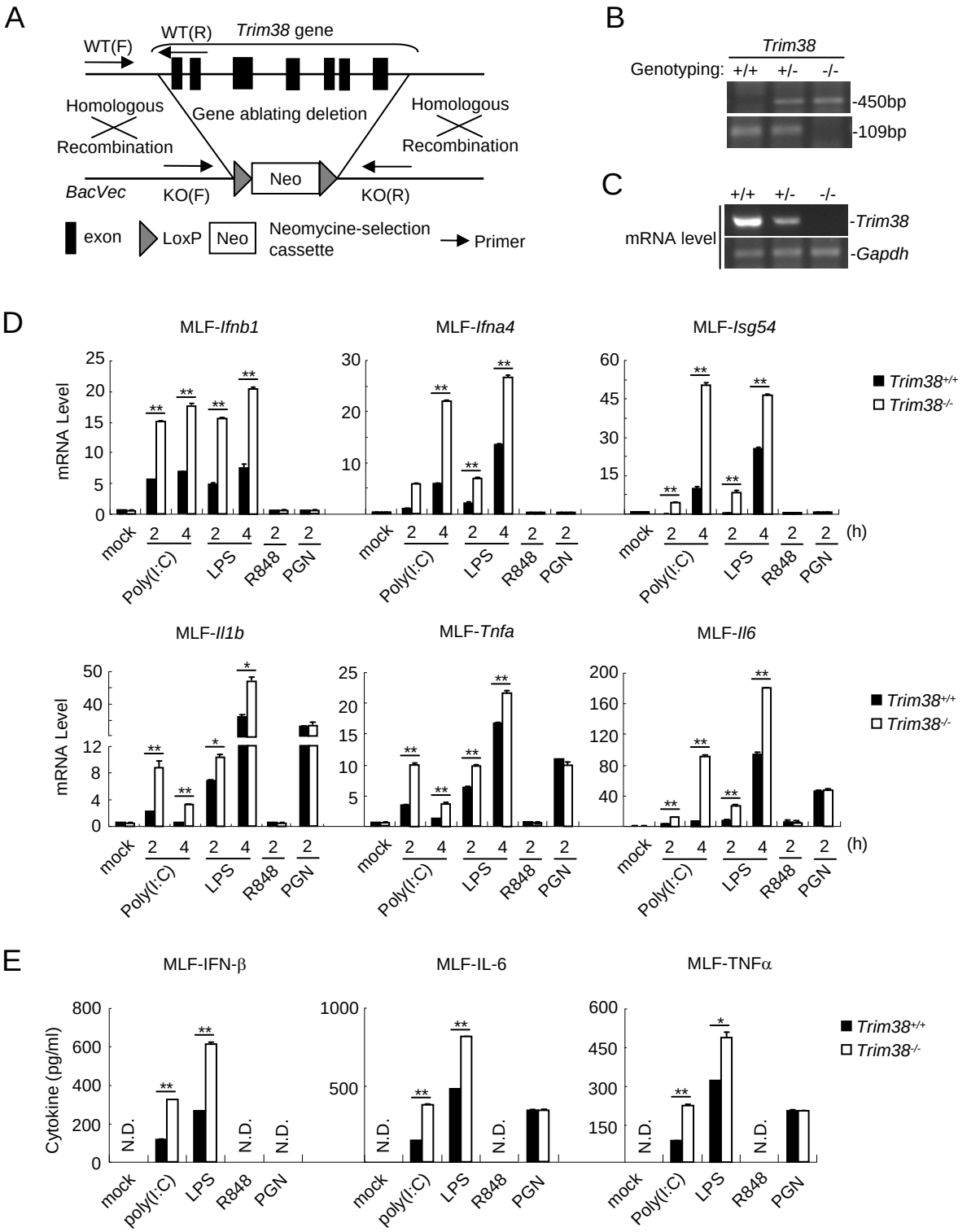

Figure S1. Trim38-deficiency potentiates TLR3/4-mediated signaling in MLFs.

(A), *Trim38* targeting strategy. The fragment containing all exons of *Trim38* gene was deleted by homogenous recombination.

