Supporting Information

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SI Materials and Methods

Chromatin Immunoprecipitation Assay. After fixation and lysis of cells, DNA was sheared by using a Covaris sonicator and equal amounts of lysates were used for immunoprecipitation of chromatin with Dynabeads Protein A (Invitrogen) and anti-PLZF (Calbiochem), NF-κB p65 (sc-372, Santa Cruz; or 06-418, Millipore), p50 (sc-114, Santa Cruz), HDAC3 (sc-8138; Santa Cruz), p300 (05-257; Millipore), or IgG (IgG) (sc-372 or sc-2434; Santa Cruz) as a control. After washing with salt buffer, LiCl buffer and Tris EDTA (TE) buffer, the Dynabeads Protein A complexes were mixed with 10% (vol/vol) Chelex-100 slurry, boiled for 10 min, and treated with proteinase K for 2 h. The beads were then boiled again for 10 min, and following centrifugation, the supernatant was collected and used for ChIP PCR. Sonicated chromatin (1% of the input) was used as a positive control to ensure equal input. All quantitative ChIP PCR data were normalized to those of IgG control samples, and fold enrichments were calculated. The presence of the target gene promoter sequences in both the input DNA and the recovered DNA immune complexes was detected by quantitative PCR. All primers used are listed in Table S3. PCR was performed by using a SYBR Green PCR master mix (AP Biotech) on Applied Biosystems 7700 Prism real-time PCR machine. Primer pairs spanned NF-κB sites in each of the target gene promoters. For Re-ChIP, the precipitated Dynabeads Protein A complexes were washed with TE, resuspended in TE with DTT, incubated at 37 °C for 30 min, and centrifuged at 800 × g for 2 min, then the supernatant was diluted for the next round of ChIP.

ChIP-seq. Cells (2×10^6) were cross-linked with 1% formaldehyde for 8 min at room temperature. After cell lysis, chromatin was sheared by using a Covaris S2 for 10 min with 2% duty cycle, 105-W peak incident power and 200 cycles per burst. One hundred microliters of diluted, sheared chromatin (equivalent to 2×10^5 cells) was used per immunoprecipitation, and 10 μL was reserved as input control. Sheared chromatin was incubated with Dynabeads Protein A (Invitrogen) coated with either 2.5 μg of anti-H3K4me3 (pAB-003–050; Diagenode) or anti-H3K27ac (ab4729; Abcam) antibody or 1 μL of rabbit-IgG (kch-504–250; Diagenode), as a negative control, and rotated at 40 rpm overnight at 4 °C. The following day, immunoprecipitates were washed and DNA was purified by using the iPure kit (Diagenode) according to the manufacturer's instructions. DNA from each ChIP (50 ng) was sheared by using a focal acoustic device (Covaris), then sequencing adapters were ligated and PCR amplified according to the manufacturer's protocol (Illumina). Libraries were quantified by using real-time PCR against a library of known concentration and then processed for cluster generation and sequencing according to standard protocols (HiSEq 2000; Illumina). High-throughput sequencing experiments were single-ended with 50 nt of read length (300 nt average insert size). The targeted minimum number of sequencing reads per sample was 20 million, which were aligned to the complete mouse genome [University of California, Santa Cruz (UCSC) mm8 mouse genome version, February 2006] by using Bowtie2 (1). ChIP-seq experimental samples were normalized to a total of 10^7 uniquely mapped sequencing tags.

ChIP-seq Analysis. To identify H3K4me3- or H3K27ac-enriched regions, normalized sequencing tags of *Zbtb16*$^{-/-}$ samples (untreated or LPS-treated for 0.5 h) were compared with their WT counterparts (threefold change over WT, Poisson enrichment *P* value of 0.0001). An unsupervised hierarchical cluster analysis of previously identified immediate/early response genes (2) (average linkage and Pearson's correlation as distance measure) was performed by using Partek Genomics Suite (Sixth Ed.; Partek).

Myeloperoxidase Activity Assay. Briefly, equal weights of liver and spleen samples were suspended in 1 mL of buffer (0.5% hexadecyltrimethylammonium bromide in 50 mM phosphate buffer, pH 6.0) and sonicated on ice. The homogenate was cleared by centrifuging, and the supernatants were used to measure MPO activity by using the assay. An aliquot of the supernatant was incubated with the substrate *o*-dianisidine hydrochloride. This reaction was carried out in a 96-well plate by adding phosphate buffer, substrate solution (containing 20 mg/mL *o*-dianisidine hydrochloride) and H_2O_2. Samples were added to each well to start the reaction. Protein content in the samples was determined by using the Bio-Rad assay kit. The absorbance was measured spectrophotometrically at 450 nm.

