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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 Pstpip2^{cmo} mice*

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ABSTRACT

Chronic recurrent multifocal osteomyelitis (CRMO) in humans can be modeled in *Pstpip2^{cmo}* 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 with caspase-8 in instigating *cmo*. However, the signaling components upstream of caspase-8 and pro–IL-1β cleavage in *Pstpip2^{cmo}* mice are not well understood. Therefore, here we investigated the signaling pathways in these mice and discovered a central role of a nonreceptor tyrosine kinase spleen tyrosine kinase (SYK) in mediating osteomyelitis. Using several mutant mouse strains, immunoblotting, and microcomputed tomography (micro-CT), we demonstrate that absent in melanoma 2 (AIM2), receptor-interacting serine/threonine protein kinase 3 (RIPK3), and caspase recruitment domain-containing protein 9 (CARD9) are each dispensable for osteomyelitis induction in *Pstpip2^{cmo}* mice, whereas genetic deletion of *Syk* completely abrogates the disease phenotype. We further show that SYK centrally mediates signaling upstream of caspase-1 and caspase-8 activation and principally up-regulates NF-κB and IL-1β signaling in *Pstpip2^{cmo}* mice and thereby induces *cmo*. These results provide a rationale for directly targeting SYK and its downstream signaling components in CRMO.

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^{cmo}* 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^{cmo}* 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-1 or NLRP3 with 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^{cmo} mice

The NLRP3 inflammasome plays a redundant role with caspase-8 to promote disease progression in *Pstpip2*^{cmo} 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$^{-/-}$* 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^{cmo}* mice by analyzing *cmo* disease progression in NLRP3 and RIPK3-deficient *Pstpip2^{cmo}* mice (*Pstpip2^{cmo}Nlrp3$^{-/-}$Ripk3$^{-/-}$*) and NLRP3 and AIM2-deficient *Pstpip2^{cmo}* mice (*Pstpip2^{cmo}Nlrp3$^{-/-}$Aim2$^{-/-}$*). All mice with both genotypes (*Pstpip2^{cmo}Nlrp3$^{-/-}$Ripk3$^{-/-}$* and *Pstpip2^{cmo}Nlrp3$^{-/-}$Aim2$^{-/-}$*) developed disease similarly to *Pstpip2*^{cmo} 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*^{cmo} mice.

