

SUPPLEMENTARY MATERIAL

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SUPPLEMENTARY METHODS

SKG mice and arthritis induction

We used SKG mice with a point mutation in the ZAP-70 gene, which predisposes them to develop autoimmune arthritis under nonspecific pathogen-free conditions.

The causative factor has been identified as a point mutation (W163C) in the SH2 domain of the Zap70 gene. This mutation alters the interaction between ZAP70 and the ITAMs of the CD3 ζ chain, thereby attenuating T-cell receptor (TCR) signal transduction. The resulting reduction in TCR signaling modifies thymic selection thresholds, permitting self-reactive T cells to escape negative selection in the thymus and populate the periphery. Arthritis or inflammatory conditions were induced via the intraperitoneal (IP) injection of 2mg zymosan (Sigma–Aldrich, Japan). Phosphate-buffered saline (PBS) was IP into SKG mice as a “Control”. Arthritis was scored every 3–4 days according to a previous study ¹.

***In vivo* treatment**

Baricitinib was purchased from ChemScene LLC, USA, and was dissolved in 0.5 w/v% Methyl Cellulose 400 Solution (Sterilized, FUJIFILM Wako Pure Chemical Corporation). Recombinant murine (rm-) GM-CSF (#315–03) and rm-IL-6 (#216–

16) were purchased from PeproTech. Purified anti-mouse GM-CSF antibody (clone: MP1-22E9) and the rat IgG2a, κ , isotype (clone: RTK2758) were purchased from Biolegend. In the arthritis treatment experiment, age-matched female SKG mice received IP injections of zymosan. At week 5, SKG mice with arthritis scores of 4 or higher were allocated to either the baricitinib treatment group or the methyl cellulose (control) group, ensuring an average arthritis score of 4 points for SKG mice in each group. Baricitinib or methyl cellulose was administered orally daily until week 8. For the arthritis prevention experiment, age-matched female SKG mice were injected intraperitoneally with 2 mg zymosan. Baricitinib or methyl cellulose was orally administered daily from week 2 until week 5. In the subsequent additional experiment with rm-GM-CSF or IL-6, age-matched female SKG mice, previously injected with 2 mg zymosan, were administered rm-GM-CSF (2 μ g) or IL-6 (2 μ g) or PBS every 2 d for a total of 4 injections starting from week 4. In an additional experiment involving anti-GM-CSF antibody treatment, age-matched female SKG mice, previously injected with zymosan, were administered anti-GM-CSF antibody (100 μ g) and a rat IgG2a, κ , isotype (100 μ g) every 7 days beginning at week 2 (4 times).

Magnetic resonance imaging of femur osteitis and data analysis

All animal procedures for magnetic resonance imaging (MRI) experiments were conducted in accordance with the guidelines for animal experiments of Kyoto University. All MRI experiments were performed at the Medical Research Support Center, Graduate School of Medicine, Kyoto University. After the induction of anesthesia with 2.5% isoflurane in air, the mice were placed prone on the animal cradle. Anesthesia was maintained via the inhalation of 1.5–3.5% isoflurane in air at a flow rate of 1 L/min through a face mask. The leg of each mouse was fixed to the cradle via surgical tape. The respiratory rate of the mice and rectal temperature were continuously monitored via a dedicated system (Model 1025, MR-compatible Small Animal Monitoring & Gating System; SA Instruments, Inc., NY, USA) and software (PC-SAM V8.04; SA Instruments). The body temperature was maintained by a flow of warm air via a heater system (MR-compatible Small Animal Heating System; SA Instruments). MRI scanning was performed on a 7 Tesla preclinical scanner (BioSpec 70/20 USR, Bruker BioSpin MRI GmbH, Ettlingen, Germany). A circular polarized volume coil with an inner diameter of 35 mm (T9988, Bruker BioSpin) was used for signal transmission and reception. MRI data were acquired with ParaVision 5.1 software (Bruker BioSpin). Water–fat separation imaging ² was conducted. For this purpose, we modified a rapid

