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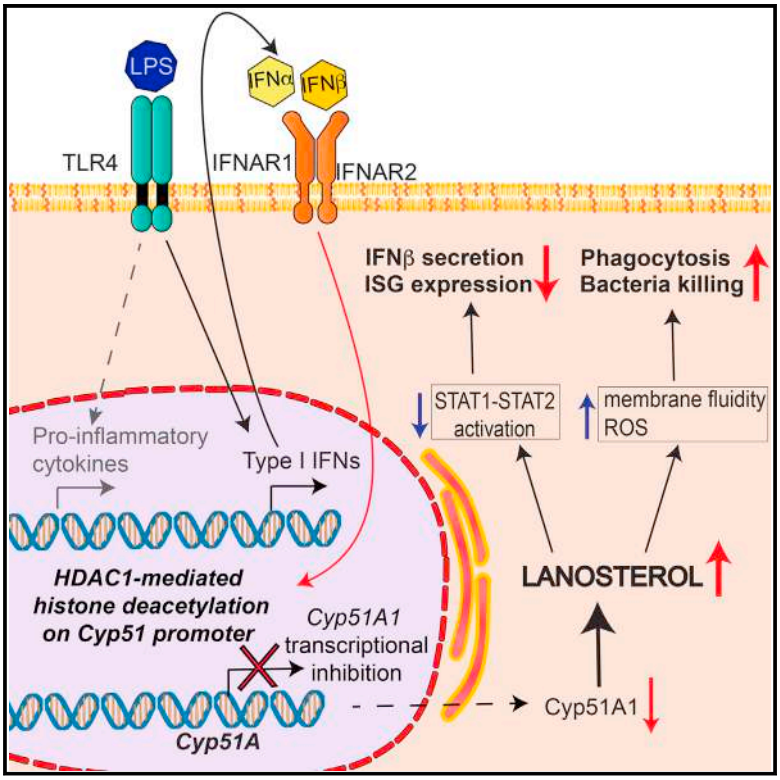
Lanosterol Modulates TLR4-Mediated Innate Immune Responses in Macrophages

Graphical Abstract



Authors

Elisa Araldi, Marta Fernández-Fuertes, Alberto Canfrán-Duque, ..., Dianqing Wu, Carlos Fernández-Hernando, Yajaira Suárez

Correspondence

carlos.fernandez@yale.edu (C.F.-H.), yajaira.suarez@yale.edu (Y.S.)

In Brief

Araldi et al. find that TLR4-mediated transcriptional downregulation of *Cyp51A1* induces lanosterol accumulation in macrophages, thus promoting antimicrobial activity and favoring negative feedback of type I IFN-mediated responses.

Highlights

- TLR4-mediated reduced expression of CYP51A1 increases lanosterol accumulation
- *Cyp51A1* transcriptional downregulation is type I IFN dependent and HDAC1 mediated
- Lanosterol decreases ISG expression and increases survival to endotoxemic shock
- Lanosterol increases phagocytic activity and resistance to *Listeria* monocytogenes infection

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Lanosterol Modulates TLR4-Mediated Innate Immune Responses in Macrophages

Elisa Araldi,[1,2] Marta Fernández-Fuertes,[1] Alberto Canfrán-Duque,[1,3] Wenwen Tang,[4] Gary W. Cline,[5] Julio Madrigal-Matute,[2] Jordan S. Pober,[6] Miguel A. Lasunción,[3] Dianqing Wu,[4] Carlos Fernández-Hernando,[1,*] and Yajaira Suárez[1,7,*]

[1]Department of Comparative Medicine, Department of Pathology, Program in Integrative Cell Signaling and Neurobiology of Metabolism and Vascular Biology and Therapeutics Program, Yale University School of Medicine, New Haven, CT 06520, USA
[2]Departments of Medicine and Cell Biology, Leon H. Charney Division of Cardiology and Cell Biology, New York University School of Medicine, New York, NY 10016, USA
[3]Servicio de Bioquímica-Investigación, Hospital Universitario Ramón y Cajal, IRyCIS, Madrid y CIBER Fisiopatología de la Obesidad y Nutrición (CIBEROBN), ISCIII, 28029 Madrid, Spain
[4]Department of Pharmacology and Vascular Biology and Therapeutics Program, Yale University School of Medicine, New Haven, CT 06520, USA
[5]Department of Internal Medicine, Yale University School of Medicine, New Haven, CT 06520, USA
[6]Department of Immunobiology and Vascular Biology and Therapeutics Program, Yale University School of Medicine, New Haven, CT 06520, USA
[7]Lead Contact
*Correspondence: carlos.fernandez@yale.edu (C.F.-H.), yajaira.suarez@yale.edu (Y.S.)
http://dx.doi.org/10.1016/j.celrep.2017.05.093

SUMMARY

Macrophages perform critical functions in both innate immunity and cholesterol metabolism. Here, we report that activation of Toll-like receptor 4 (TLR4) in macrophages causes lanosterol, the first sterol intermediate in the cholesterol biosynthetic pathway, to accumulate. This effect is due to type I interferon (IFN)-dependent histone deacetylase 1 (HDAC1) transcriptional repression of lanosterol-14α-demethylase, the gene product of *Cyp51A1*. Lanosterol accumulation in macrophages, because of either treatment with ketoconazole or induced conditional disruption of *Cyp51A1* in mouse macrophages in vitro, decreases IFNβ-mediated signal transducer and activator of transcription (STAT)1-STAT2 activation and IFNβ-stimulated gene expression. These effects translate into increased survival to endotoxemic shock by reducing cytokine secretion. In addition, lanosterol accumulation increases membrane fluidity and ROS production, thus potentiating phagocytosis and the ability to kill bacteria. This improves resistance of mice to *Listeria monocytogenes* infection by increasing bacterial clearance in the spleen and liver. Overall, our data indicate that lanosterol is an endogenous selective regulator of macrophage immunity.

INTRODUCTION

Macrophages are effector cells of innate immunity that phagocytose bacteria and secrete both pro-inflammatory and antimicrobial mediators. They can sense a variety of inflammatory and immune stimuli and respond by adapting their gene expression profile to provide protection against microbial infections and maintain tissue homeostasis (Mosser and Edwards, 2008). In response to TLR4 activation, macrophages activate molecular mechanisms that both positively and negatively regulate inflammatory responses (Iyer et al., 2010; Medzhitov and Horng, 2009). TLR4 signaling induces immediate or early gene expression through activation of transcription factors such as nuclear factor κB (NF-κB), activator protein 1 (AP-1), and interferon regulatory factor (IRF) 3 (Glass and Natoli, 2016; Medzhitov and Horng, 2009). Mediators induced by these, such as type I interferons (IFNs) can then induce secondary response genes (e.g., IFN-stimulated gene products) (Ivashkiv and Donlin, 2014) that both support innate immunity and set the stage for adaptive immunity. TLR4 signaling also results in downregulation of a broad program of gene expression, although molecular mechanisms responsible for this are less well characterized.

The crosstalk between innate immune responses and cholesterol homeostasis is instrumental for proper macrophage function (Castrillo et al., 2003; Im et al., 2011). Both sterol-regulatory element-binding proteins (SREBPs), which activate cholesterol synthesis and uptake (Jeon and Osborne, 2012) and liver X receptors (LXRs), which control of cholesterol efflux (Hong and Tontonoz, 2014), participate in regulating several immune functions (Castrillo et al., 2003; Im et al., 2011; Spann et al., 2012; York et al., 2015). Moreover, observations indicate that post-cholesterol oxysterols, including 25-hydroxycholesterol (25-HC), participate in the immune activation of macrophages in response to different inflammatory stimulus (e.g., TLR4 or type I IFN), thus emerging as important regulators of immune functions elicited by macrophages (Bauman et al., 2009; Blanc et al., 2011; Liu et al., 2013; Reboldi et al., 2014; Shibata et al., 2013). Moreover, type I IFN-induced responses produce perturbations in the intracellular homeostasis of cholesterol, which in

turn can regulate these processes (York et al., 2015). Although the downregulation of cholesterol biosynthetic enzymes and the concomitant accumulation of sterol intermediates has been reported in response to TLR4 agonists in a macrophage cell line (Dennis et al., 2010), the role of these intermediates in regulating host responses to pathogens has not been elucidated. In lymphoid cells, several precursors of cholesterol biosynthesis are essential for their development and differentiation (Hu et al., 2015; Santori et al., 2015); however, it remains unclear how sterol intermediates of the cholesterol biosynthesis influence macrophage physiology.

In the present study, we show that transcription of lanosterol-14α-demethylase (Cyp51A1) was downregulated in TLR4-activated macrophages due to a secondary repressive response that is dependent on type I IFN production and on the activation of histone deacetylases. Consequently, CYP51A1 protein levels were reduced, and lanosterol, the first sterol of cholesterol biosynthesis, accumulated intracellularly. Either pharmacological accumulation of lanosterol through administration of ketoconazole (KT), a competitive inhibitor of CYP51A1, or inducible conditional deletion of *Cyp51A1* in macrophages increased the survival of mice subjected to endotoxemic shock, which was associated with diminished pro-inflammatory cytokine secretion. Mechanistically, lipopolysaccharide (LPS)/IFNβ-triggered signal transducer and activator of transcription (STAT)1-STAT2 activation in macrophages that accumulated lanosterol was attenuated, resulting in reduced expression of IFN type I-mediated cytokines. In addition, we found that lanosterol accumulation increased membrane fluidity and reactive oxygen species (ROS) production, thus potentiating phagocytosis and the ability to kill bacteria. As such, mice treated with KT exhibited a survival advantage to *Listeria monocytogenes* infection and increased bacteria clearance in spleen and liver.

