Supporting Information

Chang et al. 10.1073/pnas.0905815106

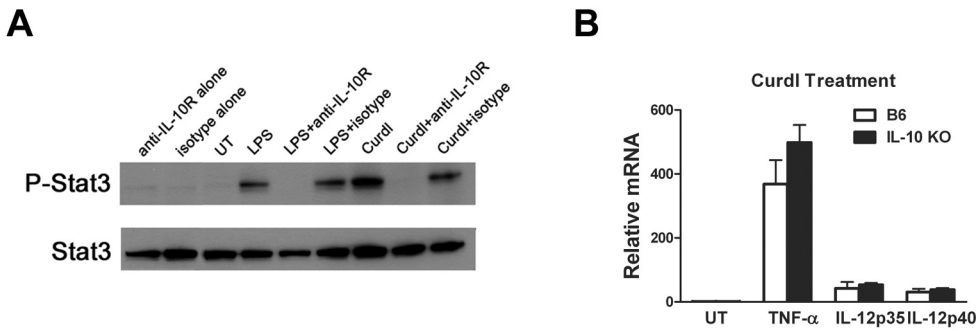

Fig. S1. Curdlan-mediated signaling pathway is not inhibited by IL-10. (*A*) Dendritic cells (DC) from C57BL/6 mice were treated as indicated for 6 h. Whole-cell lysates were prepared and Stat3 and P-Stat3 expression was analyzed by immunoblotting. (*B*) C57BL/6 and IL-10-deficient DC were treated with curdlan for 6 h. Quantitative (q)RT-PCR was performed to assess the amount of mRNA for TNF-α, IL-12p35, and IL-12p40. Values are presented as means $\pm$ SD.

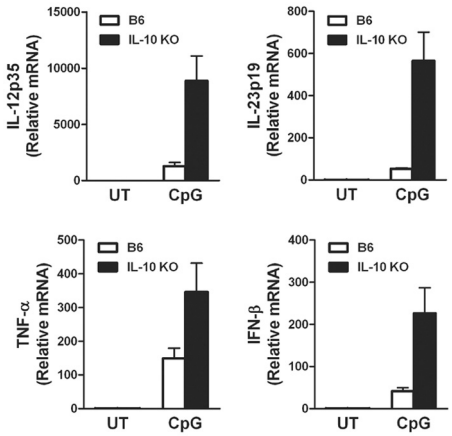

Fig. S2. IL-10 negatively regulates CpG signaling pathway. DC from C57BL/6 and IL-10-deficient mice were treated with CpG for 6 h, and qRT-PCR was performed to quantify IL-12p35, IL-23p19, TNF-α, and IFN-β mRNA expression.

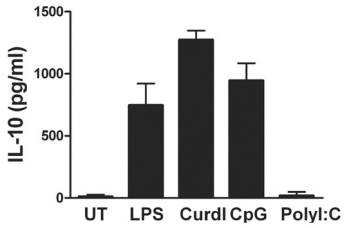

Fig. S3. IL-10 production by DC stimulated with various ligands. DC from C57BL/6 mice were stimulated as indicated for 24 h, and IL-10 level was measured by ELISA.

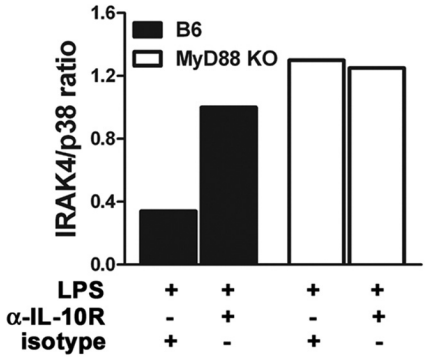

Fig. S4. IL-10 degraded IL-1 receptor-associated kinase (IRAK)4. Quantitation of IRAK4 protein levels at 48 h, measured by IRAK4/p38 ratio, was assessed by densitometric analysis using AlphaView (Alpha Innotech).

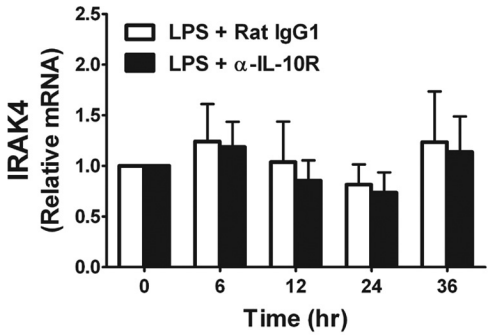

Fig. S5. The IRAK4 mRNA level was not affected by IL-10. DC from C57BL/6 mice were treated with 1 μg/mL LPS with 15 μg/mL rat IgG1 or with α-IL-10R for the indicated time. Quantitative RT-PCR was performed to quantify IRAK4 mRNA levels. The primers used for IRAK4 was described previously (1). Values are presented as mean $\pm$ SE.

1. Hatao F, et al. (2004) Prolonged Toll-like receptor stimulation leads to down-regulation of IRAK-4 protein. *J Leukoc Biol* 76:904–908.