acquisition with relaxation enhancement (RARE) sequence for incorporating the Dixon technique ³ and implemented it in ParaVision 5.1. The two-point asymmetric Dixon method in the RARE sequence was used in the present study ⁴. The acquisition details were as follows: echo time (TE): 11 ms, effective TE: 33 ms, RARE factor: 8, repetition time (TR): 3500 ms, field of view (FOV): 30 mm × 30 mm, matrix size: 256 × 256, inplane spatial resolution: 0.117 mm × 0.117 mm, number of slices: 12, slice thickness: 0.5 mm, number of averages (NA): 8, scan time: > 14 m 56 s, respiration triggering per slice. For the out-of-phase image, an angle of 140 degrees between fat and water magnetization was chosen, resulting in an echo time shift of 0.37 ms ². Water and fat images were obtained from the two complex images with (0-degree, 140-degree) sampling by via a method proposed by Xiang ⁴. All the data were processed via custom-written scripts in MATLAB (MathWorks, MA, USA). The assessment of osteitis was performed according to previous literature ⁵. Water images were analysed via Image J software. Regions of interest (ROIs) measuring 1 mm² (width: 9, height: 9) were drawn at the distal femoral bone marrow and at the center of the gastrocnemius muscle in three consecutive slices. The osteitis score was calculated by dividing the average ROI intensity of the femoral bone marrow by that of the gastrocnemius muscle.

Micro quantitative computed tomography (micro-CT) analysis

Micro-CT analyses of mouse distal femurs were performed via a micro-CT scanner (SkyScan 1275; Bruker, Billerica, MA) with a voxel size of 7.0 μm . The X-ray excitation voltage was set to 50 kV, and the current was 60 mA, without any filters⁶. The X-ray projections were obtained at 0.1-degree intervals with a scanning angular rotation of 360 degrees and four frames were averaged for each rotation. Three-dimensional reconstructions were performed via NRecon software (Bruker). Bone parameters were calculated via CTAn software (Bruker) on a volume of interest of 151 slices, each containing 75 slices distal and proximal to the center of the growth plate, for which a region of interest of trabecular bone was drawn, manually excluding cortical bone.

Histological assessment

For histological assessment, the mice were culled and their femur bones were fixed in 10% formalin at the experimental endpoints and were decalcified with 5% neutral buffered EDTA (pH 7.2) for 4 weeks before being embedded in paraffin. The paraffin-embedded blocks were cut into 4- μm sections and stained with hematoxylin and eosin (H&E) (Muto Pure Chemical Co., Ltd.). For the H&E

staining, tissue sections were stained with hematoxylin for 15 min followed by staining eosin for 1 min. To evaluate osteoclast formation, tartrate-resistant acid phosphatase (TRAP) staining was performed. For the TRAP staining, tissue sections were fixed with 10% glutaraldehyde solution for 15 min at 37 °C, and then they were covered with approximately 300 µL of TRAP staining solution for 5 to 10 min at 37 °C, followed by counterstaining with a Hoechst stain for 10 min at room temperature. A 10% glutaraldehyde solution was prepared by adding 40 mL of glutaraldehyde stock solution (25% aqueous solution) to 60 mL of H₂O. The TRAP staining mixture was prepared by dissolving 0.3 mg of Fast Red Violet LB stain per mL of TRAP buffer. TRAP buffer was prepared by mixing acetate buffer (0.1 M, 50 mL), sodium tartrate (0.3 M, 10 mL), naphthol AS-MX phosphate disodium salt (10 mg/mL, 1 mL), and Milli-Q water (39 mL). Acetate buffer (0.1 M) was prepared by mixing sodium acetate solution (0.2 M, 35.2 mL), acetic acid solution (0.2 M, 14.8 mL), and water (50 mL). Sodium acetate solution (0.2 M) was prepared by adding 16.4 g of sodium acetate per liter of H₂O. An acetic acid solution (0.2 M) was prepared by adding 11.5 mL of glacial acetic acid to 988.5 mL of H₂O. Sodium tartrate (0.3 M) was prepared by adding 6.9 g of sodium tartrate per 100 mL of H₂O. Naphthol AS-MX phosphate disodium salt (10 mg/mL) was prepared by

adding 10 mg of phosphate disodium salt per mL of H₂O. All the solutions were purchased from Sigma Aldrich. TRAP-positive cells are red in color.

