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Supplementary Materials for

Targeting cartilage EGFR pathway for osteoarthritis treatment

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The PDF file includes:

Table S1. Mouse real-time PCR primer sequences.
References (*55*–*59*)

Other Supplementary Material for this manuscript includes the following:

(available at stm.sciencemag.org/cgi/content/full/13/576/eabb3946/DC1)

Data file S1. Individual subject-level data (Excel file).
Data file S2. Western blotting films (PDF).

Supplementary Materials:

Materials and Methods

TGFα-NP synthesis

Synthesis of azido-terminated Poly(ε-caprolactone) (PCL-N_3)

All chemicals were purchased from either Fisher Scientific or Sigma-Aldrich unless otherwise specified. PCL-OH (1.6 g, 0.40 mmol, Mw 4000) was dissolved in anhydrous chloroform (15 ml) followed by addition of triethanolamine (TEA, 202 mg, 2 mmol, Thermo Fisher Scientific). The mixture was then added to a solution of MsCl (229 mg, 2 mmol, Sigma Aldrich) in chloroform (3 ml) at 0 °C under N_2 stream. The reaction was carried out overnight, under stirring at room temperature. After the reaction, the polymer was recovered as a white solid by precipitating into ethyl ether (Sigma Aldrich) and vacuum-drying. This mesylated copolymer (1.08 g, 0.27 mmol) was dissolved in dimethylformamide (DMF, 12 ml, Sigma Aldrich), and reacted with sodium azide (800 mg, 12.30 mmol, Sigma Aldrich) at 45 °C under stirring for 3 days. After the reaction, DMF was evaporated and the concentrate was diluted with chloroform (40 ml), and then washed five times with water and brine (Sigma Aldrich). The organic layer was dried over $MgSO_4$ (Sigma-Aldrich), filtered, concentrated, and then precipitated into ethyl ether (0.97 g, 90%).

Synthesis of copolymers poly(ε-caprolactone)-block-poly(L-lysine) (PCL4K-b-PLL3.3K)

The PCL-b-PLL was synthesized by click reaction between poly(ε-caprolactone)-azide (PCL-N_3, Mw: 4000) and propargyl-poly(L-lysine) (propargyl-PLL, Mw: 3300). Briefly, PCL-N_3 (60 mg, 0.015 mmol), propargyl-PLL (55 mg, 0.0167 mmol), CuSO4 (0.375 mg, 0.0015 mmol, Sigma Aldrich), sodium ascorbate (0.594 mg, 0.003 mmol, Sigma Aldrich) and 10 ml degassed DMF were added into a 30 ml Schlenk flask under a nitrogen atmosphere. The flask was sealed and

placed into an oil bath. The reaction was carried out at 45 °C with magnetic stirring for 3 days, and the mixture was dialyzed against water to remove the residual propargyl-PLL. The resulting copolymer was lyophilized to get the powder.

Synthesis of TGFα-GGG and TGFα-DBCO

The human TGFα gene sequence (50 amino acids) was ordered from Integrated DNA Technologies (IDT) and cloned into the Sortase-Tag Expressed Protein Ligation (STEPL) system (*28*). Briefly, TGFα was fused in series with the sortase A (Srt A) substrate sequence (LPXTG), Srt A enzyme, and a His12-tag. The sequence-confirmed plasmid construct was heat-shot transformed into *E. coli* T7 express competent cells (New England BioLabs). On the next day, colonies were cultured in autoinduction medium (Formedium) with 100 ug/ml Ampicillin (Corning) and were shaken at 150 rpm at 25 °C for 2 days. Afterwards, the cultures were pelleted by centrifugation at 5000×g for 15 mins and the cells were lysed with 1% (g/v) octylthioglucoside (OTG, GoldBio) in PBS, with protease inhibitor. The lysate was centrifuged again at maximum speed for 20 minutes and then loaded into a cobalt resin (Thermo Fisher Scientific) for capturing the TGFα fusion protein. After washing with 10mM Imidazole and 1×PBS buffer, the resin was incubated with 1×PBS+50 μM $CaCl_2$+2 mM Gly-Gly-Gly (GGG, Santa Cruz Biotechnology) at 37 °C for 1 hour or 1×PBS+50 μM $CaCl_2$+200 μM Gly-Gly-Gly-Ser-Cys-DBCO (GGGSC-DBCO, LifeTein) at 37 °C for 4 hours. The excess GGG or GGG-DBCO was removed by spin filter (Amicon Ultra-4, 3000 MWCO). The TGFα concentration was quantified by bicinchoninic acid (BCA, Thermo Fisher Scientific) assay according to the manufacturer's instructions.

Synthesis of fluorescently labeled TGFα

We synthesized rhodamine-TGFα for penetration assay. Rhodamine-TGFα was prepared using a molar ratio of 1/10 of 5-(and 6)-carboxytetramethylrhodamine, succinimidyl ester (NHS-rhodamine, Thermo Fisher Scientific)/TGFα-DBCO. Specifically, 1.5 ml 60 μM TGFα-DBCO (in 0.1 M PBS) was mixed with 18 μl of 50 μM NHS-Rhodamine (in DMF). After shaking at room temperature for 2h, unconjugated NHS-Rhodamine was removed by centrifugal filter devices (Amicon Ultra-4, 3000 MWCO, Millipore Corp.). IRDye 800CW-labeled TGFα was prepared for retention assay. It was synthesized utilizing a molar ratio of 1/10 of IRDye 800CW NHS Ester (LI-COR, Inc)/TGFα-DBCO. Specifically, 200 μl of 85 μM TGFα-DBCO (in 0.1 M PBS) was mixed with 17 μl of 10 mM IRDye 800CW NHS Ester (in DMSO). After shaking at room temperature for 2h, unconjugated IRDye 800CW NHS Ester was removed by centrifugal filter devices (Amicon Ultra-4, 3000 MWCO, Millipore Corp.).

Synthesis of TGFα-conjugated nanoparticles

TGFα-conjugated nanoparticles (TGFα-NPs) were prepared via click reaction (*27*). Briefly, stock solutions of poly(ethylene glycol) (4000)-polycaprolactone (3000) copolymer (denoted PEG-PCL), polylysine (3300)-polycaprolactone (4000) copolymer (denoted PLL-PCL) and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[azido(polyethylene glycol)-5000] (ammonium salt) (denoted DSPE-PEG5K-N_3) in chloroform were mixed in the following molar ratios: PEG-PCL/PLL-PCL/DSPE-PEG5K-N_3 (55/20/25). The total amount of PEG-PCL for each of the nanoparticle compositions was 1 mg. For non PLL-PCL doped nanoparticles, 75 mol% PEG-PCL/25 mol% DSPE-PEG5K-N_3 was used. The chloroform was removed using a direct stream of nitrogen prior to vacuum desiccation for overnight. Nanoparticles were formed by adding an aqueous solution (0.1 M PBS, pH 7.4) to the dried film and incubating in a 60 °C water bath for

3 minutes and then sonicating for another 3 minutes at the same temperature. Samples were filtered through a 0.22 μm cellulose acetate membrane filter (Thermo Fisher Scientific) and stored in the dark at 4 °C.

To prepare TGFα-NPs, azide-modified nanoparticles were mixed with TGFα-DBCO at a molar ratio of 1 to 1 in 0.1 M PBS (pH 7.4). Reactions were mixed overnight at room temperature and then purified by centrifugal filter devices (Amicon Ultra-4, 50K MWCO, Millipore Corp.). Similar methods were used to prepare Rhodamine or IRDye 800CW-labeled TGFα-NPs. The diameter and size distribution of the nanoparticles were measured with dynamic light scattering (DLS, Malvern, Zetasizer, Nano-ZS). Zeta potential was also determined by Zetasizer Nano analyzer (Malvern). The morphology of the nanoparticles was characterized by transmission electron microscope (TEM, JOEL 1010) using a negative-staining technique (i.e. phosphotungstic acid). Fluorescence spectra measurements were made on a SPEX FluoroMax-3 spectrofluorometer (Horiba Jobin Yvon).

TGFα-NP characterization

Stability study

For stability assay, TGFα-NPs were stored in 0.1×PBS at 4 °C. Measurement of nanoparticle structural integrity was acquired by monitoring the hydrodynamic diameter over the course of one week by dynamic light scattering (DLS). In addition, the in vitro stability of TGFα-NPs was also measured by DLS in 50% bovine synovial fluid (Vendors, Lampire biological laboratories) at 37 °C for 24 hours. TGFα-NP stability was tested in triplicate.

