Supporting Information

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SI Materials and Methods

Mice. The DNase $II^{-/-}IFN\text{-}IR^{-/-}$ and DNase $II^{flox/-}Mx1\text{-}Cre^{T}$ mice were described previously (1). To delete the DNase II gene, the 4- to 5-wk-old DNase $II^{flox/-}Mx1\text{-}Cre^{T}$ mice were given three times i.p. injections of poly(I):poly(C) at 0.8 μg/g bodyweight at 1-d intervals, and are referred to as DNase $II^{\Delta/-}$ mice. The induced deletion of the DNase II gene in the bone marrow or joint of the DNase $II^{\Delta/-}$ mice was confirmed by real-time PCR. The $TLR9^{-/-}$ (2), $Rag2^{-/-}$ (3), $IL\text{-}18^{-/-}$ (4), and $IL\text{-}6^{-/-}$ (5) mice on a B6 background were provided by S. Akira (Osaka University, Osaka), Y. Shinkai (Kyoto University, Kyoto), K. Nakanishi and H. Tsutsui (Hyogo College of Medicine, Nishinomiya, Japan), and M. Kopf (Eidgenössische Technische Hochschule, Zurich), respectively. $TNF\alpha^{-/-}$ (6) mice on a B6/129 mixed background were provided by K. Sekikawa (National Institute of Agrobiological Sciences, Tsukuba).

Antibodies. The following mAbs and reagents were used for staining in FACS: anti-CD3 (clone KT3), CD4 (clone GK1.5), CD8 (clone 53-6.7), CD11c (clone HL3), CD69 (clone H1.2F3), B220 (clone RA3-6BC), Gr-1 (clone RB6-8C5), Mac1 (clone M1/70), CD45.1 (clone A20), CD45.2 (clone 104), TER119 (clone TER119), Fcγ receptor III/II (clone 2.4G2), streptavidin-APC, and streptavidin-Alexa 488. All Abs and reagents were from BD Pharmingen or Biolegend.

Primers Used for Genotyping. Primers used for the mouse genotypes were as follows:

DNase II: WT sense, 5′-CAGTGCCACAGAGGACCACT-3′
mutant sense, 5′-GATTCGCAGCGCATCGCCTT-3′
common antisense, 5′-GAGTCTTAGTCCTTTGCTCCG-3′.

IFN-IR: WT antisense, 5′-AAGATGTGCTGTTCCCTTCCT-CTGCTCTGA-3′
mutant antisense, 5′-CCTGCGTGCAATCCATCTTG-3′
common sense, 5′-ATTATTAAAAGAAAAGACGAGGCG-AAGTGG-3′.

TLR9: WT antisense, 5′-GAGTCTTAGTCCTTTGCTCCG-3′
mutant antisense, 5′-ATCGCCTTCTATCGCCTTCTTGAC-GAG-3′
common sense, 5′-GCAATGGAAAGGACTGTCCACTTT-GTG-3′.

Mx1-Cre: sense, 5′-GTGGGCACAGCAAGCTCAGG-3′
antisense, 5′-TCCATGAGTGAACGAACCT-3′.
flox allele: sense, 5′-CGCACGTCTAAGAAACCATT-3′
antisense, 5′-CAGTACTAGTGAACCTCTTC-3′.
Δ allele: sense, 5′-TAGGGATACTCCAAAGGGAC-3′
antisense, 5′-CAGTACTAGTGAACCTCTTC-3′.

Rag2: WT antisense, 5′-CTGATTTCAATCGTGTTGTCCC-3′
mutant antisense, 5′-TGTGCCCAGTCATAGCCGAA-3′
common sense, 5′-GATTCCTGCTACCTCCCACCT-3′.

TNFα: WT antisense, 5′-AGATGGAGAAGGGCAGTTAG-3′
mutant antisense, 5′-GATTCGCAGCGCATCGCCTT-3′
common sense, 5′-ATACCAGGGTTTGAGCTCAG-3′.