SYK, but not CARD9, is required for inflammatory disease progression in Pstpip2^{cmo} *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*^{cmo} mice. First, we monitored disease progression in *Pstpip2*^{cmo}*Nlrp3*$^{–/–}$*Syk*$^{fl/fl}$*LysM*^{cre} mice and *Pstpip2*^{cmo}*Nlrp3*$^{–/–}$*Card9*$^{–/–}$ mice. While *Pstpip2*^{cmo}*Nlrp3*$^{–/–}$*Card9*$^{–/–}$ mice did not show protection from disease, *Pstpip2*^{cmo}*Nlrp3*$^{–/–}$*Syk*$^{fl/fl}$*LysM*^{cre} mice displayed nearly complete protection (Fig. 2, A and B). Next, we investigated whether deletion of SYK in *Pstpip2*^{cmo} mice with intact NLRP3 would be sufficient to provide protection from disease. We found that myeloid-specific deletion of SYK alone in *Pstpip2*^{cmo} mice (*Pstpip2*^{cmo}*Syk*$^{fl/fl}$*LysM*^{cre}) provided complete protection from disease (Fig. 2C). The structural bone lesions found by micro-CT and the popliteal lymphomegaly observed in *Pstpip2*^{cmo}, *Pstpip2*^{cmo}*Nlrp3*$^{–/–}$, and *Pstpip2*^{cmo}*Nlrp3*$^{–/–}$*Card9*$^{–/–}$ mice were rescued in *Pstpip2*^{cmo}*Nlrp3*$^{–/–}$*Syk*$^{fl/fl}$*LysM*^{cre} and *Pstpip2*^{cmo}*Syk*$^{fl/fl}$*LysM*^{cre} 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*^{cmo} mice. Footpads from *Pstpip2*^{cmo} 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*^{cmo} 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*^{cmo} mice relative to that of BMDMs from wild type mice (Fig. 3B). The increased pro–IL-1β expression in *Pstpip2*^{cmo} mice correlated with activation of SYK. The myeloid-specific deletion of SYK in *Pstpip2*^{cmo} mice abolished the increased induction of pro–IL-1β in BMDMs upon LPS stimulation relative to *Pstpip2*^{cmo} 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*^{cmo} 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*^{spin} 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*^{cmo} 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^{cmo}* 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^{cmo}*, and *Pstpip2^{cmo}Syk$^{fl/fl}$LysM^{cre}* mice in response to the classical NLRP3 inflammasome trigger LPS + ATP, which was futher 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^{cmo}* 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^{cmo}* 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*^{cmo} 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^{cmo}* 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 SYK licenses MyD88 to induce IL-1α–mediated inflammatory disease in *Ptpn6*^{spin} 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*^{cmo} 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*^{cmo} mice indicate that SYK signaling potentially mediates neutrophil recruitment in *Pstpip2*^{cmo} 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*^{cmo} 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*^{cmo} 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^{cmo} (35), *Nlrp3*$^{-/-}$ (36), *Ripk3*$^{-/-}$ (37), *Aim2*$^{-/-}$ (38), *Card9*$^{-/-}$ (39), and *Syk*$^{fl/fl}$*LysM*^{cre} (25) mice were described previously. *Pstpip2*^{cmo}*Nlrp3*$^{-/-}$ mice were generated by crossing *Pstpip2*^{cmo} and *Nlrp3*$^{-/-}$ mice; then *Pstpip2*^{cmo}*Nlrp3*$^{-/-}$*Ripk3*$^{-/-}$, *Pstpip2*^{cmo}*Nlrp3*$^{-/-}$*Aim2*$^{-/-}$, *Pstpip2*^{cmo}*Nlrp3*$^{-/-}$*Card9*$^{-/-}$, and *Pstpip2*^{cmo}*Nlrp3*$^{-/-}$*Syk*$^{fl/fl}$*LysM*^{cre} mice were generated by crossing *Pstpip2*^{cmo}*Nlrp3*$^{-/-}$ mice onto *Ripk3*$^{-/-}$, *Aim2*$^{-/-}$, *Card9*$^{-/-}$, and *Syk*$^{fl/fl}$*LysM*^{cre} backgrounds, respectively. *Pstpip2*^{cmo}*Syk*$^{fl/fl}$*LysM*^{cre} mice were generated by crossing *Pstpip2*^{cmo} and *Syk*$^{fl/fl}$*LysM*^{cre} mice. *Pstpip2*^{cmo} 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 1×10^6 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

#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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Figures

