

Supporting Information

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Reprogramming of the LXR α Transcriptome Sustains Macrophage Secondary Inflammatory Responses

*Juan Vladimir de la Rosa, Carlos Tabraue, Zhiqiang Huang, Marta C. Orizaola, Patricia Martin-Rodríguez, Knut R. Steffensen, Juan Manuel Zapata, Lisardo Boscá, Peter Tontonoz, Susana Alemany, Eckardt Treuter and Antonio Castrillo**

FIGURE S1

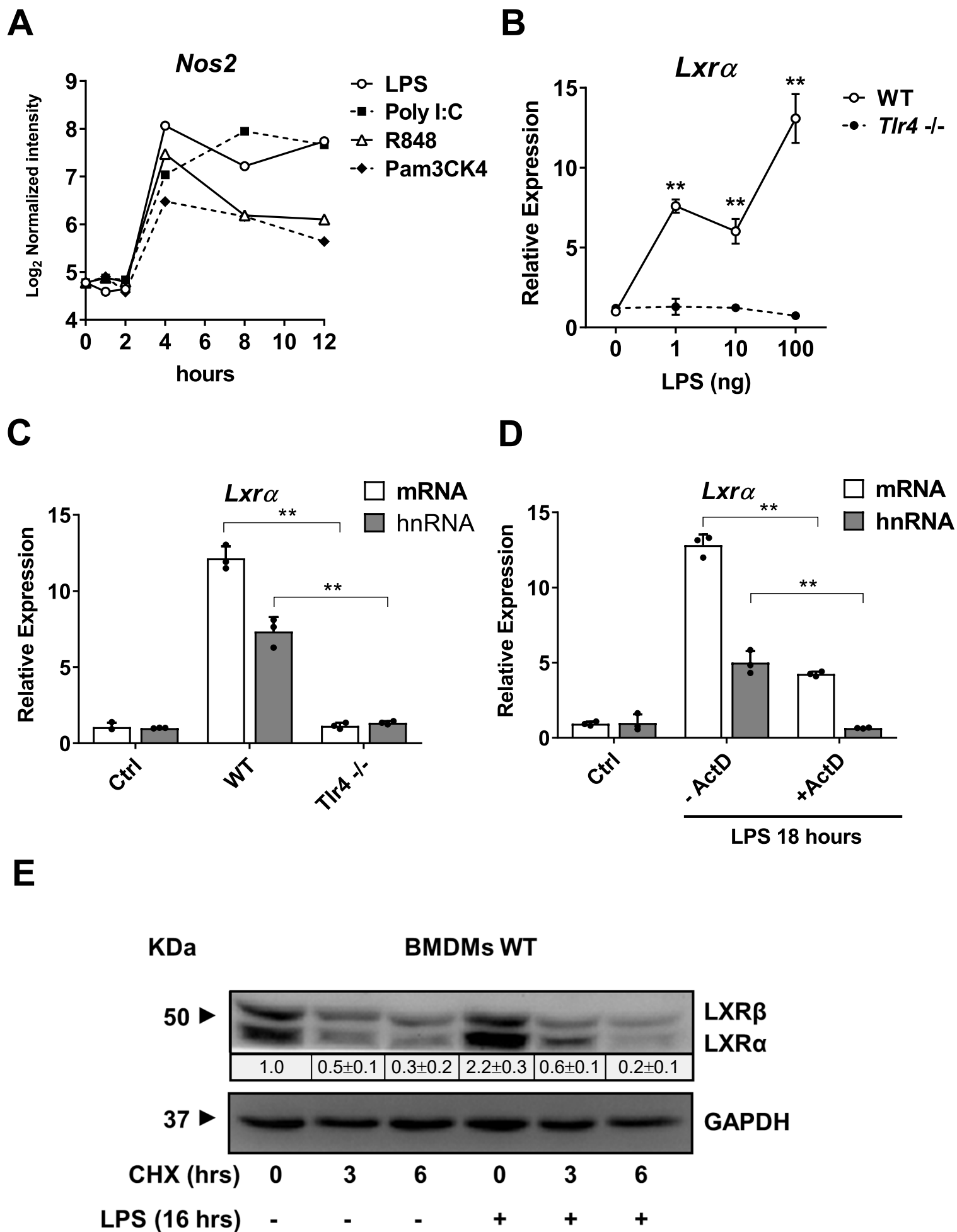


Figure S1.