(B), Genotyping of mice. The genomic DNA was exacted from mice for PCR assays.

(C), mRNA levels of *Trim38* in BMDMs from wild-type, heterozygous and *Trim38*$^{-/-}$ mice were analyzed by qPCR with the indicated primers.

(D), Effects of Trim38-deficiency on poly(I:C)-, LPS-, R848- or PGN-induced expression of IRF3-dependent (upper panel) or NF-κB-dependent (lower panel) genes in MLFs. *Trim38*$^{+/+}$ and *Trim38*$^{-/-}$ MLFs were stimulated with Poly(I:C) (50 μg/ml), LPS (50 ng/ml), R848 (20 nM) or PGN (20 μg/ml) for the indicated times before qPCR was performed.

(E), Effects of Trim38-deficiency on Poly(I:C)-, LPS-, R848- or PGN-induced production of IFN-β, IL-6 and TNFα in MLFs. *Trim38*$^{+/+}$ and *Trim38*$^{-/-}$ MLFs were stimulated with Poly(I:C) (50 μg/ml), LPS (50 ng/ml), R848 (20 nM) or PGN (20 μg/ml) for 18 hours. The concentrations of IFN-β, IL-6 and TNFα in the supernatants were determined by ELISA.

Graphs show mean±s.d., n=3. *, p<0.05; **, p<0.01.