Cell isolation from BM

SKG mice were sacrificed via decapitation under deep anesthesia with isoflurane inhalation and their femurs were isolated and flushed with D-PBS. The bone marrow cells were harvested in RPMI 1640 tissue medium (Thermo Fisher Scientific) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 50 U/mL penicillin, and 50 µg/mL streptomycin and passed through a 70-µm filter. The single-cell suspension was subjected to centrifugation (2 min, 1000 × g, 4 °C), followed by resuspension in 2 mL of red blood cell lysis solution for 2 min at room temperature. Lysis was stopped by the addition of 10 mL of RPMI 1640 tissue medium supplemented with 10% heat-inactivated FBS, 50 U/mL penicillin, and 50 µg/mL streptomycin. The cells were subsequently centrifuged (2 min, 1000 × g, 4 °C), resuspended in 1 mL of RPMI 1640 tissue medium and were counted. Before flow cytometry and fluorescence-activated cell sorting analysis, the cells were subjected to cell counting.

Flow cytometry and fluorescence-activated cell sorting (FACS)

Single-cell suspensions from femur BM were prepared as outlined above. A total of $1-2 \times 10^6$ cells were blocked with anti-CD16/32 antibodies for 15 min in the dark at 4 °C. (BioLegend, CA, USA) (except for CD16/32 staining). The extracellular antigens were then stained for 15 min in the dark at 4 °C in FACS buffer (D-PBS supplemented with 2% FBS and 2 mM EDTA). For intracellular antigens, the cells were fixed and permeabilized for 30 min at room temperature via fixation/permeabilization buffer concentrate (Invitrogen, REF:00-5123-43) mixed with diluent (Invitrogen, REF:00-5223-56) at a 1:3 ratio, and FcγR blockade with anti-CD16/32 antibodies, followed by staining for intracellular cytokines. Flow cytometric data acquisition and cell sorting (>95% purity) were carried out via the FACS Melody cell sorter system (BD Biosciences). Analysis was performed via FlowJo v10.8.1 (<https://www.flowjo.com/>). Each cell subset was distinguished by comparison with its isotype control. The antibody sets, used for flow cytometry, are shown in Supplementary Table S4.

Real-time quantitative PCR (RT-qPCR)

The bone marrow cells from femur bone were lysed, and the RNA was extracted via the RNeasy Micro Kit (Qiagen) and converted into cDNA via the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems) via Mastercycler

(Eppendorf). To analyze the gene expression of JAK-STAT signaling-related cytokines, we used quantitative RT-PCR, following a previously described method

7. The following primers were purchased from Applied Biosystems: *Csf2*

(Mm01290062_m1), *Csf2ra* (Mm00438331_g1), *Il6* (Mm00446190_m1), *Irf9*

(Mm00492679), *Ifng* (Mm01168134_m1), and *Hprt* (Mm01545399_m1). To analyze

osteoclast-related genes, RT-qPCR was performed on the basis of a previous report

6. Briefly, cDNA was synthesized with the ReverTra Ace qPCR RT Master Mix

(TOYOBO, Osaka, Japan). RT-qPCR was conducted in a Step One Plus system

(Applied Biosystems, Foster City, CA) with the THUNDERBIRD® SYBR qPCR

Mix (TOYOBO). The primer sequences are presented in Supplementary Table S5.

Gene expression was calculated via the comparative $\Delta\Delta C_t$ method and normalized

to the housekeeping gene, *Hprt*.

