SUPPLEMENTAL DATA

Supplemental Experimental Procedures

Reagents and antibodies
Mouse IFNα and IFNβ (PBL Interferon Source), Lanosterol (Avanti Lipids), Ketoconazole, Simvastatin, Zaragozic acid, Actinomycin D, DMSO (all from Sigma). Antibodies against Cyp51 (ProteinTech), Cox2 (Cayman Chemical), Nos2, Dhcr24, Histone H3 (96C10), PhosphoStat1 (Tyr701)(D4A7), Phospho-IKKa/b (Ser176/180), Phospho-IKBa (Ser32) (14D4), Phospho-NF-kB p65 (Ser536)(93H1), Phospho-TBK1/NAK (Ser172)(D52C2), TBK1/NAK (D1B4) and Stat2 (all from Cell Signaling), HSP90 (#610418 BD Bioscience), SREBP2 (from culture supernatant of hybridoma IgG-1D2 from ATCC), Anti-acetyl-Histone H3 (#06-599) and Anti-acetyl-Histone H4 (#06-866)(Millipore), Stat1 p84/p91 (B-9), IKB-a (C-21) (Santa Cruz) and pStat2 (Abcam, ab53132). The following antibodies were used for ChIP: Pol2 (Abcam, ab5408), Pol2 S5 (Abcam, ab5131), Pol2 S2 (Abcam ab5095) and normal IgG (Cell Signaling, #2729).

Cell culture
Bone marrow derived macrophages (BMDM) were differentiated as described previously (Rotllan et al. 2015) in Iscove's Modified Dulbecco's medium (IMDM) containing 20% of FBS and supplemented with 20% of L-929 cells conditioned. After 7 d in culture, contaminating non-adherent cells were eliminated, and adherent cells were treated and harvested for the assays. BMDM were cultured in RPMI media containing 20% FBS unless specifically indicated that the media contained 20% LPDS. Lipoprotein-deficient serum (LPDS) was prepared from FBS as described (Suarez et al., 2005). When experiments were performed with BMDMs obtained from
Peripheral blood mononuclear cells were isolated from whole human blood (Ficoll-Paque) and plated on fibronectin-coated plates in RPMI supplemented with pen/strep and L-glutamine at a density of $5x10^7$ PBMC per 75 cm^2. Monocytes were allowed to adhere for 1-2 hours at 37°C in a 5% CO_2 atmosphere. Supernatant with non-adherent cells was discarded and adherent cells were washed and supplemented with 10% FBS/RPMI with pen/strep and L-glutamine. Recombinant human M-CSF (100ng/mL) was added the following day and cells were allowed to differentiate into macrophages for 7 days(Martinez et al., 2006). In some instances, peritoneal macrophages that were collected after intraperitoneal injection of aged sterile 3% thioglycollate for 3 days as described previously (Aryal et al., 2016)

Mice
All the experiments conformed to the ethical principles and guidelines approved by the Institutional Animal Care and Use Committee (IACUC) Yale university School of Medicine. Wild type (WT) C57BL/6 and *Ch25h-/-* 6-12 weeks old mice were purchased from The Jackson Laboratory (Bar Harbor, ME, USA). *Ifna1r*$^{-/-}$, and *Myd88*$^{-/-}$ animals were a kind gift from Dr. A. Iwasaki and Dr. D. Goldstein. *Cyp51A1*$^{f/f}$*~ Csf1r-Mer-iCre-Mer* (Cyp51A1iMΦKO) mice were generated by crossing B6.129P2-Cyp51tm1Bfro/J (i.e. *Cyp51A1*$^{f/f}$) females animals with FVB-Tg(Csf1r-cre/Esr1*)1Jwp/J (i.e.Csf1r-Mer-iCre-Mer)males. Littermates from breeding *Cyp51A1*$^{f/f}$ females with Cyp51A1iMΦKO male mice were used for experiments. At 8 week of age were treated with intraperitoneal injection of 60mg tamoxifen/kg in peanut oil for 5 consecutive days. Experiments were performed at 3 days after the first injection. For inhibition of CYP51A1 in WT mice sex- and age-matched 6-12 weeks old C57BL/6 WT mice were injected with 25mg/kg of Ketoconazole (Sigma) every other day for 14 days. DMSO was used as vehicle control. Animals were housed under constant temperature and humidity in a 12h controlled dark/light cycle in a specific pathogen-free environment.

Microarray analysis.
BMDM were stimulated with 10ng/mL of LPS and 20ng/mL of IFNγ for 8 hours prior to harvesting cells in Trizol reagent (Invitrogen). RNA was purified RNAeasy Isolation Kit (Qiagen). The purity and integrity of total RNA sample were verified using the Agilent Bioanalyzer (Agilent Technologies, Santa Clara, CA). RNA was hybridized to Illumina expression profiling microarrays according to the manufacturer's directions. Three biological replicates for each condition were used for microarray analysis GEO Submission (GSE89559). Raw data were normalized and analyzed by GeneSpring GX software version 11.5 (Agilent Technologies). mRNAs showing an altered expression in LPS/IFNγ treatment compared with control were identified using a *t* test unpaired with $p<0.05$ and fold change ≥1.5, asymptotic *P* value computation. Ingenuity pathway analysis of downregulated genes was performed with Ingenuity Pathways Analysis software (www.Ingenuity.com) according to software instructions.

RNA extraction and qRT-PCR analysis
Cells were treated as indicated and total RNA was isolated using TRIzol reagent (Invitrogen) according to the manufacturer's protocol. One microgram of total RNA was reverse transcribed using the iScript RT Supermix (Bio-Rad, Hercules, CA), following the manufacturer's protocol. qRT-PCR was performed in triplicate using SsoFast EvaGreen Supermix (BioRad) on a Real-Time Detection System (Eppendorf, Hauppauge, NY, USA or Bio-Rad, Hercules, CA)(Rayner et al., 2010). The mRNA levels were normalized to 18S RNA as a housekeeping gene.

Western blot
Cells were lysed in ice-cold buffer containing 50 mM Tris-HCl, pH 7.5, 125 mM NaCl, 1% NP- 40, 5.3 mM NaF, 1.5 mM NaP, 1 mM orthovanadate and 1 mg/ml of protease inhibitor cocktail (Roche) and 0.25 mg/ml AEBSF (Roche). Cell lysates were rotated at 4^{o}C for 1 h before the insoluble material was removed by centrifugation at 12000 x *g* for 10 min. After normalizing for equal protein concentration, cell lysates were resuspended in SDS sample buffer before separation by SDS-PAGE (Goedeke et al., 2015; Rayner et al., 2010). Following overnight transfer of the proteins onto nitrocellulose membranes, the membranes were probed with the primary antibodies at a dilution of 1:1000 (hybridoma supernatant at a dilution of 1:10). Protein bands were visualized using the Odyssey Infrared Imaging System (LI-COR Biotechnology). Densitometry analysis of the gels was carried out using ImageJ software from the NIH (http://rsbweb.nih.gov/ij/).