IL-6: WT antisense, 5′-TTCTCATTTCCACGATTTCCCAG-3′
mutant antisense, 5′-CCGGAGAACCTGCGTGCAATCC-3′
common sense, 5′-TTCCATCCAGTTGCCTTCTTGG-3′.

IL-18: WT sense, 5′-GGCATTTGCGAGCTGCCAAATTCC-3′
mutant sense, 5′-TGTGCCCAGTCATAGCCGAA-3′
common antisense, 5′-CTTGTTGTGTCCTGGAACACG-3′.

Real-Time PCR and ELISA for MMP-3. The limbs, including the ankle joints, were separated from the skin and homogenized with a Polytron homogenizer in ISOGEN (Nippon Gene). Total RNA was prepared from the homogenates according to the manufacturer's instructions and purified using an RNeasy kit (Qiagen). RNA was reverse transcribed using SuperScript III (Invitrogen) with an oligo (dT) primer, and real-time PCR was carried out using a Light-Cycler480 (Roche Diagnostics). The specific mRNA was quantified at the upstroke of the exponential phase of PCR accumulation, compared with the standard curve, and normalized to the GAPDH or β-actin mRNA level. The serum MMP-3 level was determined with an ELISA kit from R&D Systems.

Primers Used for Real-Time PCR. Primers used for real-time PCR were as follows:

TNFα: sense primer, 5′-CACAGAAAGCATGATCCGCGA-CGT-3′
antisense primer, 5′-CGGCAGAGAGGAGGTTGACTTT-CT-3′.

TGFβ: sense primer, 5′-GACCGCAACAACGCCATCTA-3′
antisense primer, 5′-GGCGTATCAGTGGGGGTCAG-3′.

IL-1β: sense primer, 5′-CCAGCTTCAAATCTCACAGCAG-3′
antisense, 5′-CTTCTTTGGGTATTGCTTGGGATC-3′ or
sense primer, 5′-CTGATGAGAGCATCCAGCTTCA-3′
antisense, 5′-CTTCTTTGGGTATTGCTTGGGATC-3′.

IL-6: sense primer, 5′-TCCAGTTGCCTTCTTGGGAC-3′
antisense primer, 5′-GTACTCCAGAAGACCAGAGG-3′.

IL-10: sense primer, 5′-TGGCCCAGAAATCAAGGAGC-3′
antisense primer, 5′-CAGCAGACTCAATACACACT-3′.

IL-17A: sense primer, 5′-AACACTGAGGCCAAGGACTT-3′
antisense primer, 5′-ACCCACCAGCATCTTCTC G-3′.

DNase II: sense primer, 5′-GAGACGGTGATCAAGAAC-CAA-3′
antisense primer, 5′-AATTTTGCCAGAACTGGACCT-3′.

MMP-3: sense primer, 5′-ACTCTACCACTCAGCCAAGG-3′
antisense primer, 5′-TCCAGAGAGTTAGACTTGGTGG-3′.

β-actin: sense primer, 5′-TGTGATGGTGGGAATGGGT-CAG-3′
antisense primer, 5′-TTTGATGTCACGCACGATTTCC-3′.

GAPDH: sense primer, 5′-AACGACCCCTTCATTGAC-3′
antisense primer, 5′-TCCACGACATACTCAGCAC-3′.

G-CSF: sense primer, 5′-GTTGTGTGCCACCTACAA GC-3′
antisense primer, 5′-CCATCTGCTGCCAGATGGTGGT-3′.

CCL20: sense primer, 5′-AGAAGCAGCAAGCAACTACG-3′
antisense primer, 5′-ACATCTTCTTGACTCTTAGGC-3′.

FACS Analysis. Spleens were squashed between slide glasses and passed through a screen. To collect $CD11c^{+}$ cells, the debris trapped on the screen was dispersed in RPMI buffer, incubated with collagenase, and returned to the cell suspension. After a preincubation for 10 min at 4 °C with rat anti-Fcγ receptor III/II Ab, the cells were stained with Abs for 30 min at 4 °C in PBS containing 2% FCS, 0.05% NaN_3, and 0.1 mg/mL propidium iodide and analyzed by FACScalibur (BD Biosciences) using Cell Quest software (Quest Software).