Cell viability study

For cell viability assay, we used mouse primary chondrocytes isolated from the distal femoral and proximal tibial epiphysis of mice (3-6 days old) via enzymatic digestion as described previously (*43*). Cells (5000/well) were seeded in 96-well plates and incubated overnight. The diluted TGFα-NPs were added to wells at five different concentrations ranging from 10 μM to 0.3125 μM (10, 5, 2.5, 1.25, 0.625, 0.3125 μM). After 24 h incubation, the cells grew in 100 μl of fresh DMEM/F12 medium with 10 μl of MTT assay (Thermo Fisher Scientific) stock solution added to each well and incubated for 4 h. The formazan was dissolved by adding 100 μl of detergent to each well and then incubated for another 4 h. Finally, the absorbance of formazan product was measured on a Tecan microplate reader (BioTek Instruments, Inc.) at 570 nm. Cell viability was calculated using the following equation:

$$\text{Cell viability (\%)} = \frac{\text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{control}}} \times 100$$

TGFα-NP activity study

Mouse primary chondrocytes were seed in 6-well plates and reached to 80% confluency. Cells were then incubated in fresh medium containing TGFα (0 or 15 ng/ml TGFα content), Ctrl-NPs (i.e. NP with no TGFα conjugation), TGFα-NPs (15 or 100 ng/ml TGFα content) for 15 minutes at 37 °C. The cells were washed twice with PBS and then lysed with lysis buffer for Western blot analysis. Primary chondrocytes seeded in 6-well plate were also incubated in fresh medium with or without TGFα-NPs (15 ng/ml TGFα content) for 48 hours. Cell morphology was observed under bright field of inverted microscope.

Cell binding study

Mouse primary chondrocytes plated in 24-well plates were washed once with PBS and then incubated in fresh medium containing TGFα-NPs (10 nM TGFα content) for 2 hours. For competitive inhibition experiments, cells were treated with the same amount of TGFα-NPs but in the presence of 100 μg/ml free TGFα in the media. Prior to acquisition of fluorescence images, cells were washed with PBS two times, and then fixed and mounted with DAPI Fluoromount-G Mounting Medium (Southern Biotech). Confocal images were taken using confocal microscope (Zeiss LSM 710).

Cartilage explant penetration and uptake study

For bovine cartilage explant penetration assay, we obtained young (1-2 weeks old) bovine knee joints from Lampire biological laboratories, harvested cartilage explants from the trochlear groove using biopsy punch (6 mm in diameter and 2 mm in thickness), cultured them in chemically defined medium (DMEM, 100 μg/ml streptomycin and 100 U/ml penicillin, 1% ITS+Premix, 40 μg/ml L-proline, 0.1 μM dexamethasone, 100 μg/ml sodium pyruvate and 50 μg/ml ascorbate 2-phosphate) in 48-well plate. The cartilage explants were then incubated with rhodamine labeled-TGFα-DBCO (~10 μM rhodamine and 5 μM TGFα), TGFα-NPs (without PLL-PCL, ~10 μM rhodamine and 5 μM TGFα) or TGFα-NPs (with PLL-PCL, ~10 μM rhodamine and 5 μM TGFα) in 500 μl of culture medium for 48, 96 or 144 hours at 37 °C under gentle agitation with medium replacement every other day. After incubation, cartilage explants were washed three times with PBS, fixed with 4% Paraformaldehyde (Sigma Aldrich), dehydrated with 20% sucrose (Sigma Aldrich)+2% Polyvinylpyrrolidone (PVP, Sigma Aldrich) followed by embedding with 20% sucrose+2% PVP+8% gelatin (Sigma Aldrich). Sections were mounted with DAPI Fluoromount-G Mounting Medium on glass slides and immediately

observed under confocal microscope (Zeiss LSM 710). All images are taken under the same laser power, intensity and offset. Quantitative analysis was performed on maximum intensity projections of Z-stack images taken from 100 μm thick sections. The diffusivity (*D*) of nanoparticle was calculated by using non-linear regression in MATLAB to fit to the classical error function solution of Fick's second law

$$\frac{\partial c(x,t)}{\partial t} = D \cdot \frac{\partial^2 c}{\partial x^2}$$

where fluorescence values were substituted for concentration values as described previously (*55*). The normalized axial fluorescence intensity in cartilage sections taken from the center of the cartilage samples was fitted to a 1-dimensional model of diffusion (*17*).

For bovine cartilage explant uptake assay, 300 μl of IRDye 800CW-labeled TGFα-DBCO, TGFα-NPs (without PLL-PCL, ~10 μM IRDye 800CW and 5 μM TGFα) or TGFα-NPs (with PLL-PCL, ~10 μM IRDye 800CW and 5 μM TGFα) was added to bovine cartilage explants. The explants were incubated for 48 hours at 37 ℃ and 5% CO_2 under gentle agitation. The explants were then removed from the medium, washed tree times with PBS, imaged by in vivo imaging system (IVIS, Spectrum, PerkinElmer). All images are taken under the same laser power, intensity and offset. Radiant efficiency within a fixed anatomical region of interest (ROI) was measured using Living Image software.

Intra-articular retention and systemic biodistribution study

For in vivo retention assay, we injected 10 μl IRDye 800CW-labeled TGFα-DBCO (~20 μM IRDye 800CW and 10 μM TGFα) or TGFα-NPs (with PLL-PCL, ~20 μM IRDye 800CW and 10 μM TGFα) in healthy and OA (8 weeks post DMM surgery) mouse knees (3 months old). IVIS was used to serially acquire fluorescence images within each joint over a period of 4 weeks. All

images are taken under the same laser power, intensity and offset. Using Living Image software, radiant efficiency within a fixed anatomical region of interest (ROI) was measured.

For in vivo biodistribution assay, we injected 10 μl of PBS or IRDye 800CW-labeld TGFα-NPs (~20 μM IRDye 800CW and 10 μM TGFα) in *WT* mouse knees (3 months old). At 24 hours or 1 month after injection, the mice were euthanized and the knee joints, blood, and major organs (heart, liver, spleen, lung, kidney) were harvested. Knees were dissected to isolate the major joint components, including the surrounding tissues (quadriceps, patella, patellar ligament, synovium, fat pad), femoral condyles, tibial plateau and meniscus. All the major joint components, blood and organs were imaged using the IVIS under the same laser power, intensity and offset. The data was analyzed as described above.

Micro-computed tomography (microCT) analysis

After euthanasia, mouse knee joints were harvested, fixed in 4% paraformaldehyde for 2 days, rinsed with running water, and stored in 1×PBS. Bones were then scanned at a 6-μm isotropic voxel size with a microCT 35 scanner (Scanco Medical AG, Brüttisellen, Switzerland). All images were smoothened by a Gaussian filter (sigma = 1.2, support = 2.0).

Bone structure was calculated as we previously described (*9*). Briefly, sagittal images from distal femurs were contoured for subchondral bone plate (SBP) followed by generating a 3D color map of thickness for the entire SBP. This map was converted to a grayscale thickness map, whose histogram was then used for the quantification of the average SBP thickness at any defined area. For analysis of trabecular bone, sagittal images from distal femurs were thresholded, corresponding to 472.1 mg HA/cm^3, and contoured for subchondral trabecular bone between SBP and growth plate and metaphyseal trabecular bone at 0.6-1.8 mm next to growth plate. The bone parameters of bone mineral density (BMD), bone volume fraction/total volume

(BV/TV), trabecular thickness (Tb.Th), trabecular separation (Th.Sp), trabecular number (Tb.N), and structure model index (SMI) were calculated by 3-D standard microstructural analysis (22).

