The nonreceptor tyrosine kinase SYK induces autoinflammatory osteomyelitis in a mouse model of chronic recurrent multifocal osteomyelitis

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Running title: Targeting SYK prevents disease in Pstpip2cmo mice

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ABSTRACT

Chronic recurrent multifocal osteomyelitis (CRMO) in humans can be modeled in Pstpip2cmo mice, which carry a missense mutation in the proline-serine-threonine phosphatase-interacting protein 2 (Pstpip2) gene. As cmo disease in mice, the experimental model analogous to human CRMO, is mediated specifically by interleukin (IL)-1β, and not by IL-1α, delineating the molecular pathways contributing to pathogenic IL-1β production is crucial to developing targeted therapies. In particular, our earlier findings support redundant roles for NLR family pyrin domain-containing 3 (NLRP3) and caspase-1, caspase-8, chronic multifocal osteomyelitis (cmo), autoimmunity.

Autoinflammatory bone diseases including chronic recurrent multifocal osteomyelitis (CRMO), osteoporosis, Paget’s disease, arthritis, and periodontal disease are increasingly pervasive contributors to severe chronic pain, physical disabilities, and morbidity (1). CRMO is primarily a pediatric chronic inflammatory bone disease, with at least 80% of patients experiencing primary symptoms including osteomyelitis and debilitating bone pain (2). Treatment of CRMO is currently limited to nonsteroidal anti-inflammatory drugs with escalation to corticosteroids or bisphosphonates for pain relief (3). However, all current therapeutic options have limited specificity to the pathophysiology underlying CRMO.
To study the molecular mechanisms underpinning disease manifestation, CRMO in humans can be modeled in mice that carry the L98P missense mutation in the Pstpip2 gene. Proline-serine-threonine phosphatase-interacting protein 2 (PSTPIP2), a Fes/CIP4 homology domain and Bin-Amphiphysin-Rvs (F-BAR) family protein involved in regulating membrane and cytoskeletal dynamics (4) is encoded by Pstpip2 on chromosome 18 in both humans and mice and is predominantly expressed in the myeloid lineage (5). The L98P mutation in mice is termed chronic multifocal osteomyelitis (cmo), and Pstpip2<sup>cmo</sup> mice are phenotypically characterized by autoinflammatory disease involving the bones and skin, resulting in osteomyelitis and bone deformities. The bone lesions in both cmo disease and CRMO are associated with increased IL-1 signaling, osteoclast-mediated resorption, and an elevation of osteoclast precursors (6), but the specific inflammatory pathways critical for disease are not known.

IL-1β has been established as the principle driver of dysregulated cellular homeostasis, extracellular matrix composition, proinflammatory cytokine production, and osteolysis in a diverse array of autoinflammatory, hematologic, and bone diseases including osteoarthritis (7) and multiple myeloma (8). Inhibition of IL-1β and IL-1 receptor (IL-1R) signaling has been shown to completely protect against disease in Pstpip2<sup>cmo</sup> mice (9), suggesting that inhibition of IL-1β, IL-1R, or their upstream regulators could provide significant benefit to patients with autoinflammatory bone disease. It is known that caspase-1–mediated cleavage of pro–IL-1β is activated by the nucleotide-binding oligomerization domain (NOD)-like receptor family, pyrin domain-containing 3 (NLRP3) inflammasome (10), and previous studies have established a redundant role for caspase-8 in mediating this cleavage and disease progression (11,12). However, the signaling cascade involved in caspase-8 activation remains not well understood.

The nonreceptor tyrosine kinase SYK is a central regulatory molecule in innate immune toll-like receptor and NOD-like receptor signaling pathways (13,14) and inflammatory cytokine secretion (15). SYK is also known to play a role in activating caspase-8, thereby resulting in IL-1β processing (16). Based on the involvement of SYK in the caspase-8 pathway and the importance of caspase-8 in mediating cmo disease, we sought to determine the role of SYK signaling in regulating cmo disease. Here, we have discovered the mechanistic basis underpinning SYK-dependent induction of autoinflammatory osteomyelitis. Specifically, we show that SYK critically up-regulates pro–IL-1β production responsible for cmo disease progression and proinflammatory NF-κB signaling which contributes to pro–IL-1β upregulation.

