**Online Supplementary Materials and Methods**

**Mice**

*Nrdc–/–* mice (Accession No. CDB0466K, http://www.clst.riken.jp/arg/mutant%20mice%20list.html) were generated as described previously,[1] and backcrossed to the BALB/c background (> 98%). BALB/c wild-type (WT) mice were purchased from Japan SLC. *NrdcdelM* mice (C57BL/6 background) were generated by mating *Nrdcflox/flox* mice (Accession No. CDB1019K, <http://www.clst.riken.jp/arg/mutant%20mice%20list.html>) [2] with mice expressing Cre recombinase under the control of the *Lyz2* promoter that were obtained from The Jackson Laboratory (Bar Harbor, ME, USA). KRN mice were kindly provided by Diane Mathis and Christophe Benoist (Harvard Medical School, Boston, MA, USA). K/BxN mice were obtained by crossing KRN with NOD mice (CLEA Japan Inc., Tokyo, Japan). All animal experiments were performed according to the procedures approved by the Animal Ethics Committee of the Institute of Laboratory Animals, Kyoto University. Mice were maintained in environmentally controlled rooms.

**Induction of CAIA and K/BxN STA.**

Eight to twelve-week-old female BALB/c WT and *Nrdc–/–* mice were used for CAIA. Eight to twelve-week-old sex-matched *Nrdcflox/flox* and *NrdcdelM* mice were used for CAIA and K/BxN STA. Mean body weight of Balb/c WT, *Nrdc–/–*, *Nrdcflox/flox* and *NrdcdelM* mice were 19.95 g, 11.08 g, 21.94 g and 21.18 g, respectively. A five-clone cocktail of monoclonal antibodies against type II collagen was purchased from Chondrex (Redmond, WA, USA). BALB/c WT and *Nrdc–/–* mice were injected i.p. with 100 mg/kg body weight of antibodies, while *Nrdcflox/flox* and *NrdcdelM* mice were injected i.p. with 250 mg/kg body weight of antibodies. Three days after the antibody injection, 25 μg of LPS (Chondrex) was administered i.p. K/BxN serum was harvested from 8–10-week-old K/BxN mice, pooled, and stored at –30°C until use. For K/BxN STA induction, 200 μl of serum was injected i.p. to *NrdcdelM* and *Nrdcflox/flox* mice on day 0 and day 3.

**Evaluation of arthritis.**

The severity of arthritis in each limb was scored daily between day 1 and 14 after the induction as previously described [3] : 0 = normal; 1 = mild redness, slight swelling of ankle or wrist; 2 = moderate swelling of ankle or wrist; 3 = severe swelling including some digits, ankle, and foot; 4 = maximally inflamed. Ankle thickness was measured daily with a digital caliper.

**Histological evaluation.**

Mice were euthanized 14 days after CAIA or K/BxN STA induction and the ankle joints were cut and fixed with 4% paraformaldehyde before being paraffin embedded after decalcification with 20% EDTA. Sections 5 μm thick were stained with hematoxylin and eosin.

**Cell culture and viral infection.**

BMDM were separated as follows: mouse femurs and tibia were cut at both ends and bone marrow was washed out with PBS, then cells were seeded at 2 x 106/ml in RPMI-1640 medium containing 10% FBS, 10 μM Hepes, 10 ng/ml gentamicin, and 20 ng/ml macrophage colony stimulating factor (R&D Systems, Minneapolis, MN, USA). An equal volume of culture medium was added on day 3 and adherent cells were detached on day 7 using Accutase (Innovative Cell Technologies, San Diego, CA, USA. THP-1 cells were purchased from the JCRB cell bank (Osaka, Japan) and cultured in RPMI-1640 containing 10% FBS, 1 μM Hepes, 1 mM sodium pyruvate, 1% nonessential amino acids, 10 ng/ml gentamicin, and 50 μM 2-ME. THP-1 cells were infected with a lentivirus expressing an miRNA targeting *Nrdc* (BLOCK-iT miR RNAi Select; Thermo Fisher Scientific, Waltham, MA, USA) [4] and GFP-positive cells were sorted using a FACSAria IIu (BD Biosciences, San Jose, CA, USA). Mouse embryonic fibroblasts (MEFs) were isolated from C57BL/6 WT embryos at embryonic day 14.5 (E14.5), and were maintained in DMEM containing 5% FBS, 100 U/ml penicillin, 100 mg/ml streptomycin, and 2 mM L-glutamine. MEFs were passaged according to the 3T3 protocol and immortalized.[1] WEHI 13 VAR cells and RAW 264.7 cells were purchased from ATCC (Manassas, VA, USA) and cultured in DMEM containing 10% FBS and 10 ng/ml gentamicin.