(A) Relative *Nos2* mRNA expression in BMDM cultured with TLR agonists: LPS (TLR4), Poly I:C (TLR3), PAM3CSK4 (TLR2) and R848 (TLR7 and TLR8); normalized values of RNA expression were obtained from database ArrayExpress E-TABM-310^[30]. **(B)** Relative *Lxra* mRNA levels in WT and *Tlr4*^{-/-} BMDMs cultured with LPS (1-100 ng ml⁻¹) for 24 hours. **(C)** Relative *Lxra* mRNA and nascent hnRNA in WT and *Tlr4*^{-/-} BMDMs cultured with LPS (1-100 ng ml⁻¹) for 24 hours (left panel). **(D)** Relative *Lxra* mRNA and nascent hnRNA in WT BMDM cultured with or without LPS (100 ng ml⁻¹) for 18 hours and then challenged with Actinomycin D (10ug ml⁻¹) for 6 hours (panel on the right). **(E)** Expression levels of LXR α , LXR β and GAPDH proteins in BMDM control or LPS stimulated for 16 hours and then either untreated or cultured with Cycloheximide (CHX, 10ug ml⁻¹) for 3 or 6 hours as indicated. Levels of LXR α protein were quantified by densitometry of band intensity. mRNA or nascent hnRNA expression data were represented as mean $\pm$ SD from 3 experiments. Significant differences between mean values of mRNA or nascent hnRNA are denoted (** p < 0.01)

FIGURE S2

□ WT □ *Lxrα*^{-/-} ■ *Lxrβ*^{-/-}

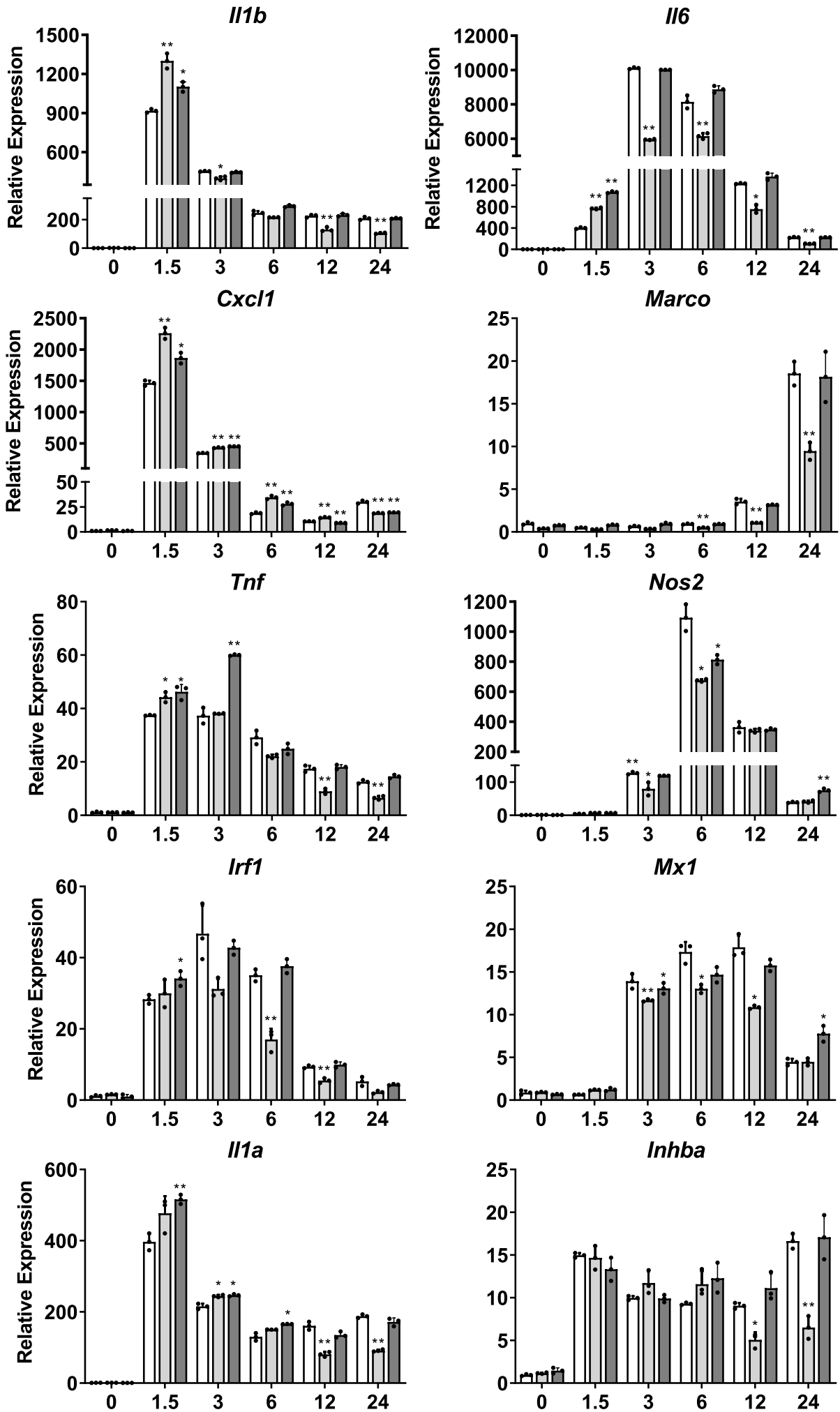


Figure S2.