Our data indicate that innate immune transcriptional downregulation of CYP51A1 induces lanosterol accumulation in macrophages, promoting antimicrobial activity and favoring anti-inflammatory response in macrophages. We further identify lanosterol as an endogenous mediator of innate immune responses of macrophages.

RESULTS

CYP51A1 Downregulation in LPS/IFNγ-Treated Macrophages Promotes the Accumulation of Lanosterol

To better understand the role of non-immune related genes on innate immune responses, we performed an unbiased whole genome expression analysis on LPS/IFNγ-activated bone marrow-derived macrophages (BMDMs) (Figure 1A). Ingenuity pathway analysis on significantly downregulated genes (Figure S1A) revealed that the expression of key enzymes of the cholesterol synthesis, such as the lanosterol-using enzymes lanosterol-14α-demethylase (*Cyp51A1*) and 24-dehydrocholesterol reductase (*Dhcr24*) was reduced in LPS/IFNγ-treated macrophages (Figure 1B) and in agreement with a previous report (Dennis et al., 2010). This effect was translated to protein levels, which decreased over time after LPS/IFNγ stimulation (Figure 1C). CYP51A1 catalyzes the demethylation of the 14α-methyl group from lanosterol, an obligatory step of cholesterol synthesis, while DHCR24 converts all sterols from the Bloch to the Kandutsch-Russell pathway and desmosterol into cholesterol (Sharpe and Brown, 2013) (Figure S1B). The downregulation of CYP51A1 and DHCR24 was also observed in human peripheral blood monocyte-derived macrophages, suggesting that this process is relevant in humans (Figure 1D).

Classical activation of macrophages is mediated by a combined effect of type II IFN (IFNγ) and LPS (Schroder et al., 2004). The effect of the LPS + IFNγ on CYP51A1 or DHCR24 protein expression did not differ from that observed with LPS alone, while the stimulation with IFNγ alone did not produce an effect on the expression of these enzymes (Figures S2A and S2B). Therefore, co-stimulation with IFNγ is not necessary to promote the downregulation of CYP51A1 or DHCR24 and indicates an IFNγ-independent mechanism is responsible for the changes in the expression of these enzymes. Thereafter, experiments were performed in the absence of IFNγ.

We next determined whether the effect of LPS on the downregulation of these enzymes is a physiological response that also occurs in vivo. Thus, macrophages were elicited via intraperitoneal injection of thioglycolate. After 3 days, mice were injected with LPS, and 24 hr later, macrophages were isolated and the protein levels of CYP51A1 and DHCR24 were analyzed. Thioglycolate-recruited macrophages of mice injected with LPS showed reduced expression of CYP51A1 and DHCR24 compared with their vehicle (PBS)-injected counterparts (Figure 1E).

We then evaluated whether the reduced levels of CYP51A1 and DHCR24 were associated with alterations in the de novo synthesis of cholesterol. Previous studies in RAW 264.7 cells treated with the TLR4 ligand Kdo2-Lipid A (KLA) reported a decrease in *Cyp51A1* and *Dhcr24* expression and lanosterol accumulation over 24 hr of stimulation (Andreyev et al., 2010; Dennis et al., 2010). However, cholesterol content analyzed by mass spectrometry was increased. This latter effect was attributed to increased lipoprotein uptake from the cholesterol-containing media. We first tested whether the uptake of exogenous cholesterol through lipoproteins present in the culture media was involved in the downregulation of these enzymes. As shown in Figure 1F, incubation of macrophages in media containing lipoprotein-deficient serum (LPDS) did not alter the TLR4-mediated downregulation of CYP51A1 or DHCR24 at either mRNA or protein levels (Figure 1F; Figure S2C), indicating that the effect is not due to a negative cholesterol-mediated feedback regulation but rather is mediated by the inflammatory stimulus per se. We next analyzed the incorporation of radioactive acetate into non-saponifiable lipids after TLR4 stimulation. As shown in Figure 1G, LPS treatment produced a slight reduction of [1-2-^{14}C]-acetate incorporation into non-saponifiable lipids (corresponding mainly to cholesterol, 7-dehydrocholesterol, dehydrodesmosterol, and desmosterol) and to a significant increase of radioactivity incorporation into lanosterol (Figure 1G). General reduction of the flux of carbon into sterols is supported by the accumulation of non-sterol isoprenoids in LPS-treated macrophages (Figure 1G). Mass spectrometry analysis confirmed the accumulation of lanosterol (~4-fold increase), while total cellular cholesterol content was not significantly altered after 24 hr of stimulation (Figure 1H). Because dehydrolanosterol, the product of DHCR24-mediated

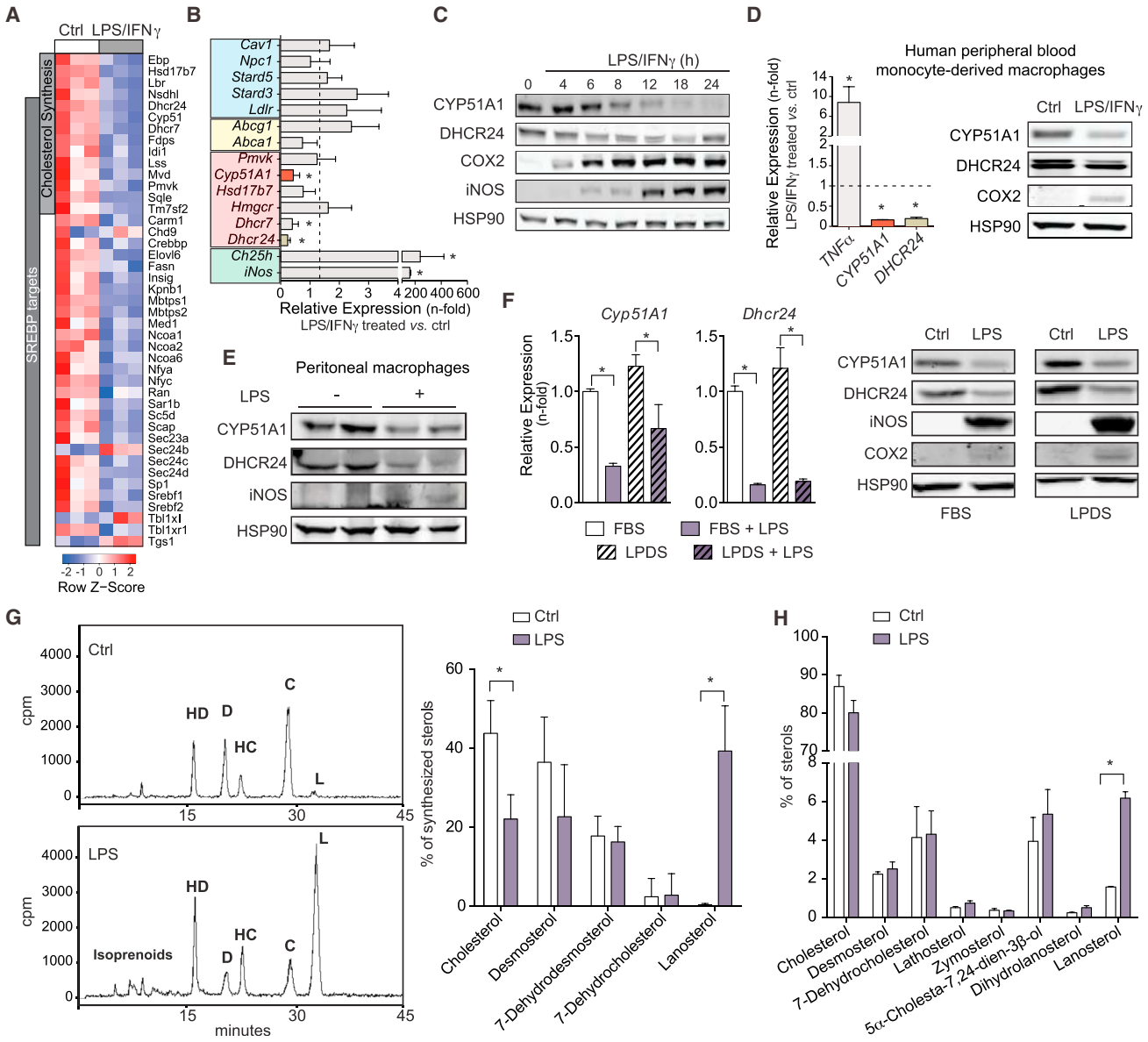


Figure 1. CYP51A1 Downregulation in LPS/IFNγ-Treated Macrophages Promotes the Accumulation of Lanosterol

(A) Heatmap of differentially expressed cholesterol biosynthesis genes and SREBP2 targets in BMDMs stimulated with LPS, 10 ng/mL, and IFNγ, 20 ng/mL, for 8 hr.

