Supporting Information

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SI Methods

Mice were injected in the left hind footpad with 1×10^6 promastigotes of *Leishmania major* MHOM/S U/73-5-ASKH strain. The thickness of the infected and contralateral uninfected footpad was measured as described (1).

To measure the immune responses to pathogens, draining popliteal lymph node cells ($3–10 \times 10^4$) were cultured in the presence of *L. major* antigen for 48 h as described (1).

1. Tominaga N, *et al*. (2003) Development of Th1 and not Th2 immune responses in mice lacking IFN-regulatory factor-4. *Int Immunol* 15:1–10.

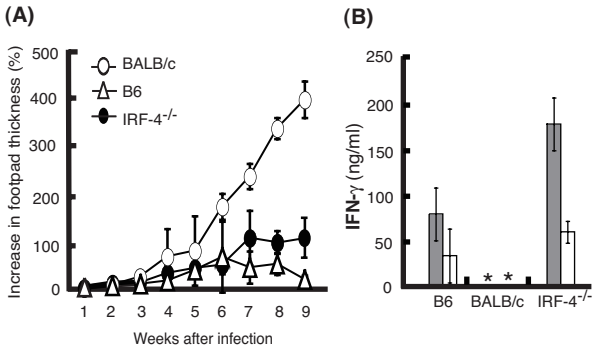

Fig. S1. IRF-4$^{-/-}$ mice are resistant to *L. major* infection. Mice were injected in the left hind footpad with 1×10^6 promastigotes of *L. major* MHOM/SU/73-5-ASKH strain. The thickness of the infected and contralateral uninfected footpad was measured as described (1). (*A*) BALB/c (open circles, $n = 8$), B6 (open triangles, $n = 4$), and IRF-4$^{-/-}$ mice (closed circles, $n = 14$) were infected with *L. major* promastigotes, and the increase in footpad thickness (percentage) was determined. The data represent mean ± SD of each group. (*B*) Four weeks after infection, 1×10^5 (gray bars) and 3×10^4 (open bars) draining popliteal lymph node cells were cultured for 48 h in the presence of *L. major* antigen. The levels of IFN-γ in the supernatant were determined by ELISA. Representative results of three independent experiments are shown. *, not detectable.

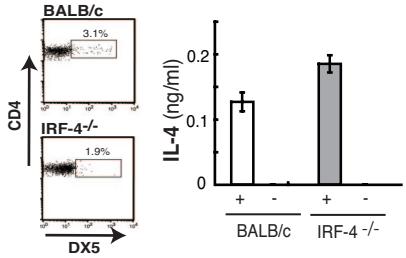

Fig. S2. $CD4^+$ NK T cells from IRF-$4^{-/-}$ mice produce IL-4 in response to α-GalCer. (*Left*). Splenic $CD4^+$ T cells were stained with FITC–anti-CD4 and with biotin–anti-DX5 mAb plus streptavidin-phycoerythrin and analyzed by using FACS Aria. The proportion of cells within the square is indicated. (*Right*) Sorted $CD4^+DX5^+$ cells (5×10^4) from BALB/c (open bar) and IRF-$4^{-/-}$ (filled bar) mice were cultured with splenic dendritic cells ($CD11c^+$ cells, 1×10^4) in the presence (+) and absence (−) of α-GalCer (200 ng/ml) for 48 h. The cytokine levels in the supernatant were determined by ELISA. The purity of $CD4^+DX5^+$ cells from BALB/c and IRF-$4^{-/-}$ mice was 93.3 and 70.6%, respectively. The lower purity of $CD4^+DX5^+$ cells from IRF-$4^{-/-}$ mice was caused by the lower expression of DX5 marker and their small numbers.

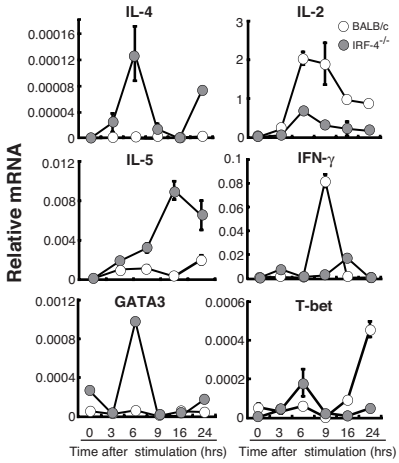

Fig. S3. RNA expression of Th2 cytokines by naïve CD4$^+$ T cells. Naïve (CD62L$^+$) CD4$^+$ T cells from BALB/c (open circles) or IRF-4$^{-/-}$ (closed circles) mice were cultured with plates coated with anti-TCR mAb for 0–24 h. Messenger RNA levels were determined by real-time PCR and were expressed as the ratio of DNA to glucose-3-phosphate dehydrogenase. Representative results of three independent experiments are shown.

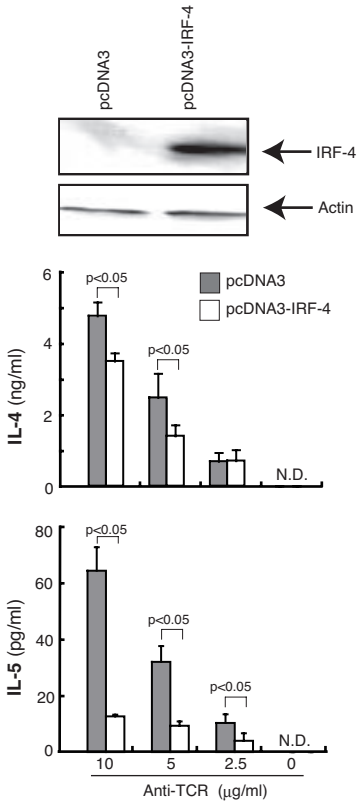

Fig. S4. The forced expression of IRF-4 in IRF-4$^{-/-}$CD4^{+} T cells enhanced their Th2 cytokine production. CD4^{+} T cells from IRF-4$^{-/-}$ mice were cotransfected with pcDNA3 or pcDNA3-mIRF-4 together with pmaxEGFP at a ratio of 5:1, cultured for 3 h, and stimulated with plate-bound anti-TCR mAb (0–10 μg/ml) for 48 h. The proportion of cells expressing GFP reached ≈40% 24 h after the gene transfer. Cell lysate was prepared from unstimulated cells, separated by 12.5% SDS/PAGE, blotted, and probed with anti-IRF-4 Ab. The blot was stripped and reprobed with anti-actin Ab. The levels of IL-4 and IL-5 in the supernatant were determined by ELISA. The proportion of EGFP^{+} cells was 30–45%. The Mann–Whitney *U* test for unpaired observations was used to calculate *P* values. Representative results of three independent experiments are shown.

Table S1. Primer sequences used for real-time RT-PCR

Name	5′ primer	3′ primer
IL-4	TCATCGGCATTTTGAACGAG	TTTGGCACATCCATCTCCG
IL-5	CTCTCAGCTGTGTCTGGGCC	GCTTGTCAACAGAGCTCGGTG
IFN-γ	GCATTCATGAGTATTGCCAAGTTT	GATTCCGGCAACAGCTGGT
IRF-1	ATTCCAACCAAATCCCAGGG	CTCCGGAACAGACAGGCATC