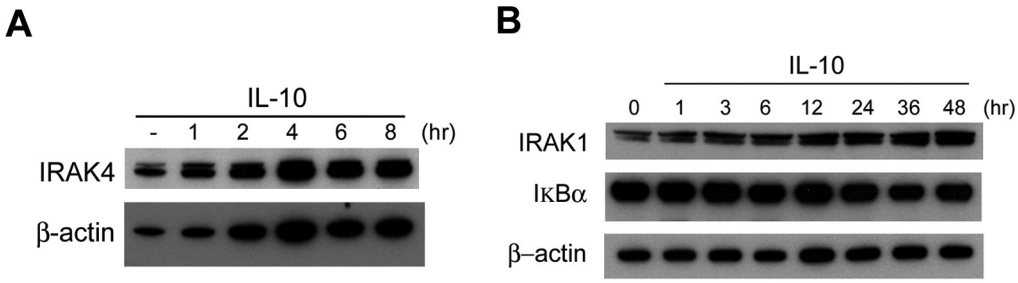

Fig. S6. Exogenous IL-10 could not induce protein degradation. DC from C57BL/6 mice were treated with 10 ng/mL of IL-10 for the indicated time. IRAK4 and β-actin (*A*) and IRAK1, IκBα, and β-actin (*B*) expression was analyzed by immunoblotting.

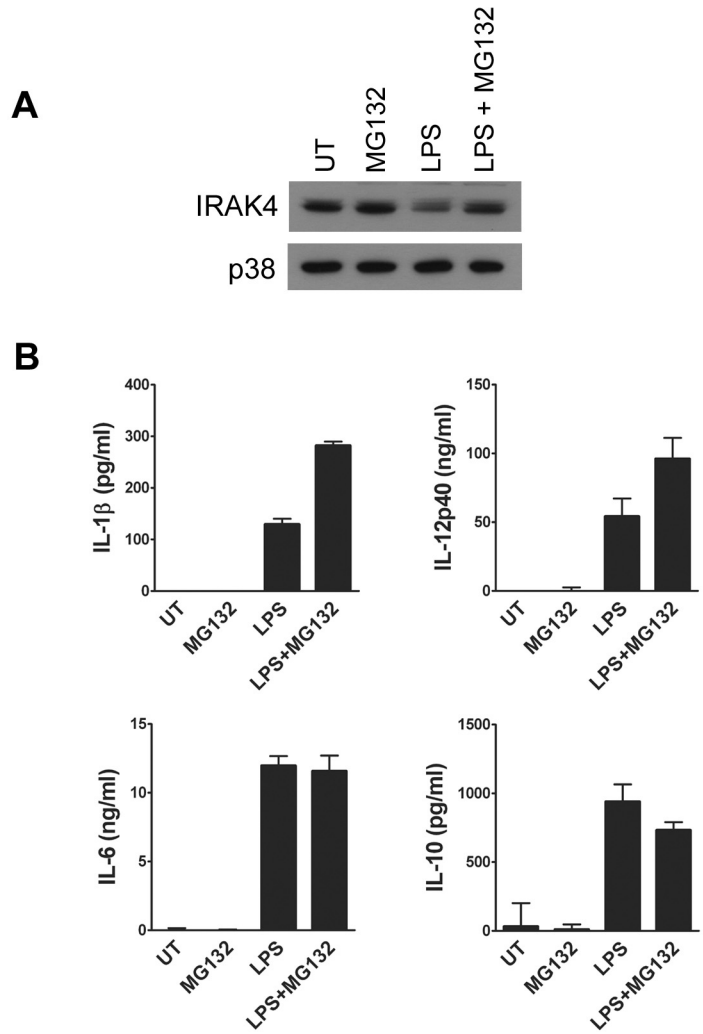

Fig. S7. MG132 alone does not enhance cytokine production. DC were treated with MG132 (0.5 μM), LPS (1 μg/mL), LPS together with MG132, or left untreated for 48 h. IRAK4 and p38 expression was analyzed at by immunoblotting (*A*); and IL-1β, IL-12p40, IL-6, and IL-10 proteins in the supernatants were quantified by ELISA (*B*).

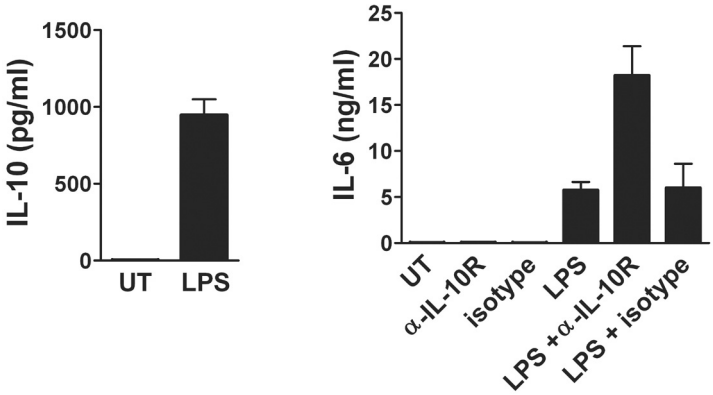

Fig. S8. RAW264.7 produced IL-10 on prolonged Toll-like receptor (TLR)4 stimulation. RAW264.7 cells were treated with LPS alone (1 μg/mL) or together with anti-IL-10R or rat IgG1 (both at 15 μg/mL) for 48 h. IL-10 and IL-6 levels were quantified by ELISA in culture supernatants.