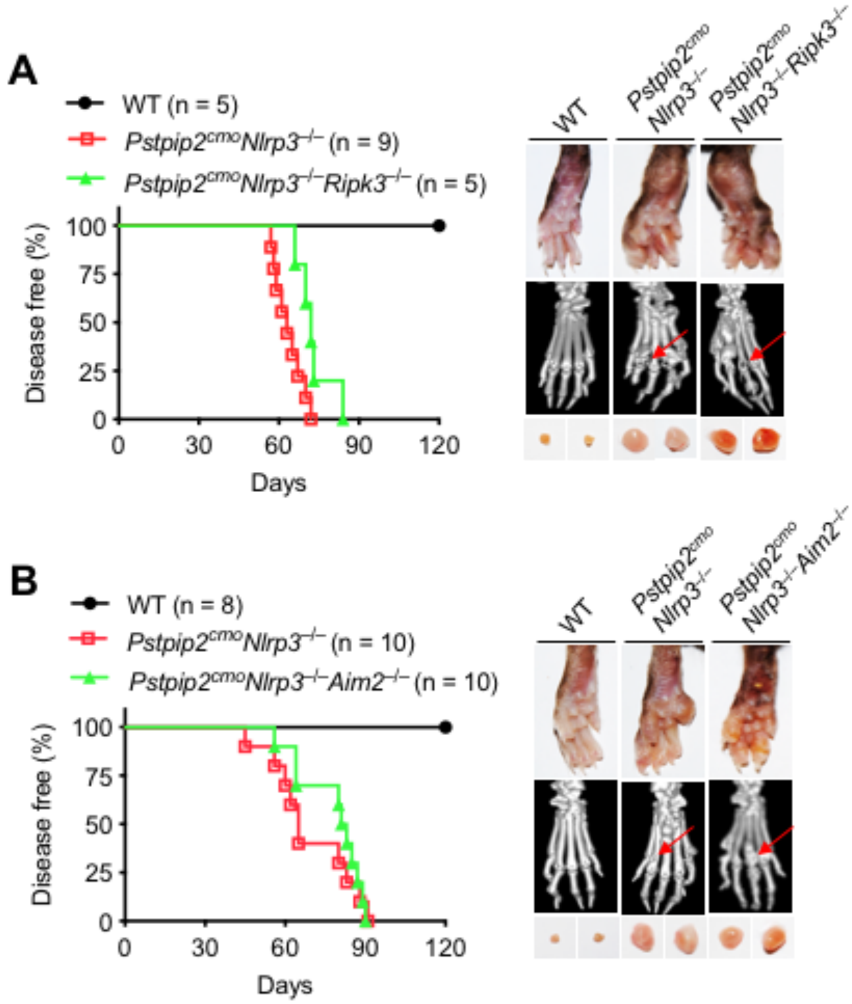

Fig. 1. RIPK3 and AIM2 are dispensable for disease progression in *Pstpip2*^{cmo} mice. (**A**) Incidence of disease in wild type (WT; n = 5), *Pstpip2*^{cmo}*Nlrp3*$^{-/-}$ (n = 9), and *Pstpip2*^{cmo}*Nlrp3*$^{-/-}$*Ripk3*$^{-/-}$ (n = 5) mice over the experimental course and representative footpad images, footpad CT scans, and popliteal lymph nodes from these respective mice. (**B**) Incidence of disease in WT (n = 8), *Pstpip2*^{cmo}*Nlrp3*$^{-/-}$ (n = 10), and *Pstpip2*^{cmo}*Nlrp3*$^{-/-}$*Aim2*$^{-/-}$ (n = 10) mice over the experimental course and representative footpad images, footpad CT scans, and popliteal lymph nodes from these respective mice.