**Results**

**RIPK3 and AIM2 are dispensable for disease progression in Pstpip2<sup>cmo</sup> mice**

The NLRP3 inflammasome plays a redundant role with caspase-8 to promote disease progression in Pstpip2<sup>cmo</sup> mice, indicating NLRP3 is an upstream regulator of caspase-1 activation (12), but understanding of the upstream regulation of caspase-8 activation remains incomplete. Although caspase-8 deficiency is embryonically lethal, caspase-8-deficient mice can be completely rescued through the knockout of receptor-interacting serine/threonine kinase (RIPK) 3 (17-19). In addition, reduced IL-1β production and abolished caspase-8 activation in Ripk3<sup>−/−</sup> bone marrow-derived dendritic cells (BMDCs) suggest that RIPK3 is required for caspase-8 activation and subsequent release of IL-1β (20). Absent in melanoma 2 (AIM2) acts as an inflammasome sensor for cytosolic DNA, and it activates caspase-1 through the adaptor protein apoptosis-associated speck-like protein containing a caspase activation and recruitment domain (ASC). AIM2 induces caspase-8 activation in caspase-1–deficient macrophages in the context of several bacterial infections, including Burkholderia (21), Francisella (22), and Legionella (23). Given their established functions in caspase-8 activation under various conditions, we explored the roles of RIPK3 and AIM2 in mediating caspase-8 activation in Pstpip2<sup>cmo</sup> mice by analyzing cmo disease progression in NLRP3 and RIPK3-deficient Pstpip2<sup>cmo</sup> mice (Pstpip2<sup>cmo</sup>Nlrp3<sup>−/−</sup>Ripk3<sup>−/−</sup>) and NLRP3 and AIM2-deficient Pstpip2<sup>cmo</sup> mice (Pstpip2<sup>cmo</sup>Nlrp3<sup>−/−</sup>Aim2<sup>−/−</sup>). All mice with both genotypes (Pstpip2<sup>cmo</sup>Nlrp3<sup>−/−</sup>Ripk3<sup>−/−</sup> and Pstpip2<sup>cmo</sup>Nlrp3<sup>−/−</sup>Aim2<sup>−/−</sup>) developed disease
similarly to Pstpip2<sup>cmo</sup> mice (Fig. 1, A and B). Microcomputed tomography (micro-CT) scans of the inflamed areas revealed extensive reduction in bone density and structural malformation in the feet of these mice (Fig. 1, A and B). Further, massive lymphomegaly was observed in the popliteal lymph nodes draining inflamed footpads (Fig. 1, A and B). These data suggest that RIPK3 and AIM2 are dispensable for disease progression in Pstpip2<sup>cmo</sup> mice.

**SYK, but not CARD9, is required for inflammatory disease progression in Pstpip2<sup>cmo</sup> mice**

In addition to the role of SYK in innate immune signaling pathways (13,14) and inflammatory cytokine secretion (15), recent evidence has indicated the involvement of SYK in a diverse range of biological functions including cellular adhesion, platelet activation, and osteoclast maturation (24). The SYK adaptor protein caspase recruitment domain-containing protein 9 (CARD9) is expressed primarily in lymphoid tissues and contributes to innate immune signaling in response to fungal, viral, and bacterial infections (25-27). Given that SYK and CARD9 are involved in caspase-8 activation and subsequent IL-1β processing in BMDCs during fungal infection (16), we explored the respective contributions of SYK and CARD9 to disease progression in Pstpip2<sup>cmo</sup> mice. First, we monitored disease progression in Pstpip2<sup>cmo</sup>Nlrp3<sup>–/–</sup>Syk<sup>β<sup>fl/fl</sup></sup>LysM<sup>cre</sup> mice and Pstpip2<sup>cmo</sup>Nlrp3<sup>–/–</sup>CARD9<sup>–/–</sup> mice. While Pstpip2<sup>cmo</sup>Nlrp3<sup>–/–</sup>CARD9<sup>–/–</sup> mice did not show protection from disease, Pstpip2<sup>cmo</sup>Nlrp3<sup>–/–</sup>Syk<sup>β<sup>fl/fl</sup></sup>LysM<sup>cre</sup> mice displayed nearly complete protection (Fig. 2, A and B). Next, we investigated whether deletion of SYK in Pstpip2<sup>cmo</sup> mice with intact NLRP3 would be sufficient to provide protection from disease. We found that myeloid-specific deletion of SYK alone in Pstpip2<sup>cmo</sup> mice (Pstpip2<sup>cmo</sup>Syk<sup>β<sup>fl/fl</sup></sup>LysM<sup>cre</sup>) provided complete protection from disease (Fig. 2C). The structural bone lesions found by micro-CT and the popliteal lymphomegaly observed in Pstpip2<sup>cmo</sup>, Pstpip2<sup>cmo</sup>Nlrp3<sup>–/–</sup>, and Pstpip2<sup>cmo</sup>Nlrp3<sup>–/–</sup>CARD9<sup>–/–</sup> mice were rescued in Pstpip2<sup>cmo</sup>Nlrp3<sup>–/–</sup>Syk<sup>β<sup>fl/fl</sup></sup>LysM<sup>cre</sup> and Pstpip2<sup>cmo</sup>Syk<sup>β<sup>fl/fl</sup></sup>LysM<sup>cre</sup> mice (Fig. 2, A-C). Taken together, these data suggest that SYK functions upstream of both caspase-1 and caspase-8 in inducing cmo disease, that SYK is sufficient and necessary for cmo disease induction, and that NLRP3 and CARD9 are dispensable for cmo disease progression.