1. Langmead B, Salzberg SL (2012) Fast gapped-read alignment with Bowtie 2. *Nat Methods* 9(4):357–359.

2. Escoubet-Lozach L, et al. (2011) Mechanisms establishing TLR4-responsive activation states of inflammatory response genes. *PLoS Genet* 7(12):e1002401.

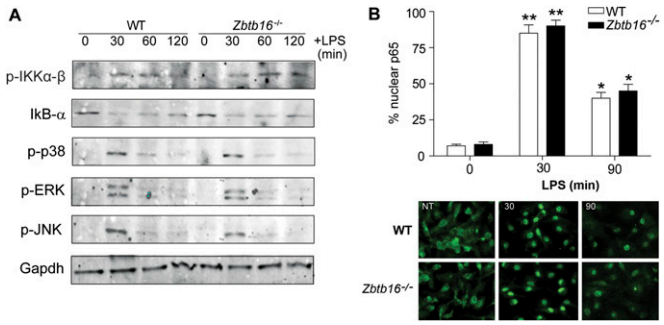

Fig. S1. Receptor proximal cell signaling is not affected by PLZF. (*A*) Immunoblots measuring the activation kinetics of the indicated cell signaling molecules, using antibodies specific for the active state of each protein, in WT and *Zbtb16*$^{-/-}$ BMDM stimulated with LPS (100 ng/mL) for the indicated times. An anti-GAPDH antibody is used as a loading control. (*B*) Quantitation of the activation of NF-κB p65 by measuring the nuclear localization of the protein after immunofluorescent detection in WT and *Zbtb16*$^{-/-}$ BMDM treated with LPS (100 ng/mL) for the indicated times. The graph is a quantitation of the percentage of nuclei that are positive for the fluorescently labeled protein shown in the micrographs below. Mean ± SEM from independent experiments; **P* < 0.05, ***P* < 0.01 by Student's *t* test; *n* = 3. Also, see Fig. S3 *B* and *C* for additional comparisons of cell signaling events in WT and *Zbtb16*$^{-/-}$ BMDM.