Mouse bone marrow cell osteoclast differentiation assay

Bone marrow cells isolated from femur bone were seeded in a 96-well plate

(0.2×10^5 cells/well) and cultured for 4 days in alpha modified essential medium

(SIGMA, St. Louis, USA) containing 10% FBS (MP Biomedicals, Irvine, USA), 1%

penicillin-streptomycin (Nacalai Tesque, Kyoto, Japan), and 1% L-glutamine

(Nacalai Tesque) supplemented with 30 ng/mL M-CSF (PeproTech, Rocky Hill,

USA) to generate bone marrow-derived macrophages (BMMs). BMMs were additionally cultured for 5 to 6 d and supplemented with 30 ng/mL M-CSF and 100 ng/mL RANKL in the absence or presence of 0.1, 1, or 10 ng/mL rm-GM-CSF (PeproTech, Rocky Hill, USA), or 0.1, 1, or 10 μ M baricitinib (ChemScene LLC, USA). The culture medium was changed to fresh medium and cytokines were supplemented every other day. TRAP staining was conducted via a TRAP staining kit from Sigma–Aldrich, following the guidelines provided by the manufacturer. TRAP-positive osteoclasts were counted under a BZ-X800L microscope (Keyence).

Human monocyte-derived osteoclast differentiation assay

Peripheral blood mononuclear cells (PBMCs) from healthy donors were obtained according to a protocol approved by the ethics committee of Kyoto University Graduate School and the Faculty of Medicine (approved number: R1166-3). The CD14⁺ monocytes were obtained from PBMCs via the MACS system with CD14 MicroBeads (Miltenyi Biotec, Bergisch Gladbach, Germany). CD14⁺ monocytes from healthy individuals were cultured in α modified essential medium (SIGMA, St. Louis, USA) with 10% FBS (MP Biomedicals, Irvine, USA), penicillin–streptomycin (Nacalai Tesque, Kyoto, Japan), and L-glutamine (Nacalai Tesque) supplemented with 20 ng/mL M-CSF (PeproTech, Rocky Hill, USA) overnight to

obtain osteoclast precursors (OCPs). For osteoclast differentiation, OCPs were cultured with 20 ng/mL M-CSF and 40 ng/mL RANKL (PeproTech, Rocky Hill, USA) for 4 days, after which human GM-CSF (PeproTech, Rocky Hill, USA) was added in a dose-dependent manner. The cells were fixed with 4% paraformaldehyde. TRAP staining was conducted via a TRAP staining kit from Sigma–Aldrich, following the guidelines provided by the manufacturer. TRAP-positive osteoclasts were counted under a BZ-X800L microscope (Keyence).

Public database analysis

The associations between genetic variants near *CSF2* (GM-CSF gene) and the risk of RA were analyzed via the largest ever genome-wide association study ⁸. To identify the causal gene, we used an optimized SNP-to-gene approach ⁹. We further tested the effects of this SNP on hematological traits ¹⁰. The region plot was generated via LocusZoom ¹¹. To compare the expression of GM-CSF-related genes from human RA patients, we utilized public data of RA (n = 9) and osteoarthritis (n = 10) patients ¹² via limma (ver. 3.46.0) ¹³. The GM-CSF score was calculated as the average expression level of 36 genes defined in the Pathway Interaction Database ¹⁴ under the identifier “PID_GMCSF_PATHWAY” (Supplementary Table S4). The granulocyte–macrophage progenitor score was calculated as the average expression

level of 16 genes exhibiting increased expression in progenitors according to a previous study ¹⁵ (Supplementary Table S5).

Statistical analysis

Statistical analysis was performed via Prism 9.0 (GraphPad Software Inc., San Diego, CA, USA). The nonparametric Mann–Whitney test was used to compare two groups. The Kruskal–Wallis test and post-hoc Dunn's multiple comparison test were performed for multiple-group comparisons. Simple linear regression was used to investigate the relationships between two continuous variables. A P value less than 0.05 was considered to indicate statistical significance.

SUPPLEMENTARY TABLES

Supplementary Table S1. Associations between rs657075 and the risk of RA

development and hematological traits

SNPID	REF	ALT	Trait	BETA	SE	P value
rs657075	G	A	Rheumatoid arthritis	0.07	0.01	4.50×10^{-7}
			White blood cell count	1.65×10^{-3}	4.47×10^{-3}	0.71
			Red blood cell count	-1.02×10^{-3}	4.47×10^{-3}	0.82
			Platelet count	1.08×10^{-3}	4.46×10^{-3}	0.81
			Neutrophil count	-1.49×10^{-3}	0.01	0.80
			Eosinophil count	0.02	0.01	1.24×10^{-3}
			Basophil count	0.01	0.01	0.32
			Monocyte count	0.01	0.01	0.08
			Lymphocyte count	4.44×10^{-3}	0.01	0.46

Beta is based on the ALT allele.