(B) qPCR validation of microarray data. Relative mRNA expression levels of cholesterol trafficking (blue), efflux (yellow), biosynthesis (red), and positive controls of inflammatory stimulation (green) (n = 3).

(C) CYP51A1 and DHCR24 protein levels of LPS/IFNγ-treated BMDMs for the indicated times (n = 3).

(D) Relative mRNA expression (left) and protein levels of CYP51A1 and DHCR24 (right) in human macrophages treated with LPS/IFNγ for 8 hr (left) or 18 hr (right) (n = 3).

(E) CYP51A1 and DHCR24 protein levels of elicited peritoneal macrophages collected 24 hr after intraperitoneal injection of LPS (20 mg/kg), which was performed 3 days after initial 3% thioglycolate injection (n = 2).

(F) Left: relative mRNA expression. Right: CYP51A1 and DHCR24 protein levels of BMDMs cultured for 24 hr in regular media containing 20% FBS or in media containing 20% LPDS and then treated with LPS (100 ng/mL) for 8 hr (left) or 18 hr (right) (n = 3).

(G) Sterol intermediate analysis by high-performance liquid chromatography (HPLC) and online radioactivity of BMDM-treated LPS (100 ng/mL) or PBS (Ctrl) for 24 hr. Left: representative plots of [1-2^{14}C]-acetate incorporation into sterols. Right: quantification of total synthesized sterols, expressed as the percentage of total synthesized sterols versus Ctrl (n = 3).

(H) Composition of sterols by GC-MS of BMDMs as in (G) (the percentage of each sterol species within the total sterol) (n = 3).

(C–F) Cyclooxygenase (COX2), inducible nitric oxide synthase (iNOS), or *TNFα* are positive controls of inflammatory activation. Heat shock protein 90 (HSP90) is a loading control. Results are expressed as mean ± SEM; *p < 0.05 versus Ctrl unless otherwise indicated. See also Figure S1.

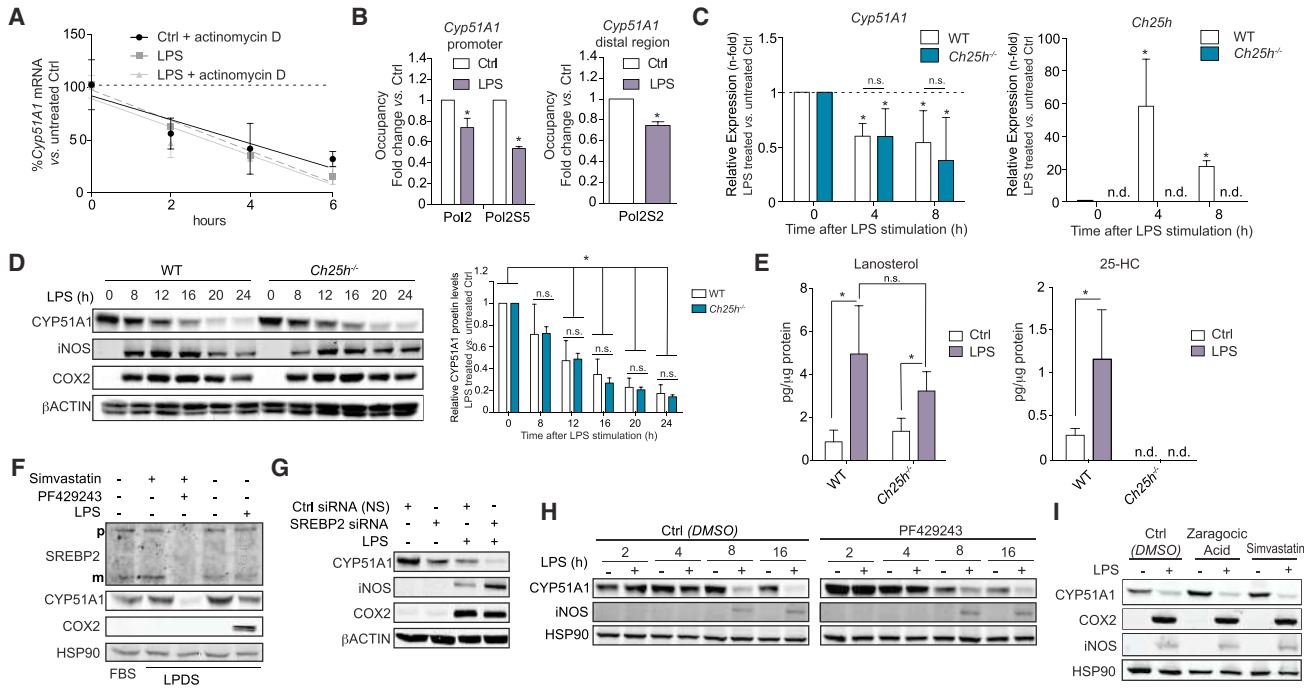


Figure 2. CYP51A1 Is Transcriptionally Downregulated Independently of Newly Synthesized 25-HC and of SREBP Activation

(A) Cyp51A1 mRNA analysis by qPCR of BMDMs treated with actinomycin-D (30 min) before LPS (100 ng/mL) stimulation for indicated times (n = 3).

(B) ChIP analysis with Pol II and Pol II S5 (left) and Pol II S2 (right) of BMDMs cultured in media containing 20% LPDS for 24 hr and treated with LPS for 1 hr. Quantification of promoter-specific bound antibody by qPCR with primers proximal to the transcription start site (TSS) of *Cyp51A1*. Data are fold change versus Ctrl (PBS treated) and normalized to input chromatin (n = 3).

(C) Relative mRNA expression of *Cyp51A1* (left) or *Ch25h* (right) of WT of *Ch25h*$^{-/-}$ BMDMs incubated with LPS for the indicated times (n = 4).

(D) Left: CYP51A1 protein levels of LPS-treated BMDMs for the indicated times. Right: quantification of CYP51A1 protein levels normalized by β-actin (n = 4).

(E) Quantification of lanosterol (left) or 25-HC (right) by GC-MS, normalized by protein content, of WT of *Ch25h*$^{-/-}$ BMDMs incubated with LPS for 24 hr (n = 3).

(F) CYP51A1 protein levels of precursor (p) and mature (m) forms of SREBP2 of BMDMs incubated in regular media containing 20% FBS or 20% LPDS as indicated, treated with simvastatin (5 μM) for 12 hr, PF429243 (10 μM) for 12 hr, or LPS (100 g/mL) for 4 hr, respectively (n = 4).

(G) CYP51A1 protein levels of BMDMs transfected with 30 nM of non-silencing (NS) control siRNA or *Srebp2* siRNA and treated or not with LPS, as indicated, for 8 hr (n = 3).

(H) CYP51A1 protein levels of BMDMs treated with PF429243 (10 μM) or vehicle (DMSO) Ctrl stimulated with LPS for the indicated times.

(I) CYP51A1 protein levels of BMDM incubated with DMSO (Ctrl), zaragozic acid (10 μM), or simvastatin (5 μM) for 2 hr before LPS stimulation for 8 hr (n = 3).

(D and F–I) COX2 and iNOS are positive controls of activation. β-actin or HSP90 are loading controls. Results are expressed as mean ± SEM; *p < 0.05 versus Ctrl unless otherwise indicated. NS, not significant; ND, not detectable. See also Figures S1 and S2.

conversion of lanosterol, was not significantly affected upon TLR4 stimulation (Figure 1H), we concluded that the accumulation of lanosterol after TLR4 stimulation is mainly a consequence of the LPS-mediated downregulation of CYP51A1 expression.

CYP51A1 Is Transcriptionally Downregulated Independently of Newly Synthesized 25-HC and of SREBP Activation

To elucidate the mechanism responsible in the downregulation of CYP51A1, we first tested whether TLR4 activation induces *Cyp51A1* mRNA instability by inhibiting transcription with actinomycin D. *Cyp51A1* mRNA levels in LPS-treated macrophages were similar to those observed in presence of actinomycin D independently of the stimulation (Figure 2A). mRNA transcription requires promoter recruitment of RNA polymerase II (Pol II) followed by phosphorylation at Ser5 at the CT domain of Pol II S5 for transcriptional initiation and subsequently at Ser2 on actively elongating Pol II S2 (Phatnani and Greenleaf, 2006). Pol II and Pol II S5 chromatin immunoprecipitation (ChIP) from LPDS-cultured BMDMs showed that Pol II and Pol II S5 occupancy on the promoter of *Cyp51A1* is decreased upon LPS treatment, which was accompanied by reduction of Pol II S2 recruitment (Figure 2B; Figure S2D). ChIP-sequencing analysis of LPS-stimulated BMDMs also showed the decreased of Pol II occupancy on the *Cyp51A1* promoter (Figure S2E).

