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Supporting Information

for

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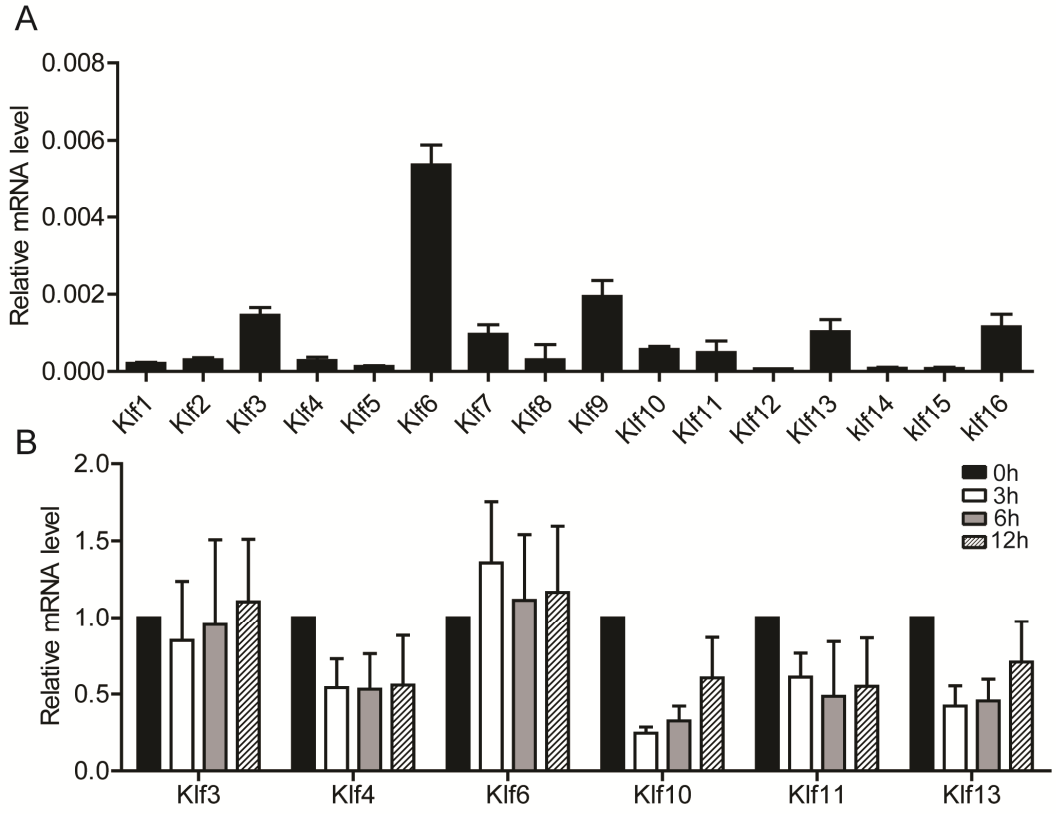
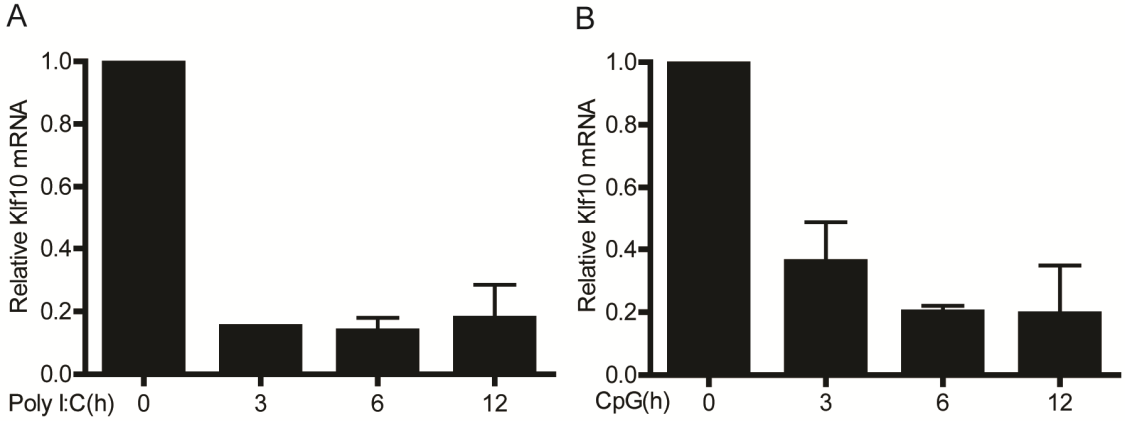
Klf10 inhibits IL-12p40 production in macrophage colony-stimulating factor-induced mouse bone marrow-derived macrophages

