Figure S1: L-arginine ameliorates inflammation and bone loss in the serum transfer arthritis model

(A-B) Representative hind paw H&E (top) and tartrate-resistant acid phosphatase (TRAP) (bottom) staining (A) and quantification of hind paw bone erosion area, number of osteoclasts per paw (N. Oc) and inflammation area (B) at day 10 post serum transfer in control, control/L-arginine, SIA, SIA/L-arginine Day 0 and SIA/L-arginine Day 4 groups (n=5-9/group). Scale bars: 500 μm; **(C)** mRNA expression of *Ctsk* and *Mmp9* in the paw lysate of controls, control/L-arginine, SIA, SIA/L-arginine Day 0 and SIA/L-arginine Day 4 groups (n=5-9/group); **(D-E)** Representative vertebral μCT images (D) and quantification of vertebral bone volume/total volume (BV/TV), bone density trabecular numbers (Tb. N), trabecular thickness (Tb. Th), and trabecular separation (Tb. Sp) (E) in controls, control/L-arginine, SIA, SIA/L-arginine Day 0 and SIA/L-arginine Day 4 groups (n=5-9/group). Scale bars: 1 mm; **(F)** Ratio of RANKL/osteoprotegerin (OPG) level in the bone marrow supernatant of control, control/L-arginine, SIA, SIA/L-arginine Day 0 and SIA/L-arginine Day 4 groups (n=5-9/group). Graph points indicate individual mice. Data are shown as mean ± s.e.m. Asterisks mark statistically significant difference (*$p<0.05$; **$p<0.01$; ***$p<0.001$; ****$p<0.0001$), one-way ANOVA.

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Figure S2: L-arginine resolves inflammation and generalized bone loss in collagen induced arthritis model and human TNF-transgenic (hTNFtg) mice

(A-B) Representative hind paw H&E (top) and TRAP (bottom) staining (A) and quantification of hind paw bone erosion area, N. Oc and inflammation area (B) at day 39 after first immunization in control, control/L-arginine, CIA and CIA/L-arginine groups (n=4-9/group). Scale bars: 500 μm; **(C)** mRNA expression of *Ctsk* and *Mmp9* in the paw lysate of control, control/L-arginine, CIA and CIA/L-arginine groups (n=4-9/group), 39 days after first immunization; **(D-E)** Representative vertebral μCT images (D) and quantification of vertebral BV/TV, Tb. N, Tb. Th, and Tb. Sp (E) in control, control/L-arginine, CIA and CIA/L-arginine groups (n=4-9/group), 39 days after first immunization. Scale bars: 1 mm; **(F-G)** TNFα level (F) and ratio of RANKL/osteoprotegerin (OPG) level (G) in the bone marrow supernatant of control, control/L-arginine, CIA and CIA/L-arginine groups (n=4-9/group), 39 days after first immunization; **(H-I)** Representative hind paw H&E (top) and TRAP (bottom) staining (H) and quantification of hind paw bone erosion area, N. Oc and inflammation area (I) 12 weeks after birth in hTNFtg$^{-/-}$, hTNFtg$^{+/-}$, hTNFtg$^{-/-}$/L-Arginine and hTNFtg$^{+/-}$/L-Arginine groups (n=5–10/group). Scale bars: 500 μm; **(J-K)** Representative vertebral μCT images (J) and quantification of BV/TV, Tb. N, Tb. Th, and Tb. Sp (K) in hTNFtg$^{-/-}$, hTNFtg$^{+/-}$, hTNFtg$^{-/-}$/L-Arginine and hTNFtg$^{+/-}$/L-Arginine groups (n=5–10/group). Scale bars: 1 mm. Graph points indicate individual mice. Data are shown as mean ± s.e.m. Asterisks mark statistically significant difference (*p<0.05; **p<0.01; ***p<0.001; ****p<0.0001), one-way ANOVA.

Figure S3: L-arginine reduces osteoclastogenesis and resorption activity in osteoclasts derived from hTNFtg$^{+/-}$ mice

