Supporting Information

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SI Materials and Methods

shRNA/Lentivirus Infection. Lentivirus incorporating with shRNA was generated in HEK293T packaging cells by combining a pLKO.1 plasmid containing shRNA sequences, packing plasmid pCMV-dR8.91, and envelope plasmid VSV-G/pMD2.G (Broad Institute, MIT). Synovial fibroblasts were infected with lentivirus containing control shRNA (mission; Sigma-Aldrich) or either of the cad-11 shRNAs (clone ID: TRCN0000054334 and TRCN0000054335) targeting different sequences of human cad-11 (Open Biosystems). After selecting cells in the presence of 2 μg/mL puromycin (Sigma-Aldrich) for 3 d, cells were reseeded and rested in complete synovial fibroblast medium (SF medium). Two days later, experiments were carried out.

Quantification of Cytokine Secretion by ELISA. IL-6 and MCP-1 levels were determined in cell-free culture supernatants using cytokine-specific ELISA kits (R&D Systems). Results are shown in picograms per milliliter (as mean ± SEM). To measure p-p65 and p-65 by ELISA, synovial fibroblasts (150,000 cell/1 mL/well in six-well plates) were stimulated with 20 μg/mL hCad-11-Fc or 10 ng/mL TNF-α for 15 or 30 min. Total cell lysates were determined for p-p65 (#7173) and p-65 (#7174) by using ELISA kits (Cell Signaling Technology, Inc.).

Immunoblotting. After stimulating serum-starved synovial fibroblasts (150,000 cells/1 mL/well in six-well plates) with or without 20 μg/mL hCad-11 or 10 ng/mL TNF-α for the indicated time points, cells were lysed using lysis buffer [50 mM Tris (pH 7.4), 150 mM NaCl, 1% Triton X-100, 0.1% SDS, 1 mM EDTA] supplemented with 1 mM sodium orthovanadate, 1 mM PMSF, and a mixture of protease inhibitors (Complete; Roche Applied Science). After clearing insoluble material, total cell lysates were subjected to SDS/PAGE and transferred to Immobilon-P membranes (Bio-Rad). Membranes were blocked in TBS-T [25 mM Tris·HCl (pH 7.2), 150 mM NaCl, 0.05% Tween] supplemented with 5% nonfat skim milk or 5% BSA (for detecting phosphorylated proteins) for 1 h at room temperature. The membrane was blotted for target proteins using specific antibodies [pJNK (4668), pERK1/2 (4370), p-p38 (9211), JNK (9252), ERK1/2 (4695), and p38 (9212)] from Cell Signaling Technology, Inc., and anti–β-actin from Sigma-Aldrich) for overnight incubation at 4 °C. After washing, the membranes were incubated with horseradish peroxidase-conjugated anti-mouse IgG or anti-rabbit IgG for 1 h at room temperature. After washing, proteins in the membranes were then visualized using the SuperSignal West Pico Chemiluminescent Substrate (Fisher Scientific).

Immunofluorescence and Flow Cytometry. For confocal immunofluorescence, 5.5-μm protein A-precoated polystyrene beads (Bangs Laboratories, Inc.) were coated with hIgG1 or human cadherin (hCad)-11-Fc overnight in 0.1% BSA/Hepes buffered saline (0.78 μg hCad-11-Fc/1 × 10^6 beads). After washing three times with 0.1% BSA/HBS, beads were resuspended in 1% FBS/SF medium. GFP-cad-11-L cells (0.2 × 10^4/well in 24-well plates) were serum-starved overnight and incubated with hCad-11-Fc or hIgG1-coated beads for 40 min in 500 μL 1% FBS/SF medium. Cells were fixed with 4% paraformaldehye (Electron Microscopy Sciences) and were mounted with FluorPreserve Reagent (Calbiochem). The GFP images were taken using a TE2000-U inverted microscope equipped with a Nikon C1 confocal system controlled by Nikon EZ-C1 software. The Digital Image Correlation (DIC) images were taken with a SPOT-RT CCD camera (Diagnostics Instruments) controlled by SPOT Advanced software. A 60× objective was used. For flow cytometry, synovial fibroblasts were released from tissue culture flasks using 0.02% (wt/vol) irradiated and L-1-tosylamido-2-phenylethyl chloromethyl ketone-treated trypsin in HBS– Ca^{2+} and washed with FACS buffer (2% FBS and 0.05% sodium azide in HBS– Ca^{2+}). Cells were stained with anti–cad-11 mAb or MOPC21 for 30 min at 4 °C followed by FITC-conjugated $F(ab')_2$ fragment goat anti-mouse IgG (Jackson Laboratory) for 15 min at 4 °C in FACS buffer. Fixed cells with 1% paraformaldehyde were analyzed using a FACSCanto (BD Biosciences). FlowJo analysis software (Tree Star) was used.

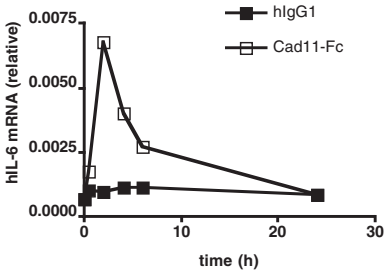

Fig. S1. Cad-11 induces IL-6 at the mRNA level. Synovial fibroblasts (100,000 cells/well in 24-well plates) were stimulated with 5 μg/mL hIgG1 or hCad-11-Fc for 0.5, 2, 4, 6, and 24 h. Then total RNA was extracted from the cells. IL-6 mRNA was measured by quantitative real-time PCR. Data shown represent two independent experiments.

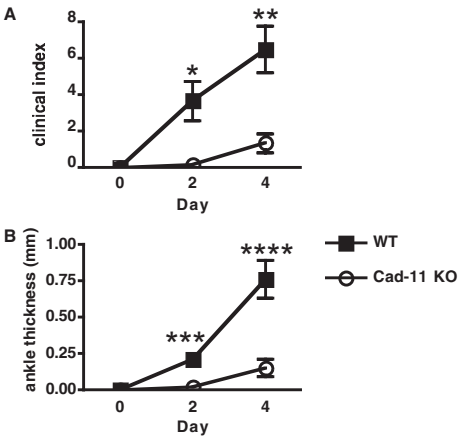

Fig. S2. Cad-11 expression impacts on clinical score and ankle thickness in K/BxN serum transfer arthritis. WT and cad-11–deficient mice were injected with 150 μL arthrtitogenic K/BxN serum at day 0 and day 2. Clinical index (*A*) and ankle thickness (*B*) were measured on days 0, 2, and 4. **P* = 0.0083, ***P* = 0.0038, ****P* = 0.0016, and *****P* = 0.0018.