In macrophages, both type I and type II IFNs stimulate the expression of cholesterol 25-hydroxylase (*Ch25h*) (Blanc et al., 2013; Liu et al., 2013; Park and Scott, 2010; Reboldi et al., 2014; Shibata et al., 2013), the enzyme responsible for the conversion of cholesterol to 25-HC (Lund et al., 1998), which inhibits cholesterol synthesis via SREBP inactivation (Adams et al., 2004). Therefore, we examined whether LPS-mediated accumulation of this oxysterol could be responsible for the observed downregulation of CYP51A1 expression. To do so, we analyzed CYP51A1 expression over time after LPS stimulation in BMDMs isolated from wild-type (WT) and *Ch25h*$^{-/-}$ mice. As expected, LPS induced the

expression of *Ch25h* in WT BMDMs (Figure 2C, right) while downregulating CYP51A1 over time (Figures 2C, left, and 2D). *Ch25h* mRNA and 25-HC were undetectable in *Ch25h*$^{-/-}$ BMDMs (Figures 2C, right, and 2E, right). LPS-mediated decrease of CYP51A1 was comparable in WT or *Ch25h*$^{-/-}$ BMDMs (Figures 2C, left, and 2D), leading to the accumulation of lanosterol (Figure 2E, left). Thus, 25-HC does not play a major role in the early LPS-mediated transcriptional downregulation of Cyp51A1.

SREBP2 activity is tightly regulated by cellular sterol levels, and when intracellular cholesterol levels are decreased, it promotes the transcriptional activation of genes responsible for cholesterol synthesis and uptake (Jeon and Osborne, 2012). We next assessed the contribution of SREBPs in the TLR4-mediated downregulation of CYP51A1 in human macrophages after testing SREBP2 antibody on human hepatic cells (Figure S2F). As expected, human macrophages cultured under conditions of cholesterol deprivation showed the expected increase in SREBP2 processing when compared to cells incubated in media containing fetal bovine serum (FBS) with lipoproteins (Figure 2F). In this scenario, the increase in SREBP2 processing correlated with the expected increase in CYP51A1 protein expression (Figure 2F). When human macrophages were stimulated with LPS in cholesterol-free media, we did not observe a decrease in the mature form of SREBP2 (Figure 2F) that could account for the decrease of mRNA and protein levels of CYP51A1. To further explore the role of SREBP2, we knocked down its expression in BMDMs using small interfering RNA (siRNA) (Figure S2G). Protein levels of CYP51A1 were decreased upon either LPS stimulation or *Srebp2* silencing (Figure 2G). LPS stimulation in *Srebp2*-silencing conditions caused a further decrease in the protein levels of CYP51A1 when compared to LPS stimulation in non-silencing (NS) control conditions or to *Srebp2* silencing in the absence of LPS stimulation (Figure 2G). We then blocked SREBP processing with a serine protease inhibitor that is selective for SREBP site 1 protease (S1P), PF429242 (Hawkins et al., 2008). As expected, PF429243 treatment efficiently inhibited the simvastatin-induced cleavage of SREBP2 (Figure 2F). In non-LPS stimulated conditions, PF429242-treated macrophages showed the expected gradual decrease of Cyp51A1 expression, but stimulation with LPS decreased CYP51A1 protein levels regardless of the presence of PF429242 (Figure 2H). To summarize, inhibiting SREBP2 expression or its activation does not alter LPS-mediated CYP51A1 downregulation. However, when SREBP activation was induced by inhibition of cholesterol biosynthesis via incubation in the presence of either simvastatin or zaragozic acid (Figure S1B), the expression of CYP51A1 was induced (Figure 2I). In these conditions, treatment with LPS stimulation was still able to promote the downregulation of CYP51A1 independently of previous SREBP activation (Figure 2I). Therefore, induction of SREBP activation does not prevent LPS-mediated downregulation of CYP51A1. Cumulatively, SREBP activation is not directly involved in the early TLR4-mediated repression of CYP51A1.

CYP51A1 Downregulation in Macrophages Is Caused by a Type I IFN Response and Is Mediated by HDAC1 Activation

LPS-mediated TLR4 signaling elicits two parallel signaling pathways: the MyD88 pathway, which triggers NF-κB and AP-1 activation, TLR-stimulated genes (TSGs), and related inflammatory cytokine production, and the TIR-domain-containing adapter-inducing interferon-β (TRIF) pathway, which activates the IRF3 transcription factor that mediates the subsequent upregulation of genes encoding type I IFNs and co-stimulatory molecules (Takeda and Akira, 2004). Secreted type I IFNs signal through their heterodimeric receptors, IFNAR1 and IFNAR2, to form a heterotrimeric transcription factor composed by STAT1, STAT2, and IRF9, promoting an autocrine loop that induces IFN-stimulated genes (ISGs) (Ivashkiv and Donlin, 2014). We thus tested whether the stimulation of BMDMs with type I IFNs (e.g., IFNβ) (Figure S3A, positive control of treatment) reduced the expression of CYP51A1. As shown in Figure 3A, both mRNA and protein levels of CYP51A1 were reduced and lanosterol levels were increased in response to IFNβ stimulation (Figure 3B). To better characterize the signaling pathway accounting for CYP51A1 downregulation, we tested the effect LPS or IFNβ on Cyp51A1 expression in BMDMs isolated from WT, *myD88*$^{-/-}$, or *ifnar1*$^{-/-}$ mice. The downregulation of CYP51A1 at mRNA and protein levels was observed in response to LPS or IFNβ in both WT and *myD88*$^{-/-}$ BMDMs (Figures 3C and 3D). However, neither LPS nor IFNβ reduced CYP51A1 expression in *ifnar1*$^{-/-}$ BMDMs (Figures 3C and 3D). These results are consistent with RNA sequencing (RNA-seq) data of *ifnar1*$^{-/-}$ BMDMs treated with LPS over time, where *Cyp51A1* expression was not downregulated, as opposed to WT BMDMs (Figure S3B). In agreement with those data, lanosterol accumulation after LPS or IFNβ stimulation is observed in WT or *myD88*$^{-/-}$ BMDMs but prevented in *Ifnar1*$^{-/-}$ BMDMs (Figure 3E). These data indicate that autocrine secretion of type I IFNs by TLR4 activation promotes the downregulation of CYP51A1 after LPS stimulation and the type I IFNs, but not type II IFNs (Figure S2A), which is the underlying stimulus responsible for the LPS-mediated downregulation of Cyp51A1 expression.

To further understand the basis of LPS-mediated *Cyp51A1* transcriptional downregulation, we investigated the chromatin landscape of the *Cyp51A1* locus. ChIP-sequencing analysis of the Cyp51A1 locus of LPS-stimulated BMDMs (Ostuni et al., 2013) showed a diminished occupancy of transcription factor PU.1 (master regulator and pioneer transcription factor of the myeloid lineage), a marked decrease in the active enhancer markers histone 4 acetylation (H4ac) and of histone 3 lysine 4 mono-methylation (H3K4me1), as well as a reduction in the active promoter marker H3K4me3 (Figure 3F). Altogether, these data suggest that LPS alters enhancer and promoter maintenance and represses the *Cyp51A1* locus.

The observed decrease in histone acetylation in the *Cyp51A1* locus after LPS treatment led us to investigate whether histone deacetylases (HDACs) were participating in the downregulation of CYP51A1 expression. As shown in Figure 3G, pre-treatment with a pan-HDAC inhibitor prevented either LPS- or IFNβ-mediated downregulation of CYP51A1 expression. More specifically, while inhibition of HDAC1 produced an effect similar to that observed with the pan-HDAC inhibitor (Figure 3G; Figure S3C), the HDAC3-specific and/or HDAC4-specific inhibitor did not reduce the LPS or IFNβ-mediated downregulation of CYP51A1 (Figure 3G). Altogether, our data indicate that upon LPS or IFNβ stimulation, the *Cyp51A1* locus is repressed through a mechanism that involves HDAC1-mediated deacetylation.