(A) Schematic diagram of the osteoclastogenesis with bone marrow cells from hTNFtg$^{+/-}$ mice and wild-type littermates, supplemented with 5 mM or 10 mM L-arginine during the whole procedure (day 0-3); **(B)** Cell viability on day 2 of osteoclastogenesis after different doses of L-arginine (0.5 mM, 1 mM, 3 mM, 5 mM, 10 mM) in hTNFtg$^{+/-}$ cells (n=4/group, representative of three independent experiments); **(C-D)** Quantification of TRAP^{+} osteoclasts (C) and representative TRAP staining images (D) of mature osteoclasts from hTNFtg$^{-/-}$ and hTNFtg$^{+/-}$ cells at day 3, supplemented with 5 mM or 10 mM L-arginine (n=3-4/group, representative of three independent experiments). Scale bars: 100 μm; **(E)** Representative images of pit formation assay and quantification of bone resorption area of mature osteoclasts from hTNFtg$^{-/-}$ and hTNFtg$^{+/-}$ cells at day 3, supplemented with 5 mM or 10 mM L-arginine (n=3-4/group, representative of three independent experiments). Scale bars: 100 μm; **(F)** Expression of osteoclast-associated genes in mature osteoclasts from hTNFtg$^{-/-}$ and hTNFtg$^{+/-}$ cells at day 3, supplemented with 5 mM or 10 mM L-arginine (n=3/group, representative of three independent experiments). Data are shown as mean ± s.e.m. Asterisks mark statistically significant difference (*p<0.05; **p<0.01; ***p<0.001; ****p<0.0001), one-way ANOVA in **B** and two-way ANOVA in **C, E** and **F**.

Figure S4: L-arginine suppresses pathological osteoclastogenesis

(A) Schematic diagram of pathological osteoclastogenesis from the Cx3cr1^{+} cells sorted from the knee and ankle joints of C57BL/6J mice; L-arginine (10mM) was supplemented at the later phase (day 4-5), with or without stimulation with 40 ng/ml TNFα; **(B)** Gating strategy for Cx3cr1^{+} cells; **(C)** Representative TRAP staining images of mature osteoclasts and quantification of TRAP^{+} osteoclasts at day 5 from different treatments. Scale bars: 100 μm. Data are shown as mean ± s.e.m. Asterisks mark statistically significant difference (**p<0.01; ***p<0.001), two-way ANOVA.

Figure S5: Bone marrow cells cannot differentiate into osteoclasts without any L-arginine

Representative TRAP staining images of mature osteoclasts and quantification of $TRAP^+$ osteoclasts at day 3 of osteoclastogenesis using normal αMEM medium or Arg-free medium, with or without 40 ng/ml TNFα stimulation. Scale bars: 100 μm. Data are shown as mean ± s.e.m. Asterisks mark statistically significant difference (**p<0.01; ****p<0.0001), two-way ANOVA.

Figure S6: Putrescine diminishes TNFα-induced osteoclastogenesis

(A-C) Schematic diagram of osteoclastogenesis with WT bone marrow cells (above), and cell viability on day 2 of WT osteoclasts (bottom) exposed to putrescine (A), ornithine (B) or spermidine (C) at the later phase, with or without 40 ng/ml TNFα stimulation; **(D-F)** Representative TRAP staining images (above) and quantification of $TRAP^+$ osteoclasts (bottom) at day 3 supplemented with putrescine (A), ornithine (B) or spermidine (C) at the later phase, with or without 40 ng/ml TNFα stimulation. Scale bars: 100 μm; **(G-I)** Expression of osteoclast-associated genes and representative dot plot of *Mmp9* at day 3; supplementation of putrescine (A), ornithine (B) or spermidine (C) was at the later phase, with or without stimulation with 40 ng/ml TNFα. Data are shown as mean ± s.e.m. Asterisks mark statistically significant difference (*p<0.05; **p<0.01; ***p<0.001; ****p<0.0001), one-way ANOVA in **A-C** and two-way ANOVA in **D-I**.

Figure S7: L-arginine reduces osteoclastogenesis and promotes OXPHOS in TNF expressing cells

(A-B) Extracellular acidification rate (ECAR) (A) and glycolysis and glycolytic capacity (B) analyzed by extracellular flux assay in pre-osteoclasts derived from hTNFtg$^{+/-}$ mice and littermates; supplementation of L-Arginine (10mM) was done during the whole procedure (n=9-12/group); **(C-D)** Oxygen consumption rate (OCR) (C) and associated maximal respiration, non-mitochondrial oxygen consumption, ATP production and proton leak (D) analyzed by extracellular flux assay in pre-osteoclasts derived from hTNFtg$^{+/-}$ mice and littermates; supplementation of L-arginine (10mM) was done during the whole procedure (n=9-12/group); **(E)** Kinetic expression of glycolysis, OXPHOS, pentose phosphate pathway (PPP) and redox pathway-associated genes in pre-osteoclasts derived from hTNFtg$^{+/-}$ mice and littermates at day 2; supplementation of L-arginine (10mM) was done during the whole procedure (n=3/group). Data are shown as mean ± s.e.m. Asterisks mark statistically significant difference (*p<0.05; **p<0.01; ***p<0.001; ****p<0.0001), one-way ANOVA in **B** and **D** and two-way ANOVA in **A** and **C**. For panel A and C, black asterisk means group hTNFtg$^{+/-}$ vs. hTNFtg$^{-/-}$ and orange asterisk means group hTNFtg$^{+/-}$/L-arginine vs. hTNFtg$^{+/-}$.