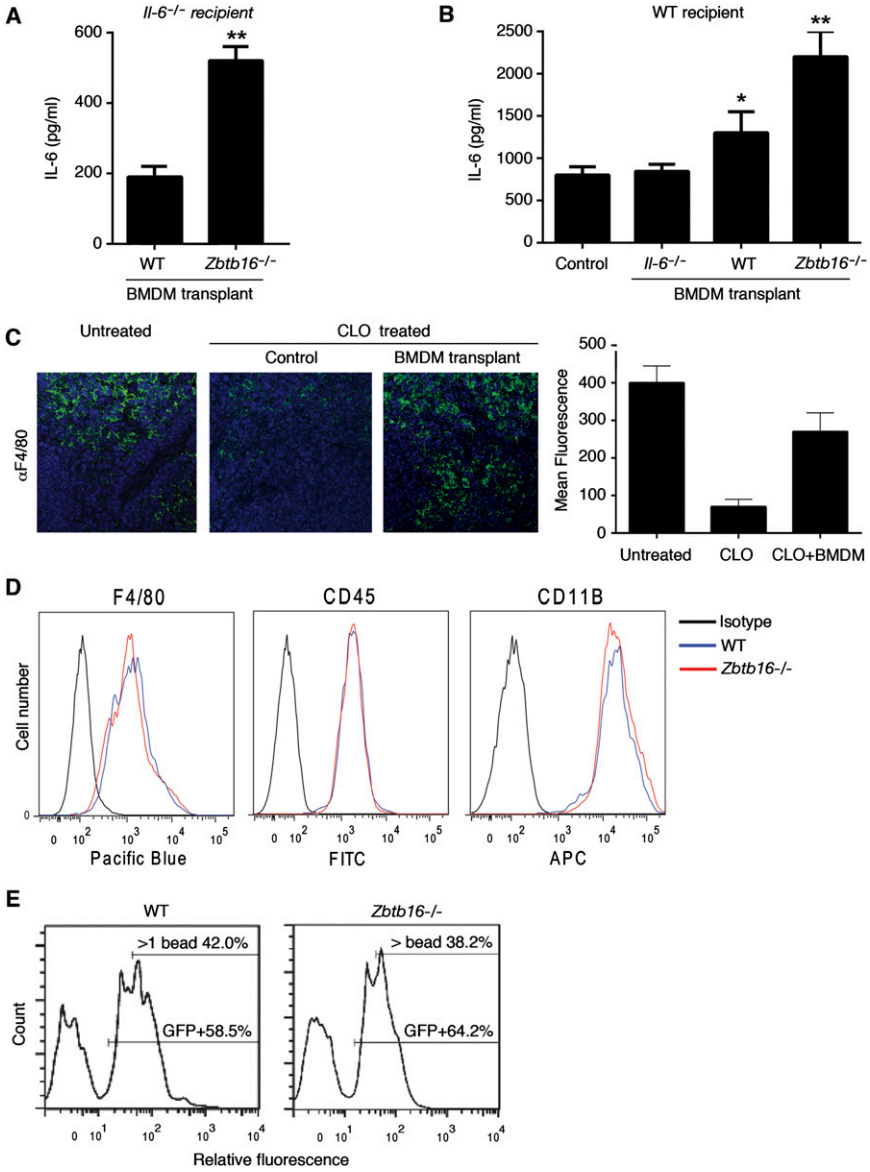

Fig. S2. Corroboration of the dichloromethylene diphosphate (CLO) treatment and cell transplantation protocols, and comparison of macrophages from WT and *Zbtb16*$^{-/-}$ mice. (*A*) To demonstrate that the elevated levels of inflammatory cytokines in the *Zbtb16*$^{-/-}$ genetic background (shown in Fig. 2*D*) is attributed to the transplanted cells, *Il6*$^{-/-}$ mice were treated with CLO, then transplanted with BMDM from either WT or *Zbtb16*$^{-/-}$ mice, and the levels of IL-6 were measured (by ELISA) after being LPS challenged (100 ng/mL) for 2 h. (*B*) To measure the relative production of cytokines from endogenous and transplanted macrophages, WT mice were treated with CLO alone (Control), then transplanted with BMDM from WT, *Il6*$^{-/-}$, or *Zbtb16*$^{-/-}$ mice and the levels of IL-6 were measured after being LPS challenged (100 ng/mL) for 2 h. (*A* and *B*) Mean ± SEM; $*P < 0.05$, $**P < 0.01$ by Student's *t* test; $n = 6$ per group. (*C*) To demonstrate the extent of the depletion and repopulation of macrophages in CLO-treated and BMDM-transplanted mice, we measured the numbers of macrophages by immunohistochemistry. The micrographs detect fluorescence in spleen tissue treated with the Hoechst nuclear stain (in blue) and a fluorescent anti-F4/80 antibody (GFP). A quantification of the number of macrophages, assessed by the relative mean GFP fluorescence intensity, is graphed to the right of the micrographs. (*D*) Graphs measuring the relative expression of the indicated cell surface markers by immunofluorescence and flow cytometry of macrophages from WT and *Zbtb16*$^{-/-}$ mice. Isotype-matched control antibodies are used as a control. (*E*) The phagocytic ability of macrophages from WT and *Zbtb16*$^{-/-}$ mice was assessed by flow cytometric measure of fluorescence (GFP) after engulfment of GFP-labeled beads.