Histology

After euthanasia, mouse knee joints were harvested and fixed in 4% paraformaldehyde overnight followed by decalcification in 0.5 M ethylenediaminetetraacetic acid (EDTA, pH 7.4, Sigma Aldrich) for 4 weeks prior to paraffin embedding. A serial of 6 μm-thick sagittal sections (about 100) were cut across the entire medial compartment of the joint until anterior cruciate ligament (ACL) junction. To measure the thicknesses of articular cartilage, chondrocyte numbers and growth plate thickness, 3 sections from each knee, corresponding to 1/4 (sections 20-30), 2/4 (sections 45-55), and 3/4 (sections 70-80) regions of the entire section set, were stained with safranin O/fast green (Sigma Aldrich) or hematoxylin and eosin (H&E, Sigma Aldrich) and quantified using BIOQUANT software. The final measurement is an average of these three sections. We defined uncalcified cartilage as the area from articular surface to tide mark and calcified cartilage as the area from tide mark to cement line. A similar approach was used on H&E stained sections to count the number of superficial chondrocytes (flat cells at the cartilage surface) and to measure synovial inflammation score as defined previously (*56*). The method to measure Mankin score was described previously (*57*). Briefly, two sections within every consecutive six sections in the entire section set for each knee were stained with safranin O/fast green and scored by two blinded observers. Each knee received a single score representing the maximal score of all its sections. In both scoring systems, higher score indicates worse disease condition.

Paraffin sections were used for immunohistochemistry. After appropriate antigen retrieval, slides were incubated with primary rabbit antibodies against EGFR (1:100, CST, 4267), p-EGFR

(1:200, Abcam, ab40815), ERK (1:200, CST, 4695), p-ERK (1:100, CST, 4370), Ki67 (1:100, Abcam, ab15580), HBEGF/DTR (1:100, Novus Biologicals, AF8239), Collagen II (1:300, Abcam, ab34712), Collagen X (1:200, LifeSpan Biosciences, LS-C667746), PRG4 (1:100, Abcam, ab28484), MMP13 (1:200, Abcam, ab39012), and ADAMTS5 (1:200, Abcam, ab41037) at 4°C overnight, followed by binding with biotinylated secondary antibodies and DAB (Vector Laboratories) color development. The terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay was carried out according to the manufacturer's instructions (Millipore, s7101). Mouse tissues, such as liver, spleen, lung etc, were also collected for paraffin sections followed by H&E staining to observe their morphology.

For EdU labeling experiment, joints were harvested and fixed in 4% paraformaldehyde overnight followed by decalcification in 0.5 M EDTA (pH 7.4) for 1 week prior to OCT embedding. Using a similar approach as described above, 3 representative sections were selected for EdU staining according to the manufacturer's instructions (Invitrogen). Positive cells within periarticular region or articular cartilage region were quantified using ImageJ software.

AFM-nanoindentation

Nanoindentation was performed on femoral articular cartilage surface as we previously described (*58*). Freshly dissected femoral condyle cartilage was indented at more than 10 locations by a borosilicate colloidal spherical tip (*The radius of the spherical tip* ≈ 5 μm, nominal spring constant k = 7.4 N/m, AIO-TL tip C, NanoAndMore) with maximum indentation depth of ~1 μm at 10 μm/s indentation rate using a Dimension Icon AFM (BrukerNano) in PBS with protease inhibitors. The effective indentation modulus, E_{ind} (MPa), was calculated by fitting the whole loading portion of each indentation force-depth curve using the Hertz model.

OA pain analysis

The knee joint pain after DMM surgery in mice was evaluated using von Frey filaments as described previously (*59*). An individual mouse was placed on a wire-mesh platform (Excellent Technology Co.) under a 4×3×7 cm cage to restrict their move. Mice were trained to be accustomed to this condition every day starting from 7 days before the test. During the test, a set of von Frey fibers (Stoelting Touch Test Sensory Evaluator Kit #2 to #9; ranging from 0.015 to 1.3 g force) were applied to the plantar surface of the hind paw until the fibers bowed, and then held for 3 seconds. The threshold force required to elicit withdrawal of the paw (median 50% withdrawal) was determined five times on each hind paw with sequential measurements separated by at least 5 min.

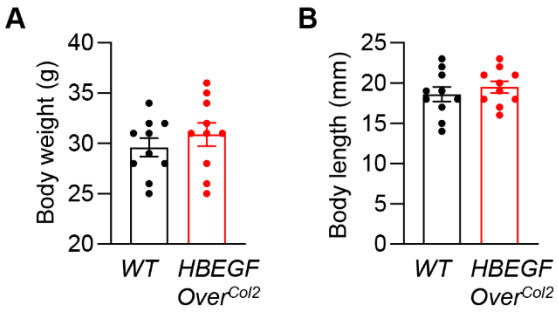

Fig. S1. *HBEGF Over*^{Col2} mice have normal body weight and body length. Male *HBEGF Over*^{Col2} and *WT* mice at 3 months of age were measured for (**A**) body weight and (**B**) length. *n* = 10 mice/group. Statistical analysis was performed using unpaired two-tailed t-test. Data presented as means ± SEM.

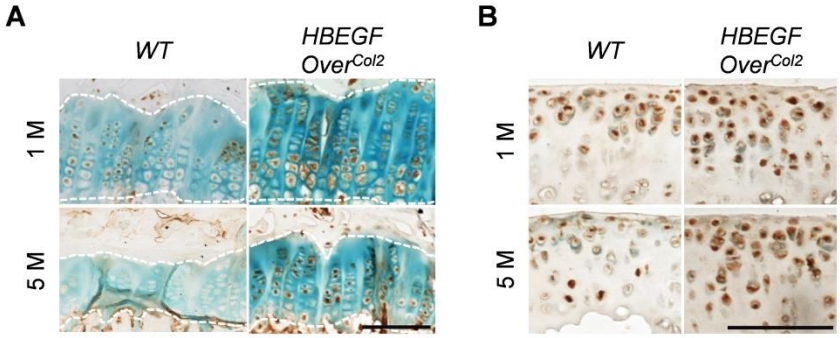

Fig. S2. *HBEGF Over*^{Col2} mice overexpress HBEGF in cartilage. Immunostaining of HBEGF in (**A**) the growth plate and (**B**) articular cartilage of *WT* and *HBEGF Over*^{Col2} mice at 1 and 5 months of age. Scale bars, 100 μm. *n* = 3 mice/group.

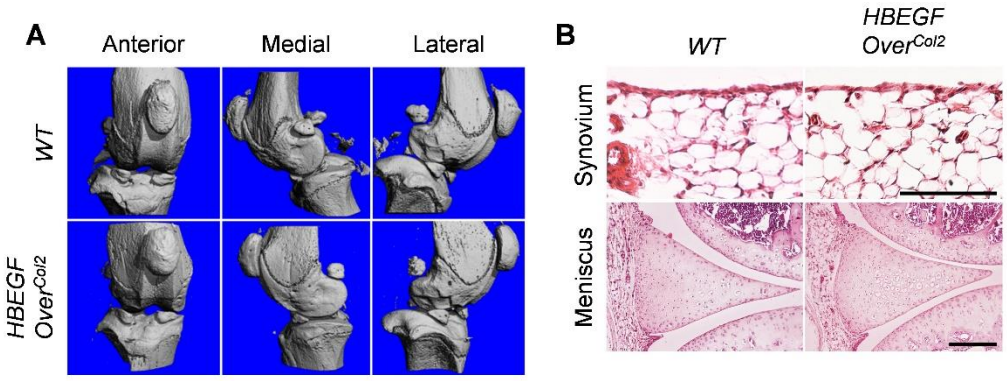

Fig. S3. *HBEGF Over*^{Col2} mice have normal joint structure. (**A**) Representative 3D microCT images of mouse joints from anterior, medial, and lateral views in 5-month-old *HBEGF Over*^{Col2} mice. *n* = 3 mice/group. (**B**) Representative H&E stained images of synovium (top) and meniscus (bottom) of knee joints from 5-month-old *WT* and *HBEGF Over*^{Col2} mice. Scale bars, 200 μm. *n* = 3 mice/group.

