Supporting Information

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SI Methods

Antibodies and Reagents. Anti-p65 (C22B4, 4764), anti–phospho-p65 at Ser536 (93H1, 3033), anti-IκBα (L35A5, 4814), anti–phospho-IκBα at Ser32 (14D4, 2859), anti-IKKα (2682), anti-IKKβ (L570, 2678), anti-MyD88 (D80F5, 4283), and anti-IRAK1 (D51G7, 4505) were purchased from Cell Signaling Technology. Anti-p65 (C-20) and anti-STAT1 (E-23) were obtained from Santa Cruz Biotechnology. Anti–β-actin (ab8224) was purchased from Abcam. Anti-STAT2 (07-140) and anti–phospho-Tyr689 STAT2 (07-224) were purchased from Upstate Biotechnology. Anti-mouse Toll-like receptor (TLR) 4 was purchased from Imgenex, and anti-mouse F4/80 antibody was purchased from eBiosciences. Anti–phospho-Y701-STAT1, Fc Block, anti-CD80, anti–Ly-6G Alexa Fluor-647, anti–Gr1-FITC, anti–CD11b-PE, anti–CD3-FITC, anti–CD19-PE, and isotype antibody controls were obtained from BD Pharmingen. Anti–alpha smooth muscle actin (aSMA)-Cy3 was purchased from Sigma-Aldrich. Anti-MRP was a gift from Nancy Kogg (Cancer Research, London). Anti-mouse ICAM-1 blocking antibody was bought from eBiosciences, and the IgG2b control was bought from Serotec. Donkey anti–rabbit-HRP antibody was purchased from Jackson ImmunoResearch Laboratories, and goat anti–mouse-HRP antibody was obtained from Dako. Recombinant murine IFN-α was purchased from PBL Laboratories, and murine IFN-β was provided by Biogen Idec. LPS (*Salmonella typhimurium*) TNF-α, RANTES, MCP1, and MIP-1α were purchased from Sigma-Aldrich.

Animal Physiology. Mice underwent echocardiography with temperature measured (rectal probe) under isoflurane (1.5%) just before and 14 h following i.p. injection. Two-dimensional images were recorded by using an echocardiograph (VIVID 7 dimension; GE Vingmed) with epicardial probe (model i13L; GE Vingmed). Ascending aortic flow velocity was determined with a probe positioned over the ascending aorta in Doppler wave mode. Beat-to-beat cycles of aortic blood flow velocity were recorded in three captured loops of five heart beat cycles. Peak aortic flow velocity and velocity time integral were determined, and stroke volume was calculated as product of velocity time integral and aortic root cross-sectional area. Immediately following the second echocardiogram, mice were euthanized, arterial blood was taken via intracardiac puncture and centrifuged (5,000 × *g* for 10 min), and plasma was stored at −80 °C for later analysis.

Immunohistochemical Analysis of Whole-Mount Cremaster. Following intravital microscopy, mice were euthanized, and cremaster muscles were then dissected away from the animals, pinned flat, and further fixed in 4% (wt/vol) PFA for 10 min. Fixed whole mounted tissues were blocked and permeabilized in PBS solution containing 10% normal goat serum, 10% FCS, 5% normal mouse serum, and 0.5% Triton X-100 for 3 h at room temperature. The tissues were then incubated with anti–αSMA-Cy3 and anti–MRP-14-488 in PBS solution containing 0.1% Triton X-100 and 0.01% sodium azide for 24 h. The samples were imaged using a Leica TCS-SP5 confocal microscope at 400 Hz by using sequential scanning of the 488-nm and 561-nm channels at a resolution of 512 × 1,024 pixels, corresponding to a voxel size of ∼0.25 × 0.25 × 0.3 μm in the *x*/*y*/*z* planes, respectively. *Z*-stacks were reconstructed into 3D images by using Imaris (Bitplane).

The level of ICAM-1 expression on venular endothelial cells was quantified by stimulating an inflammatory response with an intrascrotal injection of 300 ng LPS. Three hours later, 4 μg each of anti–PECAM-1 conjugated to Alexa Fluor-555 and anti–ICAM-1 conjugated to Alexa Fluor-488 were injected intrascrotally. After a period of 1 h labeling time, the cremasters were exteriorized and images of venules were captured by using the Leica TCS-SP5 confocal microscope as described earlier. The intensity of ICAM-1-488 labeling was quantified by using Imaris (Bitplane). An isosurface was built on the endothelial PECAM-1 labeling, and the mean intensity of ICAM-1-488 signal within this surface was recorded.

Measurement of Serum Inflammatory Mediators. Multiplex measurement of TNF-α, IL-12, IFN-γ, MCP-1, IL-10, and IL-6 was calculated by using a mouse inflammation CBA kit (BD Biosciences), and MIP1-α, TNF-α, and RANTES were measured by using a commercial ELISA kit (R&D Systems), according to the manufacturers' instructions. NO production was measured using highly sensitive nitrate/nitrite fluorimetric assay kit (Cayman) as instructed by the manufacturer. The experiments were done in triplicate wells from individual mice.

