SUPPLEMENTAL MATERIAL

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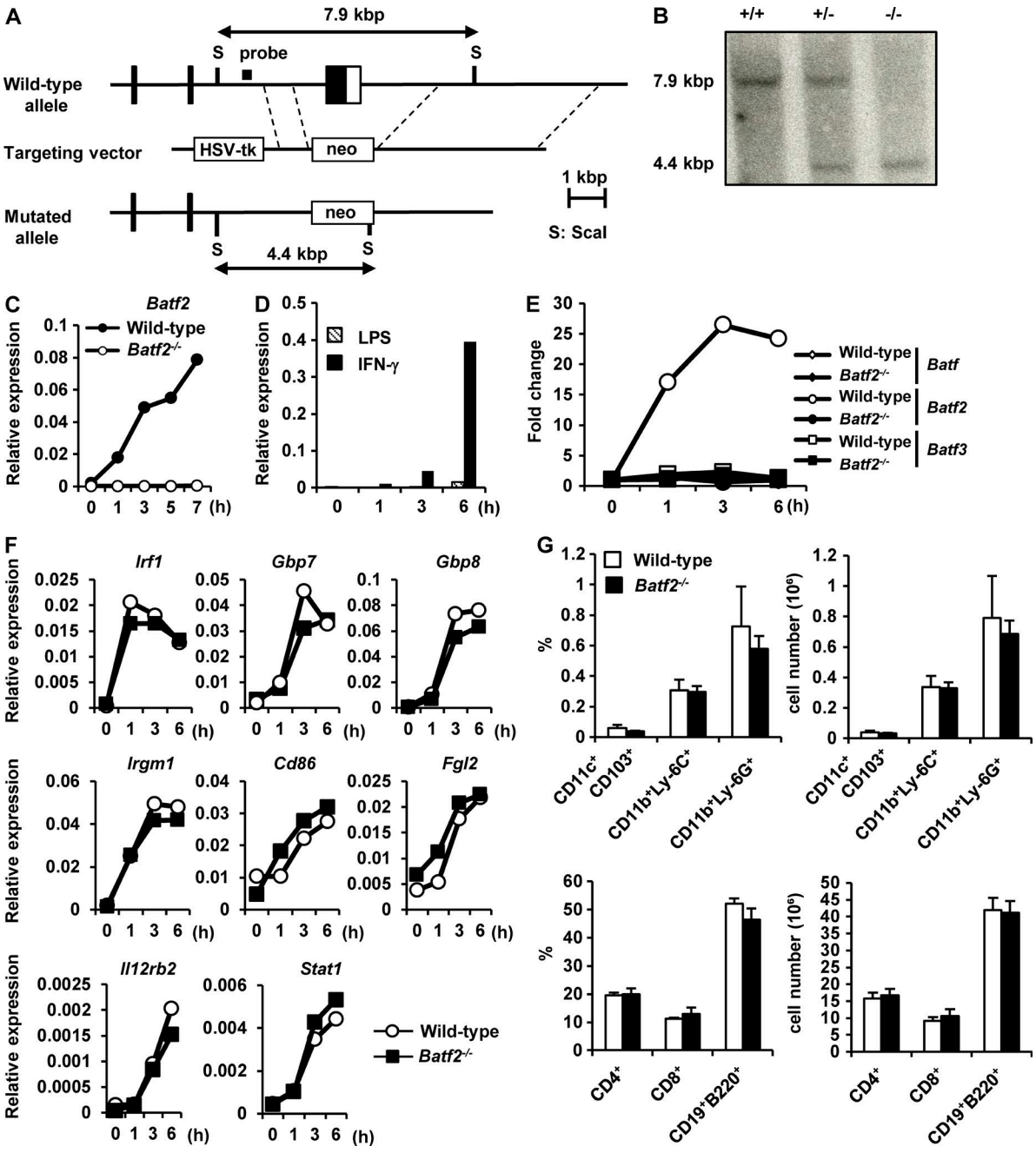


