Microbial metabolites are known to modulate immune responses of the host. The main metabolites derived from microbial fermentation of dietary fibers in the intestine, short-chain fatty acids (SCFA), affect local and systemic immune functions. Here we show that SCFA are regulators of osteoclast metabolism and bone mass in vivo. Treatment of mice with SCFA as well as feeding with a high-fiber diet significantly increases bone mass and prevents post-menopausal and inflammation-induced bone loss. The protective effects of SCFA on bone mass are associated with inhibition of osteoclast differentiation and bone resorption in vitro and in vivo, while bone formation is not affected. Mechanistically, propionate (C3) and butyrate (C4) induce metabolic reprogramming of osteoclasts resulting in enhanced glycolysis at the expense of oxidative phosphorylation, thereby downregulating essential osteoclast genes such as TRAF6 and NFATc1. In summary, these data identify SCFA as potent regulators of osteoclast metabolism and bone homeostasis.
Several reports highlighted the immunomodulatory capacities of short-chain fatty acid (SCFA). As immune activation is intimately linked to bone homeostasis, we hypothesized that SCFA may influence bone homeostasis thereby providing a direct mechanistic link between the gut microbiota and bone. In support of the clinical relevance of this concept, recent studies suggested that presence or absence of specific bacterial species influence the course of inflammatory bone diseases, including rheumatoid arthritis (RA) and ankylosing spondylitis. Furthermore, it was shown that fiber-rich diet, the main fermentable source for SCFA, can ameliorate bone destruction in RA. These observations prompted us to investigate the role of SCFA on bone during steady-state conditions as well as in the case of pathological bone loss during menopause and inflammation. Studies addressing the role of the gut microbiota on bone homeostasis by using germ-free (GF), antibiotic-treated or mono-colonized mice reported contradicting findings. These discrepancies may be explained by different microbial exposure of the mice used for the experiments. Therefore, we tested whether the secreted metabolites of the gut microbiota rather than the microbiota per se may explain these effects. To test this hypothesis, we used three independent experimental approaches (direct supplementation of SCFA, highfiber diet (HFD), and bacterial transfer) and found consistent beneficial effects on steady state and postmenopausal bone metabolism highly related to SCFA levels. It should be also mentioned that the source and biological function of SCFA is essentially different from long-chain fatty acids, which influence insulin metabolism and where effects on bone have been suggested.

Here we show that the SCFA C3 and C4 have a beneficial impact on bone homeostasis and alleviated disease in two models of inflammatory arthritis. At the molecular level, the direct impact of SCFA on bone homeostasis is dependent on early metabolic reprogramming of pre-osteoclasts. Moreover, immunosuppressive mechanisms induced by SCFA also contribute to the attenuation of inflammation by inducing regulatory T cells (Tregs). Hence, our findings indicate a crucial role of SCFA in bone metabolism and immune responses attenuating the severity of arthritis.

Results
SCFA and HFD increase systemic bone density. To determine whether SCFA affect bone metabolism, we analyzed bone mass in C57BL/6J (wild-type (WT)) mice either fed with or without C2/C3/C4 supplementation in the drinking water for 8 weeks. After 8 weeks, serum SCFA concentrations in treated mice were significantly increased (Supplementary Fig. 1a). Micro-computed tomography (μCT) analysis of tibial bone showed that SCFA treatment significantly increased bone mass as shown by increased bone volume per tissue volume (BV/TV) and decreased trabecular separation (Th.Sp) (Fig. 1a–c). CTX-I serum level, a marker for bone resorption, was significantly decreased (Fig. 1d), and SCFA treatment led to a significant decrease of bone-degrading osteoclasts as shown by histomorphometric analysis (Fig. 1e). In contrast, markers for bone formation such as osteoblast numbers, serum osteocalcin (OCN) levels (total and decarboxylated) and mineral apposition rate and bone formation rate per bone surface remained unchanged (Fig. 1f, g and Supplementary Fig. 1b, c).