Bone Marrow Transfer. Bone marrow leukocytes were isolated from WT or Stat2$^{-/-}$ donor mice by flushing the femur and tibia bones with PBS solution. Cells were then sieved, counted, and resuspended in PBS solution containing BSA (0.25%), and incubated with 10 μM Calcein-AM or PKH-26 at 37 °C for 30 min as described in the Sigma kit protocol. After two washes, the cells of different genotypes and fluorescent labels were combined and were injected i.v. into recipient WT or *Stat2*$^{-/-}$ mice via the tail vein (8 × 10^6 cells per mouse) immediately before an i.p. injection of saline solution or LPS (1 mg/kg). Labeling of cells with fluorochromes did not affect the number of transmigrated cells. Mice were killed 18 h later and subjected to a peritoneal lavage with 7 mL PBS solution containing 10 mM EDTA. The percentage of endogenous neutrophils and numbers of fluorescently labeled transferred cells present in the lavage fluid were quantified as described later.

Flow Cytometry. Cells were resuspended in FACS buffer (1× PBS solution containing 0.2% sodium azide and 2% FCS). Fc receptors were blocked by incubation with FC block for 20 min at 4 °C. Cells were stained accordingly with antibodies against CD3, Gr1, Tlr4, CD80, and F4/80. Ten thousand cells were collected with a FACScan flow cytometer (Becton Dickinson) and analyzed with FC Express software.

In the bone marrow transfer experiments, the cellular composition of the lavage was evaluated by staining cells with anti–Ly-6G to identify neutrophils. Red blood cells were lysed with ACK lysis buffer (150 mM NH_4Cl, 1 mM $KHCO_3$, 0.1 mM EDTA), and the number of endogenous and transferred (Calcein-AM or PKH-26–labeled) neutrophils was measured by flow cytometry. Leukocytes were identified by forward and side scatter characteristics, dead cells were excluded by 7-aminoactinomycin D (BD Biosciences) staining, and neutrophils were identified based on Ly-6G positivity.

EMSA. dsDNA oligonucleotide probe (5′AGTTGAGGGGACTTTCCCAGGC) containing a consensus κB binding sequence for NF-κB homodimeric or heterodimeric complexes (underlined) was 5′ end-labeled with biotin (Sigma). Binding reactions were performed at room temperature with gentle agitation for 30 min in a reaction volume of 20 μL. Nuclear extracts (6.5 μg) were mixed with reaction buffer (10 mM Tris, pH 7.5, 50 mM KCl, 1 mM DTT, 10% glycerol, 5 mM $MgCl_2$, poly I:C, and Nonidet P-40) and labeled double-stranded oligonucleotides. Specificity

of binding was demonstrated by using 200× molar excess of identical unlabeled oligonucleotide as competitor. DNA–protein complexes were run on 6% (vol/vol) DNA polyacrylamide gels in 0.5× TBE buffer (Invitrogen) for at least 1 h at room temperature and transferred onto Biodyne B nylon membranes (Thermo Scientific). Detection of signal was performed by using the chemiluminescent nucleic acid detection module according to the manufacturer's instructions (Thermo Scientific).

Fluorescent Immunocytochemical Analyses. In other experiments, WT and *Stat2*$^{-/-}$ cells were seeded onto glass coverslips (Thermo Scientific) and incubated with or without LPS. Cells were fixed in 4% (wt/vol) paraformaldehyde for 15 min and permeabilized in PBS solution/0.25% Triton X-100 for 10 min. Slides were blocked for 30 min in PBS solution/1% BSA/0.05% Tween-20 and then incubated with primary antibodies (anti-p65; 1:200) for 1 h. Goat anti-rabbit IgG (heavy + light chains) DyLight 594 (BioLegend) was used at a 1:2,000 dilution for 1 h. After several washes with PBS solution, slides were counterstained with DAPI Vectashield mounting media (Vector Laboratories) and cells were examined by using a Nikon Eclipse TE2000-U inverted fluorescent microscope (Carl Zeiss). Images were viewed and overlaid by using MetaMorph software (Molecular Devices).

Generation of Immortalized Bone Marrow-Derived Macrophages, Cell Culture, and in Vitro Stimulation. Immortalized macrophage cell lines were established by infecting bone marrow cells from WT and *Stat2*$^{-/-}$ mice with the J2 recombinant retrovirus as described earlier (1). Bone marrow-derived macrophages (BMDMs) were established as described by others (2, 3). Briefly, single cell suspensions from bone marrow harvested from femurs were grown in DMEM supplemented with 10% (vol/vol) FBS, containing 5% (vol/vol) horse serum (Sigma), 2.2 g GlutaMax-1, 1 mM sodium pyruvate, 10 U/mL penicillin G sodium, 10 μg/mL streptomycin sulfate (Invitrogen), and 10 ng/mL murine macrophage colony stimulating factor (M-CSF, Peprotech) at 37 °C in a 5% CO_2 atmosphere for 5 d. Cells were switched to M-CSF–free complete medium for 24 h before they were used for experiments.

WT and *Stat2*$^{-/-}$ cells were seeded overnight in six-well plates (1×10^6/mL) in DMEM supplemented with 2% (vol/vol) FCS. Cells were stimulated for indicated amounts of time with defined concentrations of *Salmonella typhi* LPS (Sigma), murine IL-1 (Sigma), Poly I:C, imiquimod, flagellin (Autogen Bioclear), murine TNF-α (R&D Systems), or murine IFN-α (AbD Serotech). Blockade of type I IFN signaling was achieved by adding neutralizing IFNAR1 antibody (MAR1-5A3, 0.4 μg/mL; eBioscience) to the culture for 1 h before stimulation with other agonists when required.

Cells were scraped and centrifuged at 1,500 rpm (Heraeus megafuge 1.0R) for 5 min before removal of supernatant for ELISA and resuspension of the pellet in TRIzol for RNA extraction.