Supplementary Table S2. GM-CSF-related gene set

GM-CSF-related genes			
CSF2RA	GAB2	CSF2	STAT5A
YWHAZ	PRKACB	IKBKB	PIK3CA
PIK3R1	CCL2	KRAS	JAK2
NRAS	STAT5B	STAT1	PRKACG
CSF2RB	OSM	HRAS	PTPN11
MAPK3	GRB2	STAT3	SYK
MAP2K2	PIM1	PRKACA	MAP2K1
MAPK1	LYN	RAF1	SOS1
FOS	CISH	SHC1	IRF8

Supplementary Table S3. Myeloid progenitor-related gene set

Myeloid progenitor-related genes			
HOPX	SCOC	FLT3	EIF4E3
ITGA4	SRP72	TMED2	ITGA6
ITGB1	EIF2S3	TMPO	OSM
NR1D2	FKBP5	CXCL8	PICALM

Supplementary Table S4. Information regarding the antibodies used

Target	Fluorochrome	Company	Isotype	Clone	Dilution
CD3	FITC	Biolegend	Rat IgG2b, κ	17A2	1:100
CD11b	FITC	Biolegend	Rat IgG2b, κ	M1/70	1:100
CD45R/B220	FITC	Biolegend	Rat IgG2a, κ	RA3-6B2	1:100
Gr-1	FITC	Biolegend	Rat IgG2b, κ	RB6-8C5	1:100
TER-119	FITC	Biolegend	Rat IgG2b, κ	TER-119	1:100
CD11b	PE	BD	Rat IgG2b, κ	M1/70	1:100
CD34	PE	BD	Rat IgG2a, κ	RAM34	1:100
CD138	PE	Biolegend	Rat IgG2a, κ	281-2	1:100
GM-CSF	PE	Biolegend	Rat IgG a κ	MP1-22E9	1:100
GM-CSF Rα	PE	BD	Rat IgG A	698423	1:100
IL-6	PE	Biolegend	Rat IgG1, κ	MP5-20F3	1:100
CD117 (c-kit)	PE-Cy7	Biolegend	Rat IgG2b, κ	2B8	1:100
CD267 (TACI)	APC	Invitrogen	Rat IgG2a	ebio8F10-3	1:100
Ly6A/E (Sca-1)	APC	Biolegend	Rat IgG2a, κ	D7	1:100
CD16/32	APC-Cy7	Biolegend	Rat IgG2a, λ	93	1:100
CD48	APC-Cy7	Biolegend	Arm Ham IgG	HM48-1	1:100
Ly6C	APC/Cy7	Biolegend	Rat IgG2c, κ	HK1.4	1:100
CD150	PerCP-Cy5.5	Biolegend	Rat IgG2a, λ	TC15-12F12.2	1:100
Ly6G	PerCP/Cy5.5	Biolegend	Rat IgG2a, κ	1A8	1:100

Supplementary Table S5. Sequences of the primers used

Gene	Primer	Sequence (5'→3')
Mouse <i>Hprt</i>	Forward	TCC TCA GAC CGC TTT TTG CC
	Reverse	CTA ATC ACG ACG CTG GGA CT
Mouse <i>NFATc1</i>	Forward	CCC GTC ACA TTC TGG TCC AT
	Reverse	CAA GTA ACC GTG TAG CTC CAC AA
Mouse <i>CtsK</i>	Forward	AAG ATA TTG GTG GCT TTG G
	Reverse	ATC GCT GCG TCC CTC T
Mouse <i>TRAP</i>	Forward	ACG GCT ACT TGC GGT TTC
	Reverse	TCC TTG GGA GGC TGG TC