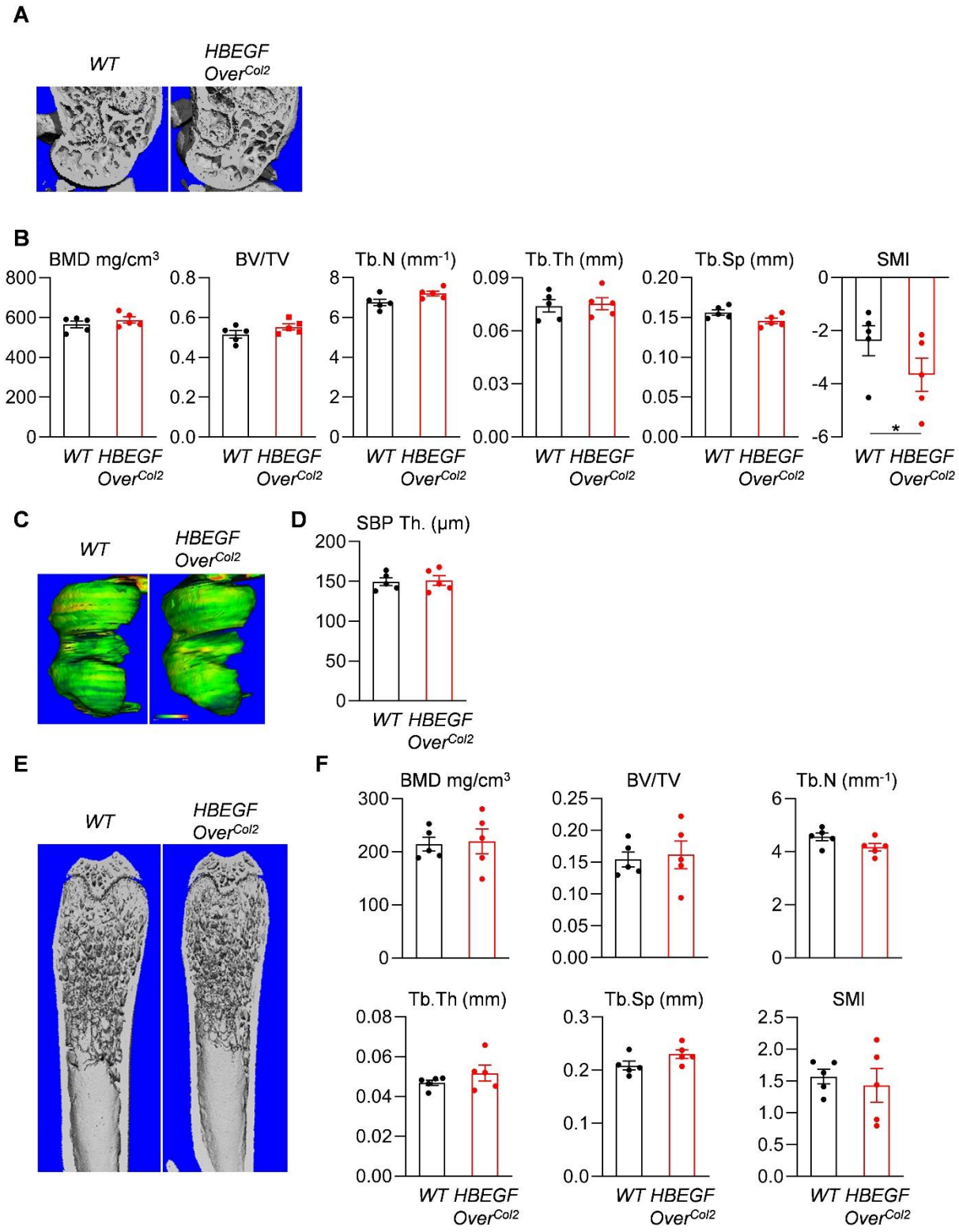

Fig. S4. Overexpressing HBEGF in articular cartilage does not affect long bone structure.

(**A)** Representative sagittal microCT images of distal femur in *WT* and *HBEGF Over*^{Col2} mice at

5 months of age. (**B**) Trabecular bone structural parameters in the subchondral trabecular bone were quantified. *n* = 5 mice/group. (**C**) Representative 3D color maps derived from microCT images show femoral subchondral bone plate thickness (SBP Th.) of *WT* and *HBEGF Over*^{Col2} mice. Color ranges from 0 (blue) to 320 μm (red). (**D**) SBP Th. at the medial posterior site of femoral condyle was calculated. *n* = 5 mice/group. (**E**) Representative longitudinal microCT images of distal femur in *WT* and *HBEGF Over*^{Col2} mice at 5 months of age. (**F**) Trabecular bone structural parameters in the secondary spongiosa were quantified. *n* = 5 mice/group. BMD: bone mineral density, BV/TV: bone volume/tissue volume, Tb.N: trabecular number, Tb.Th: trabecular thickness, Tb.Sp: trabecular separation, SMI: structure model index. Statistical analysis was performed using unpaired two-tailed t-test. Data presented as means ± SEM. **P* < 0.05.

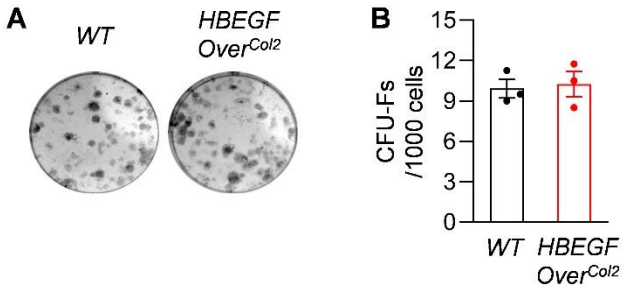

Fig. S5. Mesenchymal progenitors in synovium are not affected by HBEGF overexpression in cartilage. (**A**) CFU-F assay using synovial cells dissociated from mouse knee joints at 5 months of age. (**B**) Quantification of CFU-F frequency. *n* = 3 independent experiments. Statistical analysis was performed using unpaired two-tailed t-test. Data presented as means ± SEM.

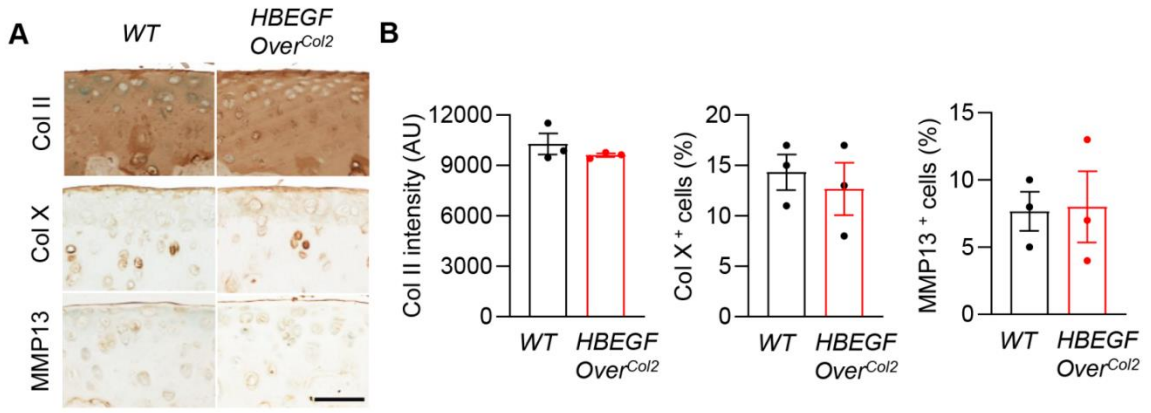

Fig. S6. Overexpressing HBEGF in cartilage does not affect cartilage matrix composition and cartilage degradation. (**A**) Immunostaining of Col II, Col X and MMP13 in 5-month-old mouse tibial articular cartilage of *WT* and *HBEGF Over*^{Col2} mice. Scale bar, 50 μm. (**B**) Quantification of Col II staining intensity, percentages of Col X^{+} and MMP13^{+} chondrocytes in the tibial articular cartilage of *WT* and *HBEGF Over*^{Col2} mice. n = 3 mice/group. Statistical analysis was performed using unpaired two-tailed t-test. Data presented as means ± SEM.