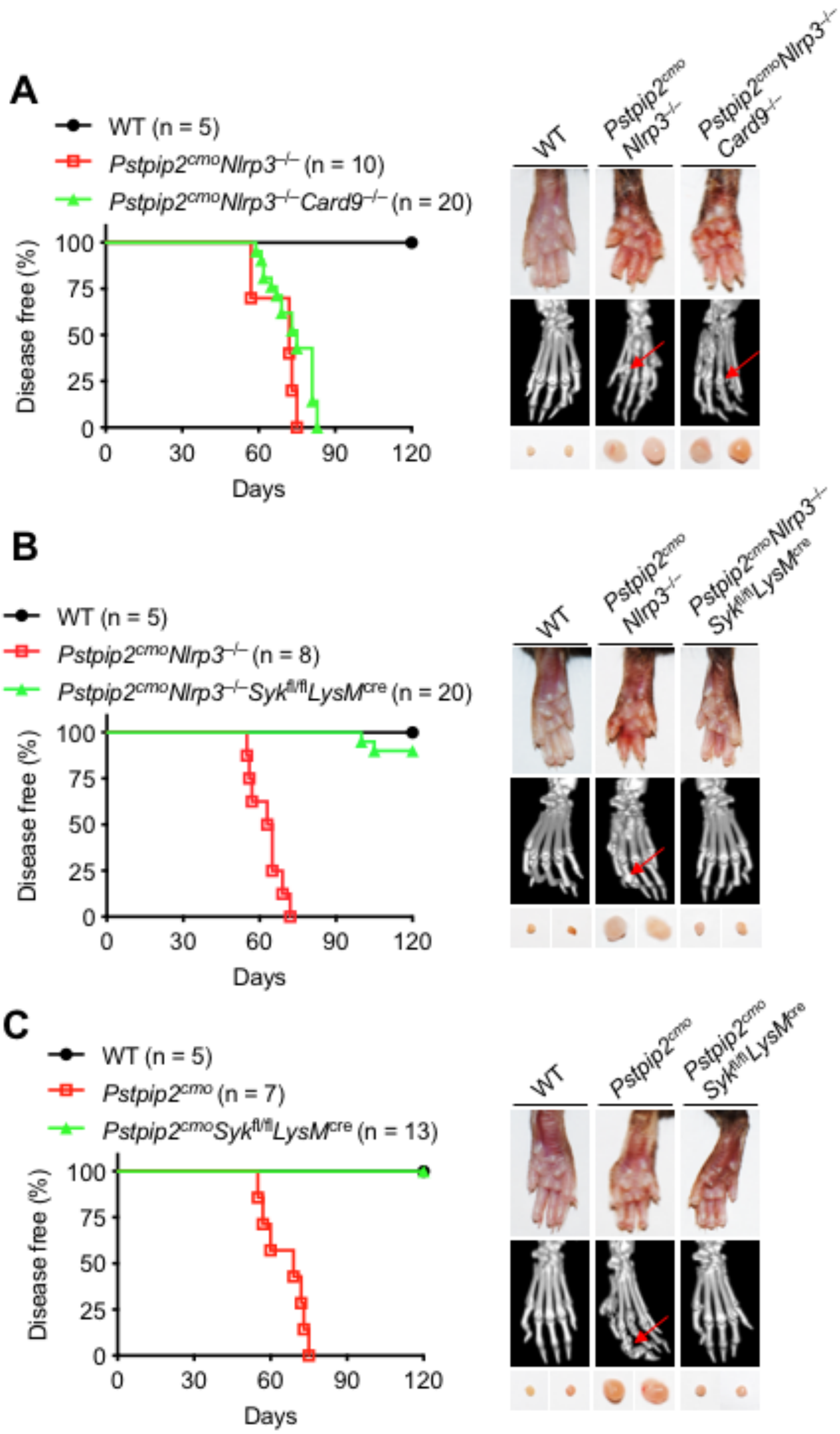

Fig. 2. CARD9, but not SYK, is dispensable for disease progression in *Pstpip2*^{cmo} mice. (**A**) Incidence of disease in wild type (WT; n = 5), *Pstpip2*^{cmo}*Nlrp3*$^{-/-}$ (n = 10), and *Pstpip2*^{cmo}*Nlrp3*$^{-/-}$*Card9*$^{-/-}$ (n = 20) mice over the experimental course and representative footpad images, footpad CT scans, and popliteal lymph nodes from these respective mice. (**B**) Incidence of disease in WT (n = 5),

Pstpip2^{cmo}*Nlrp3*$^{-/-}$ (n = 8), and *Pstpip2*^{cmo}*Nlrp3*$^{-/-}$*Syk*$^{fl/fl}$*LysM*^{cre} (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*^{cmo} (n = 7), and *Pstpip2*^{cmo}*Syk*$^{fl/fl}$*LysM*^{cre} (n = 13) mice over the experimental course and representative footpad images, footpad CT scans, and popliteal lymph nodes from these respective mice.