SUPPLEMENTARY FIGURES LEGEND

Supplementary Fig S1. Skewed myelopoiesis can be observed in the inflammatory bone marrow (BM). (A) Total counts of BM cells extracted from one femur of each mouse. (B) Gating strategy for LT-HSCs: long-term hematopoietic stem cells (Lin^- , Sca-1^+ , c-Kit^+ , CD34^- , CD150^+ , CD48^-), ST-HSCs: short-term hematopoietic stem cells (Lin^- , Sca-1^+ , c-Kit^+ , CD34^+ , CD150^+ , $\text{CD48}^{+/+}$), MPPs: multi-potent progenitors (Lin^- , Sca-1^+ , c-Kit^+ , CD34^+ , CD150^- , CD48^+) among Lineage^- , Sca-1^+ , c-Kit^+ (LSK) population, and GMPs: granulocyte–monocyte progenitors (Lin^- , Sca-1^- , c-Kit^+ , CD34^+ , $\text{CD16/32}^{\text{high}}$) and MEPs: megakaryocyte–erythroid progenitors (Lin^- , Sca-1^- , c-Kit^+ , CD34^- , CD16/32^-) via representative images. (C) Schematic illustrating the hierarchy of hematopoietic stem cells, progenitor cells, and granulocyte–monocyte lineage cells in the BM. (D) Staining of BM cells showing the frequencies of progenitor cells (Sca-1^- , cKit^+) and LSK cells (Sca-1^+ , cKit^+) among Lin^- cells. The graphs display the frequencies of LSKs (LSK , CD34^+ , CD150^- , CD48^+) within the total BM population. (E-F) Staining of bone marrow cells showing the frequencies of (E) ST-HSCs and (F) LT-HSCs among the Lin^- Sca-1^+ c-Kit^+ CD34^- and Lin^- Sca-1^+ c-Kit^+ CD34^+ populations, respectively. Lin^- ; Lineage^- ($\text{CD3/TER-119/B220/Gr-1/CD11b}$) negative. The graphs display the frequencies of ST-HSCs and LT-HSCs

within the total BM population. (G) BM staining showing frequencies of GMPs (Lin⁻, Sca-1⁻, c-Kit⁺, CD34⁺, CD16/32^{high}) and MEPs (Lin⁻, Sca-1⁻, c-Kit⁺, CD34⁻, CD16/32⁻) among progenitor cells. Graphs display frequencies of GMPs and MEPs within the total BM population. Lin⁻: Lineage (CD3/TER-119/B220/Gr-1/CD11b) negative. (H-K) BM staining showing the T cells (CD3⁺), B cells (B220⁺), plasma cells (TACI⁺, CD138⁺), and erythroid cells (TER-119⁺) among live single cells. The graphs display the frequencies of T cells, B cells, plasma cells, and erythroid cells within the total BM population. The data are the means $\pm$ SDs. Each data point represents an independent biological sample. Groups were compared via the Mann–Whitney tests (a, c, d). *P < 0.05; **P < 0.01. ns, not significant.

Supplementary Fig S2. Effects of baricitinib on zymosan-SKG mice. (A)

Comparison of arthritis scores among groups treated with various concentrations of baricitinib or vehicle. The groups were adjusted to achieve an average arthritis score of 2 at week 5, followed by treatment once daily for three weeks. (B) Bone marrow (BM) staining showing the frequencies of LSK cells (Lin⁻, Sca-1⁺, c-Kit⁺) and multi-potent progenitors (MPPs) (LSK, CD34⁺, CD150⁻, CD48⁺) among Lin⁻ cells. The graphs display the frequencies of LSK cells and MPPs within the total BM population. Lin⁻: Lineage (CD3/TER-119/B220/Gr-1/CD11b) negative. (C) BM