Preparation of Synovial Cells. Synovial cells were prepared essentially as described (7). In brief, joint capsules were harvested from knee joints of 5-wk-old to 8-wk-old C57/BL6 mice with razor blade,

and attached muscles were removed. The tissues including synovial membrane were minced and incubated at 37 °C for 5 h with 2.0 mg/mL of collagenase D (Roche Diagnostics) and 2.0 mg/mL dispase (Gibco BRL) in serum-free DMEM. After passing through the dispersed cells through mesh, the cells were washed twice, seeded onto 96-well microtiter plates (8×10^4 cells per well), and cultured in αMEM supplemented with mouse M-CSF for 2–3 d.

Staining for Active Caspase 1. Staining for active caspase 1 was carried out using a FLICA Caspase 1 detection kit (Immunochemistry Technologies) according to the manufacturer's instructions. In brief, the cell suspension from mouse bone marrow was treated with FAM-YVAD-FMK and Hoechst. After washing, the cells were fixed, cytospun, and observed by fluorescence microscopy (BioRevo BZ-9000, Keyence).

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Fig. S1. Accumulation of DNA in the bone marrow but not the joints of DNase II-null mice. (*A*) The DNase II$^{\Delta/-}$, DNase II$^{\Delta/-}$TNFα$^{-/-}$, and DNaseII$^{\Delta/-}$IL-6$^{-/-}$ mice were generated by injecting poly(I):poly(C). Six weeks after the injection, their bone marrow sections were stained with H&E. (Scale bar, 50 μm.) (*B*) Joint sections from 2- and 12-mo-old DNase II$^{-/-}$IFN-IR$^{-/-}$ mice were stained with H&E. (Scale bar, 50 μm.)

Fig. S2. IL-18–independent arthritis. (*A*) Arthritis scores of the DNaseII$^{\Delta/-}$IL-18$^{+/+}$, DNaseII$^{\Delta/-}$IL-18$^{+/-}$, and DNaseII$^{\Delta/-}$IL-18$^{-/-}$ mice (n = 3–12 for each genotype) after the injection of poly(I):poly(C) are plotted with SD. (*B*) A section from the joint of a DNaseII$^{\Delta/-}$IL-18$^{-/-}$ mouse 10 mo after the poly(I):poly(C) injection stained with H&E. (Scale bar, 0.1 mm.) (*C*) Real-time PCR analysis of the joint RNA from WT, DNaseII$^{\Delta/-}$IL-18$^{+/+}$, DNaseII$^{\Delta/-}$IL-18$^{+/-}$, and DNaseII$^{\Delta/-}$IL-18$^{-/-}$ mice 9–10 mo after the poly(I):poly(C) injection for the indicated mRNAs. The mRNA level is expressed relative to the GAPDH mRNA, and the mean value is indicated by horizontal bars. *P* values are shown.

Fig. S3. Arthritis in mice with BALB/c background. (*A*) Macroscopic view of the fore and hind paws of DNase II$^{-/-}$IFN-IR$^{-/-}$ or control (DNase II$^{+/-}$IFN-IR$^{-/-}$ littermates) BALB/c mice at the age of 16 mo. (*B*) Average arthritis scores with SD (n = 3 for each group). *indicates that P value was <0.05. (*C*) RNA was prepared from the joints of 16-mo-old DNase II$^{-/-}$IFN-IR$^{-/-}$ mice or control BALB/c mice, and the indicated mRNA level was quantified by real-time PCR. Values are expressed relative to the GAPDH mRNA level, and the mean value is indicated by horizontal bars. P values are shown.

Fig. S4. A model for the development of arthritis. In the affected joints, synoviocytes, macrophages, and fibroblasts make up the pannus-like structure. A set of inflammatory cytokines (at least TNFα, IL-6, and IL-1β) produced in these cells activate each other's gene expression as well as its own, establishing a "cytokine storm." These cytokines stimulate the fibroblasts and macrophages to express chemokines and colony-stimulating factors to recruit neutrophils and lymphocytes to the joints. They also stimulate the MMP-3 gene expression to destroy the tissue matrix and enhance the maturation of osteoclasts to destroy bones.