As SCFA were shown to act on immune cells, especially to induce Tregs, we analyzed Treg numbers in the spleens and found increased numbers after SCFA treatment while serum cytokine levels remained largely unchanged, except for C4 treatment that impacted on interleukin (IL)-10, interferon-γ (IFNγ), transforming growth factor-β (TGFβ) as well as on tumor necrosis factor-α (TNFα) levels (Supplementary Fig. 1d, e). Increased Tregs may explain the bone effects of SCFA since Treg were shown to suppress osteoclasts and increased bone mass.

To exclude this possibility, we treated RAG1−/− mice with SCFAs and also observed an increase in BV/TV along with decreased trabecular separation (Th.Sp) and osteoclast numbers (Fig. 1h–j). Interestingly, osteoclast numbers were not decreased in RAG1−/− mice after C2 treatment, pointing toward a different suppressive mechanism. Although we cannot exclude other possible indirect mechanisms on innate immune cells, we can clearly exclude the impact of T and B cells with these experiments. Also, a 14-day nutritional supplementation of sodium propionate (Propicum) in healthy humans significantly decreased serum parameters for bone resorption (Fig. 1k) while leaving OCN levels unchanged (Supplementary Fig. 1f).

Next, we investigated whether diets rich in fermentable, indigestible fibers, which increase SCFA levels in the cecum and serum (Fig. 1l and Supplementary Fig. 1g), exert similar effects on bone. In accordance with direct SCFA supplementation, an 8-week exposure to fiber-rich diet (Supplementary Table 1) led to an increased bone mass (Fig. 1m) and decreased trabecular separation (Fig. 1n) associated with decreased osteoclast numbers and CTX-I serum levels (Fig. 1o and Supplementary Fig. 1h). At the same time, OCN serum levels and osteoblast numbers remained unchanged (Supplementary Fig. 1i, j). Although Treg numbers were also increased after HFD treatment, serum cytokine levels did not show significant changes (Supplementary Fig. 1k, l). Of note, no differences in weight gain were observed in the feeding experiments, either with SCFA or HFD (Supplementary Fig. 1m).

We next addressed whether the transfer of specific bacterial species and respective changes of gut microbiota and its secreted metabolites could also impact bone mass. Enhanced abundance of Prevotella copri has been described in early RA. We therefore transferred three representative species from the genus Prevotella separately into WT mice and analyzed systemic bone mass 8 weeks later. Transfer of all Prevotella spp. significantly increased osteoclast numbers (Supplementary Fig. 1n) and the relative abundance of Prevotella spp. durably for 8 weeks (Supplementary Fig. 1o, p) but decreased SCFA levels (Fig. 1p) and systemic bone mass in the recipient mice (Fig. 1q, r), thus highlighting the impact of disease-relevant changes in the microbiome and subsequently in SCFA levels on bone homeostasis. This change in bone mass after bacterial transfer seems to be specific for Prevotella spp., as transfer of representative bacteria from taxonomically related families Bacteroidaceae and Muribaculaceae did not show similar effects (Supplementary Fig. 1q).

SCFA change the metabolic state of pre-osteoclasts. Our in vivo data indicated that SCFA modulate bone metabolism, resulting in significant decreased bone resorption. In vitro, osteoclast differentiation assays showed that concentrations from 0.03 mM of C2 and 0.015 mM of C3/C4 suppressed osteoclast differentiation (Fig. 2a, b), and this range of concentration was found in pooled bone marrow fluids from two individual experiments after SCFA feeding, namely, around 0.4 mM for C3 and 0.15 mM for C4 after adjusting measured concentrations in flushed bone marrow samples to what we experimentally found in the bone marrow fluid volume from repeated measurements of 5.5 ± 1.34 μl (n = 5) (Supplementary Fig. 2a, b). The suppressive capacity of C2/C3/C4 on osteoclast differentiation and bone resorption was independent of the free fatty acid surface receptors FFA3 (GPR41) and FFA2 (GPR43) (Supplementary Fig. 2c, d). Mechanistically, C3 and C4 significantly suppressed two essential osteoclastogenic
signaling components, TRAF6 and NFATc1, at early time points after receptor activator of nuclear factor-κB ligand (RANKL) stimulation (Fig. 2c–e). Previous in vitro studies using C4 as HDAC inhibitor for influencing bone cells showed inhibition of osteoclasts while results were inconsistent in osteoblasts23–26. While our in vivo data showed no consistent effects of SCFA on osteoblasts and bone formation, C3 and C4 strongly suppressed osteoclast differentiation.

