Dietary modulation of the microbiome affects autoinflammatory disease

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Figure 1 | Changes in diet limit the development of inflammatory bone disease in Pstpip2cmo mutant mice. a–d, Wild-type (WT) and Pstpip2cmo mutant mice were fed a low-fat diet (LFD) or a high-fat and cholesterol diet (HFD). a, Incidence of inflammatory bone disease. Combined data from three independent experiments. b–d, Representative in vivo magnetic resonance imaging (MR) of hind paw samples from WT, LFD Pstpip2cmo and HFD Pstpip2cmo mice. Each point represents an individual mouse, and the line represents the mean ± s.e.m. ***p < 0.001; Student’s t-test.

The incidences of chronic inflammatory disorders have increased considerably over the past three decades1. Recent shifts in dietary consumption may have contributed importantly to this surge, but how dietary consumption modulates inflammatory disease is poorly defined. Pstpip2cmo mice, which express a homozygous Leu98Pro missense mutation in the Pombe Cdc15 homology family protein PSTPIP2 (proline-serine-threonine phosphatase interacting protein 2), spontaneously develop osteomyelitis that resembles chronic recurrent multifocal osteomyelitis in humans2–4. Recent reports demonstrated a crucial role for interleukin-1β (IL-1β) in osteomyelitis, but deletion of the inflammatory components caspase-1 and NLRP3 failed to rescue Pstpip2cmo mice from inflammatory bone disease5–6. Thus, the upstream mechanisms controlling IL-1β production in Pstpip2cmo mice remain to be identified. In addition, the environmental factors driving IL-1β-dependent inflammatory bone erosion are unknown. Here we show that the intestinal microbiota of diseased Pstpip2cmo mice was characterized by an outgrowth of Prevotella. Notably, Pstpip2cmo mice that were fed a diet rich in fat and cholesterol maintained a normal body weight, but were markedly protected against inflammatory bone disease and bone erosion. Diet-induced protection against osteomyelitis was accompanied by marked reductions in intestinal Prevotella levels and significantly reduced pro–IL-1β expression in distant neutrophils. Furthermore, pro–IL-1β expression was also decreased in Pstpip2cmo mice treated with antibiotics, and in wild-type mice that were kept under germ-free conditions. We further demonstrate that combined deletion of caspases 1 and 8 was required for protection against IL-1β-dependent inflammatory bone disease, whereas the deletion of either caspase alone or of elastase or neutrophil proteinase 3 failed to prevent inflammatory disease. Collectively, this work reveals diet-associated changes in the intestinal microbiome as a crucial factor regulating inflammatory disease in Pstpip2cmo mice.

Changes in diet are known to determine susceptibility to common autoimmune diseases such as atherosclerosis, coronary heart disease and type 2 diabetes7. To address whether dietary intake affects osteomyelitis in Pstpip2cmo mice, a cohort of animals was fed ad libidum a diet rich in saturated fats and cholesterol (high-fat diet, or HFD), and disease progression was compared to that of Pstpip2cmo mice placed on a regular low-fat diet (LFD). As expected, all animals on a LFD (n = 40) had developed inflammatory bone disease by day 100 (Fig. 1a), as evidenced by the red and swollen appearance of their hind paws (Extended Data Fig. 1a), the significant bone erosion and deformity seen in representative in vivo magnetic resonance imaging micrographs (Fig. 1b), and the increased size of draining popliteal lymph nodes (Extended Data Fig. 1b). In marked contrast, Pstpip2cmo mice that were fed a HFD (n = 22) were largely protected from osteomyelitis, and these mice resembled healthy wild-type mice in terms of hind paw inflammation, bone erosion and lymph node size (Fig. 1a, b and Extended Data Fig. 1b). In agreement, haematoxylin and eosin-stained sections of the hind paws (Fig. 1c, d and Extended Data Fig. 1c, d). Conversely, Pstpip2cmo mice that were fed a regular LFD diet showed significant bone destruction and inflammatory cell infiltration in stained paw (Fig. 1b–d) sections. In agreement, profound reductions in the numbers of infiltrating neutrophils and macrophages were evident in the footpads of HFD-fed Pstpip2cmo mice compared to LFD-fed Pstpip2cmo mice (Extended Data Fig. 1e). Consumption of a HFD was also found to rescue hyperinflammatory cytokine production in Pstpip2cmo mutant mice (Extended Data Fig. 2a, b). As expected for mice on a BALB/cJ genetic background, Pstpip2cmo mice retained a normal body weight during these studies, regardless of whether they were fed a lean or high-fat diet (Extended Data Fig. 3a, b). Collectively, these observations demonstrate that the dietary composition determines to a large extent whether genetically susceptible Pstpip2cmo mice develop osteomyelitis independently of gross changes in body weight.

