Supporting Information

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SI Materials and Methods

RT-PCR. Cells were treated as described for individual experiments, and total RNA was isolated using TRIzol (Invitrogen) and treated with DNase (Promega) in the presence of 20 units of RNase Block (Invitrogen). Following DNase treatment, the RNA was phenol: chloroform extracted to remove the DNase. Two micrograms of RNA was used to make cDNA using SuperScript II (Invitrogen). One microliter of this cDNA reaction was then used to perform PCR, using Taq polymerase (Invitrogen).

Enzyme-Linked Immunosorbant Assays. ELISAs were performed as per the manufacturer's instructions. IL6 was from BD Biosciences, IL12 was from Peprotech, and TNFα was from Peprotech.

Intravital Measurements. The average rolling velocity was measured in each vessel by monitoring the average length of time required for 20 randomly selected leukocytes to roll a distance of 100 μm (μm/sec).

Internalization of FITC-FSL1/GML. Bone marrow-derived macrophages were isolated from wild-type and $TLR2^{-/-}$ mice and cultured on glass coverslips. The cells were incubated on ice for 10 min in the presence of 10 μg/mL FITC-FSL1 or FITC-GML to allow ligands binding and then returned to 37 °C for 60 min. Cells were then fixed for 10 min in 4.5% paraformaldehyde, stained for the golgi marker Giantin (1:250, Abcam), and imaged using confocal microscopy.

Neutrophil Whole Blood and Lymph Node Counts. Male LysM-eGFP mice, which express green fluorescent protein predominantly in neutrophils, were given an intrascrotal injection of 150 μL of saline (4.5 h) or an i.p. injection of 300 μL of saline (16 h). Whole blood and lymph nodes were isolated from these animals and the percent neutrophils per total cells was evaluated using flow cytometry for GFP^{hi} cells.

Cremaster Histology. Wild-type male C57/B6 mice were given an intrascrotal injection of 150 μL of saline or saline containing various proinflammatory ligands, or superfused with buffer containing proinflammatory ligands, as described in the results. Four hours following ligand administration the cremaster muscle was exteriorized, cut off, and fixed in 10% formalin. The cremaster tissue was embedded in paraffin and 5-μm sections were cut and stained with hematoxylin and eosin. The numbers of lymphocytes, monocytes, and neutrophils in the post capillary venules were counted, and differential percentages were scored.

In Vitro Bacterial Culture. In vitro, *E. coli* overnight cultures were diluted 1:20 in Luria Broth and distributed in 100-μL aliquots in a 96-well fluorescent plate, along with increasing amounts of LTA or GML in 5 μl of saline and grown at 37 °C. The OD_{600} and luminescence were monitored every 20 min using a Wallac Victor^2 1420, Multilabel Counter (Perkin-Elmer).