Cell metabolism governs different cell functions27 and during osteoclast differentiation several sequential metabolic changes happen20,28,29. For example, differentiation from precursors to mature osteoclasts depends on oxidative phosphorylation,
SCFA protect from postmenopausal bone loss. To test whether these findings could be exploited as novel strategy in the treatment of pathological bone loss, we investigated the impact of SCFA on postmenopausal bone loss induced after ovariectomy (OVX). Treatments with C3 and C4 effectively prevented OVX-induced bone loss (Fig. 3a–c). At the cellular level, we observed a complete rescue of OVX-induced osteoclast formation after C3 and C4 treatment (Fig. 3d). The OVX-induced increase in CTX-I concentrations in the serum was also completely blocked (Fig. 3e). In contrast, OCN levels remained unchanged (Fig. 3f and Supplementary Fig. 3a). Serum SCFA concentrations were significantly increased to the range observed for in vitro suppressive capacities of osteoclast differentiation (Supplementary Figs. 3b and 2a). Again, no significant changes in body weight or serum cytokine levels were detected (Supplementary Fig. 3c, d). Of note, no positive effects on BV/TV were observed after HFD feeding experiments during the OVX mouse model (Supplementary Fig. 3e).

SCFA mitigate arthritis and protect from bone loss. As early as 1979, it was shown that GF rats developed exacerbated arthritis\(^{33}\). Later reports revealed that not all types of bacteria have regulatory effects on arthritis. Pro-arthritisogenic effects of certain bacterial strains were shown after mono-colonization of GF mice\(^{32,33}\). Furthermore, studies performed in gnotobiotic mice showed discrepant results with either anti-arthritogenic\(^{34}\) or pro-arthritogenic\(^{35}\) effects of defined intestinal microbes. Therefore, we addressed the role of SCFA and HFD on inflammatory bone loss in two experimental arthritis models. C3/C4 treatment significantly attenuated the severity of inflammation in the collagen-induced arthritis (CIA) mouse model (Fig. 4a, b), and systemic bone mass was increased after C3/C4 treatment (Fig. 4c). Decreased osteoclast numbers in the affected paws and serum CTX-I levels were decreased, whereas osteoblast numbers and OCN serum levels remained unchanged (Fig. 4d, e). SCFA serum concentrations were significantly increased, whereas no effect on weight gain was observed (Supplementary Fig. 4a, b). Furthermore, osteoclast-specific gene expression in the bones was significantly downregulated after treatment with C3/C4 (Supplementary Fig. 4c). Of note, the observed effects seem to be largely independent of GPR43 as treatment with C3/C4 also maintained bone mass and suppressed osteoclast-specific genes in GPR43\(^{−/−}\) mice (Supplementary Fig. 4d–h). Similar effects were observed in the CIA mouse model after HFD feeding (Fig. 4i–l) and in the K/BxN serum-induced mouse (SIA) model after SCFA supplementation in the drinking water (Fig. 4k–m), which significantly increased serum SCFA concentrations (Supplementary Fig. 4i). Furthermore, HFD feeding also protected from inflammatory bone loss during the SIA mouse model (Fig. 4n–p). In all performed experimental arthritis models, no change in weight gain was observed (Supplementary Fig. 4j, k).