Analysis of sterol de novo biosynthesis by HPLC.
BMDMs were incubated RPMI medium containing 10% LPDS. 30 min before LPS stimulation medium was supplemented with 3 μC/ml of [2-^{14}C] and incubated for 24h. BMDM were washed twice with ice-cold PBS and lysed with 10% (w/v) KOH, as described (Canfran-Duque et al., 2013; Suarez et al., 2002). Briefly, samples were treated sequentially with chloroform-methanol (2:1, v/v) and distilled water to obtain the lipid and water-soluble fractions. The lipid extract was further sub-fractionated into the saponifiable and non-saponifiable fractions. Nonsaponifiable lipids were resuspended in hexane and used for sterol separation by reverse-phase HPLC using a Mediterranea SEA 18.5 μm pore size C18 (250 × 4.6 mm, Teknokroma) column and radioactivity counting as described previously (Fernandez et al., 2005).
Lipids were eluted with acetonitrile:water (95:3.5 v/v) at a flow rate of 1.2 ml/min. The eluent was monitored simultaneously for UV absorption (Diodo Array 168 detector, Beckman Coulter) and online radioactivity counting (Radioactivity detector LB 509, Berthold Technologies). The eluting sterols were identified by comparison of the retention time and the UV spectrum with those of pure standards. The HPLC method could not resolve zymostenol from cholesterol or zymosterol from desmosterol. Radioactivity incorporation into sterols was performed using batch integration functions of the RadioStar software package (version 4.6, Berthold Technologies). Briefly, background signal or noise was determined in area free of sterols in the chromatogram (between 50-55 min). Average Background was similar amongst the samples and subtracted to all the samples for the base line reach 0 (Threshold ,TH). CPM analysis was selected and Peak Width (PW), Minimal Height (MH), Minimal Area (MA) and TH were fixed to PW=35, TH=0, MA=0 y MH=10. Horizontal integration was performed to calculate the area of the pick. Qualitatively similar results were obtained when valley to valley integration method was utilized.

Analysis of total sterols by GC/MS.
Extracted sterols were analyzed as previously described (Canfran-Duque et al., 2013; Haskins et al., 2015). Total sterols were dissolved in tert-butylmethylether (TBME) and derivatized with silylation reagent (N-methyl-N-trimethylsilyl-trifluoroacetamide / trimethylsilylimidazole 9:1, v/v). Derivatized sterols were analyzed using an Agilent 6890N GC and an Agilent 5975C MS detectors (Agilent Technologies) with an Agilent DB-5ms column (30m x 0,25mm x 0,1μm) or a HP-1 column (12m x 0,2mm x 0,33μm). Helium was used as carrier gas at a flow rate of 1.0 ml/min and variable pressure according to retention time locked for 5-α cholestane. The inlet temperature was maintained at 260 ºC. The oven temperature was initially held at 55 ºC for two minutes and was increased to 260 ºC at a rate of 55 ºC/min, held 10 minutes, and then to 310 ºC at a rate of 7ºC/min. Total runtime was 22.35min. In other instances (using HP-1 column), the oven temperature was initially held at 225 ºC for two minutes and was increased to 280 ºC at a rate of 25 ºC/min, held 10 minutes for a total runtime of 15min. The injector was settled to splitless (injection volume 2 μl). GC/MS was carried out using in electron ionization at 70 eV. For quantization purposes MS detector were operated in selective ion monitoring (SIM) mode following at least one quantifier and two qualifying ions for each sterol. Peak identification was achieved both by retention time comparison with known external standards and by monitoring characteristics fragment ions according to **RRT Table**. The intensities of the quantifier ion for each sterol were directly interpolated on the calibration curves constructed from pure standards.

The limit of quantification for the different sterols ranged between 0.02 ng for lanosterol to 0.5 ng for 5α-Cholesta-7,24-dien-3b-ol, directly injected on column Agilent DB-5ms column.

Substance	**RRT** *(Agilent DB-5ms column)*	**Quantifier & qualifier ions** *(Agilent DB-5ms column)*	**RRT** *(HP-1 column)*	**Quantifier & qualifier ions** *(HP-1 column)*
5α-Cholestane (Int. Std)	1	372, **217**, 149	1	372, **217**, 149
Cholesterol	1.414	458, **368**, 329	1.33	458, **368**, 329
Cholestanol	1.436	460, **445**, 215	n.a.	n.a.
Desmosterol	1.483	456, **129**, 456	1.884	**131**, 369
7-Dehydrocholesterol	1.497	456, **351**, 325	n.a.	n.a.
Lathosterol	1.523	**458,** 255**,** 213	n.a.	n.a.
Zymosterol	1.530	**456**, 441, 351	n.a.	n.a.
5α-Cholesta-7,24-dien-3b-ol	1.592	**456**, 441, 343	n.a.	n.a.
Dihydrolanosterol	1.663	500, 486, **395**	n.a.	n.a.
Lanosterol	1.723	498, **393**, 241	1.723	498, **393**, 241
25-OH-Cholesterol	n.a.	n.a.	1.884	**131**, 369

RRT Table. Relative retention times (RRT), quantifier and quantifier ions employed in the analysis of sterols by GC/MS. *The relative retention (RRT) was normalized to the retention time obtained for the internal standard (5α-Cholestane, 10.885 min) with the two different columns used and as noted. The qualifier ions chosen were those of the molecular ion (M+) and two of the most abundant fragments present in the MS spectra obtained from standards. The quantifier ion is highlighted in bold, n.a. (not analyzed)*

Chromatin immunoprecipitation

ChIP was performed as previously described(Bhatt et al., 2012; Nicodeme et al., 2010). Briefly, BMDM were treated with 100ng/mL of LPS for the indicated times. To cross-link proteins to the DNA, cells were treated with 1% formaldehyde for 10 minutes and the cross-linking reaction was terminated by addition of glycine to a final concentration of 0.125 M. Cells were then collected by centrifugation, washed twice in cold 1x PBS plus protease inhibitors and incubated in 2 ml cold ChIP lysis buffer 1 (50 mM HEPES pH 7.6, 140 mM NaCl, 1 mM EDTA, 10% glycerol, 0.5% NP-40, 0.25% Triton X-100) for 10 min at 4°C. The samples were then centrifuged at 3000 xg at 4°C for 5 min, incubated with 2 ml cold ChIP lysis buffer 2 (10 mM Tris-HCl pH 8, 200 mM NaCl, 1 mM EDTA, 0.5 mM EGTA) for 10 min at 4°C, and centrifuged at 3000 xg for 5 min at 4°C. The supernatant was removed and nuclei pellet resuspended in 270 µl ChIP lysis buffer 3 (10 mM Tris-HCl pH 8, 0.5% Sarkosyl, 0.5 mM EGTA, 1 mM EDTA, 100 mM NaCl, 0.1 % Na-Deoxycholate). Nuclear lysates were sonicated 2 x 5 min and 1 x 7.5 min (30 sec ON/OFF) on high using a Diagenode Biorupter (Diagenode, UCD-200 TO) to generate DNA-fragments of approximately 200 to 600 bp. After checking chromatin size by agarose gel electrophoresis, extracts were clarified by centrifugation at max speed for 10 min at 4°C, pre-cleared with 60 µl of Protein G beads (Millipore #16-201) for 1h at 4°C, and then incubated overnight 4°C at specific antibodies (Pol2, Pol2 S5) or normal rabbit IgG. ChIP assays were performed using $1x10^6$ cells and 2 µg of antibody coupled to 60 µL beads. Antibody-bound complexes were then captured by incubation with 60 µl of Protein G beads for 1h at 4°C. Beads were washed once with low-salt immune complex wash buffer (SDS 0.1%, Triton X-100 1%, EDTA 2mM, Tris-HCl pH8.1 20 mM, NaCl 150 mM), once with high salt immune complex wash buffer (SDS 0.1%, Triton X-100 1%, EDTA 2mM, Tris-HCl pH8.1 20 mM, NaCl 500 mM), twice with LiCl immune complex wash buffer (LiCl 0.25M, NP-40 1%, deoxycholic acid 1%, EDTA 1mM and Tris-HCl pH 8.1 10 mM) and twice with TE buffer. Antibody-bound complexes were then eluted by incubation with 200 µl of Elution buffer (100 mM $NaHCO_3$, 1% SDS) for 15 min with gentle rotation followed by a second 15 min elution with 200 µl of Elution buffer. To reverse crosslinks, the eluates were combined, treated with 5 M NaCl, and incubated overnight at 65°C. Samples were then incubated with 1 µl RNase A, incubated for 30 min at 37°C and treated with 4 µl 0.5 M EDTA, 8 µl 1M Tris-HCl, and 1 µl Proteinase K for 1h at 45°C. DNA was purified using the QIAquick PCR Purification Kit (QIAGEN) according to the manufacturer's instructions and eluted in 50 µl of TE buffer(Goedeke et al., 2015).