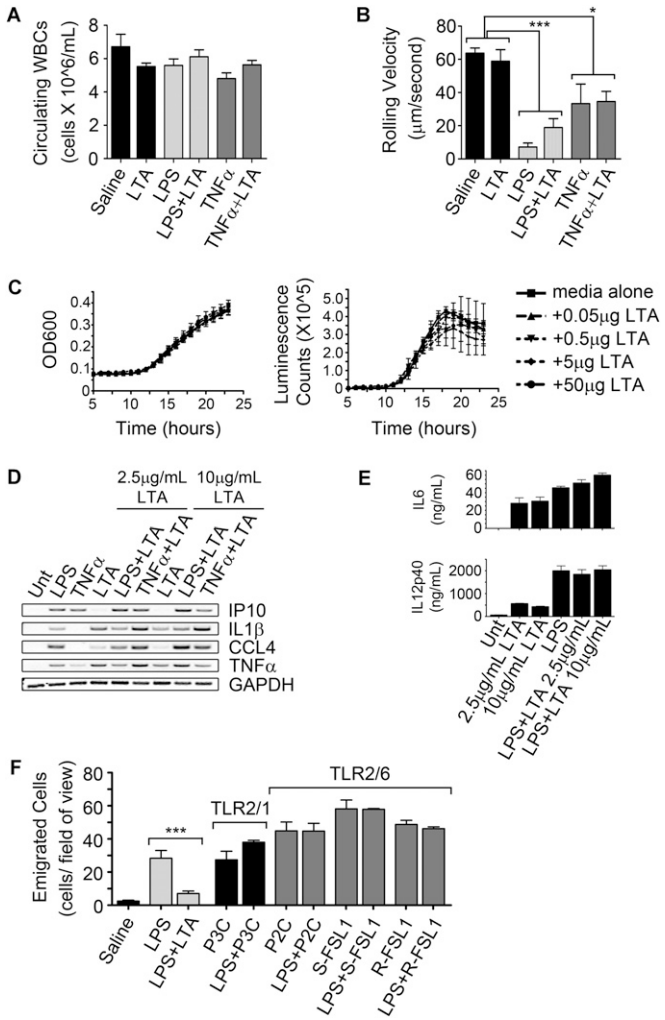

Fig. S1. The inhibitory capacity of LTA preparations evaluated in vivo and in vitro. (*A*) Wild-type mice were given intrascrotal injections of 150 μL of saline, or saline containing LTA (5 ng/g), LPS (10 ng/g), or TNFα (20 ng/g) in the indicated combinations. Four and a half hours after these injections, the total number of circulating leukocytes were determined from whole blood. (*B*) The velocity of the rolling cells within the cremaster tissue was determined via intravital microscopy 4.5 h following intrascrotal injections of 150 μL of saline containing LTA (5 ng/g), LPS (10 ng/g), or TNFα (20 ng/g) in the indicated combinations. (*C*) Luminescent *E. coli* were grown in culture in the presence of increasing amounts of LTA and monitored for any differences in growth kinetics or luminescence as a result of the presence of LTA. (*D* and *E*) Bone marrow-derived macrophages were treated with either LPS (100 ng/mL) or TNFα (20 ng/mL) in the presence or absence of LTA as indicated. (*D*) mRNA isolated from these cells was used in RT-PCR reactions to determine mRNA transcript amounts for IP10, IL1β, CCL4, TNFα, and as a loading control GAPDH. (*E*) Supernatants taken from these cells were used to detect secreted IL6 and IL12p40 by ELISA. (*F*) Intravital microscopy was used to evaluate emigrated neutrophils 4.5 h following an intrascrotal injection of 150 μL of saline or saline containing LPS (10 ng/g), or LPS plus LTA, Pam3CSK3, Pam2CSK4, S-FSL1 or R-FSL1 (each at 5 ng/g).

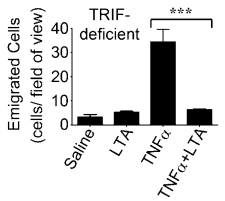

Fig. S2. Inhibitory effect of the LTA preparations is still evident in TRIF-deficient mice. TRIF-deficient mice were given an intrascrotal injection of 150 μL of saline containing LTA (5 ng/g), TNFα (20 ng/g), or both together and evaluated after 4.5 h for the number of emigrated cells in the cremaster tissue using intravital microscopy.

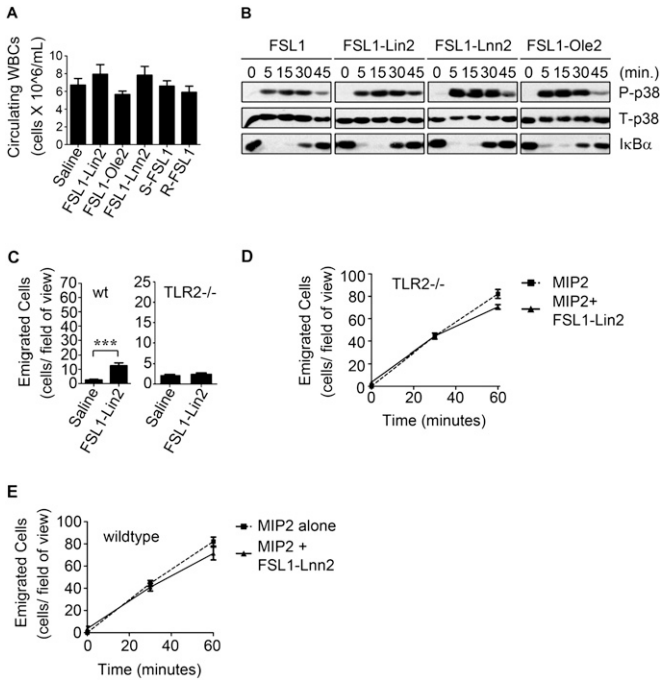

Fig. S3. Evaluating the in vitro and in vivo responses to the modified FSL1 ligands. (*A*) Wild-type mice were given intrascrotal injections of 150 μL of saline containing FSL1-Lin2, FSL1-Ole2, FSL1-Lnn2, R-FSL1, or S-FSL1 (all at 5 ng/g) as indicated, and 4.5 h later the number of circulating leukocytes was determined from whole blood. (*B*) Raw264.7 cells were treated with FSL1, FSL1-Lin2, FSL1-Lnn2, or FSL1-Ole2 (all at 100 ng/mL) for the indicated times and lysates were then subjected to Western blotting for IκBα and p38MAPK (P-phospho and T-total). (*C*) The number of emigrated cells was determined using intravital microscopy 4.5 h following intrascrotal injections containing FSL1-Lin2 (5 ng/g) into TLR2$^{-/-}$ mice compared with wild-type mice. (*D* and *E*) Intravital microscopy was used to determine the number of cells that had emigrated into the cremaster muscle during a 60-min exposure to MIP2 (2.5 μM) in TLR2$^{-/-}$ mice in the presence or absence of FSL1-Lin2 (200 ng/mL) (*D*) or in wild-type mice in the presence or absence of FSL1-Lnn2 (200 ng/mL) (*E*).