**Discussion**

In previous studies, we and others could show that microbiota, or their metabolites, regulate innate and adaptive immune responses\(^{36–38}\). Here we show a hitherto undiscovered role of SCFA on bone homeostasis. Our data suggest that microbial homeostasis in the gut associated with adequate production of SCFA is an important regulatory element in determining bone composition in mice. Hence, not necessarily the microbial species per se but rather the composition of secreted microbial metabolites, in particular SCFA, appear to link gut and bone homeostasis.

There are only few studies yet having addressed the role of the gut microbiota on bone homeostasis studying GF, antibiotic-treated or mono-colonized mice and reporting contradictory findings\(^{10–15,39}\). These discrepancies may be explained by different microbial reconstitution, exposure time, concentrations of nutritional supplements, and mice strains used for the experiments. Earlier reports discussed the possibility that SCFA might be responsible for the observed beneficial effects on bone after consumption of fiber-rich diets (inulin-type fructans)\(^{40}\) and...
Fig. 2 SCFA suppress osteoclastogenesis by changing cellular metabolism. a Quantification and b representative microphotographs of multinucleated TRAP-positive osteoclasts from in vitro bone marrow cell cultures stimulated with M-CSF and RANKL and treated with 0.5 mM C2, C3, C4, or left untreated (not treated, NT). Scale bar, 1000 µm. c–d Real-time PCR analysis of osteoclast marker genes c TRAF6 and d NFATc1 during osteoclast differentiation after 24 and 48 h of SCFA exposure. e Western blot analysis of TRAF6 and NFATc1 expression after C3 and C4 stimulation for 48 h. f, g Analysis of the extracellular acidification rate (ECAR) by glycolysis measured with a Seahorse real-time metabolic analyzer. f Glycolytic capacity and g glycolytic reserve. h Ratio of ECAR to oxygen consumption rate (OCR), the latter resembling mitochondrial activity. i Time course of ECAR values in osteoclasts expose to SCFA. j Representative western blots of total AMPK and phosphorylated AMPK (pAMPK) in osteoclasts not treated (NT) or treated with SCFA (C2/C3/C4) (left panel) and quantification of the pAMPK/AMPK ratio (right panel). k Quantification of multinucleated TRAP-positive osteoclasts from in vitro bone marrow cell cultures stimulated with M-CSF and RANKL and treated with 0.5 mM C3 or 0.25 mM C4 or left untreated (not treated, NT) and incubated with 0.1 mM 2-deoxy-D-glucose (2DG), 2.5 nM oligomycin, 5 nM trichostatin A (TSA), or 10 µM anacardic acid. Pictures are representative of at least three independent experiments. Data are expressed as the mean ± s.d. Statistical difference was determined by one-way or two-way ANOVA. *p < 0.5; **p < 0.01; ***p < 0.001
galactooligosaccharides\textsuperscript{[41]}. Here we used three independent experimental approaches (direct supplementation of SCFA, HFD, and bacterial transfer) and found consistent beneficial effects on steady state, postmenopausal, and inflammatory bone metabolism highly correlated to increased SCFA levels.

Used concentrations in the in vitro experiments proved to be physiological as they were in the range of the increased SCFA concentrations in the serum and bone marrow samples after direct supplementation of SCFA or HFD feeding. Interestingly, steady-state SCFA concentrations in the bone marrow were higher than the lowest dilution used in our in vitro osteoclast suppression assays pointing toward a potential persistent suppressive capacity of SCFA during steady-state conditions. But this clearly needs to be further evaluated as our in vitro assays certainly neglect the complex situation found in the bone marrow niche with its multitude of factors impacting on the osteoclast differentiation. This is why we argue here that the balance between pro-osteoclastic and antiosteoclastic factors need to be taken into account. The mere steady-state concentrations are not that revealing, but what makes the difference is the increase over the steady-state concentrations.

In consequence, therapeutic supplementation of SCFA or diets increasing the endogenous production of SCFA may provide a powerful instrument to balance osteoclast activity and inhibit bone resorption. Even more importantly, in case of inflammatory bone loss the combination of anti-inflammatory immune-regulatory properties of SCFA together with the direct inhibition of osteoclast activity may be particularly useful. In this context, the benefits of the so-called Mediterranean-type diets, which are rich in fibers causing elevated levels of SCFA\textsuperscript{[42]} in managing human inflammatory arthritis, may be explained.