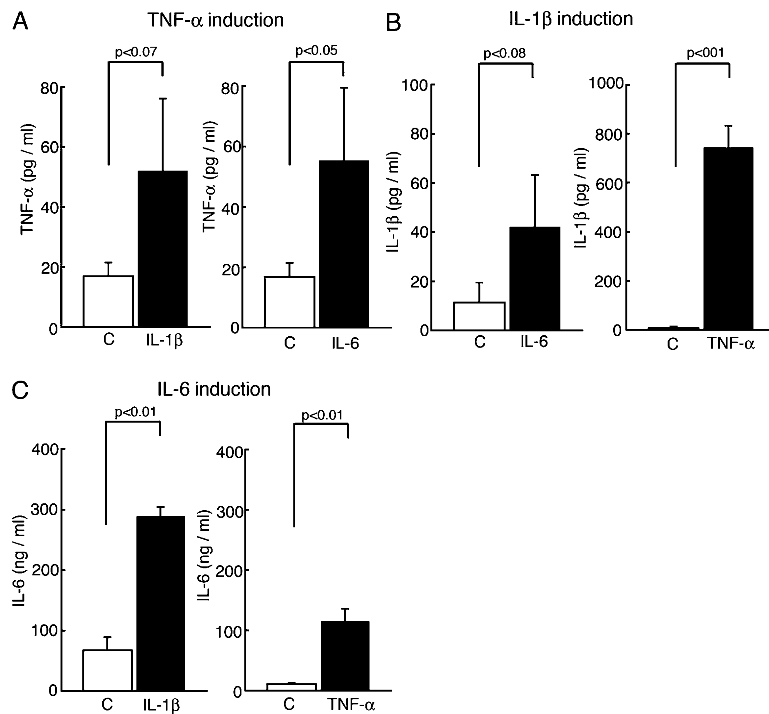

Fig. S5. Mutual activation of TNFα, IL-1β, and IL-6 genes in synovial cells. Synovial cells in 96-microtiter plates were treated in triplicate in αMEM with or without 20 ng/mL of mouse IL-1β (Peprotech) or 25 ng/mL of mouse IL-6 (BD Bioscience) plus 25 ng/mL of human IL-6Rα (R&D Systems) for 24 h (*A*), with or without 15 ng/mL of mouse TNFα (Peprotech) or 25 ng/mL of mouse IL-6 plus 25 ng/mL of human IL-6Rα for 23 h followed by stimulation with 6.5 mM ATP for 1 h (*B*), or with or without 15 ng/mL of mouse TNFα or 20 ng/mL of mouse IL-1β for 10 h (*C*). The levels of TNFα (*A*), IL-1β (*B*), and IL-6 (*C*) in the supernatants were determined by ELISA using kits from BD Bioscience (for TNFα and IL-6) and R&D Systems (for IL-1β), and are plotted with SD. *P* values are shown.