Figure S8: L-arginine suppresses mitochondrial ROS level in pre-osteoclast under inflammatory condition

(A) Percentage of ATP produced by glycolysis and OXPHOS analyzed by real-time ATP rate assay in pre-osteoclasts from WT cells along the time line of osteoclastogenesis, with or without 40 ng/ml TNFα stimulation (n=6-12/group); **(B)** Gating strategy for pre-osteoclasts ($CD45^+$ $CD11b^+$ $Ly6G^-$ $Mcsfr^+$ $RANK^+$ cells) in SCENITH assay; **(C)** Comparison of glucose dependence, fatty acid oxidation (FAO) and amino acid oxidation (AAO) capacity, mitochondrial dependence and glycolytic capacity in pre-osteoclasts at day 2 in TNFα and TNFα/L-arginine groups (n=3-6/group, representative of three independent experiments); **(D)** Percentage of glycolysis capacity among 4 groups (n=3-6/group, representative of three independent experiments); **(E-F)** Gating strategy for osteoclast-precursors (CD45+ CD11b+ Mcsfr+ cells) with MITOSOX staining (E) and ratio of MitoSOX mean fluorescence intensity (MFI) (compared to control group without TNFα stimulation) in osteoclast-precursors (F) on day 2; supplementation of L-Arginine (10mM) was done at the later phase, with or without 40ng/ml TNFα stimulation for 0.5h; **(G)** Representative images of whole cells, lysosomes and mitochondria via transmission electron microscopy in mature osteoclasts at day 3; supplementation of L-Arginine (10mM) was done at the later phase, with or without 40 ng/ml TNFα. Scale bar as indicated. Data are shown as mean ± s.e.m. Asterisks mark statistically significant difference (*$p<0.05$; **$p<0.01$), student t-test in **C**, two-way ANOVA in **A**, **D** and **F**.

Figure S9: Urea Levels are Unchanged After L-arginine Supplementation in Arthritis Models

(A) *Arg2* expression levels in mature osteoclasts at day 3 from cJun-deficient cells or littermate controls, with or without 40 ng/ml TNFα (n=3-6/group, representative of three independent experiments); **(B)** Normalized urea level and nitrite level in the paw lysate of mice from Control, Control/L-Arginine, CIA and CIA/L-Arginine groups (n=4-9/group) with CIA model. Data are shown as mean ± s.e.m. Asterisks mark statistically significant difference (*p<0.05; **p<0.01; ***p<0.001), one-way ANOVA in **B** and two-way ANOVA in **A**.

Figure S10: Spermidine altered purine metabolism especially in TNFα-stimulated condition

(A) Schematic diagram of purine metabolism and genes encoding the associated enzymes; **(B)** Expression of genes encoding enzymes in purine metabolism at day 3; supplementation of putrescine, ornithine or spermidine was at the later phase, with or without stimulation of 40 ng/ml TNFα.

Tabel S1: Demographics and clinical data of the study cohort.

Feature	Value
Healthy Controls	
Age, years, median (IQR)	52.1 (44.0-57.0)
Gender, n (%)	
Female	26 (89.7%)
Male	3 (10.3%)
Patients with rheumatoid arthritis	
Age, years, median (IQR)	53.3 (44.8-58.5)
Gender, n (%)	
Female	21 (91.3%)
Male	2 (8.7%)
DAS28, median (IQR)	
Low activity (<3.2), n (%)	2 (8.7%)
Medium activity (3.2-5.1), n (%)	13 (56.5%)
High activity (>5.1), n (%)	8 (34.8%)
CRP (mg/L), median (IQR)	12.8 (2.2-10.8)
ESR (mm/h), median (IQR)	31.0 (15.5-39.8)

IQR, interquartile range; RF, rheumatoid factor; Anti-CCP, anti-cyclic citrullinated peptide; DAS28, disease activity score 28; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; DMARDs, disease-modifying anti-rheumatic drugs.

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Tabel S2: Antibodies used for FACS staining

Name	Lot Number	Clone	Company
Anti CD45-Alexa Fluor 700	103128	30-F11	Biolegend
Anti Ly6G-BV650	127641	1A8	Biolegend
Anti CD16/32	101320	93	Biolegend
Anti F4/80-APC	123116	BM8	Biolegend
Anti CD11b-APC/Cyanine7	101225	M1/70	Biolegend
Anti Ly6C-PerCP/ Cyanine5.5	128011	HK1.4	Biolegend
Anti CD265(RANK)-PE	119805	R12-31	Biolegend
Anti CD115-Brilliant Violet 421	135513	AFS98	Biolegend
Anti-Puromycin-FITC	MABE343	12D10	Sigma-Aldrich
MitoSOX	M36008	/	Invitrogen