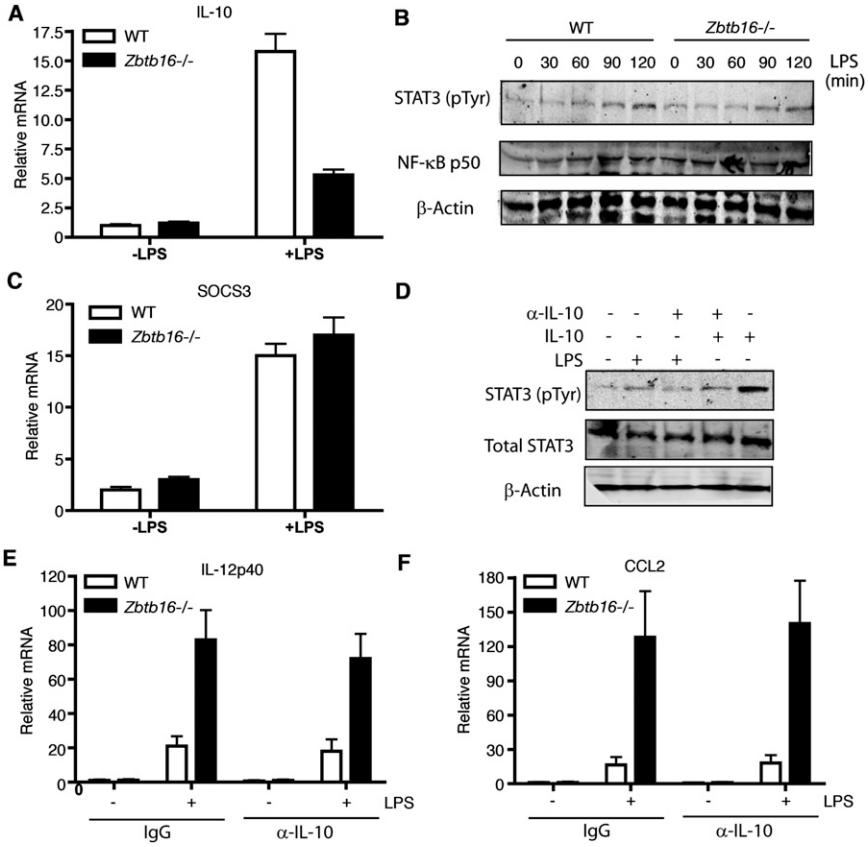

Fig. S3. PLZF-dependent control of TLR-regulated early response genes is independent of IL-10. (*A*) The induction of IL-10 is reduced in *Zbtb16*$^{-/-}$ compared with WT BMDMs treated with LPS (100 ng/mL) for 1 h. (*B* and *C*) PLZF-dependent regulation of the TLR4-mediated activation of STAT3 and induction of the *Socs3* transcript is equivalent as measured by immunoblot with a phospho-tryosine antibody (pTyr), and by RT-PCR, respectively. The levels of NF-κB p50 are equivalent between the WT and *Zbtb16*$^{-/-}$ BMDMs by immunoblot. (*D–F*) TLR4-mediated activation of STAT3 and induction of *IL-12-p40* and *Ccl-2* transcripts, measured by immunoblot or quantitative PCR, respectively, are independent of IL-10 in WT and *Zbtb16*$^{-/-}$ BMDMs treated with an IL-10 neutralizing antibody before LPS (100 ng/mL) stimulation for 1 h. The levels of each amplicon in the quantitative PCR were normalized to 18S RNA in each sample.

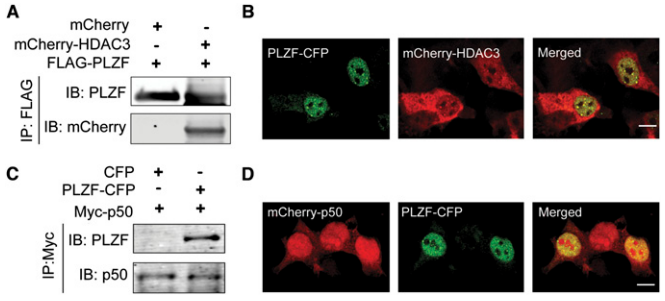

Fig. S4. PLZF interacts with both HDAC3 and NF-κB p50. (*A*) An immunoblot showing coimmunoprecipitation (co-IP) of FLAG-PLZF with mCherry-HDAC3. (*B*) Micrograph showing colocalization of the fluorescent signals of PLZF-CFP and mCherry-HDAC3. (*C*) An immunoblot showing coimmunoprecipitation of PLZF-CFP and Myc-NF-κB p50. (*D*) Micrograph showing colocalization of the fluorescent signals of PLZF-CFP and mCherry–NF-κB p50. The specific IP and detection antibodies are as indicated. These experiments were conducted in HEK293T cells.

Table S1. Gene ontology classification of PLZF-regulated genes identified by ChIP-seq analysis