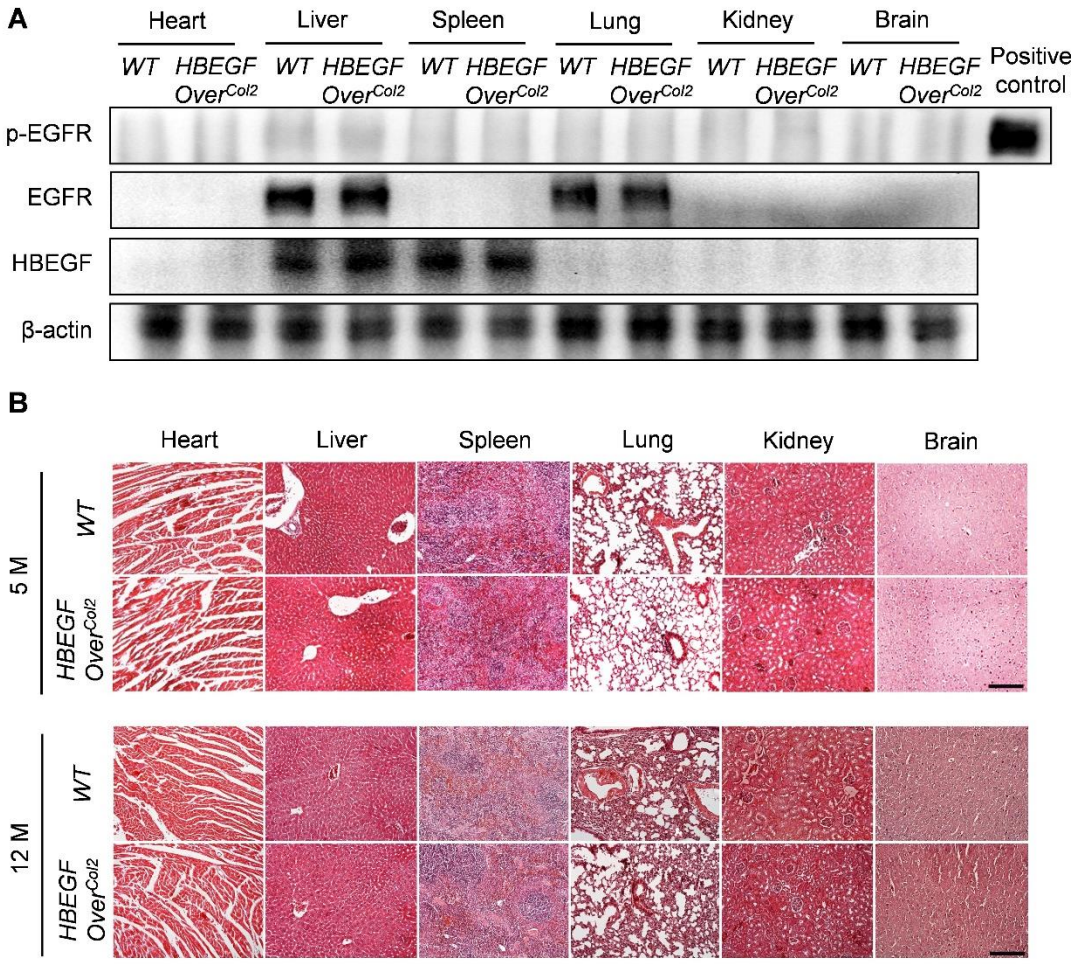

Fig. S7. Overexpressing HBEGF in cartilage does not affect vital internal organs. (A) Western blot of HBEGF, EGFR, and p-EGFR in the main organs of *WT* and *HBEGF Over*^{Col2} mice. Positive control was protein sample from TGFα-activated chondrocytes. *n* = 3 mice/group. **(B)** H&E staining of representative organ sections from 5- or 12-month-old *WT* and *HBEGF Over*^{Col2} mice. Scale bars, 200 μm. *n* = 5 mice/group.

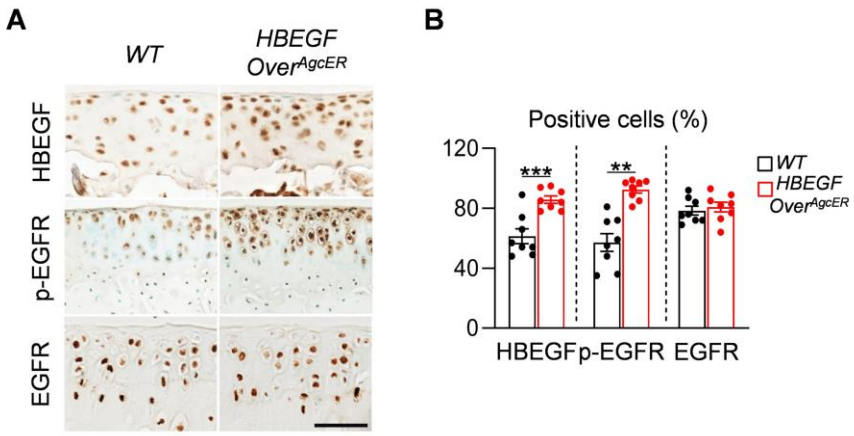

Fig. S8. *HBEGF Over*^{AgcER} mice have increased HBEGF expression and EGFR activity in knee articular cartilage. (**A**) Immunostaining of HBEGF, p-EGFR and EGFR in tibiae of 4-month-old *WT* and *HBEGF Over*^{AgcER} mice with tamoxifen injections at 3 months of age. Scale bar, 50 μm. (**B**) The percentages of HBEGF^{+}, p-EGFR^{+} and EGFR^{+} cells within uncalcified articular cartilage were quantified. n = 8 mice/group. Statistical analysis was performed using unpaired two-tailed t-test. Data presented as means ± SEM. ** $P < 0.01$, *** $P < 0.001$.

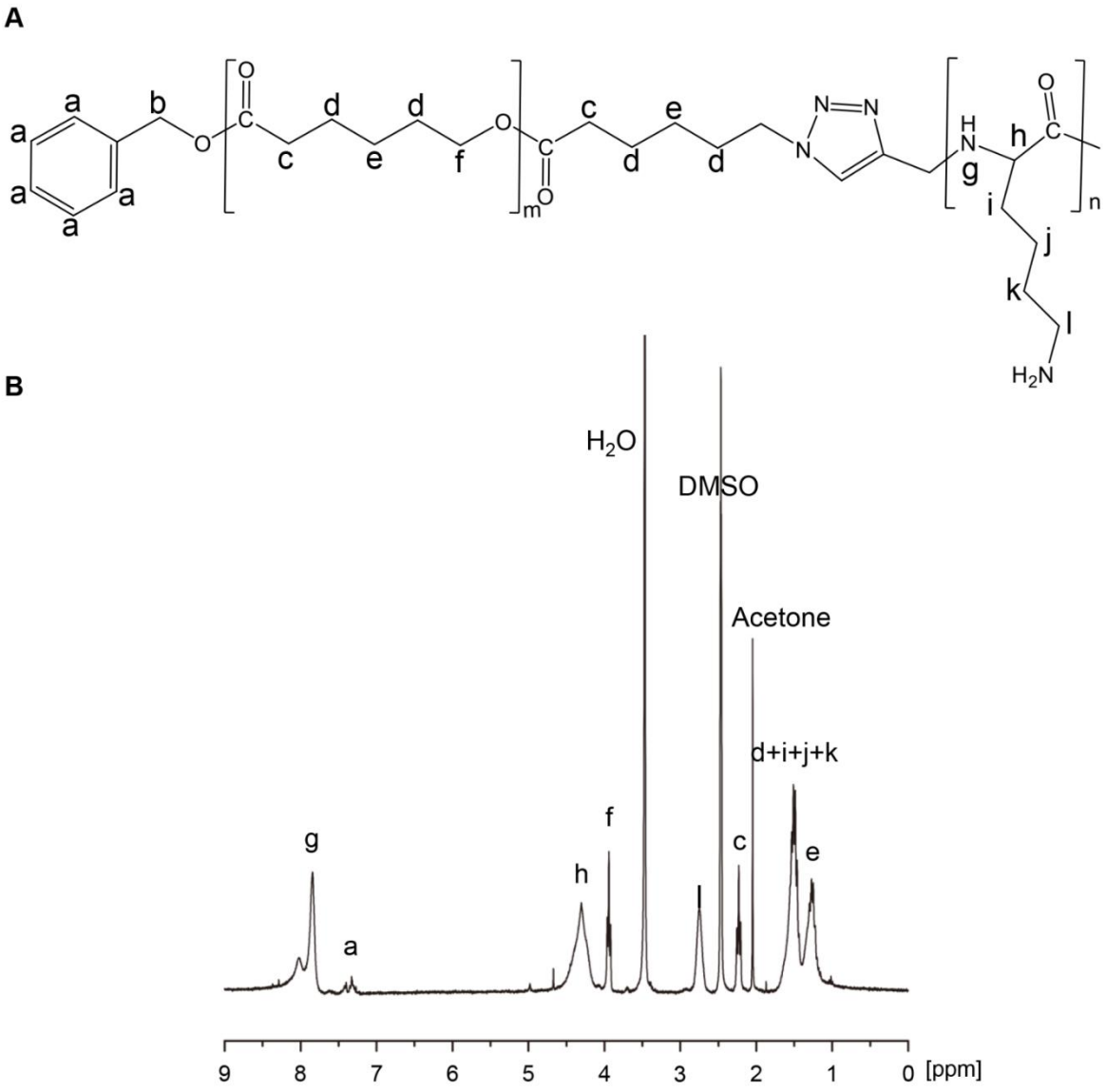

Fig. S9. Synthesis and characterization of PLL-PCL. Chemical structure (**A**) and ^{1}H NMR spectrum (**B**) of PLL-PCL are shown.

