Supplementary File

Interferon Regulatory Factor 4 controls T_{H1} cell effector function and metabolism

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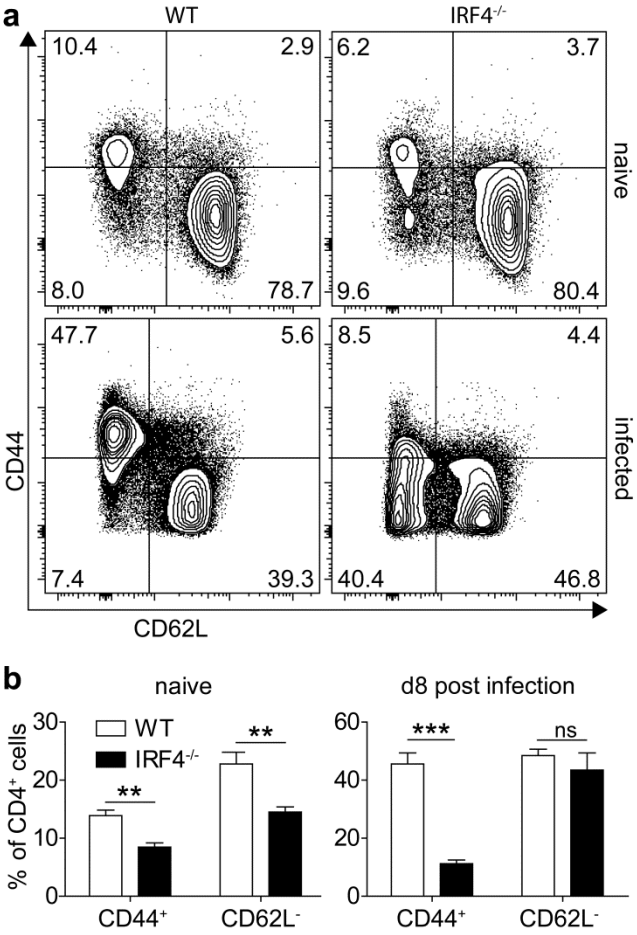
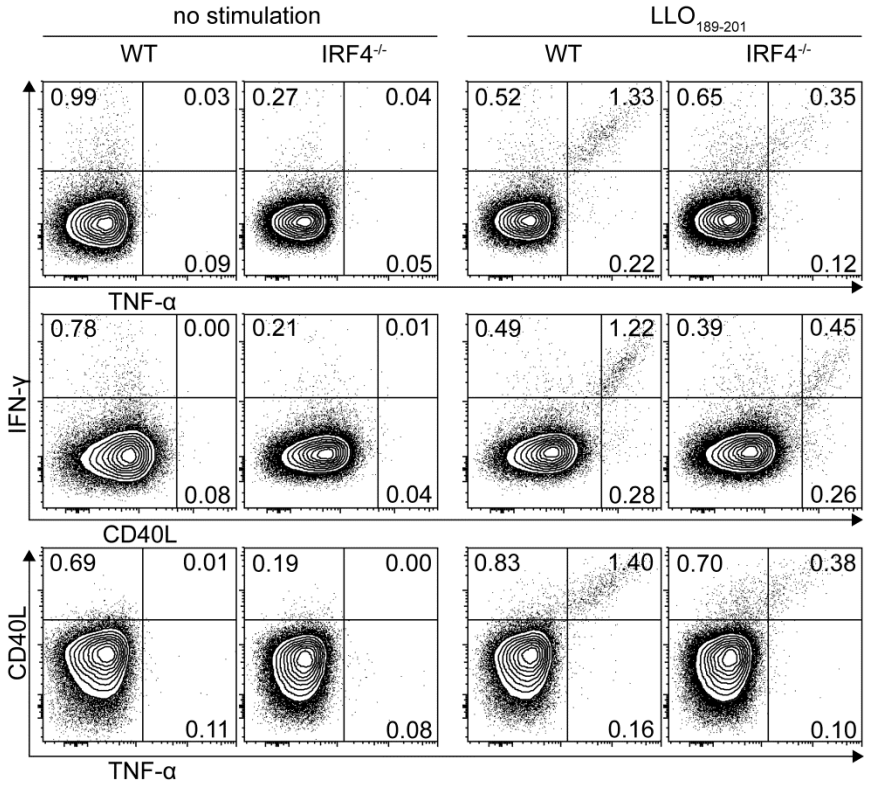
Supplementary Figures

Figure S1



IRF4$^{-/-}$ mice show impaired CD4^{+} T cell response to infection with *L. monocytogenes*.
WT and IRF4$^{-/-}$ mice were i.v. infected with 5×10^{4} LmOVA. Eight days post infection, CD4^{+} T cells from spleen were analyzed for CD44 and CD62L expression. (A) Representative dot plots for CD4-gated cells from naive and infected mice. (B) CD44 and CD62L expression of CD4^{+} T cells from naive and infected mice. Bars represent mean ± SEM from 4 individually analyzed mice per group. The result is representative for 2 independent experiments.