**Methods**

**Mice and treatments.** For all experiments, except the OVX model, 8-week-old female mice were used unless stated otherwise. Five-to-six-week-old WT C57BL/6 and DBA/1J female mice (Charles River) were acclimated for 1 week, followed by a 2-week co-housing period before the experiments started. WT C57BL/6 mice from the Helmholtz Centre for Infection Research, Braunschweig, Germany as indicated in the respective methods section. GPR41\textsuperscript{[43]}/43 double KO mice were kindly provided by Professor Stefan Offermanns (Max-Planck Institute, Bad Nauheim, Germany) and Dr. Tobias Junot (Novartis Pharma AG, Basel, Switzerland). Both GPR41 and GPR43 single KO mice. All mice were maintained under specific pathogen-free conditions at the Präklinisches Experimentelles Tierzentrum (PETZ) Erlangen, Germany and approved by the local ethics authorities of the Regierung von Unterfranken (\#55.2-2532-2-424). Supplementation of SCFA (all Sigma Aldrich, Germany) was done in the drinking water at a final concentration of 150 mM and changed every 3 days; control mice received pH and sodium-matched water. Specially adjusted fiber-rich diet (Supplementary Table 1) and control diet were purchased from Ssniff (Ssniff Spezialitäten GmbH; Germany) and were provided ad libitum starting 2 weeks before the experiment and throughout the study.

**Bacteria growth and mice colonization.** Three novel Prevotella species (Prevotella nov. P1, Prevotella nov. P2 and Prevotella nov. P3), Bacteroides sartorii, and Muribaculum sp. nov. have been isolated from the intestinal content of mice (data unpublished). All isolates were cultured anaerobically in Brain Heart Infusion broth, supplemented with 10% fetal bovine serum and 0.5 g/l vitamin K, for 48 h at 37 °C. WT C57BL/6N mice devoid of Prevotella spp., Bacteroides sartorii, or Muribaculum sp. nov. in their intestinal microbiota were bred and maintained in a specific-pathogen-free facility at Helmholtz Centre for Infection Research for colonization experiments. 6–7-week-old female mice were colonized with one of each isolates via oral gavage. After 6–8 weeks, cecum content and serum samples were collected and stored at –80 °C until processing. Tibia and femurs were fixed in 4% formalin for 24 h and stored in 70% ethanol.

**DNA isolation and 16S rRNA microbial community analysis.** Fresh stool samples of mice were collected and immediately stored at –20 °C. For DNA-based 16S
rRNA gene sequencing. DNA was extracted according to established protocols using a method combining mechanical disruption (bead-beating) and phenol/chloroform-based purification. Briefly, sample was suspended in a solution containing 500 µl of extraction buffer (20 mM Tris, 20 mM EDTA, 200 mM NaCl, pH 8.0), 200 µl of 20% sodium dodecyl sulfate (SDS), 500 µl of phenol:chloroform:isoamyl alcohol (24:24:1), and 100 µl of 0.1 mm zirconia/silica. Samples were homogenized twice with a bead beater (BioSpec) for 2 min. After precipitation of DNA, crude DNA extracts were resuspended in TE Buffer with 100 µg/ml RNase and column purified to remove PCR inhibitors (BioBasic). Amplification of the V4 region (F515/R806) of the 16S rRNA gene was done. Samples were sequenced on an Illuminia MiSeq platform (PE250). Sequence assembly, quality control, and clustering were performed using the usearch8.1 software package (http://www.drive5.com/usearch/). Reads were clustered into 97% ID operational taxonomic units (OTUs) using UPARSE algorithm followed by taxonomy assignment using the Silva database v128 and the RDP Classifier with a bootstrap confidence cutoff of 80%. The OTU absolute abundance table and mapping file were used for
statistical analyses and data visualization in the R statistical programming envi-
ronment package phyloseq23.