Hu et al. Figure S2

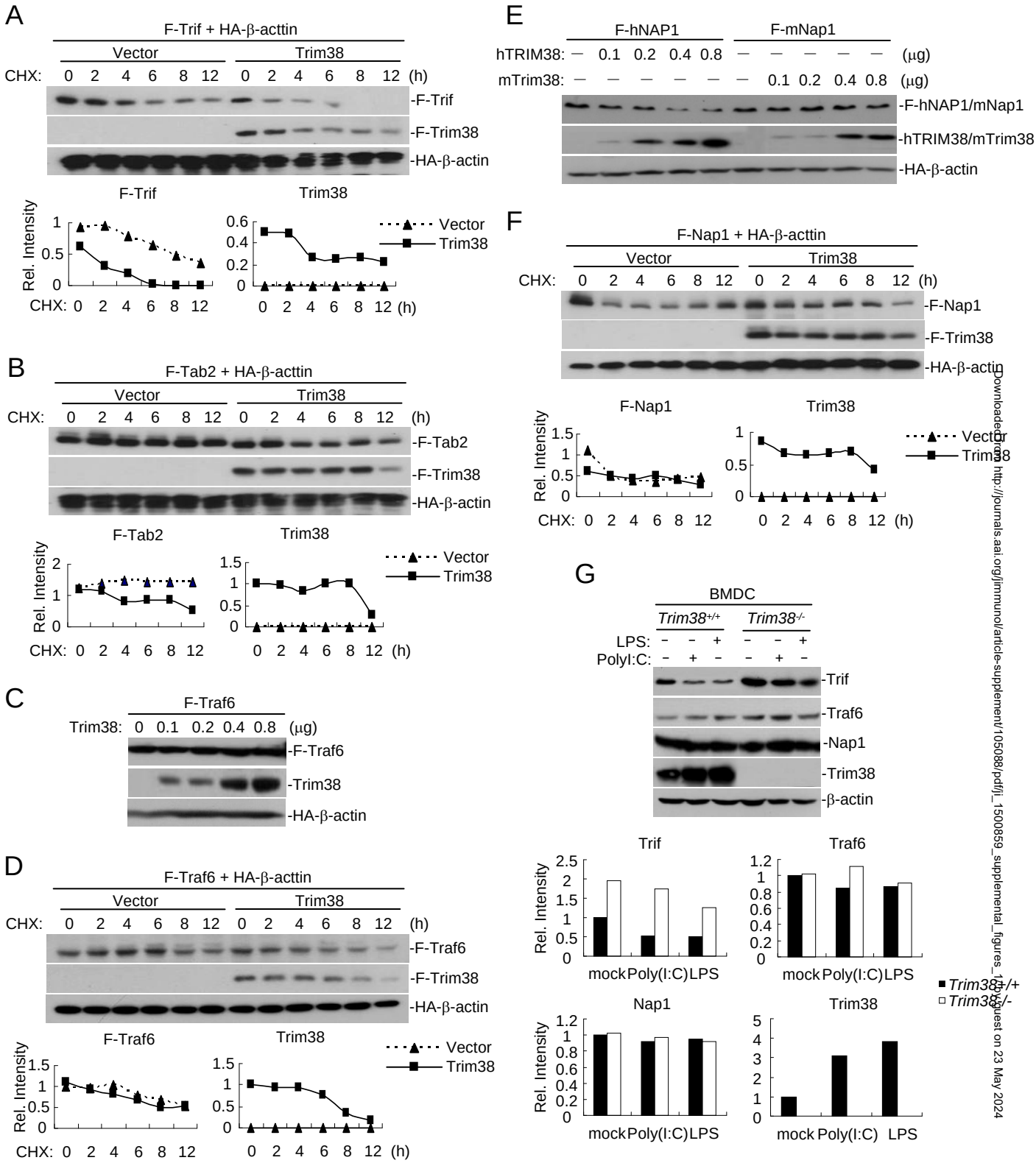

Figure S2. Murine Trim38 promotes degradation of Trif and Tab2.

(A), Murine Trim38 destabilizes Trif. HEK293 ($4x10^5$) cells were transfected with the indicated plasmids for 24 hours and then CHX(100 μg/ml) was added to the medium for the indicated time points before immunoblotting analysis was performed with the indicated antibodies. The histographs show the relative intensities of the bands, which were quantitated by densitometry with Image J program and normalized by the β-actin levels.

(B), Murine Trim38 destabilizes Tab2. Experiments were performed similarly as in (A).

(C), Murine Trim38 had no marked effect on the level of Traf6. HEK293 ($4x10^5$) cells were transfected with the indicated plasmids for 24 hours, followed by immunoblotting analysis with the indicated antibodies.

(D), Murine Trim38 had no marked effect on the level of Traf6. Experiments were performed similarly as in (A).

(E), Murine Trim38 had no marked effect on the level of Nap1. Experiments were performed similarly as in (C).

(F), Murine Trim38 had no marked effect on the level of Nap1. Experiments were performed similarly as in (A).

(G), Analysis of expression levels of the indicated proteins in *Trim38*$^{+/+}$ and *Trim38*$^{-/-}$ BMDCs. BMDCs were left untreated or treated with Poly(I:C) or LPS for 4h. Immunoblotting analysis was performed with the indicated antibodies. The histographs show the relative intensities of the bands, which were quantitated by densitometry with Image J program and normalized by the β-actin levels.

