Supporting Information

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SI Materials and Methods

Reagents. LPS (0111:B4), poly(I:C), and CpG oligodeoxynucleotide (ODN) were described previously (1). *Escherichia coli* 0111:B4 was obtained from the China Center for Type Culture Collection. *Listeria monocytogenes* was provided by H. Shen (University of Pennsylvania Perelman School of Medicine, Philadelphia). Antizinc finger and bric-a-brac domain-containing 20 (anti-ZBTB20) antibody used for immunoblot and ChIP assays was from Santa Cruz. Antibodies specific to IκBα and p65, phosphospecific antibodies against ERK (Thr202/Tyr204), JNK (Thr183/Tyr185), p38 (Thr180/Tyr182), inhibitor of κB kinase α/β (IKKα/β; Ser177/181), p65 (Ser536), and IFN regulatory factor 3 (Ser396) were from Cell Signaling Technology. Antibodies specific to Toll-like receptor 4 (TLR4), TLR3, and TLR9 were from Abcam. Antibodies specific to lamin A and β-actin were from Santa Cruz. Recombinant murine IFN-β and TGF-β1 were from R&D Systems. The inhibitor of MAPK kinase 1/ERK (PD98059), JNK (SP600125), p38 (SB203580), and NF-κB (pyrrolidinecarbodithoic acid) were from Merck.

Plasmid Constructs. The recombinant vectors encoding mouse *Zbtb20* (GenBank accession no. NM_019778.2) were constructed by PCR-based amplification from cDNA of mouse macrophages and then subcloned into the pcDNA3.1 eukaryotic expression vector (Invitrogen). The promoter of *IκBα* and its deletion mutants as well as the promoter of the *IκBβ*, *IKKα*, and *IKKβ* genes were amplified from genome of mouse macrophages by PCR and then subcloned into the PGL3–enhance luciferase vector (Promega) to construct promoter luciferase reporter vector as described previously (2). All constructs were confirmed by DNA sequencing.

Cell Culture and Transfection. HEK293 cells and thioglycolate-elicited mouse peritoneal macrophages were prepared, cultured, and transfected with DNA plasmids as described previously (1).

RNA Quantification. The extraction of total cellular RNA and quantitative real-time RT-PCR analysis (Q-PCR) were performed as described previously (1). The primers used for IκBα were 5′-TGAAGGACGAGGAGTACGAGC-3′ (sense) and 5′-TGCAGGAACGAGTCTCCGT-3′ (antisense). The primers used for TNF, IL-6, IFN-β, and β-actin were described previously (1). Data were normalized to β-actin expression.

Cytokine Detection. TNF, IL-6, and IFN-β in the supernatants and serum were measured with ELISA Kits (R&D Systems).

Assay of Luciferase Reporter Gene Expression. HEK293 cells were cotransfected with a mixture of indicated luciferase reporter plasmid, pRL-TK-Renilla-luciferase plasmid, and indicated other constructs. Total amounts of plasmid DNA were equalized with empty control vector. After 24 h, luciferase activities were measured using the Dual Luciferase Reporter Assay System (Promega) as described previously according to the manufacturer's instructions. Data are normalized for transfection efficiency by dividing *Firefly* luciferase activity with activity of *Renilla* luciferase.

Immunoblot. Cells were lysed with cell lysis buffer (Cell Signaling Technology) supplemented with protease inhibitor mixture. Protein concentrations of the extracts were measured with bicinchoninic acid assay (Pierce). The immunoblot assays were performed as described previously (1).

Establishment of the Endotoxin Shock Model and the Bacterial Sepsis Model. The endotoxin shock mouse model was established by i.p. injection of 15 mg/kg LPS as described previously (1). For bacterial infection, *E. coli* serotype 0111:B4 and *L. monocytogenes* in midlogarithmic growth were collected, counted on agar plates, and then resuspended in PBS. Mice were given i.p. injection of 1×10^7 *E. coli* or i.v. injection of 1×10^4 *L. monocytogenes*. Serum was collected for measurement of cytokines (or cfu for *E. coli*), and spleens or livers were lysed for measurement of cfu (*L. monocytogenes*) as described (3).

Macrophage Reconstitution. Bone marrow cells from myeloid cell-specific ZBTB20 KO (MZB20KO) and control mice were cultured for 7 d in murine macrophage colony-stimulating factor (50 ng/mL; PeproTech) for the preparation of bone marrow-derived macrophages (BMDMs). Clodronate liposomes (Sigma) were injected i.p. into WT mice (50 mg in 200 μL per mouse) to deplete endogenous macrophages; 2 d later, BMDMs from MZB20KO or control mice (1×10^7) were transplanted into the macrophage-depleted mice by tail vein injection 6 h before challenge with LPS (3).

