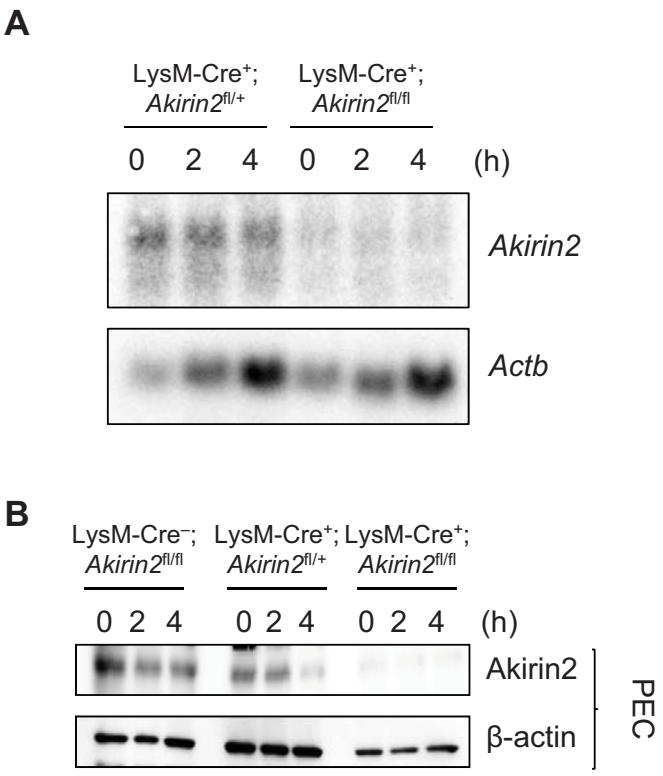
Supplementary Figures S1 – S11

Akirin2 is critical for inducing inflammatory genes by bridging IκB-ζ and the SWI/SNF complex

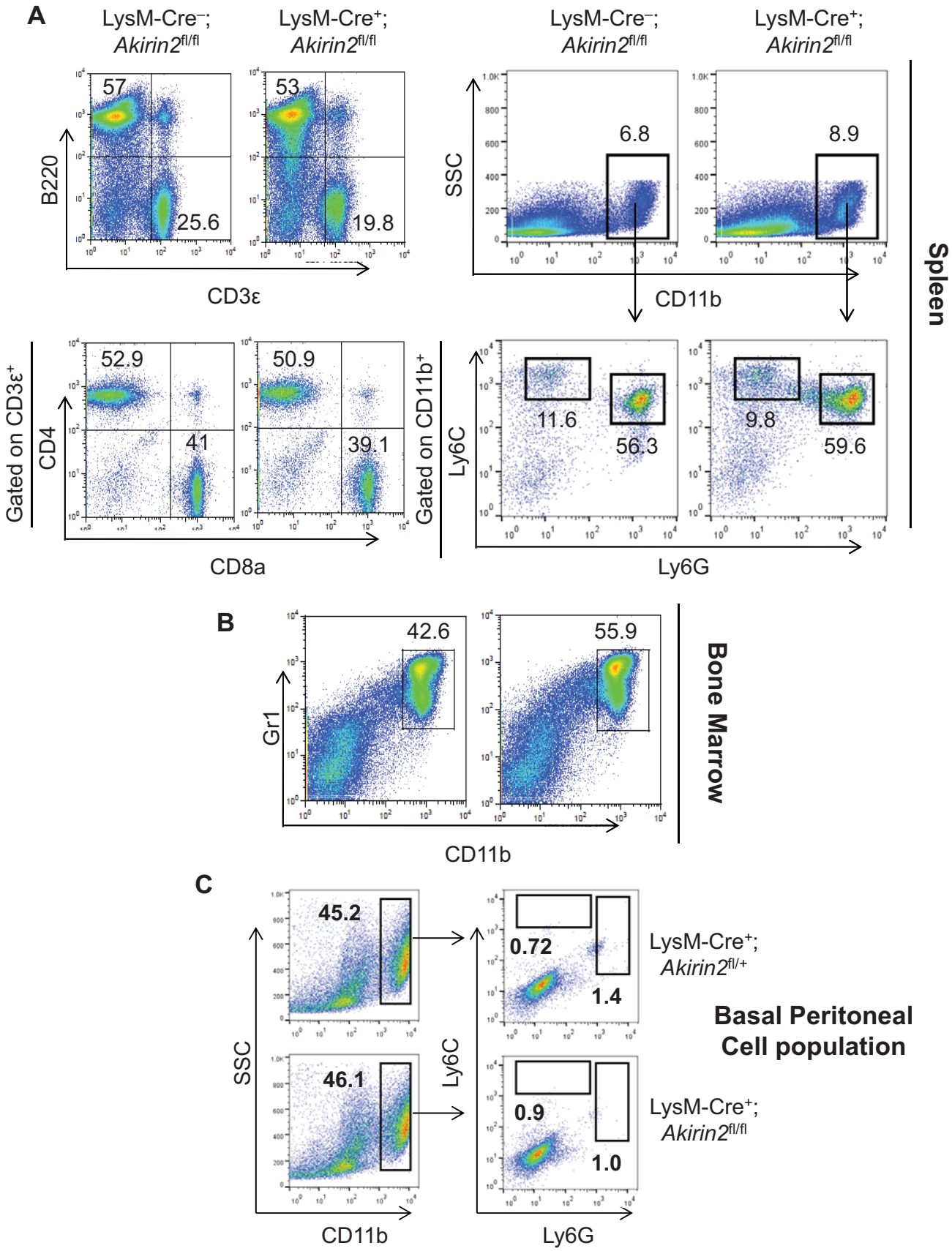
Sarang Tartey[1,2,3,5], Kazufumi Matsushita[6], Alexis Vandenbon[4], Daisuke Ori[1,2], Tomoko Imamura[1,2], Takashi Mino[1,2], Daron M. Standley[4], Jules A. Hoffmann[7], Jean-Marc Reichhart[7], Shizuo Akira[3,5] and Osamu Takeuchi[1,2,3]

