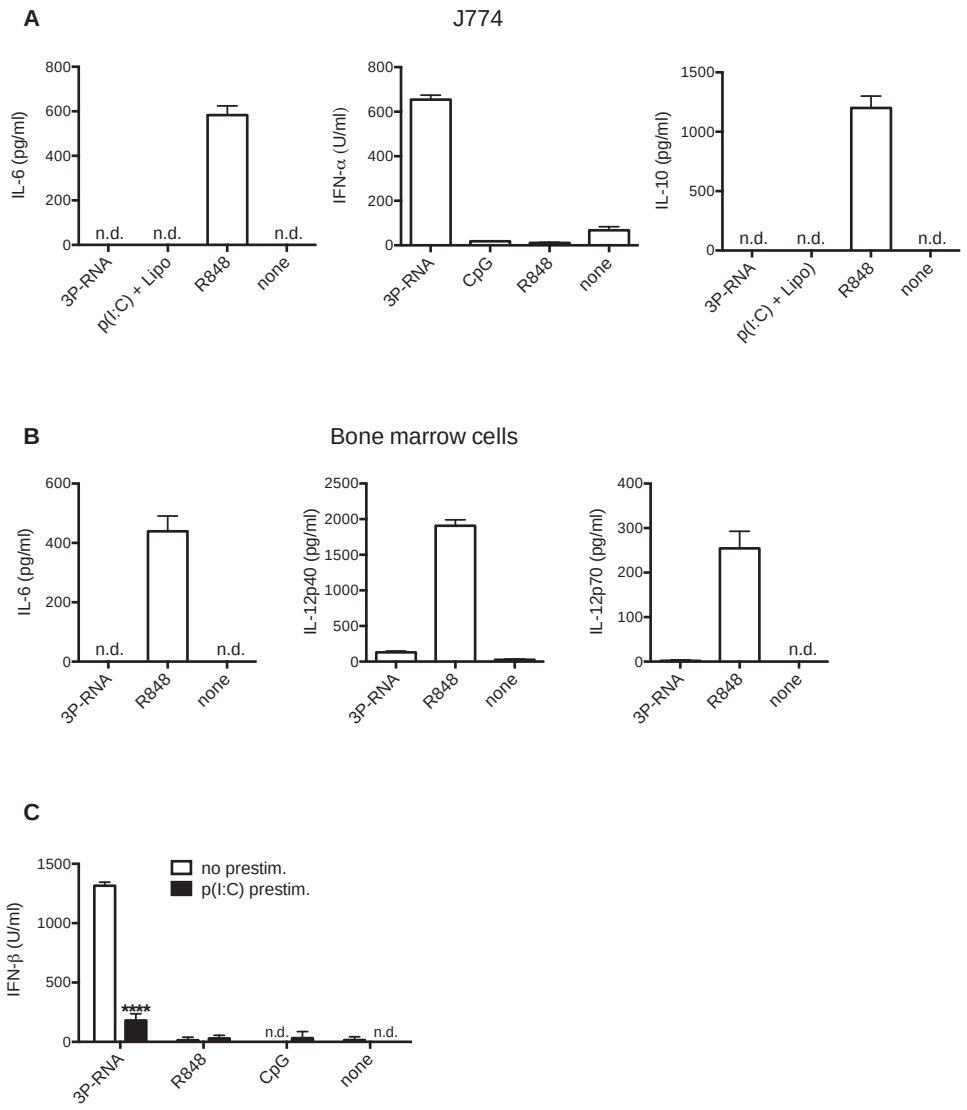


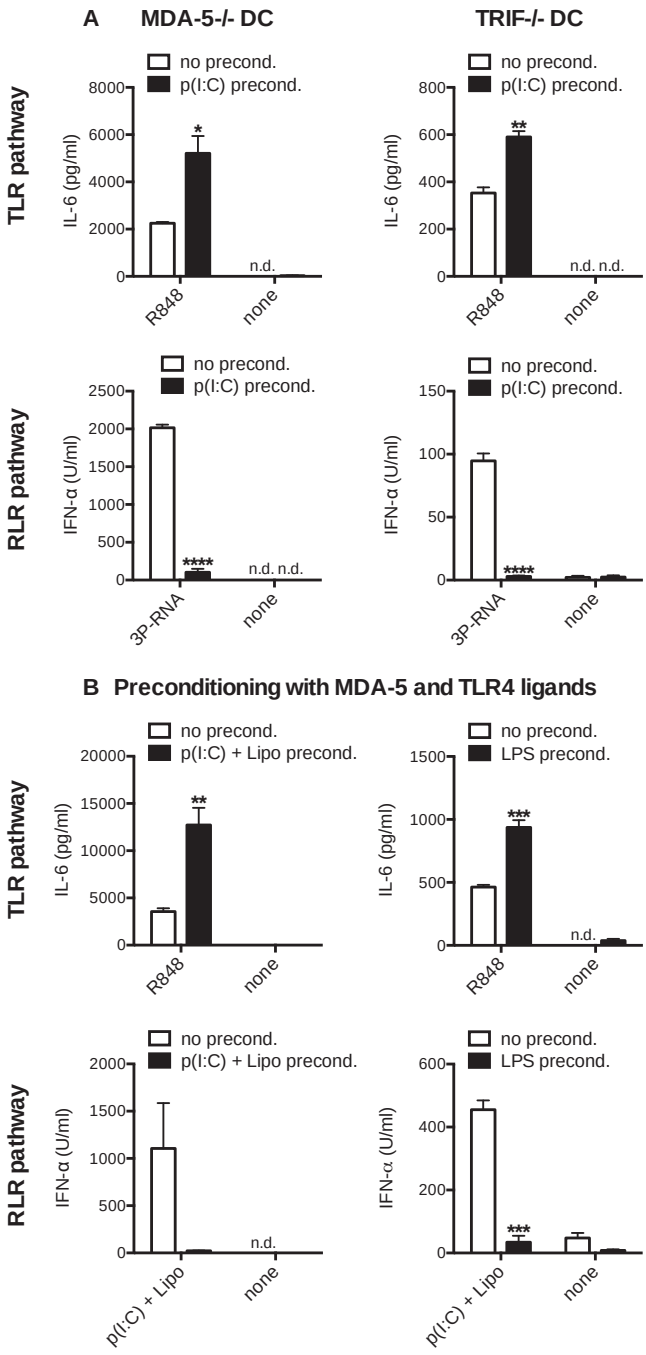
SUPPLEMENTAL FIGURE 1

(A) IL-6, IL-10 and IFN-α in supernatants from J774 stimulated for 24 h with CpG, R848, 3'-triphosphate RNA complexed with Lipofectamine (3P-RNA) or poly(I:C) complexed with Lipofectamine (p(I:C)+Lipo).

(**B**) IL-6, IL-12p40 and IL-12p70 in supernatants from bone marrow cells stimulated for 24 h with 3P-RNA or R848.

(**C**) IFN-β in supernatants from DC preconditioned for 24 with poly(I:C) h and stimulated for 24 h with CpG, R848 and 3P-RNA.

****, $p < 0.0001$; "n.d." cytokine levels below detection limit. Black bars vs. white bars. Student's T-Test. Data are representative of at least 2 independent experiments.

