Online supplementary text

S1. Studies were conducted at Bolder BioPATH, Inc. (Boulder, Colorado, USA); study designs and animal usage were approved by their Institutional Animal Care and Use Committee (IACUC) prior to study initiation (IACUC protocols BBP13-029 [MMT followed by amputation] and BBP12-004 [all other procedures]). Animal care, including room, cage and equipment sanitation, conformed to accepted guidelines.[34]

Male Lewis rats (Charles River Laboratories, Malvern, Pennsylvania, USA; approximately 80–90 days old, 275–300 g) were acclimated for 7 days upon arrival at the facility and housed four animals per cage.

S2. On each day of analysis, the rear feet of rats were placed in ink. Rats were placed at the entrance of a tunnel with a dark chamber at the far end and allowed to walk to the chamber, across an 8.5 × 14 inch sheet of paper. This process was repeated as necessary to generate four clear, evenly-linked footprint pairs representing the overall gait pattern. Footprints were analysed digitally using Photoshop (Adobe, Inc., San Jose, California, USA) to measure the average ink lightness (0, fully inked to 255, pure white) in a 400 × 400 pixel area around each footprint on a 300 dpi scan. The inking level (255 minus the value of the footprint area) was determined for each foot and the ipsilateral inking level was expressed as a percentage of the mean of both limbs. This percentage was subtracted from 100 to determine the gait deficiency.

S3. Following 3 days in Immunocal (Decal Chemical Corporation, Tallman, NY), the right knee and surrounding tissue were cut into approximately equal halves along

the frontal plane, and were embedded in paraffin. Three sections were cut from each right knee at ~200-µm intervals, stained with toluidine blue, and analysed microscopically. Additionally, a single toluidine blue-stained section from each of the 5 left knees from isotype control animals was collected and analysed. The worst-case scenario for the 2 halves on each slide was determined for general cartilage degeneration, proteoglycan loss, collagen damage, and osteophyte formation and the values for each parameter averaged across the 3 sections to determine overall subjective scores.

General cartilage degeneration includes decreased proteoglycan, chondrocyte death/loss, and/or cartilage matrix fibrillation/loss. Cartilage degeneration in the tibia was scored on a 6-point numeric scale (0 = no degeneration to 5 = severe degeneration). Each zone (outside, middle, and inside) was evaluated separately and a 3-zone sum for cartilage degeneration was calculated. The width of the cartilage affected by any degeneration (cell loss, proteoglycan loss or collagen damage) was measured by ocular micrometer. This measurement extends from the origination of the osteophyte with adjacent cartilage degeneration (outside 1/3) across the surface to the point where tangential layer and underlying cartilage appear histologically normal. Substantial Cartilage Degeneration Width (µm) reflects areas of tibial cartilage degeneration in which both chondrocyte and proteoglycan loss extend through greater than 50% of the cartilage thickness. In general, the collagen damage is mild (25% depth) or greater for this parameter but chondrocyte and proteoglycan loss extend to at least 50% or greater of the cartilage depth. Growth plate thickness was measured in all knees on medial and lateral sides (2 measurements per joint) at the approximate

midpoint of the medial and lateral physis (assuming a non-tangential area of the section). The difference between the 2 sides was determined by subtracting the lateral value from the medial. Measurements were made of the thickness of the medial synovial/collateral ligament repair in a non-tangential area of the section. Synovial reaction, if abnormal, was described (mainly fibrosis) and characterised with respect to the inflammation type and degree, but was not scored. Damage to calcified cartilage and subchondral bone was scored on a 6-point numeric scale (0 = no changes to 5 = increased basophilia; marked to severe fragmentation of calcified cartilage, mesenchymal change in marrow involves up to three-fourths of the total area and articular cartilage has collapsed into the epiphysis to a depth of >250 µm from tidemark). Medial tibial subchondral/epiphyseal bone sclerosis was scored on a 6-point numeric scale (0 = no changes to 5 = 76-100% increase in subchondral or epiphyseal trabecular bone thickness in medial versus lateral; very little marrow space remains in medial tibia). Marginal zone proliferative changes and osteophytes were measured with an ocular micrometer. Proliferative changes had to be ≥200 µm in order to be designated as osteophytes. Scores were assigned to the largest osteophyte in each section based on the measurement on a 6-point numeric scale (0= <200 µm to 5= ≥600 µm).

SS4. In the 0.1 mg/kg tanezumab group, 11/30 (36.7%) animals were ADA-positive (day 7 terminal group: 4/10; day 14 terminal group: 4/10; day 28 terminal group: 3/10, (online supplementary table S2). In the 1 and 10 mg/kg tanezumab groups there were no ADA-positive animals. PK sampling confirmed exposure in all tanezumab-treated animals. All

animals maintained exposure through the last time point collected just prior to necropsy except one animal (ADA-positive) in the 0.1 mg/kg day 14 group had measurable drug levels on day 7 but was BLQ on days 10 and 14. Although some ADA-positive animals had lower tanezumab concentrations than ADA-negative animals, mean exposures across the 0.1 mg/kg dose groups were similar among all animals and ADA-negative animals within a study/dose group. In delayed treatment, plasma concentrations of tanezumab confirmed exposure and tanezumab levels were observed through necropsy for 9 out of 10 animals in the 0.1 mg/kg tanezumab-treated dose groups (mean ADA-negative animals had drug exposure levels of 405 ± 223 µg/mL and 499 ± 254 µg/mL for the Day 23 and Day 57 treatment onset groups, respectively). ADA did not impact the interpretation of the data or the overall conclusion.