Figure S1. **Generation of *Batf2*$^{-/-}$ mice.** (A) Map of the BATF2 wild-type genome, targeting vector, and predicted targeted gene. Open boxes, noncoding exons; closed boxes, coding exons; S, ScaI. (B) Southern blot analysis of offspring from heterozygote intercrosses. Genomic DNA from the mouse tails was digested with ScaI, separated electrophoretically, and then hybridized with the probe indicated in A. Proximal size of the wild-type band is 7.9 kbp; of the mutated band is, 4.4 kbp. (C) BMMφs were stimulated with or without 100 ng/ml LPS plus 10 ng/ml IFN-γ for the indicated periods and then analyzed for the expression of BATF2 mRNA with real-time RT-PCR. The data are representative of two independent experiments. (D) BMMφs were stimulated with or without either LPS or IFN-γ and analyzed for expression of BATF2 mRNA after the indicated periods. Data are representative of two independent experiments. (E) Expression of *Batf*, *Batf2*, and *Batf3* in wild-type and *Batf2*$^{-/-}$ BMMφs stimulated with IFN-γ for the indicated periods. Data are representative of two independent experiments. (F) Expression of a subset of IFN-γ–dependent genes in wild-type and *Batf2*$^{-/-}$ BMMφs stimulated with IFN-γ for the indicated periods. All the data are representative of at least two independent experiments. (G) Frequency (left) and number (right) of innate immune cell subsets, including CD11c$^+$ CD103$^+$, CD11b$^+$ Ly-6G$^+$, and CD11b$^+$ Ly-6C$^+$ and lymphocytes, including CD3$^+$ CD4$^+$, CD3$^+$ CD8$^+$, and CD19$^+$ B220$^+$ in the spleens from wild-type ($n$ = 4) and *Batf2*$^{-/-}$ ($n$ = 3) mice (mean values ± SD).