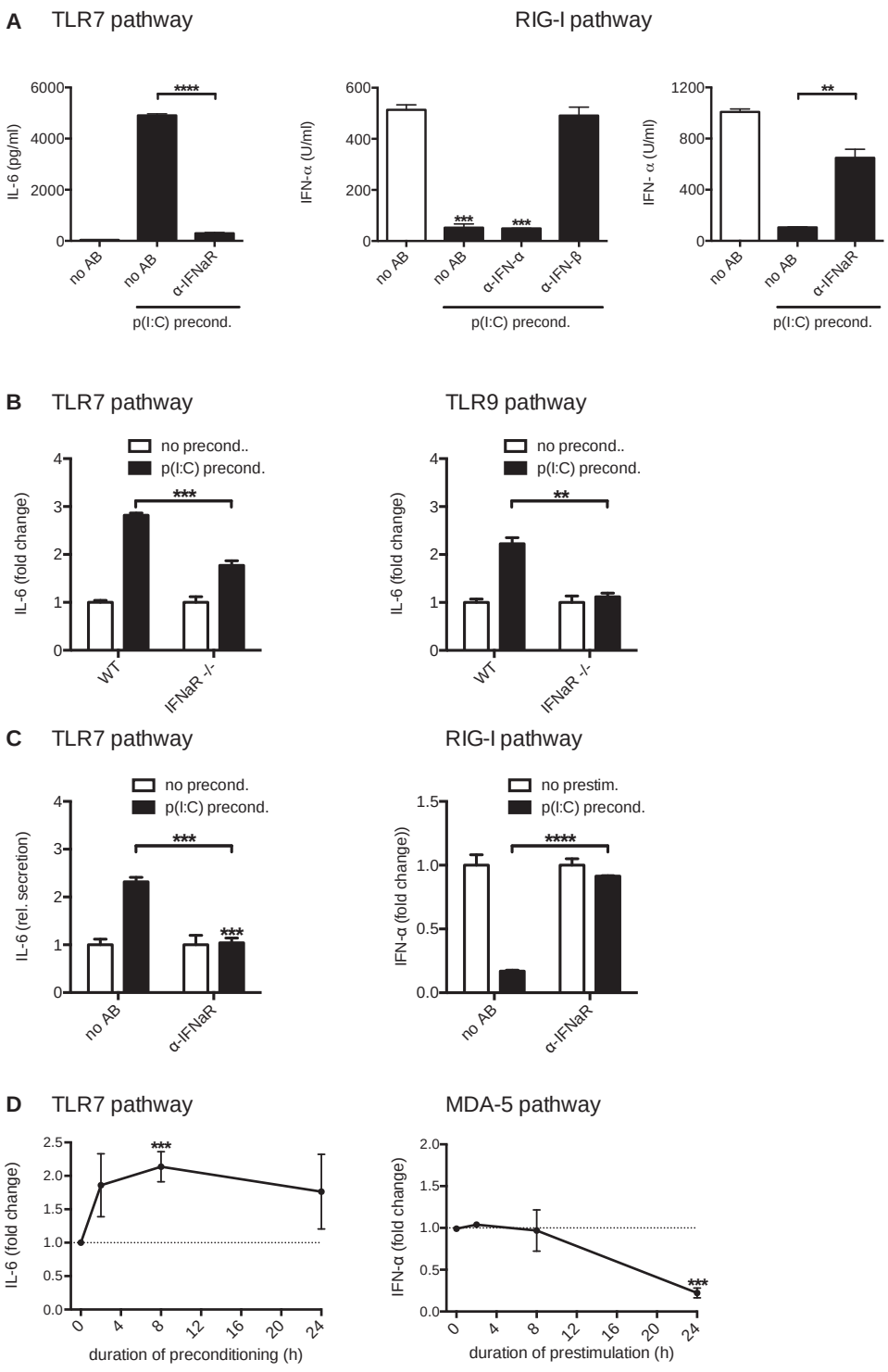


SUPPLEMENTAL FIGURE 2

(A) IL-6 and IFN-α in supernatants from TRIF or MDA-5-deficient DC preconditioned for 24 h with poly(I:C) and stimulated for 24 h with R848 and 3'-triphosphate RNA complexed with Lipofectamine (3P-RNA).

(B) IL-6 and IFN-α in supernatants from J774 cells preconditioned for 24 h with comnplexed poly(I:C) or LPS and stimulated for 24 h with R848 and 3'-triphosphate RNA.

*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ and ****, $p < 0.0001$; “n.d.” cytokine levels below detection limit. Black bars vs. white bars. Student’s T-Test. Data are representative of at least 2 independent experiments (mean + s.e.m. of triplicates).



