Supplemental Data

PGLYRP-2 and Nod2 Are Both Required for Peptidoglycan-Induced Arthritis and Local Inflammation

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Supplemental Results

Significance of differences in arthritis severity and incidence between WT and KO mice

Arthritis scores (severity) and incidence were significantly higher for the data presented in Figure 1B in: PGN-injected WT than PGLYRP-2$^{-/-}$ mice and Nod2$^{-/-}$ mice at P<0.0001 on days 2 and 3, and at P=0.001-0.05 on days 4 through 11; PGN-injected WT than PGLYRP-1$^{-/-}$PGLYRP-2$^{-/-}$ mice at P= 0.0004-0.004 on days 2 and 3, and P=0.02-0.05 (scores only) on days 4 and 5; MDP-injected WT than PGLYRP-2$^{-/-}$ mice and Nod2$^{-/-}$ mice at P<0.0001 on days 1 and 2, and at P=0.005-0.03 on days 3 and 4; MDP-injected WT than PGLYRP-1$^{-/-}$PGLYRP-2$^{-/-}$ mice at P<0.0001 on day 1; PGN+MDP-injected WT than PGLYRP-2$^{-/-}$ mice and Nod2$^{-/-}$ mice at P<0.0001 on days 2 and 3, and at P=0.004-0.05 on days 1 and 4 through 11; PGN-injected PGLYRP-1$^{-/-}$PGLYRP-2$^{-/-}$ than PGLYRP-2$^{-/-}$ mice at P=0.003-0.01 on days 3 and 4; and MDP-injected PGLYRP-1$^{-/-}$PGLYRP-2$^{-/-}$ than PGLYRP-2$^{-/-}$ mice at P=0.01-0.04 on day 1 (and incidence only on day 2). Arthritis scores and incidence were not significantly different in PGN-injected or MDP-injected WT than PGLYRP-1$^{-/-}$, PGLYRP-3$^{-/-}$, and PGLYRP-4$^{-/-}$ mice (for the data presented in Figure 1C), except for PGLYRP-1$^{-/-}$ mice, which after MDP injection had significantly higher scores than WT mice at P=0.03-0.05 on days 3, 4, and 5.

Serum PGLYRP-2 is not sufficient for induction of arthritis

We tested the contribution of serum PGLYRP-2 to the development of MDP-induced arthritis, because PGLYRP-2 is constitutively produced in the liver and is secreted into blood (Xu et al., 2004; Zhang et al., 2005; Royet and Dziarski, 2007). Intravenous injection of 500 µg of purified recombinant PGLYRP-2 into WT mice did not produce arthritis, which indicates that increasing serum concentration of PGLYRP-2 in WT mice is not sufficient for induction of arthritis (Figure S5A). Intravenous injection of 500 µg of purified recombinant PGLYRP-2 protein into PGLYRP-2$^{-/-}$ mice, immediately followed by injection of 100 µg of MDP, did not produce arthritis either (Figure S5A), which indicates that following MDP stimulation PGLYRP-2 is not provided to the local cells in the foot from the serum, and thus it likely has to be produced by the local cells themselves (which does not happen in PGLYRP-2$^{-/-}$ mice). PGLYRP-2 is a large protein (140 kD dimers) and our results indicate that in the absence of inflammation it does not leak out of the vasculature into the foot tissues. Our PGLYRP-2 protein is active, as shown by the in vitro experiments with fibroblasts (Figure 5B) and by the amidase activity assays (Supplemental Experimental Procedures). Although we cannot completely exclude the possibility that the recombinant PGLYRP-2 protein is unstable when injected in vivo, this is unlikely, because it was sufficiently stable to be active in vitro and we injected a large excess of the protein, compared to the concentration that is active in vitro. Injection of a lower amount of PGLYRP-2 protein (50 µg/mouse) did not result in the arthritis either (data not shown). These results are consistent with the requirement for MDP stimulation for induction of arthritis

and with our finding that MDP does not increase PGLYRP-2 production in the liver. Therefore, our results indicate that PGLYRP-2 protein acts locally and has to be produced locally in the foot, and for induction of local inflammation and arthritis it is not obtained from the serum.

Macrophages do not express PGLYRP-2 and are not defective in PGLYRP-2$^{-/-}$ mice

Because macrophages often mediate innate immune responses to bacteria and their components, we tested whether macrophages from PGLYRP-2$^{-/-}$ mice had defective pro-inflammatory responses that could account for the low MDP-induced gene activation and inability to induce arthritis with MDP or peptidoglycan in PGLYRP-2$^{-/-}$ mice. Peritoneal and bone marrow-derived macrophages from PGLYRP-2$^{-/-}$ mice responded normally to Nod2, TLR2, and TLR4 stimulants (MDP, peptidoglycan, Pam3CSK4, and LPS, and intact bacteria) with activation of NF-κB, MAP kinases, and secretion of chemokines (data not shown). This is consistent with the lack of constitutive and induced expression of PGLYRP-2 in WT macrophages (Liu et al., 2001; Wang et al., 2005; Li et al., 2006). These results, therefore, support our conclusion that macrophages are not responsible for the lack of local inflammation and arthritis in peptidoglycan- or MDP-injected PGLYRP-2$^{-/-}$ mice. They also further demonstrate that this model of arthritis is different from the macrophage-dependent intraarticular injection model (Joosten et al., 2003, 2008).

Amidase activity of PGLYRP-2 is not required for its pro-inflammatory activity

Mouse and human PGLYRP-2 are amidases that hydrolyze the lactyl bond between the MurNAc and L-Ala (the first amino acid) of the stem peptide in bacterial peptidoglycan (Gelius et al., 2003; Wang et al., 2003). We considered the question whether the amidase activity of PGLYRP-2 was required for its pro-inflammatory activity. This question was important because the amidase activity has been so far the only known function of mammalian PGLYRP-2, and also because hydrolysis of peptidoglycan could possibly generate biologically active peptidoglycan fragments, such as stem peptides, which are known Nod1 stimulants if they contain meso-diaminopimelic acid.

Our results, however, indicate that the amidase activity is not required for the pro-inflammatory activity of PGLYRP-2, because acute arthritis and induction of pro-inflammatory gene expression was induced by MDP in a PGLYRP-2-dependent manner (Figures 1-3 and S1), yet MDP does not bind to PGRPs (Kumar et al., 2005; Swaminathan et al., 2006; Guan et al., 2006) and is not hydrolyzed by PGLYRP-2 (Wang et al., 2003). MDP is too small to have any measurable affinity for PGRPs, and the smallest peptidoglycan fragment that binds to PGRPs and is hydrolyzed by PGLYRP-2 is muramyl tripeptide, and muramyl terta- and penta-peptides have higher affinity for PGRPs than muramyl tripeptides (Guan et al., 2004, 2006; Wang et al., 2003). Moreover, this point is further supported by lower responsiveness of paw fibroblasts from PGLYRP-2$^{-/-}$ mice to various stimulants and reconstitution of the responsiveness of these cells by recombinant PGLYRP-2 protein, regardless whether these stimulants are or are not amidase substrates (peptidoglycan, bacteria, ReLPS, IL-1 – Figure 5A,B). These fibroblasts from PGLYRP-2$^{-/-}$ mice also have lower baseline (unstimulated) secretion of cytokines and chemokines (Figure 5A). Also, in the in vitro transfection model enhanced responses induced by transfection with PGLYRP-2 are also induced independently of amidase substrates, i.e., following transfection with PGLYRP-2 without any additional stimulation (Figure 5G,H), or following co-transfection with

TLR4+MD2+CD14 and stimulation with ReLPS (Figure 5H), which further supports that amidase activity of PGLYRP-2 is irrelevant for its immunoenhancing effect.

To further determine whether amidase activity of PGLYRP-2 was required for its pro-inflammatory activity, we used amidase-inactive C530S PGLYRP-2 mutant (Wang et al., 2003) to transfect HEK293 cells. This mutant stimulated IL-8 transcription and also in co-transfected cells enhanced responsiveness to TLR2 and TLR4 in a similar manner to WT PGLYRP-2 (Figure S6, compare to Figure 5G,H). These results further indicate that the amidase activity of PGLYRP-2 is not required for its pro-inflammatory activity and support the notion that mammalian PGLYRP-2 has two functions: amidase activity demonstrated previously and pro-inflammatory activity demonstrated in this paper.

The pro-inflammatory activity of PGLYRP-2 based on generation of biologically active peptidoglycan fragments, such as Nod1-stimulatory stem peptides, is also unlikely, because again MDP is also active, yet it is not hydrolyzed by PGLYRP-2 and even if it was, its dipeptide alone has no activity. Moreover, staphylococcal peptidoglycan, which is arthritogenic, does not have meso-diaminopimelic acid that is necessary for Nod1 stimulation. Furthermore, involvement of Nod1 and generation of Nod1-stimulatory peptides by PGLYRP-2 is not a significant mechanism in our model of peptidoglycan-induced arthritis, because *Bacillus subtilis* peptidoglycan and *Escherichia coli* peptidoglycan, both of which contain the Nod1-stimulatory peptide, when injected intravenously into WT mice did not produce arthritis of higher incidence or severity than *S. aureus* peptidoglycan (data not shown). These results, therefore, further indicate that neither amidase activity of PGLYRP-2 nor Nod1 are directly involved in our arthritis and inflammation model. We cannot exclude, however, a permissive role of Nod1 downstream of Nod2, independent of Nod1 stimulation by the arthritogenic compound.

MDP and peptidoglycan are not contaminated with TLR4 stimulants

While studying the role of TLR4 in peptidoglycan- and MDP-induced inflammation and arthritis, it was important to exclude direct activation of TLR4 with any possible TLR4 activators in these preparations, and especially endotoxin. In addition to showing no endotoxin contamination in our MDP and peptidoglycan preparations using Limulus assay (see Supplemental Experimental Procedures), we also demonstrated that these preparations did not contain any functional TLR4 stimulants. HEK293 cells transfected with TLR4 + MD2 + CD14 responded to ReLPS with activation of NF-κB (as expected), but did not respond to MDP and peptidoglycan (Figure S7). By contrast, cells transfected with TLR2 + CD14 responded to peptidoglycan, but not to MDP and ReLPS (as expected) (Dziarski and Gupta, 2005). These results further confirm our conclusion that MDP and peptidoglycan induce arthritis through activation of Nod2 and not TLR4 and TLR2, and that the permissive role of TLR4, which is needed for maturation of PMNs, is independent of MDP and peptidoglycan stimulation and is evident in untreated mice (Figure 6E). Also note that although peptidoglycan activates TLR2, this activation is not required for the induction of arthritis, because both peptidoglycan and MDP induce arthritis in $TLR2^{-/-}$ mice (Figure 6A,B), but not in $Nod2^{-/-}$ mice (Figure 1).

Supplemental Experimental Procedures

Peptidoglycan, MDP, PGLYRP-2, and other stimulants

Insoluble peptidoglycan was purified from *S. aureus* (or from *E. coli* or *B. subtilis*, where

indicated), analyzed, and sonicated as previously described (Dziarski and Gupta, 2005; Rosenthal and Dziarski, 1994). Synthetic MDP (N-acetylmuramyl-L-alanyl-D-isoglutamine) and MDP-LL (N-acetylmuramyl-L-alanyl-L-isoglutamine) were from Sigma. All these preparations were negative for endotoxin: contained <25 or <1 pg endotoxin/mg of peptidoglycan or MDP, respectively, by Limulus Lysate test, and did not activate TLR4-transfected cells (Figure S7). Pam3CSK4 was from InVivogen, *Salmonella minnesota* Re595 LPS (ReLPS) was from Sigma, human recombinant IL-1β was from NCI Biological Resources Branch, and *Micrococcus luteus* and *Enterobacter cloacae* were previously described (Dziarski et al., 2003; Wang et al., 2005).

Mouse PGLYRP-2 cDNAs was amplified from mouse liver RNA by reverse transcriptase with the following primers: CTGGAACTGGAGACCCACCATGAAGG and GAGAGACCCTTGAAGATCCAGGAGGT, and subcloned without the signal peptide and stop codon into the insect vector pMT/BiP/V5-His (Invitrogen) using the following primers: CTAGATCTTCCTCCTTGCCTCTGCTCATGGAC and GCCTCACTTCACAGAGGTTGAAAACTCTAGAGC. Mouse albumin was amplified without the signal peptide and stop codon from a cDNA clone (NM009654) with the following primers: GCAGATCTAGGGGTGTGTTTCGCCGAGAAG and GCTCTAGAGGCTAAGGCGTCTTTGCATCTAGTG, and inserted into the insect vector pMT/BiP/V5-His. Stable S2 cells expressing mouse PGLYRP-2 or albumin with V5 and 6xHis tags were generated and the proteins were purified from culture supernatants by Ni-NTA affinity chromatography as previously described for human PGLYRPs (Lu et al., 2006). The purified proteins yielded a single band on Coomassie blue-stained gel (Figure S5B). The amidase activity of PGLYRP-2 protein was verified as previously described (Wang et al., 2003), which confirms the correct conformation of the recombinant protein. The proteins were endotoxin-free (contained <10 pg endotoxin per mg protein measured by Limulus Lysate test).

Generation, breeding, characterization, and development of PGLYRP-1$^{-/-}$, PGLYRP-2$^{-/-}$, PGLYRP-3$^{-/-}$, PGLYRP-4$^{-/-}$, and double KO PGLYRP-1$^{-/-}$PGLYRP-2$^{-/-}$ mice

We generated PGLYRP-1$^{-/-}$ (PGRP-S$^{-/-}$) mice as previously described (Dziarski et al., 2003). We generated PGLYRP-2$^{-/-}$ mice by replacing all four amino-acid-coding exons (1 through 4, ~4.8 kb) with a neomycin-resistance (NEO) cassette (Figure S8), using a similar procedure to the one that we used previously for generation of PGLYRP-1$^{-/-}$ mice (Dziarski et al., 2003). To create *PGLYRP-2* targeting vector, *PGLYRP-2*-containing BAC clone was first identified from a BAC library screen. The short homology arm (~1.3 kb) was amplified by PCR with the primers located at the 3' end of exon 4 and 1.2 kb 3' from the end of exon 4. The long homology arm was a 7 kb *Bsi*WI fragment that starts 5' to the exon 1 ATG start codon. The short and long *PGLYRP-2* homology arms were inserted 5' and 3' to the NEO gene cassette using *Mlu*I and *Kpn*I sites, respectively. The targeting vector was confirmed by restriction analysis and sequencing using primers designed to read from the NEO cassette into the short and long homology arms. 10 μg of linearized targeting vector was transfected by electroporation into 129Sv/Ev ES cells. After selection with G418, resistant colonies were expanded and analyzed by PCR to identify recombinant clones. For *PGLYRP-2*$^{-}$, NEO primer (TGCGAGGCCAGAGGCCACTTGTGTAGC) and intron 4 primer (ATGGTTCCATCAGCAAAGTGCTGG) located 3' to the replaced region were used, and

wild-type *PGLYRP-2* gene was identified using exon 4 primer (GCTGCGCAATTCGCGCAGGTCTC) and the same intron 4 primer (Figure S8). Two correctly targeted ES cell lines were expanded and microinjected into the C57BL/6J blastocysts and produced 4 male and 2 female *PGLYRP-2*/WT chimeras (100% agouti). The chimeric mice were mated to generate heterozygous PGLYRP-2$^{-/+}$ mice, which we backcrossed to BALB/c mice for 8 generations to obtain heterozygous PGLYRP-2$^{-/+}$ mice on BALB/c background, which were then bred to obtain homozygous PGLYRP-2$^{-/-}$ mice on BALB/c background.

We generated PGLYRP-3$^{-/-}$ mice and PGLYRP-4$^{-/-}$ mice by replacing exons 2 through 5 (~5.6 kb) or exons 3 and 4 (~2.4 kb), respectively, with a NEO cassette using similar strategy, followed by backcrossing to BALB/c mice for 8 generations. The details of the generation of these mice will be reported elsewhere. PGLYRP-1$^{-/-}$PGLYRP-2$^{-/-}$ double KO mice were obtained by crossing PGLYRP-1$^{-/-}$ (Dziarski et al., 2003) with PGLYRP-2$^{-/-}$ mice. Wild-type and KO alleles were confirmed by PCR analysis of genomic DNA, and the lack of expression of PGLYRP-2, PGLYRP-3, and PGLYRP-4 mRNA was confirmed by quantitative real-time RT-PCR (Figure S8 and data not shown).