ELISA CTX-I (IDS Immunodiagnostics Systems GmbH, Germany). OCN (Alfa
Aesar, Germany) and undercarboxylated OCN (BlueGene, Japan) serum levels were
measured by enzyme-linked immunosorbent assay (ELISA) according to the
manufacturer’s instructions.

Multiplex IL-6, IL-10, IL-17, IFNγ, TGFβ, and TNFα serum levels were measured
by Multiplex using a LEGENDplex Multi-Analyte Flow Assay Kit (BioLegend),
according to the manufacturer’s instructions.

Histology. Tibial bones were fixed in 4% formalin for 24 h and decalcified in
EDTA (Sigma-Aldrich). Serial paraffin sections (2 μm) were stained for tartrate
resistant acid phosphatase (TRAP) using a Leukocyte Acid Phosphatase Kit
(Sigma) according to the manufacturer’s instructions. Osteoclast numbers were
quantified using a microscope (Carl Zeiss) equipped with a digital camera and an
image analysis system for performing histomorphometry (Osteomeasure; Osteo-
Metrics). For Calcein labeling, mice were injected with 30 mg/kg body weight of
green fluorescent Calcein (Sigma) 11 and 4 days before sacrifice. Undecalcified
bones were embedded in methacrylate and 5 mm sections were cut. Unstained
sections were used to measure fluorescent Calcein-labeled bone surfaces at a
wavelength of 495 nm. Toluidine Blue staining was performed for quantification of
osteoblasts and von Kossa staining for bone mineralization.

Micro-computed tomography μCT imaging was performed using the cone-beam
Desktop Micro Computer Tomograph μCT 40 by SCANCO Medical AG.
Bruttisellen, Switzerland. The settings were optimized for calcified tissue visual-
ization 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 was used and an evaluation script adjusted grayscale thresholds of the operating
system “Open VMS” by SCANCO Medical was used. Volume of interest: Tibia
The analysis of the bone structure was performed in the proximal metaphysis of the
tibia, starting 0.43 mm from an anatomic landmark in the growth plate and
1.72 mm above (200 tomograms) distally.

Collagen-induced arthritis. CIA was induced in 8-week-old female C57BL/6J or
DBA/1J mice by subcutaneous injection at the base of the tail with 100 μl
of 0.25 mg chicken type II collagen (Chondrex, Redmond, WA) in complete Freund
adjuvant (Dîko Laboratoire, Detroit, MI), containing 5 mg/ml killed Mycobacteri-
tuberculosis (H37Ra). Mice were rechallenged after 21 days intradermal
immunization in the base of the tail with this emulsion. The paws were evaluated
for joint swelling and grip strength three times per week. 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 limb: 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, normal grip strength; 1, mildly reduced grip strength; 2, moderately reduced
grasp strength; 3, severely reduced grip strength; 4, no grip strength at all).

K/BxN serum-induced model. K/BxN SIA was induced by intraperitoneal
injection of 200 μl pooled K/BxN serum. The swelling of fore and hind paws were
measured three times per week. Development of arthritis was evaluated for each
paw using a semiquantitative scoring system (0–4 per paw; maximum score of 16).

Postmenopausal bone loss model. At 6 weeks of age, mice were either sham-
operated or ovariectomized. Surgically removed ovaries were examined histologi-
cally to verify successful ovariectomy. Mice were kept in identical housing condi-
tions controlling for the same environment, food, light, and temperature for
8–10 weeks. Then tibial bones were excised and subjected to histological assessment
or μCT imaging.