For regular ChIP and for validation of ChIP-Seq, ChIP DNA was analyzed via qPCR as described above.

siRNA Knockdown of SREBP2 or Cyp51A1
At day 5 of differentiation, BMDMs were transfected with 30 nM *SREBP2* or *Cyp51A1* siRNA SMART pools or 30 nM control non-silencing siRNA (NS) (5'AATTCTCCGAACGTGTCACGT3') (Dharmacon) using RNAiMax (Life Technologies) as previously described (Chamorro-Jorganes et al., 2016) and assayed after 48 hours.

Endotoxemic shock model.
Mice were treated with Ketoconazole, DMSO, or tamoxifen, as described above, and then injected with 60mg/kg of LPS (Sigma). Survival was monitored every 6 hours. For plasma cytokine analysis, mice were injected with 60mg/kg of LPS and plasma collected after 3 hours.

Enzyme-linked immunosorbent assay.
IL6 or CCL2 ELISA Ready-set-go kits were purchased from eBioscience, antibodies pairs and standards for IFNβ were purchase from Biolegend. Assays were performed according to manufacturer's directions.

Phagocytosis and bacteria killing in peritoneal macrophages
Ketoconazole or DMSO treated mice were injected with 3 mL of 4% Thioglycollate for 3 days. The peritoneal cavity was then washed with 10mL of ice-cold DMEM and fluid carefully aspirated. After removal of erythrocytes with ACK lysis buffer, cells were counted and used for further experiments. Bacteria killing: 1x10^6 peritoneal cells were incubated in HBSS + 1% BSA with 1x10^7 dsRed-expressing live *E.coli* for 45 minutes at 37 °C, then pelleted at 200xg and extensively washed at least 3 times in HBSS. Peritoneal cells were then allowed to kill *E.coli* for 60 minutes. Subsequently, cells were fixed with 1% PFA and stained with Pacific Blue-CD11b and APC-Ly-6G and fluorescence acquired by FACS. Phagocytosis: 1x10^6 peritoneal cells were incubated in 20 mM HEPES HBSS with Ca2+ with 20mg of opsonized pHrodo® Red E. coli BioParticles® Conjugate for Phagocytosis (LifeTechnologies) at 37°C for one hour, then stained and analyzed as above.

Phagocytosis in BMDMs macrophages
0.5x10^6 BMDM were treated with 10 mM EDTA, 4 mg/ml Lidocaine in PBS for 10 minutes on ice and gently removed from culture plate, incubated at 37°C with gentle shaking in 20 mM HEPES HBSS with Ca2+ with 10mg of opsonized pHrodo® Red E. coli BioParticles® Conjugate for Phagocytosis (LifeTechnologies) for one hour, then analyzed as above.

Reactive oxygen species production.
BMDM were treated with LPS for 24 hours and then incubated with CellROx Deep red (Invitrogen) at 37°C for 20 minutes, gently detached from the plate and fluorescence was analyzed using a LSRII cytometer (BD).

Assessment of membrane fluidity by multiphoton microscopy
Cells were plated on glass coverslips, and then treated for 24h with ketoconazole or lanosterol. Cells were stained with 1μM Laurdan in serum-free media at 37°C for 30 minutes, washed extensively with PBS and fixed with 4% PFA for 5 minutes. Coverslips were kept in PBS and maintained at 37°C during acquisition.
Image stacks were acquired with a LaVision TriM Scope II (LaVision Biotec, Germany) microscope equipped with a tunable Chameleon Vision II (Coherent, USA) Ti:Sapphire laser set to an excitation wavelength of 800nm and a 40X water immersion lens (N.A. 1.1; Zeiss, USA). A field of view of 228 x 228 um was scanned at 600 Hz with a final image resolution of 0.22um/pixel. Resulting emission was collected with two bandpass filters: 435/90 nm and 525/50 nm.
GP images and histograms were calculated with the ImageJ script "GP images analysis macro(Owen et al., 2011).

***Listeria monocytogenes* infection, bacteria burden and survival**
Sex- and age-matched adult mice were infected with 100,000 virulent *Listeria monocytogenes*.
For bacteria burden experiments, mice were injected with 15,000 virulent Listeria (LM10403S) intravenously. To estimate the number of bacteria in mouse tissue, homogenized spleen and livers in PBS were serially diluted and plated on Brain Heart Infusion Agar plates (Becton Dickinson Microbiology Systems). Colony forming unit (CFU) numbers were counted after 24 h. For Ketoconazole toxicity assay Listeria, 1x10^6 particles of Listeria monocytogenes were inoculated with the indicated dose of Ketoconazole or controls. O.D. was measured after 24h culture.

Statistical analysis

Animal sample size for each study was chosen based on literature documentation of similar well-characterized experiments. The number of animals used in each study is listed in the figure legends. No inclusion or exclusion criteria were used and studies were not blinded to investigators or formally randomized. *In* vitro experiments were routinely repeated at least three times unless otherwise noted. Data are expressed as mean ±SEM unless otherwise indicated. Statistical differences were measured using an unpaired two-sided Student's *t*-test, one-way ANOVA with Bonferroni correction for multiple comparisons or logrank 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. A value of $p \leq 0.05$ was considered statistically significant. Data analysis was performed using GraphPad Prism Software Version 7 (GraphPad, San Diego, CA).