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Tabel S3: Primers sequences used for Real-Time PCR and ChIP-qPCR

Primers for murine Real-Time PCR		
Gene name	**Forward sequence**	**Reverse sequence**
Beta-actin	TGTCCACCTTCCAGCAGATGT	AGCTCAGTAACAGTCCGCCTAGA
Ctsk/Cath.K	ATATGTGGCCAGGATGAAAGTT	TCGTTCCCCACAGGAATCTCT
Mmp9	GCTGACTACGATAAGGACGGCA	TAGTGGTGCAGGCAGAGTAGGA
Rank	TTGTGGCAGGGGACTTTAAC	ATTGTCATCCTGCCCTCAAC
Traf6	AAAGCGAGAGATTCTTTCCCTG	ACTGGGGACAATTCACTAGAGC
Trap	GGC CGG CCA CTA CCC CAT CT	CACCGTAGCCACAAGCAGGACTCT
Nfatc1	GGTGCCTTTTGCGAGCAGTATC	CGTATGGACCAGAATGTGACGG
Atp6v0d2	CAGAGCTGTACTTCAATGTGGAC	AGGTCTCACACTGCACTAGGT
Hk2	GGAGAGCACGTGTGACGAC	GATGCGACAGGCCACAGCA
Gpi1	GTTGCCTGAAGAGGCCAGG	GCTGTTGCTTGATGAAGCTGATC
Pdk1	GATTCAGGTTCACGTCACGCT	GACGGATTCTGTCGACAGAG
Ldha	CACAAGCAGGTGGTGGACAG	AACTGCAGCTCCTTCTGGATTC
Pkm2	CAGGAGTGCTCACCAAGTGG	CATCAAGGTACAGGCACTACAC
Mct4	TGGCATCTCATATGGCATGGTG	CACCTCCTCAGGCTCTGTC
Slc2a1	TTAATCGCTTTGGCAGGCGG	CCCAGTTTGGAGAAGCCCAT
Aco1	CCATCCGTGATGTTAGGAGCAG	GACAGGTAAGGCATGACTCCAC
Idh2	GGCTGTCAAGTGTGCCACAATC	TTGGCTCTCTGAAGACGGTTCC
Acly	AGGAAGTGCCACCTCCAACAGT	CGCTCATCACAGATGCTGGTCA
Mdh2	TCACTCCTGCTGAAGAACAGCC	CCTTTGAGGCAATCTGGCAACTG
Sdhb	GTGGATCTGAATAAGTGCGGA	CCAGAGTATTGCCTCCGTTGA
Sdhc	CGACACTTGCTATGGGACCTA	AACACAGCAAGAACCACGAC
Sdhd	CCAAGCCACCACTCTGGTTC	TGCAGCCAGAGAGTAGTCCA
G6pdx	CACAGTGGACGACATCCGAAA	AGCTACATAGGAATTACGGGCAA
Nrf2	CCAGCAGGACATGGATTTGA	AGCTCATAGTCCTTCTGTCGC
Sod1	CAGGACCTCATTTTAATCCTCAC	TGCCCAGGTCTCCAACAT
Sod2	TGCTCTAATCAGGACCCATTG	GTAGTAAGCGTGCTCCCACAC
Sod3	CTCTTGGGAGAGCCTGACA	GCCAGTAGCAAGCCGTAGAA
Nox1	GGTTGGGGCTGAACATTTTTC	TCGACACACAGGAATCAGGAT
Nox2	TGTGGTTGGGGCTGAATGTC	CTGAGAAAGGAGAGCAGATTTCG
Nox3	TGGCAGTAAACGCCTATCTGT	CGGAACCCAGAATAACTCGTGTA
Arg1	CTCCAAGCCAAAGTCCTTAGAG	AGGAGCTGTCATTAGGGACATC
Arg2	GTTGATGCTCATGCCGACAT	TGGCAGTTGTGGTACCTTGTC