Quantitative RT-PCR and Primers. Total RNA (3 μg) was extracted with TRIzol, DNase-treated (DNase kit; Promega), and reverse transcribed by using Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLV RT; Promega). Quantitative PCR was performed using SYBR Green Master Mix (Applied Biosystems) and the Rotor Gene 3000 machine (Corbett). β-Actin was used as a reference gene, and the $2^{-\Delta\Delta}$Ct method was used to calculate relative gene expression levels.

Primers. Primers were as follows:

Mouse TNF-α, forward, CCAGTGTGGGAAGCTGTCTT; reverse, AAGCAAAAGAGGAGGCAACA;

Mouse Stat2, forward, GCTGTCAAGGTTCTGCAACA; reverse, CGCTTGGAGAATTGGAAGTT;

Mouse Trail, forward, TGGAGTCCCAGAAATCCTCA; reverse, TCACCAACGAGATGAAGCAG;

Mouse Egr1, forward, TGGGATAACTCGTCTCCACC; reverse, GAGCGAACAACCCTATGAGC;

Mouse Litaf, forward, GATGGTGCTGAGGAAGGC; reverse, TCCTCTTACTGCTGATGGCA;

Mouse MxA, forward, GGCAGACACCACATACAACC; reverse, CCTCAGGCTAGATGGCAAG; and

Mouse β-actin, forward, AATCGTGCGTGACATCAAAG; reverse, ATGCCACAGGATTCCATACC.

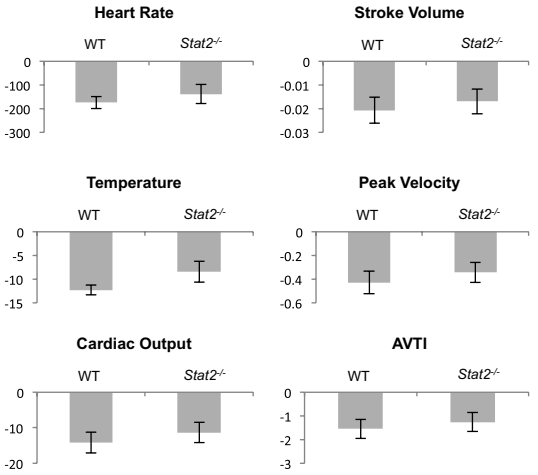
Measurement of Nitrite Concentration. Nitric oxide production was assayed by measuring nitrite in the supernatant of WT and *Stat2*$^{-/-}$ cells using the Griess Reagent System (Promega). Briefly, cells were cultured in six-well plates and treated with LPS for the indicated time points before supernatants were collected for analysis. Absorbance was measured at 570 nm in a microplate reader. Standard curves were produced by using sodium nitrite.

Protein Extraction and Immunoblotting. Cytoplasmic and nuclear extracts were prepared as described previously (4) with some modification. Buffers contained 0.1 mM sodium orthovanadate, 10 mM sodium fluoride, PhosSTOP phosphatase inhibitor, and protease inhibitor mixture (Roche). Whole cell extracts were prepared using Brij lysis buffer (10 mM Tris, pH 7.0, 2 mM EDTA, 150 mM NaCl, 1% Brij96, 0.1% IGEPAL, 0.1 mM sodium orthovanadate, 10 mM sodium fluoride) containing PhosSTOP phosphatase inhibitor and protease inhibitor mixture (Roche) and incubation on ice for 15 min. Supernatants were cleared by centrifuging at 13,793 × *g* for 15 min at 4 °C. Twenty micrograms of whole-cell or cytoplasmic extracts or 10 μg of nuclear protein extracts were separated on 4% to 12% (vol/vol) Bis-Tris polyacrylamide gels (Invitrogen) and transferred onto Hybond-P PVDF membrane (GE Healthcare). Membranes were blocked for in TBS with 0.1% Tween 20/5% (wt/vol) dry skim milk at room temperature and incubated with primary antibodies overnight at 4 °C. Membranes were then incubated with the corresponding secondary antibody (anti-HRP IgG) in blocking solution with additional Igepal (0.1%) for 1 h at room temperature. Signal was detected by using ECL-plus (GE Healthcare).

Transient Transfection. Immortalized WT and *Stat2*$^{-/-}$ BMDMs were seeded at a density of 3.5×10^5 cells in each well of a 12-well plate. Two micrograms of each DNA to be transfected was diluted in a total of 100 μL Opti-MEM (Invitrogen) combined with 4 μL Lipofectamine 2000 reagent (Invitrogen) according to the manufacturer's instructions before incubation with the cells for 3 h. After this time, media were aspirated and replaced with fresh 600 μL supplemented DMEM.

Plasmids and Luciferase Assay. pUNO-mSTAT2 (Invivogen) was purchased, and the Y689F mutation was generated by using a site-directed mutagenesis and sequencing service (Genscript). The secreted luciferase pNF-κB-MetLuc Vector kit (Clontech) was used to assess transcriptional activity. The pORF-eSEAP plasmid (Invivogen) was used to normalize luciferase activity in supernatants. Cells were incubated with and without TNF-α for 6 h, and aliquots of supernatant were harvested at various time points for luminescence measurements. Luciferase and SEAP activity were measured by using the Ready-To-Glow Secreted Luciferase Reporter Assay system according to the manufacturer's instructions (Clontech).