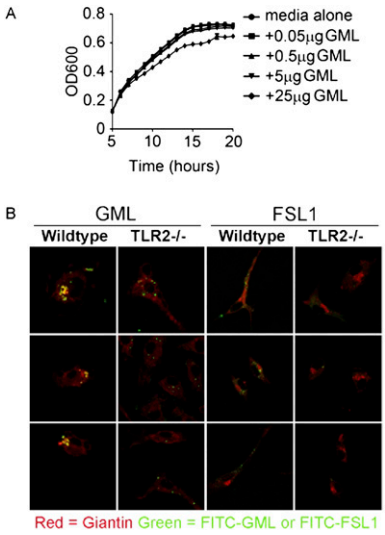

Fig. S4. Inhibitory characteristics of GML. (*A*) Luminescent *E. coli* were grown in culture in the presence of increasing amounts of GML and monitored for any differences in growth kinetics as a result of the presence of GML. (*B*) Wild-type or TLR2$^{-/-}$ bone marrow-derived macrophages were incubated for 60 min with either FITC-FSL1 (green) or FITC-GML (green) and fixed and stained for the golgi marker Giantin (red).

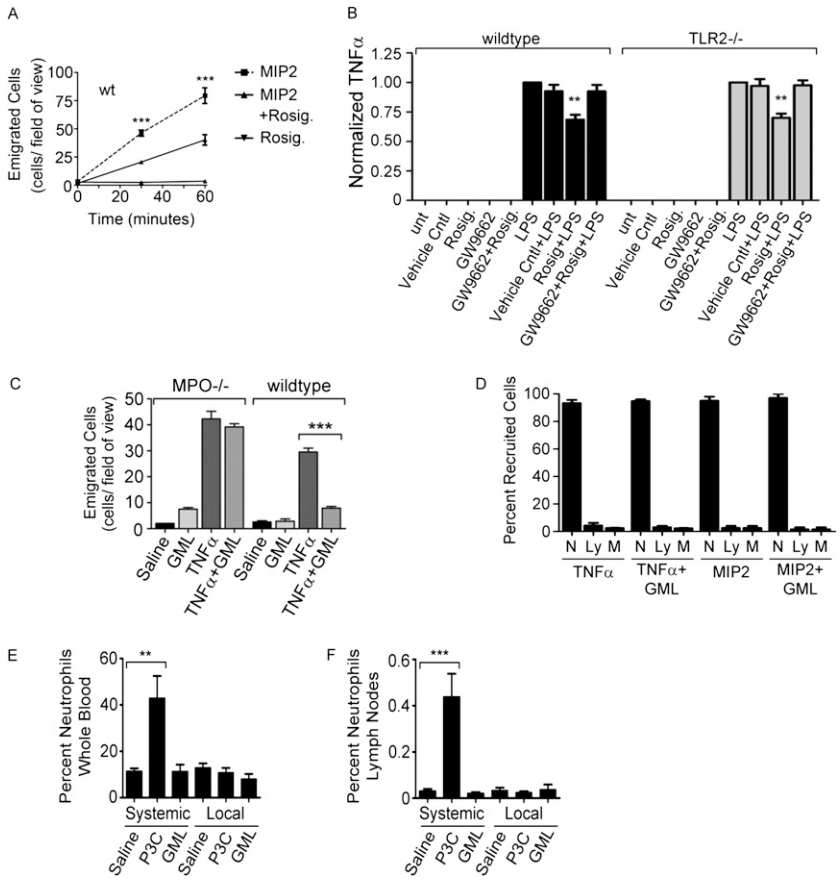

Fig. S5. (*A*) Wild-type mice were evaluated using intravital microscopy for the number of neutrophils emigrated into the cremaster tissue following superfusion of MIP2 (2.5 μM), MIP2 (2.5 μM) + Rosiglitazone (5 μM), or Rosiglitazone alone (5 μM). (*B*) Wild-type and TLR2$^{-/-}$ bone marrow-derived macrophages were treated as indicated and evaluated for the production of the cytokine TNFα in response to LPS (100 ng/mL), in the presence or absence of the PPARγ agonist Rosiglitazone (10 μM) and/or the PPARγ inhibitor GW9662 (10 μM). (*C*) MPO$^{-/-}$ mice evaluated using intravital microscopy for the number of neutrophils emigrated into the cremaster tissue following intrascrotal injections of 150 μL of saline containing GML (37.5 ng/g), TNFα (20 ng/g), or TNFα+GML. (*D*) Male mice were treated with an intrascrotal injection of TNFα (20 ng/g) in the presence or absence of GML (37.5 ng/g) for 4.5 h or the cremaster was exteriorized and treated with MIP2 (5 μM) in the presence or absence of GML (5 μg/mL) for 60 min. The cremasters were then excised and fixed and stained with hematoxylin and eosin and differential cell counts were determined. (*E* and *F*) Percentages of neutrophils (GFP^{hi} cells) were determined by flow cytometry from whole blood and isolated lymph nodes from LysM-eGFP mice following systemic administration of 300 μL of saline alone or saline containing Pam3CSK4 (100 μg) or GML (200 μg) for 16 h, or a local administration of 150 μL of saline alone or saline containing Pam3CSK4 (10 ng/g) or GML (37.5 ng/g) for 4.5 h.