Pnp	CAACACACTGAATATCGACCTCA	GCTTTGGGGAAAGTTGGGTATCT
Xdh	ATGACGAGGACAACGGTAGAT	TCATACTTGGAGATCATCACGGT
Ada	ACCCGCATTCAACAAACCCA	AGGGCGATGCCTCTCTTCT
Hprt	TCAGTCAACGGGGGACATAAA	GGGGCTGTACTGCTTAACCAG
Ampd	GTTGGCGGAGAAGGTGTTTG	CTGCGACCGGATCATCTTGAA
Nt5c1a	CTCAGGTGGGAGTTCGTCTCA	GGTAGCAGATGGGGCTATTCC
Primers for human Real-Time PCR		
Gene name	**Forward sequence**	**Reverse sequence**
B2M	AGATGAGTATGCCTGCCGTG	TTCAAACCTCCATGATGCTGC
MMP9	CCTGGAGACCTGAGAACCAA	ATTTCGACTCTCCACGCATC
OSCAR	AGATCGCTCCCCTTCTCTTC	TAGCAGCAGCGGTAACTTCC
NFATC1	GTCCTGTCTGGCCACAAC	GGTCAGTTTTCGCTTCCATC
Primers for ChIP-qPCR		
Name	**Forward sequence**	**Reverse sequence**
Arg1_ChIP_1	GGCAGGCTGCAAGGAATTTTTA	TGCCACTGTGTTTCTGCTGT
Arg1_ChIP_2	GGTGCTGGGCTAACACAGAT	CCATACACACGACGGTTCCA
Arg1_ChIP_3	TCATGAGTTTTACTTAATCAGGGCG	AGGGTGACTTTTCATCATTGGC
Arg1_ChIP_4	GCTAGTAACGGCCGTAGCAC	GTCGGGGTCTCGCAAATGTA
Arg1_ChIP_5	CCCAGCAGGTGTTGAACCTAT	TGGCCCACAAATTCCAGCTAT
Arg1_ChIP_6	CCTGAGGGACTTTGGCTTCT	TGAGGACAAAAAGCACCCAGA
Arg1_ChIP_7	GCCCCATGCTTTCCTAGACA	TTCCCTCCTGTTGAGCATCC
Arg1_ChIP_8	TCCTCTGATGGGGAGGTTCT	TTATCCAACAACTGGCTCCGC

Supplementary Methods

K/BxN SIA model and Collagen-induced arthritis model

K/BxN serum transfer arthritis was induced on day 0 by intraperitoneal (i.p.) injection of 200 µl pooled K/BxN serum. CIA was induced in 8-week-old male DBA/1J mice by subcutaneous (s.c.) injection at the base of the tail with 100 µl 0.25 mg chicken type II collagen (CII; Chondrex, Redmond, WA) in complete Freund adjuvant (CFA; Difco Laboratory, Detroit, MI), containing 4 mg/mL killed Mycobacterium tuberculosis (H37Ra). Mice were re-challenged after 21 days by intradermal immunization in the base of the tail with this emulsion.

The swelling of fore and hind paws was evaluated every other day by digital caliper (Mitutoyo, Japan). Each paw was individually scored using a 4-point scale: 0, normal paw; 1, minimal swelling or redness; 2, redness and swelling involving the entire forepaw; 3, redness and swelling involving the entire limp; 4, joint deformity or ankylosis or both. In addition, grip strength of each paw was analyzed on a wire 3 mm in diameter, using a score from 0 to −4 (0, normal grip strength; −1, mildly reduced grip strength; −2, moderately reduced grip strength; −3, severely reduced grip strength; −4, no grip strength at all).

Histology

Tibia were fixed overnight in 4% formalin and decalcified in EDTA (Sigma-Aldrich) until bones were pliable. Serial paraffin sections (2 µm) were stained with haematoxylin and eosin and tartrate resistant acid phosphatase (TRAP) using a Leukocyte Acid Phosphatase Kit (Sigma) according to the manufacturer's instructions. Bone volume/total volume (BV/TV), bone density trabecular numbers (Tb. N), trabecular thickness (Tb. Th), trabecular separation (Tb. Sp), osteoclast surface/bone surface (Oc.S/BS), osteoclast number/tissue area (N.Oc/T.Ar) and osteoclast number/bone perimeter (N.Oc./B.Pm.) were quantified using a microscope (Carl Zeiss) equipped with a digital camera and an image analysis system for performing histomorphometry (Osteomeasure; OsteoMetrics).

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Micro-computed tomography

µCT imaging was performed using the cone-beam Desktop Micro Computer Tomograph µCT 40 by SCANCO Medical AG, Bruettisellen, Switzerland. The settings were optimized for calcified tissue visualization at 55 kVp with a current of 145 µA and 200 ms integration time for 500 projections/180°. For the segmentation of 3D-Volumes an isotropic voxel size of 8.4 µm and an evaluation script with adjusted greyscale thresholds of the operating system Open VMS by SCANCO Medical was used.