PGLYRP-1$^{-/-}$, PGLYRP-2$^{-/-}$, PGLYRP-3$^{-/-}$, PGLYRP-4$^{-/-}$, and PGLYRP-1$^{-/-}$PGLYRP-2$^{-/-}$ mice were viable and fertile, bred normally, and yielded the expected male:female ratios and similar litter size as the wild type and heterozygous mice. They had the same weight as the WT and heterozygous mice and developed normally with no obvious defects. Their major internal organs had normal macroscopic appearance, and normal histological appearance on hematoxylin/eosin-stained sections.

Nod2$^{-/-}$, TLR-2$^{-/-}$, TLR-4$^{-/-}$, and MyD88$^{-/-}$ mice

Nod2$^{-/-}$ mice (Kobayashi et al., 2005) and TLR-2$^{-/-}$, TLR-4$^{-/-}$, and MyD88$^{-/-}$ mice (De Trez et al., 2005) were previously described.

Maintenance and testing of mice

WT BALB/c breeder mice were from Harlan-Sprague-Dawley. All mice were bred and kept under conventional conditions. All KO mice were backcrossed to BALB/c mice for 8 generations and their BALB/c background was confirmed by SNP analysis (performed by the Jackson Laboratory). All mice were 98%-99.9% BALB/c; all SNP markers typed as BALB/c, except for occasional few SNPs near the targeted gene that were from 129 mice and likely originated from the targeting vector. We also confirmed selective lack of responsiveness of Nod2$^{-/-}$, TLR-2$^{-/-}$, TLR-4$^{-/-}$, and MyD88$^{-/-}$ mouse macrophages to appropriate ligands (MDP, peptidoglycan, Pam3CSK4, ReLPS, data not shown). All experiments on mice were approved by the Indiana University School of Medicine-Northwest Institutional Animal Care and Use Committee.

Evaluation of arthritis

The development of arthritis in the hind legs (ankles and feet, where it was the most prominent) was evaluated using: (a) severity of arthritis, which is based on the arthritis score, which is the sum of inflammation scores for both hind paws, each graded on a scale from 0 to 4: 0, normal paw, no redness or swelling; 1, some swelling of ankle; 2, moderate swelling and redness of ankle; 3, moderate swelling and redness of ankle and some swelling of foot pad and/or digits; 4, pronounced swelling and redness of the whole paw; (b) incidence of arthritis (percent of mice with arthritis score of at least 1); and (c) histological analysis, for which hind feet were cut off above ankle joints, fixed in Bouin's fixative, postfixed

in 70% ethanol, de-calcified, embedded in paraffin, sectioned in the sagittal plane, stained with hematoxylin/eosin, and evaluated microscopically.

Calculation of quantitative real-time PCR results and additional primers

For each gene, ΔCt was calculated followed by normalization to 5 housekeeping genes included in each array, followed by calculation of ΔΔCt for each gene from two arrays: ΔΔCt = ΔCt1 – ΔCt2, where ΔCt1 is the experimental group (mice injected with the tested preparation, e.g., MDP) and ΔCT2 is the control group (mice injected with buffer), using the program provided by SA Biosciences (formerly SuperArray). This calculation gives the fold increase in expression of each gene in mice injected with the test preparation versus mice injected with the buffer alone. The genomic DNA contamination controls, reverse transcription controls, and positive PCR controls were included in each array and were all passed. For all experiments, 6 to 12 mice per group were individually assayed, and the means ± SE of the fold increase in each gene expression from 6-12 mice were then calculated. When comparing baseline gene expression in uninjected WT vs KO mice, the average of ΔCt from 6 WT mice was used as ΔCT1 and ΔCTs from 6 individual KO mice were used as ΔCT2.

In some experiments, the expression of additional genes was measured by the same procedure using sets of qPCR primers from SA Biosciences (IL-6, PGLYRP-1, PGLYRP-3, PGLYRP-4) or designed by us (PGLYRP-2, exons 1 and 2 primers, GATCGTGCTTGGATTGCTGCTGTG and TCTTGGCTGACAGAATCCATGCAG, or exons 3 and 4 primers, ACCAGGATGTGCGCAAGTGGGAT and AGTGACCCAGTGTAGTTGCCCA).

Chemokine and cytokine assays

The amounts of CCL-2, CCL-12, CXCL-1, and IL-6 in the serum of mice or in culture supernatants were measured by ELISA using paired capture and detection antibodies from R&D Systems. The results are expressed as means ± SE pg/ml. TNF-α was measured as described (Dziarski et al., 2003). The significance of differences was determined by two-sample t-test.

Cell cultures and transfections

Primary paw fibroblasts were isolated from minced paw tissues of WT or PGLYRP-$2^{-/-}$ mice following digestion with collagenase (type I, 100 μg/ml, from Sigma) and DNase I (type IV, 150 μg/ml, from Sigma) in the presence of 33 μg/ml polymyxin B and 3 mM $CaCl_2$ at 37°C for 4 hrs, followed by overnight adherence to tissue culture plates in DMEM with 10% FCS, and subsequent culture of adherent cells in the same medium at 37°C 5% CO_2 (4-5 days). Paw fibroblasts were then seeded into 24-well plates (for ELISA) or 10-cm plates (for RNA), grown to 80% confluence in DMEM with 10% FCS, washed, grown without FCS for 12-14 hrs, and then stimulated with peptidoglycan (100 μg/ml), ReLPS (100 ng/ml), *Enterobacter* (40 μg/ml), *Micrococcus* (40 μg/ml), or IL-1β (100 ng/ml) in DMEM with 2% FCS for 12 hrs for cytokine and chemokine ELISA assays, or for 2 hrs for RNA isolation. Purified recombinant mouse serum albumin or mouse PGLYRP-2 protein (1 μg/ml) were added for the entire stimulation period in some experiments. RNA was isolated by TRIZOL method, followed by DNase digestion and purification using RNeasy Minikit, as described for RT^2 arrays. Paw fibroblasts were identified and checked for purity by morphology and immunocytochemical staining for fibroblast cytoplasmic antigen marker (Van Vliet et

al., 1986) using ER-TR7 rat mAb (Cedarlane, Hornby, Ontario, Canada) and Vectastain Elite ABC kit, followed by counterstaining with hematoxylin as described (Lu et al., 2006). Mouse L929 fibroblasts and RAW264 macrophage cells were used as a positive and negative control, respectively. Paw fibroblasts were identified as pure fibroblasts, judging by morphology and staining with ER-TR7 mAb. Their morphology and staining were identical to L929 fibroblasts (Figure S9). RAW264 cells incubated with ER-TR7 mAb or paw fibroblasts incubated with control rat IgG (negative controls) showed no peroxidase staining.

Human HEK293 cells were transfected with the following plasmids (individually or in combination): PGLYRP-2 (Liu et al., 2001), amidase-inactive C530S PGLYRP-2 (Wang et al., 2003), Nod2 (Inohara et al., 2003), TLR2, TLR4, CD14, MD-2, and NF-κB-, IL-8-, and 2 kb PGLYRP-2 promoter-luciferase reporters (Dziarski and Gupta, 2005; Li et al., 2006). Transiently transfected cells were then cultured in medium alone or stimulated with heat-killed *Micrococcus luteus* or *Enterobacter cloacae* (40 μg/ml) and assayed for luciferase activity as described (Li et al., 2006). Mouse peritoneal or bone marrow macrophages were cultured and stimulated with MDP, peptidoglycan, Pam3CSK4, ReLPS, *Micrococcus*, or *Enterobacter*, and IL-6 concentration in the supernatants or activation of NF-κB or ERK, p38, and JNK MAP kinases were determined as described (Li et al., 2006; Kim et al., 2008).

PMN isolation and CD11b expression

Blood PMNs were isolated as described (Liu et al., 2000) and lysed in 1% SDS sample buffer. The lysates from $3x10^6$ PMNs were subjected to polyacrylamide gel electrophoresis, and CD11b was detected on Western blots using anti-mouse CD11b (integrin αM) Abs. To confirm equal loading, each sample was diluted 30 times and actin was detected on parallel Western blots with anti-actin Abs (both Abs from Santa Cruz Biotechnology).

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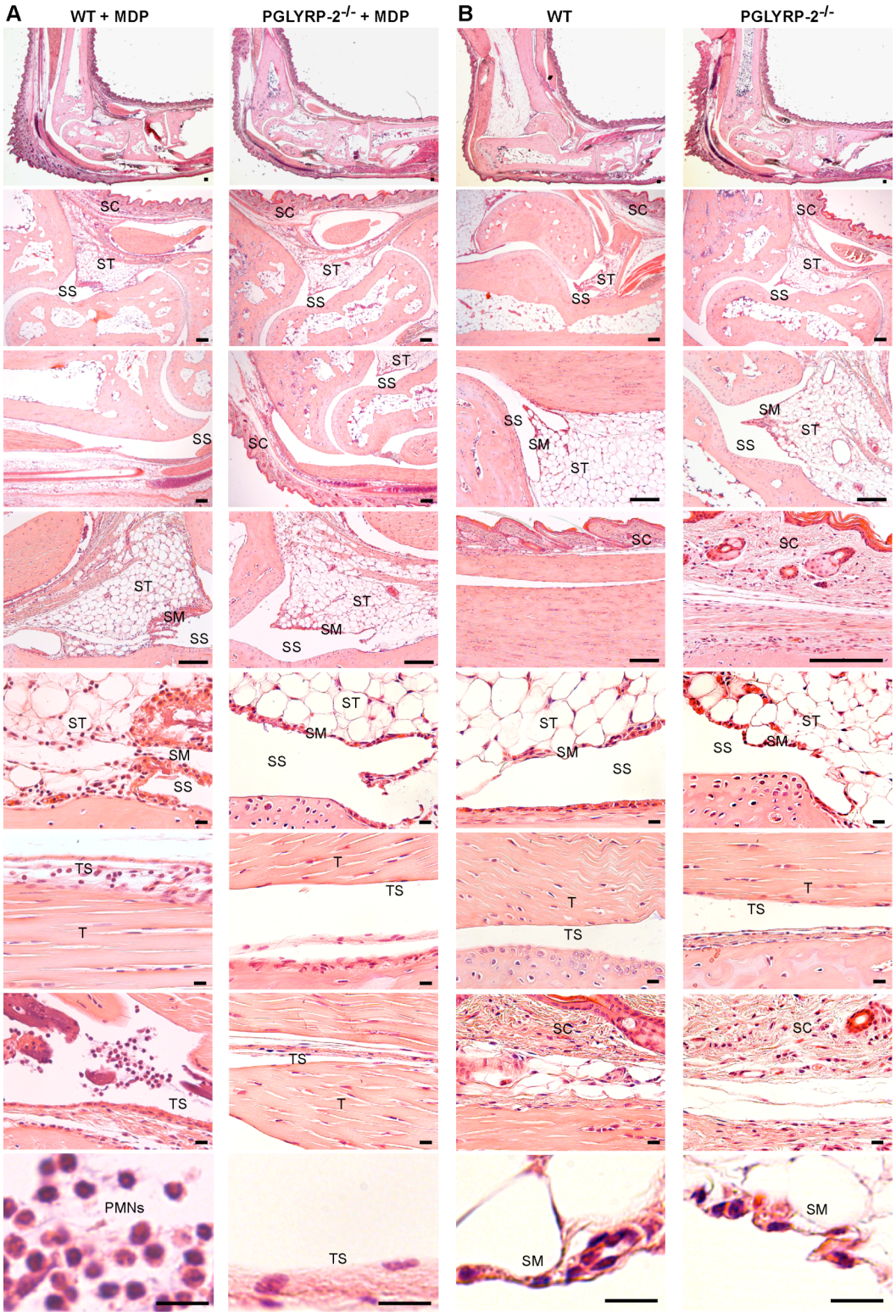

Figure S1. PGLYRP-2 is required for the development of MDP-induced pathologic changes characteristic of acute arthritis. (**A**) Edema and cellular infiltrations of synovial and sub-synovial tissues (ST) and tendon sheaths (TS) and other surrounding tissues in ankles and feet in WT, but not in PGLYRP-2$^{-/-}$ mice, 1 day after intravenous injection of 100 µg of MDP. PMNs were the predominant infiltrating cells in WT mice (bottom left panel in **A**). Ankles and feet in injected PGLYRP-2$^{-/-}$ mice appeared normal. (**B**) Normal histology of feet of untreated WT and PGLYRP-2$^{-/-}$ mice. Size bars and abbreviations are as in Figure 2.

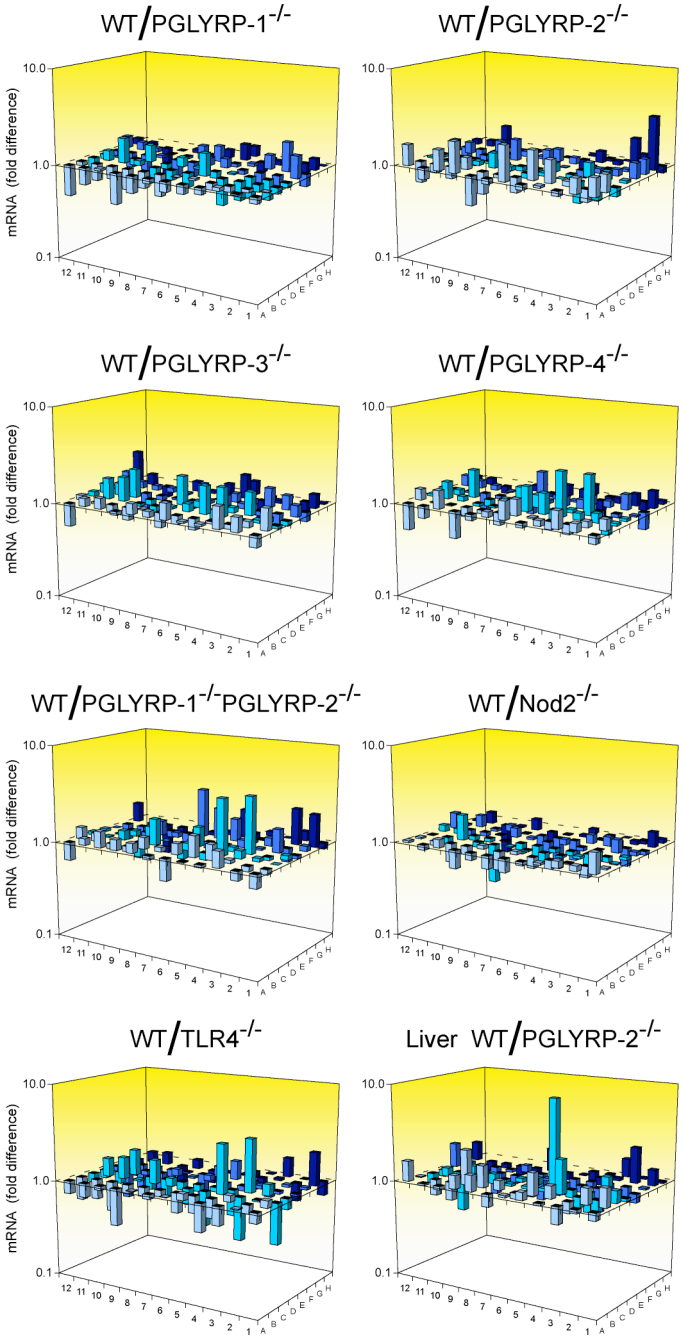

Figure S2. Background gene expression is mostly similar in WT and KO mice. The ratios in the gene expression in the feet (or liver where indicated) in untreated WT to untreated KO mice are shown (which represents fold difference in the background gene expression in WT versus KO mice, WT/KO), determined by qPCR as in Figure 3. The results are means of 6 mice/group (6 separate qPCR assays). The means, SE, and the significance of differences are given in Supplemental Table S4.

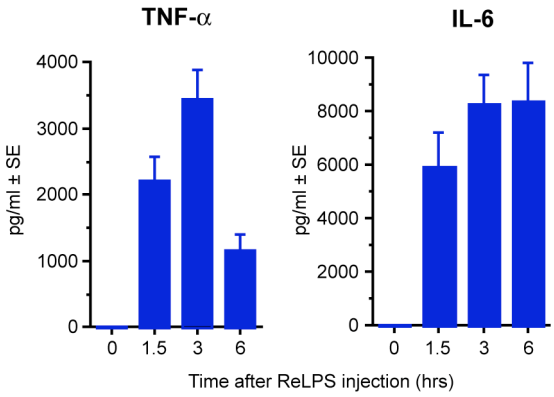

Figure S3. ReLPS induces high levels of TNF-α and IL-6 in blood of WT mice. Serum levels of TNF-α and IL-6 after intravenous injection of 10 μg of ReLPS, means ± SE of 6 mice per time point.