SUPPLEMENTAL FIGURE 3

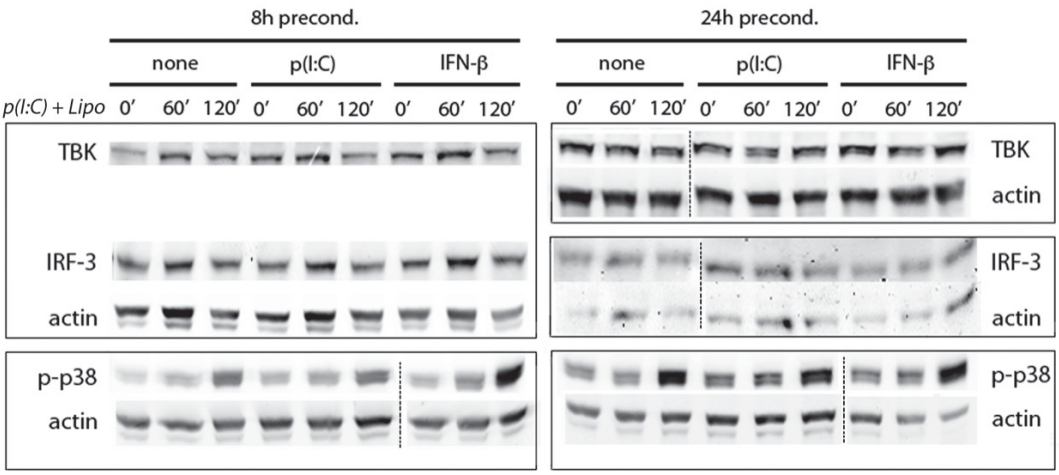
(**A**) IL-6 and IFN-α in supernatants from J774 cells preconditioned for 24 h with poly(I:C) and stimulated with R848 (left) or 3P-RNA (middle, right) for 24 h. In some conditions α-IFN-α, β or IFNaR antibodies were added in the preconditioning phase.

(**B**) IL-6 and IFN-α in supernatants from J774 preconditioned for different durations with recombinant IFN-β prior to stimulation for 24 h with R848 (left) or complexed poly(I:C) (right). Relative expression to the 0 h time-point is shown.

(**C**) IL-6 and IFN-α in supernatants from DCs stimulated from WT or IFNaR -/- 129/Sv mice preconditioned for 24 h with poly(I:C) and stimulated with R848 (left) or CpG (right) for 24 h. Data are expressed as fold change compared to the WT control. and are representative of 3 independent experiments (mean + s.e.m. of triplicates).

(**D**) IL-6 and IFN-α in supernatants from WT DCs preconditioned for 24 h with poly(I:C) and stimulated with R848 (left) or 3P-RNA (right) for 24 h. In some conditions α-IFNaR antibodies were added in the preconditioning phase. Data are expressed as fold change compared to non-antibody-treated, non-preconditioned control and are representative of 2 independent experiments (mean + s.e.m. of triplicates).

**, $p < 0.01$; ***, $p < 0.001$ and ****, $p < 0.0001$. (A,C,D): black bars vs. white bar or as indicated. Student's T-Test (2-3 groups) or 1-way ANOVA, Dunnett's Multiple Comparison Test (4 groups); (B): Zero h time-point vs. other conditions, 1-way ANOVA, Dunnett's Multiple Comparison Test. Data are representative of 2-3 independent experiments (mean + s.e.m. of triplicates in A,C,D). Mean ± s.e.m. of 6 independent experiments are shown in (B).



SUPPLEMENTAL FIGURE 4

Immunoblot analysis of p38 phosphorylation, TBK-1 and IRF-3 expression in lysates of J774 cells preconditioned for 8 and 24 h with poly(I:C) or IFN-β prior to stimulation for 60 and 120 min with complexed poly(I:C). Individual blots are depicted by rectangles. Some blots were sliced for clarity as indicated by dashed lines.