[1]Laboratory of Infection and Prevention, Institute for Virus Research, Kyoto University, [2]CREST, JST, 53 Shogoin Kawara-cho, Sakyo-ku, Kyoto 606-8507, Japan
[3]Laboratory of Host Defense, [4]Laboratory of Systems Immunology, WPI Immunology Frontier Research Center (IFReC), [5]Research Institute for Microbial Diseases, Osaka University, 3-1 Yamada-oka, Suita, Osaka 565-0871, Japan
[6]Laboratory of Allergic Diseases, Institute for Advanced Medical Sciences, Hyogo College of Medicine, 1-1 Mukogawa-cho, Nishinomiya, Hyogo 663-8501, Japan
[7]IBMC UPR 9022 CNRS, 15, rue Rene Descartes, 67084 - Strasbourg Cedex, FRANCE

Correspondence and requests for materials should be addressed to O.T. (otake@virus.kyoto-u.ac.jp)

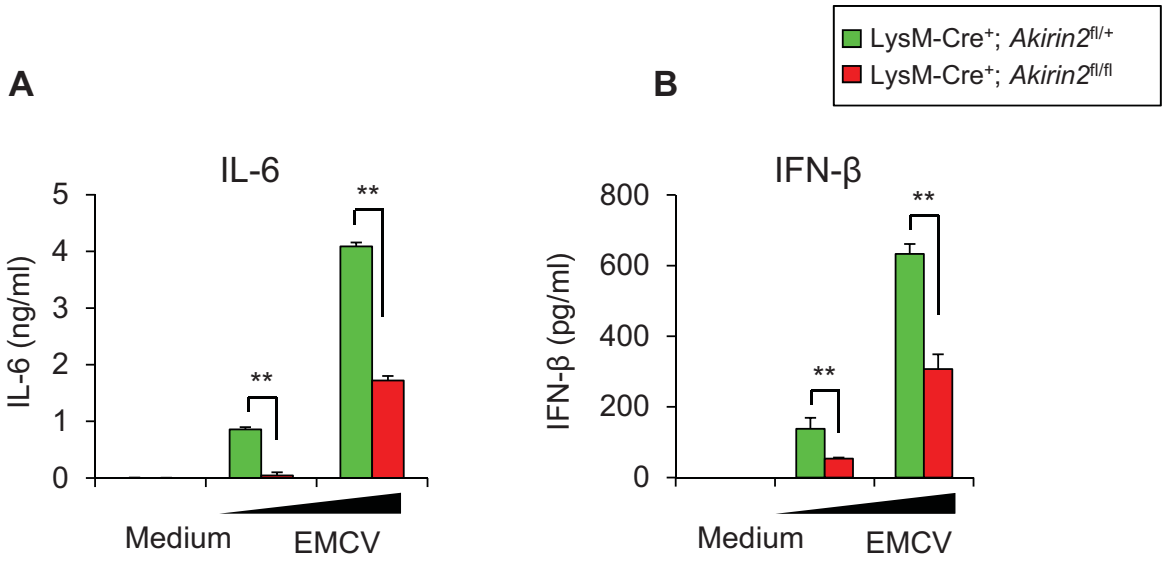


Supplementary Figure S1 Myeloid specific deletion of Akirin2.
Peritoneal macrophages from LysM-Cre^{+};*Akirin2*$^{fl/+}$ and LysM-Cre^{+};*Akirin2*$^{fl/fl}$ mice were stimulated with 100 ng/ml LPS, and the expression of *Akirin2* and *Actb* was determined in the total RNA by northern blot analysis (**A**) and in the whole cell lysate by western blot analysis (**B**).