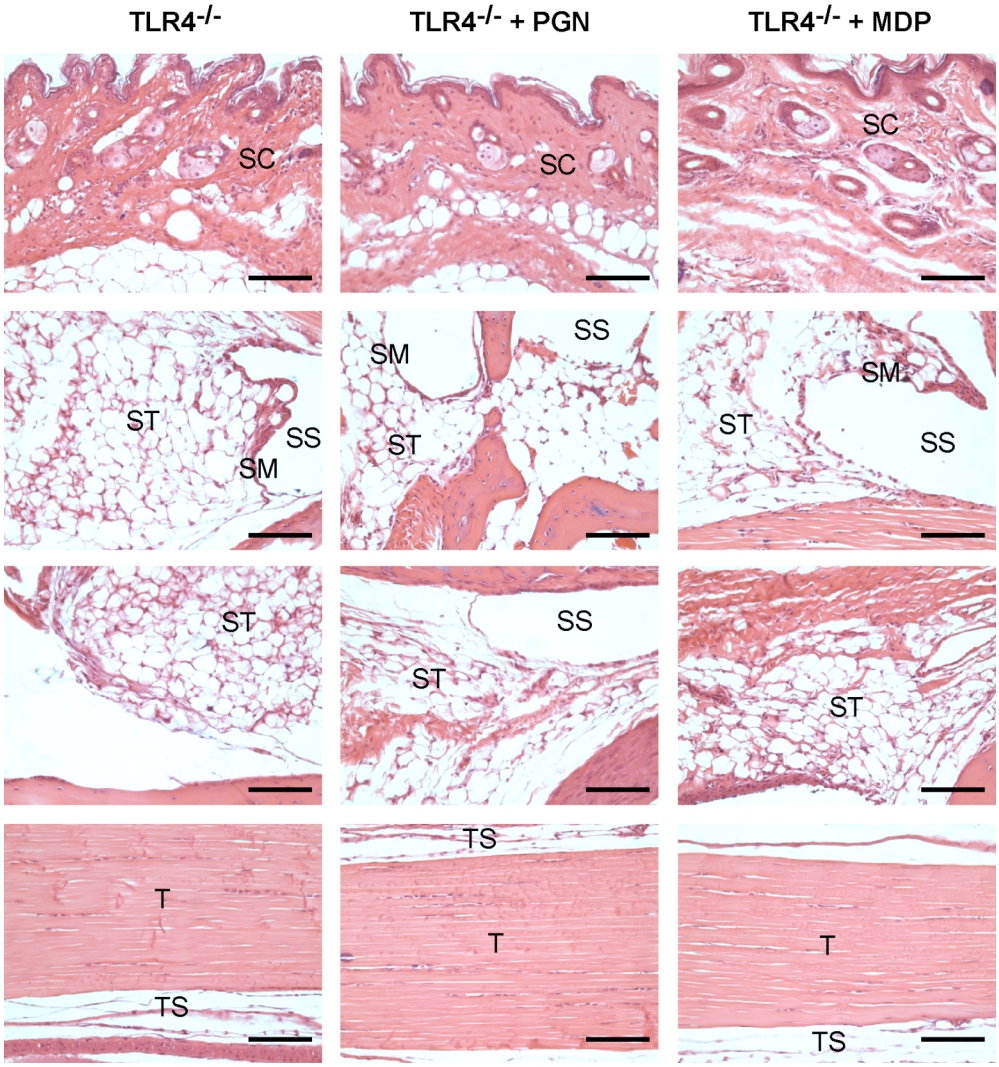

Figure S4. Normal foot histology and no PMN infiltrations in peptidoglycan- or MDP-injected TLR4$^{-/-}$ mice. The histology of ankles and feet in TLR4$^{-/-}$ mice that were untreated or injected intravenously with 200 μg of PGN or 100 μg of MDP (3 days or 1 day earlier, respectively) is normal, and there are no PMN infiltrations (compare to PMN infiltrations in WT mice in Figures 2 and S1). Bar = 100 μm, abbreviations as in Figure 2.

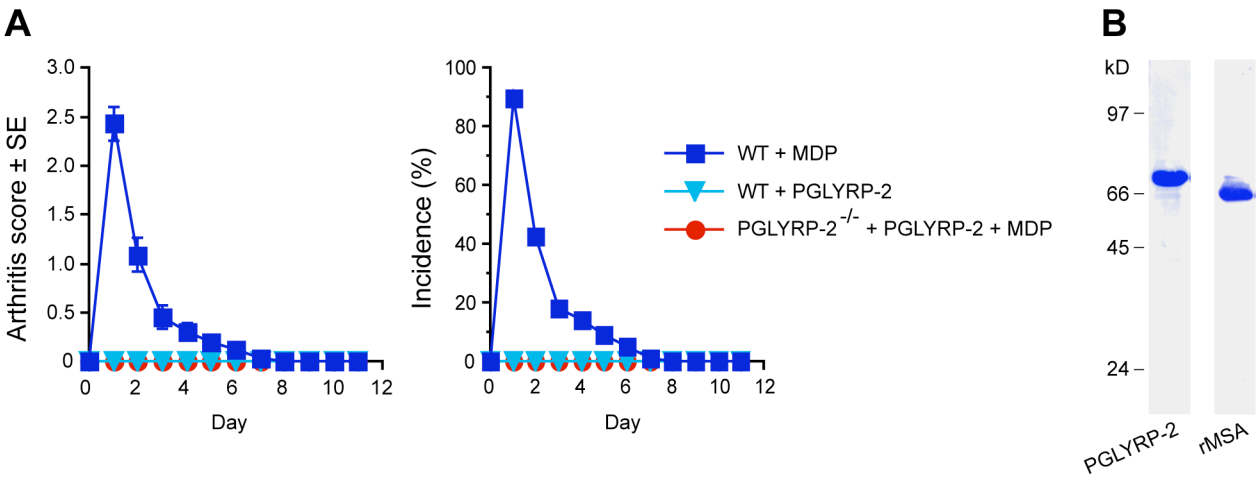

Figure S5. Serum PGLYRP-2 is not sufficient for induction of arthritis. (**A**) Arthritis scores and incidence in WT mice after intravenous injection of 100 µg of MDP or 500 µg of mouse recombinant PGLYRP-2; or in PGLYRP-2$^{-/-}$ mice after intravenous injection of 500 µg of mouse recombinant PGLYRP-2 plus 100 µg of MDP; means ± SE of 76 (WT + MDP) or 10 (WT + PGLYRP-2 protein, or PGLYRP-2$^{-/-}$ + PGLYRP-2 protein + MDP) mice/group. (**B**) Coomassie blue staining of 20 µg/lane of purified mouse recombinant PGLYRP-2 and mouse recombinant serum albumin (rMSA).

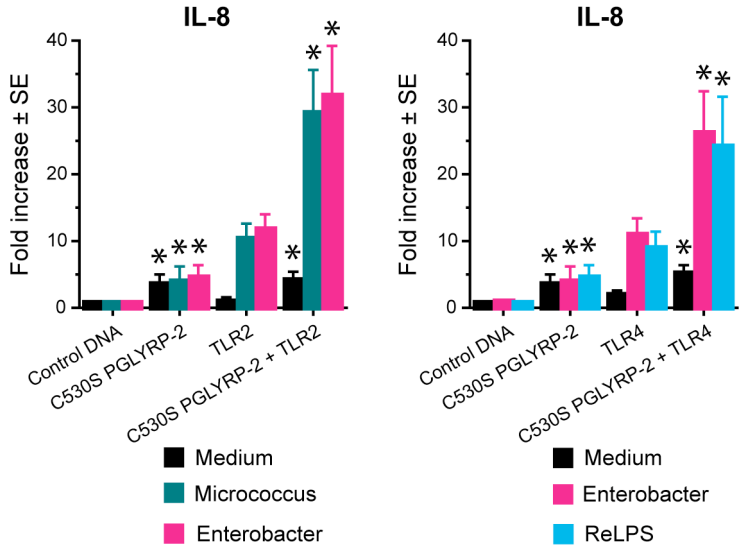

Figure S6. Amidase activity is not required for PGLYRP-2-induced stimulation of IL-8 and synergism with TLR2 and TLR4 in HEK293 cells. Induction of IL-8 transcription in HEK293 cells transfected with control DNA, amidase-inactive C530S PGLYRP-2, and TLR2 (left), or TLR4 (plus CD14 and MD-2) (right), alone or in combination, after stimulation with *Micrococcus*, *Enterobacter*, or ReLPS, or no stimulation; means ± SE of 3 experiments, significance of differences of C530S PGLYRP-2 vs control DNA or C530S PGLYRP-2+TLR vs TLR is indicated by an asterisk ($P \leq 0.05$).

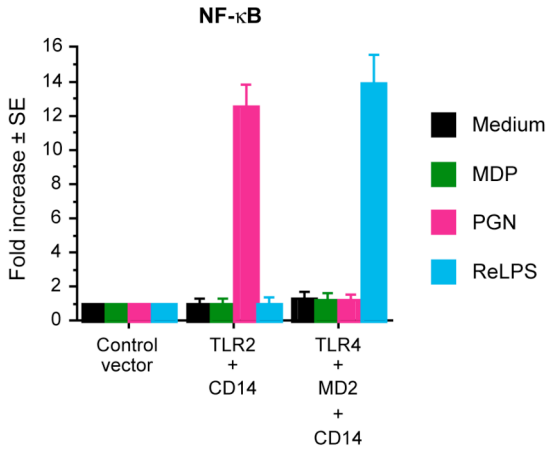

Figure S7. MDP and peptidoglycan do not activate TLR4-transfected cells. Induction of NF-κB in HEK293 cells transfected with vector DNA, TLR2 + CD14, or TLR4 + MD2 + CD14 after incubation in the medium alone or with 100 μg/ml MDP, 100 μg/ml peptidoglycan, or 10 ng/ml ReLPS. MDP and peptidoglycan do not activate cells transfected with TLR4 + MD-2 + CD14, which indicates lack of contamination with LPS; means ± SE of 3 experiments.

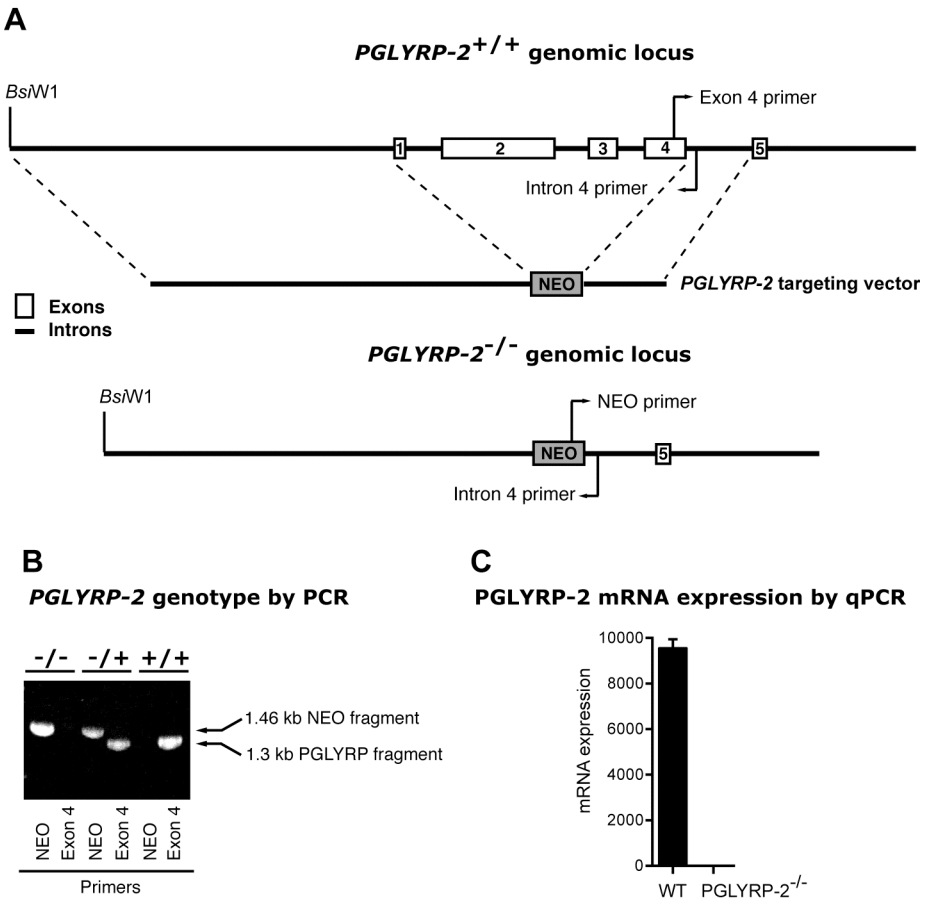

Figure S8. Generation and characterization of PGLYRP-2$^{-/-}$ mice. (**A**) Genomic organization of *PGLYRP-2*$^{+/+}$ and *PGLYRP-2*$^{-/-}$ loci and *PGLYRP-2* targeting vector, and locations of relevant primers. (**B**) Genotypes of PGLYRP-2$^{-/-}$, PGLYRP-2$^{-/+}$, and PGLYRP-2$^{+/+}$ mice determined by typing of genomic DNA by PCR with the indicated primers showing replacement of PGLYRP-2 exons with NEO cassette. (**C**) Phenotypes of WT and PGLYRP-2$^{-/-}$ mice by qPCR showing expression of PGRLRP-2 mRNA in the liver in WT but not in PGLYRP-2$^{-/-}$ mice.

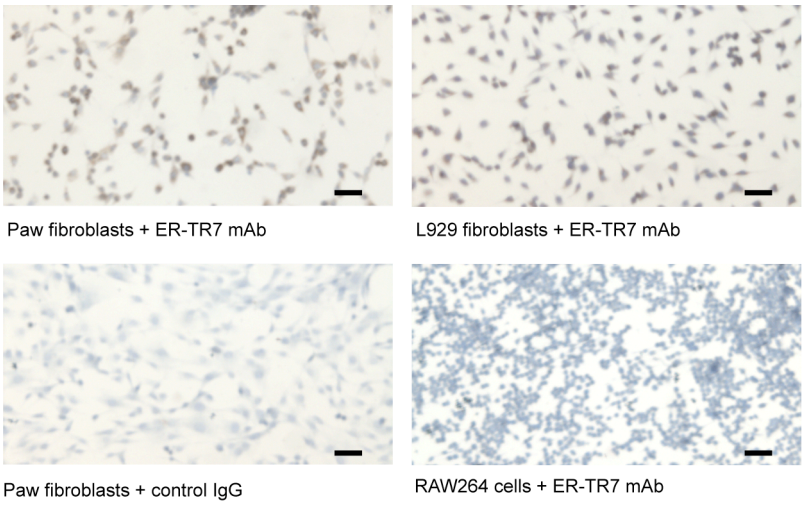

Figure S9. Morphology and immunoperoxidase staining of mouse paw fibroblasts. Primary mouse (WT) paw fibroblasts are positive for fibroblast cytoplasmic marker detected with ER-TR7 mAb (brown color); positive control (L929 fibroblasts) and two negative controls (RAW264 macrophage cell line and paw fibroblasts incubated with control IgG, counterstained blue) are also shown. Bar = 100 µm.