Diets high in fat and cholesterol induce large-scale changes in the host microbiota composition8,9. We made use of 16S ribosomal RNA (rRNA)
Moreover, we failed to detect bacteria in the peripheral organs of LFD-induced changes in the microbiota composition were not accompanied by changes in diet. a, Heat map of fold differences in LFD-fed Pstpip2cmo mice that were fed a regular LFD that was characterized by a suppression of disease-associated commensals (Fig. 2b, c). Most notably, LFD-fed Pstpip2cmo mice displayed a time-dependent increase in Prevotella levels (Fig. 2d), which was significantly reduced in Pstpip2cmo mice that were kept on a HFD (Fig. 2e). The latter group of HFD mice was further characterized by an expansion of Lactobacillus species in their intestinal tract (Fig. 2c). Diet-induced changes in the microbiota composition were not accompanied by readily detectable intestinal inflammation (Extended Data Fig. 3c–e). Moreover, we failed to detect bacteria in the peripheral organs of LFD-fed Pstpip2cmo mice (Extended Data Fig. 3f). Together, these results show that inflammatory bone disease in Pstpip2cmo mice is specifically characterized by an outgrowth of inflammation-associated intestinal commensals, which is suppressed by a HFD regimen.

We and others have previously shown that inflammatory bone disease in Pstpip2cmo mice crucially relies on IL-1β (refs 5, 6). Given that Pstpip2cmo mice on a HFD were markedly resistant to disease progression, we addressed whether HFD dampened IL-1β levels. Pstpip2cmo mice that were fed a LFD had Il1b (which encodes the precursor protein pro-IL-1β) messenger RNA levels that were on average 60-fold higher than in footpads of healthy wild-type mice (Fig. 3a). In sharp contrast, HFD-fed Pstpip2cmo mice had markedly suppressed local Il1b transcript levels that were comparable to those of healthy wild-type mice (Fig. 3a). In agreement with these observations, IL-1β protein concentrations were significantly increased in the footpads of LFD-fed Pstpip2cmo mice, whereas those of HFD-fed Pstpip2cmo mice were comparable to healthy controls (Fig. 3b and Extended Data Fig. 4a). Together, this suggests that HFD suppressed osteomyelitis in Pstpip2cmo mice by dampening pro-IL-1β expression.