staining showing the frequencies of granulocyte–monocyte progenitors (GMPs) (Lin⁻, Sca-1⁻, c-Kit⁺, CD34⁺, CD16/32^{high}) and megakaryocyte–erythroid progenitors (MEPs) (Lin⁻, Sca-1⁻, c-Kit⁺, CD34⁻, CD16/32⁻) among progenitor cells. The graphs display the frequencies of GMPs and MEPs within the total BM population. (D) Experimental schema of the baricitinib treatment experiment. (E) Comparison of arthritis scores between the baricitinib-treated and vehicle control groups. (F) Representative T2 fat-suppressed MR images and osteitis scores of femurs from the baricitinib-treated and vehicle control groups at week 5. (G) Representative micro-CT images of femurs in the baricitinib-treated and vehicle control groups at week 5. (H) Micro-CT quantification of femoral trabecular bone mass, including trabecular bone volume/total volume (BV/TV), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), and bone mineral density (BMD), in baricitinib-treated and vehicle control groups at week 5. The data are the means ± SDs. Each data point represents an independent biological sample. Groups were compared via multiple Mann–Whitney test (E) and Mann–Whitney test (B, F, H). *P < 0.05; ns, not significant.

Supplementary Fig S3. Effects of granulocyte–macrophage colony-stimulating factor (GM-CSF) on the development of osteitis and bone microstructural changes

(BMCs) under inflammatory conditions. (A) Representative bone marrow (BM) staining of GM-CSF⁺, GM-CSF receptor (GM-CSFR)⁺, IL-6, and TNF α -positive cells among live cells in the osteitic BM at week 5. (B) mRNA expression of *Irf9* (left) and *Ifng* (middle), and *Trfaip3* (right) in BM cells from zymosan-SKG mice at week 5. (C) Representative T2 fat-suppressed MR images and osteitis scores of femurs in the rm-IL-6⁻ and phosphate buffered saline (PBS)-treated groups at week 5. (D) Representative micro-CT images of femurs from the rm-IL-6⁻ and PBS-treated groups at week 5. (E) Micro-CT quantification of femoral trabecular bone mass, including trabecular bone volume/total volume (BV/TV), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), and bone mineral density (BMD), in the rm-IL-6⁻ and PBS-treated groups at week 5. (F) BM staining showing the granulocytes (Ly6G⁺) and monocytes (Ly6C⁺, CD11b⁺) among Lin⁻ lineage (CD3/B220/TER-119)⁻ negative and (Lin⁻, Ly6G⁻) cells, respectively. The data are presented as the means $\pm$ SDs. Each data point represents an independent biological sample. Groups were compared via Mann–Whitney test. ns, not significant.

Supplementary Fig S4. Effects of granulocyte–macrophage colony-stimulating factor (GM-CSF) on the development of osteitis and bone microstructural changes (BMCs) under noninflammatory conditions. (A) Experimental schema for

administering rm-GM-CSF or phosphate buffered saline (PBS) to nonarthritic SKG mice (noninflammatory conditions). (B) Comparison of arthritis scores between PBS and rmGM-CSF-treated non zymosan-SKG mice. (C, D) Representative T2 fat-suppressed MR images and osteitis scores of femurs in the rm-GM-CSF- and PBS-treated groups at week 1. (E) Representative micro-CT images of femurs from the rm-GM-CSF- and PBS-treated groups at week 1. (F) Micro-CT quantification of trabecular bone mass in femurs, including trabecular bone volume/total volume (BV/TV), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), and bone mineral density (BMD), in the rm-GM-CSF- and PBS-treated groups at week 1. The data are the means $\pm$ SDs. Each data point represents an independent biological sample. Groups were compared via Mann–Whitney test. * $P < 0.05$; ns, not significant.

Supplementary Fig S5. Anti-granulocyte-macrophage colony-stimulating factor

(anti-GM-CSF) treatment suppresses osteitis and bone microstructural changes

(BMCs) under inflammatory conditions. (A-B) Bone marrow (BM) staining showing

the frequencies of granulocytes (Ly6G⁺), monocytes (CD3⁻, B220⁻, TER119⁻, Ly6G⁻,

CD11b⁺/Ly6C⁺), and osteoclast precursor cells (CD11b^{low}, Ly6C^{high}) among lineage-

negative cells.

Supplementary Fig S6. Regional association plots of RA for the CSF2 region. The calculated r^2 values for the lead variant in the 1000 Genomes Project Phase 3 East Asian population are colored.

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