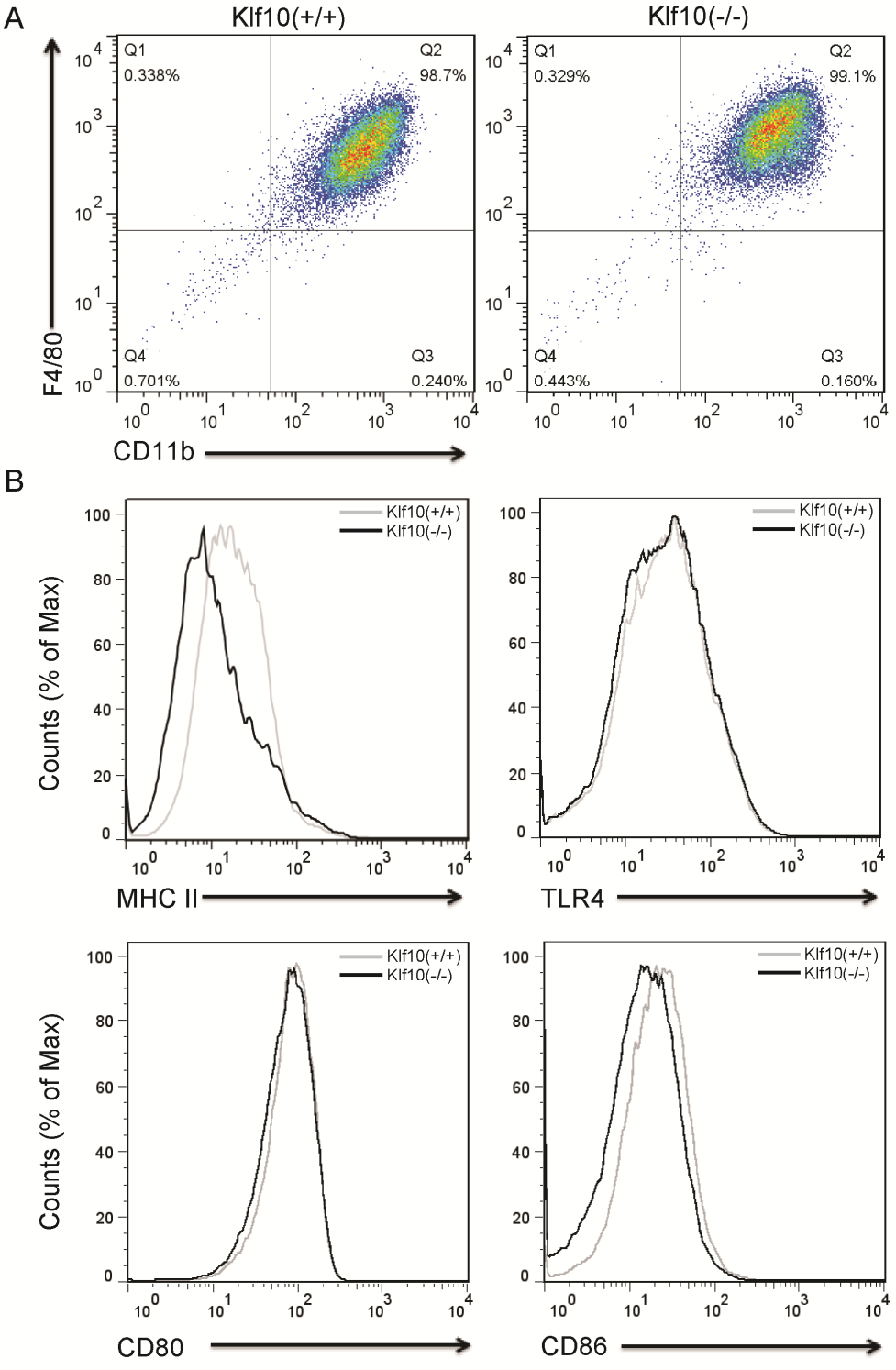


Supporting Information Figure 1. Down-regulation of Klf10 expression upon TLR4 activation by LPS in GM-BMMs. (A) The mRNA levels of KLFs in GM-BMMs. GM-BMMs on day 5 were harvested and the gene expression levels of KLFs were assessed by qPCR. The actin mRNA level in these cells was set as 1. (B) Response of KLFs to LPS stimulation in GM-BMMs. GM-BMMs on day 5 were treated with 1 μg/ml LPS for the indicated time, then gene expression levels were assessed by qPCR and normalized to those in untreated cells. Data are shown as mean±SD of three samples pooled from three independent experiments.