Figure 3 | Microbiota-mediated regulation of IL-1β expression shapes inflammatory bone disease. a, Quantitative PCR with reverse transcription (qRT–PCR) analysis of relative Il1b expression in the footpads of 12–16-week-old wild-type, LFD Pstpip2cmo and HFD Pstpip2cmo mice. Each point represents an individual mouse, and the line represents the mean ± s.e.m. Combined data from three independent experiments. b, Protein levels of IL-1β in the hind paws. Combined data from two independent experiments. c, Relative Il1b mRNA expression levels in CD45+ cells isolated from the colon of specific-pathogen-free (SPF) and germ-free (GF) WT mice. Two biological replicates, with two technical replicates each. d, e, Pstpip2cmo mice were treated with a cocktail of broad-spectrum antibiotics in their drinking water (ABX). d, qRT–PCR analysis of colonic Il1b expression levels from 12–14-week-old Pstpip2cmo mice that received either regular drinking water (n = 15) or antibiotics water (n = 9). e, Incidence of inflammatory bone disease. f, g, Young Pstpip2cmo mice (3 weeks old) received PBS or faecal microbiota from disease LFD Pstpip2cmo mice or disease-free HFD Pstpip2cmo mice by oral transplantation. f, 16S rRNA analysis of Prevotella copy numbers. g, Incidence of inflammatory bone disease. Combined data from three independent experiments. h, i, Representative footpad images (h) and haematoxylin and eosin micrographs (original magnification, ×2) (i). NS, not significant; *P < 0.05; **P < 0.01; ***P < 0.001; Student’s t-test.
Given that HFD skewed the intestinal microbiota composition of Pstpip2cmo mice (Fig. 2), we next asked whether the microbiota controlled Il1b expression. We found that Il1b levels in CD45+ cells that were isolated from the colons of germ-free wild-type mice were significantly lower than mice that were kept under specific pathogen-free conditions (Fig. 3c). Moreover, the levels of pro-IL-1β protein were considerably reduced in the hind paws of germ-free wild-type mice (Extended Data Fig. 4b). However, the expression of Il1b mRNA by CD45.2+ cells isolated from germ-free mice was greatly enhanced following in vitro stimulation with lipopolysaccharide (LPS), suggesting that these germ-free mice do not have any intrinsic defects in Il1b mRNA expression (Extended Data Fig. 4c). Notably, broad-spectrum antibiotics that significantly reduced Prevotella and Flexispira levels in LFD-fed Pstpip2cmo mice (Extended Data Fig. 5a) also substantially decreased the levels of colonic Il1b in these mice (Fig. 3d). In addition, broad-spectrum antibiotics significantly protected LFD-fed Pstpip2cmo mice from developing osteomyelitis (Fig. 3f), and significantly accelerated the development of osteomyelitis. Mice deficient in caspase-8 are embryonic lethal19–21, and this lethality is rescued by further deleting the necroptosis-regulating kinase RIPK3 (refs 22, 23). We thus bred Pstpip2cmo, Casp8−/− and silica (12 h). Left untreated (UT) or were first primed with LPS derived macrophages (e), Secretion of IL-1β was measured by ELISA. Western blot analysis of IL-1β (f) and caspase-8 were also recently shown to convert pro-IL-1β into its bioactive form12–18. Genetic deletion of caspase-1 and the related protease caspase-11 failed to rescue Pstpip2cmo mice from inflammatory bone disease. We therefore addressed the role of additional proteases in IL-1β-dependent osteomyelitis. To this end, Pstpip2cmo mice were bred onto wild-type, and Il1b-deficient Pstpip2cmo mice (Extended Data Fig. 6a, b). However, deletion of neither neutrophil proteinase 3 nor elastase rescued or delayed inflammatory bone disease in Pstpip2cmo mice (Extended Data Fig. 6a, b). We next sought to examine the role of caspase-8 in Pstpip2cmo-associated osteomyelitis. Mice deficient in caspase-8 are embryonic lethal19–21, and this lethality is rescued by further deleting the necroptosis-regulating kinase RIPK3 (refs 22, 23). We thus bred Casp8−/− × Ripk3−/− on Pstpip2cmo mice. Caspase-8 may act redundantly with caspase-1 in pro-IL-1β protease responsible for IL-1β maturation1.