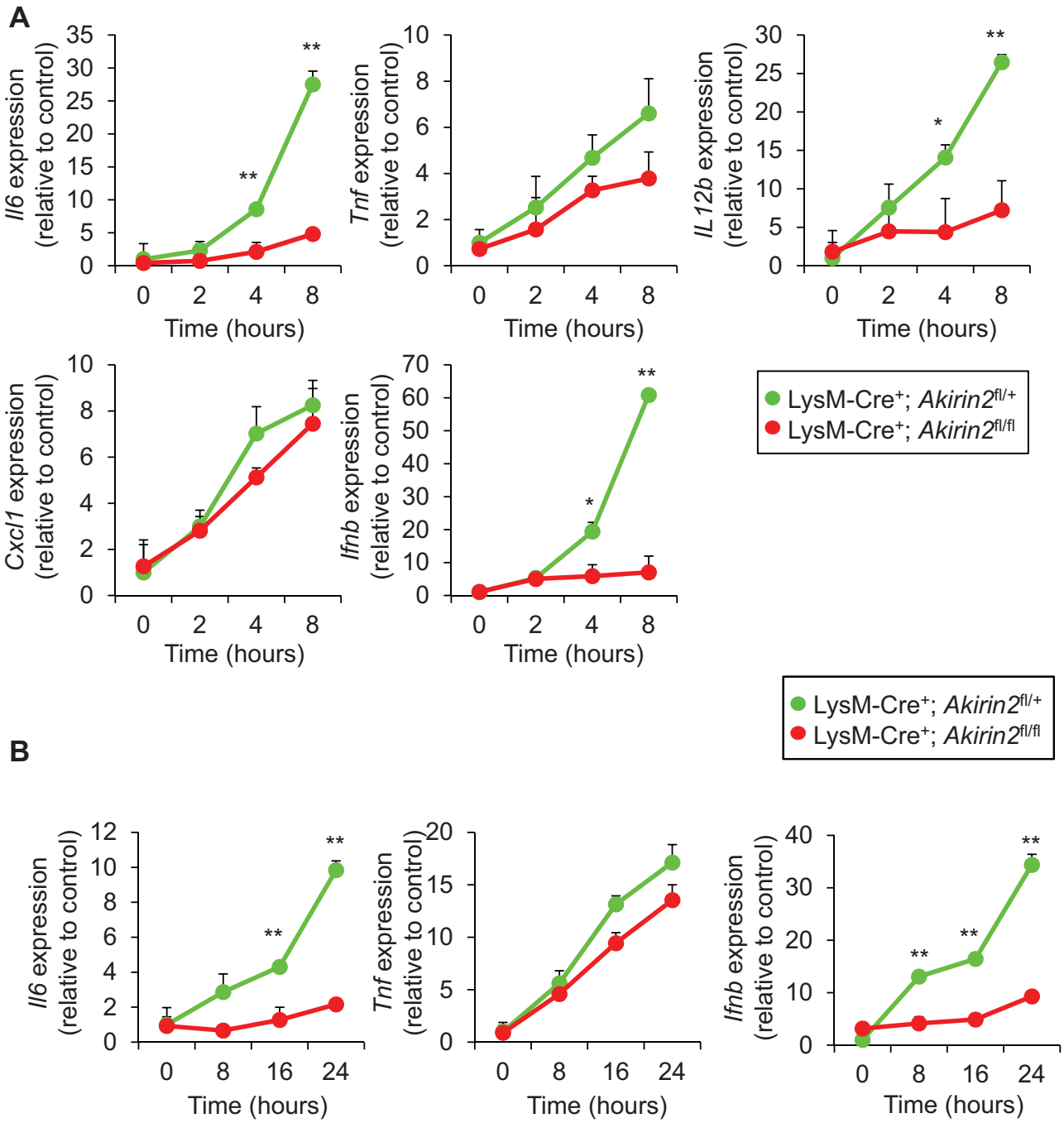
Supplementary Figure S2 Flow cytometric analysis of LysM-Cre+;*Akirin2*fl/fl mouse splenocytes and Bone Marrow Cells.

Splenocytes (**A**) Bone Marrow (**B**) and Peritoneal (**C**) cells from LysM-Cre⁻;*Akirin2*fl/fl and LysM-Cre+;*Akirin2*fl/fl mice were isolated and stained with indicated antibodies and were analyzed by flow cytometry. Numbers indicate the percentages of cells in the defined gates. Results are representative of two to three independent experiments.