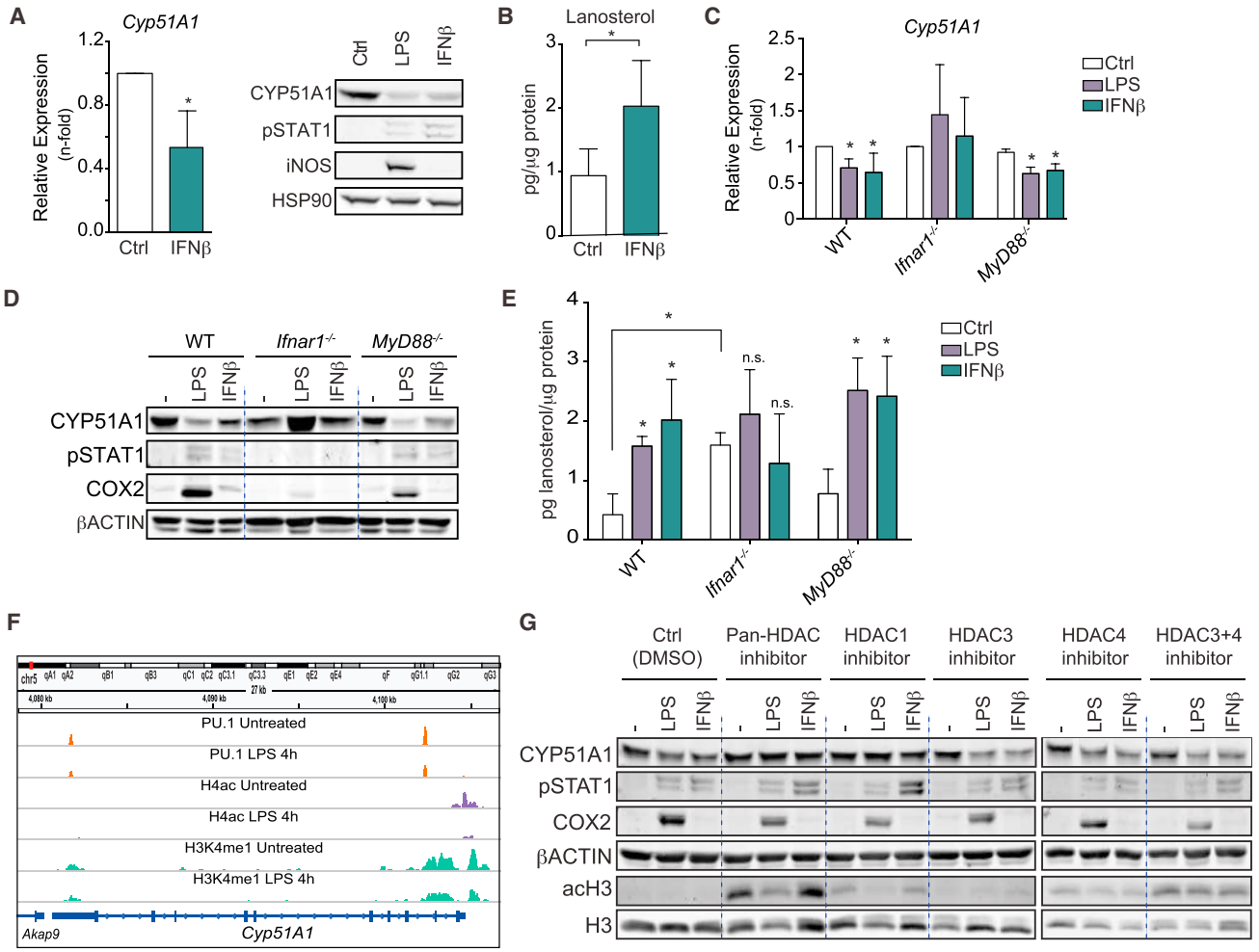


Figure 3. CYP51A1 Downregulation in Macrophages Is Caused by a Type I IFN Response and Is Mediated by HDAC1 Activation

(A) Relative mRNA expression of *Cyp51A1* (left) and CYP51A1 protein levels (right) of BMDMs stimulated with IFNβ (1,000 U/mL) for 4 hr (left) or with LPS (100 ng/mL) or IFNβ for 12 hr (right) (n = 3).

(B) Quantification of lanosterol by GC-MS, normalized by protein content, in BMDMs incubated with IFNβ for 24 hr (n = 3).

(C and D) Relative mRNA expression (C) or protein levels (D) of CYP51A1 of WT, *Ifnar1*$^{-/-}$, or *myD88*$^{-/-}$ BMDMs stimulated with LPS or INFβ for 4 hr (C) or 12 hr (D) (n = 3).

(E) Quantification of lanosterol by GC-MS, normalized by protein content, in BMDMs WT, *Ifnar1*$^{-/-}$, or *myD88*$^{-/-}$ BMDMs as in (B).

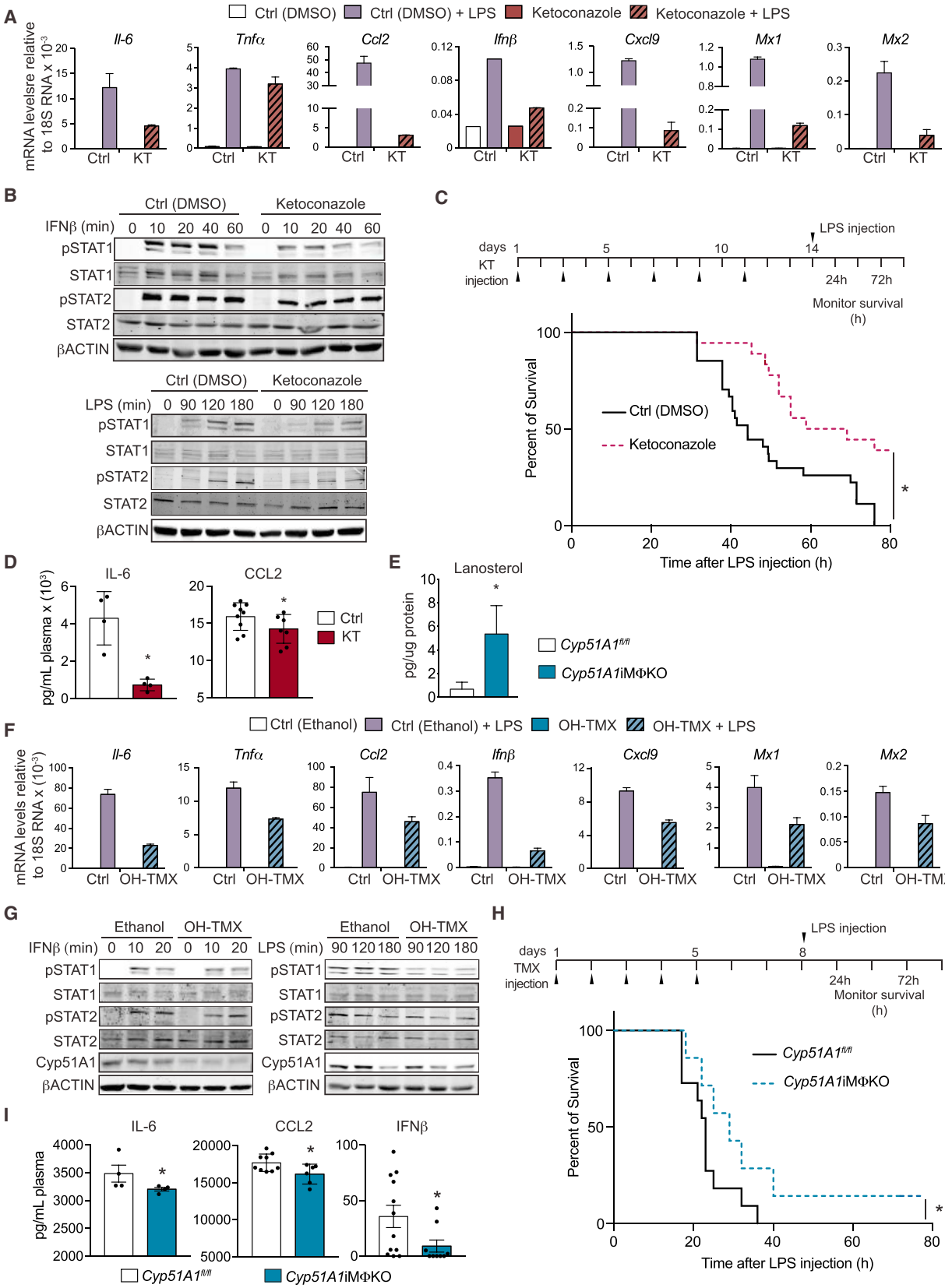
(F) ChIP sequencing analysis of PU.1, H4ac, or H3K4me1 pull-down in BMDM treated with LPS for 4 hr or control. Pioneer transcription factor of the myeloid lineage (PU.1), active enhancer markers histone 4 acetylation (H4ac), histone 3 lysine 4 mono-methylation (H3K4me1), and active promoter marker H3K4me3 (Ostuni et al., 2013).

(G) CYP51A1 protein levels of BMDMs treated with different HDAC inhibitors. Pan-HDAC: panobinostat (50 nM), HDAC1: CAY10398 (10 μM), HDAC3: RGFP966 (5 μM), HDAC4: tasquinimod (10 mM) for 1 hr before LPS or IFNβ stimulation for 12 hr (n = 3). Dashed blue lines are for treatment group separation and do not indicate cropped blots. H3 and acetylated histone H3 (AcH3) are controls for HDAC inhibitor action.

(A, D, and G) pSTAT1, iNOS, and COX2 are controls of activation of inflammatory activation. HSP90 and β-actin are loading controls. Results are expressed as mean ± SEM; *p < 0.05 versus Ctrl unless otherwise indicated. See also Figure S3.

Lanosterol Accumulation in Mice Improves Survival to Endotoxemic Shock via Reduced STAT1/STAT2-Mediated Expression of Pro-inflammatory Cytokines

To determine whether there is a functional role of lanosterol in TLR4-induced immune responses in macrophages, we first investigated the effect of its accumulation on the expression of several pro-inflammatory cytokines. To this end, we exploited the CYP51A1 inhibitor KT, which blocks the demethylation of lanosterol, thus promoting its accumulation (Iglesias and Gibbons, 1989). This azole drug is commonly used as an antimycotic agent, because it inhibits the conversion of lanosterol to ergosterol (Van Den Bossche et al., 1979). As expected, treatment of BMDMs with KT resulted in increased lanosterol content (Figure S4A). We found that, in response to LPS, KT-treated macrophages showed reduced expression of several inflammatory mediators of TLR4 and/or IFNβ activation in macrophages (Thomas et al.,



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2006), such as of *IL-6*, *Tnfα*, *Ccl2*, *Infβ*, *Cxcl9*, *Mx1*, or *Mx2* (Figure 4A). KT, as well as other azoles, has been described to have pleotropic anti-inflammatory effects in different cell types (Kanda and Watanabe, 2006; Tsuji et al., 2012). Thus, to avoid potential unspecific effects of KT not related with the accumulation of lanosterol, we silenced the expression *Cyp51A1* in macrophages (Figure S4B). Similarly to KT-treated macrophages, LPS-induced expression of pro-inflammatory mediators was diminished in BMDMs silenced for *Cyp51A1* (Figure S4C).

