Appendix

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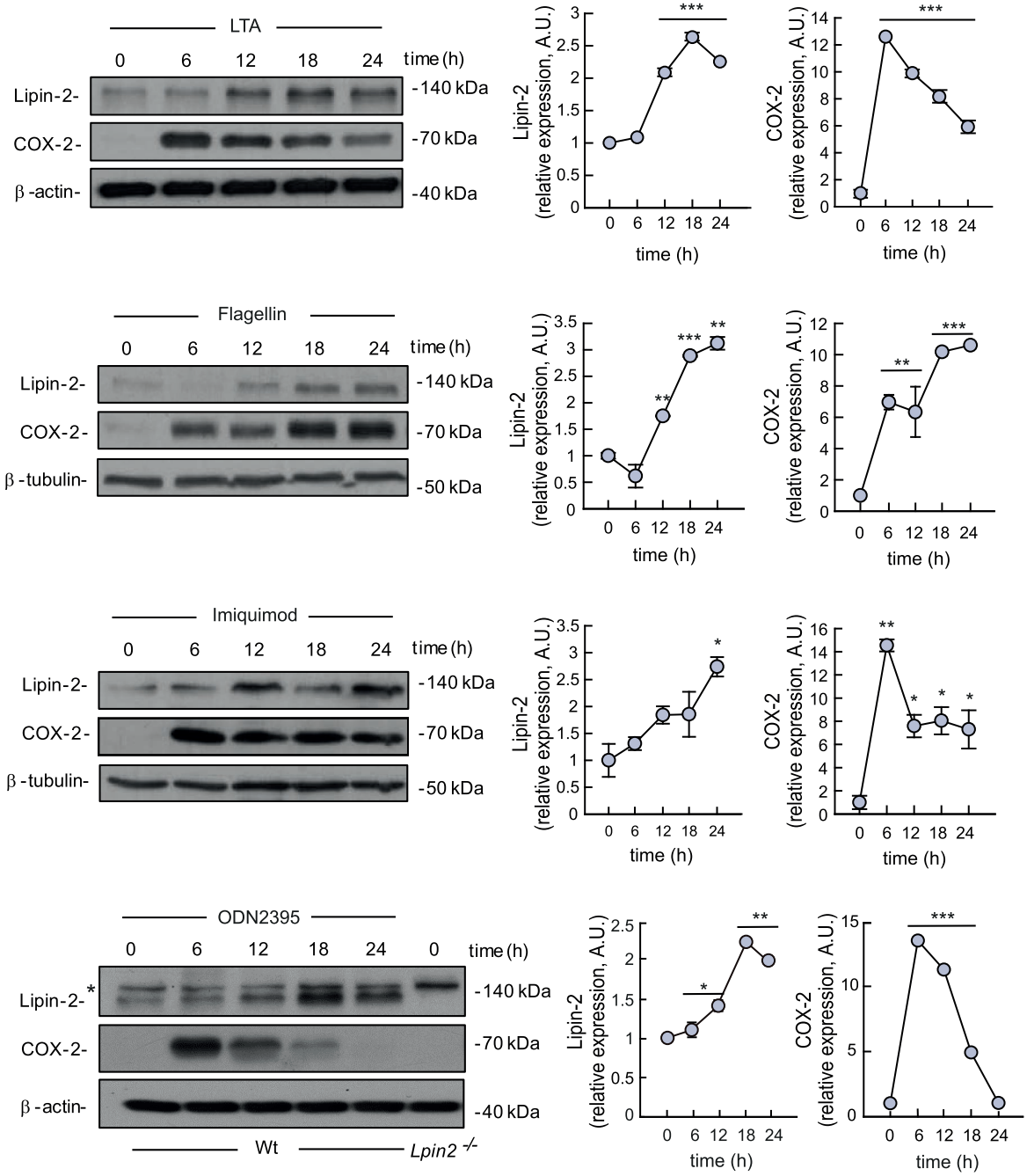