1. Liu X, et al. (2011) Intracellular MHC class II molecules promote TLR-triggered innate immune responses by maintaining activation of the kinase Btk. *Nat Immunol* 12(5): 416–424.
2. Ma F, et al. (2011) The microRNA miR-29 controls innate and adaptive immune responses to intracellular bacterial infection by targeting interferon-γ. *Nat Immunol* 12(9):861–869.
3. Han C, et al. (2010) Integrin CD11b negatively regulates TLR-triggered inflammatory responses by activating Syk and promoting degradation of MyD88 and TRIF via Cbl-b. *Nat Immunol* 11(8):734–742.

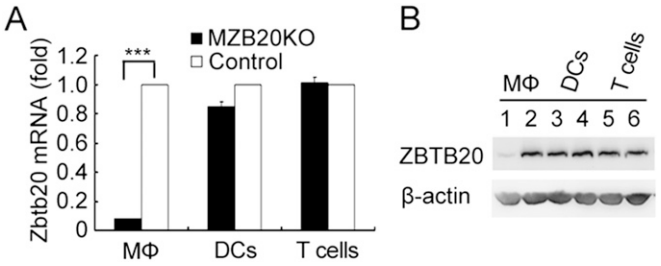

Fig. S1. ZBTB20 deficiency in macrophages from myeloid cell-specific ZBTB20 KO mice. (*A*) Q-PCR analysis of ZBTB20 mRNA level or (*B*) immunoblot analysis of ZBTB20 protein level in peritoneal macrophages (Mϕ), splenic dendritic cells (DCs), or T cells from MZB20KO ($ZBTB20^{flox/flox/LysM\text{-}Cre}$) and control ($ZBTB20^{flox/flox}$) mice. (*B*) Lanes 1, 3, and 5 are MZB20KO mice; lanes 2, 4, and 6 are control mice. ***$P < 0.001$. Data are (*A*) from three independent experiments (mean ± SEM) or (*B*) representative of three independent experiments with similar results.

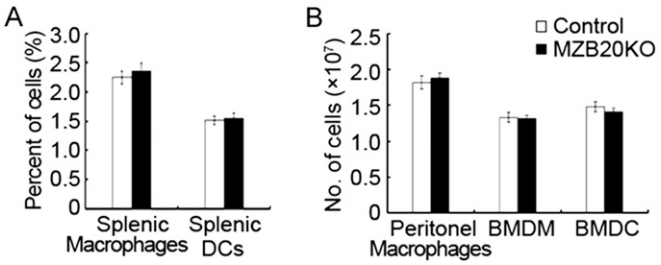

Fig. S2. Normal myeloid development and macrophage differentiation in *Zbtb20*-deficient mice. (*A*) Analysis of the percentage of macrophages ($CD11b^+$ $F4/80^+$) and DCs ($CD11c^+$) in splenocytes from MZB20KO and control mice by flow cytometry. (*B*) Analysis of numbers of peritoneal macrophages (pMϕ), BMDMs, and bone marrow-derived DCs (BMDCs) from MZB20KO and control mice by flow cytometry. Data are shown as mean ± SEM of three independent experiments.

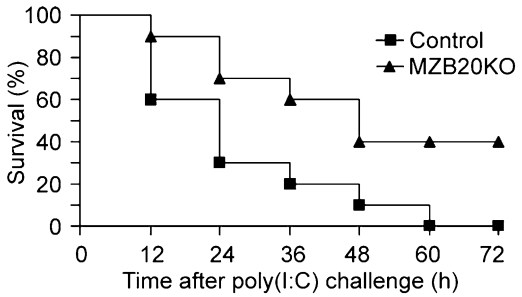

Fig. S3. ZBTB20 deficiency protects mice from challenge with poly(I:C). Survival of MZB20KO or control mice ($n = 10$ per genotype) given i.p. injection of poly (I:C) (20 mg/kg body weight). $P < 0.01$ (Wilcoxon test). Similar results were obtained in three independent experiments.

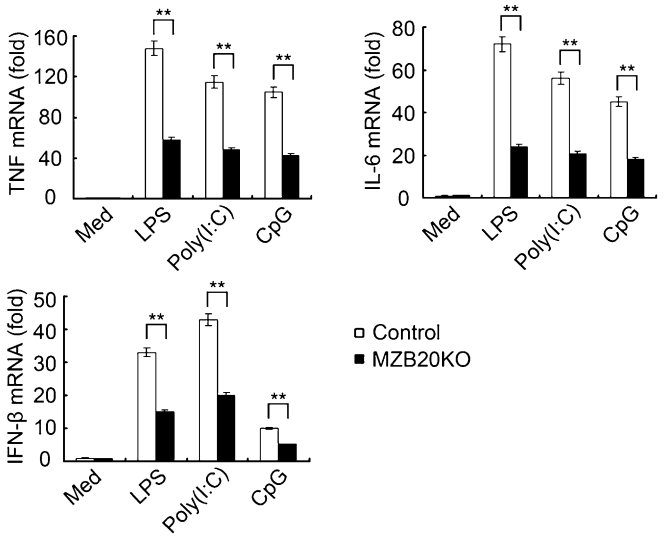

Fig. S4. ZBTB20 deficiency attenuates TLR-triggered mRNA expression of proinflammatory cytokines and IFN-β in macrophages. Q-PCR analysis of TNF, IL-6, and IFN-β mRNA expression levels in MZB20KO or control macrophages left unstimulated (Med) or stimulated with LPS (100 ng/mL), poly(I:C) (10 μg/mL), or CpG ODN (CpG; 0.3 μM) for 4 h. Q-PCR results are presented as fold induction relative to control cells without agonist stimulation. $^{**}P < 0.01$. Data are shown as mean ± SEM of three independent experiments.