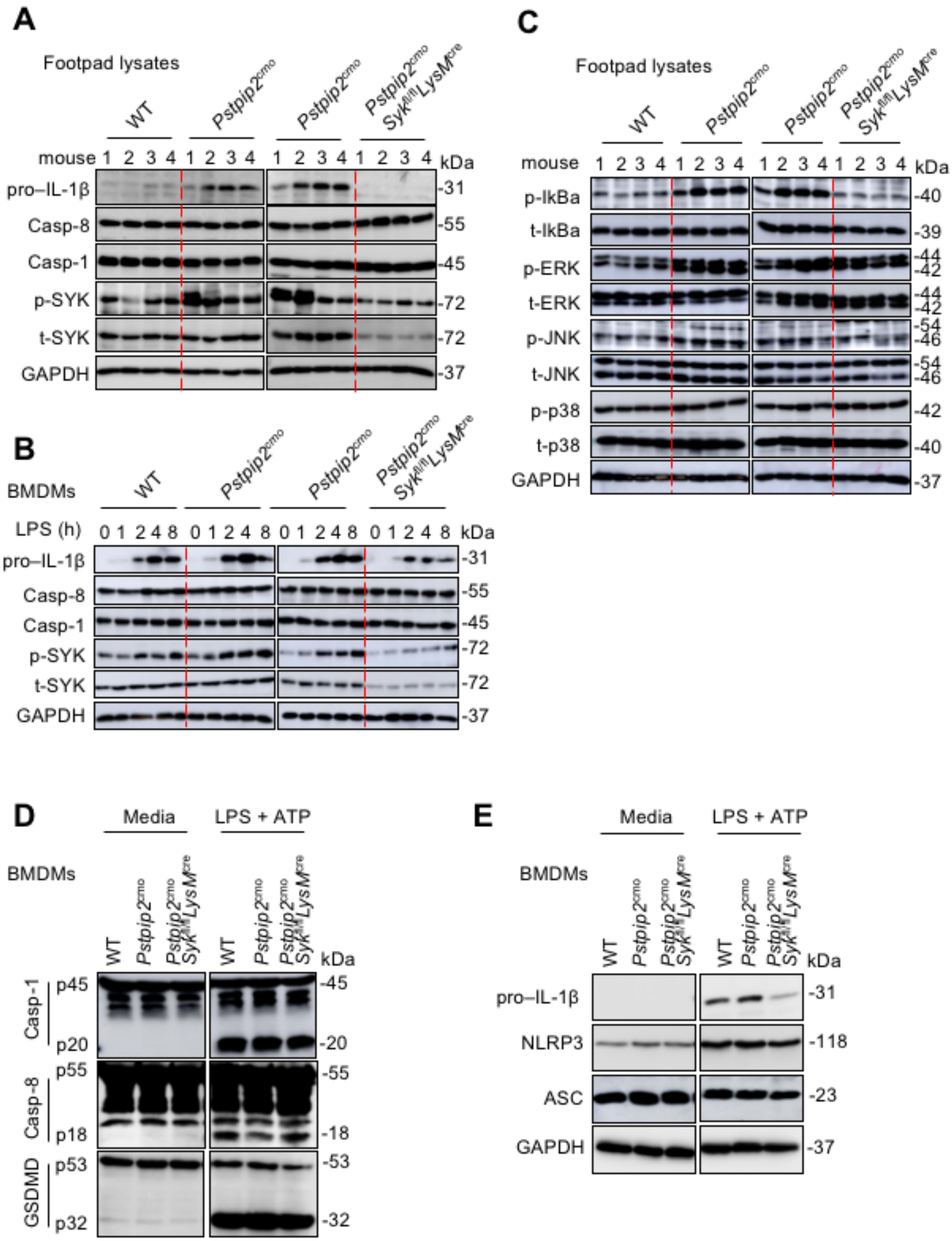
A
Footpad lysates
WT
Pstpip2cmo
Pstpip2cmo
Pstpip2cmo Sykfl/fl LysMcre
mouse 1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4 kDa
pro–IL-1β
Casp-8
Casp-1
p-SYK
t-SYK
GAPDH
-31
-55
-45
-72
-72
-37
B
BMDMs
LPS (h) 0 1 2 4 8 0 1 2 4 8 0 1 2 4 8 0 1 2 4 8 kDa
C
Footpad lysates
p-IkBa
t-IkBa
p-ERK
t-ERK
p-JNK
t-JNK
p-p38
t-p38
GAPDH
-40
-39
-44
-42
-54
-46
-42
-40
-37
D
Media
LPS + ATP
BMDMs
WT
Casp-1
p45
p20
Casp-8
p55
p18
GSDMD
p53
p32
-45
-20
-55
-18
-53
-32
E
pro–IL-1β
NLRP3
ASC
GAPDH
-31
-118
-23
-37