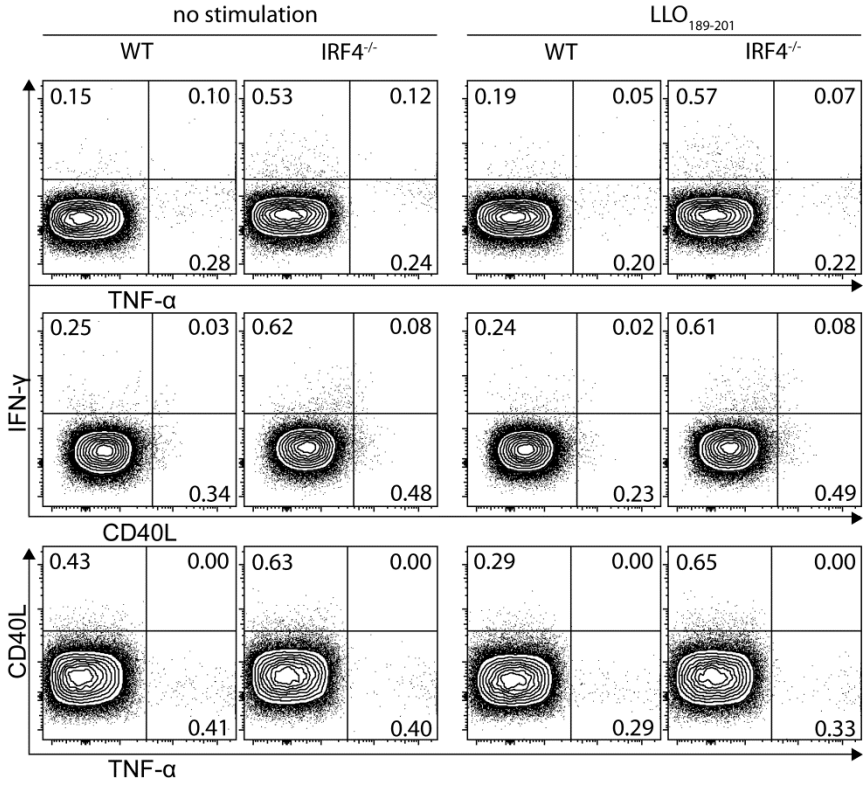
Figure S2



IRF4$^{-/-}$ mice fail to mount a T_{H1} response against *L. monocytogenes*.

WT and IRF4$^{-/-}$ mice were i.v. infected with 5×10^4 *L. monocytogenes*. Eight days post infection, spleen cells were stimulated for 4h with 10^{-5}M $LLO_{189\text{-}201}$ or were incubated without stimulation. Intracellular expression of IFN-γ, TNF-α and CD40L was determined by flow cytometry. Dot blots show results for CD4-gated cells of infected WT and IRF4$^{-/-}$ mice. Results are representative for 4 individually analyzed mice per group and 2 independent experiments.

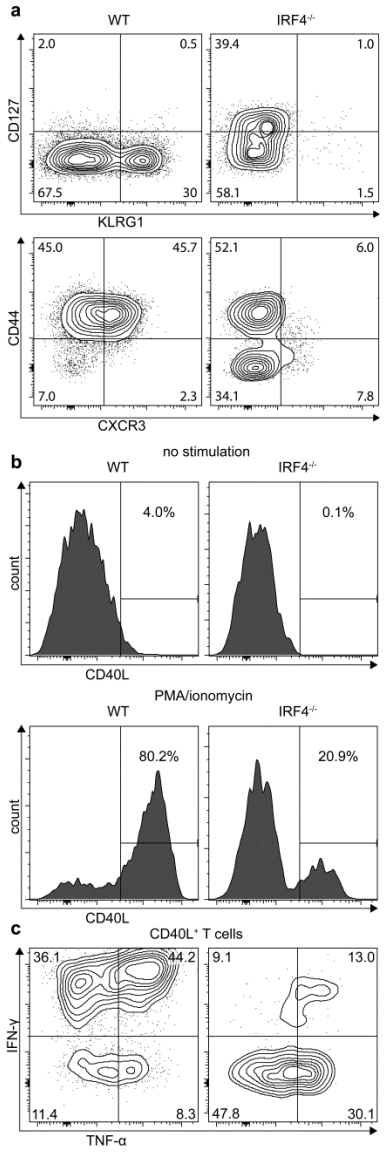
Figure S3



Control stimulation of spleen cells from naive WT and IRF4$^{-/-}$ mice.

