**SUPPLEMENTAL FIGURE 1**

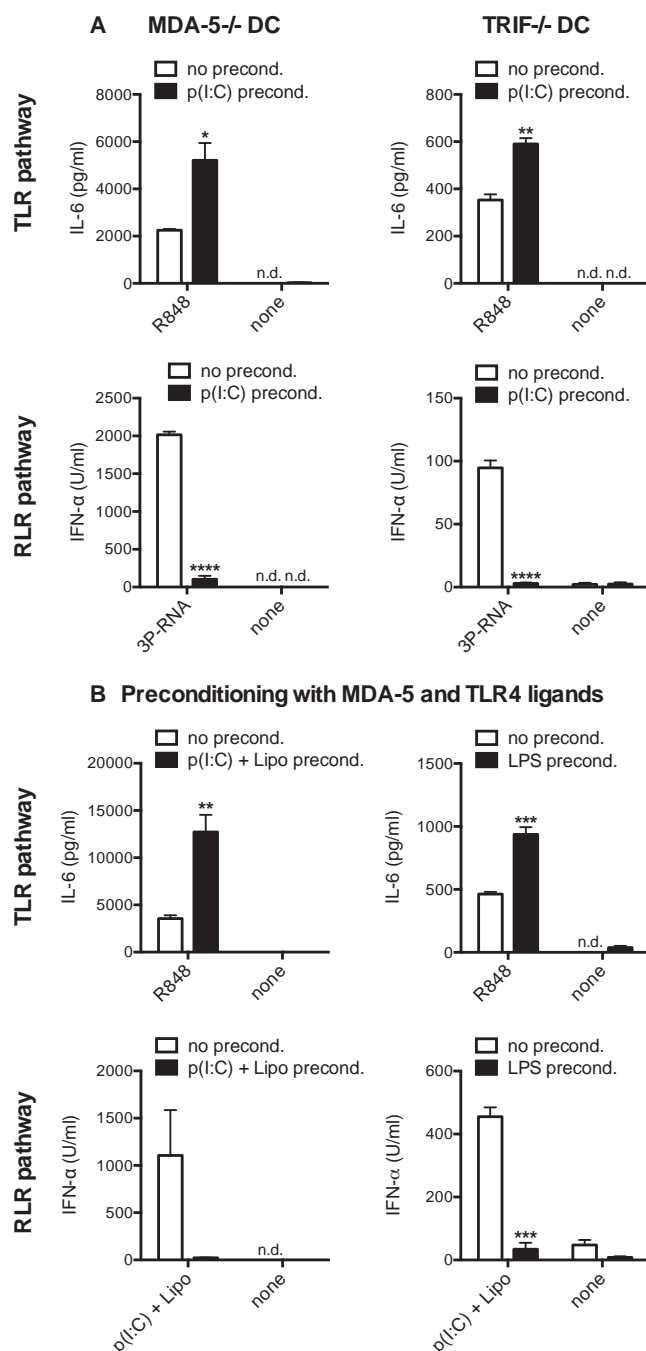
**(A)** IL-6, IL-10 and IFN- $\alpha$  in supernatants from J774 stimulated for 24 h with CpG, R848, 3'-triphosphate RNA complexed with Lipofectamine (3P-RNA) or poly(I:C) complexed with Lipofectamine (p(I:C)+Lipo).

**(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- $\beta$  in supernatants from DC preconditioned for 24 h with poly(I:C) 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.