**TNF-α shedding assay.**

BMDMs (5 x 104) were seeded in 96-well plates and stimulated with 10 ng/ml of LPS (Sigma-Aldrich, St. Louis, MO, USA) for 3–6 h. THP-1 cells (3 x 105) were seeded in 24-well plates and stimulated with 3 ng/ml of LPS for 3–6 h. Cell extracts were obtained using Cell Lysis Buffer (Cell Signaling Technology, Danvers, MA, USA) supplemented with Halt Protease Inhibitor Cocktail (Thermo Fisher Scientific), according to the manufacturer’s instructions. Total RNA was isolated using an RNeasy Mini Kit (Qiagen, Venlo, Netherlands) and cDNA was synthesized using ReverTra Ace (Toyobo, Osaka, Japan).

**Reverse passive Arthus reaction.**

Following i.v. injection of 20 mg/kg of chicken egg albumin (Wako, Tokyo, Japan), 800 μg of rabbit anti-chicken egg albumin antibody (Sigma-Aldrich) was injected i.p. Mice receiving i.p. antibody injection without i.v. injection served as controls.[5] Mice were euthanized 3 h after injection and PMs were collected using 3 ml of PBS.

**Flow cytometric analysis.**

Anti-mouse CD16/32 antibody (93), anti-mouse TNF-α antibody (MP6-XT22), PE-conjugated anti-mouse TNF-α antibody (MP6-XT22), PE-indotricarbocyanine (Cy7)–conjugated anti-F4/80 antibody (BM8), and irrelevant isotype controls were purchased from BioLegend (San Diego, CA, USA). Alexa Fluor 488-conjugated anti-rat IgG antibody was purchased from Thermo Fisher Scientific. BMDMs (1 x 105) were stimulated with 10 ng/ml of LPS for 3 h and fixed with 4% paraformaldehyde. PMs were harvested from peritoneal lavage fluid using centrifugation and fixed with 4% paraformaldehyde. BMDMs and PMs were treated with anti-CD16/32 antibody for Fc receptor blocking. After the Fc receptor blocking, BMDMs were stained with PE-conjugated anti-mouse TNF-α antibody and PMs were incubated with anti-mouse TNF-α antibody followed by staining with Alexa Fluor 488-conjugated anti-rat IgG antibody. Samples were processed on a FACSAria IIu (BD Biosciences). Data were analyzed with FlowJo (V. 7.6.5 FlowJo LLC, Ashland, OR, USA) software.

**Western blots.**

A rat anti-mouse NRDC monoclonal antibody (clone #135) was raised in our laboratory against recombinant mouse NRDC. Antibody to β-actin (sc47778) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA) and antibody to human ADAM17 was purchased from Cell Signaling Technology. Cell extracts were separated by SDS–PAGE and transferred to nitrocellulose filters. After blocking, filters were incubated with primary antibodies, followed by HRP-conjugated secondary antibodies (GE Healthcare Life Sciences, Marlborough, MA, USA). The immobilized peroxidase activity was detected with an enhanced chemiluminescence system (Thermo Fisher Scientific or EMD Millipore, Darmstadt, Germany).

**Reverse transcription and quantitative PCR** (q**RT-PCR).**

qRT-PCR was carried out using the LightCycler 96 system (Roche, Basel, Switzerland) and Thunderbird SYBR qPCR (Toyobo) following the manufacturers’ directions. The levels of TNF-α mRNA were standardized for comparison with the level of β-actin mRNA in each sample. The sequences of the primers were as follows: mouse TNF-α forward: 5’-AGCACAGAAAGCATGATCCG-3’, mouse TNF-α reverse: 5’-CCCGAAGTTCAGTAGACAGAAGAG-3’, human TNF-α forward: 5’-CCTGCCCCAATCCCTTTATT-3’, human TNF-α reverse: 5’-CCCTAAGCCCCCAATTCTCT-3’, mouse ADAM17 forward: 5’-CAGTAGGGTTTTGCGACA-3’, mouse ADAM17 reverse: 5’-CTTATCCACACAGTGGACA-3’, mouse β-actin forward: 5’-CTGACTGACTACCTCATGAAGATCCT-3’, mouse β-actin reverse: 5’-CTTAATGTCACGCACGATTTCC-3’, human β-actin forward: 5’-CATGTACGTTGCTATCCAGGC-3’, human β-actin reverse: 5’-CTCCTTAATGTCACGCACGA-3’.