**SYK mediates cmo disease by promoting proinflammatory signaling but not inflammasome activation**

Disease in cmo mice is mediated by the cytokine IL-1β (9). To investigate the role of SYK in regulating IL-1β upregulation in cmo, we first measured pro–IL-1β expression and SYK activation in the footpads of wild type and Pstpip2<sup>cmo</sup> mice. Footpads from Pstpip2<sup>cmo</sup> mice had increased pro–IL-1β expression and SYK activation with respect to those of wild type mice (Fig. 3A). The myeloid-specific deletion of SYK in Pstpip2<sup>cmo</sup> mice reduced the expression of pro–IL-1β in footpads to a level similar to that of wild type mice without affecting the expression of caspase-1 or caspase-8 (Fig. 3A). Consistent with these data, the expression of pro–IL-1β induced by lipopolysaccharide (LPS) treatment was increased in bone marrow-derived macrophages (BMDMs) derived from Pstpip2<sup>cmo</sup> mice relative to that of BMDMs from wild type mice (Fig. 3B). The increased pro–IL-1β expression in Pstpip2<sup>cmo</sup> mice correlated with activation of SYK. The myeloid-specific deletion of SYK in Pstpip2<sup>cmo</sup> mice abolished the increased induction of pro–IL-1β in BMDMs upon LPS stimulation relative to Pstpip2<sup>cmo</sup> BMDMs without affecting the expression of caspase-1 and caspase-8 (Fig. 3B). These findings suggest a primary role for SYK in mediating pro–IL-1β production and cmo disease progression.

We next sought to identify additional intracellular signaling pathways mediated by SYK signaling contributing to the induction of pro–IL-1β expression and excessive inflammation in Pstpip2<sup>cmo</sup> mice. Recent evidence has demonstrated that mitogen-activated protein (MAP) kinases ASK1 and ASK2 centrally regulate NF-κB and downstream MAP kinases, including JNK, ERK, and p38, to drive autoinflammatory disease progression in the Ptpn6<sup>spin</sup> mouse model of neutrophilic dermatosis (28). We hypothesized that NF-κB and MAP kinase signaling promote cmo disease progression and that SYK plays a role in regulating this signaling. Although there was more activation of NF-κB and ERK in the footpads of Pstpip2<sup>cmo</sup> mice compared with wild type mice,
JNK and p38 were similarly activated (Fig. 3C). However, deletion of SYK reversed the elevated NF-κB, but not ERK, activation in Pstpip2<sup>cmo</sup> mice, suggesting that NF-κB plays an important role downstream of SYK to mediate persistent inflammation in cmo disease.

Furthermore, SYK has been shown to regulate inflammasome activation and IL-1β maturation downstream of dectin-1 signaling (16). We therefore asked whether SYK regulates both NLRP3 inflammasome and caspase-8 activation upstream of IL-1β production. We observed similar caspase-1 and caspase-8 cleavage in BMDMs derived from wild type, Pstpip2<sup>cmo</sup>, and Pstpip2<sup>cmo</sup>Syk<sup>fl/fl</sup>LysM<sup>cre</sup> mice in response to the classical NLRP3 inflammasome trigger LPS + ATP, which was further supported by the similar gasdermin D (GSDMD) activation observed among these genotypes (Fig. 3D). In addition, we further noticed that SYK deficiency did not affect the expression of GSDMD, NLRP3, and ASC, all of which are crucial components for inflammasome signaling (Fig. 3E). These data suggest that SYK does not regulate the caspase-1 and caspase-8 activation mediated by the classical NLRP3 trigger.