The pro-IL-1β precursor protein is produced as a biologically inactive molecule that resides in the cytosol and needs to be proteolytically converted into mature IL-1β to gain biological activity. Caspase-1, a protease that is activated by inflammasome complexes, is the principal protease responsible for IL-1β maturation1. Neutrophil protease 3, elastase and caspase-8 were also recently shown to convert pro-IL-1β into its bioactive form12–18. Genetic deletion of caspase-1 and the related protease caspase-11 failed to rescue Pstpip2cmo mice from inflammatory bone disease. We therefore addressed the role of additional proteases in IL-1β-dependent osteomyelitis. To this end, Pstpip2cmo mice were bred onto wild-type, and Il1b-deficient Pstpip2cmo mice (Extended Data Fig. 6a, b). However, deletion of neither neutrophil proteinase 3 nor elastase rescued or delayed inflammatory bone disease in Pstpip2cmo mice (Extended Data Fig. 6a, b). We next sought to examine the role of caspase-8 in Pstpip2cmo-associated osteomyelitis. Mice deficient in caspase-8 are embryonic lethal19–21, and this lethality is rescued by further deleting the necroptosis-regulating kinase RIPK3 (refs 22, 23). We thus bred Casp8−/− × Ripk3−/− on Pstpip2cmo mice. Caspase-8 may act redundantly with caspase-1 in pro-IL-1β protease responsible for IL-1β maturation1.

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conversion under particular conditions, which we addressed by further deleting caspase-1 in Casp8/Ripk3-deficient Pstpip2<sup>−/−</sup> mice. As expected, Pstpip2<sup>−/−</sup> mice gradually developed inflammatory bone disease, with all mice being affected by 80 days (Fig. 4a). As reported, Pstpip2<sup>−/−</sup> mice lacking IL-1β were fully resistant to osteomyelitis development (Fig. 4a). Ripk3-deficient, Casp1-deficient and Casp8/Ripk3-deficient Pstpip2<sup>−/−</sup> mice developed osteomyelitis with similar kinetics to Pstpip2<sup>−/−</sup> mice (Fig. 4a and Extended Data Fig. 7a), which was also reflected in the extent of bone erosion and histopathology seen in these mice (Extended Data Fig. 7b, c). Notably, the combined deletion of caspase-1 and -8 provided significant protection against osteomyelitic disease (Fig. 4a, b). In agreement, pro-IL-1β expression levels were reduced and IL-1β maturation was virtually blunted in the footpads of Pstpip2<sup>−/−</sup> mice lacking both caspases (Fig. 4c and Extended Data Fig. 7d). In marked contrast, we observed spontaneous IL-1β maturation in footpads of Pstpip2<sup>−/−</sup> mice, as well as in mice lacking either caspase-1 or -8 (Fig. 4c).

Pstpip2<sup>−/−</sup> haematopoietic cells were recently shown to be sufficient to induce osteomyelitis in wild-type donor mice, suggesting that bone-marrow-derived cell populations are probably responsible for aberrant IL-1β production in Pstpip2<sup>−/−</sup> mice. We first evaluated the production of IL-1β by macrophages and neutrophils because these are the predominant immune cell types found in active osteomyelitis lesions (Extended Data Fig. 1e). As reported, stimulation of LPS-primed Pstpip2<sup>−/−</sup> macrophages with NLPR3 inflammasome triggers such as ATP and silica triggered normal levels of secreted IL-1β (Fig. 4d and Extended Data Fig. 8a). In contrast, levels of IL-1β secreted by Pstpip2<sup>−/−</sup> neutrophils that were stimulated with these agents were at least fourfold higher than those of wild-type cells (Fig. 4e, f). Importantly, neutrophils of HFD-fed Pstpip2<sup>−/−</sup> mice expressed less pro-IL-1β (Extended Data Fig. 8b), and IL-1β maturation was markedly affected when compared to neutrophils of LFD-fed Pstpip2<sup>−/−</sup> mice (Extended Data Fig. 8c). By contrast, pro-IL-1β production and IL-1β maturation were not significantly different in macrophages of LFD- and HFD-fed Pstpip2<sup>−/−</sup> mice (Extended Data Fig. 8d). To ascertain the role of neutrophils in IL-1β-dependent osteomyelitis further, Pstpip2<sup>−/−</sup> mice were treated with anti-Ly6G antibodies to deplete neutrophils. Anti-Ly6G treatment led to marked reductions in circulating neutrophil counts (Extended Data Fig. 9a–c). Notably, neutrophil ablation conferred significant protection from clinical disease progression (Fig. 4g, h) and histopathological tissue damage (Fig. 4i).