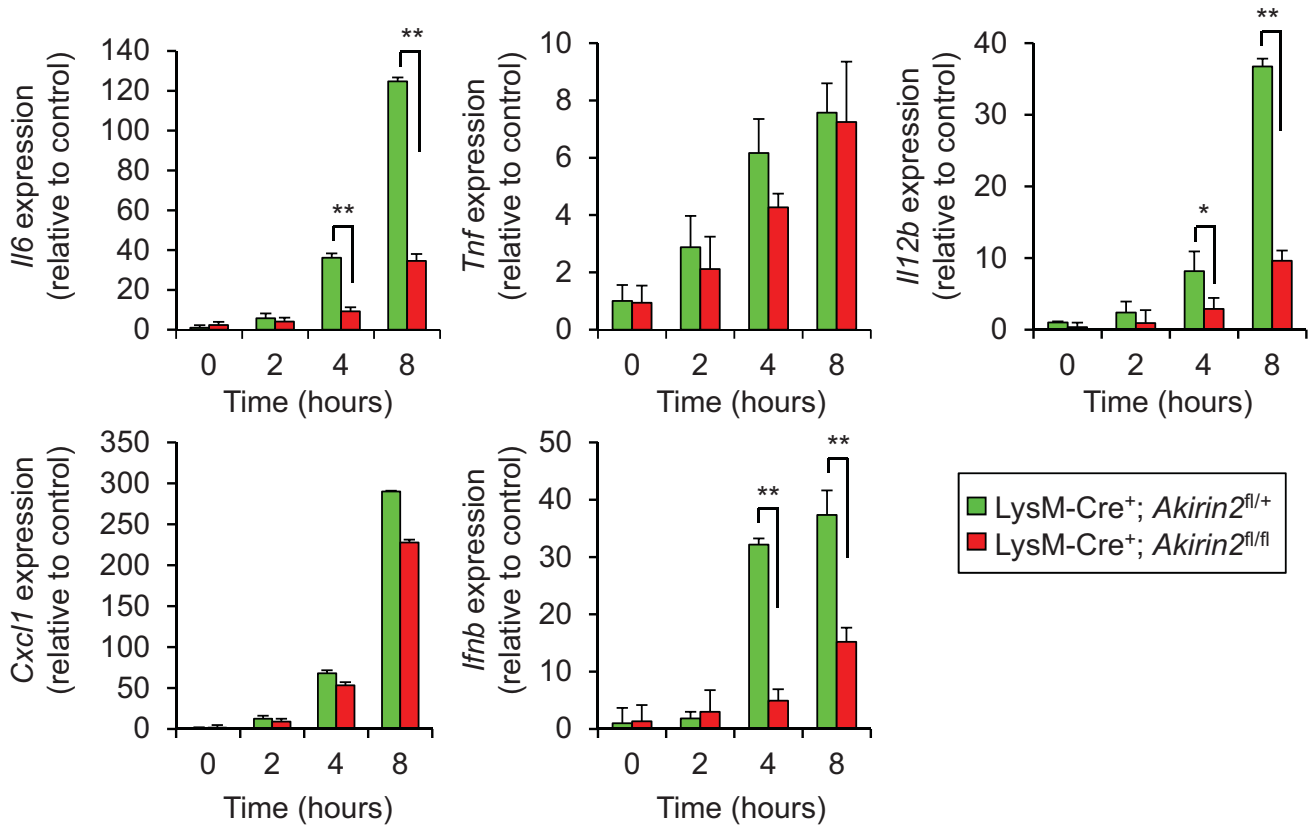
Supplementary Figure S3 Virus infection induced cytokine production in Akirin2-deficient macrophages.

LysM-Cre^{+};*Akirin2*$^{fl/+}$ and LysM-Cre^{+};*Akirin2*$^{fl/fl}$ mice were injected with 4% thioglycollate intra-peritoneally. Peritoneal exudate cells were collected three days after injection and stimulated with EMCV (MOI 1 and 5) for 24 h. IL-6 (**A**) and IFN-β (**B**) concentrations in the culture supernatants were determined by ELISA. The results are representative of three independent experiments (error bars represent s.d.). Statistical significance was determined using the Students t-test. **$p < 0.01$.



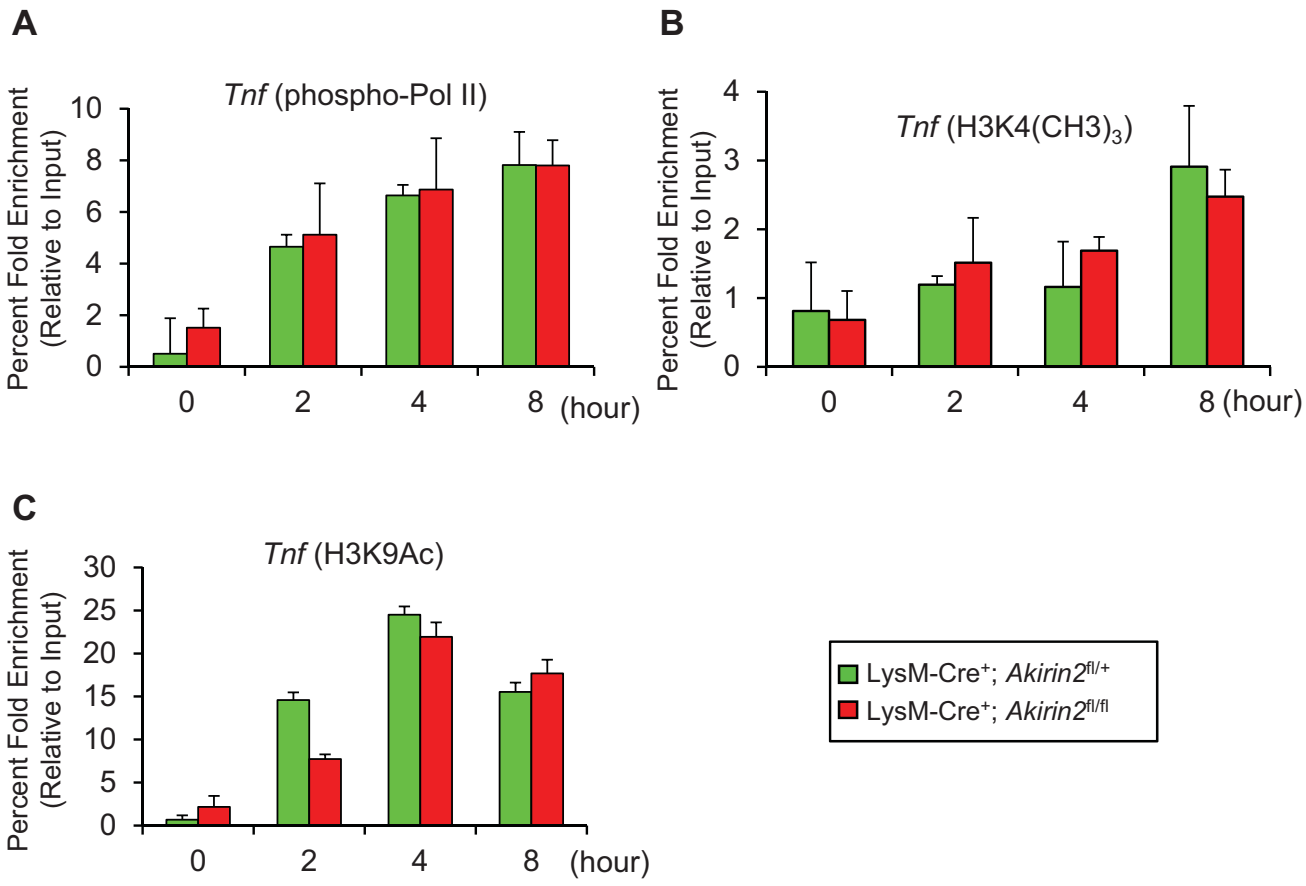
Supplementary Figure S4 LPS and Poly I:C induced gene expression in Akirin2-deficient peritoneal macrophages.