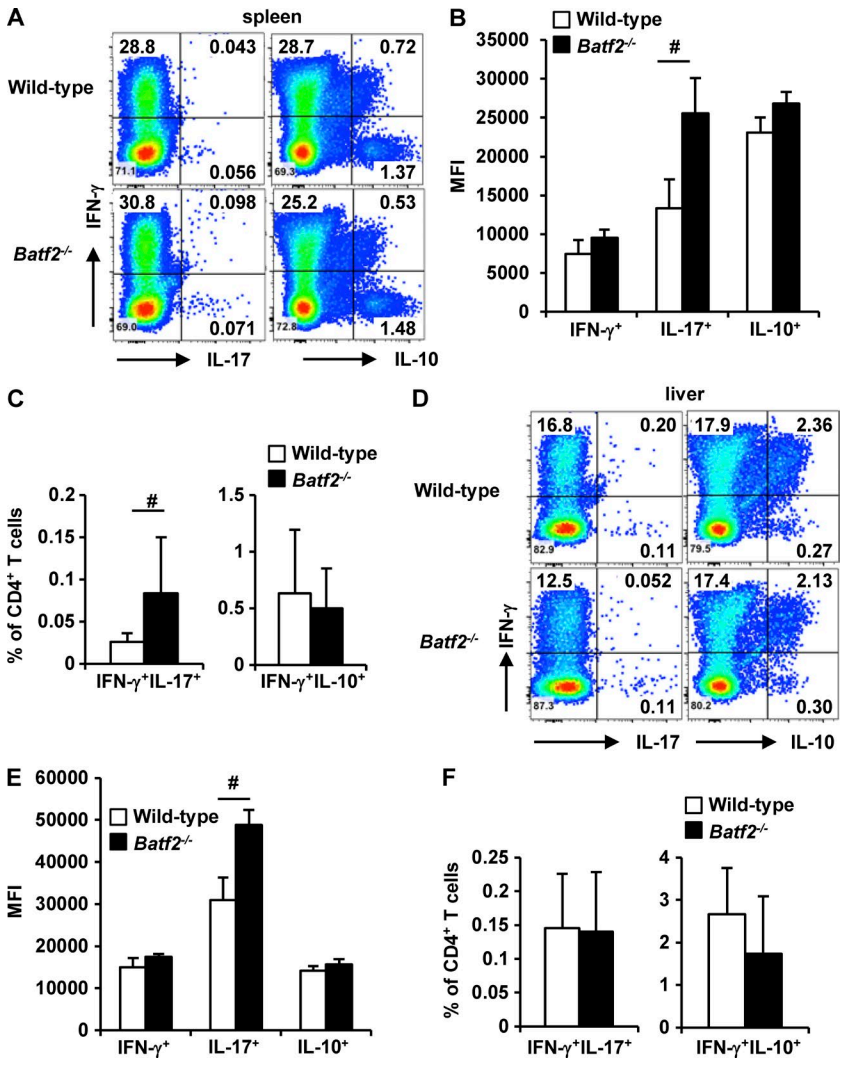


Figure S2. **Increased production of IL-17, but not IL-10 and IFN-γ, in CD4⁺ T cells in *T. cruzi*–infected *Batf2*$^{-/-}$ mice.** Wild-type ($n$ = 6) and *Batf2*$^{-/-}$ ($n$ = 6) mice were infected with *T. cruzi* for 20 d, and the spleens and livers were collected. (A and D) Flow cytometric blot of IFN-γ–, IL-17–, and IL-10–producing CD4⁺ T cells from the spleens (A) and livers (D). Data are representative of six independent experiments. (B and E) Mean fluorescence intensity (MFI) of IFN-γ, IL-17, and IL-10 in CD4⁺ T cells from the spleens (B) and livers (E). Data show mean values ± SEM. #, $P < 0.05$. (C and F) Frequency of IFN-γ⁺ IL-17⁺ and IFN-γ⁺ IL-10⁺ cells among CD4⁺ T cells from the spleens (C) and livers (F). Data are mean values ± SD. #, $P < 0.05$.