Collectively, our findings presented here show that dietary intake determines the composition of the intestinal microbiota, and greatly influences disease outcome in osteomyelitis-susceptible Pstpip2<sup>−/−</sup> mice by upregulating pro-IL-1β levels. We further show that activation of caspases 1 and 8 in these mice result in spontaneous induction of IL-1β-driven neutrophilic osteomyelitis in Pstpip2<sup>−/−</sup> mice (Extended Data Fig. 10). These results suggest that diet-induced changes in the intestinal microbiota composition may promote autoinflammatory disease in susceptible individuals by increasing pro-IL-1β levels available for conversion by caspases 1 and 8.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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**METHODS**

Mice. *Psippip2m* (ref. 3), *Il1b−/−* (ref. 24), *Casp1−/−* (ref. 25), *Casp8−/−* (ref. 26), *Ripk3−/−* (ref. 27), *Elane−/−* (ref. 28) and *Elane−/−* *Prtn3−/−* (ref. 29) mice were previously described. *Psippip2m* were purchased from The Jackson Laboratory and are on the BALB/c background. All other mutant mice are on the C57BL/6 background. To generate the necessary controls and experimental mice for these experiments, mice that were heterozygous for both the *Psippip2* and knockout allele(s) were used as breeders. Littermate controls were used to evaluate whether genetic deletions influence immune responses, IL-1β regulation and osteomyelitis disease development. Germ-free mice were obtained from Taconic. The number of mice per group used in an experiment is annotated in the corresponding figure legend as *n*. No gender differences were observed. In vivo experiments were controlled with age-matched littermates. The sample sizes were chosen to validate statistical analyses. All mice were housed in specific pathogen-free conditions within the Animal Resources Center at St Jude Children’s Research Hospital. Animal studies were conducted under protocols approved by the Institutional Animal Care and Use Committee of St Jude Children’s Research Hospital.

**Diet.** Feed that was high in fat and cholesterol was purchased from Research Diets (in stock number D12107 and consisted of 40% fat and 0.5% cholesterol. Standard low fat diet was obtained from LabDiet (stock number 5013) and consisted of 5% fat and 0% cholesterol.

**Histopathology.** Formalin-preserved paws and tails were processed and embedded in paraffin according to standard procedures. Haematoxylin and eosin (H&E) staining was done on sections (5 μm) were examined by a pathologist blinded to the experimental groups. Tail and paw sections were scored based on the extent and severity of inflammation.

**Micro-computed tomography.** Micrographs of paws and tails fixed in formalin were made using an ex vivo micro-computed tomography scanner (LocusSP Specimen CT, GE Healthcare) at 28-μm isotropic voxel size, with 720 projections, an integration time of 1,700 ms, photon energy of 80 keV, and a current of 70 μA.

**16S RNA microbiome analysis.** Fifty nanograms of purified DNA was prepared using Nextflex 16S v4 Amplicon-seq kit according to the manufacturer’s instructions (Bioo Scientific). In brief, PCR primers targeted the fourth hypervariable domain of microbial 16S ribosomal RNA genes and simultaneously introduced sequences required for sequencing demultiplexing. Ampure XP PCR purification was used to clean up the PCR reactions (Beckman Coulter). PCR products were quantified using the Quant-iT PicoGreen assay (Invitrogen), normalized and pooled. Pooled samples were sequenced on a MiSeq sequencer (Illumina San Diego) according to manufacturer’s instructions with modifications specified in the Nextflex 16S v4 kit. The 16S primers targeting the V4 region were aligned to the full set of sequences from the Greengenes database v13.5 using exonerate. Each sequence was truncated to include only the V4 region, the primer-matching regions, and an additional 40 bases on either side. Duplicate V4 regions were removed from the data set. All taxa labels from the removed duplicates were associated with the remaining representative V4 region sequence. Reads from each sample were aligned exhaustively to the non-redundant V4 sequences using USEARCH allowing a minimum sequence identity of 90%. All taxon labels associated with the top-scoring V4 region(s) were used to determine the taxon assignment of each read. The highest resolution non-conflicting taxon from all taxa associated with the top-scoring V4 region was assigned as the taxon for a read.