In vitro osteoclastogenesis assay

Total bone marrow cells were isolated from WT C57BL/6 or respective transgenic mice and littermates by flushing femur and tibia. Cells were incubated overnight with α-MEM supplemented with 5 ng ml^{-1} M-CSF (Peprotech) (Day -1). Non-adherent cells were collected, washed and further cultured (Day 0) in α-MEM supplemented with 10% heat-inactivated FCS, 1% glutamine, 1% penicillin and streptomycin (all from Invitrogen), 20 ng/ml M-CSF and 20 ng/ml RANKL (Peprotech) in 96-well plate at the concentration of 2×10^5 cells per ml, in 48-well plate at the concentration of 5×10^5 cells per ml or in 24-well plate at the concentration of 1×10^6 cells per ml. Medium was changed every 2 days. On day 2 of culture, cells were stimulated with or without 40ng/ml TNFα, supplemented or not with 10mM L-arginine in the osteoclast medium. In some of the experiments, pre-osteoclasts on Day2 were also treated with OXPHOS inhibitors antimycin A and rotenone at the final concentration of 5-20 nM, or putrescine at the final concentration of 5/10 mM, or ornithine at the final concentration of 12.5/25mM, or spermidine at the final concentration of 2.5/5µM, or Inosine at the final concentration of 20 mM, or Hypoxanthine at the final concentration of 1 mM, or ADA inhibitor Pentostatin at the final concentration of 20/40 µM or erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA) at the final concentration of 2.5/5 µM. All the compounds were supplemented in the osteoclast medium. Osteoclast differentiation was evaluated at day 3 by tartrate-resistant acid phosphatase staining using the leukocyte acid phosphatase kit 386A (Sigma-Aldrich) according to the manufacturer's instructions.

In arginine-free experiment, bone marrow cells were cultured from Day -1 in SILAC medium

(Thermo, #88368) supplemented with 0.4mM L-lysine (Roth, # 39665-12-8), 10% heat-inactivated FCS, 1% glutamine, 1% penicillin and streptomycin.

In vitro osteoclastogenesis of synovial inflammatory macrophages were performed as shown in the previous study (1). Briefly, knee and ankle joints from WT C57BL/6 mice were cut into small pieces (1mm^3) and digested with 0.05% Collagenase A in αMEM medium (4mL/mouse) at 37°C, 250 rpm for 30min. Digested joints were then filtered twice through 70 μm cell strainers and isolated single cells were then stained with the surface markers: Zombie Aqua, CD45, Cx3cr1 (all from BioLegend) for 30min in the dark at 4°C. After washing, cells were resuspended in FACS buffer for sorting on MoFlo Astrios EQ (Beckman Coulter). Zombie Aqua$^-$CD45$^+$Cx3cr1$^+$ inflammatory macrophages were sorted into 1x PBS with 0.1% NaN3, 0.1% BSA and 0.5mM EDTA. After sorting, cells were resuspended in αMEM with 10% FCS, 1% glutamine, 1% PS, 10 ng/ml M-CSF in 96-well plate at the concentration of 4 × 10^4 cells/well (Figure S4). On day 2 of culture, medium was changed with αMEM supplemented with 10 ng/ml M-CSF and 100 ng/ml RANKL. On day 4 of culture, cells were stimulated with or without 40ng/ml TNFα, supplemented or not with 10mM L-arginine in the osteoclast medium.

Cell viability test

Cell viability test was performed on Day 2 as described previously (2). Briefly, 2 × 10^5 cells from Day 0 were cultured in 200 μL/well osteoclast medium with 20 ng/mL M-CSF and 20 ng/mL RANKL in 96-well plates at 37°C and 5.5% CO2. 48h after different doses of L-arginine (0.5mM, 1mM, 3mM, 5mM and 10mM), putrescine (5mM, 10mM, 20 mM, 40 mM and 80mM), ornithine (6.25mM, 12.5mM, 25mM, 50mM and 100mM), or spermidine (6.25μM, 12.5μM, 25μM, 50μM and 100μM) supplementation, adherent cells in 96-well plate were washed twice in a gentle stream of tap water. Then the cells were stained with 50 μL 0.5% crystal violet staining solution and incubate for 20 min at room temperature on a bench rocker with a frequency of 20 oscillations per minute. The plate was then washed four times and air-dried overnight. 200 μL methanol was added to each well. The plate was incubated for 20 min at room and then measured at 570 nm (OD570) with a plate reader. In some experiments, cell

viability was also tested with Zombie Violet™ Fixable Viability Kit (BioLegend) according to the manufacturer's instructions.

Immunofluorescence in osteoclasts

For immunofluorescence, 1×10^6 cells from Day 0 were cultured in 1 mL/well osteoclast medium with supplements on 12-mm circle coverslips (Thermo Fisher Scientific) in 24-well plates with 20 ng/mL M-CSF and 20 ng/mL RANKL at 37°C and 5.5% CO_2 until fully differentiated. On day 2 of culture, cells were stimulated with or without 40ng/ml TNFα, supplemented or not with 10mM L-arginine in the osteoclast medium. When the osteoclasts were fully differentiated, they were washed with PBS and fixed with 4% Histo-fix (Roth). After fixation, the cells were stained with F-actin Visualization Biochem Kit (Cytoskeleton, Inc.) according to the manufacturer's instructions to visualize F-actin ring formation.

