Supplementary Materials:

EXPERIMENTAL SECTION.

Nanoparticle and peptide synthesis and characterization were performed as previously reported with no modifications.[16]

Materials. N-Isopropylacrylamide (≥ 98%, NIPAm) was acquired from Polysciences Inc. (Warrington, PA, USA). Dialysis membrane tubing was purchased from Spectrum Laboratories (Dominguez, CA). (1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (≥ 98%, HBTU), N-hydroxybenzotriazole (> 97%, HOBT), N,N'-methylenebisacrylamide (99%, MBA), sodium dodecyl sulfate (SDS; 10% w/v in water), 2- acrylamido-2-methyl-1-propanesulfonic acid (99%, AMPSA), dithiothreitol (98%, DTT), fluorescein o-acrylate (98%, F), N,N-Diisopropylethylamine (99%, DIPEA), potassium persulfate (99%, $K_2S_2O_8$), N,N'-bis(acryloyl)cystamine (98%, BAC), and dimethyl sulfoxide (DMSO) were acquired from Sigma-Aldrich (St. Louis, MO, USA). NIPAm, MBA, and AMPSA were stored under nitrogen at 4 °C. All water used in synthesis, dialysis, and testing was treated by a Milli-Q system (Millipore, Billerica, MA, USA; 18.2 MΩ•cm resistivity). Acrylate-PEG2000 was purchased from Laysan Bio (AL).

Peptide Synthesis and Purification. Therapeutic peptide KAFAK was synthesized on knorr resin by standard 9-fluorenylmethyloxycarbonyl (Fmoc) solid phase methodology[16]. Knorr amine resin was swollen with dichloromethane (DCM) followed by dimethylformamide (DMF). A solution of 25% piperidine in DMF was added to the resin, bubbling under nitrogen for 15 min, and then washed with DMF (3x) and isopropyl alcohol (i-PrOH, 3x). For amino acid

coupling, a solution of Fmoc-amino acid (3 equiv), (1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU, 3 equiv), N-hydroxybenzotriazole (HOBT, 3 equiv) and N,N-Diisopropylethylamine (DIPEA, 5 equiv) in DMF was added to the resin bubbling under nitrogen for 2 h, and then washed with DMF (3x), DCM (3x) and i-PrOH (3x). To deprotect Fmoc, 25% piperidine in DMF was added to the resin, and nitrogen was bubbled for 20 min. The solvent was then removed and the resin washed with DMF (3x) and i-PrOH (3x). The above sequence was repeated for coupling and deprotection of each amino acid. The final compound was cleaved from the resin with a cocktail of trifluoroacetic acid (Sigma-Aldrich, St. Louis, MO, U.S.A.), triisopropyl silane (TCI America, Boston, MA, U.S.A.), ethane dithiol (Alfa Asara, Ward Hill, Massachusetts, U.S.A.), and Milli-Q water. The cleaved mixture was concentrated under vacuum and then immediately precipitated in diethyl ether, recovered by centrifugation, solubilized in Milli-Q water, and lyophilized (Supporting Scheme 1). Peptides were purified on an FPLC AKTA Explorer (GE Healthcare, Pittsburgh, PA, U.S.A.) with a 22/250 C18 prep-scale column (Grace Davidson, Deerfield, Illinois, U.S.A.) and an acetonitrile gradient with 0.1% trifluoroacetic acid. Purity of peptides were determined by HPLC to be > 98%. Peptide molecular weight was confirmed by matrix-assisted laser desorption ionization time-of-flight (MALDI TOF) mass spectrometry with a 4800 Plus MALDI TOF/TOF Analyzer (Applied Biosystems, Foster City, CA, U.S.A)(Supplemental Figure S1).

Nanoparticle Synthesis. NIPAM-AMPS nanoparticles were synthesized similar to previous protocols. Briefly, the nanoparticle compositions were formed by dissolving 192.1 mg NIPAM and 5 mol% AMPS in 18 mL degassed MilliQ water in a three-neck round bottom flask. 3 mol% PEG-acrylate and 2 mol% cross-linker (BAC or MBA) was pre-dissolved in 3% (v/v) of

dimethyl sulfoxide (DMSO) in water for 10 mins before addition to polymer mixture. 41 μL of a 10% SDS in MilliQ water solution was added, and the mixture was heated to 65°C under nitrogen. Potassium persulfate (8.4mg) was dissolved in 2ml degassed MilliQ water and added after a 35 mins equilibration to initiate polymerization. After 5 h, the reaction was removed from heat and allowed to cool to room temperature. Particles were dialyzed against MilliQ water for 5 days using a 15,000 MWCO membrane.