Relative proportions (P) of microbial taxa for each sample were assembled from the highest resolution sequence counts into a matrix with samples as columns and taxa as rows with proportions in cells. Columns were also assigned to a wild-type/ knockout group according to the design. All relative proportions are transformed to near normality with a shifted logit-P transformation.

**Statistical analysis.** All results are presented as mean ± standard error. We performed statistical analysis using the two-tailed Student’s *t*-test. *P* values are denoted by *P* < 0.05, **P** < 0.01, ***P*** < 0.001, ****P*** < 0.0001.

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Extended Data Figure 1 | Placing *Pstpip2*<sup>−/−</sup> mice on a high-fat and cholesterol diet limits the development of inflammatory bone disease. a–e, Wild-type and *Pstpip2*<sup>−/−</sup> mutant mice were fed a LFD or HFD. Representative hind paw images (a) and representative pictures of popliteal lymph nodes (b) from wild-type, LFD *Pstpip2*<sup>−/−</sup> and HFD *Pstpip2*<sup>−/−</sup> mice at 12–14 weeks of age. c, d, Haematoxylin and eosin staining (original magnification, ×20) (c) and pathology scores (d) of tail samples from 12–14-week-old wild-type, LFD *Pstpip2*<sup>−/−</sup> and HFD *Pstpip2*<sup>−/−</sup> mice. Pathology scores were assigned in a blinded fashion by a veterinary pathologist based on the extent and severity of inflammation, osteolysis and osteogenesis. e, Representative immunostaining of neutrophils and macrophages in hind paw sections from 14–18-week-old *Pstpip2*<sup>−/−</sup> mice that were fed either a LFD or a HFD (original magnification, ×60). ***P < 0.001; Student’s t-test.
Extended Data Figure 2 | Consumption of a HFD limits hyperinflammatory cytokine production in Pstpip2<sup>−/−</sup> mutant mice.