**Blood and synovial fluid collection.**

Synovial fluid was obtained during joint surgery from 20 patients with RA and 17 patients with OA, who had been admitted to Kyoto University Hospital. Written informed consent was obtained from all patients and the protocol was approved by the Ethics Committee of Kyoto University. All of the RA patients met 2010 ACR/EULAR classification criteria for RA. Peripheral blood samples were obtained and the level of CRP, ESR, RF, and ACPA was examined before surgery. DAS-28 was calculated according to the results of physical examinations and blood tests.[6]

**Detection of TNF-α and NRDC in culture media, cell extracts, and synovial fluid.**

TNF-α in culture media and cell extracts was measured by ELISA using mouse TNF-α Duoset (R&D Systems) or human TNF-α Duoset (R&D Systems) kits following the manufacturer’s protocol. For TNF-α detection in synovial fluid, Human TNF-alpha Quantikine ELISA Kit (R&D Systems) was used according to the manufacture’s protocol. Data were obtained using a Multiskan JX (Thermo Fisher Scientific). For NRDC detection, synovial fluid was treated with 0.5 mg/ml hyaluronidase (Wako) for 30 min at 37°C and then quantified by a chemiluminescent enzyme immunoassay as follows. The capture antibody (clone #231) was conjugated to glass beads, while the detection antibody (clone #304) was labeled with HRP. Then, 80 μl of each patient’s synovial fluid mixed with 60 μl of the reaction buffer was incubated with one antibody-coated glass bead for 14 min at 37°C. After washing, the bead was incubated with HRP-labeled antibody solution (5 μg/ml) for 14 min at 37°C, followed by washing. The bead was then incubated with the substrate solution for 1 min at 37°C. The HRP activity of the IC was measured as the luminescence intensity using a SphereLight 180 analyzer (Olympus, Tokyo, Japan). The values were compared with the luminescence intensity of known NRDC standards to determine the NRDC concentration in the synovial fluid.

**Intra-articular siRNA injection.**

Three different siRNA duplexes against mouse *Nrdc* were synthesized by Thermo Fisher Scientific (Stealth siRNA duplex oligoribonucleotides). The sequences of the siRNA duplexes were as follows: siRNA1 sense: 5’-GGAACACAUCUGGAAUUCUAGGAUU-3’, antisense: 5’-AAUCCUAGAAUUCCAGAUGUGUUCC-3’, siRNA2 sense: 5’-GGCUCACAGAGGACCUGGAGGUAAA-3’, antisense: 5’-UUUACUUCCAGGUCCUCUGUGAGCC-3’ and siRNA3 sense: 5’-GCUCAGCGUUCAUGUUGUUGGAUAU-3’, antisense: 5’-AUAUCCAACAACAUGAACGCUGAGC-3’. Stealth RNAi negative control duplex with medium GC content, the sequence of which is not publically available, was purchased from Thermo Fisher Scientific. Then, 25 nmol of siRNA duplexes and negative control were transfected to MEF cells, WEHI 13 VAR cells, and RAW 264.7 cells using Viromor Blue (Lipocalyx GmbH, Halle, Germany) according to the manufacturer’s protocol, and the silencing efficacy was checked 24 h after the transfection. SiRNA3 had the highest silencing efficacy. Then, 200 pmol of siRNA3 and negative control siRNA were mixed with Invivofectamin 3.0 (Thermo Fisher Scientific) according to the manufacturer’s protocol. Mice were anesthetized with pentobarbital and immobilized in a supine position. Their ankle joints were punctured anteriorly and anti-*Nrdc* siRNA was injected into the left joint and negative control siRNA was injected into the right joint.

**Statistical analysis.**

An unpaired two-tailed Welch’s *t* test and Spearman’s rank correlation test were performed using Prism (GraphPad Software, La Jolla, CA, USA) or Excel (Microsoft, Redmond, WA, USA).

1. Ohno M, Hiraoka Y, Matsuoka T, et al. Nardilysin regulates axonal maturation and myelination in the central and peripheral nervous system. *Nat Neurosci* 2009;**12**:1506-13. doi: 10.1038/nn.2438

2. Nishi K, Sato Y, Ohno M, et al. Nardilysin is Required for maintaining Pancreatic beta-Cell Function. *Diabetes* 2016;**65**:3015-27. doi: 10.2337/db16-0178

3. de Fougerolles AR, Sprague AG, Nickerson-Nutter CL, et al. Regulation of inflammation by collagen-binding integrins alpha1beta1 and alpha2beta1 in models of hypersensitivity and arthritis. *J Clin Invest* 2000;**105**:721-9. doi: 10.1172/JCI7911

4. Hiraoka Y, Matsuoka T, Ohno M, et al. Critical roles of nardilysin in the maintenance of body temperature homoeostasis. *Nat Commun* 2014;**5**:3224. doi: 10.1038/ncomms4224

5. Issuree PD, Maretzky T, McIlwain DR, et al. iRHOM2 is a critical pathogenic mediator of inflammatory arthritis. *J Clin Invest* 2013;**123**:928-32. doi: 10.1172/JCI66168

6. Prevoo ML, van 't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL. Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. *Arthritis Rheum* 1995;**38**:44-8.