We then investigated the mechanism behind the diminished expression of pro-inflammatory mediators in response to TLR4 activation. KT treatment of BMDMs did not produce a significant difference in the activation of IκB kinase (IKK)α/β, NF-kappa-B inhibitor alpha (IκBα), or TANK binding kinase (TBK) in response to LPS stimulation (Figure S4D). Thus, it appears that the alteration in TLR4-mediated responses caused by lanosterol accumulation does not affect either MyD88 or TRIF signaling but instead acts upon the autocrine-paracrine loop after IFNβ induction. LPS strongly induces IFNβ expression through the TRIF pathway, and IFNβ signaling in turn triggers STAT1-STAT2 phosphorylation and activation (Ivashkiv and Donlin, 2014). In KT-treated BMDMs, phosphorylation of STAT1 and STAT2 in response to IFNβ or LPS stimulation was considerably reduced when compared to vehicle-treated macrophages (Figure 4B). Similar effects on the phosphorylation of STAT1 and STAT2 in response IFNβ or LPS were obtained when *Cyp51A1* was knocked down by siRNA (Figure S4E).

We then analyzed the effect of lanosterol accumulation in endotoxin shock in vivo. We injected KT-treated mice with a lethal dose of LPS and monitored their survival. KT-treated mice had a survival advantage over control DMSO-injected mice (Figure 4C). Furthermore, plasma concentrations of interleukin (IL) 6 or C-C motif chemokine ligand 2 (CCL2) were reduced compared to control-injected mice (Figure 4D). Although it is well established that LPS-induced lethality is caused by factors other than overproduction of cytokines, these data show the importance of lanosterol in preventing death from endotoxemia.

To further explore how CYP51A1 downregulation might participate in the regulation of LPS-mediated responses in macrophages, we generated a transgenic mouse expressing a tamoxifen (TMX)-inducible Mer-iCre fusion protein driven by the *Csf1r* promoter (Qian et al., 2011) and crossed with *Cyp51A1*$^{fl/fl}$ mice (Keber et al., 2011) to ablate *Cyp51A1* postnatally in the monocyte or macrophage compartment (i.e., *Cyp51A1*$^{fl/fl}$;~*Csf1r-Mer-iCre-Mer*, referred as Cyp51A1iMΦKO) and thus avoiding possible developmental effects of early embryonic conditional deletion (Gomez Perdiguero et al., 2015). 4-hydroxyTMX (OH-TMX)-induced ablation of *Cyp51A1* in cultured BMDMs obtained from Cyp51A1iMΦKO mice (Figure S4F) had increased lanosterol levels compared to BMDMs from *Cyp51A1*$^{fl/fl}$ control mice (Figure 4E). We then evaluated the effect on the expression of pro-inflammatory mediators in OH-TMX- versus ethanol-treated Cyp51A1iMΦKO BMDMs. In line with our previous results, in response to LPS stimulation, OH-TMX-treated Cyp51A1iMΦKO BMDMs showed reduced expression of *IL-6*, *Tnfα*, *Ccl2*, *Infβ*, *Cxcl9*, *Mx1*, or *Mx2* when compared to ethanol-treated ones (Figure 4F). Furthermore, STAT1-STAT2 phosphorylation was attenuated in response to IFNβ or LPS (Figure 4G). Treatment with OH-TMX of control *Cyp51A1*$^{fl/fl}$ BMDMs did not affect expression of inflammatory genes, CYP51A1 protein levels, or STAT1-STAT2 activation in response to inflammatory stimulation (Figures S4G and S4H). Consistently, when challenged with a lethal dose of LPS, TMX-treated Cyp51A1iMΦKO mice were more resistant to LPS-induced lethality than TMX-treated control *Cyp51A1*$^{fl/fl}$ mice (Figure 4G) and plasma levels of IFNβ and CCL2 were reduced when compared to that of control mice (Figure 4I).

Cumulatively, these data indicate that lanosterol accumulation through enzymatic inhibition or inducible genetic ablation of *Cyp51A1* reduces LPS/IFNβ-triggered STAT1-STAT2 activation in macrophages, which results in reduced expression of type I IFN-mediated cytokines and increased survival to endotoxemic shock.

Lanosterol Improves Phagocytosis by Increasing Membrane Fluidity and Bacteria Clearance and Confers Survival Advantage to *Listeria monocytogenes* Infection

Besides the important role of microphages in immunomodulation through the secretion of cytokines, their phagocytic activity

Figure 4. Lanosterol Decreases Inflammatory Cytokine Secretion and Improves Survival to Endotoxemic Shock

(A) qPCR analysis of mRNA levels of indicated genes of BMDMs treated with KT (10 μM) or DMSO 12 hr before LPS (100 ng/mL) for 4 hr.

(B) pSTAT1/STAT1 and pSTAT2/STAT2 protein levels of BMDMs treated with KT 12 hr before IFNβ (1,000 U/mL) (upper panels) or LPS (lower panels) for the indicated times (n = 3).

(C) Survival of WT mice treated with (25 mg/kg) of KT as indicated and subjected to a lethal dose of LPS (60 mg/kg). KT-treated mice (n = 18), DMSO-treated Ctrl mice (n = 25).

(D) Plasma levels IL-6 or CCL2 by ELISA 3 hr after LPS injection (60 mg/kg) of mice injected with KT as in (C). Each dot represents the mean of triplicate measurements of sample of individual animals.

(E) Quantification of lanosterol by GC-MS normalized protein content of BMDMs isolated from *Cyp51A1*$^{fl/fl}$ or *Cyp51A1iMΦKO* 3 days after of TMX Cre-mediated induction as described in Experimental Procedures (n = 3 per group).

(F) qPCR analysis of mRNA levels of indicated genes of *Cyp51A1iMΦKO* BMDMs and treated at day 5 of differentiation with OH-TMX (10 μg/mL) or ethanol for 2 days and then stimulated with LPS 100 ng/mL for 4 hr.

(G) pSTAT1/STAT1 and pSTAT2/STAT2 protein levels of *Cyp51A1iMΦKO* BMDMs treated with OH-TMX or ethanol as in (F) before IFNβ (upper panels) or LPS (lower panels) for the indicated times (n = 3). CYP51A1 protein levels are show as control of TMX-induced deletion.

(H) Survival of *Cyp51A1*$^{fl/fl}$ or *Cyp51A1iMΦKO* treated as indicated and subjected to a lethal dose LPS (60 mg/kg). *Cyp51A1*$^{fl/fl}$ (n = 11), *Cyp51A1iMΦKO* (n = 6).

(I) Plasma levels IL-6 or CCL2 determined by ELISA 3 hr after LPS injection (60 mg/kg) of mice of *Cyp51A1*$^{fl/fl}$ or *Cyp51A1iMΦKO* mice injected with TMX as indicated in (H). Each dot represents the mean of triplicate measurements of samples of individual animals.

(A and G) β-actin is a loading control. (A and F) Data are mean of duplicate samples ± SD of one representative experiment out of four with similar results. (C and H) Kaplan-Meier survival curves compared by log-rank test. *p < 0.05 (C) or *p < 0.1 (H). (D, E, and I) Results are expressed as mean ± SEM; *p < 0.05 versus Ctrl. See also Figure S4.

