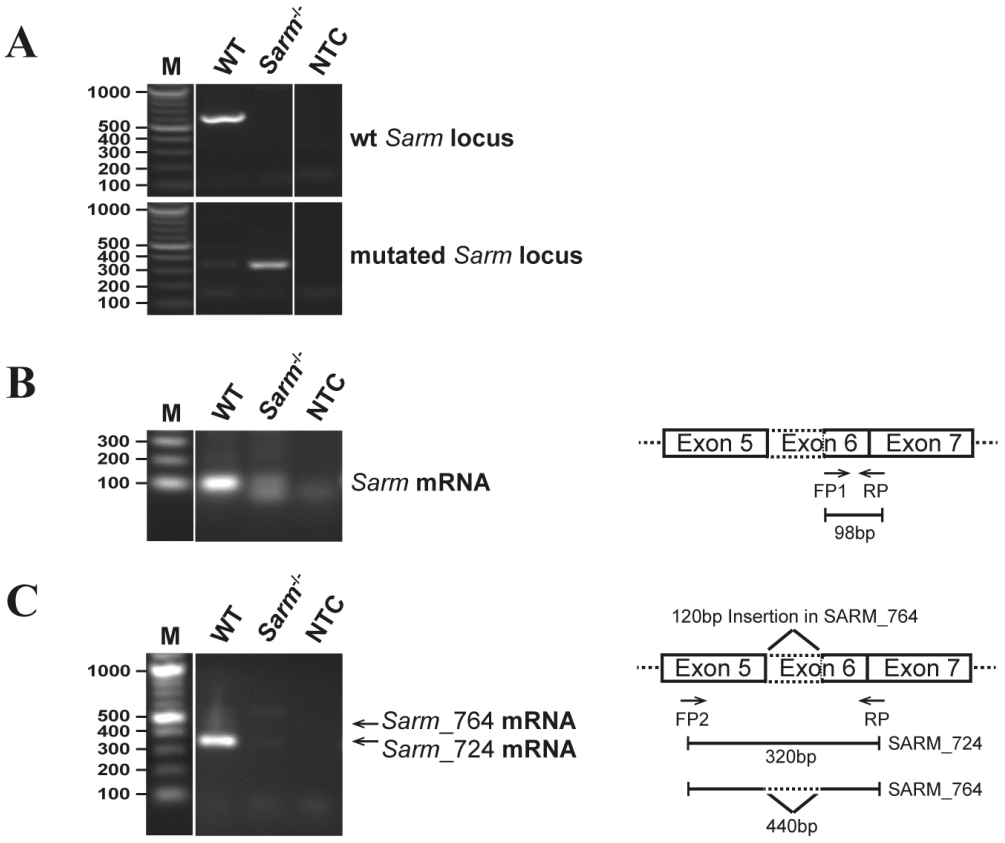
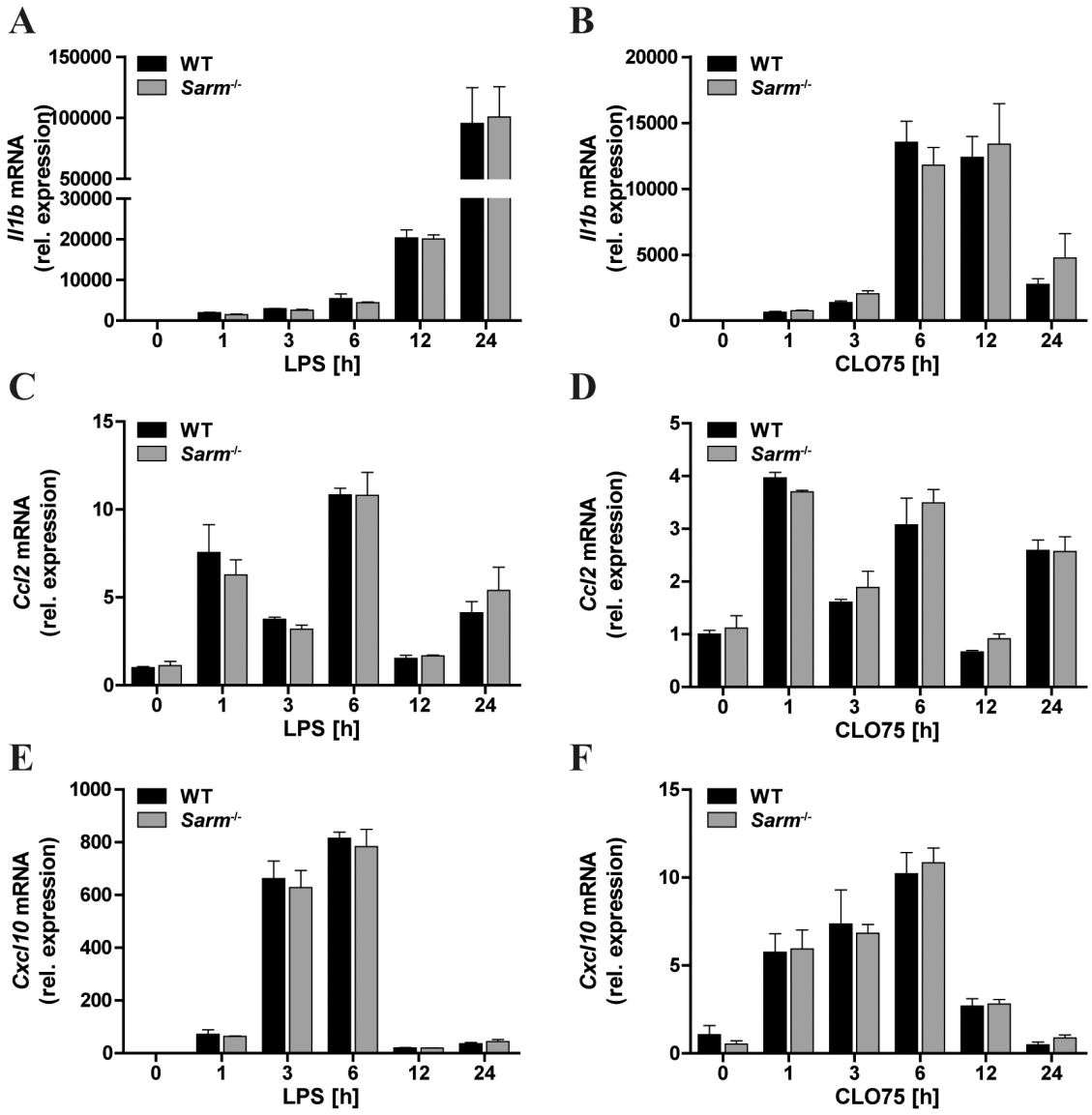
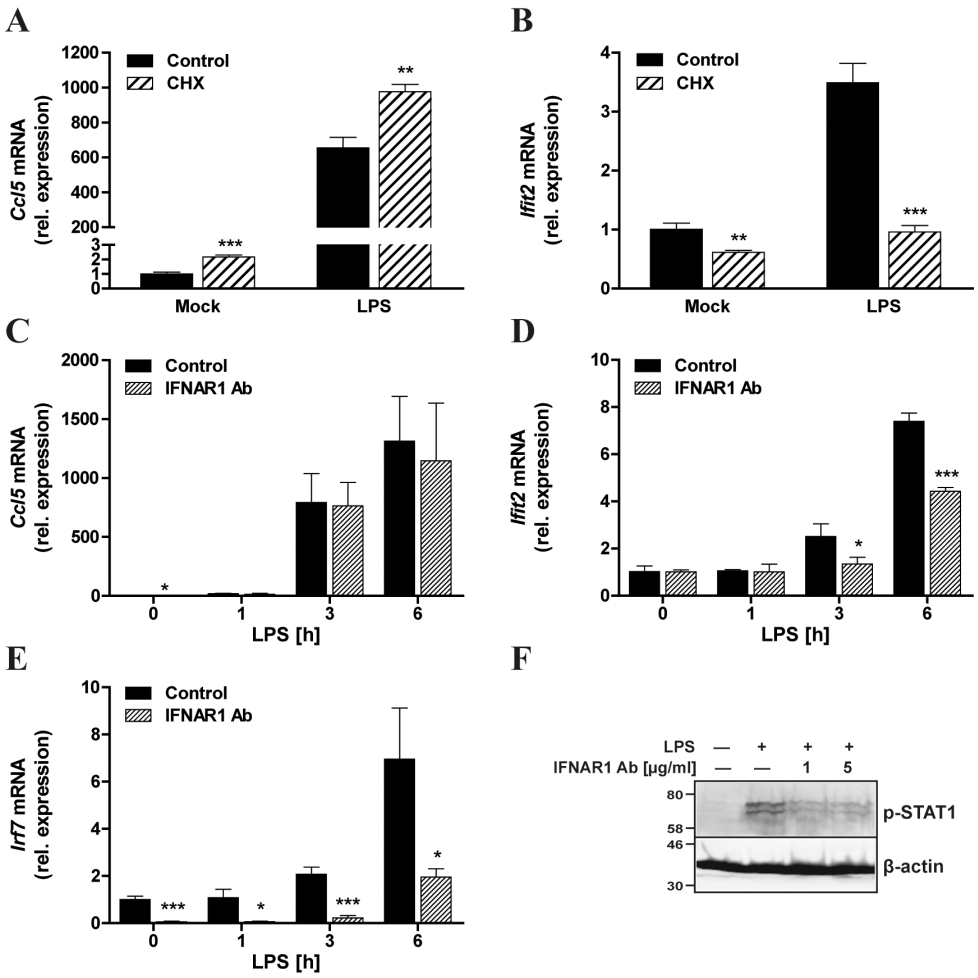


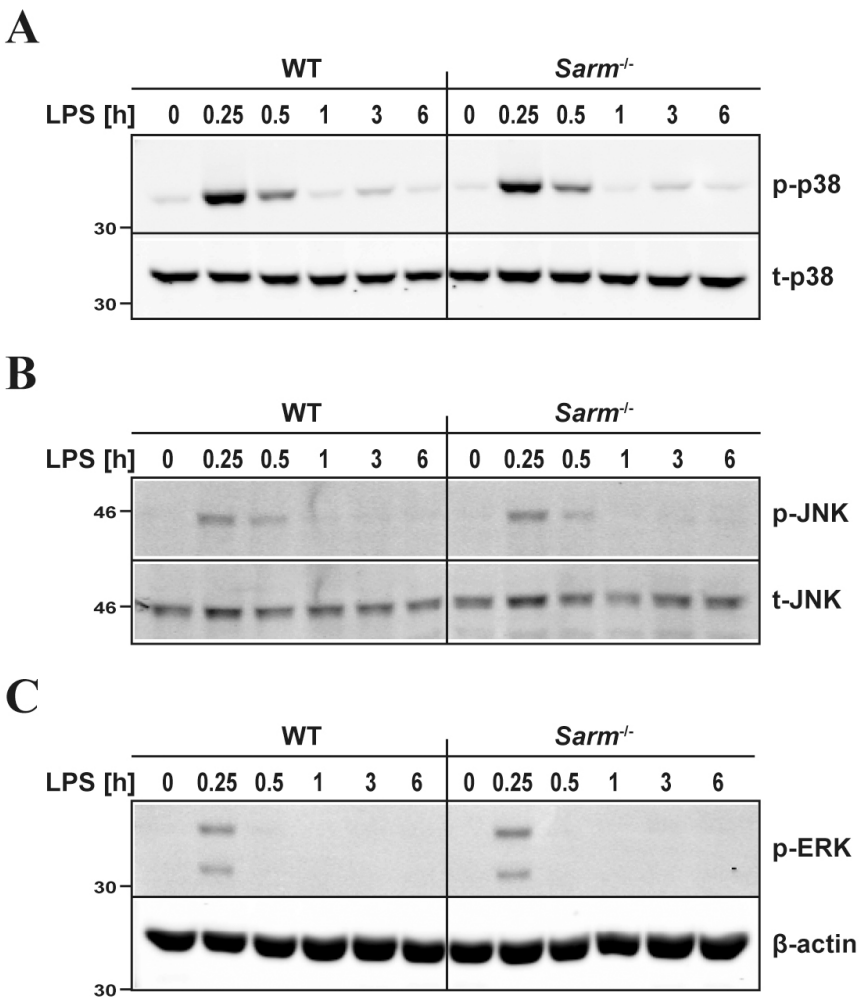
Supplemental Figure S1. SARM is expressed in murine BMDMs. *A*, Genomic DNA from primary WT and *Sarm*$^{-/-}$ BMDMs was extracted and genotyped by PCR for the presence of the WT and mutated (knockout) *Sarm* locus using specific primer pairs generating an approximately 550bp or 200bp PCR product, respectively. PCR products were resolved on an agarose gel and visualized by ethidium bromide staining and UV illumination. A no template control (NTC) was included, and a DNA marker (M) is shown to the left of the gel (in bp). *B,C*, cDNA prepared from RNA of primary WT and *Sarm*$^{-/-}$ BMDMs was assayed by PCR for the presence of *Sarm* mRNA. Total *Sarm* mRNA was amplified with forward primer 1 (FP1) and reverse primer (RP) (*B*), while the mRNA expression