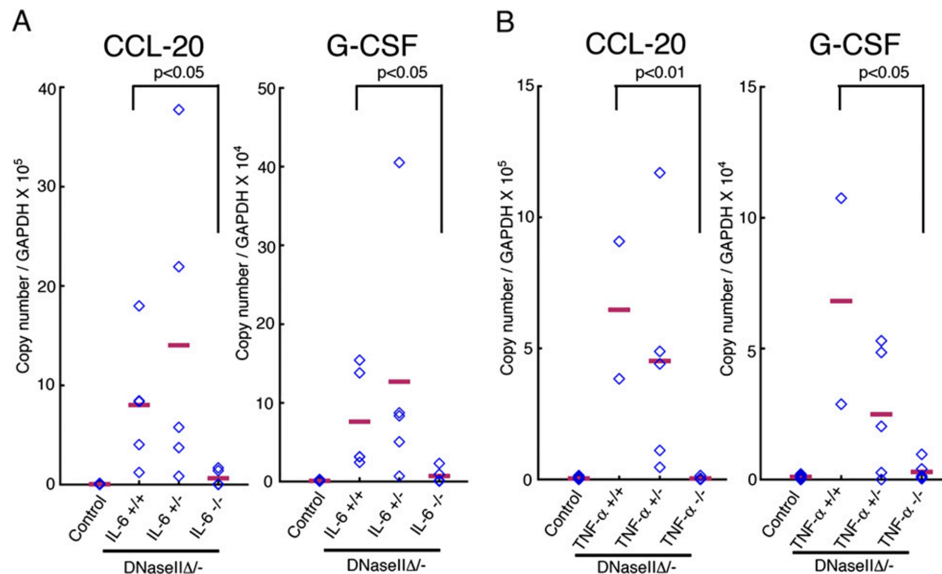

Fig. S6. Expression of CCL-20 and G-CSF genes in the joints of DNase II-null mice. DNaseII$^{\Delta/-}$IL-6$^{-/-}$ (*A*) and DNase II$^{\Delta/-}$TNFα$^{-/-}$ mice (*B*) were generated by treating with poly(I):poly(C). Nine to 12 mo after the treatment, the mRNAs for CCL-20 and G-CSF in the joints were quantified by real-time PCR and are expressed relative to the GAPDH mRNA level. Values are expressed relative to the GAPDH mRNA level, and the mean value is indicated by horizontal bars. *P* values are shown.

Fig. S7. Activation of inflammation in the bone marrow of DNase II-null mice. (*A*) Up-regulation of the TNFα gene in DNase II-null bone marrow. RNA was prepared from the bone marrow of 2-mo-old control, DNase II$^{-/-}$IFN-IR$^{-/-}$Rag2$^{+/-}$, DNase II$^{-/-}$IFN-IR$^{-/-}$Rag2$^{-/-}$, DNase II$^{-/-}$IFN-IR$^{-/-}$TLR9$^{+/-}$, and DNase II$^{-/-}$IFN-IR$^{-/-}$TLR9$^{-/-}$ mice, as well as DNaseII$^{\Delta/-}$IL-6$^{+/-}$ and DNaseII$^{\Delta/-}$IL-6$^{-/-}$ mice, 6 wk after the poly(I):poly(C) injection. TNFα mRNA was quantified by real-time PCR and expressed relative to the β-actin mRNA level. *P* values are shown. (*B*) Activation of caspase 1 in macrophages carrying undigested DNA. Bone marrow macrophages from 2-mo-old DNase II$^{-/-}$IFN-IR$^{-/-}$ mice were stained with carboxyfluorescein-YVAD-fluoromethyl ketone (FAM-YVAD-FMK, ImmunoChemistry Technologies), and counterstained with Hoechst. (Scale bar, 20 μm.)

Fig. S8. TLR9-independence of arthritis in DNase II-null mice. (*A*) Arthritis scores for DNase II$^{-/-}$IFN-IR$^{-/-}$TLR9$^{+/+}$, DNase II$^{-/-}$IFN-IR$^{-/-}$TLR9$^{+/-}$, and DNase II$^{-/-}$IFN-IR$^{-/-}$TLR9$^{-/-}$ mice at the indicated time were plotted as means with the SD (n = 5–7 for each group). (*B*) A joint section from 13-mo-old DNase II$^{-/-}$IFN-IR$^{-/-}$TLR9$^{-/-}$ mice were stained with H&E. (Scale bar, 0.1 mm.) (*C*) RNAs were prepared from the joints of 12–14-mo-old WT, DNase II$^{-/-}$IFN-IR$^{-/-}$TLR9$^{+/+}$, DNase II$^{-/-}$IFN-IR$^{-/-}$TLR9$^{+/-}$, and DNase II$^{-/-}$IFN-IR$^{-/-}$TLR9$^{-/-}$ mice. The indicated mRNA levels were quantified by real-time PCR and are expressed relative to the level of GAPDH mRNA. Horizontal bars indicate the mean values. *P* values are shown.