Overall, our data indicate that SYK regulates NF-κB signaling, but not inflammasome activation, for the induction of pro–IL-1β to mediate disease progression in Pstpip2<sup>cmo</sup> mice.

Discussion

Cmo has been shown to be mediated by pathological IL-1β production downstream of NLRP3/caspase-1 and caspase-8 (9,11,12). The disease progression occurs despite single deficiency of either caspase-1 or caspase-8 (12), which suggests the caspases function as part of distinct complexes that are independently activated. Although caspase-1 and caspase-8 have both been shown to colocalize with the AIM2/ASC speck to mediate pro–IL-1β cleavage (22), AIM2 deficiency did not provide protection in Pstpip2<sup>cmo</sup> mice, further supporting that in cmo disease, caspase-1 and caspase-8 operate and are activated independently in distinct complexes. In this study, we demonstrated that deficiency of SYK in Pstpip2<sup>cmo</sup> mice prevented the induction of osteomyelitis. SYK signaling upstream of caspase-1 and caspase-8 to promote pro–IL-1β production centrally mediates cmo disease induction. Thus, it is interesting that deficiency of the SYK adaptor protein, CARD9, did not provide protection in Pstpip2<sup>cmo</sup> mice. In addition to promoting pro–IL-1β synthesis, SYK, but not CARD9, has been shown to regulate NLRP3 inflammasome activation during fungal infection (29). This suggests that the CARD9 pathway selectively transduces SYK signaling to promote pro–IL-1β synthesis but not inflammasome activation. Additionally, several reports have highlighted the role of SYK in the regulation of the NLRP3- and caspase-8–mediated inflammasomes (16,29,30). However, our data with the canonical NLRP3 trigger LPS + ATP did not reveal a dependency of caspase-1 and caspase-8 processing on SYK, suggesting an exclusively diverse yet specific role for SYK in mediating cmo disease. In this regard, SYK primarily acts as a pivotal regulator of pro–IL-1β synthesis but not as a regulator of inflammasome activation; however, these two processes both converge towards the production of active IL-1β. Recent evidence has also established central roles for the NLRP3 inflammasome and IL-1β signaling in several additional related disorders of nonbacterial osteomyelitis, including Majeed syndrome, synovitis, acne, pustulosis, hyperostosis, and osteitis (SAPHO) syndrome, and deficiency of IL-1R antagonist (DIRA) (3,9,12). Our findings provide important context for evaluating the role SYK plays in mediating these related autoinflammatory bone disorders and for the therapeutic potential of SYK inhibitors in this disease spectrum.

The central regulatory role of SYK is not confined to IL-1β–mediated autoinflammatory disease. We have previously reported that SYKlicenses MyD88 to induce IL-1α–mediated inflammatory disease in Ptpn6<sup>pin</sup> mice (31). Similarly, we observed increased activation of SYK in the absence of PSTPIP2, suggesting that PSTPIP2 functions to suppress SYK signaling. However, the regulatory mechanisms behind SYK activation by PSTPIP2 require further investigation. Recent evidence has established that PSTPIP2 interacts with SHIP1, which is encoded by Ptpn6 (32), suggesting that SHIP1 may be able to modulate SYK activation through its phosphatase activity.

SYK signaling is known to be activated downstream of various cell surface receptors including CD74, integrins, C-type lectin receptors
(dectin-1 and dectin-2), and Fc receptors (27). Identification of the specific triggers of SYK activation in these Pstpip2™ mice would further clarify the signaling mechanism and provide a deeper understanding of the progression of cmo disease. SYK signaling has also been strongly associated with the recruitment of neutrophils to areas of inflammation (33). The marked reductions in inflammation and lymphomegaly seen in SYK-deficient Pstpip2™ mice indicate that SYK signaling potentially mediates neutrophil recruitment in Pstpip2™ mice. Although T-cell dysregulation has been associated with inflammatory bone diseases, previous studies have characterized the osteomyelitis in cmo disease by increased neutrophil numbers without T-cell abnormalities (9,34). As neutrophils have been implicated as major contributors to IL-1β production in cmo (11), our findings suggest SYK-mediated recruitment and activation of neutrophils may also play a role in promoting the bony inflammation characterizing Pstpip2™ mice. Previous studies have shown inhibition of signaling pathways highly associated with caspase-8 activation and inflammatory bone disease, such as TNF signaling, fails to protect against cmo disease (9,12). This also indicates that current guidelines for the therapeutic use of TNF inhibitors in the subset of patients with CRMO and concurrent autoimmune diseases may not be effective in treating CRMO. Therapeutic options for the largely pediatric and adolescent CRMO population are limited by nonspecificity and inadequate control of pain and disease progression, which can result in physical disabilities or permanent deformities. As genetic deletion of Syk in the myeloid compartment of Pstpip2™ mice resulted in the complete prevention of disease induction and progression, SYK and its downstream signaling components represent promising, novel therapeutic targets in CRMO.