Hu et al. Figure S3

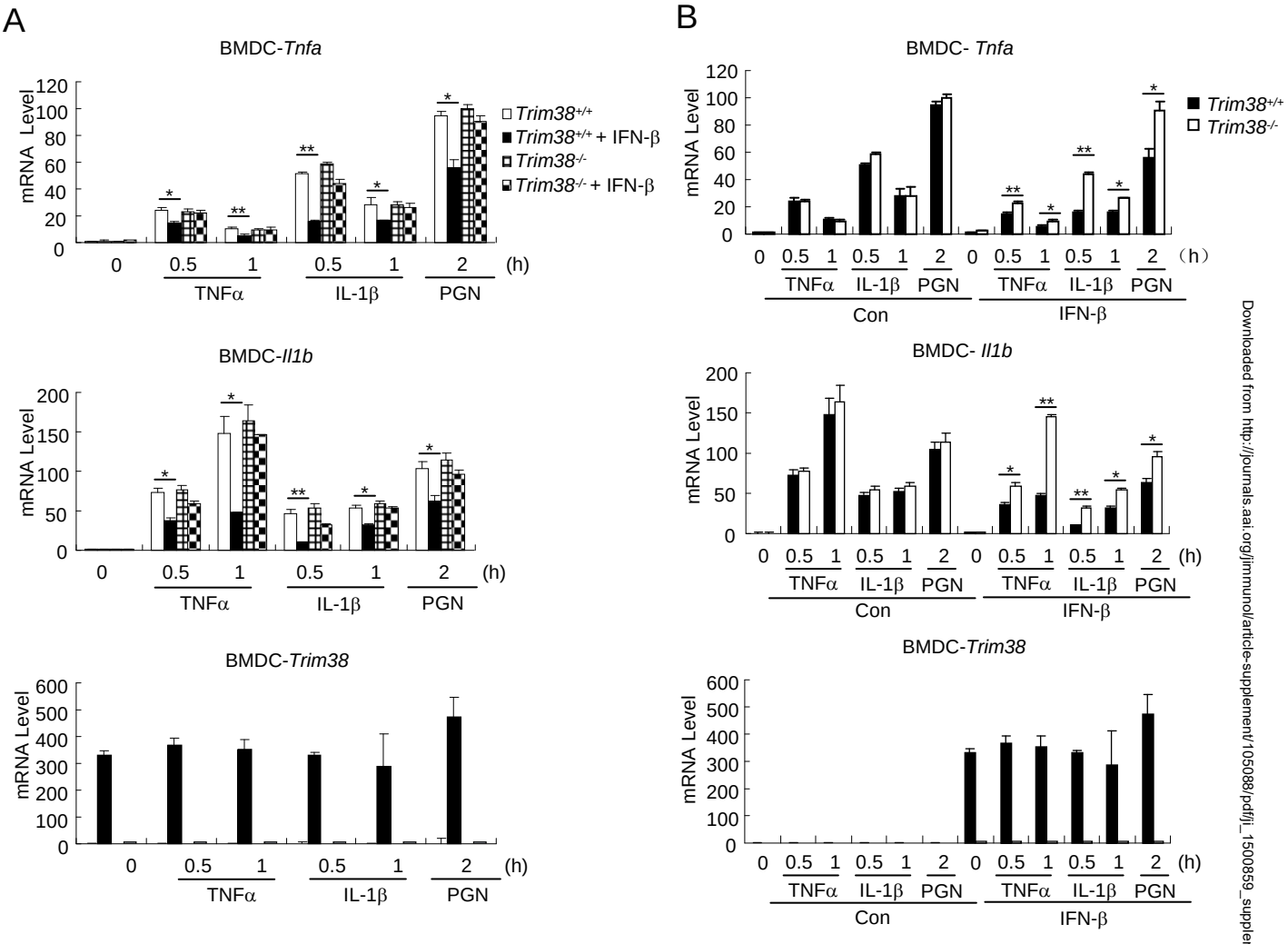

Figure S3. Trim38-deficiency potentiates TNFα- and IL-1β-triggered expression of proinflammatory cytokines in IFN-β-primed BMDCs.

(A), Effects of IFN-β treatment on TNFα- or IL-1β-induced expression of proinflammatory cytokines in *Trim38*$^{+/+}$ and *Trim38*$^{-/-}$ BMDCs. *Trim38*$^{+/+}$ and *Trim38*$^{-/-}$ BMDCs were left untreated or treated with IFN-β (10 ng/ml) for 12 hours followed by TNFα or IL-1β stimulation for the indicated times before qPCR was performed.

(B), Effects of Trim38-deficiency on TNFα- and IL-1β-induced expression of inflammatory cytokines in IFN-β-primed or un-primed BMDCs. The original data are the same as in (A) but displayed in a different way.

Graphs show mean±s.d., n=3. *, p<0.05; **, p<0.01.