Fig. 3. SYK is involved in regulating levels of pro–IL-1β and NF-κB in *Pstpip2*^{cmo} 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), *Pstpip2*^{cmo}, and *Pstpip2*^{cmo}*Syk*$^{fl/fl}$*LysM*^{cre} footpad lysates. (**B**) Immunoblot analysis of pro–IL-1β, Casp-8, Casp-1, p-SYK, t-SYK, and GAPDH in WT, *Pstpip2*^{cmo}, and *Pstpip2*^{cmo}*Syk*$^{fl/fl}$*LysM*^{cre} bone marrow-derived macrophages (BMDMs) at several timepoints after lipopolysaccharide (LPS) treatment. (**C**) Immunoblot analysis of phospho-IκBα (p-IkBa), total IκBα (t-IkBa), 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, *Pstpip2*^{cmo}, and *Pstpip2*^{cmo}*Syk*$^{fl/fl}$*LysM*^{cre} footpad lysates. (D) Immunoblot analysis of activated (cleaved) Casp-1, Casp-8, and gasdermin D (GSDMD) in WT, *Pstpip2*^{cmo}, and *Pstpip2*^{cmo}*Syk*$^{fl/fl}$*LysM*^{cre} 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, *Pstpip2*^{cmo}, and *Pstpip2*^{cmo}*Syk*$^{fl/fl}$*LysM*^{cre} BMDMs treated with LPS + ATP or left untreated with media. Representative blots from three independent experiments are shown.

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

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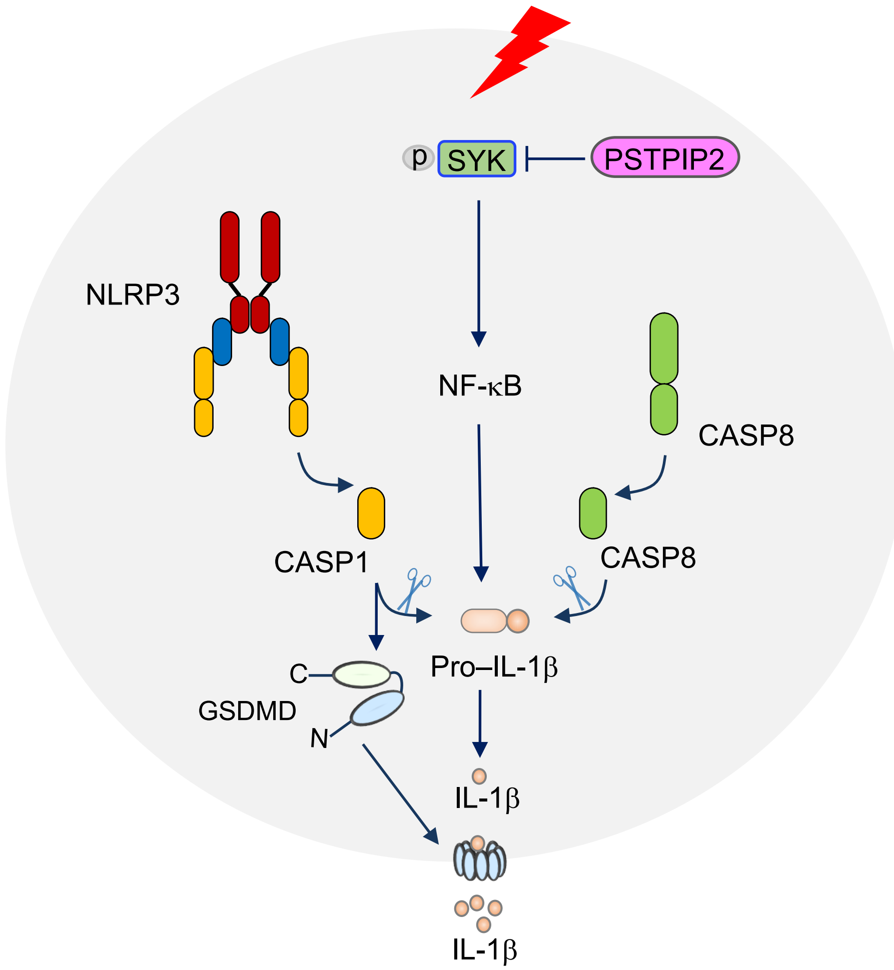

Graphical Abstract