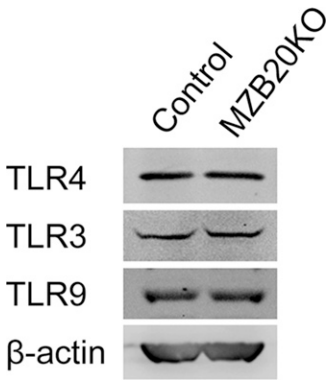

Fig. S5. Normal expression of TLRs in macrophages from MZB20KO mice. Immunoblot analysis of the protein levels of TLRs in MZB20KO or control macrophages. Similar results were obtained in three independent experiments.

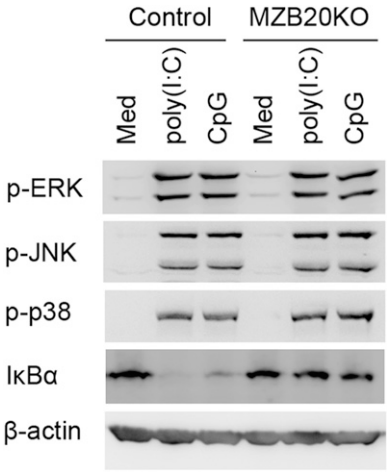

Fig. S6. ZBTB20 deficiency impairs TLR3- and TLR9-triggered activation of NF-κB but not MAPK pathway in macrophages. Immunoblot analysis of phosphorylated (p-) or total protein in lysates of MZB20KO or control macrophages left unstimulated (Med) or stimulated with poly(I:C) (10 μg/mL) or CpG ODN (CpG; 0.3 μM) for 40 min. Data are representative of three independent experiments with similar results.

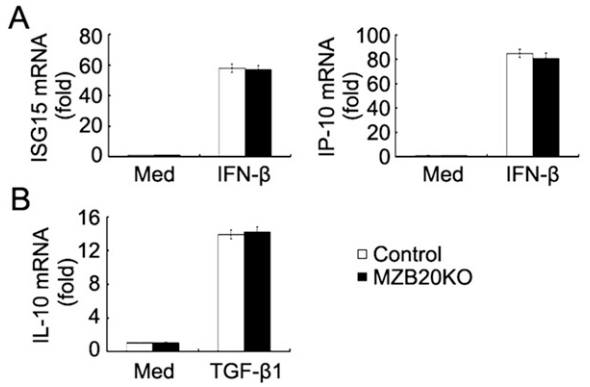

Fig. S7. ZBTB20 deficiency does not affect the mRNA expression of IFN-β– or TGF-β–induced genes in macrophages. Q-PCR analysis of (*A*) IFN-stimulated gene 15 (ISG15) and inflammatory protein 10 (IP-10) mRNA or (*B*) IL-10 mRNA expression levels in MZB20KO or control macrophages (*A*) left unstimulated (Med) or stimulated with recombinant murine IFN-β (100 U/mL) for 8 h or (*B*) stimulated with TGF-β1 (10 ng/mL) for 12 h, respectively. Results are presented as fold induction relative to control cells without IFN-β or TGF-β1 stimulation. Data are shown as mean ± SEM of three independent experiments.

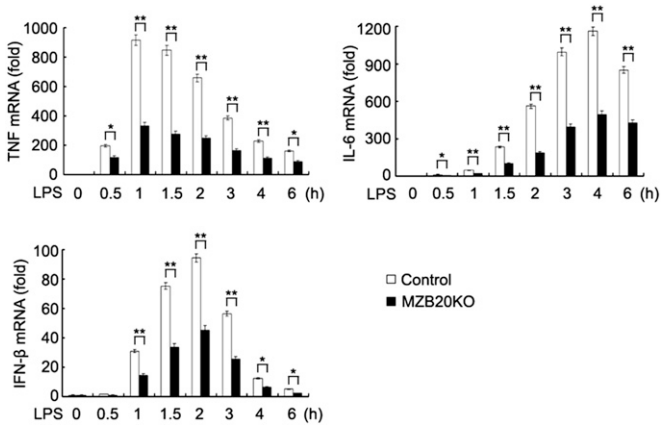

Fig. S8. ZBTB20 deficiency attenuates LPS-induced mRNA expression of proinflammatory cytokines and IFN-β in macrophages. Q-PCR analysis of TNF, IL-6, and IFN-β mRNA expression levels in MZB20KO or control macrophages stimulated with LPS (100 ng/mL) for the indicated times. Results are presented as fold induction relative to control cells without LPS stimulation. $*P < 0.05$, $** P < 0.01$. Data are shown as mean ± SEM of three independent experiments.