Key Resources Table

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Cyp51A1	Proteintech	13431-1-AP
Dhcr24	Cell Signaling	#2033
Nos2	Cell Signaling	#13120
Cox2	Cayman Chemical	*160126*
Histone H3 (96C10)	Cell Signaling	# 3638
PhosphoStat1 (Tyr701)	Cell Signaling	# 7649
Phospho-IKKa/b (Ser176/180)	Cell Signaling	# 2687
Phospho-IKBa (Ser32) (14D4)	Cell Signaling	# 2859
Phospho-NF-kB p65 (Ser536)(93H1)	Cell Signaling	# 3033
Phospho-TBK1/NAK (Ser172)(D52C2)	Cell Signaling	# 5483
TBK1/NAK (D1B4)	Cell Signaling	# 3504
Stat2	Cell Signaling	# 4597
HSP90	BD Bioscience	610418
Anti-acetyl-Histone H3	Millipore	06-599
Anti-acetyl-Histone H4	Millipore	06-866
Stat1 p84/p91 (B-9)	Santa Cruz	sc-271661
IKB-a (C-21)	Santa Cruz	# sc-371
Pol2	Abcam	ab5408
Pol2 S5	Abcam	ab5131
Pol2 S2	Abcam	ab5095
PhosphoStat2	Abcam	ab53132
Normal IgG	Cell Signaling	#2729
Biotin anti-mouse IFN-β (clone MIB-5E9.1)	BioLegend	508105
Purified anti-mouse IFN-β (clone Poly5192)	BioLegend	519202
Bacterial and Virus Strains		
Listeria monocytogenes	Dr. Dianqing Wu	LM10403S
Biological Samples		
Chemicals, Peptides, and Recombinant Proteins		
Simvastatin	Sigma	S6196
Zaragozic acid	Sigma	Z2626
ActinomycinD	Sigma	A1410

Parthenolide	Sigma	P0667-5MG
Mifepristone	Sigma	M8046-100MG
Ketoconazole	Sigma	K1003
Acetic Acid, Sodium Salt, [1,2-14C]	Perkin Elmer	NEC553001MC
Lipopolysaccharides from E.coli O26:B6	Sigma	L 8274
RGFP966	Cayman Chemicals	16917
Panobinostat	Cayman Chemicals	13280
Tasquinimod	Cayman Chemicals	17692
CAY10398	Cayman Chemicals	*89740*
Lanosterol	Avanti Polar Lipids	700063
IFN β	PBL	12410-1
Recombinant Mouse IFN β (ELISA Std.)	BioLegend	581309
Laurdan	Molecular Probes	D250
Tamoxifen	Sigma	T5648-1G
4-Hydroxytamoxifen	Sigma	H7904-5MG
Critical Commercial Assays		
Mouse CCL2 (MCP-1) ELISA Ready-SET-Go!®	eBioscience	88-7391
Mouse IL-6 ELISA Ready-SET-Go!®	eBioscience	88-7064
pHrodo® Red E. coli BioParticles® Conjugate for Phagocytosis	LifeTechnologies	P35361
Deposited Data		
Microarray data of BMDM treated with LPS or untreated	Illumina	GSE89559
Experimental Models: Cell Lines		
hybridoma IgG-1D2 (producing SREBP2 monoclonal antibody)	ATCC	ATCC® CRL-2545
NCTC clone 929 (L-929) cells	ATCC	ATCC CCL-1.1
Experimental Models: Organisms/Strains		
FVB-Tg(Csf1r-cre/Esr1*)1Jwp/J	The Jackson Labortory	019098
B6.129P2-Cyp51tm1Bfro/J	The Jackson Labortory	021790
B6(Cg)-Ifnar1tm1.2Ees/J	The Jackson Labortory	028288
B6.129P2(SJL)-Myd88tm1.1Defr/J	The Jackson Labortory	009088
B6.129S6-Ch25htm1Rus/J	The Jackson Labortory	016263
C57BL/6J (Wild type)	The Jackson Labortory	000664
Oligonucleotides		
ChIP_TNFa Fw: GGACTAGCCAGGAGGGAGAA Rv: TGTCTTTTCTGGAGGGAGATGT	This paper	
ChIP_NF-kB Fw: CCACTTACGAGTCTCCGTCCT Rv: AGGAGTACGAGCAAATGGTGA	This paper	

ChIP_Cyp51 promoter Fw: GCCACCGTCTAGATAATTCC Rv: TATACAGCAGTGTCCATCCT	This paper	
ChIP_Cyp51-distal region Fw: GGAAATTGTGATGAAAGGAGTG Rv: CGATGTGACAGTAGAGCC	This paper	
qPCR_MmuCcl2 Fw: TGCTGCTACTCATTCACCAG Rv: TCATTTGGTTCCGATCCAGG	This paper	
qPCR_MmuMx1 Fw: ATTTGTTTGGCTTGTGTTG Rv: TTACATCAAGGCGTTCTATTC	This paper	
qPCR_MmuIfnb1 Fw: AAGTACAACAGCTACGCCTG Rv: CTGCAACCACCACTCATTCT	This paper	
qPCR_MmuMx2 Fw: CGGGAGGGAGTACCCAAC Rv: CCGCACCTTCTCCTCATAC	This paper	
qPCR_MmuCxcl9 Fw: AGTTCGAGGAACCCTAGTGA Rv: AGTTTTGTTGCAATTGGGGC	This paper	
qPCR_MmuCyp51A1 Fw: CTGCCCGCTGGAGCGAAAAG Rv: CACAGGTGTTGTCAGCCGACC	This paper	
qPCR_Mmu18S Fw: TTC CGA TAA CGA ACG AGA CTC T Rv:TGG CTG AAC GCC ACT TGT C	This paper	
qPCR_MmuIL6 Fw: AGTTGCCTTCTTGGGACTGA Rv: TCCACGATTTCCCAGAGAAC	This paper	
qPCR_MmuTNFa Fw: CCCTCACACTCAGATCATCTTCT Rv: GCTACGACGTGGGCTACAG	This paper	
qPCR_MmuNos2 Fw: TCCTGGACATTACGACCCCT Rv: AGGCCTCCAATCTCTGCCTA	This paper	
qPCR_MmuCox2 Fw: ACCTCTCCACCAATGACGTG Rv: GGGAGAGAGTTCATCCCTGA	This paper	
qPCR_MmuDhcr24 Fw: ACGGACGACGTAGAGCCCAG Rv: CCACGTGGTGCTGCTCGTAC	This paper	
Recombinant DNA		
Software and Algorithms		
Ingenuity Pathway Analysis	QIAGEN Bioinformatics	
GeneSpring GX	Agilent Genomics	
Prism 7	GraphPad	
Integrative Genomics Viewer _2.3.68	Broad Institute	
ImageJ	NIH	