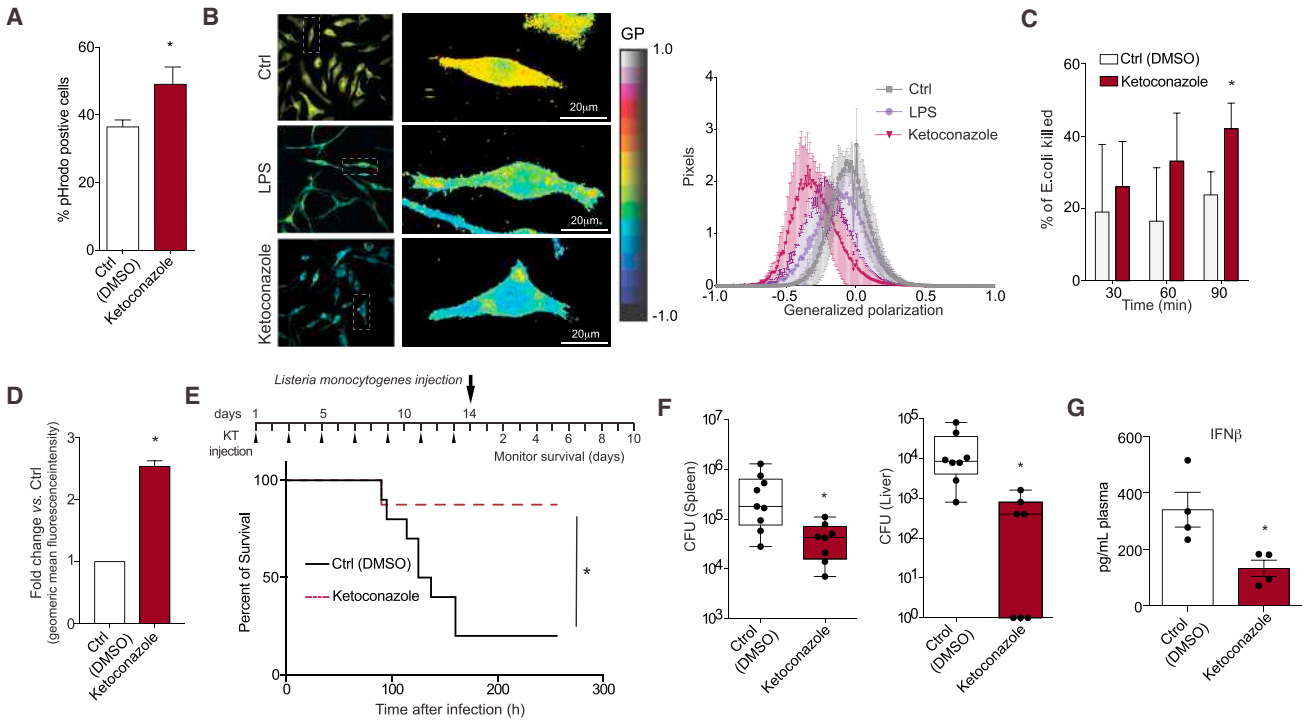

Figure 5. Lanosterol Improves Phagocytosis and Bacteria Clearance and Confers Survival Advantage to *Listeria monocytogenes* Infection

(A) Fluorescence-activated cell sorting (FACS) analysis of uptake of opsonized *E. coli* pHrodo particles in CD11b+/Ly-6G− cells from thioglycolate elicited peritoneal cells from KT- or DMSO-treated mice as in Figure 4C. Data are the percentage of pHrodo positive cells (n = 3).

(B) Representative micrographs of BMDMs treated with DMSO vehicle control, LPS (100 ng/mL) plus DMSO or KT (10 μM) for 12 hr, stained with laurdan (left) and analyzed as described in Experimental Procedures. Higher generalized population (GP) value indicates that membranes are more ordered and less dynamic or fluid (right). The GP value of each pixel was used to generate a pseudocolor GP image (left). Representative experiment out of three with similar results.

(C) Bacteria killing assay of tdTomato-*E. coli* in cells obtained as in (A) and analyzed by FACS. Data are the percentage of killed bacteria in CD11b+/Ly-6G− cells (n = 3).

(D) ROS determination with CellRox by FACS in BMDMs treated with KT (10 μM) for 12 hr. Data (geometric mean fluorescent intensity) are expressed as fold change versus DMSO Ctrl (n = 3, by triplicate).

(E) Survival of WT mice treated with 25 mg/kg of KT as in indicated and infected by retro-orbital injection with 1×10^5 *Listeria* particles. KT-treated mice (n = 10), DMSO-treated Ctrl mice (n = 15). Kaplan-Meier survival curves compared by log-rank test.

(F) Bacteria burden in spleen and liver 48 hr post-infection with 1.5×10^4 *Listeria* particles. Colony-forming units (CFUs) were determined from spleen and liver. Each dot represents data obtained from individual animals.

(G) Plasma levels of IFNβ by ELISA of mice treated with KT as indicated in (E) and then infected with 1.5×10^4 *Listeria* particles for 48 hr. Each dot represents the mean of a triplicate sample of individual animals.

Results are expressed as mean ± SEM; *p < 0.05 versus Ctrl. See also Figure S5.

is key to their microbicidal function (Mosser and Edwards, 2008). KT-treated macrophages exhibited improved phagocytosis of pHrodo-conjugated *E. coli* bacterial particles (Figure 5A). KT might also inhibit CYP3A4 (Svecova et al., 2008). Thus, to exclude off-targets effects, we used mifepristone, a non-azole CYP3A4 inhibitor, and did not observe any significant effect on pHrodo particle uptake (Figure S5A). When *Cyp51A1* was silenced, improved phagocytosis was observed (Figure S5B).

Plasma membrane composition affects lateral mobility of lipids and membrane-associated proteins, as well as phagocytosis. Previous reports suggest that lanosterol, because of its less planar structure compared to cholesterol, increases membrane fluidity (Miao et al., 2002), thus favoring phagocytosis (Berlin and Fera, 1977). Incubation of macrophages with LPS or KT produced an increase in membrane fluidity, as indicated by the decrease in generalized polarization assessed by the shift of laurdan emission spectrum (Figure 5B) and consistent with the accumulation of lanosterol observed in these conditions. Although additional mechanisms might be in play, accumulation of lanosterol (Figure S5C) is sufficient to cause an increase in membrane fluidity (Figure S5D). Upon KT treatment, macrophages displayed improved ability to kill bacteria (Figure 5C), which was associated with increased ROS production (Figure 5D). This effect was also observed in macrophages isolated from Cyp51A1iMΦKO mice (Figure S5E).

We then assessed the role of lanosterol accumulation in a model of bacterial infection in vivo. After assessing that KT did not affect the growth of *Listeria* (Figure S5F), we treated mice with KT and tested their survival upon infection. KT-treated mice were resistant to death following *Listeria* infection (Figure 5E). This effect was explained by a significantly lower bacteria burden in spleen and liver (Figure 5F). Macrophages also play

a major role in the early innate defense against *Listeria*. Increased production of type I IFNβ increases susceptibility to *Listeria* (Solodova et al., 2011), while *ifnar1*$^{-/-}$ mice are resistant (Auerbuch et al., 2004). Consistently, we found that plasma levels of IFNβ were significantly reduced in KT-treated mice (Figure 5G).

Our results indicate that innate immune transcriptional downregulation of *Cyp51A1* induces lanosterol accumulation in macrophages, promoting antimicrobial activity and favoring negative feedback of type I IFN-mediated responses.

DISCUSSION

In the present study, we identified lanosterol as an endogenous modulator of macrophage innate immune responses, expanding the connection between the roles of macrophages in host defense and those in cholesterol metabolism (Castrillo et al., 2003; Im et al., 2011). The key findings of the present study are that host responses to pathogens mediated by TLR4 through production of type I IFN reprogram lipid metabolism by genetically downregulating the cholesterol biosynthetic pathway, specifically affecting *Cyp51A1* expression and resulting in accumulation of lanosterol. In particular, we show that lanosterol reduces the capacity of macrophages to secrete inflammatory cytokines but enhances their phagocytic activity. Consequently, lanosterol accumulation reduces mortality to endotoxemia while increasing protection from infection by *Listeria.*

Previous studies have shown that macrophages respond to cholesterol loading by accumulating desmosterol, which suppresses inflammatory cytokine secretion (Spann et al., 2012). Conversely, in response to viral infections, which is integral to the protective immune response requiring a type I IFN, reduction of cholesterol biosynthesis has been is linked to activation of innate immunity (York et al., 2015), while geranylgeraniol, a non-sterol intermediate of the mevalonate pathway, reduces the antiviral effects of IFNs (Blanc et al., 2011). Here we provide evidence that accumulation of the first sterol of the cholesterol biosynthetic pathway, lanosterol, is a physiological response to TLR4 activation through a mechanism that requires the repression of the *Cyp51A1* gene and that is dependent on type I IFN production. In agreement with previous studies, we observed decreased expression of several enzymes of the synthesis of cholesterol after TLR4 stimulation of macrophages (Dennis et al., 2010), a phenomenon that is also observed upon type I IFN activation (Blanc et al., 2011), and this leads to a slight reduction of flux through the cholesterol biosynthetic pathway (Dennis et al., 2010; York et al., 2015) and the accumulation of lanosterol (Dennis et al., 2010). Lanosterol is the substrate of both CYP51A1 in the Bloch pathway and DHCR24 in the Kandutsch-Russell pathway. The expression of both genes is reduced after TLR4 activation in vitro in human macrophages and both in vitro and in vivo in murine macrophages. In most tissues, DHCR24 preferentially reduces more distal sterols (Mitsche et al., 2015), and lanosterol is the least preferred substrate for DHCR24 (Bae and Paik, 1997), which indicates that the accumulation of lanosterol observed upon TLR4 stimulation is primarily a consequence of a transcriptional downregulation of *Cyp51A1*.

In agreement with previous reports, we show that in addition to the downregulation of *Cyp51A1*, TLR4 agonists and type I IFN stimulate the expression *Ch25h* and production of 25-HC in macrophages (Blanc et al., 2013; Dennis et al., 2010; Liu et al., 2013; Lund et al., 1998; Reboldi et al., 2014; Shibata et al., 2013), which is a well-recognized type I IFN effector response (Blanc et al., 2013; Blanc et al., 2011; Reboldi et al., 2014) and a negative regulator of cholesterol biosynthesis enzymes through the repression of SREBP processing in vitro (Jeon and Osborne, 2012; Strömstedt et al., 1996). 25-HC-mediated modulation of the type I IFN inflammatory responses of BMDMs has been linked to 25-HC-mediated decreased nuclear localization and expression of SREBP2 (Blanc et al., 2011). In line with this finding, RNA-seq analysis on LPS-treated BMDMs obtained from *Ch25h*$^{-/-}$ showed elevated expression of SREBP targets when compared to LPS-treated WT BMDMs (Reboldi et al., 2014). However, *Ch25h*$^{-/-}$ mice exhibit intact cholesterol metabolism (Diczfalusy, 2013; Russell, 2003). In the absence of endogenous 25-HC synthesis, we found that the kinetics of the downregulation of CYP51A1 in response to LPS stimulation is indistinguishable from the one observed in WT macrophages and that lanosterol levels, in response to LPS stimulation, are increased in in both *Ch25h*$^{-/-}$ and WT BMDMs. These observations suggest that newly synthesized 25-HC is not responsible for the early transcriptional downregulation of *Cyp51A1* expression and subsequent lanosterol accumulation. TLR4 activation Pol II occupancy in the Cyp51A1 promoter was observed at 1 hr after LPS stimulation, which is consistent with a 25-HC-independent mechanism.