**a**, Wild-type and Pstpip2<sup>−/−</sup> mutant mice were fed a LFD or HFD for 12 weeks. Relative expression of Cxcl1 (wild type n = 8; LFD Pstpip2<sup>−/−</sup> n = 4; HFD Pstpip2<sup>−/−</sup> n = 9) and Il6 (wild type n = 11; LFD Pstpip2<sup>−/−</sup> n = 10; HFD Pstpip2<sup>−/−</sup> n = 8) in the hind paws was determined by qRT–PCR. The bar graphs depict combined data from two independent experiments. Data are shown as mean ± s.e.m. **b**, Wild-type and Pstpip2<sup>−/−</sup> mutant mice were fed a LFD or a HFD for 12 weeks and cytokines levels in the hind paws were determined by ELISA. Combined data are from two independent experiments. Each point represents an individual mouse, and the line represents the mean ± s.e.m. *P < 0.05, **P < 0.01, ***P < 0.001; Student’s t-test.
Extended Data Figure 3 | Placing Pstpip2<sup>tm1m</sup> mice on a HFD does not cause abnormal weight gain, intestinal inflammation or extraintestinal translocation of commensal bacteria. a, b, Wild-type BALB/cJ and Pstpip2<sup>tm1m</sup> mice were fed ad libidum a LFD or a HFD. Body weight was measured in age-matched female (a) and male (b) mice at 12–16 weeks of age. Each point represents an individual mouse and the line represents the mean ± s.e.m. Data were combined from three independent experiments. c–e, Colon length (c), colitis score based on rectal bleeding and stool consistency (d) and representative haematoxylin and eosin-stained sections (original magnification, ×20) (e) of the intestinal tract of LFD- and HFD-fed Pstpip2<sup>tm1m</sup> mice aged 14–18 weeks. f, Presence of commensal bacteria in the spleen, liver, mesenteric lymph nodes and bone of wild-type and diseased LFD-fed Pstpip2<sup>tm1m</sup> mice was evaluated by Gram staining and 16S rDNA qPCR analysis of eubacteria.
Extended Data Figure 4 | Dietary- and microbiota-associated factors influence the production of pro-IL-1β. a, Footpad homogenates of 12–16-week-old wild-type, LFD-fed Pstpip2<sup>Δmo</sup> and HFD-fed Pstpip2<sup>Δmo</sup> mice were immunoblotted for IL-1β. Data are representative of three independent experiments. b, Footpad samples were collected from 10–14-week-old specific pathogen-free wild-type, germ-free wild-type and Pstpip2<sup>Δmo</sup> x Il1b<sup>−/−</sup> mice and pro-IL-1β protein levels were determined by western blotting. c, CD4<sup>+</sup> cells were isolated from the colons of germ-free wild-type mice and cells were left untreated or stimulated with LPS for 1 h. Relative Il1b mRNA expression levels were determined by qRT–PCR. Two biological replicates, with two technical replicates each.
Extended Data Figure 5 | Co-housing does not alter disease progression in LFD-fed Pstpip2^{cmo} mice. a, Pstpip2^{cmo} mice were treated with a cocktail of broad-spectrum antibiotics in their drinking water. Faecal samples were collected from wild-type (n = 5) and Pstpip2^{cmo} mice that received either regular drinking water (n = 5) or antibiotics water (n = 11) 5–7 weeks later. *Prevotella* and *Flexispira* 16S rDNA copy numbers were quantified and normalized to total bacteria. The bar graphs depict the mean ± s.e.m. b, Faecal microbiota from diseased Pstpip2^{cmo} mice was orally transplanted into wild-type mice (Pstpip2^{cmo} microbiota ≥ wild type) and the incidence of inflammatory bone disease in control Pstpip2^{cmo} and faecal transplantation mice was evaluated. c, d, Pstpip2^{cmo} mice were singly housed or co-housed with wild-type (c) or Il1b-deficient Pstpip2^{cmo} (d) mice. Clinical development of bone deformity and arthritic inflammation in hind paws and tails was monitored over time. **P < 0.01, ***P < 0.001; Student’s t-test.
Extended Data Figure 6  | The neutrophil associated proteases elastase and proteinase 3 are not required for Pstpip2<sup>cmo</sup>-mediated bone disease.

**a**, Incidence of inflammatory bone disease in Pstpip2<sup>cmo</sup>, Pstpip2<sup>cmo</sup>×Elane<sup>−/−</sup>, Pstpip2<sup>cmo</sup>×Elane<sup>−/−</sup>Prtn3<sup>−/−</sup> and Pstpip2<sup>cmo</sup>×Il1b<sup>−/−</sup> mice.

**b**, Representative footpad images from wild-type, Pstpip2<sup>cmo</sup>, Pstpip2<sup>cmo</sup>×Elane<sup>−/−</sup>, Pstpip2<sup>cmo</sup>×Elane<sup>−/−</sup>Prtn3<sup>−/−</sup> and Pstpip2<sup>cmo</sup>×Il1b<sup>−/−</sup> mice.
Extended Data Figure 7 | Combined deletion of RIPK3 and caspase-8 does not provide protection against Pstpip2<sup>lox/lox</sup>-mediated osteomyelitis.