Supplemental Tables

Supplemental Tables S1-S4. qPCR Array Data

Table S1. Changes in pro-inflammatory gene mRNA levels in the feet of WT, PGLYRP-2 KO, and Nod2 KO mice injected intravenously with MDP

Gene					WT/Buffer [a]			PGLYRP-2 KO/Buffer [a]				WT/PGLYRP-2 KO [b]			Nod2 KO/Buffer [a]				WT/Nod2 KO [b]		
Symbol	Position	Unigene	GeneBank	Description	Mean	SE	P [c]	Mean	SE	P [c]	P [d]	Mean	SE	P [e]	Mean	SE	P [c]	P [d]	Mean	SE	P [e]
Abcf1	A01	Mm.329022	NM_013854	ATP-binding cassette, sub-family F (GCN20)	**0.90**	0.12		**1.22**	0.13			**0.78**	0.09		**1.53**	0.16		0.002	**0.60**	0.06	0.02
Bcl6	A02	Mm.347398	NM_009744	B-cell leukemia/lymphoma 6	**1.15**	0.10		**1.58**	0.21			**0.73**	0.06	0.002	**1.54**	0.06		0.0000	**0.55**	0.03	0.002
Blr1	A03	Mm.6246	NM_007551	Burkitt lymphoma receptor 1	**1.19**	0.09		**1.15**	0.11			**1.04**	0.08		**1.18**	0.05			**1.07**	0.05	
C3	A04	Mm.19131	NM_009778	Complement component 3	**1.51**	0.10	0.0004	**1.08**	0.03		0.009	**1.40**	0.09	0.005	**1.31**	0.06			**1.04**	0.09	
Casp1	A05	Mm.1051	NM_009807	Caspase 1	**0.96**	0.06		**0.93**	0.14			**1.03**	0.06		**0.93**	0.06			**0.92**	0.11	
Ccl1	A06	Mm.1283	NM_011329	Chemokine (C-C motif) ligand 1	2.80	0.46	0.003	2.68	0.58	0.05		**1.04**	0.17		**1.40**	0.37			**0.99**	0.18	
Ccl11	A07	Mm.4686	NM_011330	Small chemokine (C-C motif) ligand 11	**1.06**	0.06		**1.02**	0.07			**1.03**	0.06		**0.83**	0.06			**1.06**	0.05	
Ccl12	A08	Mm.867	NM_011331	Chemokine (C-C motif) ligand 12 (MCP-5)	9.55	0.76	0.0000	**1.56**	0.37		0.0000	6.11	0.49	0.0000	**1.41**	0.36		0.0002	7.71	0.74	0.0004
Ccl17	A09	Mm.41988	NM_011332	Chemokine (C-C motif) ligand 17	9.42	1.09	0.0000	3.58	0.73	0.02	0.004	2.63	0.31	0.0000	**1.34**	0.23		0.02	7.06	1.53	0.02
Ccl19	A10	Mm.379051	NM_011888	Chemokine (C-C motif) ligand 19 (MIP-3β)	2.88	0.30	0.0000	**1.21**	0.08		0.002	2.38	0.25	0.0000	**1.14**	0.23		0.03	2.01	0.29	0.02
Ccl2	A11	Mm.290320	NM_011333	Chemokine (C-C motif) ligand 2 (MCP-1)	19.63	1.45	0.0000	2.69	0.61	0.03	0.0000	7.30	0.54	0.0000	**0.95**	0.41		0.0000	23.15	1.68	0.0000
Ccl20	A12	Mm.116739	NM_016960	Chemokine (C-C motif) ligand 20 (MIP-3α)	13.71	2.96	0.002	6.02	0.72	0.03	0.04	2.28	0.49	0.03	**1.98**	0.63		0.04	2.43	0.44	0.03
Ccl22	B01	Mm.12895	NM_009137	Chemokine (C-C motif) ligand 22	2.45	0.15	0.0000	2.76	0.52	0.04		**0.89**	0.06		**1.03**	0.08		0.0005	2.62	0.21	0.0009
Ccl24	B02	Mm.31505	NM_019577	Chemokine (C-C motif) ligand 24 (Eotaxin-2)	**1.13**	0.10		**0.86**	0.09			**1.32**	0.12	0.02	**1.09**	0.11			**0.80**	0.08	
Ccl25	B03	Mm.7275	NM_009138	Chemokine (C-C motif) ligand 25	**1.03**	0.08		**0.98**	0.06			**1.05**	0.08		**0.98**	0.07			**0.81**	0.05	
Ccl3	B04	Mm.1282	NM_011337	Chemokine (C-C motif) ligand 3 (MIP-1α)	3.36	0.47	0.0000	2.31	0.38	0.03		**1.45**	0.21		**0.80**	0.11		0.004	2.45	0.29	0.006
Ccl4	B05	Mm.244263	NM_013652	Chemokine (C-C motif) ligand 4 (MIP-1β)	3.28	0.48	0.0000	**1.36**	0.17		0.02	2.41	0.35	0.003	**1.32**	0.15			**1.69**	0.43	
Ccl5	B06	Mm.284248	NM_013653	Chemokine (C-C motif) ligand 5 (RANTES)	2.97	0.34	0.0000	**1.09**	0.13		0.002	2.72	0.31	0.0002	**0.78**	0.11		0.0004	4.99	0.49	0.0007
Ccl6	B07	Mm.137	NM_009139	Chemokine (C-C motif) ligand 6	**1.32**	0.08		**1.02**	0.05		0.03	**1.30**	0.08	0.004	**0.86**	0.04		0.03	**1.28**	0.09	
Ccl7	B08	Mm.341574	NM_013654	Chemokine (C-C motif) ligand 7 (MCP-3)	7.60	0.88	0.0000	**0.86**	0.12		0.0001	8.88	1.03	0.0000	**0.70**	0.12		0.003	12.76	2.00	0.003
Ccl8	B09	Mm.42029	NM_021443	Chemokine (C-C motif) ligand 8 (MCP-2)	**1.17**	0.18		**1.05**	0.06			**1.12**	0.17		**0.88**	0.11			**1.30**	0.08	
Ccl9	B10	Mm.416125	NM_011338	Chemokine (C-C motif) ligand 9	2.32	0.18	0.0000	**1.20**	0.15		0.002	**1.93**	0.15	0.0001	**0.94**	0.10		0.004	2.76	0.34	0.005
Ccr1	B11	Mm.274927	NM_009912	Chemokine (C-C motif) receptor 1	**1.67**	0.15	0.002	**1.39**	0.17			**1.20**	0.11		**0.77**	0.08		0.0003	2.73	0.22	0.0008
Ccr2	B12	Mm.6272	NM_009915	Chemokine (C-C motif) receptor 2	**1.07**	0.10		**0.73**	0.07		0.04	**1.46**	0.13	0.006	**0.73**	0.03		0.002	**1.85**	0.13	0.002
Ccr3	C01	Mm.57050	NM_009914	Chemokine (C-C motif) receptor 3	**0.97**	0.08		**0.81**	0.06			**1.19**	0.10		**1.13**	0.25			**0.96**	0.13	
Ccr4	C02	Mm.1337	NM_009916	Chemokine (C-C motif) receptor 4	**1.43**	0.09	0.0007	**0.61**	0.07	0.003	0.0000	2.34	0.14	0.0000	**1.27**	0.05			**1.25**	0.09	
Ccr5	C03	Mm.14302	NM_009917	Chemokine (C-C motif) receptor 5	2.17	0.34	0.007	**0.87**	0.08		0.02	2.51	0.39	0.004	**0.95**	0.07		0.008	3.20	0.47	0.008
Ccr6	C04	Mm.8007	NM_009835	Chemokine (C-C motif) receptor 6	2.03	0.36	0.02	**1.12**	0.21			**1.81**	0.32	0.03	**1.99**	0.69			**1.02**	0.11	
Ccr7	C05	Mm.2932	NM_007719	Chemokine (C-C motif) receptor 7	**1.33**	0.11	0.01	**1.12**	0.16			**1.19**	0.09		**0.86**	0.14			**1.55**	0.18	0.04
Ccr8	C06	Mm.139129	NM_007720	Chemokine (C-C motif) receptor 8	**1.67**	0.19	0.006	**1.25**	0.10			**1.34**	0.15	0.05	**1.38**	0.17		0.03	**1.51**	0.14	0.02
Ccr9	C07	Mm.299367	NM_009913	Chemokine (C-C motif) receptor 9	**0.99**	0.05		**1.23**	0.28			**0.81**	0.04		**1.16**	0.07			**0.76**	0.05	
Crp	C08	Mm.28767	NM_007768	C-reactive protein, pentraxin-related	13.96	3.64	0.006	5.50	1.77	0.04		2.54	0.66	0.05	**1.74**	0.31		0.05	11.03	3.63	0.05
Cx3cl1	C09	Mm.103711	NM_009142	Chemokine (C-X3-C motif) ligand 1	2.46	0.16	0.0000	**1.37**	0.15		0.0008	**1.80**	0.12	0.0000	**1.39**	0.12		0.007	**1.77**	0.17	0.010
Cxcl1	C10	Mm.21013	NM_008176	Chemokine (C-X-C motif) ligand 1 (KC)	14.27	1.18	0.0000	4.96	1.30	0.04	0.0003	2.88	0.24	0.0000	**1.03**	0.28		0.002	11.48	1.57	0.002
Cxcl10	C11	Mm.877	NM_021274	Chemokine (C-X-C motif) ligand 10 (IP-10)	9.25	1.87	0.0000	4.76	1.44	0.03		**1.94**	0.39	0.04	**0.97**	0.28		0.03	12.19	3.51	0.01
Cxcl11	C12	Mm.131723	NM_019494	Chemokine (C-X-C motif) ligand 11 (I-TAC)	2.07	0.19	0.0002	**1.15**	0.10		0.007	**1.81**	0.17	0.0000	**1.15**	0.11			**1.50**	0.27	
Cxcl12	D01	Mm.303231	NM_021704	Chemokine (C-X-C motif) ligand 12	**1.22**	0.10		**1.01**	0.08			**1.20**	0.09		**0.96**	0.05			**0.99**	0.06	
Cxcl13	D02	Mm.10116	NM_018866	Chemokine (C-X-C motif) ligand 13 (BCL)	3.05	0.24	0.0000	**1.79**	0.26	0.04	0.007	**1.70**	0.13	0.0004	**1.15**	0.06		0.002	2.64	0.27	0.003
Cxcl15	D03	Mm.64326	NM_011339	Chemokine (C-X-C motif) ligand 15	2.35	0.45	0.02	**1.22**	0.25			**1.92**	0.37	0.04	**1.98**	0.64			**0.84**	0.29	
Cxcl4	D04	Mm.332490	NM_019932	Chemokine (C-X-C motif) ligand 4	**1.33**	0.04	0.0000	**1.13**	0.16			**1.18**	0.04		**0.95**	0.04		0.001	**1.45**	0.07	
Cxcl5	D05	Mm.4660	NM_009141	Chemokine (C-X-C motif) ligand 5 (ENA-78)	2.75	0.20	0.0000	**0.98**	0.09		0.0000	2.80	0.20	0.0000	**0.73**	0.07		0.0008	3.63	0.36	0.001
Cxcl9	D06	Mm.766	NM_008599	Chemokine (C-X-C motif) ligand 9 (Mig)	2.93	0.32	0.0000	**1.08**	0.07		0.002	2.71	0.30	0.0002	**0.85**	0.12		0.03	3.46	0.73	0.03
Cxcr3	D07	Mm.12876	NM_009910	Chemokine (C-X-C motif) receptor 3	**0.85**	0.07		**0.76**	0.09			**1.12**	0.09		**0.96**	0.08			**0.99**	0.05	
Gpr2	D08	Mm.103813	XM_894898	Chemokine (C-C motif) receptor 10	**1.03**	0.07		**0.65**	0.08	0.01	0.007	**1.57**	0.11	0.0003	**1.38**	0.06		0.02	**0.82**	0.04	
Ifng	D09	Mm.240327	NM_008337	Interferon gamma	**1.31**	0.16		**0.88**	0.19			**1.49**	0.18	0.02	**0.88**	0.13			**1.38**	0.21	
Il10	D10	Mm.874	NM_010548	Interleukin 10	2.42	0.23	0.0001	2.22	0.41	0.05		**1.09**	0.10		**0.84**	0.14		0.01	3.11	0.49	0.01
Il10ra	D11	Mm.26658	NM_008348	Interleukin 10 receptor, alpha	**1.21**	0.08		**1.15**	0.08			**1.06**	0.07		**1.12**	0.12			**1.02**	0.10	
Il10rb	D12	Mm.4154	NM_008349	Interleukin 10 receptor, beta	**1.09**	0.05		**0.92**	0.07			**1.19**	0.06		**1.07**	0.06			**0.95**	0.07	
Il11	E01	Mm.35814	NM_008350	Interleukin 11	**0.69**	0.06		**1.27**	0.08		0.0001	**0.54**	0.05	0.0000	**1.54**	0.13		0.0006	**0.45**	0.06	0.0005
Il13	E02	Mm.1284	NM_008355	Interleukin 13	**1.56**	0.20	0.02	**1.14**	0.08			**1.37**	0.17		**1.57**	0.28			**0.59**	0.08	
Il13ra1	E03	Mm.24208	NM_133990	Interleukin 13 receptor, alpha 1	**1.19**	0.10		**1.20**	0.05			**0.99**	0.09		**0.94**	0.06			**1.14**	0.20	
Il15	E04	Mm.4392	NM_008357	Interleukin 15	**1.80**	0.14	0.0002	**0.91**	0.07		0.0009	**1.98**	0.15	0.0000	**0.86**	0.17		0.01	**1.87**	0.17	0.005
Il16	E05	Mm.10137	NM_010551	Interleukin 16	**0.93**	0.05		**1.13**	0.09			**0.82**	0.04		**1.11**	0.07			**0.81**	0.08	
Il17b	E06	Mm.59313	NM_019508	Interleukin 17B	**1.05**	0.11		**1.09**	0.09			**0.97**	0.10		**0.98**	0.09			**0.73**	0.09	
Il18	E07	Mm.1410	NM_008360	Interleukin 18	**0.82**	0.06		**0.93**	0.06			**0.89**	0.07		**1.16**	0.11		0.01	**0.70**	0.11	
Il1a	E08	Mm.15534	NM_010554	Interleukin 1 alpha	**1.35**	0.13	0.03	**1.27**	0.09			**1.06**	0.10		**1.10**	0.12			**0.91**	0.12	
Il1b	E09	Mm.222830	NM_008361	Interleukin 1 beta	4.42	0.53	0.0000	**1.39**	0.15		0.002	3.17	0.38	0.0002	**1.23**	0.10		0.03	2.78	0.57	0.04
Il1f6	E10	Mm.133095	NM_019450	Interleukin 1 family, member 6	**1.14**	0.07		**0.97**	0.07			**1.17**	0.07		**0.97**	0.04			**1.07**	0.13	
Il1f8	E11	Mm.45901	XM_130058	Interleukin 1 family, member 8	**0.90**	0.05		**0.99**	0.05			**0.91**	0.05		**0.94**	0.05			**0.86**	0.08	
Il1r1	E12	Mm.896	NM_008362	Interleukin 1 receptor, type I	**1.19**	0.07		**1.85**	0.19	0.01	0.002	**0.64**	0.04	0.0000	**0.81**	0.04			**1.45**	0.17	
Il1r2	F01	Mm.1349	NM_010555	Interleukin 1 receptor, type II	**1.15**	0.09		**1.44**	0.10	0.01		**0.80**	0.06		**0.92**	0.06			**0.95**	0.08	
Il20	F02	Mm.103794	NM_021380	Interleukin 20	3.28	0.76	0.01	**0.92**	0.06		0.05	3.55	0.82	0.01	2.19	0.23	0.005	0.02	**1.38**	0.12	
Il2rb	F03	Mm.35287	NM_008368	Interleukin 2 receptor, beta chain	**1.22**	0.09		**1.01**	0.13			**1.21**	0.09		**1.16**	0.09			**1.09**	0.11	
Il2rg	F04	Mm.2923	NM_013563	Interleukin 2 receptor, gamma chain	2.20	0.10	0.0000	**1.22**	0.11		0.0000	**1.81**	0.08	0.0000	**1.36**	0.15		0.002	**1.71**	0.11	0.002
Il3	F05	Mm.983	NM_010556	Interleukin 3	2.11	0.48	0.05	**1.10**	0.15			**1.92**	0.44		**1.06**	0.18			**1.81**	0.33	0.05
Il4	F06	Mm.276360	NM_021283	Interleukin 4	**1.33**	0.21		**1.18**	0.21			**1.13**	0.18		**1.81**	0.62			**0.73**	0.09	
Il5ra	F07	Mm.3448	NM_008370	Interleukin 5 receptor, alpha	**1.07**	0.09		**1.21**	0.11			**0.89**	0.07		**0.96**	0.14			**1.07**	0.11	
Il6ra	F08	Mm.2856	NM_010559	Interleukin 6 receptor, alpha	**1.43**	0.07	0.0002	**1.72**	0.19	0.02		**0.83**	0.04		**1.71**	0.13		0.03	**0.74**	0.04	
Il6st	F09	Mm.4364	NM_010560	Interleukin 6 signal transducer	**1.29**	0.05		**1.16**	0.06			**1.11**	0.05		**1.10**	0.06			**1.13**	0.09	
Il8rb	F10	Mm.234466	NM_009909	Interleukin 8 receptor, beta	**1.49**	0.18	0.02	**1.48**	0.22			**1.00**	0.12		**1.05**	0.07		0.0006	**1.96**	0.14	
Itgam	F11	Mm.262106	NM_008401	Integrin alpha M	**1.15**	0.08		**0.93**	0.09			**1.23**	0.08		**1.23**	0.10			**1.10**	0.08	
Itgb2	F12	Mm.1137	NM_008404	Integrin beta 2	**1.18**	0.07		**1.06**	0.10			**1.12**	0.07		**1.29**	0.11			**0.98**	0.09	
Lta	G01	Mm.87787	NM_010735	Lymphotoxin A	**0.72**	0.07	0.003	**1.47**	0.10	0.009	0.0000	**0.49**	0.05	0.0000	**0.92**	0.15			**0.76**	0.10	
Ltb	G02	Mm.1715	NM_008518	Lymphotoxin B	**1.30**	0.03	0.0000	**1.15**	0.19			**1.13**	0.03		**0.83**	0.12		0.01	**1.56**	0.06	0.0003
Mif	G03	Mm.2326	NM_010798	Macrophage migration inhibitory factor	**1.00**	0.02		**1.09**	0.06			**0.92**	0.02		**1.13**	0.03			**0.86**	0.02	
Scye1	G04	Mm.235137	NM_007926	Small inducible cytokine subfamily E	**1.23**	0.13		**1.24**	0.08			**0.99**	0.10		**0.97**	0.09			**0.87**	0.05	
Spp1	G05	Mm.288474	NM_009263	Secreted phosphoprotein 1	**1.67**	0.24	0.02	**1.26**	0.13			**1.33**	0.19		**0.84**	0.17		0.0003	2.90	0.24	0.0009
Tgfb1	G06	Mm.248380	NM_011577	Transforming growth factor, beta 1	**1.09**	0.08		**1.03**	0.03			**1.06**	0.08		**1.16**	0.07			**1.13**	0.07	
Tnf	G07	Mm.1293	NM_013693	Tumor necrosis factor	4.73	0.28	0.0000	2.24	0.45	0.05	0.0003	2.05	0.11	0.0001	**1.26**	0.32		0.0001	3.52	0.24	0.0002
Tnfrsf1a	G08	Mm.1258	NM_011609	Tumor necrosis factor receptor superfamily	**0.92**	0.04		**1.06**	0.05			**0.87**	0.04		**1.17**	0.04			**0.83**	0.06	
Tnfrsf1b	G09	Mm.235328	NM_011610	Tumor necrosis factor receptor superfamily	**1.24**	0.05		**1.34**	0.13			**0.93**	0.04		**1.05**	0.08			**1.15**	0.06	
Cd40lg	G10	Mm.4861	NM_011616	CD40 ligand	**1.28**	0.28		**1.60**	0.18	0.03		**0.80**	0.17		**0.74**	0.11			**0.96**	0.28	
Tollip	G11	Mm.103551	NM_023764	Toll interacting protein	**0.84**	0.04		**1.21**	0.05		0.0002	**0.70**	0.03	0.002	**1.20**	0.11			**0.78**	0.05	
Xcr1	G12	Mm.89202	NM_011798	Chemokine (C motif) receptor 1	**1.20**	0.15		**0.83**	0.11			**1.45**	0.19	0.04	**0.64**	0.10			**1.71**	0.39	
Gusb	H01	Mm.3317	NM_010368	Glucuronidase, beta	**1.03**	0.04		**1.00**	0.07			**1.02**	0.04		**0.99**	0.05			**1.10**	0.06	
Hprt1	H02	Mm.299381	NM_013556	Hypoxanthine guanine phosphoribosyl transferase 1	**0.98**	0.07		**1.03**	0.08			**0.95**	0.07		**0.78**	0.03			**1.00**	0.05	
Hspcb	H03	Mm.2180	NM_008302	Heat shock protein 90kDa alpha (cytosolic)	**0.98**	0.03		**1.05**	0.02			**0.94**	0.03		**1.17**	0.02			**0.90**	0.02	
Gapdh	H04	Mm.343110	NM_008084	Glyceraldehyde-3-phosphate dehydrogenase	**1.17**	0.03		**1.06**	0.05			**1.10**	0.03		**1.05**	0.04			**1.18**	0.04	
Actb	H05	Mm.328431	NM_007393	Actin, beta, cytoplasmic	**0.91**	0.03		**0.90**	0.01			**1.00**	0.03		**1.08**	0.03			**0.86**	0.03	