of the two isoforms *Sarm*_724 and *Sarm*_764 were distinguished using the primer pair FP2 and RP (*C*), as indicated in the schematics. PCR products were visualized as in *A*. Each agarose gel is representative of at least three independent experiments.



Supplemental Figure S2. SARM is dispensable for the expression of other proinflammatory cytokines and chemokines. Primary WT and *Sarm*$^{-/-}$ BMDMs were stimulated with 100ng/ml LPS (*A,C,E*) or 5µg/ml CLO75 (*B,D,F*) for the indicated times, or medium as control. *Il1b* (*A,B*), *Ccl2* (*C,D*) or *Cxcl10* (*E,F*) mRNA were assayed by quantitative RT-PCR, normalized to the housekeeping gene *β-actin* and are presented relative to the untreated WT control. The data are mean ± SD of triplicate samples and are representative of at least three independent experiments.



Supplemental Figure S3. TLR4-induced CCL5 is a primary response gene and independent of type I IFN signaling. *A,B*, Primary WT BMDMs were pretreated for 1h with 5μg/ml cycloheximide (CHX) or DMSO as control, and then stimulated for 3h with 100ng/ml LPS or medium (mock). *Ccl5* (*A*) or *Ifit2* (*B*) mRNA were assayed by quantitative RT-PCR, normalized to the housekeeping gene *β-actin* and are presented relative to mock control. *C-E*, Immortalized WT BMDMs were pretreated for 16h with IFNAR1 Ab or medium as control, and then stimulated with 100ng/ml LPS for the indicated times or medium. *Ccl5* (*C*), *Ifit2* (*D*) or *Irf7* (*E*) mRNA were assayed by quantitative RT-PCR, normalized to the housekeeping gene *β-actin* and are presented relative to medium control. The data *A-E* are mean ± SD of triplicate samples and are representative of at least two independent experiments. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with control (Student's *t*-test). *F*, Immortalized WT BMDMs were treated for 16h with the indicated concentrations of IFNAR1 Ab or medium as control (—), and then stimulated for 3h with 100ng/ml LPS or medium (—). Cell lysates were subjected to SDS-PAGE and immunoblotted with antibodies specific to phosphorylated STAT1 (p-STAT1) or β-actin as a loading control. The molecular weight markers are indicated to the left of the gel (in kDa).



Supplemental Figure S4. SARM is dispensable for the activation of MAPKs. Primary WT and *Sarm*$^{-/-}$ BMDMs were stimulated with 100ng/ml LPS for the indicated times, or medium as control. Cell lysates were subjected to SDS-PAGE and immunoblotted with specific antibodies to phosphorylated p38 (p-p38) (*A*), p-JNK (*B*) or p-ERK (*C*), with loading controls of total p38 (t-p38), t-JNK or β-actin, respectively. The molecular weight markers are indicated to the left of the gel (in kDa). Each immunoblot is representative of at least three independent experiments.