1. Blasi E, Radzioch D, Merletti L, Varesio L (1989) Generation of macrophage cell line from fresh bone marrow cells with a myc/raf recombinant retrovirus. *Cancer Biochem Biophys* 10(4):303–317.
2. Zhao W, Cha EN, Lee C, Park CY, Schindler C (2007) Stat2 Dependent Regulation of MHC Class II Expression. *J Immunol* 179(1):463–471.
3. Plumlee CR, et al. (2009) Interferons direct an effective innate response to Legionella pneumophila infection. *J Biol Chem* 284(44):30058–30066.
4. Steen HC, et al. (2013) Identification of STAT2 serine 287 as a novel regulatory phosphorylation site in type I interferon-induced cellular responses. *J Biol Chem* 288(1): 747–758.



	Baseline Physiology	
	WT	*Stat2*$^{-/-}$
Peak Velocity	0.8	0.7
AVTI	3.4	3.1
SV (ml)	0.046	0.042
HR (/min)	484.0	486.0
CO	22.3	20.5
Temp (°C)	37.7	37.3
Weight (g)	21.1	22.0

Fig. S1. Effect of LPS on physiology in WT and *Stat2*$^{-/-}$ mice. Effect of LPS (20 mg/kg) on cardiovascular performance [heart rate (in beats per minute), stroke volume (in milliliters), peak velocity (in meters per second), cardiac output (in milliliters per minute), aortic velocity time integral (AVTI)] and temperature (in °C) in WT and *Stat2*$^{-/-}$ mice. All values are presented as difference between before (time 0) and after (18 h) LPS administration as mean ± SEM in WT ($n = 8$) and *Stat2*$^{-/-}$ ($n = 5$) mice. Table shows baseline physiological values for WT and *Stat2*$^{-/-}$ mice.

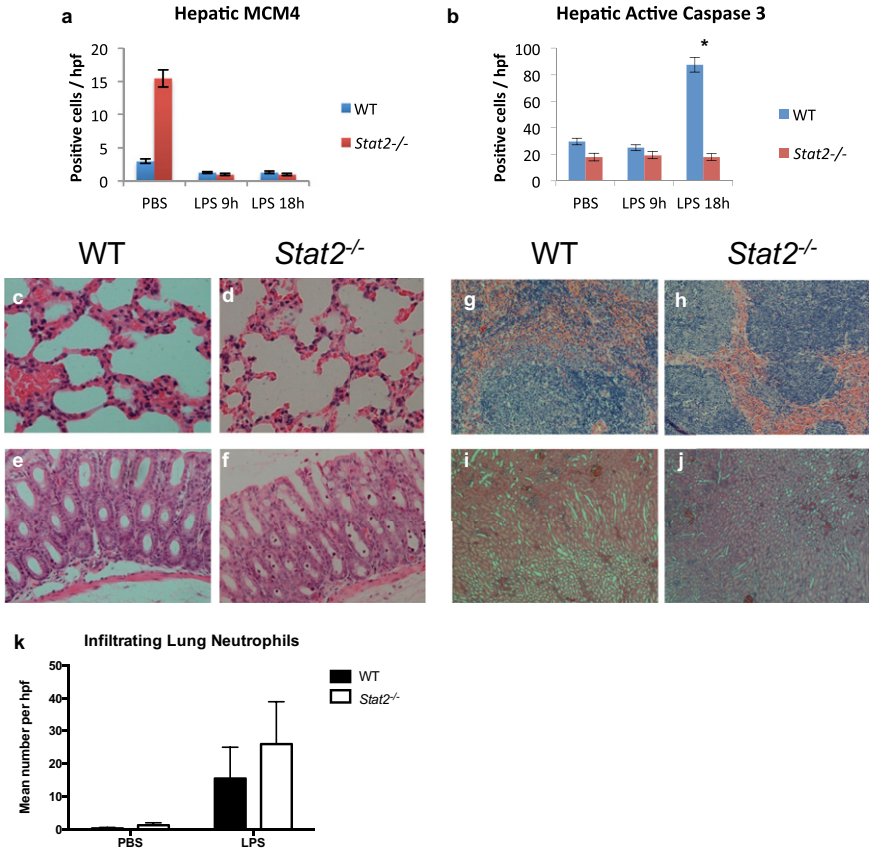


Fig. S2. Effect of LPS on pathology in WT and *Stat2*$^{-/-}$ mice. Mean number of (*A*) MCM4-positive and (*B*) active caspase 3-positive hepatocytes per high-power field (hpf) in WT and Stat2$^{-/-}$ liver tissue assessed by immunohistochemistry following i.p. injection with PBS solution or LPS for 9 h and 18 h ($n = 4$–5 for each time point). (*C* and *D*) Representative H&E-stained sections of lung (magnification of 40×), (*E* and *F*) colon (magnification of 40×), (*G* and *H*) spleen (magnification of 100×), and (*I* and *J*) kidney (magnification of 40x) from WT (*C*, *E*, *G*, and *I*) and *Stat2*$^{-/-}$ (*D*, *F*, *H*, and *J*) mice following 18 h LPS. (*K*) Mean number of neutrophils per high-power field in lung tissue assessed by histology following i.p. injection with PBS solution or LPS for 18 h ($n = 4$–5 for each group).