**Experimental procedures**

**Mice**

Pstpip2™ (35), Nlrp3™ (36), Ripk3™ (37), Aim2™ (38), Card9™ (39), and Syk™ LysM™ mice were generated by crossing Pstpip2™ mice onto Ripk3™, Aim2™, Card9™, and Syk™ LysM™ backgrounds, respectively. Pstpip2™ Syk™ LysM™ mice were generated by crossing Pstpip2™ and Syk™ LysM™ mice. Pstpip2™ mice were purchased from The Jackson Laboratory and are on the BALB/c background. All other mutant mice are on the C57BL/6 background. Littermate controls were utilized to evaluate the influence of genetic deletions on immune responses, IL-1β regulation, and cmo disease progression. All mice were kept within the Animal Resource Center at St. Jude Children’s Research Hospital. Animal studies were conducted according to protocols approved by the St. Jude Animal Care and Use Committee.

**Cell culture and stimulation**

Primary BMDMs were grown for 5 to 6 days in IMDM (Gibco) supplemented with 10% fetal bovine serum (FBS) (Atlanta Biologicals), 30% L929-conditioned media, 1% non-essential amino acids (Gibco), and 1% penicillin/streptomycin (Sigma). BMDMs were seeded at a concentration of 1 × 10⁶ cells onto 12-well plates. After incubating overnight, cells were stimulated with LPS (100 ng/mL; InvivoGen) for the indicated amount of time (0–8 hours) or treated with LPS + ATP (LPS, 4 h; ATP [5 mM; Roche], 30 min) (38) before cell harvest.

**Western blotting**

For immunoblotting, BMDMs and footpad protein lysates were prepared by tissue homogenization in RIPA lysis buffer supplemented with a protease inhibitor cocktail (Roche) and PhosSTOP (Roche). A Pierce BCA Protein Assay Kit was used to quantify samples. A total of 40 µg of protein was resolved using SDS-PAGE and transferred onto PVDF membranes (40). The membranes were blocked in 5% skim milk before primary antibodies were added and incubated overnight at 4°C. Afterward, membranes were incubated with horseradish peroxidase (HRP)-tagged secondary antibodies for 1 hour at room temperature. Primary antibodies were anti-GAPDH (Cell Signaling Technologies [CST] #5174), anti–IL-1β (CST #12507), anti–phospho-ERK1/2 (CST #9101), anti–total ERK1 (CST #9102), anti–phospho-p38 (CST
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#9211), anti-total p38 (CST #9212), anti–phospho-IκBα (CST #2859), anti-total IκBα (CST #9242), anti–phospho-SYK (CST #2717), anti-total SYK (CST #2712), anti–phospho-JNK (CST #9251), anti-total JNK (CST #9252), anti–caspase-1 (Adipogen #AG-20B-0044-C100), anti-ASC (Adipogen #AG-25B-0006-C100), anti-NLRP3 (Adipogen #AG-20B-0014-C100), anti-gasdermin D (Abcam #Ab155233), and anti–caspase-8 (Adipogen #AG-20T-0138-C100). Secondary HRP antibodies were purchased from Jackson ImmunoResearch Laboratories.

Microcomputed tomography (micro-CT)
A Siemens Inveon µCT scanner (Siemens Healthcare) was used to capture micro-CT images. Mouse footpads were imaged with a 672 x 1344 mm matrix and a field of view of 30.04 x 60.08 mm with 1 bed position. Projections were obtained at 80 kVp and 500 µA (1050 ms exposure; 1000 ms settle time) over half rotation (440 projections), giving an isotropic resolution of 44.7 µm. Inveon Research Workplace (IRW) software was used to process the data.