Resorption assay

For the resorption assay, 1×10^6 cells from Day 0 were cultured in 1 mL/well osteoclast medium in 24-well plates coated with calcium phosphate (Corning) with 20 ng/mL M-CSF and 20 ng/mL RANKL at 37°C and 5.5% CO_2. On day 2 of culture, cells were stimulated with or without 40ng/ml TNFα, supplemented or not with 10mM L-arginine in the osteoclast medium. When the osteoclasts were fully differentiated, the osteoclasts were bleached with 5% sodium hypochlorite (Roth) for 5 minutes. Afterwards, the plates were incubated with 100 µL of 5% (w/v) aqueous silver nitrate solution at room temperature covered with foil for 30 minutes. The plates were then soaked in distilled water for 5 minutes. Thereupon, 100 µL of 5% sodium carbonate (w/v in commercial buffered formalin) were added into the plates and incubated for 4 minutes at room temperature. The carbonate/formalin solution was then discarded and the plates were dried at 50°C overnight.

All images were acquired with the BZ-X 710 All-in-One Fluorescence Microscopes (Keyence, Osaka, Osaka Prefecture, Japan) and quantification of osteoclast numbers and percentage of the resorbed area were performed with ImageJ.

JC-1 staining

For JC-1 staining, 2×10^5 cells from Day 0 were cultured in 200 μL/well osteoclast medium with 20 ng/mL M-CSF and 20 ng/mL RANKL in 96-well plates at 37°C and 5.5% CO2. On day 2 of culture, cells were stimulated with or without 40ng/ml TNFα, supplemented or not with 10mM L-arginine in the osteoclast medium. When the osteoclasts were fully differentiated, they were washed with warm HBSS and then stained with MitoProbe™ JC-1 Assay Kit, according to the manufacturer's instructions. The images were acquired with the BZ-X 710 All-in-One Fluorescence Microscopes (Keyence, Osaka, Osaka Prefecture, Japan).

Mitochondrial ROS

1×10^6 cells from Day 0 were cultured in 1 mL/well osteoclast medium with 20 ng/mL M-CSF and 20 ng/mL RANKL in 24-well plates at 37°C and 5.5% CO2. On Day 2 of culture, after the stimulation with or without 40ng/ml TNFα, supplemented or not with 10mM L-arginine, pre-osteoclasts at diverse time points were washed with warm HBSS buffer and stained with MitoSOX™ Red Mitochondrial Superoxide Indicator (Invitrogen) according to the manufacturer's instructions. The cells were then detached, transferred to a V-BOTTOM 96-well plate (Greiner) and centrifuged at 500g for 2 minutes. Subsequently, the cells were stained with the surface markers of pre-osteoclasts for 30 min at 4 °C. The gating strategy was illustrated in **Fig S5E**. The details of FACS antibodies were shown in **Table S1**.

Transmission electron microscopy (TEM)

1×10^6 cells from Day 0 were cultured in 1 mL/well osteoclast medium with 20 ng/mL M-CSF and 20 ng/mL RANKL on 13-mm Thermanox plastic coverslips (Thermo Fisher Scientific) in 24-well plates at 37°C and 5.5% CO2. On day 2 of culture, cells were stimulated with or without 40ng/ml TNFα, supplemented or not with 10mM L-arginine in the osteoclast medium. When the osteoclasts were fully differentiated, they were washed with PBS and fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer for at least 48 hours. Thereupon, the cells were postfixed in 2% buffered osmium tetroxide for 2 hours and then dehydrated in graded alcohol concentrations and embedded in epoxy resin, according to standard protocols.

For orientation, 1 μm semithin sections were stained with toluidine blue. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a transmission electron microscope (EM 906E, Carl Zeiss).

Western Blot

For western blot, 2×10^6 cells from Day 0 were cultured in 2 mL/well osteoclast medium with 20 ng/mL M-CSF and 20 ng/mL RANKL in 12-well plates at 37°C and 5.5% CO2. On Day 2 of culture, cells were stimulated with or without 40ng/ml TNFα, supplemented or not with 10mM L-arginine in the osteoclast medium. When the osteoclasts were fully differentiated, the osteoclasts were lysed with RIPA buffer supplied with protease inhibitors (Roche, cOmplete™ ULTRA Tablets, 05892970001). The protein extracts were then separated by SDS-PAGE electrophoresis and semi-dry transferred to a PVDF membrane. The membrane was incubated with antibodies against cJun (#9165S, Cell Signaling Technology, 1: 1000) and β-Actin (#A5441, Sigma, 1:5000) overnight at 4°C.