[a] Ratio of the amounts of mRNA in the feet of mice injected intravenously with 100 µg MDP to the amounts of mRNA in mice injected with buffer, determined by quantitative real time PCR 6 hrs after injection; means of 12 (WT) or 6 (KO) mice.

[b] Ratio of the fold induction of mRNA in the feet of the WT to KO mice injected intravenously with 100 µg MDP, determined by quantitative real time PCR 6 hrs after injection; means of 6 mice.

[c] Significance of difference in the fold increase in the amounts of mRNA in MDP-injected over buffer-injected mice of the same genotype, determined by one-sample t-test (mean fold ratio vs 1, N = 12 WT or 6 KO); blank = not significant (P>0.05).

[d] Significance of difference between KO and WT mice in the fold increase in the amounts of mRNA in MDP-injected to buffer-injected mice, determined by two-sample t-test (mean fold MDP/Buffer WT vs mean fold MDP/Buffer KO, N = 12 WT or 6 KO); blank = not significant (P>0.05); if KO<WT P value marked in blue, if KO>WT P value marked in magenta.

[e] Significance of difference of the MDP-induced mRNA fold increase in WT/fold increase in KO mice determined by one-sample t-test (WT/KO ratio vs 1, N = 6); blank = not significant (P>0.05); if WT/KO>1 P value marked in blue, if WT/KO<1 P value marked in magenta.

Table S2. Changes in pro-inflammatory gene mRNA levels in the feet of WT and PGLYRP-1 KO, PGLYRP-3 KO, and PGLYRP-4 KO mice injected intravenously with MDP

Gene		WT/Buffer [a]			PGLYRP-1 KO/Buffer [a]				WT/PGLYRP-1 KO [b]			PGLYRP-3 KO/Buffer [a]				WT/PGLYRP-3 KO [b]			PGLYRP-4 KO/Buffer [a]				WT/PGLYRP-4 KO [b]		
Symbol	Position	Mean	SE	P [c]	Mean	SE	P [c]	P [d]	Mean	SE	P [e]	Mean	SE	P [c]	P [d]	Mean	SE	P [e]	Mean	SE	P [c]	P [d]	Mean	SE	P [e]
Abcf1	A01	0.90	0.12		1.07	0.04			0.85	0.25		0.99	0.04			0.91	0.12		1.26	0.04			0.72	0.09	0.004
Bcl6	A02	1.15	0.10		1.18	0.06			0.97	0.28		1.55	0.10	0.0000	0.03	0.74	0.07	0.003	1.32	0.06	0.01		0.87	0.08	
Blr1	A03	1.19	0.09		1.15	0.06			1.04	0.30		1.58	0.14	0.01	0.03	0.75	0.05	0.001	1.71	0.16	0.01		0.70	0.05	0.0003
C3	A04	1.51	0.10	0.0004	1.75	0.09	0.0005		0.87	0.25		1.29	0.07			1.18	0.08		1.85	0.15	0.004		0.82	0.05	
Casp1	A05	0.96	0.06		0.94	0.06			1.02	0.29		1.25	0.04		0.006	0.76	0.05	0.0006	0.97	0.14			0.98	0.06	
Ccl1	A06	2.80	0.46	0.003	3.69	0.15	0.0000		0.76	0.22		6.19	0.74	0.002	0.002	0.45	0.07	0.0000	10.65	0.62	0.0000	0.0000	0.26	0.04	0.0000
Ccl11	A07	1.06	0.06		1.35	0.06		0.01	0.79	0.23		1.64	0.04	0.0000	0.0000	0.65	0.04	0.0000	1.53	0.21			0.69	0.04	0.0001
Ccl12	A08	9.55	0.76	0.0000	11.26	0.54	0.0000		0.85	0.25		14.03	1.07	0.0001	0.006	0.68	0.05	0.0002	12.52	1.38	0.0006		0.76	0.06	0.0030
Ccl17	A09	9.42	1.09	0.0000	13.07	2.53	0.007		0.72	0.21		12.48	1.34	0.0005		0.75	0.09	0.02	9.71	2.65	0.03		0.97	0.11	
Ccl19	A10	2.88	0.30	0.0000	4.89	0.18	0.0000	0.0006	0.59	0.17	0.0000	3.62	0.26	0.0003		0.79	0.08	0.04	8.47	0.49	0.0000	0.0000	0.34	0.04	0.0000
Ccl2	A11	19.63	1.45	0.0000	23.93	2.04	0.0002		0.82	0.24		20.97	0.70	0.0000		0.94	0.07		23.25	2.54	0.0007		0.84	0.06	
Ccl20	A12	13.71	2.96	0.002	9.85	1.05	0.0008		1.39	0.40		10.51	2.03	0.01		1.31	0.28		12.38	1.23	0.0005		1.11	0.24	
Ccl22	B01	2.45	0.15	0.0000	3.35	0.17	0.0000	0.004	0.73	0.21	0.0002	4.22	0.30	0.0003	0.0001	0.58	0.04	0.0000	4.61	0.51	0.002	0.01	0.53	0.03	0.0000
Ccl24	B02	1.13	0.10		2.07	0.05	0.0000	0.0000	0.55	0.16	0.0000	1.79	0.07	0.0001	0.0008	0.63	0.06	0.0000	1.76	0.27	0.05		0.65	0.06	0.0001
Ccl25	B03	1.03	0.08		1.04	0.05			0.99	0.29		1.21	0.05			0.85	0.06		0.91	0.06			1.13	0.09	
Ccl3	B04	3.36	0.47	0.0000	4.73	0.16	0.0000		0.71	0.20	0.02	4.03	0.46	0.002		0.83	0.12		5.98	0.67	0.001	0.02	0.56	0.08	0.0002
Ccl4	B05	3.28	0.48	0.0000	5.12	0.37	0.0002	0.03	0.64	0.18	0.004	2.64	0.24	0.002		1.24	0.18		6.94	0.77	0.0009	0.005	0.47	0.07	0.0000
Ccl5	B06	2.97	0.34	0.0000	2.62	0.07	0.0000		1.13	0.33		1.41	0.17		0.009	2.11	0.24	0.001	4.53	0.79	0.01		0.66	0.07	0.0010
Ccl6	B07	1.32	0.08		1.05	0.03		0.04	1.26	0.36		1.21	0.03			1.09	0.07		1.16	0.14			1.14	0.07	
Ccl7	B08	7.60	0.88	0.0000	9.94	1.24	0.001		0.76	0.22	0.03	6.80	0.18	0.0000		1.12	0.13		10.10	1.16	0.0008		0.75	0.09	0.02
Ccl8	B09	1.17	0.18		1.25	0.11			0.93	0.27		1.33	0.10			0.88	0.13		1.39	0.16			0.84	0.13	
Ccl9	B10	2.32	0.18	0.0000	2.67	0.25	0.002		0.87	0.25		1.79	0.11	0.001		1.30	0.10		2.78	0.46	0.02		0.84	0.07	
Ccr1	B11	1.67	0.15	0.002	1.23	0.10			1.36	0.39		1.31	0.10			1.27	0.12		1.37	0.18			1.22	0.11	
Ccr2	B12	1.07	0.10		0.99	0.09			1.08	0.31		0.96	0.10			1.11	0.10		0.96	0.14			1.12	0.10	
Ccr3	C01	0.97	0.08		1.18	0.10			0.82	0.24		0.85	0.06			1.13	0.10		1.42	0.27			0.68	0.06	0.0003
Ccr4	C02	1.43	0.09	0.0007	1.04	0.06		0.01	1.38	0.40		1.71	0.10	0.001		0.84	0.05		1.90	0.11	0.0008	0.01	0.76	0.05	0.0004
Ccr5	C03	2.17	0.34	0.007	1.63	0.24	0.06		1.33	0.38		1.32	0.11			1.65	0.26	0.04	2.22	0.28			0.98	0.15	
Ccr6	C04	2.03	0.36	0.02	0.97	0.10			2.09	0.60	0.02	4.33	0.66	0.006	0.007	0.47	0.08	0.0000	1.71	0.73			1.19	0.21	
Ccr7	C05	1.33	0.11	0.01	2.36	0.31	0.01	0.002	0.56	0.16	0.0000	1.98	0.46			0.67	0.05	0.0000	2.70	0.27	0.002	0.004	0.49	0.04	0.0000
Ccr8	C06	1.67	0.19	0.006	1.29	0.06			1.30	0.38		1.70	0.13	0.004		0.98	0.11		2.77	0.28	0.0020	0.01	0.61	0.07	0.0002
Ccr9	C07	0.99	0.05		0.96	0.04			1.04	0.30		1.46	0.18		0.008	0.68	0.04	0.0000	1.06	0.07			0.93	0.05	
Crp	C08	13.96	3.64	0.006	7.01	0.58	0.0003		1.99	0.57		10.06	1.72	0.007		1.39	0.36		9.07	1.96	0.01		1.54	0.40	
Cx3cl1	C09	2.46	0.16	0.0000	2.92	0.10	0.0000		0.84	0.24		2.18	0.09	0.0000		1.13	0.07		2.94	0.19	0.0002		0.84	0.05	
Cxcl1	C10	14.27	1.18	0.0000	38.07	3.52	0.0002	0.0000	0.37	0.11	0.0000	19.45	1.54	0.0001	0.03	0.73	0.06	0.002	24.11	1.51	0.0000	0.0006	0.59	0.05	0.0000
Cxcl10	C11	9.25	1.87	0.0000	7.45	0.83	0.0008		1.24	0.36		13.29	1.68	0.001		0.70	0.14		14.37	2.39	0.004		0.64	0.13	0.02
Cxcl11	C12	2.07	0.19	0.0002	1.69	0.11	0.002		1.23	0.36		3.34	0.41	0.003	0.008	0.62	0.06	0.0000	3.29	0.81	0.04		0.63	0.06	0.0000
Cxcl12	D01	1.22	0.10		1.24	0.03			0.98	0.28		1.30	0.03			0.94	0.07		1.45	0.09	0.006		0.84	0.07	
Cxcl13	D02	3.05	0.24	0.0000	3.84	0.17	0.0000	0.05	0.79	0.23	0.01	3.06	0.32	0.002		1.00	0.08		2.47	0.37	0.02		1.23	0.10	
Cxcl15	D03	2.35	0.45	0.02	1.12	0.17			2.09	0.60	0.03	3.12	0.40	0.0000		0.75	0.15		2.61	0.62	0.04		0.90	0.17	
Cxcl4	D04	1.33	0.04	0.0000	1.47	0.07	0.002		0.91	0.26		1.56	0.08	0.001	0.02	0.86	0.03		1.15	0.07			1.16	0.04	
Cxcl5	D05	2.75	0.20	0.0000	4.67	0.76	0.007	0.009	0.59	0.17	0.0000	2.22	0.44	0.0534		1.24	0.09		3.39	0.46	0.005		0.81	0.06	
Cxcl9	D06	2.93	0.32	0.0000	2.90	0.31	0.002		1.01	0.29		5.60	0.53	0.0005	0.0006	0.52	0.06	0.0000	5.49	1.16	0.02		0.53	0.06	0.0000
Cxcr3	D07	0.85	0.07		1.18	0.19			0.72	0.21	0.001	1.47	0.24		0.009	0.58	0.05	0.0000	1.22	0.26			0.70	0.06	0.0004
Gpr2	D08	1.03	0.07		1.02	0.09			1.00	0.29		0.89	0.03			1.15	0.08		1.70	0.15	0.009	0.008	0.60	0.04	0.0000
Ifng	D09	1.31	0.16		1.53	0.25			0.86	0.25		1.45	0.19			0.90	0.11		2.07	0.41	0.05		0.63	0.08	0.0008
Il10	D10	2.42	0.23	0.0001	1.96	0.13	0.002		1.23	0.36		5.07	0.28	0.0000	0.0000	0.48	0.05	0.0000	3.85	0.64	0.01		0.63	0.06	0.0001
Il10ra	D11	1.21	0.08		1.82	0.11	0.001	0.0005	0.67	0.19	0.0000	1.80	0.06	0.0000	0.0002	0.67	0.04	0.0000	1.16	0.13			1.04	0.07	
Il10rb	D12	1.09	0.05		1.30	0.04		0.03	0.84	0.24		1.41	0.04	0.0003	0.002	0.77	0.04	0.0001	1.56	0.05	0.001	0.0000	0.70	0.03	0.0001
Il11	E01	0.69	0.06		1.46	0.10	0.008	0.0000	0.47	0.14	0.0000	1.39	0.07	0.004	0.0000	0.50	0.04	0.0000	0.99	0.07		0.01	0.70	0.06	0.0005
Il13	E02	1.56	0.20	0.02	1.25	0.06			1.24	0.36		1.30	0.09	0.03		1.20	0.15		2.32	0.40	0.03		0.67	0.09	0.003
Il13ra1	E03	1.19	0.10		0.99	0.04			1.20	0.35		1.22	0.06			0.97	0.08		1.08	0.14			1.10	0.09	
Il15	E04	1.80	0.14	0.0002	2.46	0.10	0.0000	0.01	0.73	0.21	0.001	2.44	0.15	0.0004	0.02	0.74	0.06	0.001	3.57	0.27	0.0004	0.0009	0.50	0.04	0.0000
Il16	E05	0.93	0.05		1.23	0.08		0.005	0.76	0.22	0.0000	1.38	0.07	0.005	0.0001	0.68	0.03	0.0000	1.02	0.09			0.92	0.05	
Il17b	E06	1.05	0.11		1.44	0.10	0.01	0.04	0.73	0.21	0.005	1.30	0.14			0.81	0.08		1.68	0.19	0.02	0.03	0.63	0.06	0.0001
Il18	E07	0.82	0.06		0.75	0.04			1.09	0.31		0.94	0.03			0.87	0.07		0.88	0.09			0.93	0.07	
Il1a	E08	1.35	0.13	0.03	1.52	0.12	0.01		0.89	0.26		1.72	0.10	0.001		0.79	0.08	0.02	1.74	0.27	0.05		0.78	0.07	0.02
Il1b	E09	4.42	0.53	0.0000	12.68	1.61	0.001	0.0000	0.35	0.10	0.0000	4.06	0.28	0.0002		1.09	0.13		11.26	2.00	0.006	0.03	0.39	0.05	0.0000
Il1f6	E10	1.14	0.07		0.73	0.05		0.003	1.55	0.45	0.0003	0.88	0.08			1.29	0.08		1.12	0.08			1.01	0.06	
Il1f8	E11	0.90	0.05		0.74	0.04		0.05	1.22	0.35		1.14	0.02		0.006	0.79	0.04	0.0007	1.16	0.14			0.78	0.08	0.02
Il1r1	E12	1.19	0.07		1.18	0.04			1.01	0.29		1.33	0.06	0.003		0.89	0.05		1.07	0.07			1.10	0.07	
Il1r2	F01	1.15	0.09		0.87	0.03			1.33	0.38	0.01	1.16	0.05			0.99	0.08		0.78	0.25	0.05		1.47	0.12	0.03
Il20	F02	3.28	0.76	0.01	15.21	3.36	0.01	0.0005	0.22	0.06	0.0000	2.10	0.39	0.05		1.56	0.36		3.94	0.65	0.009		0.83	0.19	
Il2rb	F03	1.22	0.09		1.68	0.16	0.01	0.02	0.72	0.21	0.0004	1.96	0.08	0.0001	0.0001	0.62	0.05		1.69	0.06	0.001	0.0008	0.72	0.05	0.0004
Il2rg	F04	2.20	0.10	0.0000	2.76	0.11	0.0000	0.005	0.80	0.23		2.17	0.07	0.0000		1.01	0.05		3.31	0.25	0.0004	0.008	0.66	0.03	0.0001
Il3	F05	2.11	0.48	0.05	3.31	0.69	0.03		0.64	0.18	0.04	2.47	0.66			0.86	0.20		4.66	1.04	0.02		0.46	0.11	0.0000
Il4	F06	1.33	0.21		2.72	0.34	0.006	0.004	0.49	0.14	0.0000	2.16	0.55			0.62	0.10	0.004	2.51	0.54	0.05		0.53	0.09	0.0003
Il5ra	F07	1.07	0.09		1.45	0.27			0.74	0.21	0.002	1.95	0.44		0.02	0.55	0.04	0.0000	1.66	0.34			0.65	0.05	0.0001
Il6ra	F08	1.43	0.07	0.0002	1.64	0.03	0.0000		0.87	0.25		1.63	0.06	0.0002		0.87	0.04		1.43	0.06	0.001		1.00	0.05	
Il6st	F09	1.29	0.05		1.37	0.03	0.0000		0.94	0.27		1.44	0.04	0.0001		0.89	0.04		1.43	0.05	0.001		0.90	0.04	
Il8rb	F10	1.49	0.18	0.02	1.34	0.10	0.03		1.11	0.32		1.44	0.05	0.0005		1.03	0.12		1.38	0.17			1.08	0.13	
Itgam	F11	1.15	0.08		1.56	0.14		0.02	0.74	0.21	0.0004	1.11	0.04			1.04	0.07		0.98	0.12			1.17	0.08	
Itgb2	F12	1.18	0.07		1.84	0.17	0.01	0.001	0.64	0.18	0.0000	1.32	0.08			0.90	0.06		1.36	0.09	0.02		0.87	0.05	
Lta	G01	0.72	0.07	0.003	1.13	0.11		0.007	0.65	0.19	0.0002	0.98	0.11			0.74	0.07	0.004	1.14	0.14		0.04	0.64	0.06	0.0001
Ltb	G02	1.30	0.03	0.0000	1.95	0.10	0.0003	0.0000	0.67	0.19	0.0000	1.40	0.06	0.001		0.93	0.02		2.32	0.16	0.0006	0.002	0.56	0.01	0.0000
Mif	G03	1.00	0.02		1.02	0.04			0.98	0.28		1.03	0.03			0.97	0.02		1.27	0.08		0.03	0.79	0.02	0.0001
Scye1	G04	1.23	0.13		0.91	0.04			1.34	0.39		1.01	0.03			1.22	0.13		1.08	0.03			1.13	0.12	
Spp1	G05	1.67	0.24	0.02	1.42	0.08	0.006		1.17	0.34		1.36	0.06	0.004		1.23	0.18		0.97	0.16		0.04	1.72	0.25	
Tgfb1	G06	1.09	0.08		1.33	0.09			0.83	0.24		1.25	0.07			0.87	0.06		1.27	0.08			0.86	0.06	
Tnf	G07	4.73	0.28	0.0000	6.96	0.41	0.0000	0.005	0.78	0.23		6.39	0.21	0.0000	0.002	0.73	0.12	0.01	6.44	0.36	0.0000	0.005	0.83	0.12	
Tnfrsf1a	G08	0.92	0.04		0.99	0.02			0.93	0.27		1.02	0.03			0.90	0.04		1.07	0.06			0.86	0.04	
Tnfrsf1b	G09	1.24	0.05		1.67	0.13	0.005	0.004	0.74	0.21	0.0000	1.23	0.07			1.01	0.04		1.41	0.09	0.01		0.88	0.04	
Cd40lg	G10	1.28	0.28		0.90	0.13			1.42	0.41		1.79	0.23	0.03		0.72	0.15		1.85	0.26	0.03		0.69	0.15	
Tollip	G11	0.84	0.04		0.88	0.02			0.95	0.27		1.07	0.05		0.006	0.79	0.04	0.0003	0.93	0.04			0.91	0.04	
Xcr1	G12	1.20	0.15		1.25	0.20			0.96	0.28		2.67	0.29	0.003	0.0003	0.45	0.06	0.0000	1.11	0.11			1.08	0.14	
Gusb	H01	1.03	0.04		1.15	0.05			0.90	0.26		0.98	0.03			1.04	0.04		0.93	0.04			1.10	0.04	
Hprt1	H02	0.98	0.07		0.94	0.05			1.04	0.30		0.98	0.04			1.00	0.07		0.87	0.05			1.13	0.08	
Hspcb	H03	0.98	0.03		0.90	0.04			1.09	0.31		0.97	0.02			1.01	0.03		1.10	0.07			0.89	0.02	
Gapdh	H04	1.17	0.03		0.92	0.03			1.27	0.37		0.98	0.01			1.19	0.03		0.97	0.20			1.21	0.03	
Actb	H05	0.91	0.03		1.15	0.05			0.79	0.23		1.10	0.06			0.82	0.13		1.20	0.16			0.78	0.09	