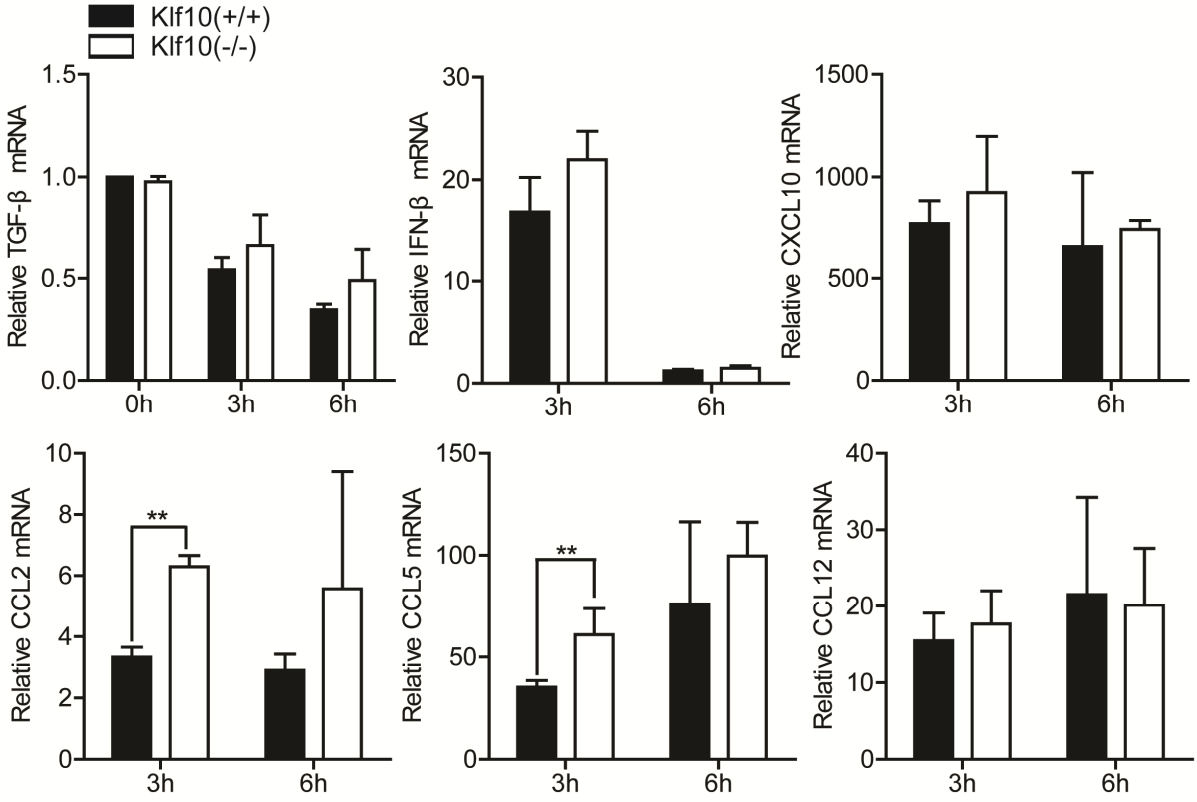


Supporting Information Figure 2. Down-regulation of Klf10 expression upon TLR3 and TLR9

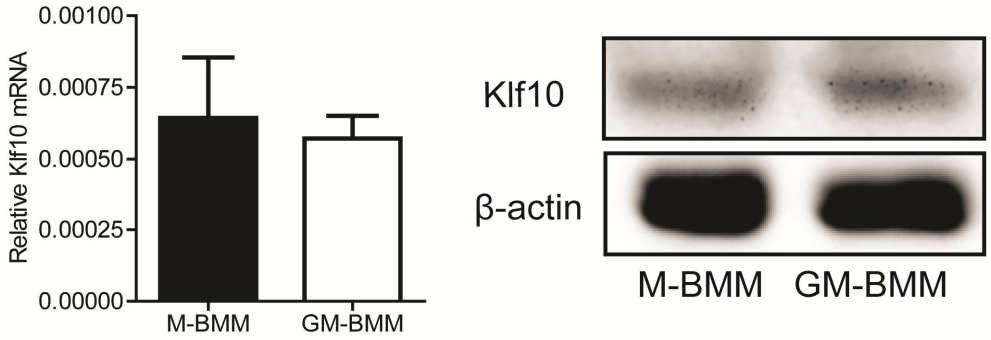
activation by Poly I: C and CpG. M-BMMs on day 5 were treated with 20μg/ml Poly I: C (A) or 0.3μM CpG (B) for indicated time, then cells were harvested for qPCR analysis and mRNA levels were normalized to those in untreated cells. Data are shown as mean±SD of three samples pooled from three independent experiments.