Time-course of mRNA expression of the indicated genes at different times (0, 1.5, 3, 6, 12, 24 h.) in *Lxrα*^{-/-} or *Lxrβ*^{-/-} BMDMs cultured with or without LPS (100 ng ml⁻¹). Data were represented as mean ± SD from 2 experiments. Significant differences between mean values of *Lxrα*^{-/-} or *Lxrβ*^{-/-} cells compared to WT within each time are denoted (** p < 0.01, *p < 0.05)

FIGURE S3

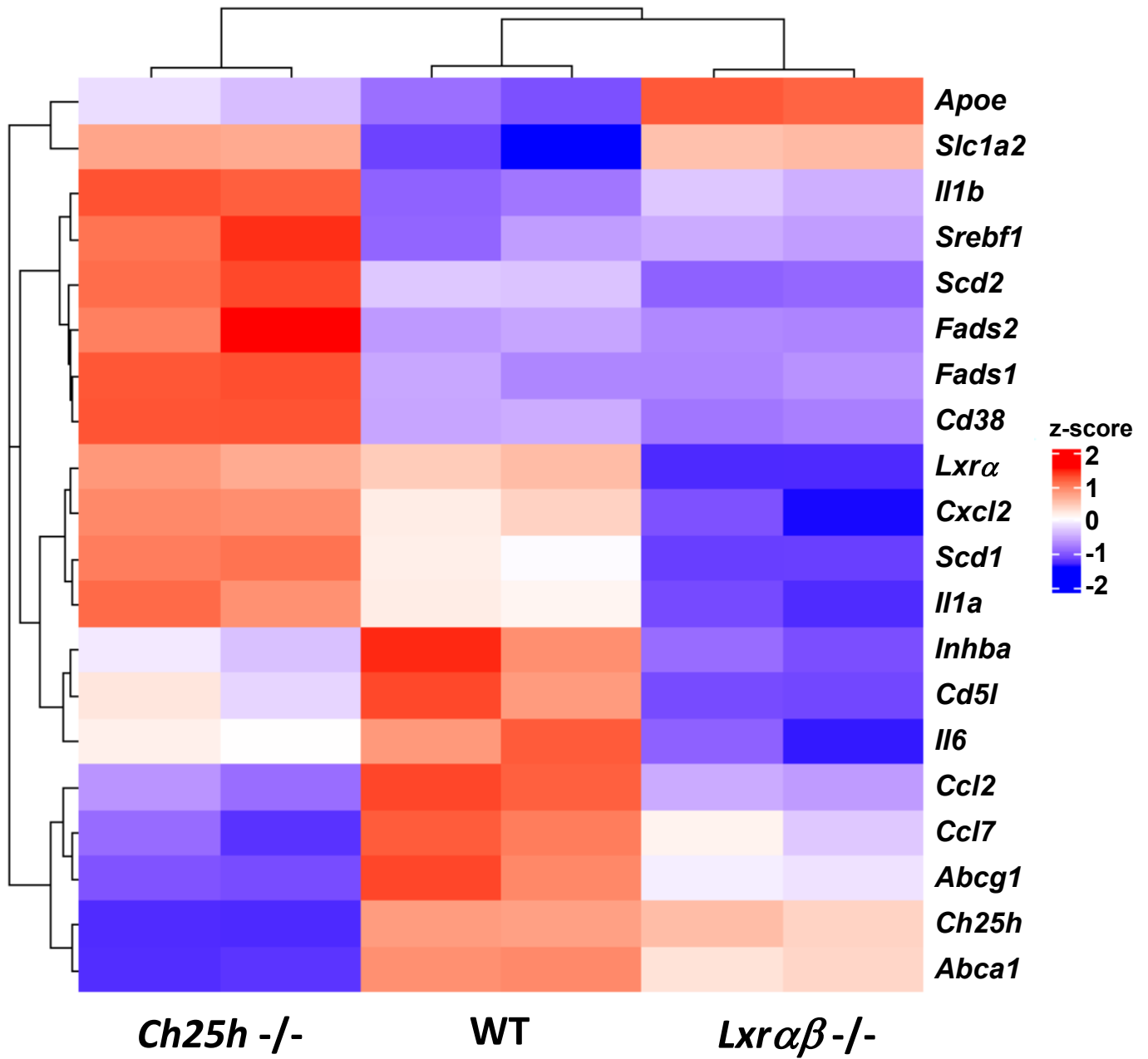


Figure S3.

Heatmap of mRNA expression of a selected panel of genes in WT and *Lxra* β ^{-/-} and *Ch25h*^{-/-} BMDMs; data analyzed from public datasets GSE58993. Each row is z-score normalized.