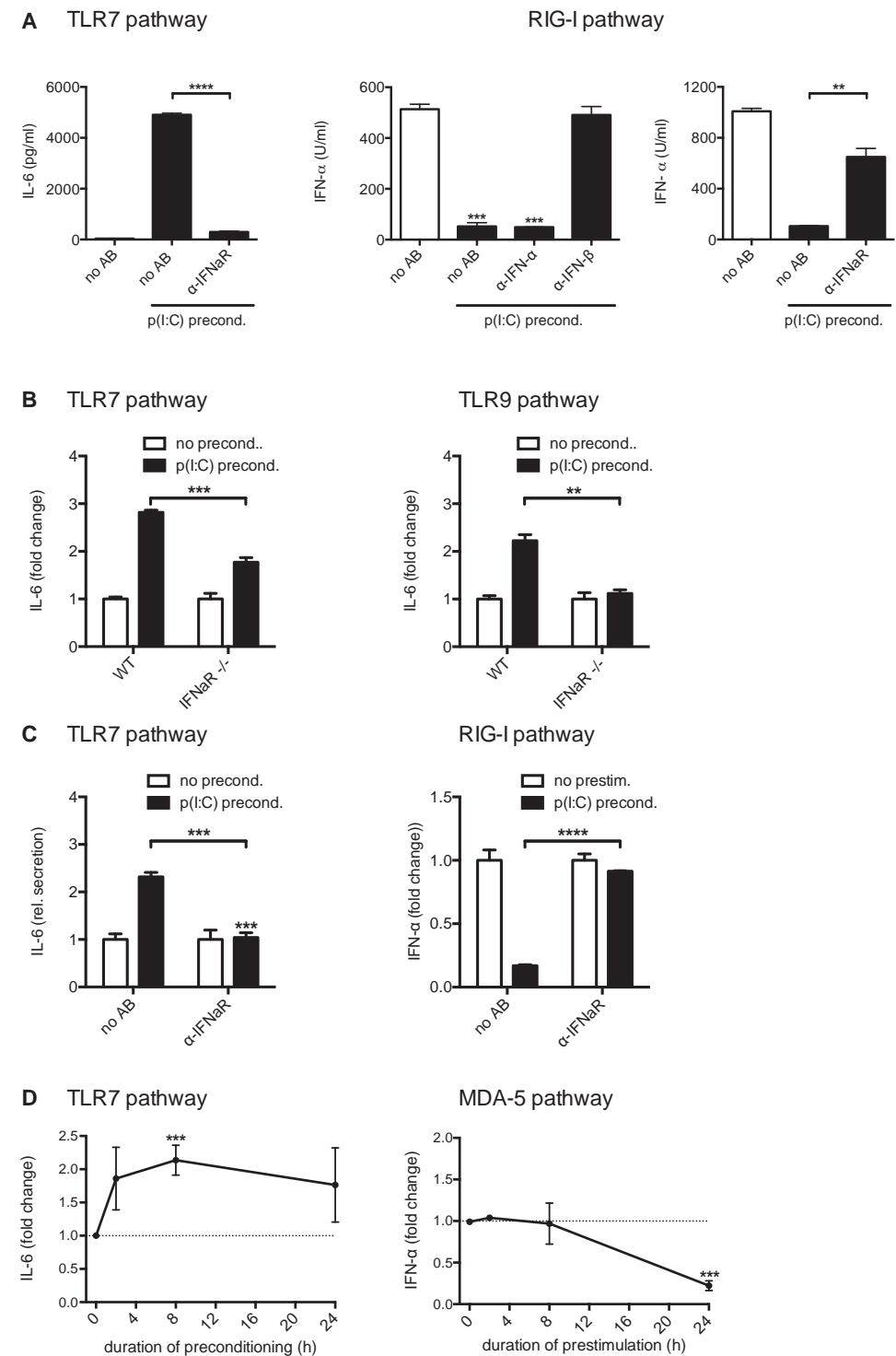
Student's T-Test. Data are representative of at least 2 independent experiments.

**SUPPLEMENTAL FIGURE 2**

(A) IL-6 and IFN- $\alpha$  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- $\alpha$  in supernatants from J774 cells preconditioned for 24 h with complexed 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).

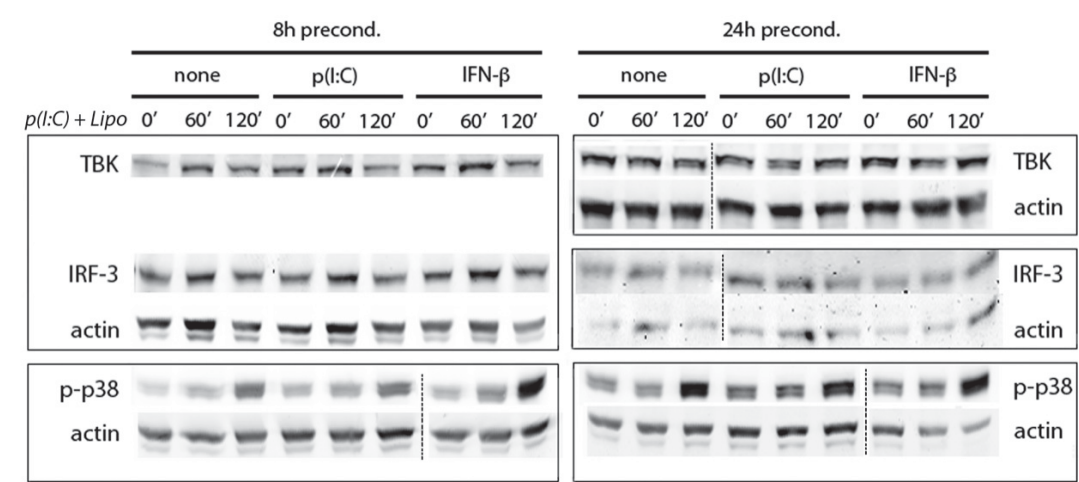


(B) IL-6 and IFN- $\alpha$  in supernatants from J774 preconditioned for different durations with recombinant IFN- $\beta$  prior to stimulation for 24 h with R848 (left) or complexed poly(I:C) (right). Relative expression to the 0 h time-point is shown.

(C) IL-6 and IFN- $\alpha$  in supernatants from DCs stimulated from WT or IFN $\alpha$ R -/- 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- $\alpha$  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  $\alpha$ -IFN $\alpha$ R 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  $\pm$  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.