GO term	*P* value	Gene
Acute inflammatory response	5.66e-05	*Ccr1, Fcer1a, Il6, Orm3, Ptgs2, Tnf, Tnfsf4, Vcam1*
Inflammatory response	8.57e-11	*Ccl12, Ccl4, Ccl7, Ccr1, Cd14, Cxcl1, Cxcl10, Cxcl13, Cxcl2, Fcer1a, Ier3, Il23a, Il33, Il6, Mefv, Ms4a2, Olr1, Orm3, Ptgs2,Tlr2, Tlr7, Tnf, Tnfaip3, Tnfsf4, Vcam1*
Regulation of leukocyte migration	5.56e-06	*Ccl7, Ccr1, Cxcl10, Cxcl13, Il23a, Il33, Tlr2, Tnfsf4*
Leukocyte migration	6.53e-09	*Ccl12, Ccl7, Ccr1, Ccr3, Cxcl1, Cxcl10, Cxcl13, Cxcl2, Il23a, Il33, Tlr2, Tnf, Tnfsf4, Vcam1*
Regulation of immunity	2.86e-07	*Ccl7, Ccr1, Cxcl10, Cxcl13, Fcer1a, H2-Aa, Il23a, Il33, Il6, Irf1, Klrk1, Ms4a2, Peli1, Sh2d1a, Tlr2, Tlr7, Tnf, Tnfaip3, Tnfsf4, Vcam1*
Regulation of response to external stimulus	4.99e-06	*Ccl7, Ccr1, Cxcl10, Cxcl13, Fcer1a, Il23a, Il33, Ptgs2, Tnf, Tnfsf4*
Defense response	9.92e-13	*Ccl12, Ccl4, Ccl7, Ccr1, Cd14, Cxcl1, Cxcl10, Cxcl13, Cxcl2, Defb4, Fcer1a, Gbp5, H2-Aa, Ier3, Ifnz, Il18r1, Il1rapl1, Il23a, Il33, Il6, Irf1, Klrk1, Lyz1, Mefv, Ms4a2, Olr1, Orm3, Peli1, Ptgs2, Rsad2, Sh2d1a, Sp110, Tlr2, Tlr7, Tnf, Tnfaip3, Tnfsf4, Vcam1*

Gene ontology classification of positively regulated genes. Gene selection was based on *P* value ($P < 0.05$) after Bonferroni's correction following multiple comparison tests of sequence enrichment for both histone marks.

Table S2. Real-time PCR primers for gene expression analysis

Primer name	Primer sequence
Ccl2 Forward	CTTCTGGGCCTGCTGTTCA
Ccl2 Reverse	CCAGCCTACTCATTGGGATCA
Ccl4 Forward	TTCCTGCTGTTTCTCTTACACCT
Ccl4 Reverse	CTGTCTGCCTCTTTTGGTCAG
Ccl7 Forward	CCAATGCATCCACATGCTGC
Ccl7 Reverse	GCTTCCCAGGGACACCGAC
Ccl9 Forward	TACTGCCCTCTCCTTCCTCA
Ccl9 Reverse	AATTTCAAGCCCTTGCTGTG
Cxcl2 Forward	GAGCTTGAGTGTGACGCCCCCAGG
Cxcl2 Reverse	GTTAGCCTTGCCTTTGTTCAGTATC
Cxcl3 Forward	GATCCATCCCAACGGTGTCT
Cxcl3 Reverse	AAGTAGATGCAATTATACCCGTAG
Ccr1 Forward	AAGGCCCAGAAACAAAGTCT
Ccr1 Reverse	TCTGTAGTTGTGGGGTAGGC
Csf3 Forward	GCTGCTGGAGCAGTTGTG
Csf3 Reverse	GGGATCCCCAGAGAGTGG
S1009a Forward	GGAGCGCAGCATAACCACCATC
S1009a Reverse	GCCATCAGCATCATACACTCCTCA

Table S3. Primers for ChIP analysis

Primer name	Primer sequence
TNFα Forward	CCCCAGATTCCCACAGAATC
TNFα Reverse	CCAGTGAGTGAAAGGGACAG
IL-6 Forward	CAGCAGCCAACCTCCTCTAA
IL-6 Reverse	CTGTGAGCGGCTGTTGTAGA
IL-12 Forward	AGTATCTCTGCCTCCTTCCTT
IL-12 Reverse	GCAACATGAAAACTAGTGTC
Cxcl10 Forward	TCCAAGTTCATGGGTCACAA
Cxcl10 Reverse	TGATTGGCTGACTTTGGAGA
Cxcl11 Forward	GCTGAGTGCTTTCACCTTCC
Cxcl11 Reverse	CGTAGCTTTCTTGCCTCCTG
IFNβ Forward	AGCTCCAAGAAAGGACGAACAT
IFNβ Reverse	GCCCTGTAGGTGAGGTTGATCT
Csf3 Forward	CCTACCTAGGGTGCTGTGGA
Csf3 Reverse	GGACAAACATCCCGAGAGAA