Isolation and culture of osteoclast precursors. Bone marrow was isolated from
8-week-old C57BL/6J mice by flushing femoral bones with complete media. Bone
marrow cells were plated in 96-well flat bottom plates (2 x 10^5/well) in aMEM
supplemented with 10% fetal calf serum, 30 ng/ml macrophages colony-stimulating
factor, and 50 ng/ml RANKL (R&D Systems, Wiesbaden-Nordenstadt, Germany).
Alternatively, osteoclast precursors were isolated from bone marrow-derived cell
suspending using CD11b microbeads (Miltenyi Biotec, Germany) according to the
manufacturer’s instructions. The purity of isolated precursors was analyzed by
flow cytometric analysis using CD11b–FITC–labeled antibodies (Miltenyi Biotec).
CD11b+ monocytes were plated in 96-well flat bottom plates (2 x 10^5/well).
Medium was changed after 72 h. Osteoclast differentiation was evaluated by staining
osteoclasts for TRAP using a Leukocyte Acid Phosphatase Kit (Sigma–
Aldrich, Taufrücken, Germany), according to the manufacturer’s instructions.
Bone resorption assays were made using an Osteo Assay Surface 96 Well (Corning),
according to the manufacturer’s instructions.

SCFA measurements. Four to five replicates of frozen cecal samples (100 mg) or
50 μl of serum were weighed into a 2 ml polypropylene tube. The tubes were kept in
a cool box under dry ice. The samples were centrifuged at 13,200g and 4°C for
30 min. Serum samples were used to measure total SCFA levels, and the supernatants
were used for gas chromatography/mass spectrometry (GCMS). The GCMS stabi-
lization was done by integration of the extracted ion chromatogram peaks for the following ion species: m/z 45 for acetate eluted at 7.8 min, m/z 74 for propionate eluted at 9.6 min, and m/z 60 for butyrate eluted at 11.5 min. GCMS solution software was used for data processing.

Real-time PCR. Tissues were stored in RNALater (Ambion) or directly transferred
to TRIzol (Invitrogen). RNA was extracted accordingly to the manufacturer’s
instructions. Gene expression results are expressed as arbitrary units relative to
expression of the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase
(GAPDH). Primer sequences are as follows: TRAF6: 5'-AAACAGGAG-
AGTCTTCTCCGT-3' and 5'-ACTGGGACAATTCACTAGAGC-3';
NFATc1: 5'-GGAATGTGACGG-3' and 5'-CCATCTCATGCCACATGC-3';
GPR41: 5'-ATGTTGGTGCAGGCAGAGTAGGA-3' and 5'-CTGATTGCAACCAACATCCG-
GAGAGCAGG-3'; RANK: 5'-GGCGAGTCTCATTGTGTCTGCG-3' and 5'-GAGAATGTGACGG-3';
PPARα: 5'-GTCGACTAGCA-
TAAGAAGCGCAGCA-3' and 5'-TAGGTGGTCGACCAGCAGTGAGA-3'; PPARγ: 5'-
ACCTGACATTGTCGACACCT-3' and 5'-CCATCTCATGCCACATGC-3'; GPR3: 5'-
GAGCAGACTGTGGTGTGCAAGATCC-3' and 5'-CAGTTGGCAAGAACAAAAAT-
GAGCAA-3'; GPR6: 5'-ATGCGAGGAGCATACTGTGTCTAGCA-3'; MMP9: 5'-
TCCGTCTGACGAGGAACAGAATAGTA-3' and 5'-GAGGTTGAGACCCACAAATCTG-
AAGG-3'; TCF7: 5'-GGTGGTGAACCCAGGAAATT-3' and 5'-CCTTCCCAAATGCGAAATG-3'.

Western blotting. Cultured cells were washed twice with phosphate-buffered
saline and subsequently lysed. Protein extracts were separated on 10% SDS-
polyacrylamide gel, transferred on a nitrocellulose membrane, and stained with
primary antibodies against TRAF6 (D-10, Santa Cruz, dilution 1:500), NFT31 (TAA, Santa
Cruz, dilution 1:500), PAK4 (40H9, CellSignal, dilution 1:1000), and AMPK (342.45,
abcam, dilution 1:1000). An antibody against β-actin (AC-15, abcam, dilution 1