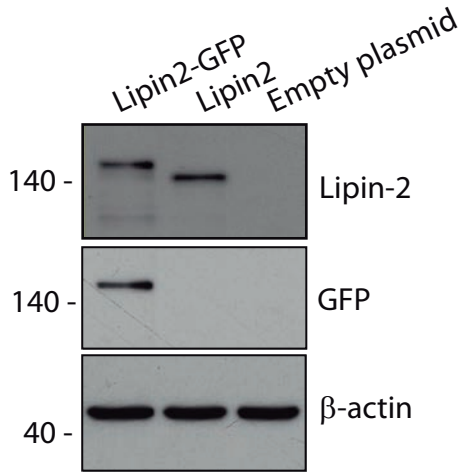
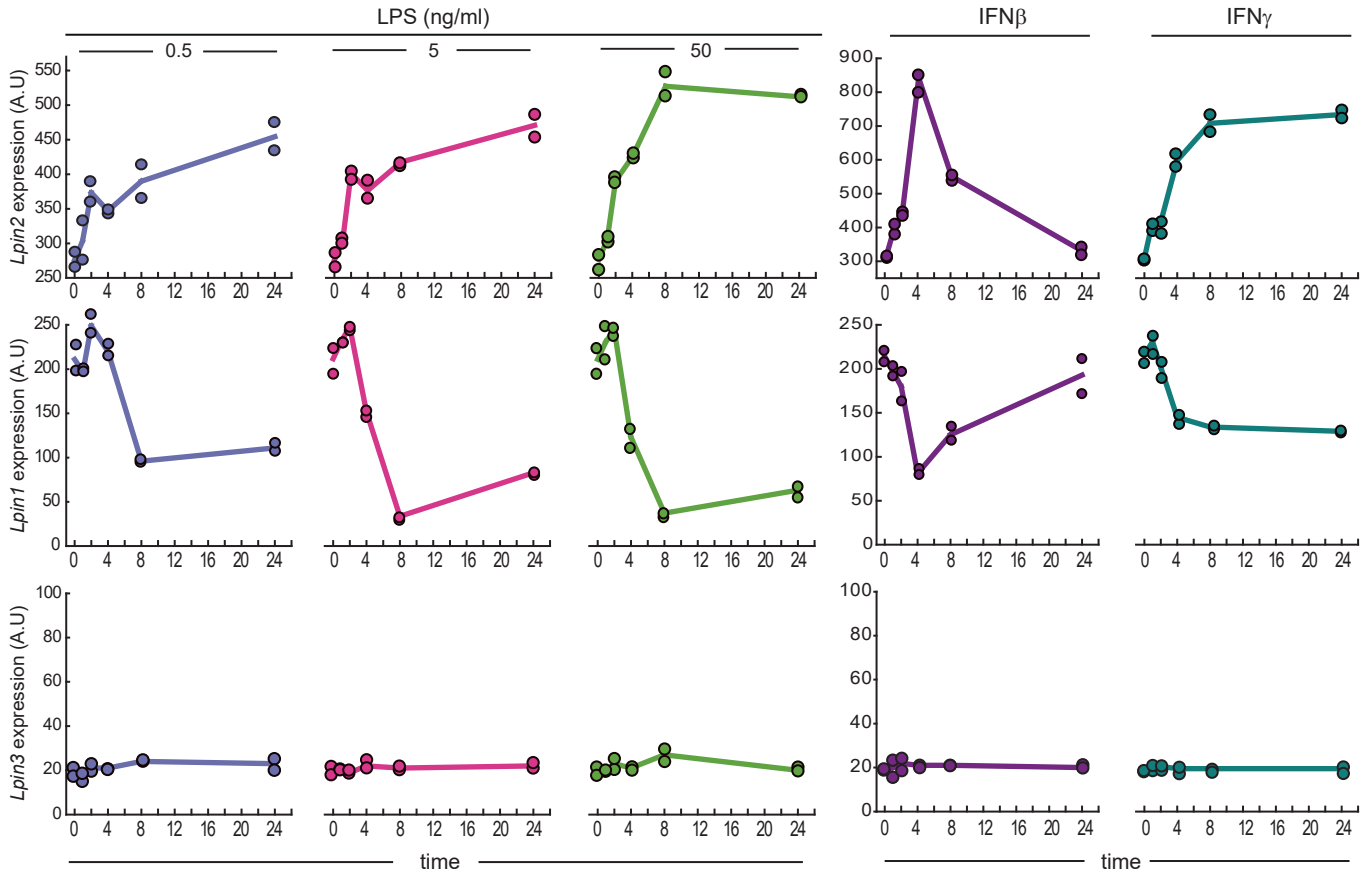