[a] Ratio of the amounts of mRNA in the feet of mice injected intravenously with 100 µg MDP to the amounts of mRNA in mice injected with buffer, determined by quantitative real time PCR 6 hrs after injection; means of 12 (WT) or 6 (KO) mice.

[b] Ratio of the fold induction of mRNA in the feet of the WT to KO mice injected intravenously with 100 µg MDP, determined by quantitative real time PCR 6 hrs after injection; means of 6 mice.

[c] Significance of difference in the fold increase in the amounts of mRNA in MDP-injected over buffer-injected mice of the same genotype, determined by one-sample t-test (mean fold ratio vs 1, N = 12 WT or 6 KO); blank = not significant (P>0.05).

[d] Significance of difference between KO and WT mice in the fold increase in the amounts of mRNA in MDP-injected to buffer-injected mice, determined by two-sample t-test (mean fold MDP/Buffer WT vs mean fold MDP/Buffer KO, N = 12 WT or 6 KO blank = not significant (P>0.05); if KO<WT P value marked in blue, if KO>WT P value marked in magenta.

[e] Significance of difference of the MDP-induced mRNA fold increase in WT/fold increase in KO mice determined by one-sample t-test (WT/KO ratio vs 1, N = 6); blank = not significant (P>0.05); if WT/KO>1 P value marked in blue, if WT/KO<1 P value marked in magenta.

Table S3. Changes in pro-inflammatory gene mRNA levels in the feet of WT, PGLYRP-1PGLYRP-2 double KO, and TLR4 KO mice, and in livers of WT and PGLYRP-2 KO mice injected intravenously with MDP