Supporting Information Figure 3. Phenotype analysis of M-BMMs in wildtype and Klf10 deficient mouse. (A) BMMs on day 5 were analyzed for CD11b and F4/80 expression by flow cytometry. (B) Expression of MHC II, TLR4, CD80, and CD86 in M-BMMs from wildtype (grey lines) or Klf10 deficient (black lines) mice. $CD11b^+$ $F4/80^+$ cells were gated from cells in A, and then analyzed for the expression of MHC II, TLR4, CD80 and CD86. Data are representative of three independent experiments.

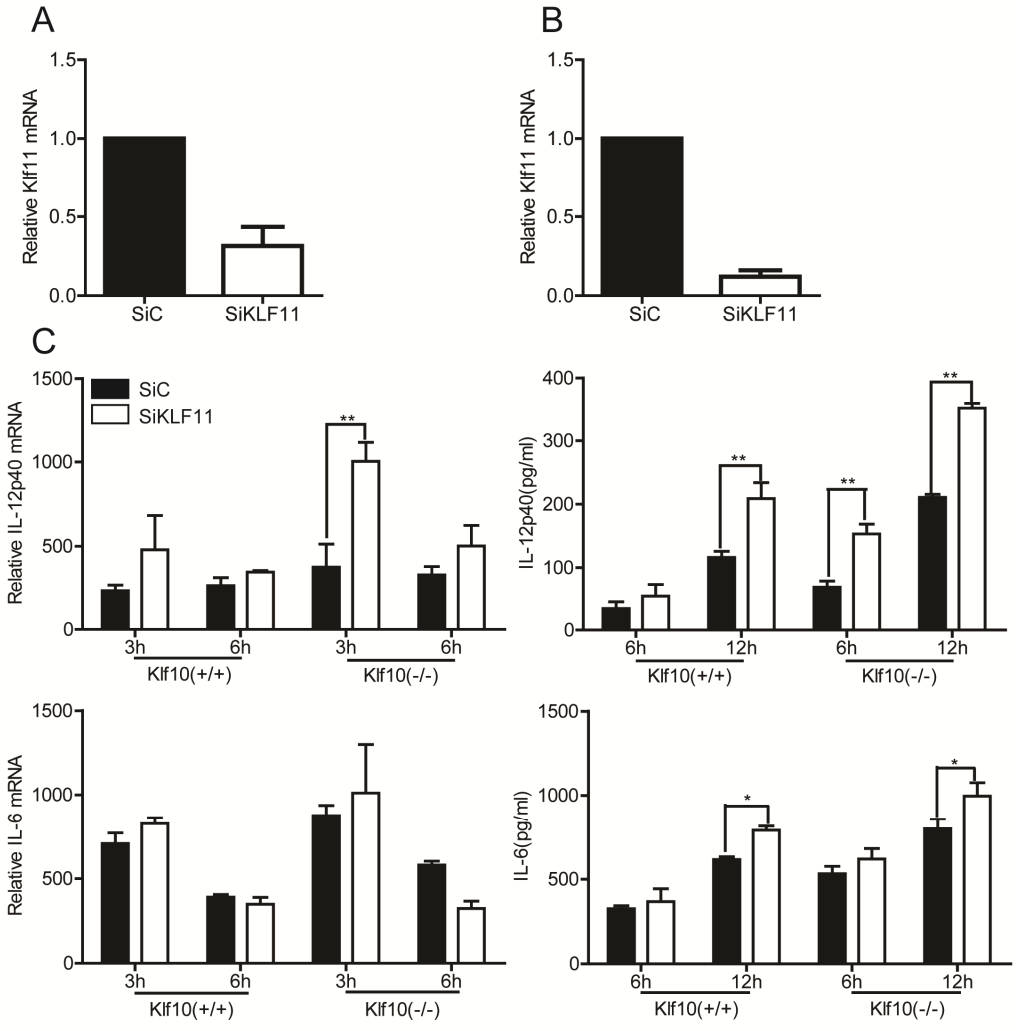


Supporting Information Figure 4. Expression of M-BMM-specific markers in wildtype and Klf10 deficient mice. M-BMMs on day 5 from wildtype or Klf10 deficient mouse were stimulated with 1 μg /ml LPS. Cells were harvested at the indicated time, and relative mRNA expression of IFN-β, TGF-β, CCL2, CCL5, CCL12, and CXCL10 were measured by qPCR and normalized to those in untreated cells. Data are shown as mean±SD of three samples pooled from three independent experiments. **, $p<0.01$, determined by Student's t-test.