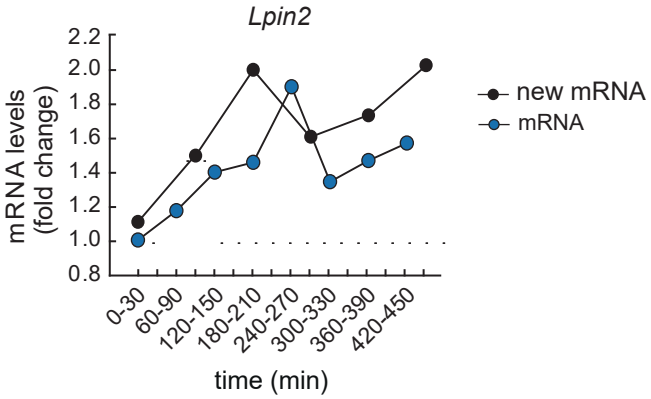
Appendix Figure S1. (continuation of Fig 1). *Effect of TLR agonists on lipin-2 expression.* BMDMs were treated with 1 μg/ml LTA (TLR2), 50 ng/ml Flagellin (TLR5), 5 μg/ml Imiquimod (TLR7) or 1 μM ODN 2395 (TLR9) for the indicated periods of time. Cells lysates were analyzed by immunoblot using specific antibodies against lipin-2, COX-2, and β-actin or tubulin as loading controls. Quantifications of the bands from technical replicates are shown on the right. Shown are representative experiments of three ones. *,P <0.05, ** P < 0.01, *** < 0.001, by Student´s t test.



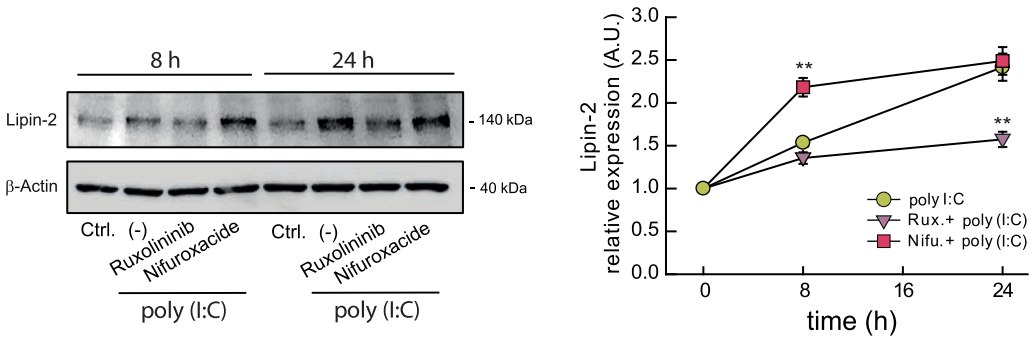
Appendix Figure S2. *Overexpression of lipin-2 in HEK-239 cells.* HEK-239 cells were transfected with 2 μg of the plasmid mlipin-2-EGFP (Valdearcos et al., 2012, J. Biol. Chem. 287: 10894-10904) containing the murine sequence of lipin-2 fused with EGFP, a plasmid containing only the lipin-2 sequence or with an empty plasmid. Lysates were analyzed by immuno-blot using antibodies against lipin-2, GFP and β-actin.



Appendix Figure S3. *Lpin mRNA expression levels in activated BMDMs.* BMDMs were treated with the indicated doses of LPS (left panels), or 10 U/ml of IFNβ or IFNγ (rigth panels). Duplicate samples were collected at 0, 1, 2, 4, 8 and 24 h. mRNA levels for the inicated lipins were analized using Affymetrix Mouse Gene 1.1ST Array (GSE44292) (Raza et al., 2014, J. Leukoc. Biol. 96:167-183).



Appendix Figure S4. New synthesis of Lpin2 mRNA induced by IFN-γ. BMDMs were treated or not with 100 U/ml INF-γ at different time points and newly transcribed mRNA and total RNA was analyzed as described elsewhere (Dölken L et al., 2008, RNA, 14:1959-1972). Fold change of newly transcribed and total levels of Lpin2 mRNA from stimulated cells is represented.