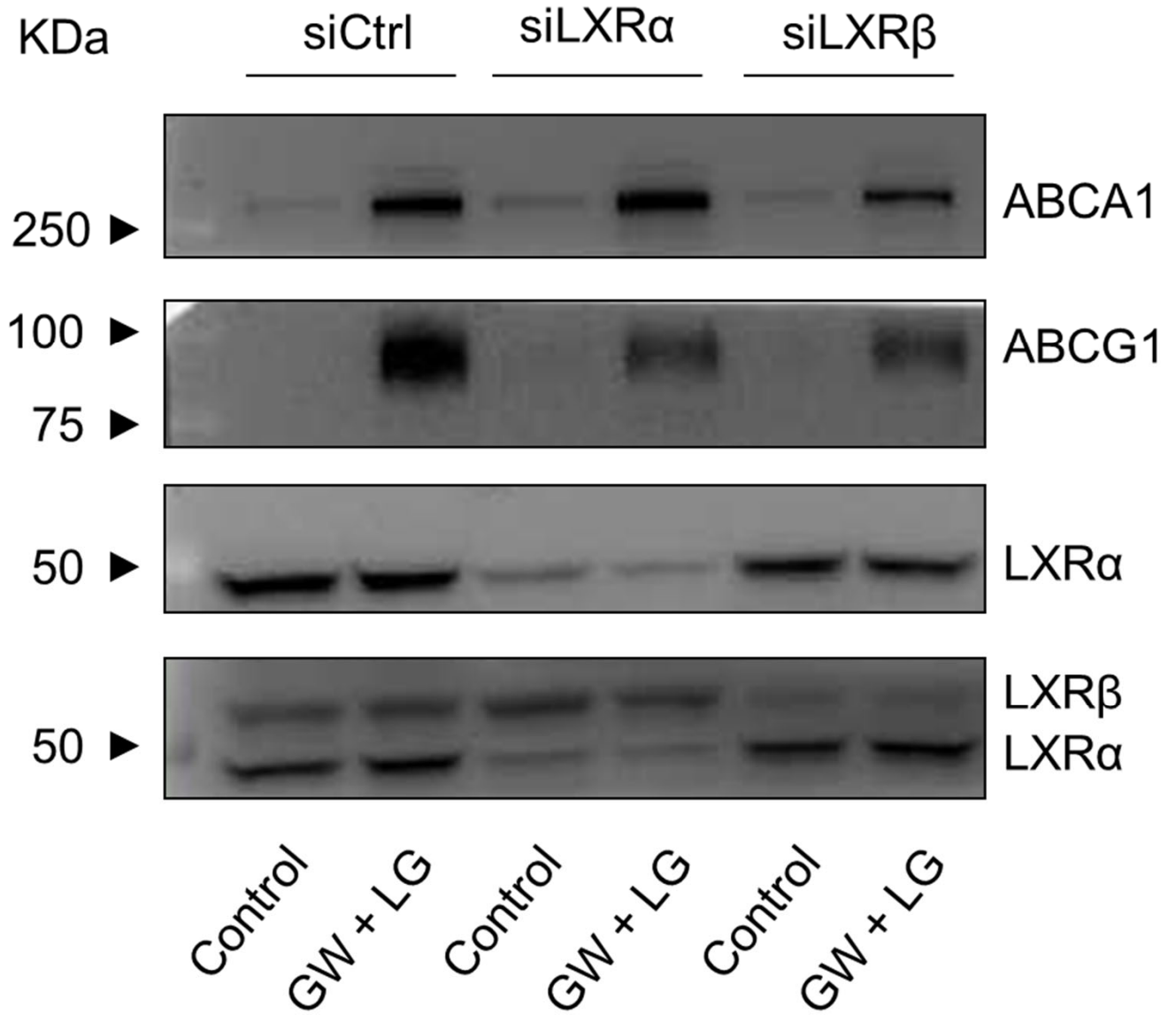
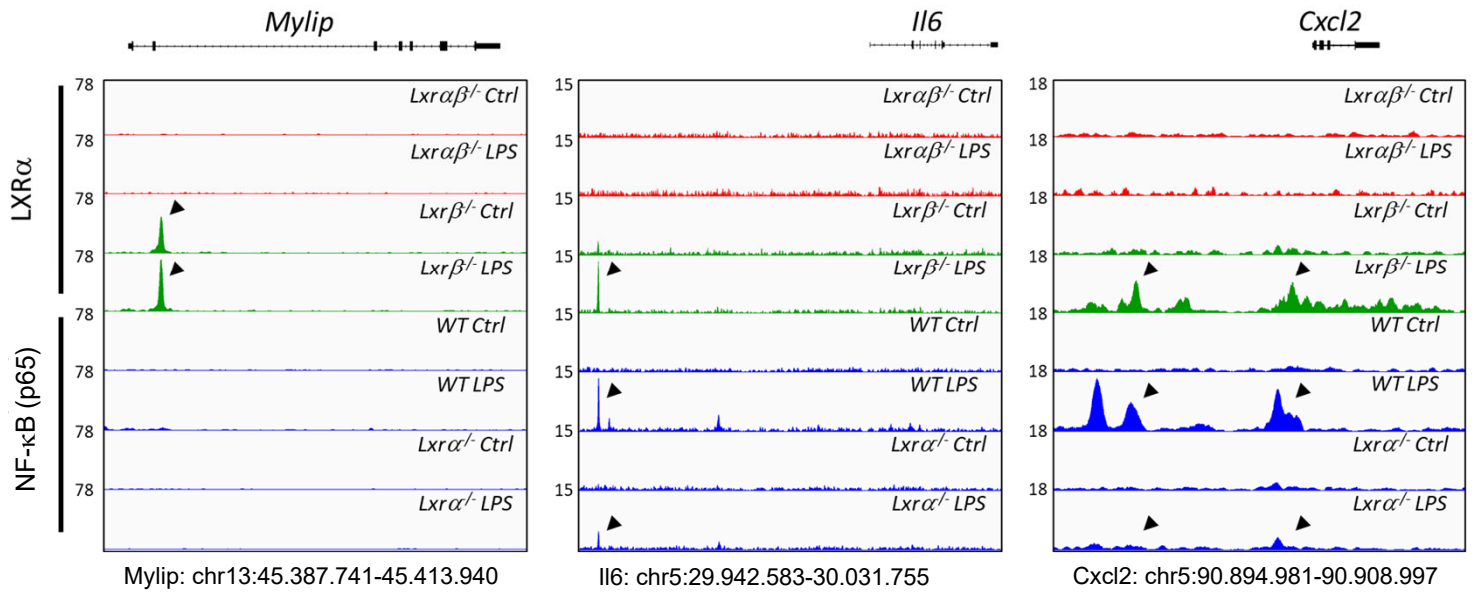
FIGURE S4**A**