SREBP1 expression is induced in macrophages upon inflammatory stimulation, which both promotes acute inflammatory responses (Im et al., 2011; Reboldi et al., 2014) and contributes to the resolution of the pro-inflammatory TLR4 signaling (Oishi et al., 2017). However, the reduction of CYP51A1 expression in response to LPS was observed in the absence of SREBP processing or SREBP2 expression, indicating that additional mechanisms must account for the early decreased expression of *Cyp51A1* observed upon inflammatory stimulation. The reported 25-HC-mediated decrease of SREBP2 processing and expression (Blanc et al., 2011) could be responsible for the maintenance of CYP51A1 downregulation or for the modulation of other enzymes of the cholesterol biosynthetic pathway after inflammatory stimulation.

Several studies have highlighted an important role for chromatin remodeling in the control of inflammatory gene expression and macrophage function (Medzhitov and Horng, 2009). In macrophages, the myeloid lineage transcription factor PU.1 establishes cell-type-specific chromatin architecture, and it maintains the accessibility of the genomic *cis*-regulatory information for constitutive and stimulus-inducible transcriptional regulation (Mancino et al., 2015). PU.1 occupancy of the *Cyp51A1* locus is reduced after LPS stimulation, and this is accompanied by a marked decrease in the active enhancer markers H4ac and H3K4me1, as well as in active promoter marker, H3K4me3. This phenomenon was not observed in other cholesterol homeostasis genes, i.e., LSS, LDLR, or Dhcr7 (Ostuni et al., 2013). In both TLR and IFN signaling, different HDACs regulate innate immune responses and mostly have a repressive role (Aung et al., 2006; Chen et al., 2012). Altogether, our data show that LPS, acting through type I IFN-recruited HDAC1, diminished histone

acetylation of the *Cyp51A1* locus and reduces Pol II-mediated transcription of *Cyp51A1*.

In response to TLR4 stimulation, activation of MyD88-dependent and MyD88-independent pathways of macrophage results in the release a variety of inflammatory cytokines such as tumor necrosis factor (TNF), IL-1 and IL-6 (MyD88 dependent), and IFNβ (MyD88 independent and TRIF dependent) (Mahieu and Libert, 2007; McNab et al., 2015). Several lines studies have indicated that type I IFNs are important mediators in endotoxemia (Mahieu and Libert, 2007) and, in synergy with other cytokines and inflammatory products, can lead to organ damage (Mahieu and Libert, 2007). Thus, to avoid excess inflammation and tissue damage, it is important that the production of proinflammatory cytokines is kept under control (Serhan et al., 2007). In line with this, mice ubiquitously deficient for *Ifnβ*, *Ifnar1*, *Tyk2*, or *Stat1* genes involved in type I IFN signaling show decreased expression of several proinflammatory cytokines and ISGs in response to LPS and exhibit a better survival to endotoxemia (Dejager et al., 2014; Karaghiosoff et al., 2003; Mahieu and Libert, 2007). One of the most intriguing results of our present work is that accumulation of lanosterol, via either inhibition or knockdown of *Cyp51A1* in macrophages, results in reduced STAT1-STAT2 activation in response to autocrine secretion of type I IFNs after TLR4 activation. This might at least partly contribute to the attenuated expression of *Infβ* and other ISGs, including *Ccl2*, *Cxcl9*, *Mx1*, and *Mx2*, as well as *Il6* or *Tnfα* in LPS-mediated autocrine IFNβ regulation in $Ifn\beta^{-/-}$ macrophages (Thomas et al., 2006). Consistently, mice treated with KT and Cyp51A1iMΦKO are less sensitive to LPS-induced mortality and have lower plasma levels of pro-inflammatory cytokines. Thus, type I IFNs, by acting as the underlying stimulus responsible for the LPS-mediated downregulation of CYP51A1, promote lanosterol accumulation, which in turn provides negative-feedback regulation for inflammatory activation.

One of the main physiological roles of macrophages is related to its phagocytic activity. Changes in phagocytosis correlate with changes in membrane composition and fluidity (Avery et al., 1995). Accumulation of lanosterol in macrophages via pharmacological inhibition of Cyp51A1, LPS-mediated Cyp51A1 downregulation or direct lanosterol loading, increased membrane fluidity. As such, we also observed an increased phagocytic activity. Lanosterol structural conformation is less planar compared to cholesterol; therefore, increased lanosterol content in membranes increases their fluidity and favors phagocytosis (Miao et al., 2002). In addition, we observe an increase in ROS production, which explains the enhanced bacterial killing and clearance thus enhancing protection form *Listeria* infection. We also found that KT-treated mice exhibit decrease secretion of IFNβ, which may also account for increased survival to *Listeria* infection. The production of type I IFN is associated with suppression of the innate response and increased susceptibility to *Listeria* infection (Solodova et al., 2011). In line with these findings, $Ifnra1^{-/-}$ is resistant to endotoxemia and to *Listeria* infection (Auerbuch et al., 2004; Dejager et al., 2014). Thus, lanosterol via decreased activation of STAT1-STAT2 is an essential part of the negative-feedback regulation mechanism regulating cytokine production during inflammatory conditions involving type I IFNs and conferring a survival advantage to endotoxemia and resistance to *Listeria* infection.

KT has been described to have pleotropic anti-inflammatory effects (Kanda and Watanabe, 2006; Tsuji et al., 2012). However, the underlying molecular mechanisms remain poorly understood (Friccius et al., 1992; Hau et al., 2014), and none of these studies have evaluated the contribution KT-mediated inhibition of CYP51A1 and accumulation of lanosterol to the reported anti-inflammatory effects. Our findings suggest that KT, which is used exclusively to treat mycotic infections in immunocompromised patients, could be of benefit in additional clinical settings by ameliorating macrophage microbicidal activity or blocking harmful cytokine overproduction.

In summary, our findings indicate that TLR4-mediated transcriptional downregulation of *Cyp51A1* induces lanosterol accumulation in macrophages, promoting antimicrobial activity and favoring negative feedback of type I IFN-mediated responses. It will therefore be of interest to investigate the role lanosterol in other disease contexts in which inflammation plays a pathogenic role.

EXPERIMENTAL PROCEDURES

Further details and an outline of resources used in this work can be found in Supplemental Experimental Procedures. Briefly, raw data from microarray experiments were normalized and analyzed by GeneSpring GX software v.11.5 (Agilent Technologies). Data are deposited in NCBI GEO: GSE89559. Other relevant data were obtained from GEO: GSE38379, GSE38892, and GSE21910. Animal studies were approved by the Institutional Animal Care and Use committee of Yale University School of Medicine. WT C57BL/6 and $Ch25h^{-/-}$ 6- to 12-week-old mice were purchased from The Jackson Laboratory. $Ifna1r^{-/-}$ and $Myd88^{-/-}$ animals were a gift from Dr. A. Iwasaki and Dr. D. Goldstein. $Cyp51A1^{fl/fl}$;$^{\sim}$*Csf1r-Mer-iCre-Mer* (Cyp51A1iMΦKO) mice were generated by crossing B6.129P2-Cyp51tm1Bfro/J (i.e., $Cyp51A1^{fl/fl}$) female animals with FVB-Tgm(Csf1r-cre/Esr1*)1Jwp/J (i.e., Csf1r-Mer-iCre-Mer) males. Endotoxemic shock experiments were performed with littermates from breeding $Cyp51A1^{fl/fl}$ females with Cyp51A1iMΦKO male mice and were used for experiments treated with TMX for 5 days before LPS injection or with WT mice treated with KT every other day for 14 days before LPS. WT mice treated with KT were also used for *Listeria monocytogenes* infection, bacteria burden, and survival experiments. Statistical analysis was performed using Student's t test, one-way ANOVA with Bonferroni correction for multiple comparisons, or the log-rank test when appropriate. Normality was checked using the Kolmogorov-Smirnov test. A nonparametric test (Mann-Whitney) was used when data did not pass the normality test. $p \leq 0.05$ was considered statistically significant.

ACCESSION NUMBERS

The accession number for the microarray data reported in this paper is GEO: GSE89559.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and five figures and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2017.05.093.

AUTHOR CONTRIBUTIONS

E.A., C.F.-H., and Y.S. conceived and designed the study. E.A., M.F.-F., A.C.-D., W.T., J.M.-M., G.W.C., and Y.S. performed experiments and analyzed data. J.S.P., G.W.C., M.A.L., D.W., C.F.-H., and Y.S. assisted with experimental design and data interpretation. E.A. and Y.S. wrote the manuscript, which was commented on by all authors.

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