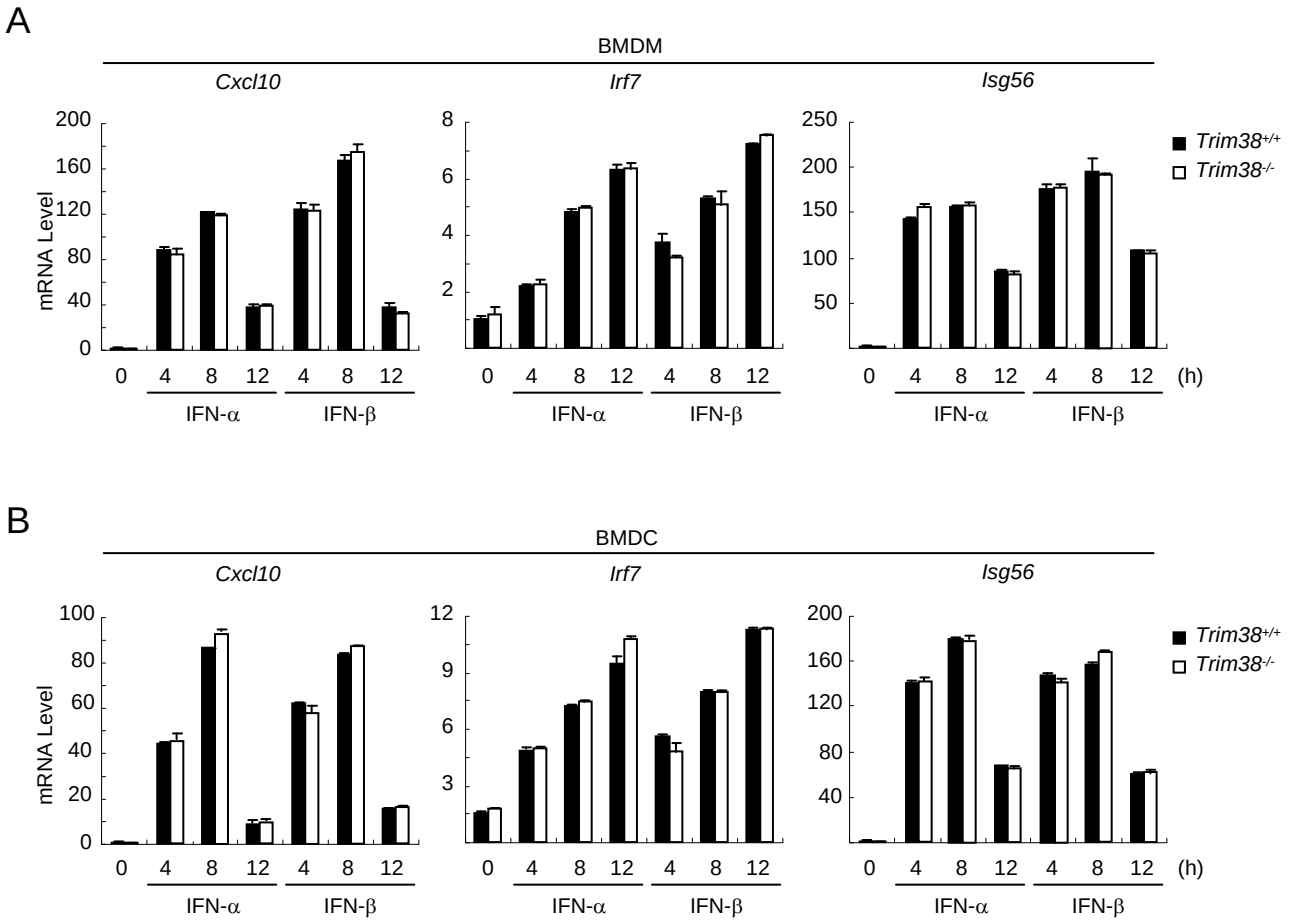

Figure S4. Trim38-deficiency does not markedly affects type I IFN-triggered signaling in mouse immune cells.

(A), Effects of Trim38-deficiency on IFN-α- and IFN-β-triggered transcription of downstream genes in BMDMs. *Trim38*$^{+/+}$ and *Trim38*$^{-/-}$ BMDMs were stimulated with IFN-α4 (20 ng/ml)) or IFN-β (10 ng/ml) for the indicated times before qPCR was performed.

(B), Effects of Trim38-deficiency on IFN-α- and IFN-β-triggered transcription of downstream genes in BMDCs. Experiments were performed similarly as in (A).

Graphs show mean±s.d., n=3.