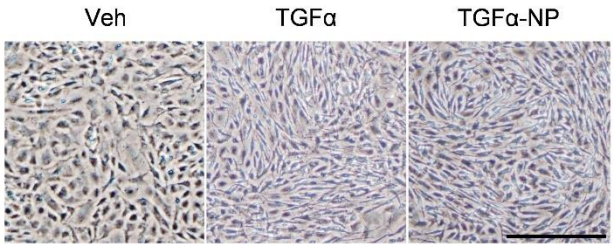

Fig. S10. TGFα-NPs result in similar morphology changes in chondrocytes as free TGFα. Mouse primary chondrocytes were treated with vehicle (PBS), free TGFα (15 ng/ml), and TGFα-NP (15 ng/ml TGFα content) for 2 days and imaged by bright field microscopy. Scale bar, 100 μm. *n* = 3 independent experiments.

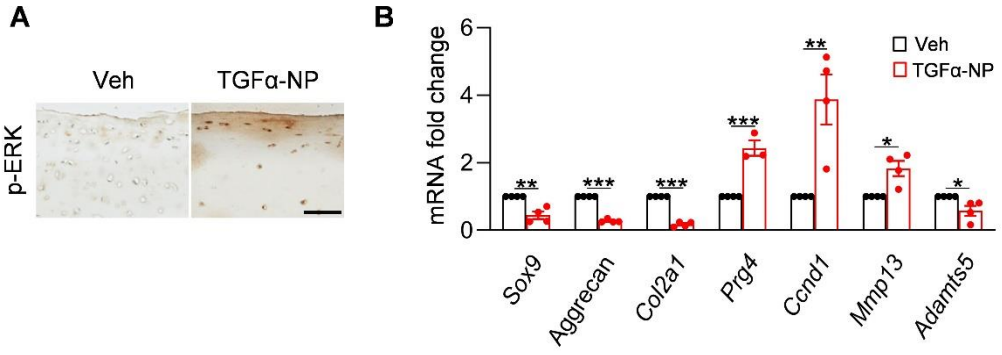

Fig. S11. TGFα-NPs are functional on human articular cartilage. (**A**) Immunostaining of p-ERK in human OA cartilage explants incubated with veh (PBS) or TGFα-NPs for 4 days. Scale bar, 200 μm. *n* = 3 samples/group. (**B**) qRT-PCR analyzes the relative gene expression. *n* = 4 independent experiments. Statistical analysis was performed using unpaired two-tailed t-test. Data presented as means ± SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

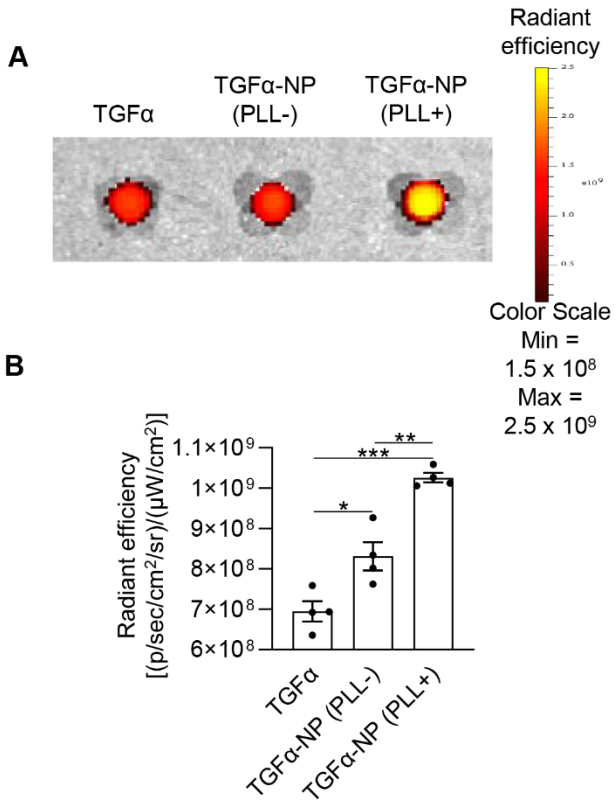

Fig. S12. TGFα-NPs doped with PLL-PCL enhance bovine cartilage uptake. (**A**) Microscopy images of bovine cartilage explants incubated with free TGFα, or TGFα-NPs with or without PLL-PCL for 24 hours. (**B**) Quantitative analysis of TGFα-NPs and free TGFα uptake by bovine cartilage explants based on images in A. $n = 4$ samples/group. Statistical analysis was performed using one-way ANOVA with Turkey's post hoc analysis. Data presented as means ± SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

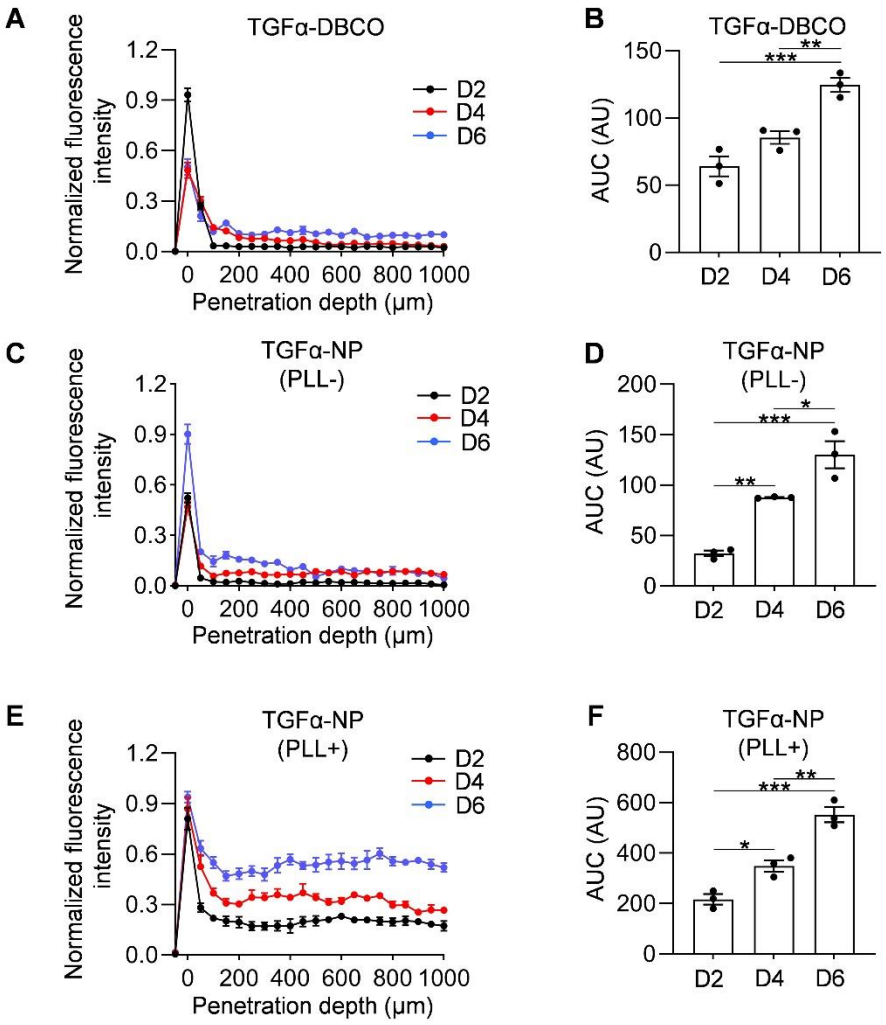

Fig. S13. TGFα-NPs doped with PLL-PCL improve their penetration and retention in bovine cartilage tissue. (**A, C, E**) Quantification of fluorescence intensity of rhodamine labeled TGFα-DBCO (A), TGFα-NPs without PLL-PCL (C) and TGFα-NPs with PLL-PCL (E) across the explant section. All images are taken under the same laser power, intensity and offset. $n = 3$ samples/group. (**B, D, F**) Area under the curve (AUC) of the corresponding fluorescence intensity from A, C and E. $n = 3$ samples/group. Statistical analysis was performed using one-way ANOVA with Turkey's post hoc analysis. Data presented as means ± SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. AU, arbitrary units.