a, Incidence of osteomyelitic bone disease in wild-type, Pstpip2<sup>lox/lox</sup>, Pstpip2<sup>lox/lox</sup>×Il1b<sup>−/−</sup> and Pstpip2<sup>lox/lox</sup>×Ripk3<sup>−/−</sup> mice. b, Representative isosurface micro-computed tomography images of hind paw samples from 12–18-week-old Pstpip2<sup>lox/lox</sup>, Pstpip2<sup>lox/lox</sup>×Ripk3<sup>−/−</sup> and Pstpip2<sup>lox/lox</sup>×Ripk3<sup>−/−</sup>×Casp8<sup>−/−</sup> mice. c, Representative haematoxylin and eosin-stained sections of inflammatory caudal vertebrae bone lesions in diseased Pstpip2<sup>lox/lox</sup>, Pstpip2<sup>lox/lox</sup>×Ripk3<sup>−/−</sup> and Pstpip2<sup>lox/lox</sup>×Ripk3<sup>−/−</sup>×Casp8<sup>−/−</sup> mice (original magnification, ×4 (top) and ×10 (bottom)). d, qRT–PCR analysis of Il1b expression in footpads of wild-type (n = 7), Pstpip2<sup>lox/lox</sup> (n = 7) and Pstpip2<sup>lox/lox</sup>×Ripk3<sup>−/−</sup>×Casp8<sup>−/−</sup>×Casp1<sup>−/−</sup> (n = 7) mice aged 12–16 weeks. Data are expressed as mean ± s.e.m. of combined data from two independent experiments. **P < 0.01, ***P < 0.001; Student’s t-test.
**Extended Data Figure 8 | Reduced pro-IL-1β expression and IL-1β maturation in neutrophils isolated from HFD-fed *Pstpip2<sup>2mo</sup>* mice.**

**a.** Wild-type, *Pstpip2<sup>2mo</sup>* and *Pstpip2<sup>2mo</sup> × Il1b<sup>−/−</sup>* bone-marrow-derived macrophages were left untreated or were primed with LPS for 3 h followed by stimulation with ATP (30 min) or silica (12 h), and IL-1β processing was evaluated by western blot. Data are representative of three independent experiments.

**b.** Western blotting for pro-IL-1β in untreated neutrophils that were purified from wild-type, LFD-fed *Pstpip2<sup>2mo</sup>* and HFD-fed *Pstpip2<sup>2mo</sup>* mice. Data are representative of two independent experiments.

**c, d.** Neutrophils (c) or macrophages (d) from wild-type, LFD-fed *Pstpip2<sup>2mo</sup>* and HFD-fed *Pstpip2<sup>2mo</sup>* mice were left untreated, or primed with LPS for 3 h and then stimulated with ATP (30 min) or silica (12 h), and IL-1β processing was evaluated by western blotting. Data are representative of two independent experiments.
Extended Data Figure 9 | Depletion of neutrophils in anti-Ly6G treated Pstpip2<sup>cmo</sup> mutant mice. Wild-type and Pstpip2<sup>cmo</sup> mice received either PBS or 500 μg per mouse anti-Ly6G antibody (clone IA8) by intraperitoneal injection every 4–5 days starting at 6 weeks of age. a–c, Two weeks after the first anti-Ly6G treatment, FACS analysis was performed on peripheral blood leukocytes (PBLs). a, Representative FACS plots of Gr-1 and CD11b expression on CD45.2<sup>+</sup> gated cells. b, Enumeration of CD45.2<sup>+</sup> Gr-1<sup>hi</sup> CD11b<sup>+</sup> neutrophils in equal volumes of peripheral blood. c, Numbers of T cells (CD45.2<sup>+</sup> TCRβ<sup>+</sup>), CD45.2<sup>+</sup> Gr-1<sup>−</sup> CD11b<sup>−</sup> monocytes/macrophages and CD45.2<sup>+</sup> Gr-1<sup>int</sup> CD11b<sup>−</sup> cells in equal volumes of peripheral blood. Each point represents an individual mouse and the line represents the mean ± s.e.m. ***P < 0.001; Student’s t-test.
Extended Data Figure 10 | Dietary modulation of the intestinal microbiota composition drives autoinflammatory osteomyelitis by setting pro-IL-1β levels available for maturation by caspases 1 and 8. Proposed model highlighting how dysbiosis and processing of IL-1β by caspases 1 and 8 contribute to the development of inflammatory bone disease.