Statistical analysis
Each experiment was repeated at least twice before inclusion in the manuscript. The log-rank (Mantel-Cox) test was used to compare statistical significance between survival curves in the two groups.
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Author contributions: T.-D.K. conceptualized the study. T.K.D., R.G., R.K., B.B., B.S., P.G., and A.B. performed the experiments. T.K.D. and R.K. wrote the manuscript. All authors discussed the results, commented on the manuscript, and approved the final version.
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References


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Fig. 1. RIPK3 and AIM2 are dispensable for disease progression in Pstpip2<sup>emo</sup> mice. (A) Incidence of disease in wild type (WT; n = 5), Pstpip2<sup>emoNlrp3</sup><sup>−/−</sup> (n = 9), and Pstpip2<sup>emoNlrp3</sup><sup>−/−</sup>Nripk3<sup>−/−</sup> (n = 5) mice over the experimental course and representative footpad images, footpad CT scans, and popliteal lymph nodes from these respective mice. (B) Incidence of disease in WT (n = 8), Pstpip2<sup>emoNlrp3</sup><sup>−/−</sup> (n = 10), and Pstpip2<sup>emoNlrp3</sup><sup>−/−</sup>Aim2<sup>−/−</sup> (n = 10) mice over the experimental course and representative footpad images, footpad CT scans, and popliteal lymph nodes from these respective mice.
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Fig. 2. CARD9, but not SYK, is dispensable for disease progression in \textit{Pstpip2}^{c/-} mice. (A) Incidence of disease in wild type (WT; \(n = 5\)), \textit{Pstpip2}^{c/-}\textit{Nlrp3}^{+/+} (\(n = 10\)), and \textit{Pstpip2}^{c/-}\textit{Nlrp3}^{+/+}\textit{Card9}^{+/+} (\(n = 20\)) mice over the experimental course and representative footpad images, footpad CT scans, and popliteal lymph nodes from these respective mice. (B) Incidence of disease in WT (\(n = 5\)),
Targeting SYK prevents disease in *Pstpip2*<sup>cmo</sup> mice

*Pstpip2<sup>cmo</sup>Nlpr3<sup>+/−</sup> (n = 8), and *Pstpip2<sup>cmo</sup>Nlpr3<sup>−/−</sup> Syk<sup>fl/fl</sup>LysM<sup>cre</sup> (n = 20) mice over the experimental course and representative footpad images, footpad CT scans, and popliteal lymph nodes from these respective mice. (C) Incidence of disease in WT (n = 5), *Pstpip2<sup>cmo</sup> (n = 7), and *Pstpip2<sup>cmo</sup>Syk<sup>fl/fl</sup>LysM<sup>cre</sup> (n = 13) mice over the experimental course and representative footpad images, footpad CT scans, and popliteal lymph nodes from these respective mice.
Targeting SYK prevents disease in \textit{Pstpip2}^{+/-} mice.
SYK is involved in regulating levels of pro–IL-1β and NF-kB in Pstpip2cmo mice. (A) Immunoblot analysis of pro–IL-1β, caspase-8 (Casp-8), caspase-1 (Casp-1), phospho-SYK (p-SYK), total SYK (t-SYK), and GAPDH in wild type (WT), Pstpip2cmo, and Pstpip2cmo Sykfl/fl LysMcre footpad lysates. (B) Immunoblot analysis of pro–IL-1β, Casp-8, Casp-1, p-SYK, t-SYK, and GAPDH in WT, Pstpip2cmo, and Pstpip2cmo Sykfl/fl LysMcre bone marrow-derived macrophages (BMDMs) at several timepoints after lipopolysaccharide (LPS) treatment. (C) Immunoblot analysis of phospho-IκBα (p-IκBα), total IκBα (t-IκBα), phospho-ERK (pERK), total ERK (t-ERK), phospho-JNK (p-JNK), total JNK (t-JNK), phospho-p38 (p-p38), total p38 (t-p38), and GAPDH in WT, Pstpip2cmo, and Pstpip2cmo Sykfl/fl LysMcre footpad lysates. (D) Immunoblot analysis of activated (cleaved) Casp-1, Casp-8, and gasdermin D (GSDMD) in WT, Pstpip2cmo, and Pstpip2cmo Sykfl/fl LysMcre BMDMs treated with LPS + ATP or left untreated with media. (E) Immunoblot analysis of inflammasome components pro–IL-1β, NLRP3, ASC, and GAPDH in WT, Pstpip2cmo, and Pstpip2cmo Sykfl/fl LysMcre BMDMs treated with LPS + ATP or left untreated with media. Representative blots from three independent experiments are shown.
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