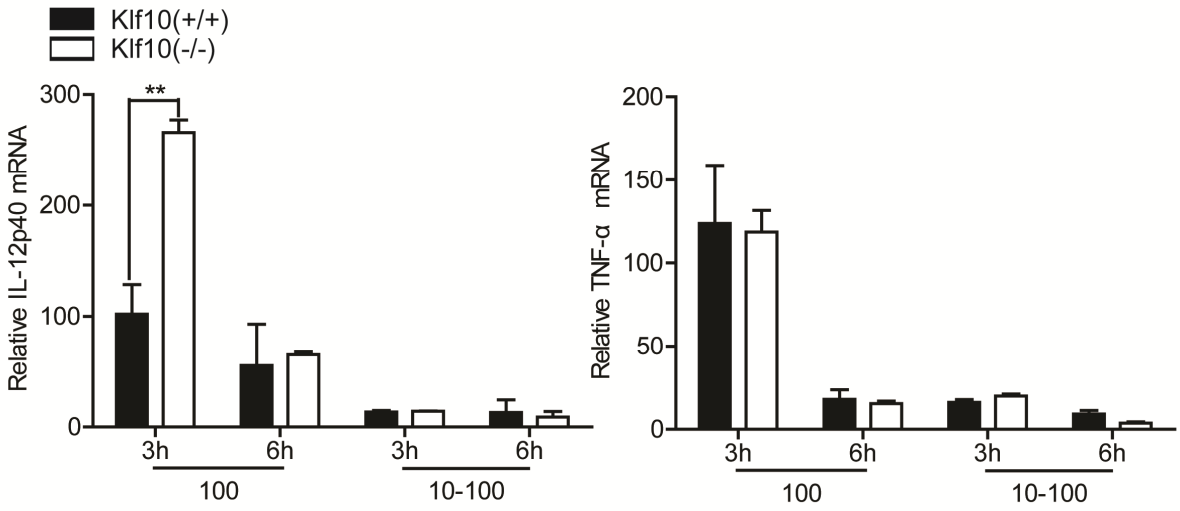


Supporting Information Figure 5. Expression of Klf10 in M-BMMs and GM-BMMs.

Bone marrow cells from wildtype or Klf10 deficient mouse were cultured with either 10 ng/ml M-CSF or 20 ng/ml GM-CSF for macrophage polarization. On day 5, cells were harvested, and the mRNA and protein levels of Klf10 were measured by qPCR and Western blot analysis. Data are shown as mean±SD of three samples pooled from three independent experiments (A) or representative of three independent experiments (B).



Supporting Information Figure 6. Silencing of Klf11 promoted production of LPS-induced IL-12p40 in M-BMMs. (A) The efficiency of Klf11 silencing in Figure 5B was evaluated by qPCR. (B) The efficiency of Klf11 silencing in Supporting Information Figure 6C was evaluated by qPCR. (C) On day 5, M-BMMs were transfected with Klf11 SiRNA or control SiRNA, cultured for 36 hours, and then stimulated with 1 μg /ml LPS. Cells were harvested at the indicated time, and relative mRNA expression of IL-12p40 and IL-6 was measured by qPCR and normalised to those in untreated cells (left). Supernatants from the cultures were collected, and the levels of IL-12p40 and IL-6 were evaluated by Elisa (right). Data are shown as mean±SD of three samples pooled from three independent experiments. *, $p<0.05$, **, $p<0.01$, determined by Student's t-test.



Supporting Information Figure 7. The roles of Klf10 in a LPS tolerance model. M-BMMs on day 5 from wildtype or Klf10 deficient mouse were pretreated with 10ng /ml LPS or not for 24 hours. Then cells were stimulated with 100ng/ml LPS and harvested at the indicated time. Relative mRNA expression of IL-12p40 and TNF-α were measured by qPCR and normalized to those in untreated cells. Data are shown as mean±SD of three samples pooled from three independent experiments. **, $p<0.01$, determined by Student's t-test.