Figure S4.

Protein levels of ABCA1, ABCG1, LXR α or LXR $\alpha\beta$ in WT BMDMs transfected with control siRNA or with LXR α or LXR β specific siRNAs smartpool. Cells were cultured with or without LXR agonists GW3965 (2 μ M) and LG268 (100 nM) for 24h.

A



B

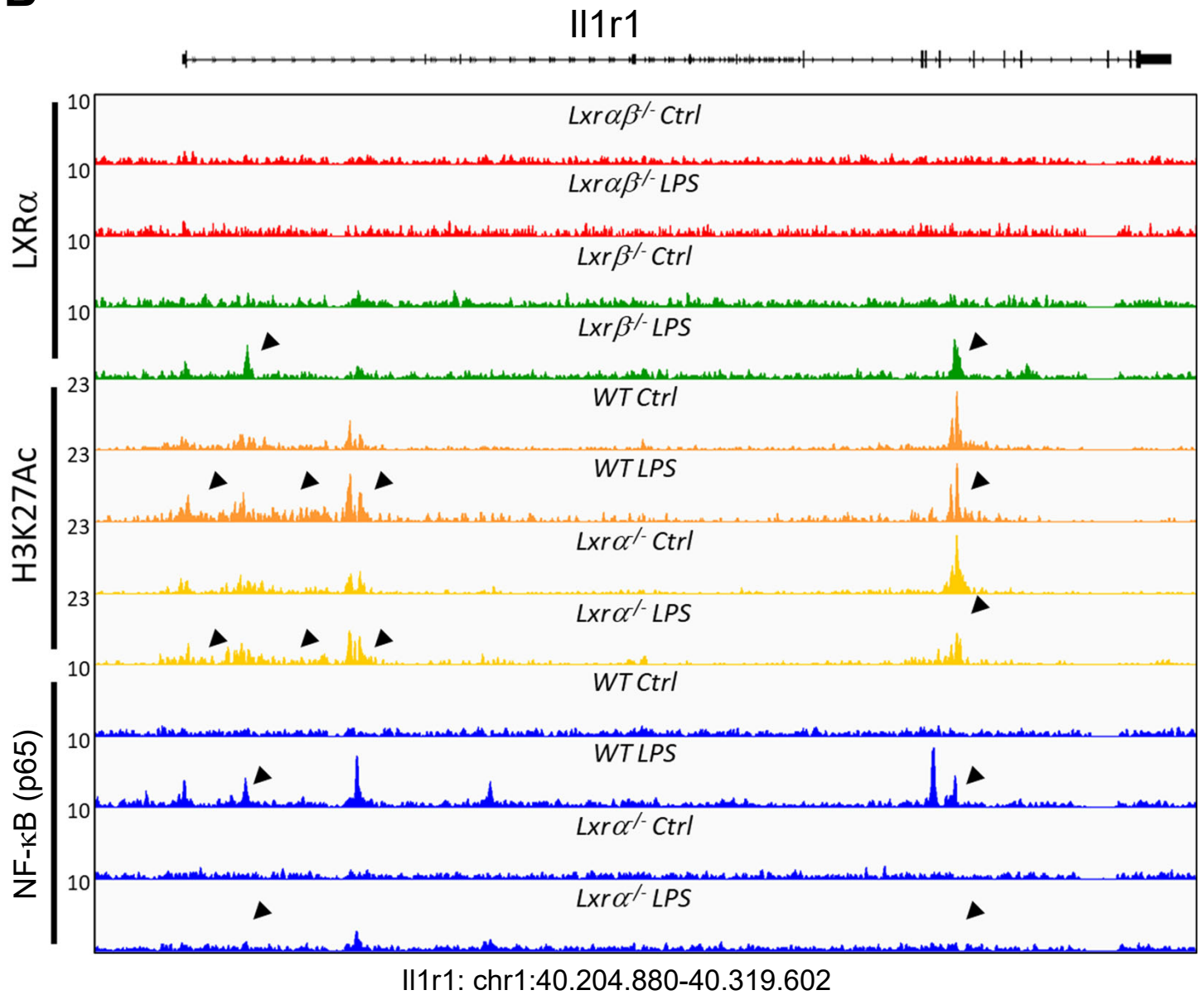


Figure S5.