		Feet																	Liver									
Gene		WT/Buffer [a]			PGLYRP-1-2 KO/Buffer [a]				WT/PGLYRP-1-2 KO [b]			TLR4 KO/Buffer [a]				WT/TLR4 KO [b]			WT/Buffer [a]			PGLYRP-2 KO/Buffer [a]				WT/PGLYRP-2 KO [b]		
Symbol	Position	Mean	SE	P [c]	Mean	SE	P [c]	P [d]	Mean	SE	P [e]	Mean	SE	P [c]	P [d]	Mean	SE	P [e]	Mean	SE	P [c]	Mean	SE	P [c]	P [d]	Mean	SE	P [e]
Abcf1	A01	0.90	0.12		0.96	0.02			0.94	0.12		1.17	0.06			0.89	0.10		1.64	0.13		1.47	0.08			1.11	0.09	
Bcl6	A02	1.15	0.10		0.99	0.04			1.17	0.10		1.23	0.07			1.05	0.07		2.76	0.52	0.033	1.37	0.34			2.01	0.38	0.05
Blr1	A03	1.19	0.09		1.33	0.07			0.90	0.07		0.92	0.05			1.26	0.11		1.29	0.23		1.01	0.40			1.28	0.23	
C3	A04	1.51	0.10	0.0004	1.56	0.12	0.0001		0.97	0.06		1.25	0.10			1.27	0.07		1.14	0.03		1.17	0.09			0.97	0.02	
Casp1	A05	0.96	0.06		0.86	0.08			1.11	0.07		1.39	0.04	0.0001	0.0003	0.72	0.03	0.0002	0.77	0.03		1.04	0.14			0.74	0.03	0.001
Ccl1	A06	2.80	0.46	0.003	2.52	0.38	0.02		1.11	0.18		5.14	0.44	0.0003		0.68	0.07		1.62	0.33		1.31	0.27			1.23	0.25	
Ccl11	A07	1.06	0.06		1.03	0.05			1.03	0.06		1.53	0.06	0.0006	0.006	0.75	0.03	0.001	0.94	0.18		3.70	0.76	0.05	0.01	0.25	0.05	0.0000
Ccl12	A08	9.55	0.76	0.0000	5.00	0.82	0.0004		1.91	0.15	0.001	13.41	0.61	0.0000	0.006	0.66	0.05	0.002	20.61	2.52	0.0009	18.72	4.91	0.02		1.10	0.13	
Ccl17	A09	9.42	1.09	0.0000	7.24	1.33	0.008		1.30	0.15		6.76	1.56	0.02		1.39	0.12	0.04	4.95	0.38	0.0002	7.31	0.86	0.001	0.04	0.68	0.05	0.0000
Ccl19	A10	2.88	0.30	0.0000	3.20	0.47	0.008		0.90	0.09		4.45	0.17	0.0000		0.71	0.06		1.88	0.18	0.006	1.61	0.31			1.16	0.11	
Ccl2	A11	19.63	1.45	0.0000	7.73	1.19	0.005	0.0001	2.54	0.19	0.0000	19.11	1.95	0.0005		0.96	0.07		10.64	1.85	0.005	8.72	2.29	0.03		1.22	0.21	
Ccl20	A12	13.71	2.96	0.002	8.59	1.23	0.003		1.60	0.34		12.30	0.71	0.0000		1.48	0.20		1.26	0.18		1.88	0.45			0.67	0.10	0.03
Ccl22	B01	2.45	0.15	0.0000	1.98	0.10	0.0003		1.24	0.08		4.53	0.33	0.0003	0.0003	0.52	0.03	0.0000	1.37	0.13	0.05	3.21	0.76	0.05	0.05	0.43	0.04	0.0000
Ccl24	B02	1.13	0.10		1.48	0.10	0.007		0.77	0.07		1.70	0.12	0.003		0.74	0.05	0.03	1.04	0.06		1.33	0.13			0.78	0.05	
Ccl25	B03	1.03	0.08		1.14	0.09			0.90	0.07		1.13	0.03			1.01	0.05		0.91	0.07		0.75	0.03			1.22	0.09	
Ccl3	B04	3.36	0.47	0.0000	3.30	0.45	0.005		1.02	0.14		3.39	0.28	0.0005		1.20	0.11		2.19	0.45	0.05	4.37	0.36	0.04	0.05	0.50	0.13	0.05
Ccl4	B05	3.28	0.48	0.0000	2.96	0.60	0.03		1.11	0.16		3.41	0.31	0.0009		1.12	0.12		1.52	0.49		4.46	0.28	0.05	0.03	0.34	0.11	0.003
Ccl5	B06	2.97	0.34	0.0000	2.54	0.33	0.008		1.17	0.13		1.83	0.17	0.007		1.38	0.13		0.79	0.07		1.03	0.25			0.77	0.07	0.02
Ccl6	B07	1.32	0.08		1.50	0.06	0.0007		0.88	0.05		1.16	0.04			1.24	0.05		4.46	0.36	0.0003	4.23	0.34	0.0003		1.05	0.09	
Ccl7	B08	7.60	0.88	0.0000	6.41	1.19	0.009		1.19	0.14		9.29	1.14	0.001		0.75	0.08	0.004	29.65	4.40	0.002	22.43	4.81	0.010		1.32	0.20	
Ccl8	B09	1.17	0.18		1.52	0.19			0.77	0.12		1.31	0.09	0.03		1.12	0.09		0.69	0.09	0.02	3.67	0.49	0.05	0.04	0.19	0.02	0.0000
Ccl9	B10	2.32	0.18	0.0000	1.82	0.13		0.05	1.40	0.11		1.59	0.14	0.01	0.04	1.38	0.08	0.01	0.96	0.08		0.79	0.08			1.22	0.10	
Ccr1	B11	1.67	0.15	0.002	1.41	0.08			1.18	0.11		1.19	0.05			1.22	0.07		4.04	0.31	0.0003	4.19	1.41	0.09		0.96	0.07	
Ccr2	B12	1.07	0.10		0.97	0.06			1.11	0.10		0.75	0.04	0.002		1.25	0.09		0.84	0.09		1.25	0.24			0.67	0.07	0.04
Ccr3	C01	0.97	0.08		1.04	0.09			0.93	0.08		0.80	0.08			1.13	0.07		1.04	0.05		1.29	0.14			0.81	0.04	
Ccr4	C02	1.43	0.09	0.0007	1.02	0.08		0.01	1.41	0.09		1.31	0.07			1.04	0.06		1.39	0.28		0.68	0.14			2.04	0.39	0.04
Ccr5	C03	2.17	0.34	0.007	1.66	0.16	0.01		1.31	0.20		0.97	0.12		0.04	1.78	0.18	0.002	1.38	0.06		1.32	0.16			1.04	0.05	
Ccr6	C04	2.03	0.36	0.02	1.90	0.69			1.07	0.19		1.37	0.34			1.83	0.23	0.05	0.81	0.31		1.04	0.48			0.78	0.30	
Ccr7	C05	1.33	0.11	0.01	1.05	0.11			1.27	0.10		0.84	0.11		0.03	1.57	0.12	0.03	0.66	0.10	0.03	1.02	0.46			0.64	0.10	0.03
Ccr8	C06	1.67	0.19	0.006	1.87	0.23			0.89	0.10		1.11	0.16			1.32	0.16		1.44	0.23		1.13	0.34			1.27	0.20	
Ccr9	C07	0.99	0.05		1.08	0.05			0.92	0.05		1.26	0.07		0.01	0.84	0.04	0.05	0.70	0.03		0.54	0.04	0.0002	0.01	1.30	0.05	0.003
Crp	C08	13.96	3.64	0.006	8.44	1.84	0.02		1.65	0.43		40.10	7.98	0.007	0.006	0.35	0.07	0.0000	1.31	0.03		1.60	0.19	0.03		0.82	0.02	
Cx3cl1	C09	2.46	0.16	0.0000	2.39	0.07	0.0000		1.03	0.07		3.44	0.10	0.0000	0.003	0.72	0.04	0.008	1.34	0.06		1.60	0.35			0.84	0.03	
Cxcl1	C10	14.27	1.18	0.0000	9.23	2.47	0.03		1.55	0.13		24.80	2.39	0.0003	0.02	0.62	0.04	0.0008	3.71	0.70	0.02	7.47	1.39	0.02	0.05	0.50	0.09	0.005
Cxcl10	C11	9.25	1.87	0.0000	8.51	2.04	0.02		1.09	0.22		10.55	0.87	0.0002		0.76	0.10		0.88	0.14		1.15	0.31			0.77	0.12	
Cxcl11	C12	2.07	0.19	0.0002	2.43	0.30	0.007		0.85	0.08		2.24	0.13	0.0004		1.00	0.06		0.70	0.08	0.02	0.54	0.06			1.30	0.15	
Cxcl12	D01	1.22	0.10		1.74	0.05	0.0000	0.003	0.70	0.05		1.13	0.02			1.19	0.07		0.76	0.08		0.64	0.07	0.005		1.20	0.12	
Cxcl13	D02	3.05	0.24	0.0000	2.63	0.40	0.01		1.16	0.09		4.00	0.28	0.0002		0.77	0.06		3.12	0.71	0.05	23.00	7.10	0.05	0.05	0.14	0.04	0.0000
Cxcl15	D03	2.35	0.45	0.02	2.11	0.50			1.12	0.22		1.96	0.42			1.31	0.22		4.31	0.59	0.004	1.06	0.19		0.004	4.08	0.56	0.004
Cxcl4	D04	1.33	0.04	0.0000	1.29	0.04			1.03	0.03		1.03	0.04		0.03	1.26	0.07		2.40	0.09	0.0000	2.19	0.40	0.05		1.09	0.04	
Cxcl5	D05	2.75	0.20	0.0000	2.43	0.28	0.006		1.13	0.08		4.29	1.05	0.040		0.66	0.05	0.007	0.29	0.04	0.0000	4.05	1.28	0.04	0.004	0.07	0.01	0.0000
Cxcl9	D06	2.93	0.32	0.0000	2.61	0.38	0.01		1.12	0.12		2.73	0.14	0.0001		1.07	0.08		0.58	0.03	0.0000	0.53	0.04	0.0000		1.10	0.06	
Cxcr3	D07	0.85	0.07		0.59	0.08	0.005	0.05	1.46	0.12		0.64	0.13	0.05		1.26	0.13		0.56	0.04	0.0002	0.54	0.03	0.0000		1.02	0.08	
Gpr2	D08	1.03	0.07		1.40	0.09	0.01	0.009	0.73	0.05	0.02	0.94	0.06			1.02	0.08		0.77	0.08	0.05	0.79	0.12			0.97	0.10	
Ifng	D09	1.31	0.16		1.89	0.47			0.69	0.08	0.005	1.23	0.09			1.11	0.13		0.70	0.06	0.005	0.49	0.09	0.004		1.44	0.12	0.02
Il10	D10	2.42	0.23	0.0001	1.92	0.14	0.003		1.26	0.12		3.46	0.27	0.00	0.02	0.67	0.05	0.001	0.66	0.10	0.02	2.32	1.24			0.29	0.04	0.0000
Il10ra	D11	1.21	0.08		1.15	0.06			1.05	0.07		1.19	0.05			1.05	0.06		0.80	0.04		0.82	0.06			0.98	0.04	
Il10rb	D12	1.09	0.05		1.39	0.06	0.002	0.005	0.79	0.04	0.001	1.12	0.03			1.01	0.04		0.96	0.06		0.88	0.04			1.09	0.07	
Il11	E01	0.69	0.06		1.05	0.07		0.003	0.66	0.06	0.0001	1.02	0.06		0.015	0.71	0.05	0.01	2.27	0.39	0.05	1.79	0.25	0.03		1.26	0.27	
Il13	E02	1.56	0.20	0.02	2.08	0.31	0.02		0.75	0.10		1.22	0.09			1.54	0.11	0.02	1.05	0.18		0.94	0.16			1.12	0.19	
Il13ra1	E03	1.19	0.10		1.10	0.06			1.08	0.09		1.41	0.08	0.004		0.88	0.04		1.52	0.10	0.006	1.01	0.17			1.51	0.10	0.007
Il15	E04	1.80	0.14	0.0002	1.64	0.27			1.10	0.09		2.34	0.06	0.0001		0.81	0.06		0.77	0.07		0.72	0.08	0.03		1.07	0.10	
Il16	E05	0.93	0.05		0.81	0.02			1.15	0.06		0.96	0.05			0.99	0.03		0.55	0.02	0.0000	0.49	0.07	0.002		1.13	0.04	
Il17b	E06	1.05	0.11		1.13	0.06			0.93	0.09		1.08	0.05			1.13	0.07		1.03	0.12		0.96	0.10			1.08	0.12	
Il18	E07	0.82	0.06		0.85	0.06			0.96	0.07		1.23	0.06		0.007	0.72	0.04	0.002	0.61	0.04	0.0002	0.35	0.04	0.0000	0.001	1.73	0.10	0.001
Il1a	E08	1.35	0.13	0.03	1.46	0.10			0.93	0.09		2.00	0.18	0.004		0.77	0.05	0.03	1.67	0.07	0.0004	1.51	0.17			1.11	0.05	
Il1b	E09	4.42	0.53	0.0000	3.07	0.48	0.01		1.44	0.17	0.03	5.32	0.67			0.93	0.08		1.75	0.24	0.04	2.62	0.60	0.05		0.67	0.09	0.02
Il1f6	E10	1.14	0.07		1.17	0.06			0.97	0.06		1.39	0.04	0.0002	0.04	0.85	0.04		5.68	1.21	0.02	2.73	0.42	0.01		2.08	0.41	0.04
Il1f8	E11	0.90	0.05		0.82	0.03			1.10	0.06		1.32	0.05		0.001	0.72	0.03	0.001	0.85	0.05		1.19	0.17			0.71	0.04	0.001
Il1r1	E12	1.19	0.07		1.09	0.05			1.09	0.06		1.38	0.10			0.87	0.09		1.99	0.21	0.01	10.90	3.15	0.04	0.03	0.18	0.02	0.0000
Il1r2	F01	1.15	0.09		1.21	0.02			0.96	0.08		1.35	0.07			0.96	0.04		4.84	0.40	0.0003	12.10	2.15	0.02	0.04	0.40	0.03	0.0000
Il20	F02	3.28	0.76	0.01	3.03	0.66	0.04		1.09	0.25		3.46	1.08			1.25	0.20		1.23	0.11		0.96	0.13			1.28	0.11	
Il2rb	F03	1.22	0.09		1.17	0.09			1.04	0.08		1.42	0.11	0.02		0.84	0.06		0.68	0.07	0.009	0.64	0.06	0.002		1.07	0.11	
Il2rg	F04	2.20	0.10	0.0000	2.08	0.25	0.01		1.06	0.05		1.92	0.20	0.008		1.12	0.05		1.43	0.07	0.002	1.15	0.12			1.24	0.06	
Il3	F05	2.11	0.48	0.05	2.01	0.47			1.05	0.24		4.05	0.75	0.01		0.55	0.14		2.73	0.54	0.03	1.69	0.34	0.05		1.83	0.25	0.03
Il4	F06	1.33	0.21		1.36	0.16			0.98	0.16		1.39	0.59			1.14	0.16		1.00	0.12		0.68	0.08			1.47	0.18	
Il5ra	F07	1.07	0.09		1.54	0.38			0.69	0.06	0.0003	0.92	0.12			1.18	0.10		0.96	0.03		0.80	0.24			1.20	0.04	
Il6ra	F08	1.43	0.07	0.0002	1.00	0.04		0.002	1.43	0.07	0.0002	1.47	0.07	0.001		1.03	0.04		1.02	0.11		1.22	0.09			0.83	0.09	
Il6st	F09	1.29	0.05		1.23	0.05			1.04	0.04		1.32	0.07			0.99	0.03		1.32	0.05		1.51	0.14			0.88	0.04	
Il8rb	F10	1.49	0.18	0.02	0.74	0.03		0.01	2.00	0.24	0.002	1.36	0.06	0.002		0.89	0.08		5.13	0.56	0.001	5.03	1.25	0.03		1.02	0.11	
Itgam	F11	1.15	0.08		0.74	0.03		0.003	1.56	0.11	0.0003	0.92	0.06			1.14	0.05		4.74	0.31	0.0001	5.21	1.21	0.02		0.91	0.06	
Itgb2	F12	1.18	0.07		0.88	0.06		0.02	1.35	0.09	0.002	0.70	0.06	0.006	0.003	1.62	0.09	0.005	0.97	0.08		1.18	0.15			0.82	0.07	
Lta	G01	0.72	0.07	0.003	0.69	0.12			1.03	0.10		1.73	0.11	0.002	0.0001	0.46	0.05	0.001	0.65	0.08	0.01	1.40	0.84		0.05	0.47	0.06	0.0004
Ltb	G02	1.30	0.03	0.0000	1.98	0.10	0.0003	0.0000	0.66	0.02	0.0000	1.48	0.07	0.001		0.88	0.02		0.49	0.04	0.0000	0.72	0.17			0.68	0.05	0.003
Mif	G03	1.00	0.02		0.86	0.03		0.003	1.17	0.03		0.96	0.02			1.06	0.03		0.80	0.06		0.83	0.06			0.97	0.07	
Scye1	G04	1.23	0.13		1.41	0.06			0.87	0.09		1.18	0.03			1.20	0.09		1.37	0.06	0.002	1.05	0.07			1.30	0.06	
Spp1	G05	1.67	0.24	0.02	1.10	0.12			1.52	0.22	0.04	1.18	0.02			1.10	0.12		0.76	0.05	0.008	0.70	0.08	0.02		1.09	0.07	
Tgfb1	G06	1.09	0.08		1.01	0.05			1.09	0.08		1.41	0.05	0.001	0.02	0.70	0.04	0.0003	1.27	0.03		1.16	0.10			1.09	0.02	
Tnf	G07	4.73	0.28	0.0000	3.79	0.53	0.005		1.28	0.10	0.05	5.24	0.38	0.0002		1.04	0.12		1.40	0.12	0.030	3.30	0.71	0.02	0.04	0.42	0.04	0.0000
Tnfrsf1a	G08	0.92	0.04		0.73	0.10			1.27	0.06		1.14	0.05		0.02	0.78	0.10		1.23	0.07		1.49	0.17			0.82	0.05	
Tnfrsf1b	G09	1.24	0.05		1.42	0.07			0.87	0.04		1.14	0.07			1.10	0.05		1.26	0.09		1.20	0.08			1.05	0.08	
Cd40lg	G10	1.28	0.28		1.15	0.28			1.12	0.24		1.21	0.17			1.29	0.23		0.51	0.08	0.002	0.43	0.08	0.002		1.17	0.18	
Tollip	G11	0.84	0.04		0.50	0.03	0.0000	0.0001	1.68	0.08	0.0003	1.02	0.03		0.02	0.78	0.03	0.02	1.19	0.06		1.06	0.09			1.13	0.06	
Xcr1	G12	1.20	0.15		1.18	0.21			1.02	0.13		1.04	0.11			1.22	0.13		0.43	0.05	0.0001	0.62	0.07	0.006		0.69	0.08	0.01
Gusb	H01	1.03	0.04		0.96	0.03			1.07	0.04		0.88	0.09			1.13	0.04		1.15	0.08		0.91	0.05			1.26	0.09	
Hprt1	H02	0.98	0.07		1.22	0.04			0.81	0.06		1.08	0.03			1.01	0.05		0.93	0.02		1.06	0.02			0.88	0.01	
Hspcb	H03	0.98	0.03		0.85	0.02			1.16	0.03		1.14	0.04			0.82	0.09		0.82	0.04		1.27	0.12			0.64	0.03	
Gapdh	H04	1.17	0.03		1.14	0.03			1.03	0.03		1.06	0.03			1.06	0.03		1.12	0.02		0.98	0.03			1.14	0.02	
Actb	H05	0.91	0.03		0.90	0.03			1.01	0.03		0.89	0.07			1.01	0.03		1.05	0.03		0.88	0.07			1.19	0.03	

[a] Ratio of the amounts of mRNA in the feet of mice injected intravenously with 100 µg MDP to the amounts of mRNA in mice injected with buffer, determined by quantitative real time PCR 6 hrs after injection; means of 12 (WT) or 6 (KO) mice.

[b] Ratio of the fold induction of mRNA in the feet of the WT to KO mice injected intravenously with 100 µg MDP, determined by quantitative real time PCR 6 hrs after injection; means of 6 mice.

[c] Significance of difference in the fold increase in the amounts of mRNA in MDP-injected over buffer-injected mice of the same genotype, determined by one-sample t-test (mean fold ratio vs 1, N = 12 WT or 6 KO); blank = not significant (P>0.05).

[d] Significance of difference between KO and WT mice in the fold increase in the amounts of mRNA in MDP-injected to buffer-injected mice, determined by two-sample t-test (mean fold MDP/Buffer WT vs mean fold MDP/Buffer KO, N = 12 WT or 6 KO); blank = not significant (P>0.05); if KO<WT P value marked in blue, if KO>WT P value marked in magenta.

[e] Significance of difference of the MDP-induced mRNA fold increase in WT/fold increase in KO mice determined by one-sample t-test (WT/KO ratio vs 1, N = 6); blank = not significant (P>0.05); if WT/KO>1 P value marked in blue, if WT/KO<1 P value marked in magenta.

Table S4. Baseline levels of pro-inflammatory gene mRNA in the feet and livers of untreated mice