LysM-Cre^{+};*Akirin2*$^{fl/+}$ and LysM-Cre^{+};*Akirin2*$^{fl/fl}$ mice were injected with 4% thioglycollate intra-peritoneally. Peritoneal exudate cells were collected three days after injection and stimulated with (**A**) LPS (100 ng/ml) and (**B**) Poly I:C (200 µg/ml) for indicated time periods. Total RNA was prepared and quantitative PCR analysis was performed for the expression of *Il6*, *Tnf*, *Il12b*, *Cxcl1* and *Ifnb* mRNAs. All the samples were normalized to 18S rRNA. Error bars indicate mean ± std. deviation. Results are representative of three independent experiments. Statistical significance was determined using the Students t-test. *p < 0.05; **p < 0.01.

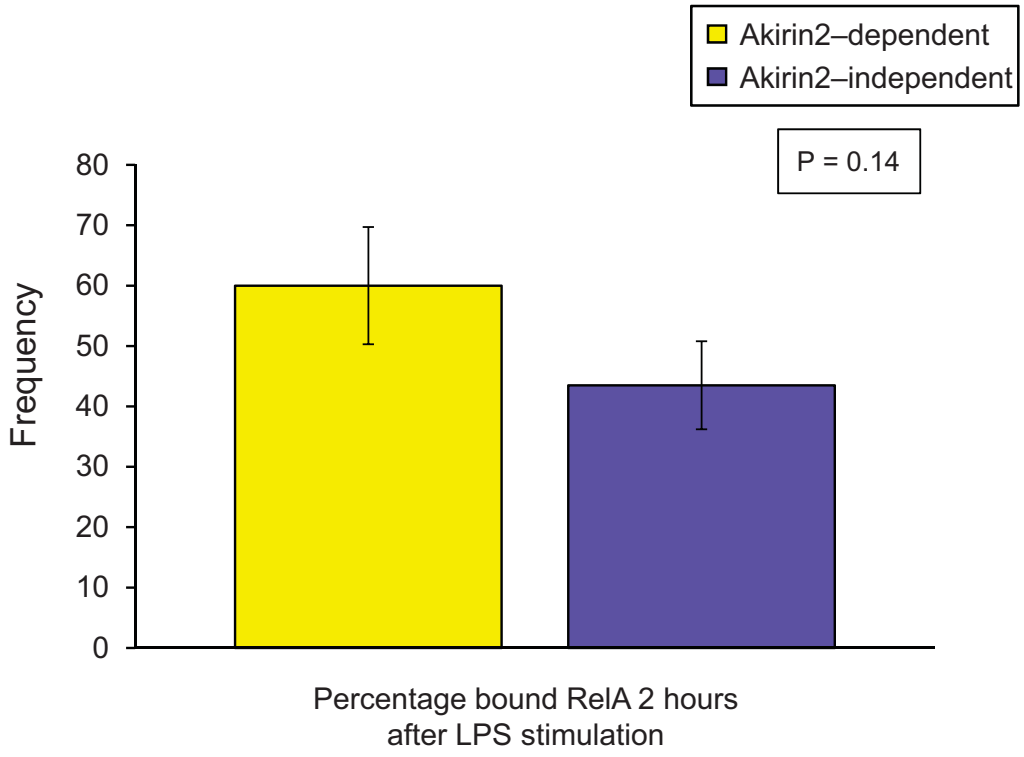


Supplementary Figure S5 LPS induced gene expression in Akirin2-deficient Bone marrow derived macrophages (BMDM).