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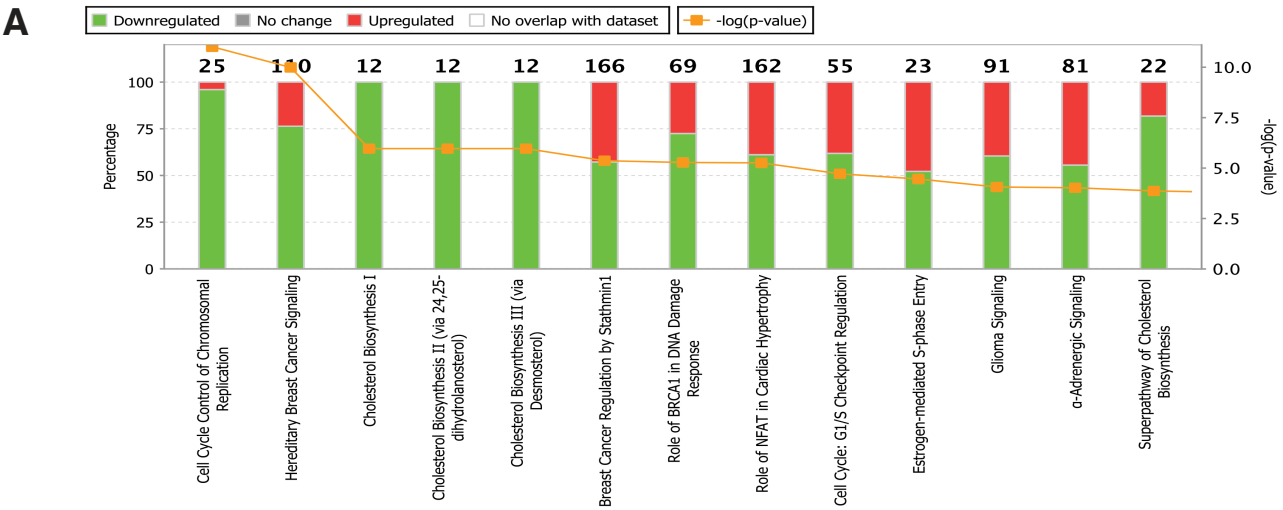
Figure S1



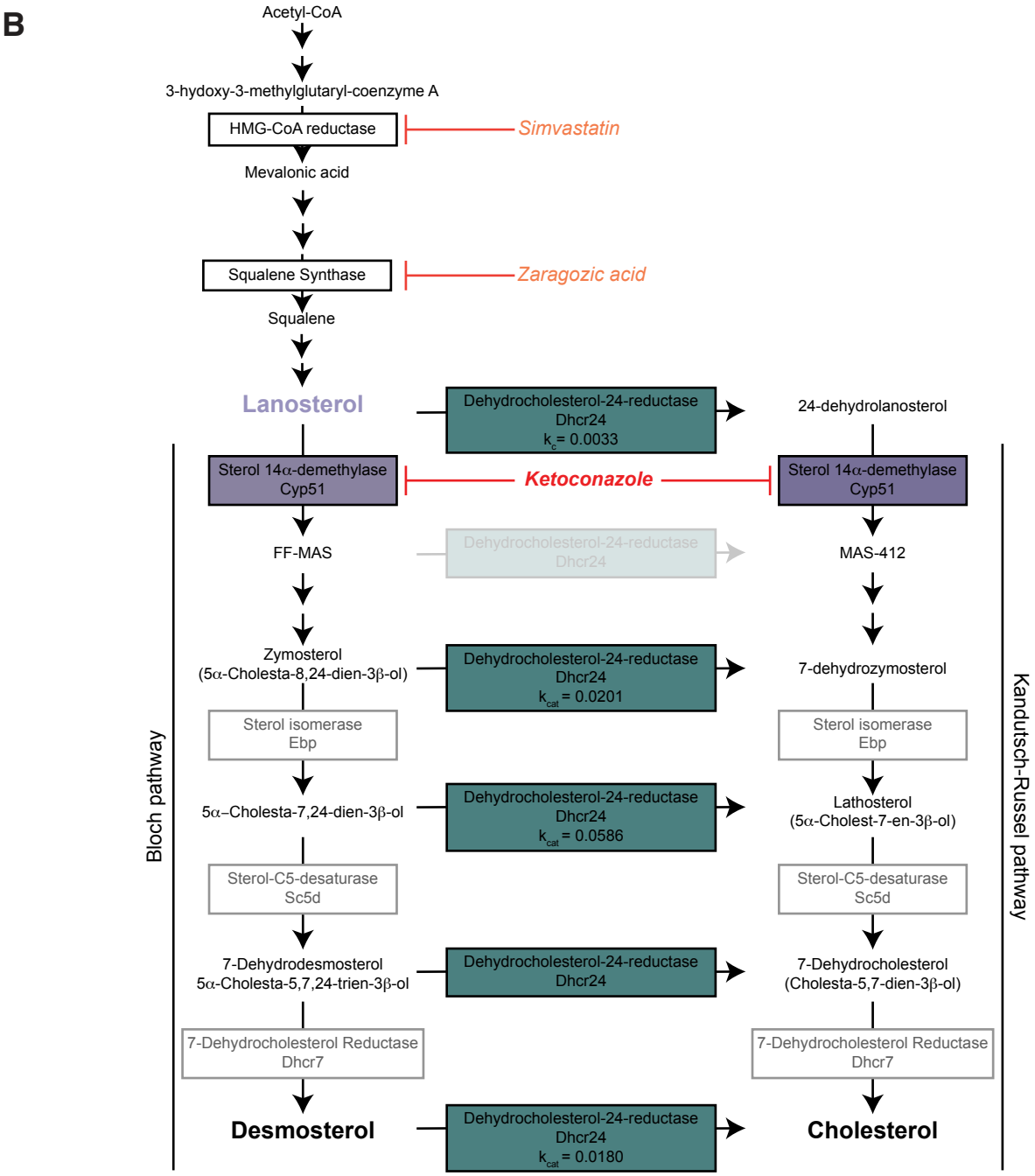


Figure S1: Ingenuity Pathways Analysis and schematic representation of cholesterol synthesis. Related to Figure 1. (A) Data generated through the use of Ingenuity Pathways Analysis (detailed description www.Ingenuity.com) of 2156 downregulated genes (fold change -1.5 and $p<0.05$) upon LPS/IFNγ treatmetn of BMDM. On the left y-axis: percentage of genes of the pathway that are downregulated in the geneset. On the right: -log(p-value). Only the first 10 pathways are represented. Total number of genes in each the pathway is above every column. (B) Inhibitors of cholesterol synthesis used are shown. Kcat data from (Bae & Paik, 1997).

