ONLINE METHODS

Human samples. We studied human samples under protocols that were approved by the Stanford Institutional Review Board (IRB) and included subjects' informed consent, and under protocols that were approved by Partners Healthcare IRB and did not require subjects' informed consent. For ELISA analysis, we obtained synovial fluids from healthy individuals with no symptoms of knee osteoarthritis; patients with early-stage knee osteoarthritis with symptoms of <1 year's duration, as assessed by the presence of arthroscopically visible cartilage lesions or by radiographic imaging; and patients with end-stage knee osteoarthritis undergoing knee-joint replacement. For transcriptional profiling, we obtained osteoarthritic synovial membranes from patients undergoing knee-joint replacement and from patients with early-stage knee osteoarthritis undergoing arthroscopy for meniscal tears with cartilage degeneration but no full-thickness cartilage loss[25].

Transcriptional profiling. We analyzed RNA from osteoarthritic synovial membranes by using Affymetrix human U133 plus 2.0 chips (GEO GSE32317). We downloaded data from healthy synovial membranes (analyzed on the same platform and array) from the US National Center for Biotechnology Information Gene Expression Omnibus (GEO GSE12021[33]). We merged these data sets[34] and computed RMA (Robust Multichip Average) expression for the genes. We organized transcript expression profiles by unsupervised hierarchical clustering (http://bonsai.hgc.jp/~mdehoon/software/cluster/software.htm).

Immunohistochemistry and immunofluorescence. Primary antibodies were as follows: mouse antibody to C5b-9 (clone aE11; Dako), rabbit antibody to MMP-13 (Abcam), rabbit antibody to ERK1/2 phosphorylated on Thr202/Tyr204 (20G11, Cell Signaling), and corresponding isotype-control antibodies. For immunohistochemical analysis, we used Vectastain Elite ABC Kits (Vector Labs). For immunofluorescent analysis, we used Alexa Fluor-555- or Alexa Fluor-488-conjugated secondary antibodies (Invitrogen).

Surgical mouse models of osteoarthritis. We performed mouse studies under protocols approved by the Stanford Committee of Animal Research and in accordance with US National Institutes of Health guidelines. We generated the medial meniscectomy[10,16,35] and DMM models[10,16-18]. In the medial meniscectomy studies, the age of the mice at the time of surgery, and the duration of the experiment after surgery, were as follows. C5 deficiency: 20-week-old mice, 16 weeks' duration; time course: 20-week-old mice; C6 deficiency: 20-week-old mice, 20 weeks' duration; Cd59a deficiency: 12-week-old mice, 16 weeks' duration. We used 15-month-old mice and continued the experiment for 12 weeks after surgery in the Cd59a DMM studies; we used 20-week-old mice and continued the experiment for 20 weeks in the C5 DMM studies. A/J congenic C5-sufficient (B10.D2-Hc1H2dH2-T18c/nSnJ) and C5-deficient (B10.D2-Hc0H2d-T18c/oSnJ) mice were from Jackson Laboratories. C6-deficient mice were backcrossed with C3H/He mice for ten generations[36]; their controls, C3H/He mice, were from Jackson Laboratories. *Cd59a*$^{-/-}$ mice were generated as described[37].

Treatment of mouse osteoarthritis. We treated 21-week-old wild-type C57Bl/6 mice *i.p.* with 750 μg of either the C5-specific monoclonal antibody BB5.1[38] or an isotype-control antibody twice per week for 18 weeks, starting 1 week after medial meniscectomy. We treated 21-week-old wild-type C57Bl/6 mice *i.v.* with 250 μg of CR2-fH[15] or with PBS once a week until the end of the experiment (12 weeks), starting 1 week after medial meniscectomy.

Scoring of cartilage degeneration in mouse osteoarthritis. We stained sections of mouse joints with toluidine blue or Safranin O. We evaluated cartilage degeneration using a modified version of a described scoring system[10]: depth of cartilage degeneration (score of 0-4) × width of cartilage degeneration (score of 1 = 1/3, 2 = 2/3, and 3 = 3/3 of surface area) in each third of the femoral-medial and tibial-medial condyles; the scores for the six regions were summed.

Gait analysis. We analyzed mouse gait with the video-based Noldus CatWalk System[39].

***In vitro* complement activation assays.** Using described methods[23], we prepared suspensions of pulverized human osteoarthritic cartilage, to which we added sepharose 4B (Pharmacia), zymosan A (Sigma), collagen II (Sigma or Chondrex), aggrecan (Sigma), matrilin-3 (R&D Systems), or fibromodulin (Novus Biologicals). We then added normal human serum (Quidel, Inc), incubated the samples at 37 °C, stopped the reactions by adding EDTA, and measured C5b-9 levels by ELISA (Quidel).

Generation of sublytic MAC on chondrocytes. We isolated chondrocytes from osteoarthritic cartilage by digesting it with trypsin and type II collagenase. We formed sublytic MAC on the chondrocytes' surface by mixing 0.8 μg ml^{-1} of C5b6 (EMD Chemicals) with 10 μg ml^{-1} of each of the remaining complement components of MAC; C7 (Quidel), C8 (EMD Chemicals), and C9 (EMD Chemicals), as described[40]. After 72 hours, we harvested culture supernatants for bead-array (Millipore) analysis of protein levels, isolated RNA for quantitative PCR analysis, and performed immunocytochemistry with a goat antibody to COX-2 (Santa Cruz Biotechnology).

Quantitative PCR. We isolated RNA from cultured chondrocytes derived from human osteoarthritic cartilage or from chondrocytes isolated from the knee joint of C5-deficient and C5-sufficient mice. We measured mRNA levels by qPCR and normalized them to those of 18s RNA or β-actin ($2^{-\Delta\Delta Ct}$). Primers and probes were from Applied Biosystems.

Statistical Analysis. We analyzed "cartilage degeneration scores" by two-tailed unpaired *t* test, and multiple comparisons by one-way analysis of variance followed by Dunnett's post-hoc test or by fixed-effect analysis of variance.