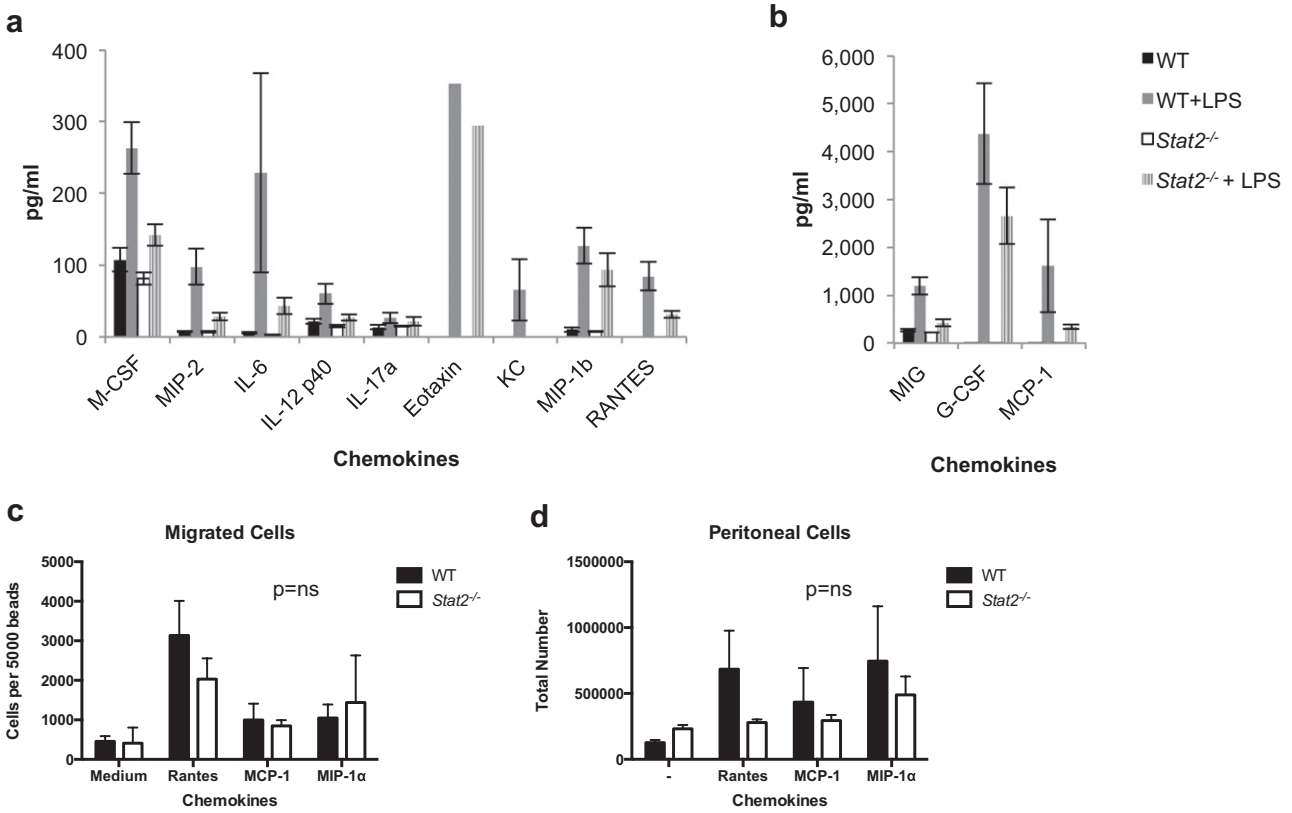


Fig. S3. Chemokine production and migration effects in the absence of Stat2. (*A* and *B*) Peritoneal lavage fluid was collected from mice at 18 h after LPS injection and assayed for the expression of 32 chemokines and cytokines by using the Luminex magnetic bead system ($n = 6$ for each group). Chemokines that were differentially produced in response to LPS (20 mg/kg) are shown. (*C*) Migration of WT and *Stat2*$^{-/-}$ immortalized BMDMs in response to different chemoattractants (RANTES 10 ng/mL; MCP1 100 ng/mL; MIP-1α 20 ng/mL). Number of cells that migrated were quantified by FACS analysis in relation to 5,000 counting beads. (*D*) Total number of cells retrieved from peritoneal lavage fluid in WT and *Stat2*$^{-/-}$ mice, 6 h following i.p. injection of RANTES 1 μg, MCP1 500 ng, or MIP-1α 500 ng.

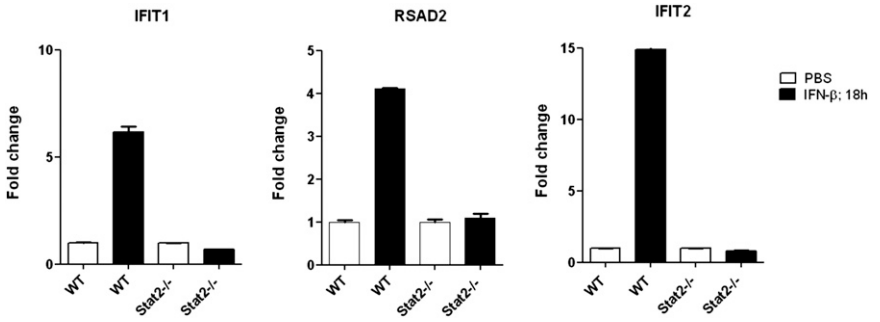


Fig. S4. Loss of Stat2 impairs transcription of IFN target genes. WT and *Stat2*$^{-/-}$ mice were injected via tail vein with IFN-β. Livers collected 18 h later were processed for RNA extraction, and gene expression was evaluated by quantitative PCR analysis.