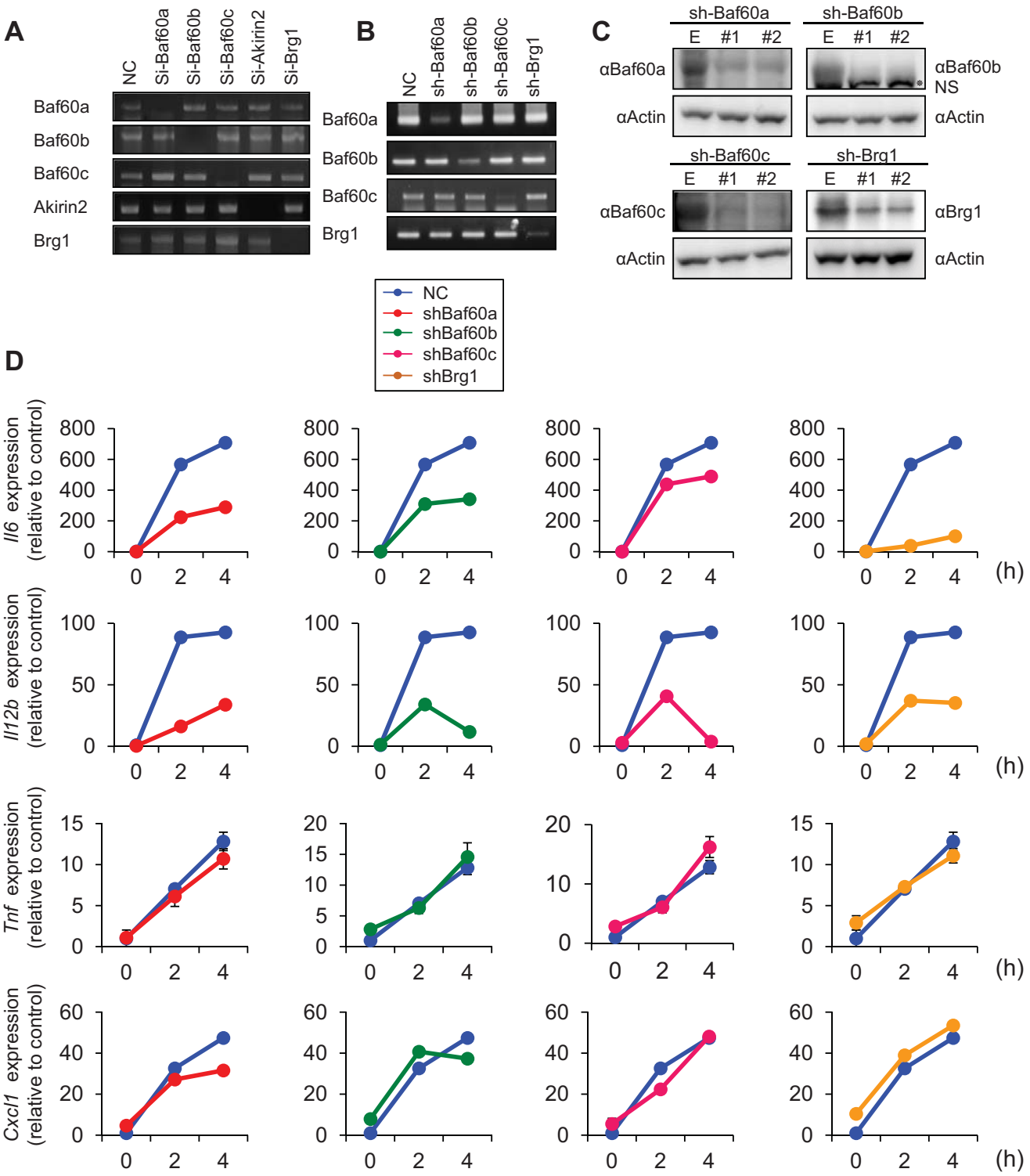
Quantitative PCR analysis for the expression of *Il6*, *Tnf*, *Il12b*, *Cxcl1* and *Ifnb* mRNAs in total RNA prepared from LPS (100 ng/ml) stimulated LysM-Cre^{+};*Akirin2*$^{fl/+}$ and LysM-Cre^{+};*Akirin2*$^{fl/fl}$ M-BMDMs. All the samples were normalized to 18S rRNA. Error bars indicate mean ± std. deviation. Results are representative of three independent experiments. Statistical significance was determined using the Students t-test. *$p < 0.05$; **$p < 0.01$.



Supplementary Figure S6 The recruitment of phospho-Pol II, H3K9 acetylation and H3K4 tri-methylation on the *Tnf* promoter was not affected by the lack of Akirin2. ChIP experiments were performed with chromatin prepared from LysM-Cre^{+};*Akirin2*$^{fl/+}$ and LysM-Cre^{+};*Akirin2*$^{fl/fl}$ PECs treated with or without LPS (1 μg/ml) for indicated time period. Antibody against phospho-RNA polymerase II (S5P) (**A**), tri-methyl Histone (H3K4) (**B**) and acetylated Histone (H3K9) (**C**) was used. Precipitated DNA was quantified by real-time PCR using primers specific for *Tnf* promoter region. ChIP values were normalized against the input and expressed as relative enrichment of the material precipitated by the indicated antibody on specific promoter (relative quantification using the comparative Ct method ($2^{-\Delta\Delta Ct}$)). Error bars indicate mean ± std. deviation. The results are representative of at least three independent experiments.