Appendix Figure S5. *Lipin-2 expression in cells treated with poly(I:C)*. BMDMs were left untreated (Ctrl.) or stimulated with 25 mg/ml poly(I:C) in the presence or absence of 5 mM Ruxolitinib or 10 mM Nifuroxacide for the indicated periods of time. Protein was analyzed by Immunoblot using specific antibodies against lipin-2 or β-actin (loading control). Quantifications of the bands are shown on the right. Shown is a representtive experiment of three ones (three biological samples). **, $P < 0.01$, by Student´s t test.

Peak 1:71527697-71528898

CAGGAGTGTAGCTGGATAGGAGAAAGAACTTCTAGAGTTTTGACTGTGGTTGGCGTCAATTAATTGCATACTT
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Peak 2:71539873-71540425

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Peak 3:71541311-71541719

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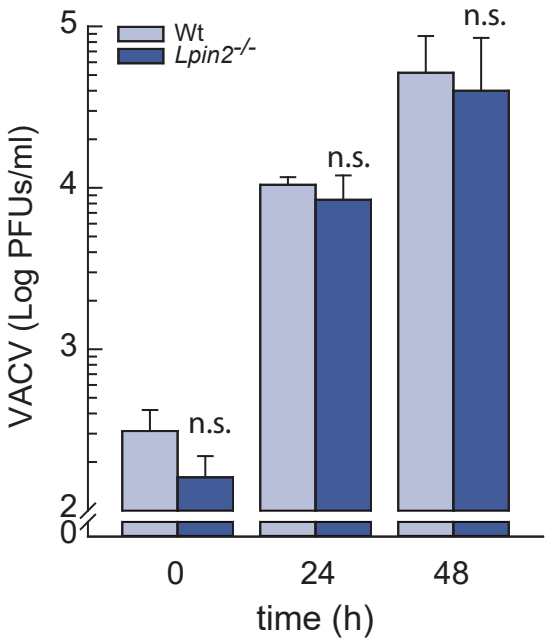
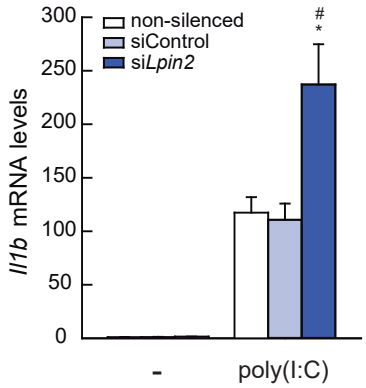
TCATGGCCTTGATCAACAGCAGTTTGAGGTTGTGTGTTTCAGATTTTTCTCTACTGACAACCTAATTTGTATT
TTTTTCTATGTATAAGAAGTTTGTAGGGGGGTCGTAAGGGGGG

Appendix Figure S6. *ISRE and GAS sequences present in Stat1 peaks in the Lpin2 gene*. DNA sequence motifs recognized by Stat1 in peaks 1, 2 and 3 called by MACS during Chip-Seq analysis were determined by the presence of ISRE (GAAANNGAAA, green) or GAS (TTCNNNGAA or TTCNNNNGAA, grey) consensus motifs within a 100-bp region centered on the peak summit. Notice that in peak 2 a GAS consensus motif was also found at 128-bp from the center of the peak.



Appendix Figure S7. *Infection of BMDMs with VACV is not affected by lipin-2 expression levels.* BMDMs from Wt or Lpin2$^{-/-}$ animals were infected with VACV (MOI = 0.01, Western Reseve strain). Cells were harvested at the indicated time points and virus yields were determined by plaque assay in BSC40 cells. Results represent the mean ± SD of three independent experiments. *P* values were calculated by two-tailed t test assuming non-equal variance. No statistical differences(n.s.) were found between Wt and lipin-2 –deficient macrophages.



Appendix Figure S8. *Effect of lipin-2 on Il1b expression in RAW264.7 macrophages.* RAW264.7 macrophages were left untreated (non-silenced), or silenced with control siRNAs (siControl) or against *Lpin2* (si*Lpin2*). After 48 h, cells were left untreated (-) or activated with 25 μg/ml poly(I:C). *Il1b* mRNA levels were analyzed by qPCR, using *Gapdh* as reference. Data from triplicate biological samples are represented as mean ± SE. *, $P < 0.05$, #, $P < 0.05$, by Student's t test. *, siLpin2 vs siControl. #, siLpin2 vs non-sileced cells.