Figure S2

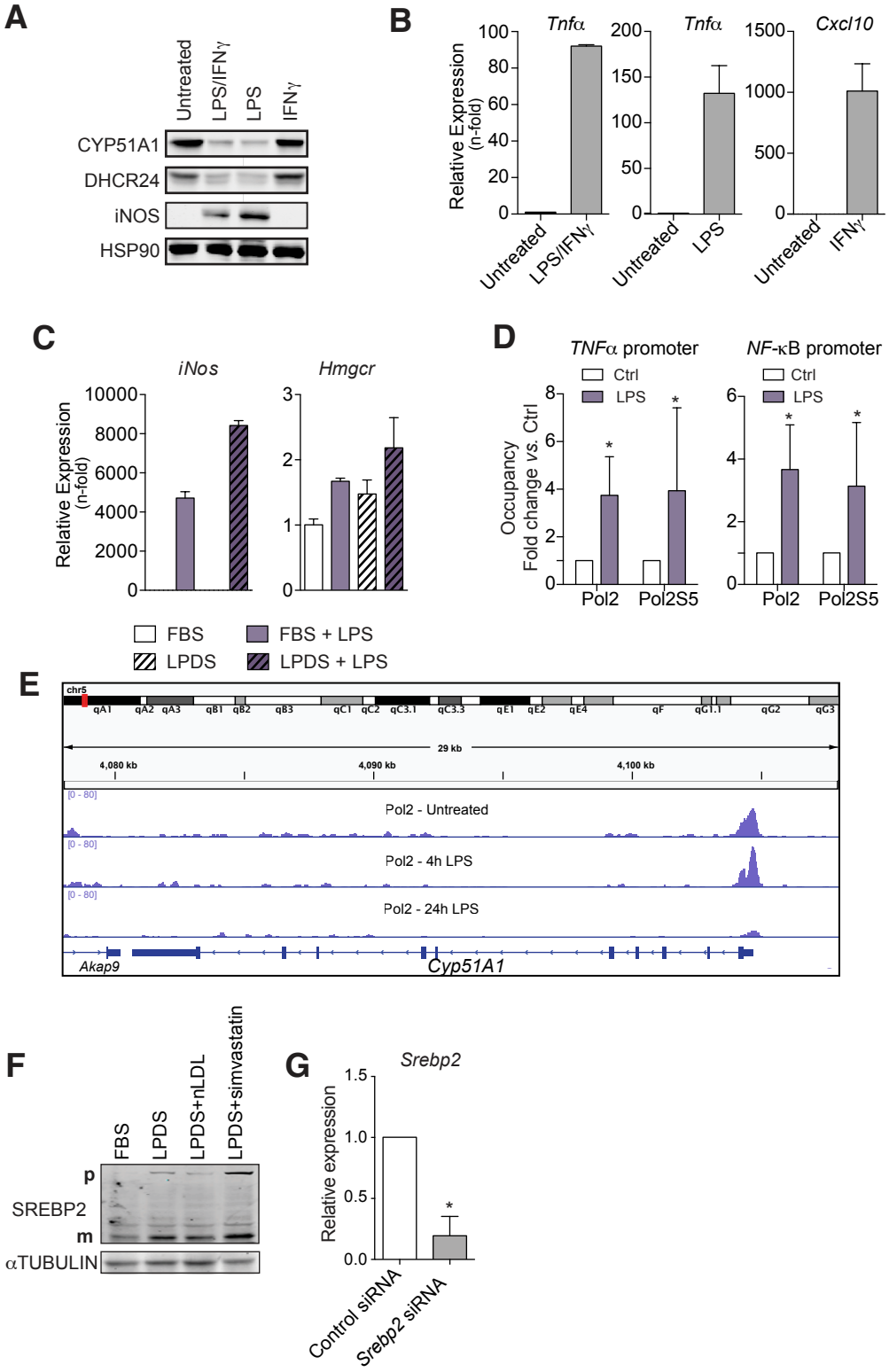


Figure S2: Lack of effect of IFNγ on CY51A1 or DHCR24 protein levels, mRNA levels of positive controls inflammatory treatments and LPDS incubation, positive control of ChIP upon LPS treatment and ChIP-seq analysis of Pol2 upon LPS treatment, control of SREBP2 antibody in Huh7 cells and effect of Srebp2 silencing on Srebp2 mRNA. Related to Figure 1 and 2. (A) Western blot analysis of CYP51A1or DHCR24 of BMDMs treated with LPS (100ng/mL), IFNγ (20ng/mL) or LPS /IFNγ for 8 h (n=3). (B) Relative mRNA expression of *Tnfα* or *Cxcl10* used as positive controls for LPS/IFNγ, LPS or IFNγ treatment as in (A) of BMDMs, respectively. (C) Relative mRNA expression of *iNos* or *Hmgcr* used as of positive controls for LPS stimulation or incubation in media containing LPDS of BMDMs cultured for 24h in regular media containing 20% FBS or in media containing 20% LPDS and then treated with LPS (100ng/ml) for 8 h, (n=3). (D) ChIP of Pol2 or Pol2S5 and analysis of genomic sequences corresponding to the promoters of *TNFα* and *NFkB* of BMDM stimulated with LPS (100ng/mL). Data are fold change *vs.* Ctrl (PBS treated) and normalized to input chromatin (n=3). (E) ChIP-sequencing of Pol2 in LPS-stimulated BMDMs (Nicodeme et al., 2010). (F) Western Blot analysis precursor (p) and mature (m) forms of SREBP2 of human hepatic cells (Huh7) cultured in regular media containing 20% FBS or 20% LPDS, as indicated in the or not of 60ng/native LDL (n-LDL) or simvastatin (5μM). αTUBULIN is a loading control. (G) Relative *Srebp2* mRNA expression of BMDM transfected with non-silencing (NS) siRNA control or *Srebp2* siRNA. $p < 0.05$ *vs.* Ctrl.