Supplementary Figure S7 The frequency of RelA binding was not significantly different between Akirin2-dependent and –independent gene promoters.
Frequency of NF-κB binding sites between Akirin2-dependent and –independent gene promoters. The Akirin2-dependent and –independent gene list was shown in Figure 4A. See Methods section for details.

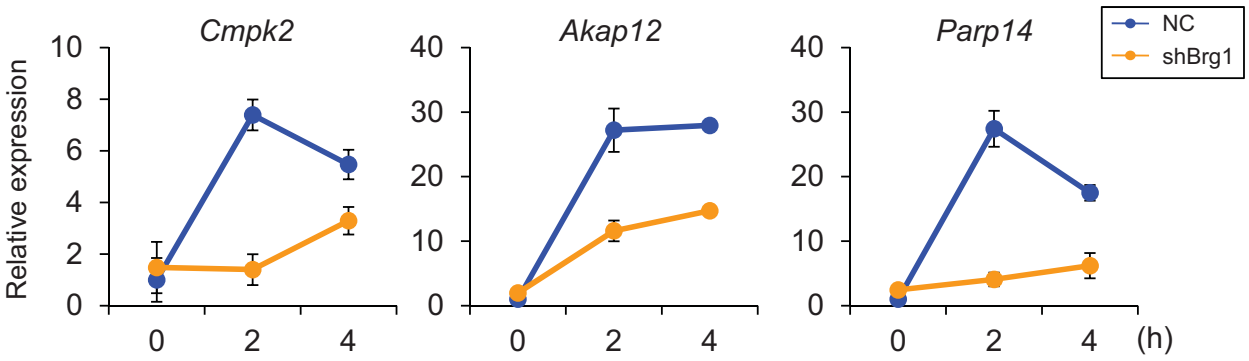


Supplementary Figure S8. Knockdown efficiency of chromatin modifiers and LPS induced gene expression

(**A**) HeLa cells were transfected with siRNA for the indicated genes. The efficiency of knockdown was determined by semi quantitative RT-PCR.

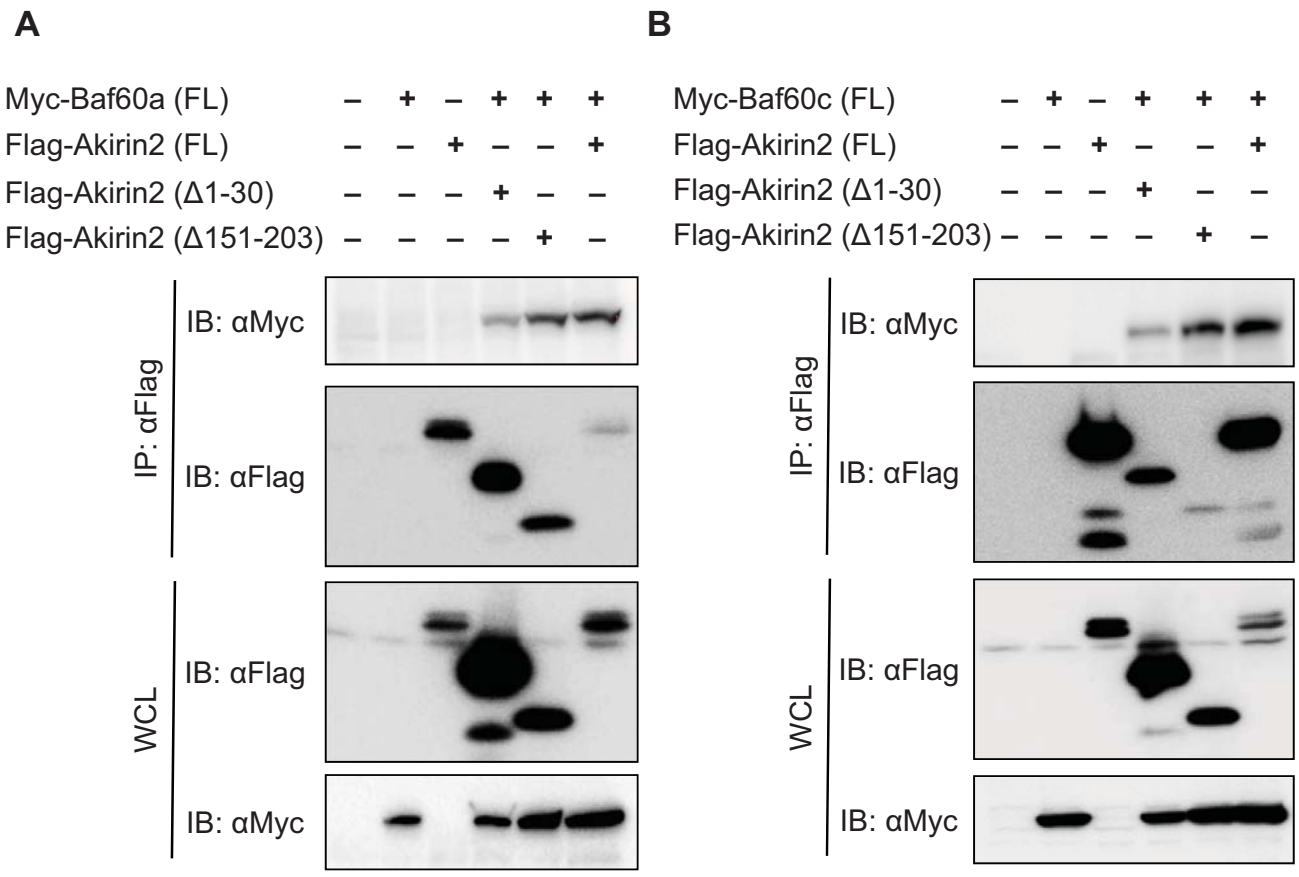
(**B and C**) J774 cells were transfected with two different shRNAs (#1 and #2) for the indicated genes. The efficiency of knockdown was determined by **(B)** semi quantitative RT-PCR and **(C)** Western blot for endogenous protein levels.

