Appendix 1

T2 mapping

The experimental animals were euthanized according to the predetermined time frame outlined in the experimental plan. Subsequently, magnetic resonance imaging (MRI) scans of the joints were immediately conducted using a Discovery MR750 3.0T MR scanner (Discovery® MR750 MR System, GE Healthcare) equipped with an 8-channel knee coil. To minimize potential anisotropic effects, each joint was placed in a closed atmosphere within a sample tube, ensuring that all samples were oriented in the same direction relative to the magnetic field (i.e., parallel to the anatomic axis, denoted as B0). Morphological assessment in the sagittal planes was performed using T2 weighted images acquired through a 2D rapid acquisition with refocused echoes RARE sequence [repetition time/echo time (TR/TE) =2,914/36 ms, matrix =384×384, field of view (FOV) = 35×35 mm^2, section thickness (ST) =0.5 mm, acquisition time (TA) = 11 min 39 s 364 ms]. Additionally, T2 maps were reconstructed using a Multi-Slice Multi-Echo (MSME) acquisition method. The T2 map images were obtained with the following parameters: TR = 3,852.4 ms; TE =–11.0, 22.0, 33, 165, and 176 ms; matrix =256×256; FOV =35 mm × 35 mm; ST =0.50 mm; TA =12 min 19 sec 666 ms; four signals acquired; flip angle = 180°. For analysis, the weight-bearing area of the femoral part of each knee joint was selected as the region of interest (ROI). The average T2 relaxation times were calculated using the Bruker ParaVision 5.0 system, and the ROI data were analyzed by a senior musculoskeletal radiologist.

Mitochondrial extraction

Fresh rat quadriceps muscle tissue weighing 100–200 mg was rinsed with PBS, washed with blood water, dried with filter paper, and then cut into pieces using scissors. The tissue fragments were placed into a small capacity glass homogenizer containing 1.0 mL of pre-cooled Lysis Buffer. Ice bath cooling was maintained at 0 ℃, and the tissue was homogenized by grinding it 20 times in an up and down motion. The resulting homogenate was transferred to a centrifuge tube and centrifuged at 1,000 g at 4 ℃ for 5 minutes. The supernatant was collected and transferred to a new centrifuge tube, which was then subjected to a second centrifugation at 1,000 g at 4 ℃ for 5 minutes. The resulting supernatant was again transferred to a new centrifuge tube and centrifuged at 12,000 g at 4 ℃ for 10 minutes. The supernatant was discarded, and the mitochondrial pellets were deposited at the bottom of the tube. To suspend the mitochondrial pellets, 0.5 mL of Wash Buffer was added, followed by centrifugation at 1,000 g at 4 ℃ for 5 minutes. The supernatant was collected and transferred to a new centrifuge tube, which was then subjected to a second centrifugation at 12,000 g at 4 ℃ for 10 minutes. The resulting supernatant was discarded, leaving behind the highly pure mitochondrial deposits at the bottom of the tube. Subsequently, the mitochondrial precipitates were suspended using a 50–100 μL volume of Store Buffer.

Articular fluid extraction and inflammatory factor assay

Following the extraction of knee fluid, the levels of inflammatory cytokines were determined using enzyme-linked immunosorbent assay (ELISA) kits for IL-1β (Cloud-Clone Corp; article No. SEA563Ra; batch No. L140116385) and TNF-α Rat ELISA Kit (Life Technologies, Lot: 1290859B; batch No. KRC3011). The results were processed using ELISA data processing software.

TNF-α is a significant inflammatory cytokine involved in immune system regulation, inflammation, and host defense. On the other hand, the pro-inflammatory cytokine IL-1β plays a crucial role in the development of inflammation and autoimmune diseases. Samples were thawed to room temperature for 30 min and centrifuged (Shanghai Lishen Scientific instrument Co., LTD.; batch No. Neofuge 15R) at 4 ℃, 2,000 rpm for 15 min. The samples were then diluted to the appropriate concentration. In each well of the assay plate, 100 mL of either standard or sample was added, and the plate was sealed with a seal membrane. Subsequently, the plate was shaken at room temperature for 2.5 hours using a micro oscillator (Medical Instrument Factory; Jintan city, model: MM-2). The membrane was carefully removed, and the plate was dried. Next, 300 mL of detergent was added to each well, removed after 2 min, and the plate was patted dry. This washing process was repeated five times. Following the washes, 100 mL of 1X Biotinylated ICAM1 detection antibody was prepared and added to each well. The plate was shaken at room temperature for 1 h, and the liquid was removed, 300 mL of detergent was added

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to each well, removed after 2 minutes, and plate was patted dry, repeating this process five times. Then, 100 mL of 1X HRP-Streptavidin solution was added to each well, and the plate was shaken for 45 minutes. The liquid was then removed, and 300 mL detergent was added to each well, removed after 2 minutes, and the plate was patted dry, repeating this process five times. Subsequently, 100 mL of TMB One-Step Substrate was added to each well, and the plate was shaken in the absence of light for 30 minutes. To terminate the reaction, 50 mL of stop solution was added to each well, and the optical density value (OD value) of each well was measured at 450 nm using a microplate reader (Bio-rad, xMarkTM) after 5 minutes.

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