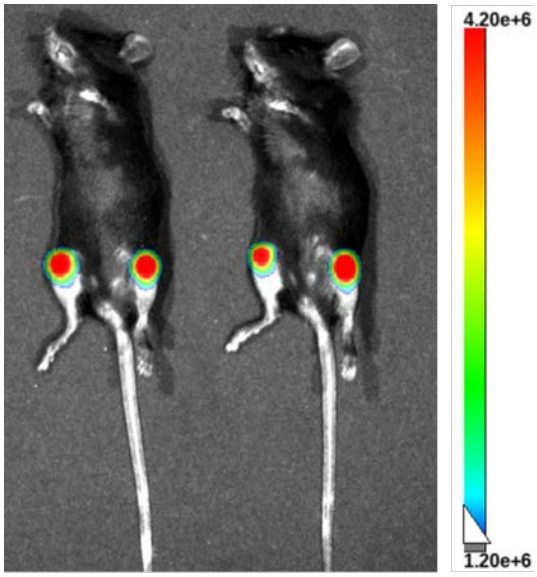

Figure S8. Distribution of fluorescence signals after 24 h of injecting PLGA nanoparticles.

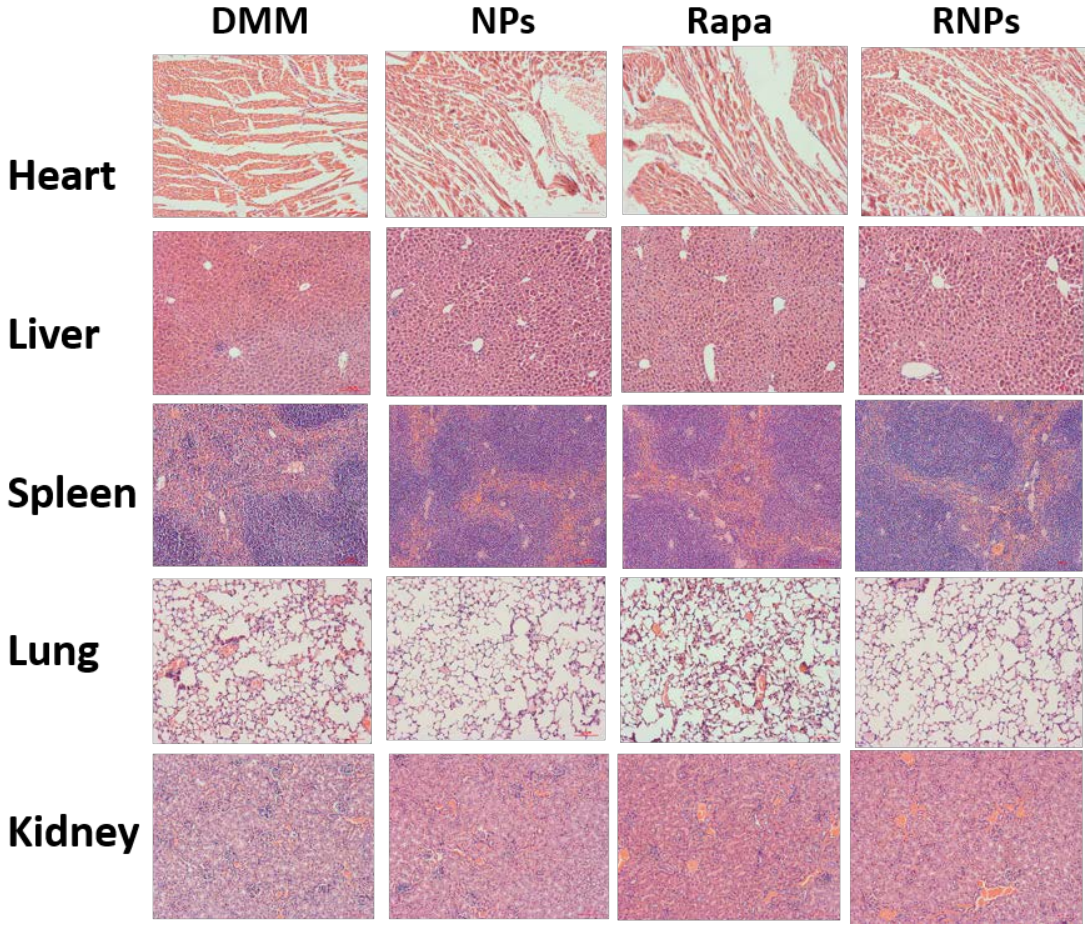

Figure S9. Hematoxylin and eosin staining of heart, liver, spleen, lung and kidney harvested from mice at week 8 post surgery.