Figure S3

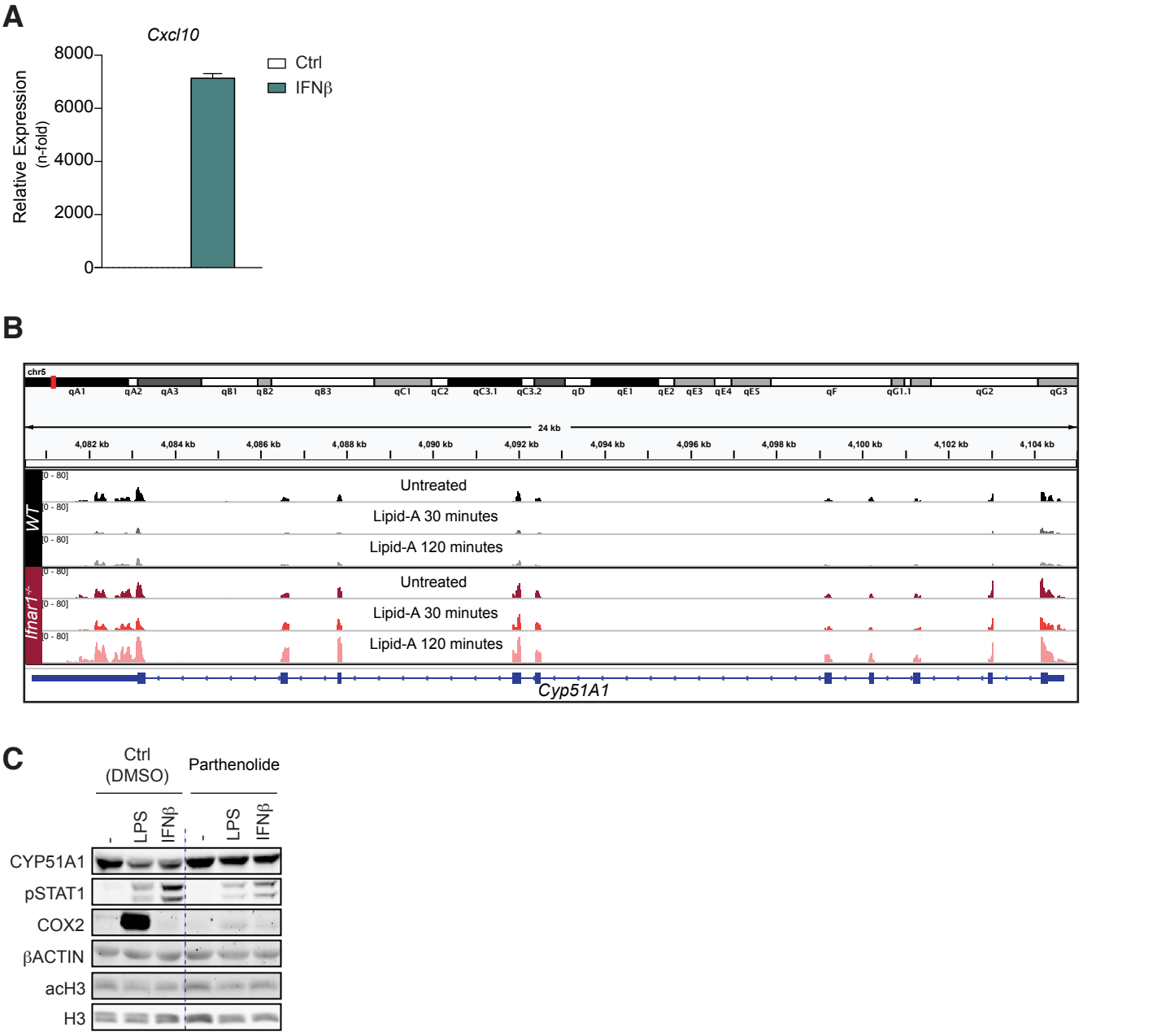


Figure S3: mRNA levels of positive controls for IFNB treatment, RNA-seq analysis of Cyp51A1 locus on Ifnar1-/- BMDMs and effect of HDAC1 inhibitor on CYP51A1 protein levels. Related to Figure 3. A) Relative mRNA expression of Cxcl10 as positive control of in BMDMs treated with IFNβ (1000U/mL). (B) RNA sequencing analysis in the genomic locus of Cyp51A1 in WT or Ifnar1-/- BMDMs over time after treatment with KLA from (Bhatt et al., 2012). (C) Western blot analysis of CYP51A1 protein levels of BMDMs treated with HDAC1 inhibitor Parthenolide (10μM) for 1 h prior LPS or INFβ stimulation for 12h. pSTAT1 and COX2 are controls of activation, H3 and AcH3 are controls for HDAC inhibitor action. βACTIN is a loading control (n=3). Dashed blue lines are for treatment group separation and do not indicate cropped blots.