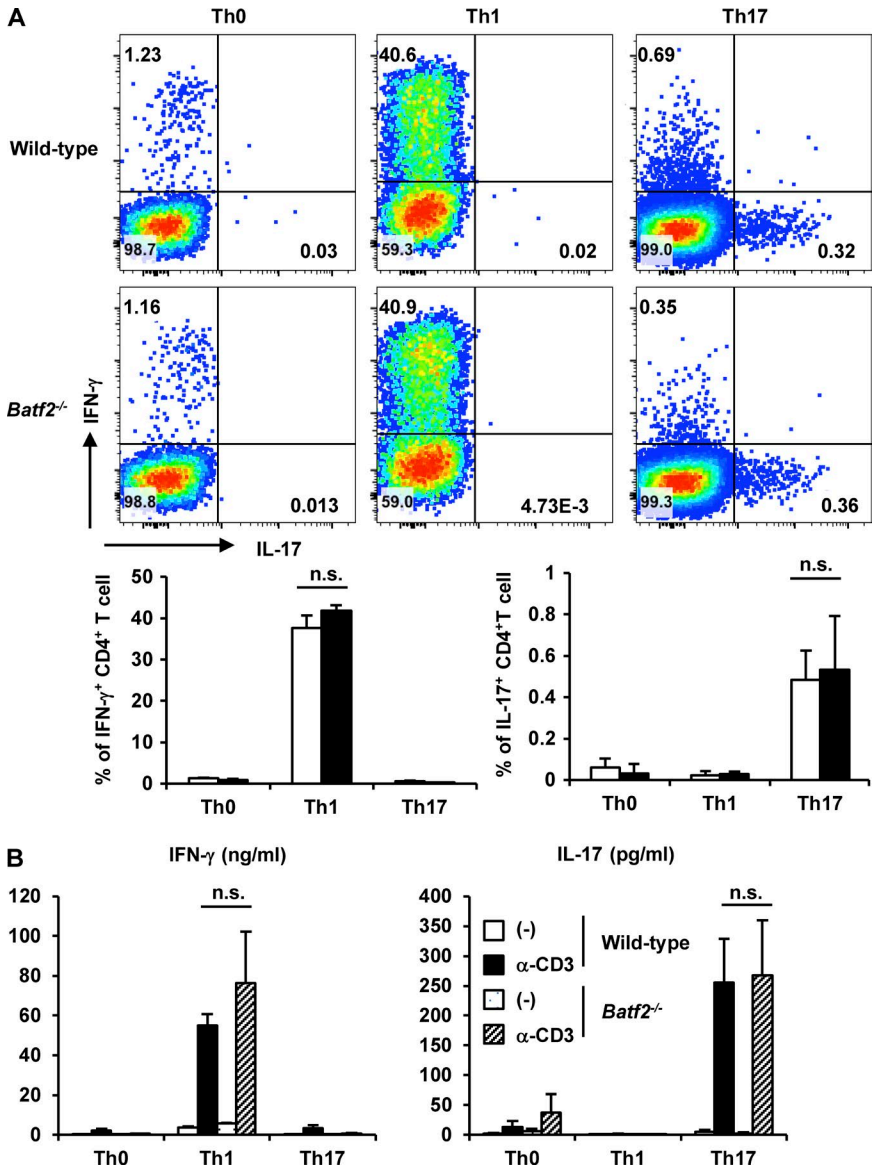


Figure S3. **Normal Th1 and Th17 cell differentiation of *Batf2*$^{-/-}$ CD4$^+$ T cells.** Naive wild-type and *Batf2*$^{-/-}$ CD4$^+$ T cells isolated from the spleen were cultured under Th0, Th1, and Th17 conditions for 4 d. (A) Flow cytometric plots of IFN-γ– and IL-17A–producing CD4$^+$ T cells (top). Frequency of IFN-γ– and IL-17A–producing CD4$^+$ T cells (bottom). (B) CD4$^+$ T cells cultured under Th0, Th1, and Th17 conditions for 4 d were stimulated with anti-CD3 antibody for 24 h. The culture supernatants were analyzed for IFN-γ and IL-17 with ELISA. n.s., not significant. Graphs show mean values ± SEM from three independent experiments.