(A) IGV genome browser images of LXR α ChIP-seq binding sites for the indicated loci of vehicle or LPS-treated *Lxr β* ^{-/-} BMDM (LXR α binding in green). As negative control, ChIP-seq in LXR $\alpha\beta$ ^{-/-} BMDM (in red). In blue, binding of p65 NF- κ B ChIP-seq in control and 24h LPS stimulated WT or *Lxr α* ^{-/-} BMDM

(B) IGV genome browser images of ChIP-seq binding sites for the *I1r1* locus of vehicle or LPS-treated *Lxr β* ^{-/-} BMDM (LXR α in green in LXR β ^{-/-} BMDMs), H3K27ac (orange/yellow in WT or *Lxr α* ^{-/-} BMDM) and p65 NF- κ B (blue in WT or *Lxr α* ^{-/-} BMDM).

FIGURE S6

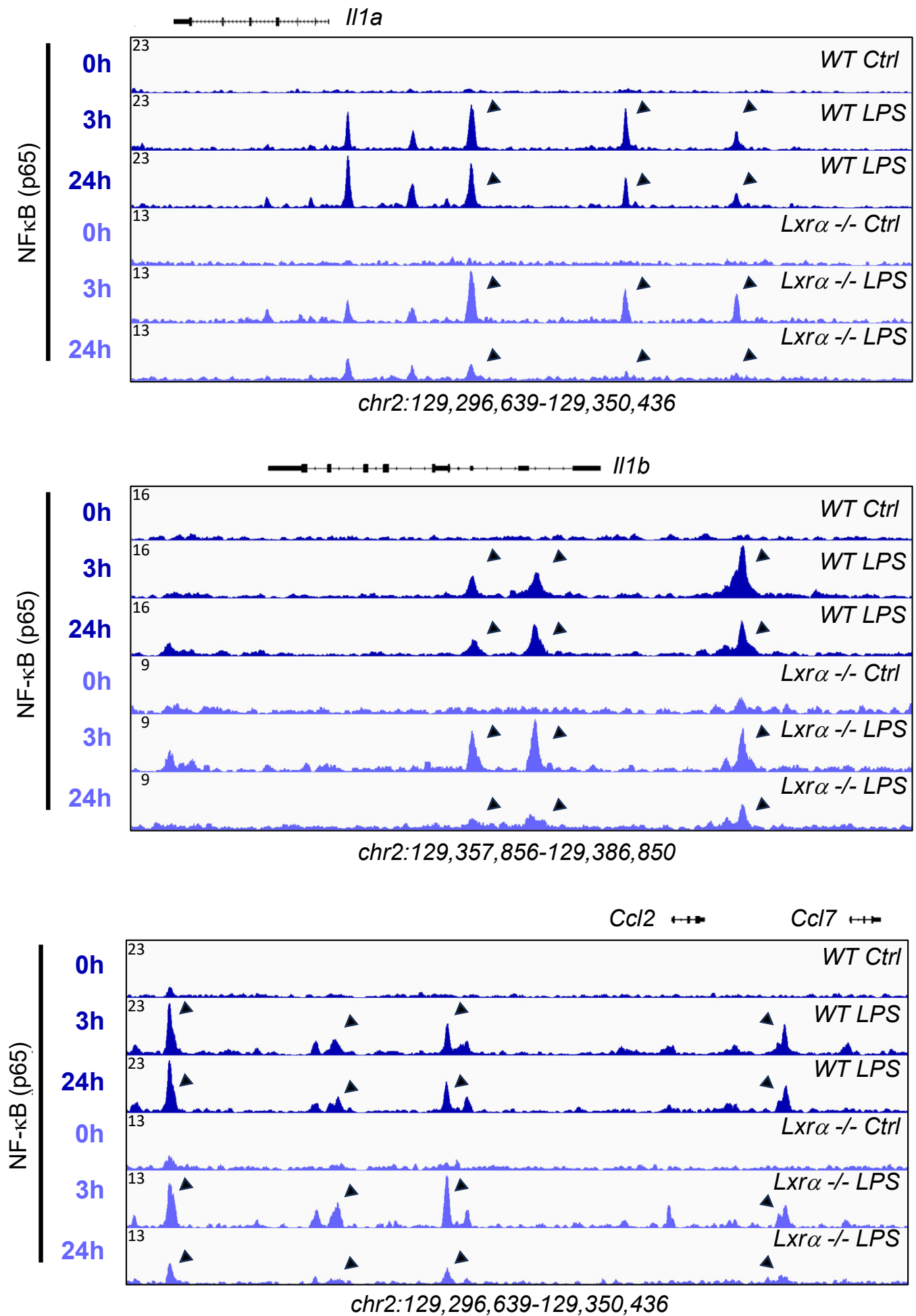


Figure S6.

IGV genome browser images for the indicated loci (*Il1a*, *Il1b* and *Ccl2*) of p65 NF- κ B ChIP-seq in control, 3h and 24h LPS stimulated in WT or *Lxr α* ^{-/-} BMDM.