For fluorescent nanoparticle synthesis, 1 mol% fluorescein o-acrylate (F) was pre-dissolved in 3% DMSO solution. The dye-DMSO solution was added to the polymer mixture and equilibrated for 30 mins. Potassium persulfate dissolved in 2 mL degassed MilliQ water was added to initiate polymerization. Particles were dialyzed against MilliQ water for 5 days using a 15,000 MWCO membrane. All sample were freeze-dried and kept in the dark for further experiments.

Nanoparticle Characterization. Size measurements were performed using dynamic light scattering (DLS) on a Nano-ZS90 Zetasizer (Malvern, Westborough, MA, USA). Zeta (ζ) potentials were measured at 25 °C using a Nano-ZS90 Zetasizer in capillary cells in PBS. To characterize their degradation, samples with an identical particle concentration were prepared in pH 7.4 PBS buffer. To one solution, an excess of DTT (1 mM) was added and allowed to incubate for 24 h. Transmission electron microscopy (TEM) was conducted at the Purdue University Life Science Microscope facility on a FEI/Philips CM-100 transmission electron microscope at 100 kV using a uranyl acetate stain (UA) at pH 4.5. Discharged TEM sample grids were placed onto the top of a droplet of sample for 2 min. Then UA stain was added, and samples were briefly dried before imaging at room temperature.

Nanoparticle Drug Loading and Release. Purified peptide was first dissolved in PBS to create a 5 mg/mL loading solution, and then this solution was added to 5 mg of lyophilized nanoparticles and allowed to incubate for 24 h at 4°C in the swollen state. After incubation, 9 mL of PBS was added and particles underwent 1 h of centrifugation at 35,000 rpm and 37°C in an Optima L-90k Ultracentrifuge (Beckman Coulter, Indianapolis, IN, USA). The nanoparticle pellet was briefly re-suspended in 2 mL PBS and lyophilized. To collect peptide release profiles, KAFAK-loaded nanoparticles were suspended in sterile PBS (Invitrogen, Grand Island, NY, USA) with or without DTT over a 96 h period. Measurement of free peptide released into the solution and the amount of peptide loaded were determined using fluorescence analysis with a fluoraldehyde ophthalaldehyde (OPA) solution (Thermo Scientific, Waltham MA, USA) according to the manufacturer's protocol. For KAFAK release studies, fluorescent measurements of drug release were taken every 15 min for the first hour and then at 1, 6, 12, and 24 h every day afterward for 4 days.

Chondrocyte Isolation and Culture. Primary chondrocytes were harvested from the load-bearing region of femoral condyles from 3-month-old calves within 24 h of slaughter (Dutch Valley Veal, South Holland, IL, USA) as described previously[15,24,25]. Cells between passage 1 and 4 were seeded with 10,000 cells/well in 8-well Lab-Tek chamber slides for experiments.

Nanoparticle Uptake. To determine intracellular nanoparticle uptake, chondrocytes were incubated with 1.5 mg/mL fluorescein-labeled nanoparticles for 4 h or 24 h at 37°C in culture medium. All samples were performed in triplicate. After washing with medium to remove any unbound nanoparticles, the cells were imaged using confocal microscopy (Olympus FV1000).

To analyze co-localization of nanoparticles in endosomal/lysosomal compartments, Lysotracker DND-99 (Life Technologies, Grand Island, NY, USA) staining was added according to the manufacturer's protocol. Semi-quantitative fluorescence measurements of confocal images were used to compare uptake between degradable and non-degradable nanoparticles. Image analysis by ImageJ determined the number of fluorescent pixels (area) and the integrated fluorescence intensity for the green channel normalized to the red channel within each field of view (n = 3). Overlapping green and red fluorescence indicated co-localization of green nanoparticles inside red endolysosomes.

Cell samples were subjected to the trypan blue dye exclusion to evaluate cell viability, and CellTiter 96 AQueous One Proliferation Assays (Promega, Madison, WI, USA) were performed on chondrocytes according to the manufacturer's protocol to quantify nanoparticle cytotoxicity.