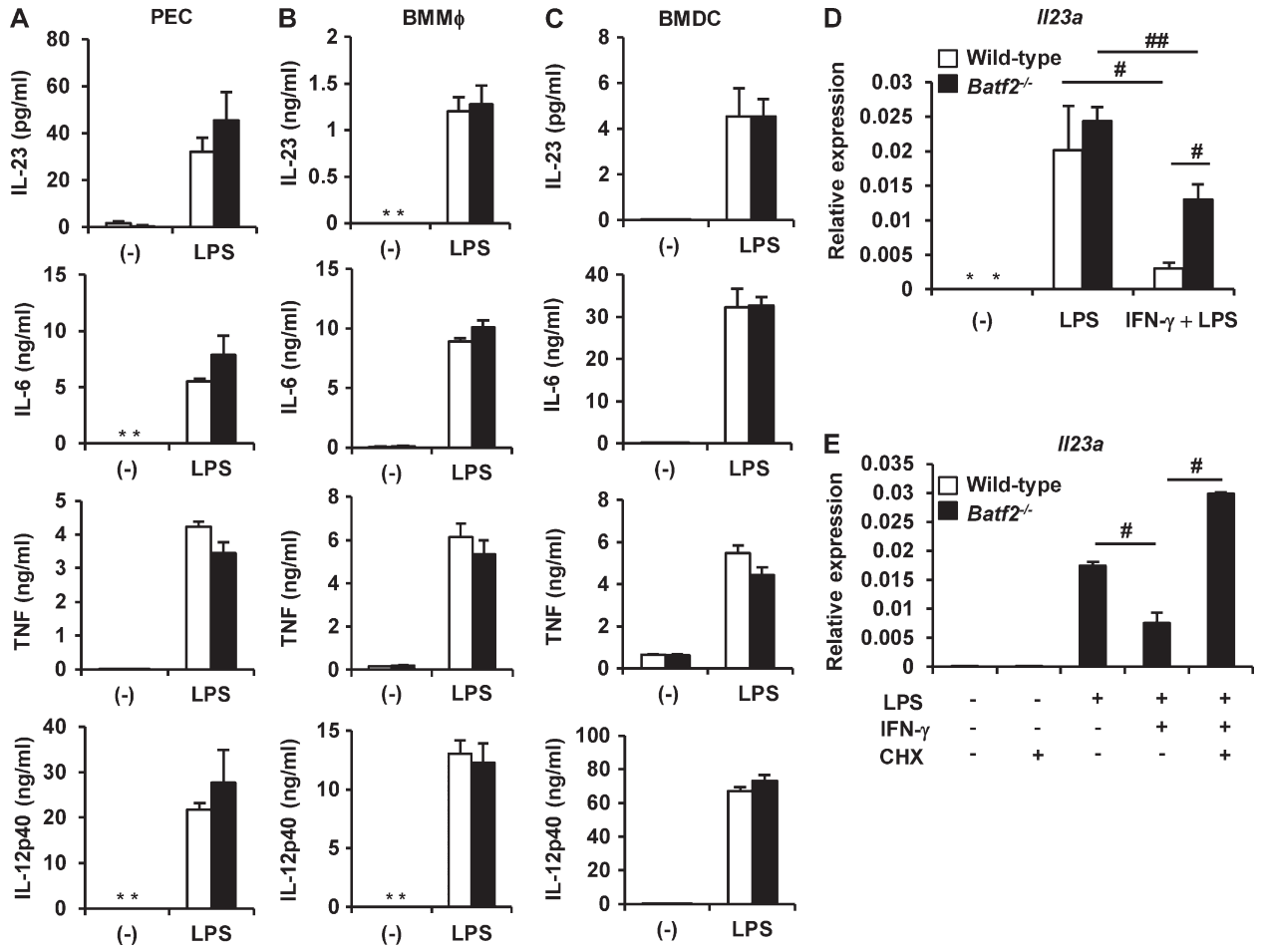


Figure S4. **IFN-γ–induced BATF2 suppressed *Il23a* expression induced by LPS.** (A–C) Wild-type and *Batf2*$^{-/-}$ PECs (A), BMMφs (B), and BMDCs (C) were stimulated with LPS for 24 h. The culture supernatants were analyzed for production of IL-6, IL-23, IL-12p40, and TNF. Data are mean ± SEM from three independent experiments. *, not detected. (D) Wild-type and *Batf2*$^{-/-}$ BMMφs pretreated with or without IFN-γ for 4 h were stimulated with LPS for 3 h, and expression of *Il23a* was analyzed. Graph shows the mean values ± SD of triplicate PCRs on identical samples. Data are representative of two independent experiments. #, $P < 0.03$; ##, $P < 0.05$; *, not detected. (E) BMMφs prepared from wild-type and *Batf2*$^{-/-}$ mice were treated with IFN-γ in the presence or absence of 1 mg/ml CHX for 4 h and then stimulated with LPS. After 3 h, expression of *Il23a* was analyzed (mean values ± SD). Representative results for three independent experiments are shown. #, $P < 0.001$.