FIGURE S7

Resident Peritoneal
Macrophages

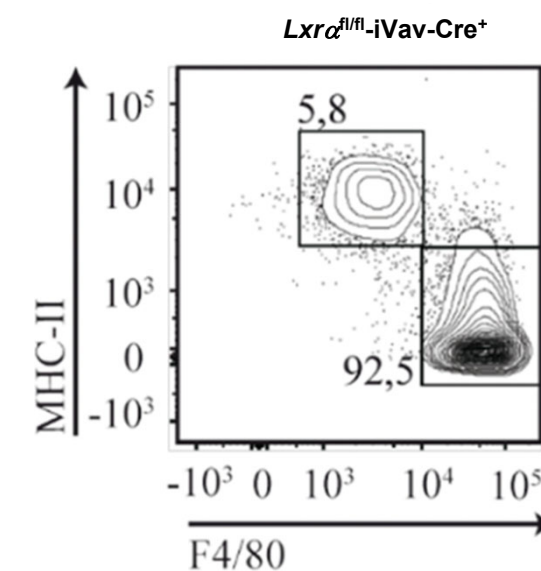
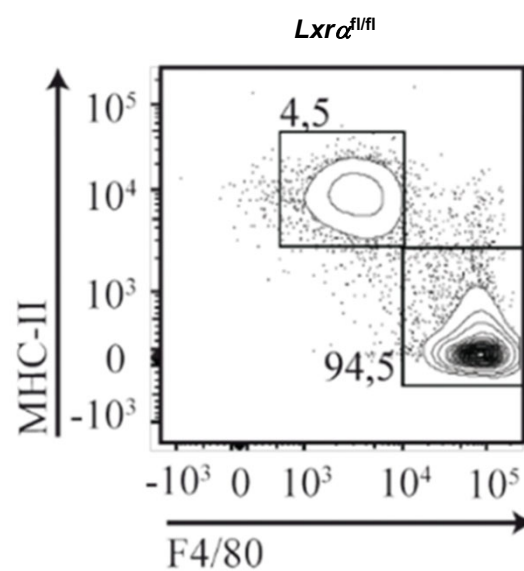
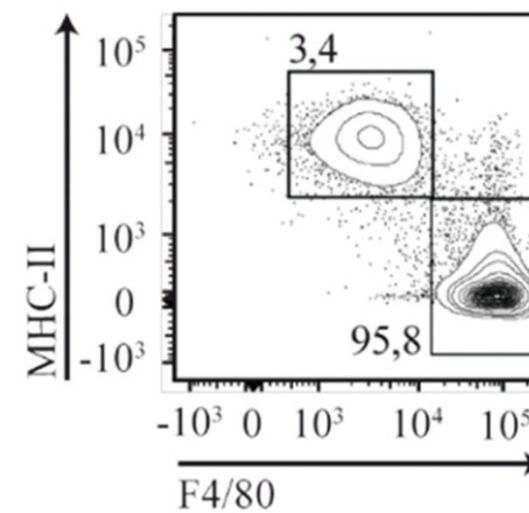
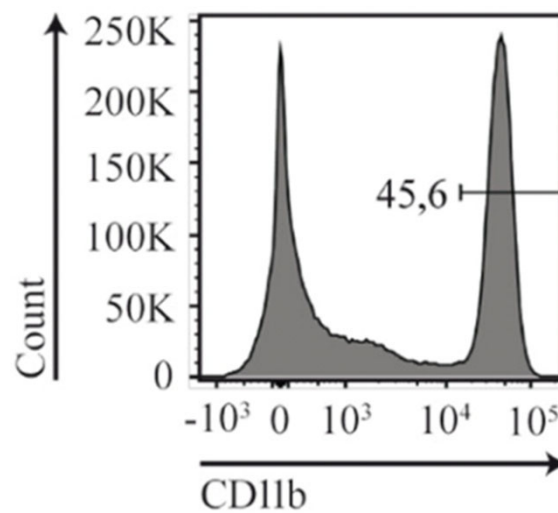
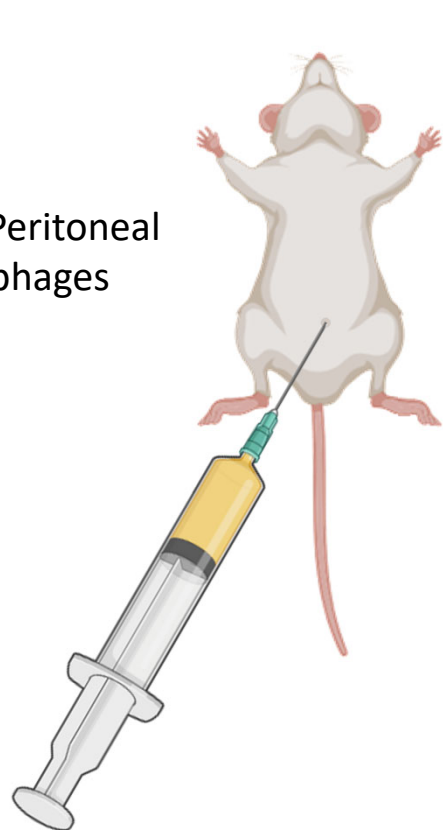


Figure S7.