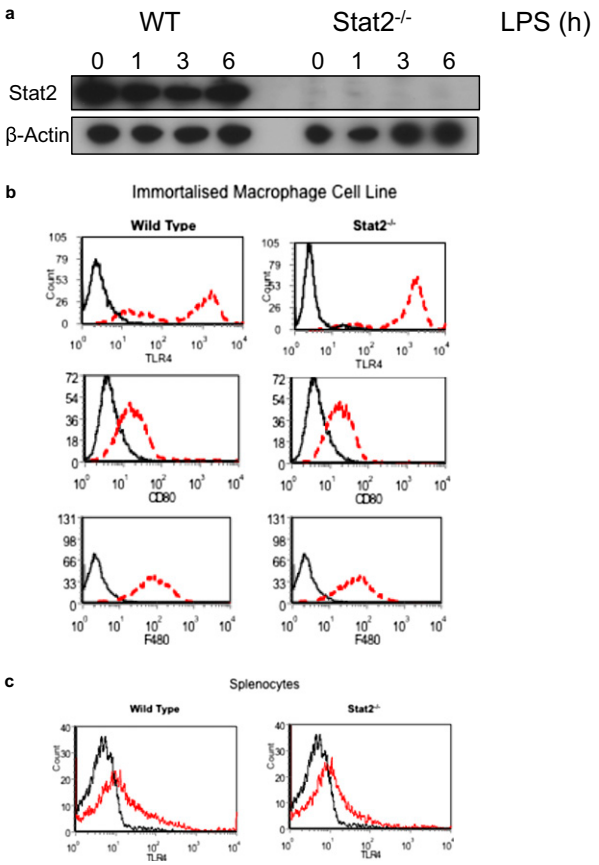


Fig. S5. Stat2 and TLR4 expression in primary BMDMs and splenocytes. (*A*) Western blot analysis confirms that Stat2 is absent in immortalized *Stat2*$^{-/-}$ BMDM even after stimulation with LPS at different hours. (*B*) Tlr4, CD80, and F4/80 expression was determined by flow cytometric analysis in immortalized BMDMs and (*C*) Tlr4 expression in splenocytes.