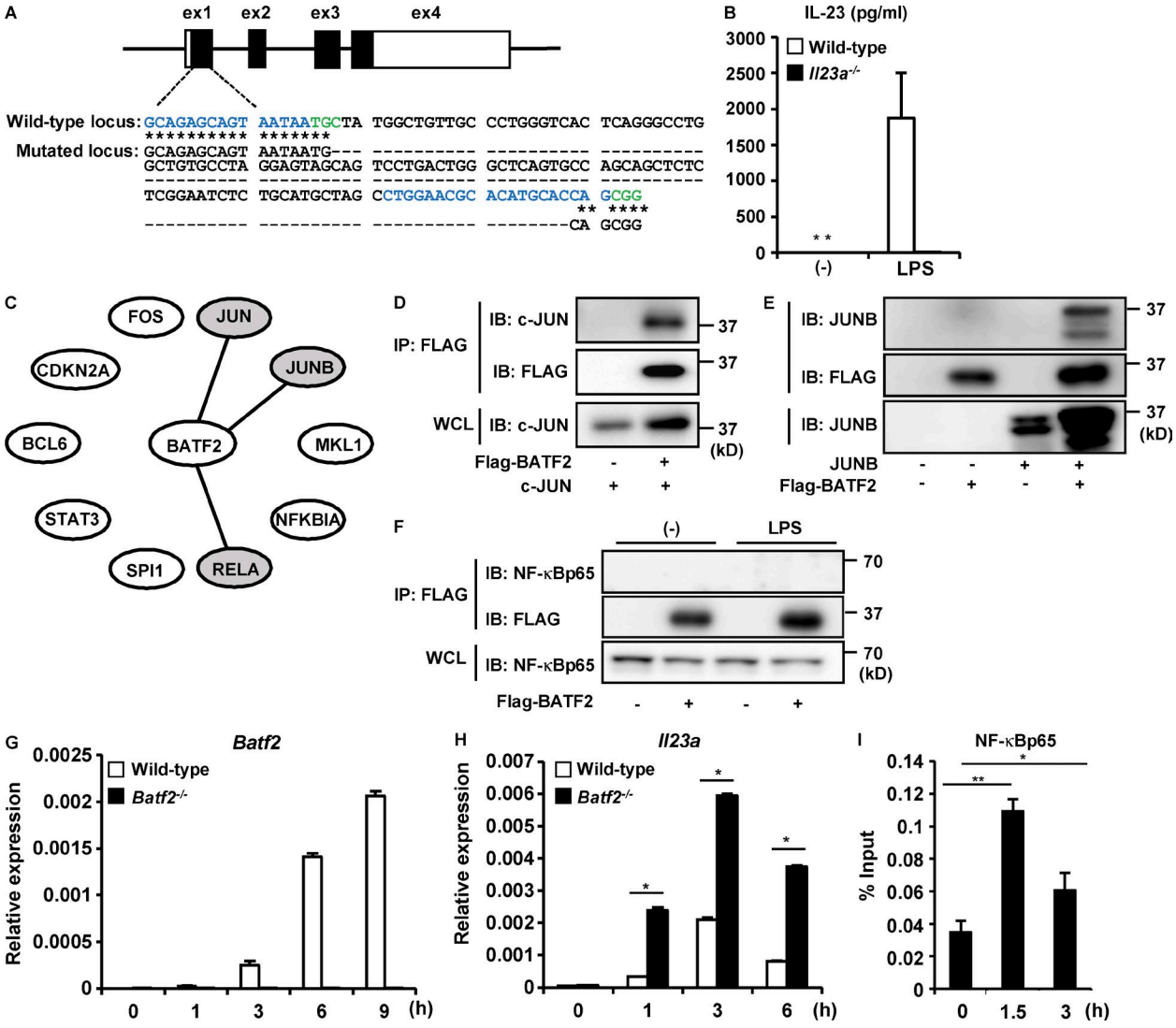


Figure S5. **BATF2-mediated down-regulation of *Il23a* expression through its interaction with c-JUN.** (A) Scheme of the Cas9/gRNA-targeting site in the first exon (ex1) of *Il23a* gene. (Top) Structure of the *Il23a* gene. Black boxes, coding exons; white boxes, noncoding exons. (Bottom) The sequence of wild type and the mutated allele. Light blue, sgRNA targeting sequence; green, PAM; black dashes, identified mutation. (B) BMMφs prepared from wild-type and *Il23a*$^{-/-}$ mice were stimulated with or without LPS. After 24 h, culture supernatants were analyzed for IL-23 by ELISA. Data are representative of two independent experiments (mean values ± SEM). *, not detected. (C) Binding partners of BATF2 predicted with IPA. (D–F) HEK293 cells were cotransfected with the Flag-BATF2 expression vector and the c-JUN (D) or JUNB (E) expression vector. TLR4-expressing HEK293 cells were transfected with Flag-BATF2 and stimulated with LPS for 4 h, 24 h after transfection (F). Total cell lysates were used for coimmunoprecipitation analysis, using anti-Flag antibody for immunoprecipitation (IP) and the indicated antibodies for immunoblotting (IB). All data are representative of three independent experiments. WCL, whole cell lysate. (G and H) Wild-type and *Batf2*$^{-/-}$ MEFs were stimulated with LPS for the indicated periods after treatment with IFN-γ and then analyzed for expression of *Batf2* (G) and *Il23a* (H). *, $P < 0.02$. Mean values ± SD for triplicate PCR on identical samples. Data are representative of at least two independent experiments. (I) Wild-type MEFs pretreated with IFN-γ were stimulated with LPS for the indicated periods, and ChIP assay was performed with anti–NF-κBp65 antibody. NF-κB–binding site in the *Il23a* promoter was analyzed in the precipitated DNA with qPCR. Means ± SD of triplicate PCRs on identical samples. Data are representative of three independent experiments. *, $P < 0.003$; **, $P < 0.0001$.

**Table S1, included in a separate Excel file, lists genes upregulated in response to LPS in *Batf2*$^{-/-}$ BMMφs pretreated with IFN-γ.**