Fig. 4 SCFA mitigate arthritis and protect from inflammatory bone loss. a–j Collagen-induced arthritis (CIA) model; k–p K/BxN serum-induced arthritis
(SIA) model. a Joint swelling and b grip strength scores in mice induced for CIA and not treated (NT) or treated with C2 (acetate), C3 (propionate), or C4
(butyrate) in the drinking water. c μCT analysis of tibial bone mass showing bone volume per total volume (BV/TV). d Immunohistochemical analysis
of TRAP-stained osteoclasts in the tibia of mice showing total osteoclast cell numbers per paw. e Serum CTX-I bone resorption marker level measured by
ELISA. f Immunohistochemical analysis showing the number of osteoblasts per analyzed bone parameter in the tibia (N.Ob./B.pm). g Osteocalcin (OCN)
in bone formation marker level measured by ELISA. h Joint swelling and i grip strength scores in mice induced for CIA and fed with special high-fiber diet
(HFD) or normal control diet (ND). j μCT analysis of tibial bone mass showing BV/TV. k Joint swelling and l grip strength scores in mice induced for SIA
and not treated (NT) or treated with C2/C3/C4 in the drinking water. m μCT analysis of tibial bone mass showing BV/TV. n Joint swelling and o grip
strength scores in SIA mice fed with special HFD or ND. p μCT analysis of tibial bone mass showing BV/TV. Data are expressed as the mean ± s.d. expect
for a, b, h, i, k, l, n, and o where mean ± s.e.m. is shown. Pictures are representative of at least two independent experiments. Statistical difference was
determined by one-way ANOVA, two-way ANOVA, or Student’s t-test. *p < 0.5; **p < 0.01; ***p < 0.001

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-25,000) was used as loading control. Uncropped scans of western blots are shown in supplementary information (Supplementary Fig. 5a).

**Extracellular flux assays.** Bioenergetics of osteoclast precursors were determined using the XF96 Extracellular Flux Analyzer (Seahorse Bioscience/Agilent Technologies, North Billerica, MA). Cells were seeded in specialized cell culture microwells at a density of 1 x 10^5 cells/well and cultured for 24 and 48 h. One hour prior to the measurement, a Seahorse XF96 cartridge (Agilent/Seahorse Bioscience) was loaded with 200 μl XF Calibrant solution (Agilent/Seahorse Bioscience) per well and incubated overnight in a CO₂-free atmosphere. One hour before the measurement, cells were incubated at 37 °C also in a CO₂-free atmosphere. The ports of the Seahorse cartridge were loaded with glucose, oligomycin, and 2DG for the glycolysis stress test and oligomycin, FCCP, and antimycin A/rotenone for the mitochondrial stress test. After sensor calibration assays were run as detailed in the manufacturer’s manual recording extracellular acidification rate and oxygen consumption rate over time. Metabolic parameters were derived from the XF Wave software (Agilent/Seahorse Biosciences) and calculated with Microsoft Excel. All experiments were performed in pentaplicates. Raw values were normalized to the total protein content for each well.

**Flow cytometry.** Spleens were smashed and filtered through a 40 μm gauze (BD Biosciences). Single-cell suspensions were stained for analysis for C-terminal collagen type I cleavage products (CTX-1) as marker for bone resorption and OCN as marker for bone formation by ELISA. A single-cell suspension was analyzed for C-terminal collagen type I cleavage products (CTX-1) as marker for bone resorption and OCN as marker for bone formation by ELISA.

**Data availability.** All relevant data are available from the authors upon reasonable request. 16S RNA sequencing data are deposited in publicly accessible database and available under the following accession code: PRJNA419741.

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Conceived and designed the experiments: T.S., G.S., and M.M.Z. Performed the experiments: S.L., Y.O., J.H., A.I., O.A., O.S., K.S., B.K., and M.M.Z. Analyzed the data: S.L., J.H., M.B., O.A., O.S., M.H., and M.M.Z. Wrote the paper: K.S., G.S., and M.M.Z.

**Additional information**

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