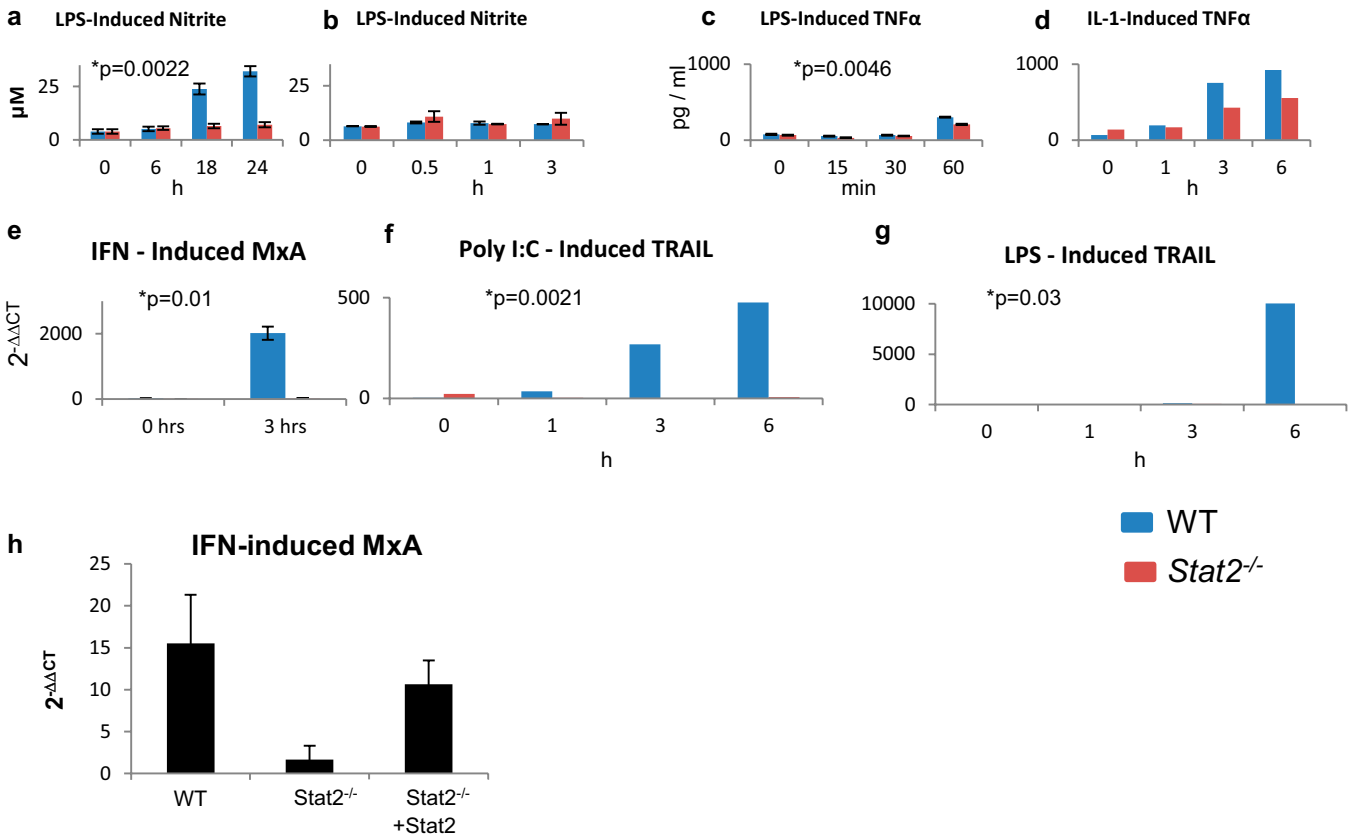


Fig. S6. Cytokine profiling in response to TLR agonists in murine cells lacking Stat2. (*A* and *B*) Production of nitrite and (*C*) TNF-α protein in murine immortalized WT (blue bars) and *Stat2*$^{-/-}$ (red bars) BMDMs in response to LPS (1 μg/mL) or (*D*) with IL-1 (400 pg/mL) stimulation. (*E*) Expression of IFN target gene, MxA, and (*F* and *G*) TLR-driven TRAIL assessed by quantitative PCR are shown as mean fold change relative to expression in unstimulated cells following normalization to β-actin. Data represent mean (±SEM) of two to four experiments. (*H*) Quantitative PCR analysis of MxA in *Stat2*$^{-/-}$ and *Stat2*$^{-/-}$ reconstituted with Stat2 immortalized BMDMs.

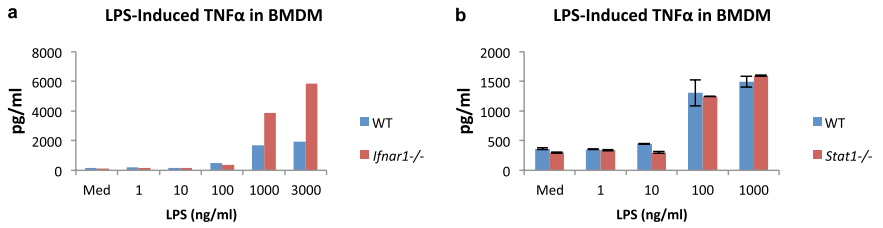


Fig. S7. LPS-induced TNF-α production in primary *Stat1*$^{-/-}$ and *Ifnar1*$^{-/-}$ BMDMs. (*A*) TNF-α production measured by ELISA in primary BMDMs from *Ifnar1*$^{-/-}$ and (*B*) *Stat1*$^{-/-}$ mice compared with WT mice following stimulation with increasing doses of LPS.

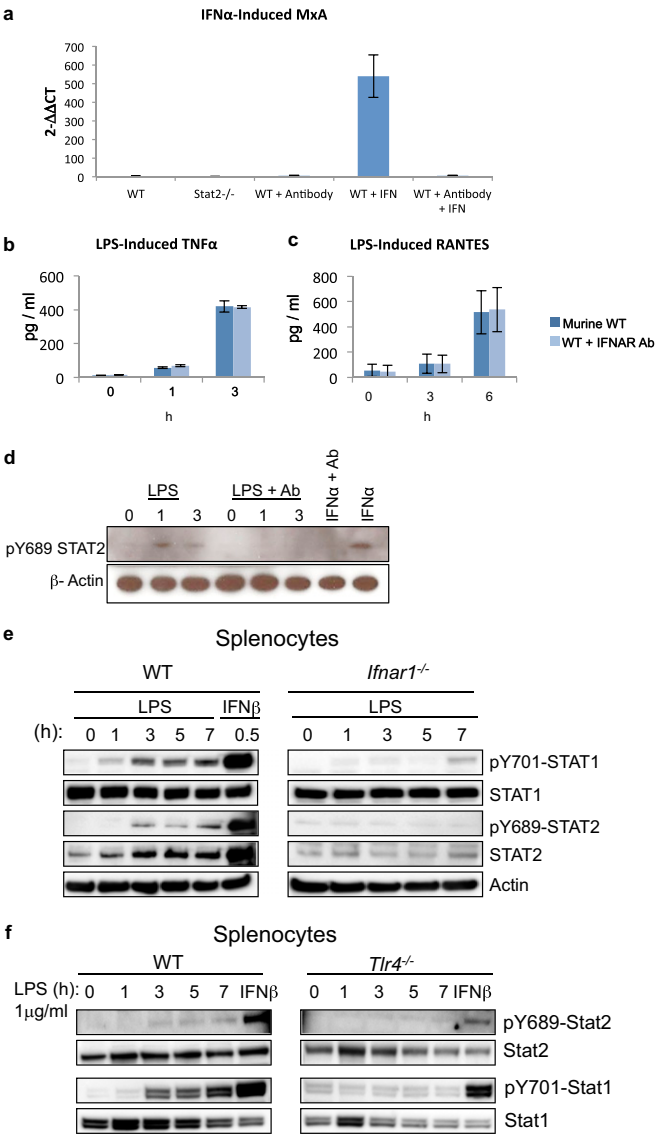


Fig. S8. Stat2-mediated TLR signaling is independent of type I IFN signaling. (*A*) IFN-induced MxA mRNA expression measured by quantitative PCR in the presence or absence of neutralizing Ifnar1 antibody. Error bars represent mean (±SEM) from duplicate samples of two independent experiments. (*B*) Production of TNF-α and (*C*) RANTES measured by ELISA in immortalized BMDMs in the presence (light blue) or absence (dark blue) of anti-Ifnar1 antibody. Error bars represent mean (±SEM) from duplicate samples of two independent experiments. (*D*) Western blot analysis of tyrosine phosphorylated Stat2 in immortalized BMDMs treated with LPS in the presence or absence of anti-Ifnar1 antibody. (*E* and *F*) Western blot analysis of tyrosine phosphorylated Stat1 and Stat2 following stimulation with LPS in primary WT, *Ifnar1*$^{-/-}$, *and Tlr4*$^{-/-}$ splenocytes.