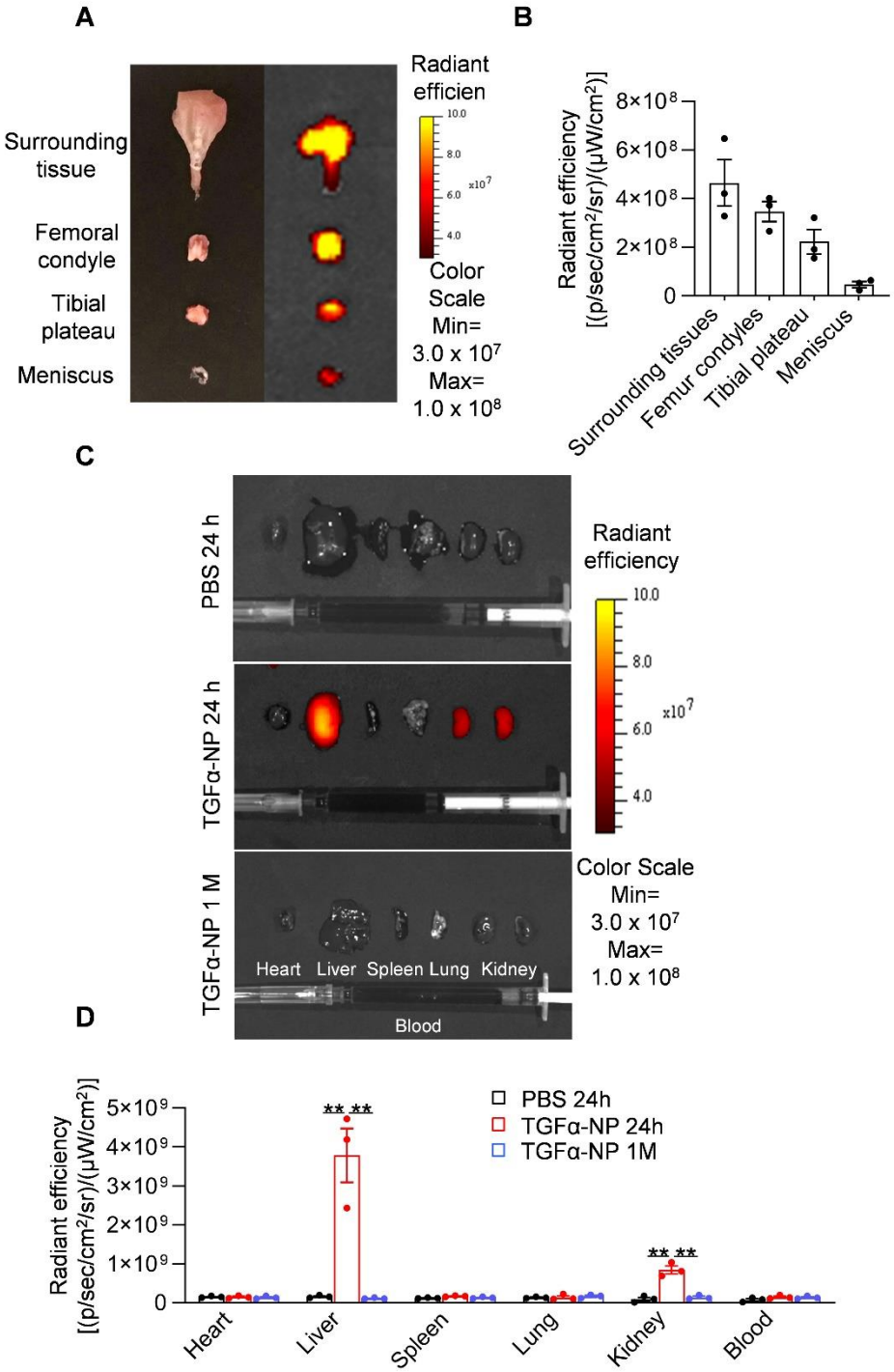

Fig. S14. Biodistribution of TGFα-NPs within the knee joints and some major organs. (**A**) Biodistribution of TGFα-NPs within mouse knee joint. Surrounding tissue include quadriceps, patella, patellar ligament, synovium, fat pad. (**B**) Quantification of fluorescent radiant efficiency on different parts of knee joints. *n* = 3 mice/group. (**C**) Fluorescence images of organs and blood

that were collected 24 hours or 1 month after PBS or TGFα-NP injection. (**D**) Quantification of fluorescent radiant efficiency on different organs and blood. *n* = 3 mice/group. Statistical analysis was performed using one-way ANOVA with Turkey's post hoc analysis. Data presented as means ± SEM. ** $P < 0.01$.

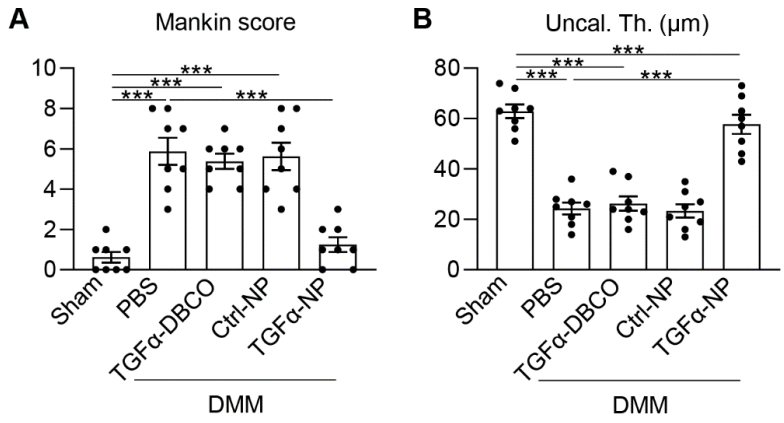

Fig. S15. TGFα-NP treatment attenuates OA progression after DMM surgery in mice.

(**A**) OA severity of knee joints and (**B**) uncalcified cartilage thickness were measured in mice received sham and DMM surgery followed by PBS, TGFα-DBCO (10 μM TGFα content), Ctrl-NP (with no TGFα conjugation) or TGFα-NP (10 μM TGFα content) treatment in the DMM group for 2 months *n* = 8 mice/group. Statistical analysis was performed using one-way ANOVA with Turkey's post hoc analysis. Data presented as means ± SEM. *** $P < 0.001$.

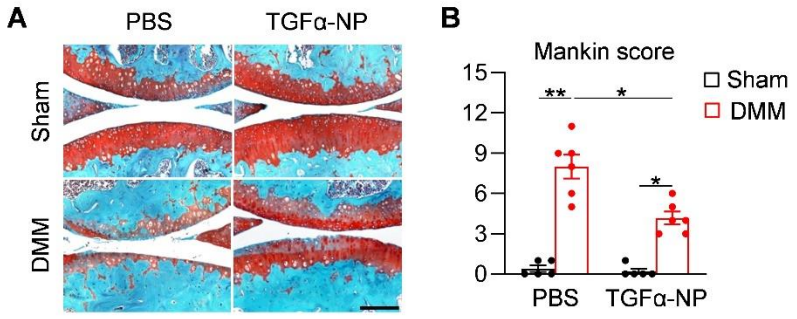

Fig. S16. TGFα-NP treatment starting at 1 month after DMM attenuates further OA progression. *WT* mice received DMM surgery at 3 months of age and intra-articular PBS or TGFα-NP (10 μM TGFα content) injections starting from 4 months of age. Knees were harvested at 6 months of age for Safranin O/Fast green staining (**A**) followed by Mankin score quantification (**B**). Scale bar, 200 μm. n = 6 mice/group. Statistical analysis was performed using two-way ANOVA with Turkey's post hoc analysis. Data presented as means ± SEM. * $P < 0.05$, ** $P < 0.01$.