Figure S4

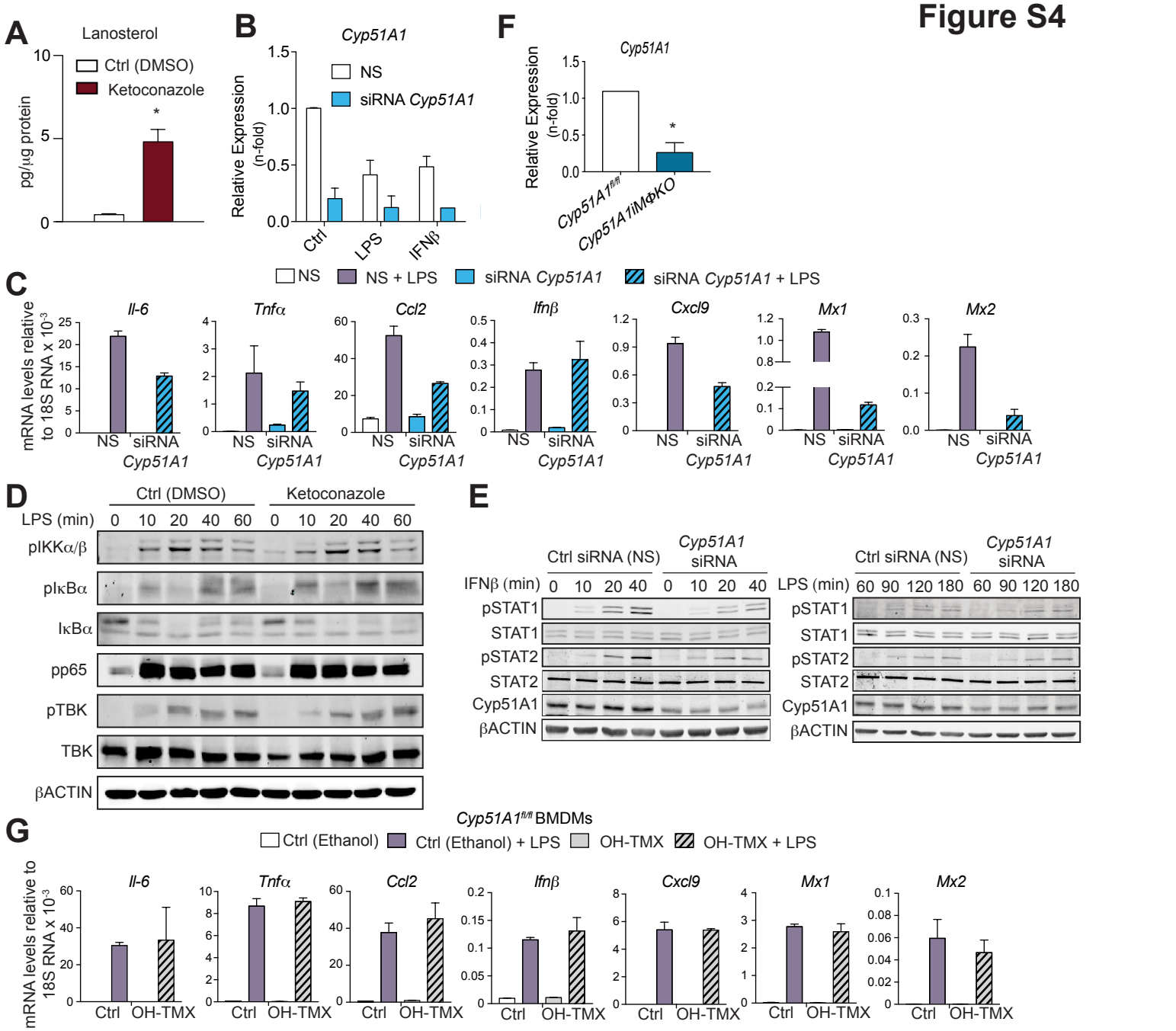


Figure S4: Lanosterol levels of BMDMs treated with Ketoconazole, expression of *Cyp51A1* on *Cyp51A1iMΦ KO*. Effect of Ketaconazlo treatment on MyD88 or TRIF signaling upon LPS stimulation, *Cyp51A1* silencing diminish STAT-STAT1 activation and expression of cytokines upon INFβ or LPS stimulation, OH-TMX-induced ablation of *Cyp51A*1 on *Cyp51A1iMΦKO* BMDMs, control of OH-TMX treatment of Cyp51A1$^{fl/fl}$ BMDMs on STAT1-STAT2 activation or mRNA levels cytokines upon INFβ or LPS stimulation. Related to Figure 4. (A) Llanosterol by GC/MS normalized protein content of BMDMs treated with KT (10µM) for 24 h, (n=3). (B) Relative mRNA expression of *Cyp51A1* on *Cyp51A1*$^{fl/fl}$ or *Cyp51A1iMΦKO* BMDMs treated with 10µg/ml OH-TMX at day 5 of differentiation for 48 h. (C) qPCR analysis of mRNA levels of the indicated genes of BMDMs transfected with non-silencing (NS) control siRNA or *Cyp51A1* siRNA prior of LPS (100ng/mL) for 4 h. (D) Western blot analysis of pIKKα/β, pIkBα, IkBα, pp65, pTBK or TBK of BMDMs treated or not with KT (10µM) 12h prior LPS stimulation for the indicate times. (n=3). (E) Western blot analysis of STAT1 and STAT2 activation (pSTAT1 and pSTAT2) of BMDMs transfected with non silencing (NS) control siRNA or Cyp51A1 siRNA prior INFβ (upper panels) or LPS (lower panels) for the indicated times. CYP51A1 blot is shown for degree of silencing. (F) Relative mRNA expression of Cyp51A1 in TMX treated *Cyp51A1iMΦ KO* or *Cyp51A1fl/fl* BMDMs. (G) qPCR analysis of mRNA levels of the indicated genes of Cyp51A1fl/fl BMDMs (no Cre) treated with 10µg/ml OH-TMX or EtOH at day 5 of differentiation for 48h prior of LPS (100ng/mL) for 4h. (G) Western blot analysis of STAT1 and STAT2 activation of BMDMs Cyp51A1fl/fl prior INFβ (upper panels) or LPS (lower panels) treatments for the indicated times. CYP51A1 blot is shown the absence of effect of OH-TMX on CYP51A1 protein levels. (D, E &G) βACTIN is a loading control (n=3). $p < 0.05$ *vs.* Ctrl.

Figure S5

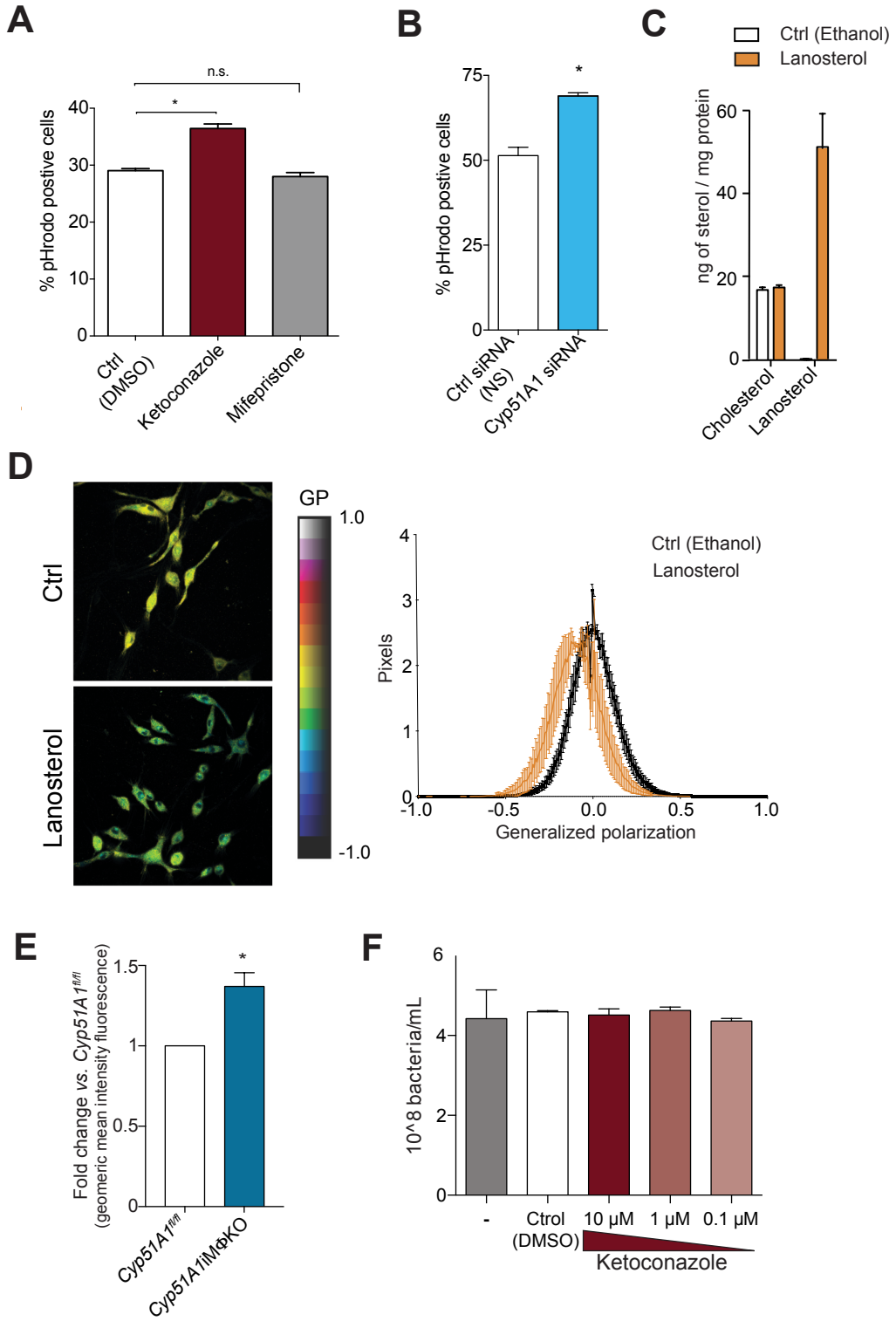


Figure S5: Phagocytic activity upon mifespristone, KT treatment or silencing of *Cyp51A1*, lanosterol content membrane fluidity analysis in lanosterol loaded BMDMs and analysis of fluidity, ROS content in *Cyp51A1iMΦKO* BMDMs and effect of Ketoconalzole in Listeria viability. Related to Figure 5. (A) BMDMs were treated with mifepristone (10µM), KT (10µM) or DMSO for 12h before analysis of pHrodo particle uptake. (B) BMDMs were silenced with *Cyp51A1* siRNA or control for 48h before analysis of pHrodo particle uptake. (C) Cholesterol and lanosterol composition was determined by GC/MS normalized protein content. BMDMs were treated with of lanosterol (5nM) for 24h. Cells were thoroughly washed with PBS and sterols were extracted as described in experimental procedures. (n=2). (D) Generalized polarization (GP) of live BMDMs incubated with Ethanol vehicle control, or Lanosterol (5nM) for 24 hours stained with laurdan. The laurdan emission spectrum was captured by a 2-photon laser-scan microscope. (GP) calculated from the emission intensities obtained from 4 images per condition. Higher GP value indicates that membranes are more ordered and less dynamic or fluidic. The size of the GP binary is 0.05. Representative experiment out of 3 independent experiments with similar results. (E) ROS determination with CellRox by FACS of *Cyp51A1$^{fl/fl}$* or ***Cyp51A1iMΦKO*** BMDMs treated with 10µg/ml OH-TMX for 48 h after day 5 of differentiation. Data (geometric mean fluorescent intensity) of are expressed as fold change vs. Ctrl *Cyp51A1$^{fl/fl}$* BMDMs treated with OH-TMX (n=3). (F) Effect of KT on Lïsteria viability (24 h) measured by optical density (n=3, mean ± SEM). $p < 0.05$ *vs.* Ctrl unless otherwise indicated