		Feet																						Liver		
Gene		WT/PGLYRP-2 KO [a]			WT/PGLYRP-1 KO [a]			WT/PGLYRP-3 KO [a]			WT/PGLYRP-4 KO [a]			WT/PGLYRP-1-2 KO [a]			WT/Nod2 KO [a]			WT/TLR4 KO [a]			WT/PGLYRP-2 KO [a]		
Symbol	Position	Mean	SE	P [b]	Mean	SE	P [b]	Mean	SE	P [b]	Mean	SE	P [b]	Mean	SE	P [b]	Mean	SE	P [b]	Mean	SE	P [b]	Mean	SE	P [b]
Abcf1	A01	1.54	0.14	0.02	0.87	0.36		0.77	0.03	0.001	0.83	0.10		0.73	0.05	0.004	1.62	0.24	0.001	0.79	0.04	0.0001	0.84	0.09	
Bcl6	A02	1.43	0.11	0.02	0.97	0.40		1.40	0.10	0.01	1.22	0.06		0.81	0.03	0.001	1.13	0.14		1.02	0.04		0.92	0.32	
Blr1	A03	0.89	0.07		1.08	0.44		1.67	0.21	0.02	1.52	0.27		1.46	0.04	0.0001	1.17	0.12		0.65	0.05	0.0009	0.67	0.10	0.02
C3	A04	1.05	0.06		0.86	0.35		1.05	0.08		1.14	0.08		0.94	0.03		1.05	0.14		0.52	0.03	0.0000	0.95	0.07	
Casp1	A05	0.89	0.02		0.81	0.33		1.04	0.05		0.90	0.09		0.98	0.02		0.92	0.08		1.17	0.06		1.21	0.09	
Ccl1	A06	2.33	0.38	0.02	0.79	0.32		1.48	0.20		1.56	0.46		0.60	0.05	0.0004	0.73	0.11	0.03	1.01	0.14		1.05	0.23	
Ccl11	A07	0.89	0.04		0.90	0.37		1.15	0.10		1.14	0.08		0.92	0.03		0.74	0.12	0.03	0.90	0.07		0.75	0.14	
Ccl12	A08	0.50	0.02	0.0001	0.67	0.27	0.0005	1.29	0.06		0.87	0.08		1.26	0.10		0.85	0.16		1.02	0.17		1.48	0.30	
Ccl17	A09	2.13	0.41	0.05	0.48	0.20	0.0001	0.92	0.09		0.53	0.11	0.006	1.29	0.32		0.65	0.07	0.0007	0.42	0.11	0.004	1.38	0.15	
Ccl19	A10	1.65	0.22	0.04	1.05	0.43		1.29	0.09		1.55	0.25		1.47	0.13	0.02	1.22	0.11		0.91	0.11		0.92	0.22	
Ccl2	A11	0.76	0.06	0.01	0.67	0.27		1.21	0.06		0.75	0.11		1.17	0.13		0.89	0.13		0.72	0.10	0.04	0.96	0.23	
Ccl20	A12	1.66	0.15	0.01	0.48	0.20	0.0001	0.58	0.07	0.002	0.53	0.06	0.0005	0.65	0.04	0.0002	1.05	0.21		0.74	0.07	0.02	1.62	0.25	
Ccl22	B01	1.50	0.12	0.02	1.01	0.41		1.65	0.13		1.29	0.06	0.004	0.89	0.06		1.03	0.10		1.18	0.09		1.56	0.17	
Ccl24	B02	0.75	0.04	0.0007	0.93	0.38		1.15	0.08		1.15	0.12		0.89	0.03		0.78	0.10	0.03	0.82	0.08		1.46	0.13	0.02
Ccl25	B03	1.12	0.05		0.88	0.36		0.90	0.06		0.80	0.07	0.03	1.08	0.05		0.98	0.03		1.16	0.05		0.77	0.06	0.01
Ccl3	B04	1.72	0.12	0.003	0.86	0.35		0.95	0.10		0.83	0.13		1.44	0.08		0.82	0.07		0.64	0.05	0.001	1.35	0.27	
Ccl4	B05	2.01	0.18	0.004	1.04	0.42		0.77	0.09	0.05	1.03	0.13		1.65	0.10	0.002	0.78	0.07	0.009	0.67	0.07	0.0004	1.71	0.40	
Ccl5	B06	0.70	0.02	0.0001	1.01	0.41		0.82	0.07		1.47	0.62		1.29	0.10		1.08	0.12		0.63	0.03	0.0000	0.87	0.05	
Ccl6	B07	0.93	0.08		0.60	0.24	0.0005	0.71	0.04	0.0007	0.75	0.09	0.03	1.11	0.06		0.65	0.05	0.0001	0.86	0.09		1.26	0.15	
Ccl7	B08	0.59	0.05	0.0008	0.68	0.28	0.004	1.03	0.07		0.92	0.16		1.32	0.12	0.04	1.09	0.14		0.81	0.13		1.68	0.70	
Ccl8	B09	1.33	0.12	0.05	0.61	0.13	0.0002	0.74	0.03	0.0006	0.80	0.07		0.88	0.04		0.82	0.13		0.81	0.05	0.02	2.29	0.36	0.02
Ccl9	B10	0.75	0.05	0.005	0.74	0.30	0.0003	0.84	0.09		0.98	0.08		0.88	0.04		0.64	0.10	0.04	0.50	0.02	0.0000	0.65	0.04	0.0002
Ccr1	B11	0.81	0.06	0.04	0.67	0.27	0.0002	0.96	0.09		0.98	0.09		1.21	0.09		0.90	0.12		0.63	0.04	0.0003	0.69	0.12	0.05
Ccr2	B12	0.60	0.02	0.0002	0.91	0.37		1.12	0.07		1.31	0.07	0.007	1.32	0.09	0.01	1.04	0.09		0.58	0.03	0.0000	1.02	0.14	
Ccr3	C01	0.82	0.12		0.87	0.36		0.94	0.16		1.06	0.09		1.00	0.10		1.02	0.12		0.39	0.01	0.0000	1.33	0.11	
Ccr4	C02	0.69	0.06	0.004	0.85	0.35		1.07	0.09		1.24	0.11		1.08	0.05		1.23	0.13		0.97	0.02		0.75	0.13	
Ccr5	C03	0.61	0.07	0.003	0.65	0.27	0.0007	0.98	0.10		1.17	0.11		1.20	0.10		1.06	0.16		0.37	0.02	0.0000	0.79	0.06	
Ccr6	C04	1.00	0.17		0.54	0.22		1.98	0.63		2.86	0.49	0.01	3.72	0.43	0.002	0.96	0.38		3.18	0.26	0.0004	2.23	0.43	0.03
Ccr7	C05	1.08	0.12		1.70	0.69	0.04	1.94	0.73		1.64	0.46		0.85	0.10		0.83	0.16		0.45	0.05	0.0000	1.08	0.05	
Ccr8	C06	0.89	0.06		0.72	0.29	0.02	1.20	0.24		1.81	0.67		1.18	0.16		0.97	0.11		0.69	0.08	0.01	1.06	0.29	
Ccr9	C07	1.10	0.10		0.80	0.33		1.05	0.07		0.97	0.06		0.98	0.02		1.03	0.09		1.21	0.09		1.06	0.08	
Crp	C08	1.43	0.23		0.70	0.29		0.79	0.21		0.96	0.18		1.85	0.67		0.41	0.08	0.0000	1.75	0.56		1.26	0.08	
Cx3cl1	C09	1.11	0.04		1.12	0.46		0.94	0.03		0.94	0.03		0.69	0.03	0.0001	0.89	0.10		1.04	0.03		1.07	0.06	
Cxcl1	C10	1.13	0.20		1.82	0.74		1.78	0.17	0.006	1.12	0.18		1.25	0.04		1.83	0.27	0.03	1.66	0.17	0.01	0.46	0.09	0.002
Cxcl10	C11	0.97	0.08		1.27	0.52		1.64	0.18	0.02	1.51	0.11	0.005	1.03	0.11		0.84	0.14		1.54	0.11	0.005	1.22	0.21	
Cxcl11	C12	1.04	0.11		0.75	0.31	0.007	1.12	0.11		1.06	0.07		1.09	0.05		0.99	0.24		0.81	0.05	0.01	0.80	0.08	
Cxcl12	D01	1.06	0.08		0.90	0.37		0.90	0.03		1.06	0.05		1.11	0.03		1.07	0.04		0.78	0.02	0.02	0.94	0.08	
Cxcl13	D02	1.22	0.19		0.63	0.26	0.0006	0.91	0.16		0.68	0.11	0.03	0.93	0.10		0.89	0.13		1.30	0.15		1.54	0.17	0.02
Cxcl15	D03	1.41	0.22		0.62	0.25	0.001	1.73	0.36		2.58	0.42	0.01	3.79	0.91	0.03	0.73	0.25		3.47	0.49	0.004	0.63	0.22	
Cxcl4	D04	0.91	0.05		1.03	0.42		1.13	0.07		1.08	0.12		1.11	0.02		0.91	0.06		0.55	0.01	0.0000	0.84	0.08	
Cxcl5	D05	1.23	0.08		0.86	0.35		0.80	0.05		0.66	0.09	0.02	1.11	0.09		0.91	0.07		0.90	0.07		7.96	1.09	0.001
Cxcl9	D06	0.86	0.08		1.05	0.43		1.16	0.11		1.35	0.15		1.57	0.11	0.004	0.78	0.11		0.85	0.10		1.17	0.10	
Cxcr3	D07	0.90	0.06		1.24	0.51		1.99	0.82		1.53	0.15	0.02	0.69	0.08		0.78	0.17		0.83	0.16		1.10	0.10	
Gpr2	D08	0.81	0.04	0.006	0.89	0.36		0.87	0.09		1.09	0.11		1.00	0.04		0.99	0.12		0.66	0.04	0.0004	0.94	0.07	
Ifng	D09	0.95	0.12		1.49	0.61		0.88	0.16		0.85	0.10		1.34	0.18		0.92	0.15		0.74	0.10		1.07	0.19	
Il10	D10	0.85	0.07		0.86	0.35		1.99	1.11		1.96	0.45		1.14	0.08		0.90	0.16		1.83	0.37		1.24	0.17	
Il10ra	D11	0.95	0.09		0.97	0.40		1.34	0.07		1.14	0.04		0.98	0.05		1.13	0.10		0.76	0.02	0.002	0.88	0.04	
Il10rb	D12	0.94	0.05		0.89	0.36		1.25	0.05		1.14	0.04		1.07	0.04		1.17	0.07		0.82	0.02		0.85	0.10	
Il11	E01	1.44	0.08	0.005	1.69	0.69		1.30	0.16		1.03	0.07		0.93	0.03		1.09	0.07		0.96	0.08		0.82	0.14	
Il13	E02	1.16	0.10		0.72	0.29	0.03	0.75	0.01	0.0001	0.92	0.17		1.72	0.14		1.36	0.16		0.94	0.05		0.85	0.20	
Il13ra1	E03	1.11	0.07		0.77	0.05	0.02	0.90	0.02		0.87	0.05		1.04	0.03		0.99	0.12		1.08	0.03		0.57	0.04	0.0002
Il15	E04	0.84	0.08		0.91	0.37		1.13	0.03		1.24	0.13		1.19	0.07		0.92	0.10		0.89	0.06		0.86	0.07	
Il16	E05	1.01	0.06		1.24	0.51		1.29	0.05		1.25	0.07		0.86	0.04		1.14	0.10		0.79	0.10		0.76	0.05	0.004
Il17b	E06	1.44	0.09		1.19	0.49		1.08	0.13		1.08	0.11		0.86	0.08		0.76	0.09		1.14	0.10		1.06	0.18	
Il18	E07	1.09	0.07		0.71	0.29	0.001	0.83	0.04		0.73	0.07	0.01	0.87	0.04		1.05	0.10		1.04	0.08		0.63	0.05	0.0006
Il1a	E08	1.66	0.12	0.004	0.69	0.05	0.001	0.96	0.04		0.86	0.07		1.12	0.08		1.25	0.18		1.18	0.04		0.83	0.11	
Il1b	E09	1.52	0.12	0.01	1.06	0.43		0.90	0.06		0.93	0.09		1.41	0.12	0.02	0.98	0.13		0.59	0.04	0.0000	0.80	0.04	
Il1f6	E10	0.87	0.04	0.02	0.62	0.25	0.0001	0.67	0.05		0.65	0.05	0.0007	0.75	0.04	0.002	0.91	0.07		0.90	0.01		0.97	0.41	
Il1f8	E11	1.31	0.08	0.02	0.70	0.05	0.001	0.99	0.07		0.96	0.04		0.91	0.04		0.87	0.10		1.30	0.06	0.004	1.18	0.10	
Il1r1	E12	1.32	0.05	0.002	0.88	0.36		1.09	0.03		0.96	0.05		1.00	0.03		1.46	0.12	0.01	0.89	0.03		1.78	0.31	0.05
Il1r2	F01	1.43	0.11	0.02	0.75	0.31	0.003	0.81	0.04		0.67	0.05	0.001	1.13	0.03		0.92	0.03		1.06	0.03		1.04	0.04	
Il20	F02	1.06	0.14		1.99	0.91		1.40	0.85		1.44	0.49		0.73	0.20		0.66	0.12	0.04	1.06	0.29		0.79	0.10	
Il2rb	F03	0.97	0.07		1.39	0.57		1.57	0.22	0.05	1.16	0.10		1.21	0.08		1.24	0.12		0.74	0.05	0.004	0.84	0.04	
Il2rg	F04	0.89	0.02		1.07	0.44		1.13	0.07		1.23	0.09		1.07	0.02		1.23	0.08		0.82	0.15		0.87	0.08	
Il3	F05	1.16	0.17		0.93	0.38		1.23	0.18		1.04	0.38		1.69	0.65		0.62	0.10	0.009	1.55	0.54		0.74	0.14	
Il4	F06	1.35	0.04	0.0008	1.45	0.59		0.99	0.11		1.11	0.32		2.07	0.36	0.03	0.47	0.05	0.0000	1.29	0.27		0.63	0.09	0.01
Il5ra	F07	1.06	0.15		1.38	0.56		1.39	0.33		1.86	0.46		3.11	0.60	0.03	0.63	0.05	0.0002	0.46	0.08	0.0008	0.53	0.60	0.0003
Il6ra	F08	1.13	0.07		0.95	0.39		1.04	0.07		1.09	0.06		0.74	0.01	0.0001	0.98	0.08		0.97	0.03		1.18	0.09	
Il6st	F09	0.90	0.05		0.99	0.40		1.12	0.05		1.03	0.05		0.86	0.03		1.17	0.11		0.84	0.04		0.94	0.09	
Il8rb	F10	0.76	0.07	0.03	1.10	0.45		1.12	0.10		1.13	0.06		0.95	0.07		0.94	0.05		0.68	0.03		0.51	0.04	0.0001
Itgam	F11	0.73	0.04	0.002	1.21	0.49		1.17	0.12		1.20	0.10		0.81	0.07		1.33	0.14		0.55	0.02	0.0000	0.88	0.08	
Itgb2	F12	0.73	0.06	0.008	1.33	0.54		1.43	0.14		1.30	0.06		0.68	0.07	0.007	0.96	0.06		0.41	0.01	0.0000	1.25	0.10	
Lta	G01	3.54	0.57	0.01	1.31	0.53		1.39	0.19		1.51	0.30		2.16	0.14	0.0004	1.43	0.18		2.19	0.37	0.02	1.45	0.35	
Ltb	G02	2.00	0.14	0.001	1.21	0.49		0.93	0.06		1.33	0.15		2.34	0.15	0.0003	1.09	0.11		0.65	0.04	0.0002	2.33	0.08	0.0000
Mif	G03	1.10	0.05		1.10	0.45		1.07	0.04		1.13	0.05		0.77	0.02	0.0001	0.87	0.05		1.05	0.03		1.06	0.06	
Scye1	G04	1.31	0.04	0.001	0.79	0.32	0.01	0.63	0.03		0.66	0.04	0.0006	0.98	0.06		0.98	0.05		0.89	0.02		0.84	0.03	
Spp1	G05	0.64	0.04	0.0003	1.47	0.60		1.77	0.11		1.23	0.12		1.95	0.13		1.05	0.13		1.03	0.05		1.02	0.12	
Tgfb1	G06	0.99	0.05		1.21	0.49		1.34	0.11		1.30	0.09		1.07	0.04		0.98	0.11		1.02	0.04		0.85	0.07	
Tnf	G07	1.06	0.08		0.90	0.37		1.06	0.02		0.91	0.08		0.93	0.05		0.74	0.08	0.01	0.74	0.06	0.009	1.73	0.32	
Tnfrsf1a	G08	1.06	0.04		1.09	0.44		1.27	0.06		1.15	0.05		0.87	0.02		1.31	0.11		1.24	0.04		0.83	0.07	
Tnfrsf1b	G09	1.01	0.04		0.99	0.40		1.05	0.08		1.00	0.07		0.87	0.05		1.04	0.08		0.62	0.01	0.0000	0.80	0.08	
Cd40lg	G10	1.76	0.42		0.48	0.20	0.001	0.77	0.12		0.84	0.28		0.49	0.07	0.0006	0.50	0.01	0.0001	1.33	0.76		0.90	0.14	
Tollip	G11	1.16	0.05		1.10	0.45		1.30	0.05		1.17	0.05		0.64	0.03	0.0001	1.33	0.15		1.29	0.08		0.80	0.04	
Xcr1	G12	1.12	0.24		1.16	0.47		2.21	0.20	0.002	1.17	0.18		1.59	0.24		1.04	0.18		0.65	0.05	0.001	1.57	0.14	0.01
Gusb	H01	0.85	0.06		1.00	0.41		1.03	0.02		1.09	0.05		0.87	0.04		1.10	0.08		0.72	0.11		0.96	0.01	
Hprt1	H02	1.25	0.03		0.80	0.33		0.78	0.02		0.81	0.04		1.25	0.04		0.92	0.04		0.99	0.03		1.03	0.04	
Hspcb	H03	1.00	0.03		1.09	0.44		1.04	0.05		1.00	0.05		1.00	0.04		1.00	0.05		1.57	0.12		1.54	0.05	
Gapdh	H04	0.94	0.03		0.93	0.38		0.88	0.03		0.88	0.04		1.10	0.01		0.93	0.04		1.02	0.01		0.85	0.03	
Actb	H05	1.04	0.03		1.28	0.52		1.32	0.12		1.28	0.11		0.85	0.02		1.11	0.08		0.88	0.08		0.78	0.02	

[a] Ratio of the amount of mRNA in the feet or livers of untreated WT to KO mice, determined by quantitative real time PCR; means of 6 mice.

[b] Significance of difference in the amounts of mRNA in untreated WT and KO mice, determined by one-sample t-test (mean ratio vs 1, N = 6); blank = not significant (P>0.05); if WT/KO>1 P value marked in blue, if WT/KO<1 P value marked in magenta.