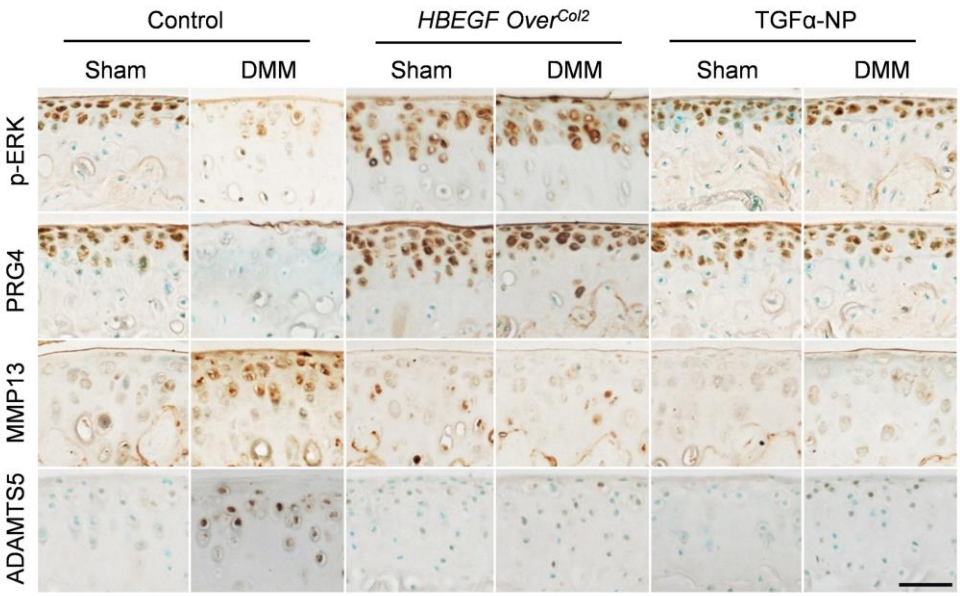

Fig. S17. The mechanism underlying the protective action of EGFR overactivation on articular cartilage after DMM surgery. Immunostaining of p-ERK, PRG4, MMP13 and ADAMTS5 in *WT*, *HBEGF Over*^{Col2} and TGFα-NP-treated *WT* knee joints at 2 months post-surgery. Scale bar, 50 μm. *n* = 3 mice/group.

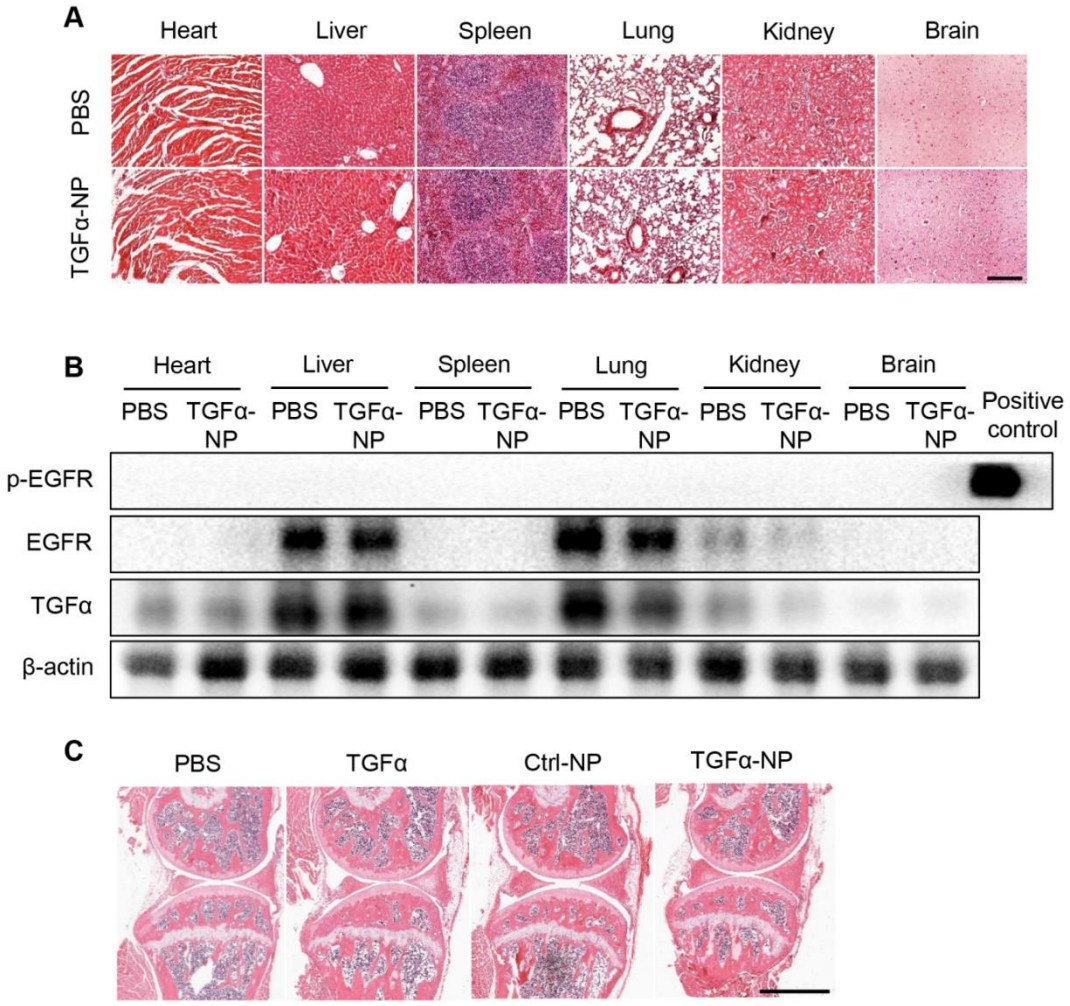

Fig. S18. Intra-articular injections of TGFα-NPs for 2 months do not affect vital internal organs and gross joint morphology. (**A**) H&E staining of representative organ sections from PBS- and TGFα-NP (10 μM TGFα content) -treated mice. Scale bar, 200 μm. *n* = 3 mice/group. (**B**) Western blots of TGFα, EGFR, and p-EGFR in organs. Positive control was protein sample

from TGFα activated chondrocytes. *n* = 3 mice/group. (**C**) H&E staining of representative knee joints from PBS-, TGFα-DBCO-, Ctrl-NP- and TGFα-NP-treated mice. Scale bar, 1mm. *n* = 3 mice/group.

Table S1. Mouse real-time PCR primer sequences.

Gene	Forward primer	Reverse primer
HBEGF	5'-CTATGACCACACAACCATCC-3'	5'-TCCTCTCCTATGGTACCTAAAC-3'
Egfr	5'-GGAAACCGAAATTTGTGCTACG-3'	5'-GCCTTGCAGTCTTTCTCAGCTC-3'
Prg4	5'-CAGGAGCCAATGAAGAAGTG-3'	5'-GAAGGTGTGTGTCTGGAAAG-3'
Col2a1	5'-CAAGAACAGCAACGAGTACCG-3'	5'-GTCACTGGTCAACTCCAGCAC-3'
Aggrecan	5'-CTACCGCTGTGAAGTGATG-3'	5'-GGTGTAGCGTGTGGAAATAG-3'
Col10a1	5'-GGGAGTTTGGCTCCAGAGTTT-3'	5'-TGTGTCTTCAGGGGTCCTTAG-3'
Mmp13	5'-TGACCTCCACAGTTGACAGG-3'	5'-ATCAGGCACTCCACATCTTGG-3'
Adamts5	5'-GCATCCCAGCATTAGGAATTCA-3'	5'-GGTGAGAGCTGCATTGGAGGTA-3'
Sox9	5'- AGGAGAGCGAGGAAGATAAG-3'	5'- ACGTGTGGCTTGTTCTTG -3'
Ccnd1	5'- CTGACACCAATCTCCTCAAC -3'	5'- GCATGGATGGCACAATCT -3'
β-actin	5'-TCCTCCTGAGCGCAAGTACTCT-3'	5'-CGGACTCATCGTACTCCTGCTT-3'