(**D**) shRNA knockdown cells were stimulated with LPS for indicated time periods. Total RNA was prepared and quantitative PCR analysis was performed for the expression of *Il6*, *Il12b*, *Tnf* and *Cxcl1* mRNAs. All the samples were normalized to 18S rRNA. Error bars indicate mean ± std. deviation. Results are representative of two independent experiments.



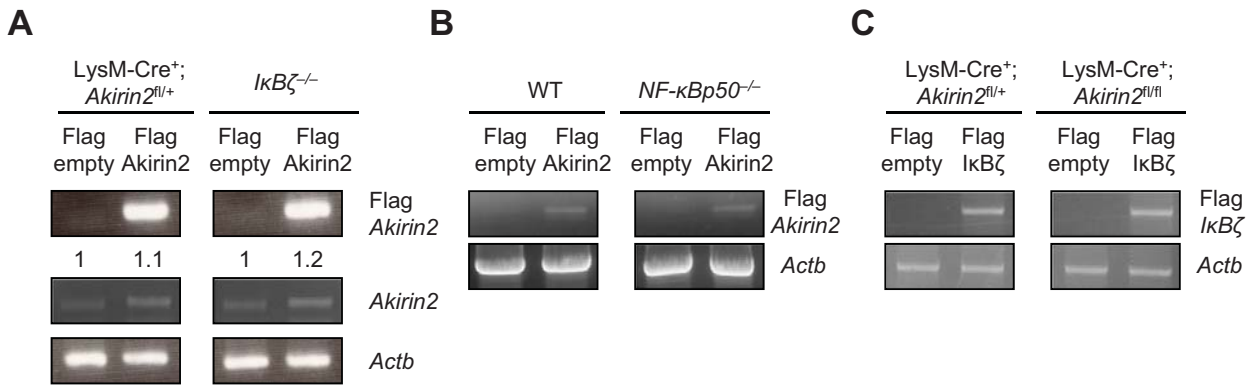
Supplementary Figure S9. SWI/SNF Dependency of CpG island harbouring Akirin2 target genes.

Brg1-shRNA knockdown J774 cells were stimulated with LPS for indicated time periods. Total RNA was prepared and quantitative PCR analysis was performed for the expression of *Cmpk2*, *Akap12* and *Parp14* mRNAs. All the samples were normalized to *Actinb* mRNA. Error bars indicate mean ± std. deviation. Results are representative of two independent experiments.



Supplementary Figure S10. Interaction of Akirin2 deletion mutants with BAF60a and Baf60c

Cell lysates prepared from HEK293 cells transfected with plasmids encoding Myc-BAF60a (**A**) or BAF60c (**B**) together with Flag-tagged Akirin2 (Full length) and a series of deletion mutants were immuno-precipitated with anti-FLAG antibody, followed by immunoblot analysis using anti-Myc and anti-Flag antibodies. The results are representative of at least three independent experiments.



Supplementary Figure S11. Retroviral expression of Flag-Akirin2 and Flag-IκB-ζ in BM macrophages

(**A**) LysM-Cre^{+};*Akirin2*$^{fl/+}$ and *IκBζ*$^{-/-}$ (**B**) WT and *NF-κBp50*$^{-/-}$ and (**C**) LysM-Cre^{+};*Akirin2*$^{fl/+}$ and LysM-Cre^{+};*Akirin2*$^{fl/fl}$ mice BM were retrovirally transduced with Flag-tagged Akirin2 (**A and B**) or Flag-tagged IκB-ζ (**C**). Total RNA was prepared and expression of Flag-tagged *Akirin2*, *IκB-ζ* and *Akirin2* mRNA levels were determined by RT-PCR. Signals were quantified, and values indicate relative density compared with the expression of *Actb*.