Arginase Activity Assay

The arginase assays were performed as described previously (3). For the preparation of the paw lysates, the skin was removed and the total paw tissues were homogenized in 500μL 40Mm Tris buffer (pH 8.0) using a Precellys® Ceramic Kit on a Precelllys®24 tissue grinder (bertin instruments, Frankfurt am Main, Germany) with the conditions 2 x 30 s at 6,500 rpm, subsequently samples were sonicated on ice (settings: cycle5, power 50%, 40 s) and centrifuged at 23,000 x g for 10 min. The values were normalized to the protein concentration of each sample, detected by the DC protein assay kit (Bio-Rad).

Griess Assay

To measure the concentration of nitrite in the paw lysates, a stable end product of the synthesized NO, Griess assay was performed with Griess Reagent System (Promega) according to the manufacturer's instructions. The values were normalized to the protein concentration of each sample, detected by the DC protein assay kit (Bio-Rad).

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Urea assay

The concentration of urea in the paw lysates was performed with Urea Assay Kit (Abcam) according to the manufacturer's instructions. The values were normalized to the protein concentration of each sample, detected by the DC protein assay kit (Bio-Rad).

ChIP-qPCR

Binding prediction of transcription factor AP-1 on the promoter sites of *Arg1* and associated primer design have been described previously (4). ChIP-qPCR on pre-osteoclasts was performed with the ChIP-IT High Sensitivity® (HS) Kit (Active Motif) following the manufacturer's instructions. Briefly, 5×10^6 cells from Day 0 were cultured in 5 mL/well osteoclast medium with 20 ng/mL M-CSF and 20 ng/mL RANKL in 6-well plates at 37°C and 5.5% CO2. On Day 2 of culture, 4h after the stimulation with or without 40ng/ml TNFα, supplemented or not with 10mM L-arginine, pre-osteoclasts were fixed with 1% formaldehyde for 15 min to cross-link the proteins to the DNA and then washed for 3 times. Cells pooled from 5 wells with the same treatment were counted as one sample. The pellet was re-suspended in ChIP lysis buffer and lysate was sonicated to shear DNA to an average fragment size of 200–1000 bp. 30 µg chromatins were incubated with antibodies against cJun (#9165S, Cell Signaling Technology, 6µL), or against H3me3K4 (#17-614, Sigma-Aldrich, 5µL), or against H3me3K27 (#ab6002, Abcam, 5µL), or Normal Rabbit IgG (#2729, Cell Signalling, 1µg/sample) overnight at 4°C. On the second day, samples were incubated with protein A/G beads for 3 hours. DNA was then eluted, de-crosslinked overnight and purified. 25 µl starting chromatins without incubation with antibodies were also de-crosslinked and purified, named as Input. The enrichment of each binding site was evaluated by ChIP-qPCR. The data was normalized and calculated with Percent Input Method: Adjust Input=Ct_{Input}-log2($V_{Chromatin}/V_{Input}$), V means volume. % Input= $100*2^{(\text{Adjusted Input - Ct Chromatin})}$. The ChIP-qPCR primer sequences are listed in **Table S3**.

Human osteoclast differentiation

Human peripheral blood mononuclear cells (PBMCs) were isolated from EDTA-blood of normal, healthy donors, using a Ficoll gradient (Lymphoflot, Bio-Rad). PBMCs (3×10^5/well) were plated in 96-well plates (TRAP) or 7.5×10^5 PBMCs/well in 48-well plates (5) in 100 µL/well or 250 µL/well osteoclast medium (1% FCS and 1% pen/strep), respectively, for 2 hours at 37°C and 5.5% CO2 to purify monocytes by plastic adhesion. Afterwards, the cells were washed and differentiated into osteoclasts in 200 µL/well or 500 µL/well osteoclast medium (10% FCS and 1% pen/strep) with 30 ng/mL M-CSF, 3 ng/mL RANKL, and 1 ng/mL TGF-β (all from PeproTech) at 37°C and 5.5% CO2. L-arginine was supplemented into the medium from Day 0. The medium was changed on day 3 and again on day 6 of differentiation. In some of the wells, cells were stimulated with 20pg/ml human TNFα initiated from Day 3. Osteoclast differentiation was evaluated by TRAP staining on Day 7 when osteoclasts were fully differentiated. Images were acquired with the Keyence microscope and quantification of osteoclast number was performed with ImageJ.

RNA extraction and quantitative real-time PCR

RNA from murine and human osteoclasts was isolated with RNA-Solv® Reagenz (VWR) according to standard protocols. cDNA was synthesized from 1 µg of total RNA with a High-Capacity cDNA Reverse Transcription Kit (ThermoFisher). Real-time PCR analysis was then performed and analyzed with a SYBR label (SYBR Select Mastermix, Applied Biosystems) in QuantStudio™ 6 Flex Real-Time PCR System. Samples were analyzed in duplicate and normalized to the level of *Actb* (β-actin) mRNA for murine and *B2M* (β-2-microglobulin) mRNA for human samples. Human and murine qPCR primer sequences are listed in **Table S3**, respectively.

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