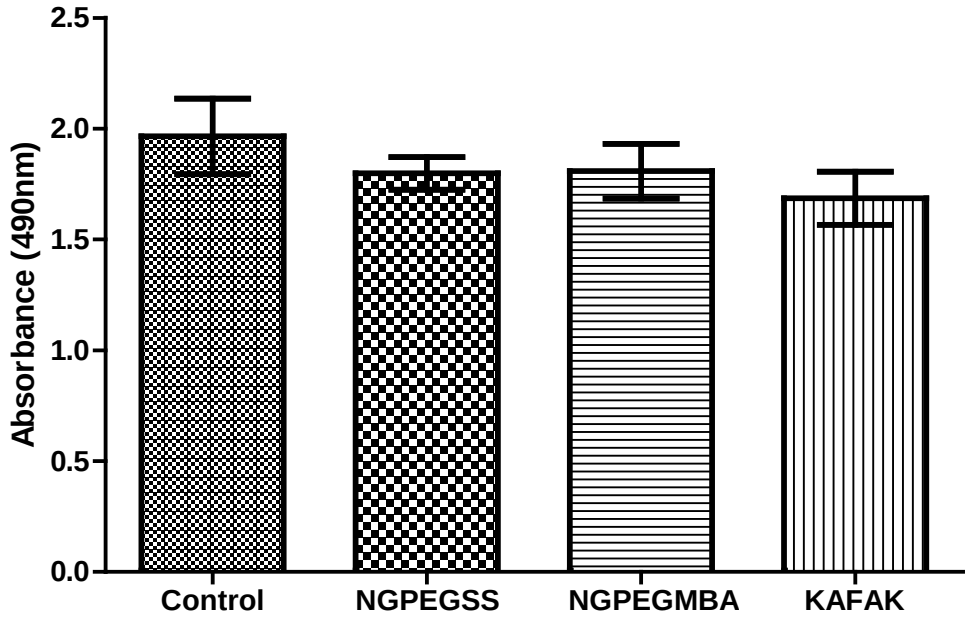
***Ex vivo* osteoarthritis model.** Cartilage plugs (3mm diameter isolated as described above) were washed three times in serum-free medium and equilibrated for 3 days in 5% FBS supplemented media. Osteoarthritis conditions were simulated by removal of native aggrecan and then inducing inflammation using a previously described protocol[25]. After culturing for 2 days, nanoparticle treatments were added. Fresh IL-1β and nanoparticles were added every 2 days for an 8-day culture period. Media aliquots were collected and stored in low bind tubes at -80°C until further analysis.

Analysis of *ex vivo* IL-6 Secretion. IL-6 cytokine production from cartilage plugs was determined with a bovine IL-6 ELISA development kit (PIESS0029, Thermo Scientific, Rockford, IL, USA) according to the manufacturer's protocol. IL-6 production was normalized

to individual plug weight and to the negative control where healthy plugs with intact aggrecan were IL-1β stimulated.

Cartilage Penetration by Nanoparticles. Bovine cartilage plugs were either trypsin treated to simulate osteoarthritis-like conditions or left in cell culture media to maintain healthy tissue-like environment. Non-fluorescent nanoparticles (as control) and fluorescent nanoparticles were re-suspended at 0.5 mg/mL in PBS and added to cartilage plugs for 24 h. After treatment, plugs were washed 3 times with PBS and incubated at 37°C for 30 mins to remove any unbound nanoparticles. A mid-sagittal cut (30 μm thickness) was made through the plug using a cryotome. Sections were imaged by confocal microscopy at 488 nm excitation (Olympus FV1000) to examine the depth of fluorescent nanoparticle penetration into the plugs from the articular surface. Fluorescent intensity was quantified using ImageJ, and the intensity in three independent areas near the articular surface of each image was measured with values represented as average intensity ± SEM.

Statistical Analysis. Student's t-tests were used to determine statistical significance between treatment groups ($\alpha = 0.05$). Data is expressed as mean values ± standard deviation unless otherwise noted.



Supplementary Figure 1. CellTiter absorbance of untreated chondrocytes treated with NGPEGSS, NGPEGMBA, and KAFAK over a 48 h period at 37 °C