Gating strategy for the identification of mouse peritoneal macrophages. Peritoneal exudate was obtained by injecting PBS into peritoneal cavity and cells were stained with CD11b, F4/80 and MHC-II antibodies. A representative flow cytometry plot from *Lxra*^{fl/fl}-iVav-Cre⁻ (WT) and *Lxra*^{fl/fl}-iVav-Cre⁺ mice is represented.

TABLE S1. PRIMERS FOR REAL-TIME QPCR

Transcript	Orientation	Sequence
36b4	FWD	5'- GGC CCT GCA CTC TCG CTT TC -3'
	REV	5'- TGC CAG GAC GCG CTT GT -3'
Abca1	FWD	5'- GCA GAT CAA GCA TCC CAA CT-3'
	REV	5'- CCA GAG AAT GTT TCA TTG TCC A-3'
Abcg1	FWD	5'- TCA CCC AGT TCT GCA TCC TCT T -3'
	REV	5'- GCA GAT GTG TCA GGA CCG AGT -3'
Apoe	FWD	5'- AAC AGA CCC AGC AAA TAC GCC -3'
	REV	5'- CTC ATT GAT TCT CCT GGG CC -3'
Ccl2	FWD	5'- CAT CCA CGT GTT GGC TCA-3'
	REV	5'- GAT CAT CTT GCT GGT GAA TGA GT-3'
Cd38	FWD	5'- AAA ACT TCT CCA TTC CAT CTG TTA CA -3'
	REV	5'- CAT GTG TGT CCA AGG CAT TAA AC -3'
Cd5l	FWD	5'-TTT GTT GGA TCG TGT TTT TCA GA -3'
	REV	5'- CTT CAC AGC GGT GGG CA -3'
Ch25h	FWD	5'-GCGACGCTACAA GATCCA-3'
	REV	5'-CACGAACACCAGGTGCTG-3'
Cxcl1	FWD	5'-ATCCAGAGCTTGAAGGTGTTG-3'
	REV	5'-GTCTGTCTTCTTTCTCCGTTACTT-3'
Fads1	FWD	5'- TCA GCG ACT TCA GCC -3'
	REV	5'- AAA AGG ATC CGT GGC AT -3'
Fads2	FWD	5'- AAG GGA GGT AAC CAG GGA GAG -3'
	REV	5'- CCG CTG GGA CCA TTT GGT AA -3'
Il1a	FWD	5'- TTG GTT AAA TGA CCT GCA ACA -3'
	REV	5'- GAG CGC TCA CGA ACA GTT G -3'
Il1b	FWD	5'-TCT TCT TTG GGT ATT GCT TGG-3'
	REV	5'-TGT AAT GAA AGA CGG CAC ACC-3'
Il6	FWD	5'- CCA GGT AGC TAT GGT ACT CCA GAA -3'
	REV	5'- GCT ACC AAA CTG GAT ATA ATC AGG A -3'
Inhba	FWD	5' - ATC ATC ACC TTT GCC GAG TC - 3'
	REV	5' - TCA CTG CCT TCC TTG GAA AT - 3'
Irf1	FWD	5'- CTT TGA ACA GTC TGA GTG GCA G -3'
	REV	5'- CCC ATC AGG AGG TTT CCT CG -3'

<i>Lxrα</i> (hnRNA)	FWD	5'- CAG CTC AGT AAA GTG GGC AA -3'
	REV	5'- TCG ACT CAC AGC ACT TTA CCT -3'
<i>Lxrα</i> (mRNA)	FWD	5'- CCT TCC TCA AGG ACT TCA GTT ACA T-3'
	REV	5'- CAT GGC TCT GGA GAA CTC AAA GAT-3'
<i>Lxrβ</i>	FWD	5'- CCC CAC AAG TTC TCT GGA CAC T-3'
	REV	5'- TGA CGT GGC GGA GGT ACT G-3'
<i>Marco</i>	FWD	5'- GGC ACC AAG GGA GAC AAA -3'
	REV	5'- TCC CTT CAT GCC CAT GTC -3'
<i>Mx1</i>	FWD	5'- AAA CCT GAT CCG ACT TCA CTT CC -3'
	REV	5'- TGA TCG TCT TCA AGG TTT CCT TGT -3'
<i>Nos2</i>	FWD	5'- GCA GCT GGG CTG TAC AAA -3'
	REV	5'- AGC GTT TCG GGA TCT GAA T -3'
<i>Scd1</i>	FWD	5'- TCA GCA CTG GGA AAG TGA GG -3'
	REV	5'- AAC TGG AGA TCT CTT GGA GCA T -3'
<i>Scd2</i>	FWD	5'- CTG GAA ATG CAA AGA CCG GA -3'
	REV	5'- AGA CGC GGA GAG GTA CAT AA -3'
<i>Tnf</i>	FWD	5'- ATC ATC TTC TCA AAA TTC GAG TGA -3'
	REV	5'- TTG AGA TCC ATG CCG TTG G -3'