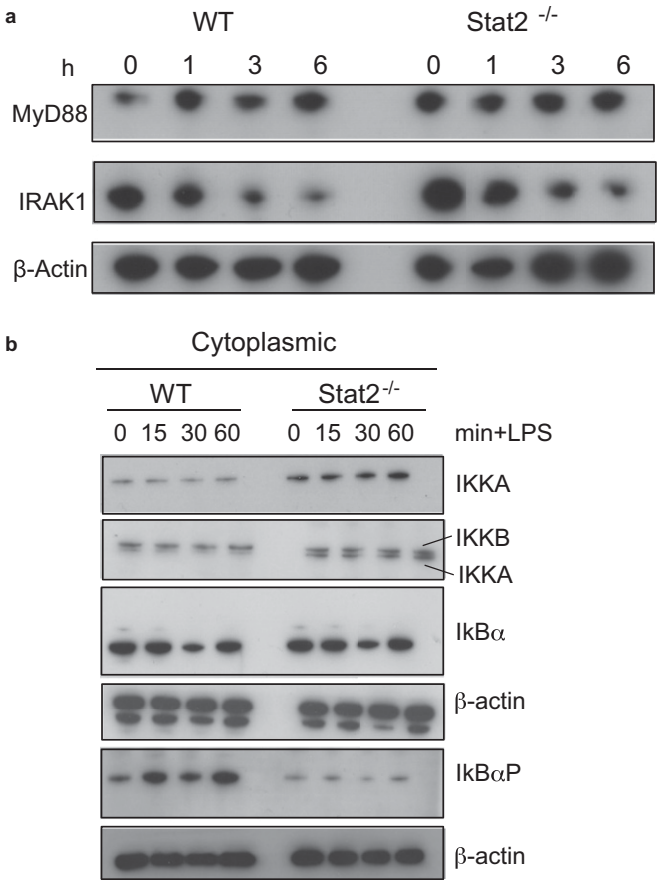


Fig. S9. Loss of Stat2 does not affect the protein expression of early components of TLR signaling and inhibitory kinases. Western blots of (*A*) Myd88, IRAK4 and β-actin, and (*B*) IKKA, IKKB, IκBα, and phospho-IκBα using whole-cell protein extracts from immortalized WT and *Stat2*$^{-/-}$ BMDMs after stimulation with LPS at different times. Images shown are representative of at least two to three independent experiments.

Table S1. Profile of chemokine and cytokine production in peritoneal lavage fluid

Cytokine	Assay range	WT	WT + LPS	*Stat2*$^{-/-}$	*Stat2*$^{-/-}$ + LPS
IL-15	5.7–37,501	397.8	368.2	366.9	304.6
IL-18	81.4–29,761	4,260.8	3,950.5	3,313.6	4,199.0
bFGF	4.8–35,500	28.9	46.3	40.4	40.5
LIF	3.5–57,366	27.5	33.2	26.1	29.5
M-CSF	1.5–24,221	107.5	263.2	81.7	142.2
MIG	183–46,393	276.3	1,196.2	224.7	422.3
MIP-2	32–8,574.2	7.8	98.0	6.9	28.4
VEGF	27.2–32,771	81.2	68.1	35.9	53.9
IL-1α	1.84–21,093	UD	35.2	UD	26.7
IL-1β	10.36–60,631	149.1	163.3	138.5	171.2
IL-2	3.72–51,857	47.4	35.9	26.1	51.3
IL-5	3.57–13,315	UD	11.6	UD	3.5
IL-6	0.74–12,053	6.0	228.8	3.2	43.3
IL-10	2.95–12,066	28.1	19.3	11.7	25.5
IL-12 p40	1.53–25,024	21.9	60.3	15.5	26.8
IL-12 p70	1.62–26,507	75.1	63.5	55.2	68.2
IL-13	47.2–57,011	323.0	319.7	280.5	294.2
IL-17α	2.65–43,337	13.7	26.1	14.8	21.4
Eotaxin	257.9–4,636	1,178.4	1,058.9	UD	882.8
IFN-γ	1.84–30,164	7.8	7.5	UD	8.8
KC	3.2–18,202	UD	131.2	UD	UD
MCP-1	22.4–41,873	UD	1,622.4	UD	345.8
MIP-1α	256.2–15,565	UD	153.2	UD	172.2
MIP-1β	3.33–24,798	10.4	127.1	7.6	93.7
RANTES	2.78–8,759	UD	84.7	UD	31.3
TNF-α	5.8–59,626	107.6	124.8	103.5	118.4
PDGF-BB	10.4–36,939	UD	UD	UD	UD
IL-3	1.55–21632	UD	UD	UD	UD
IL-4	6.98–9,372	UD	UD	UD	UD
IL-9	6.89–28,208	UD	UD	UD	UD
GM-CSF	21.2–3,401	UD	UD	UD	UD

Mean chemokine expression (in picograms per milliliter) in WT and *Stat2*$^{-/-}$ mouse peritoneal lavage fluid collected after 6 h of LPS treatment, $n = 6$ for each group. UD, undetectable. bFGF, basic fibroblast growth factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; LIF, leukaemia inhibitory factor; MIG, monokine induced by gamma interferon; MIP, macrophage inflammatory protein; PDGF, platelet-derived growth factor; UD, undetectable; VEGF, vascular endothelial growth factor.