Spleen cells from WT and IRF4$^{-/-}$ mice were stimulated for 4h with 10^{-5}M $LLO_{189-201}$ or were incubated without stimulation. Intracellular expression of IFN-γ, TNF-α and CD40L was determined by flow cytometry. Dot blots show results for CD4-gated cells of naive WT and IRF4$^{-/-}$ mice. Results are representative for 4 individually analyzed mice per group in one experiment.

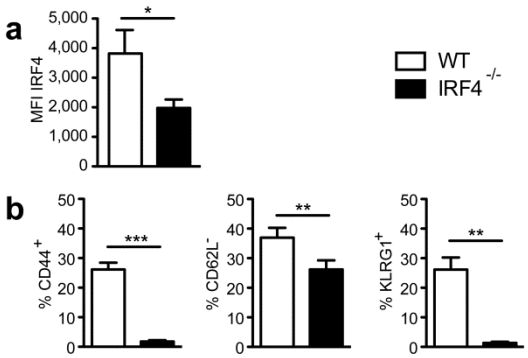
Figure S4



Impaired T_{H1} differentiation of IRF4$^{-/-}$ CD4^{+} T cells.

Purified CD4^{+} T cells from CD90.1^{+}CD90.2^{-} WT and CD90.1^{-}CD90.2^{+} IRF4$^{-/-}$ mice were mixed in a 1:1 ratio and 2×10^{6} cells were i.v. transferred into RAG1$^{-/-}$ mice, which had been infected with 10^{5} Lm 4h earlier. Eight days post infection, spleen cells of mice were analyzed. (A) Representative expression profile for CD127, KLRG1, CD44 and CXCR3 for CD90.1^{+}CD90.2^{-} WT CD4^{+} T cells and CD90.1^{-}CD90.2^{+} IRF4$^{-/-}$ CD4^{+} T cells. (B, C) Spleen cells from infected recipients were incubated for 4h with PMA/ionomycin or without stimulation and stained intracellularly for CD40L, TNF-α, and IFN-γ. (B) CD40L expression in CD4^{+} T cells. (C) IFN-γ and TNF-α expression in CD40L^{+} cells from stimulated WT and IRF4$^{-/-}$ CD4^{+} T cells. Representative results are shown.

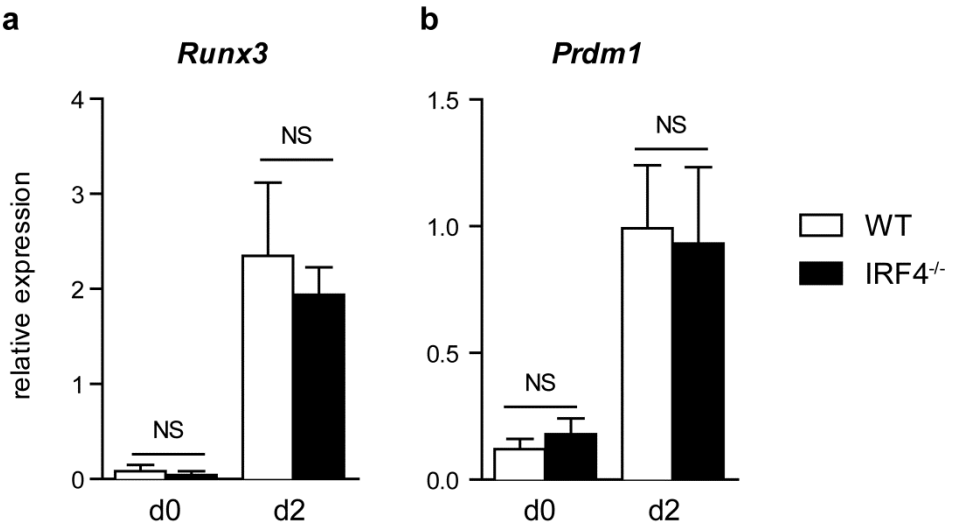
Figure S5



Impaired response of IRF4$^{-/-}$ OT-II cells.

Purified CD4^{+} T cells from WT CD90.1^{+}CD90.2^{+} or IRF4$^{-/-}$ CD90.1^{-}CD90.2^{+} OT-II mice were mixed at a 1:1 ratio and 3×10^{6} cells were i.v. transferred into CD90.1^{+}CD90.2^{-} WT mice. Recipient mice had been i.v. infected with 10^{5} LmOVA one day before. Spleen cells were analyzed 4d post transfer by extra- and intracellular mAb staining and flow cytometry. (A) IRF4 expression in WT and IRF4$^{-/-}$ OT-II cells. (B) Surface expression of CD44, CD62L and KLRG1 on WT and IRF4$^{-/-}$ OT-II cells. Bars give the mean ± SEM of 6 individually analyzed mice. Results are representative for 4 experiments.

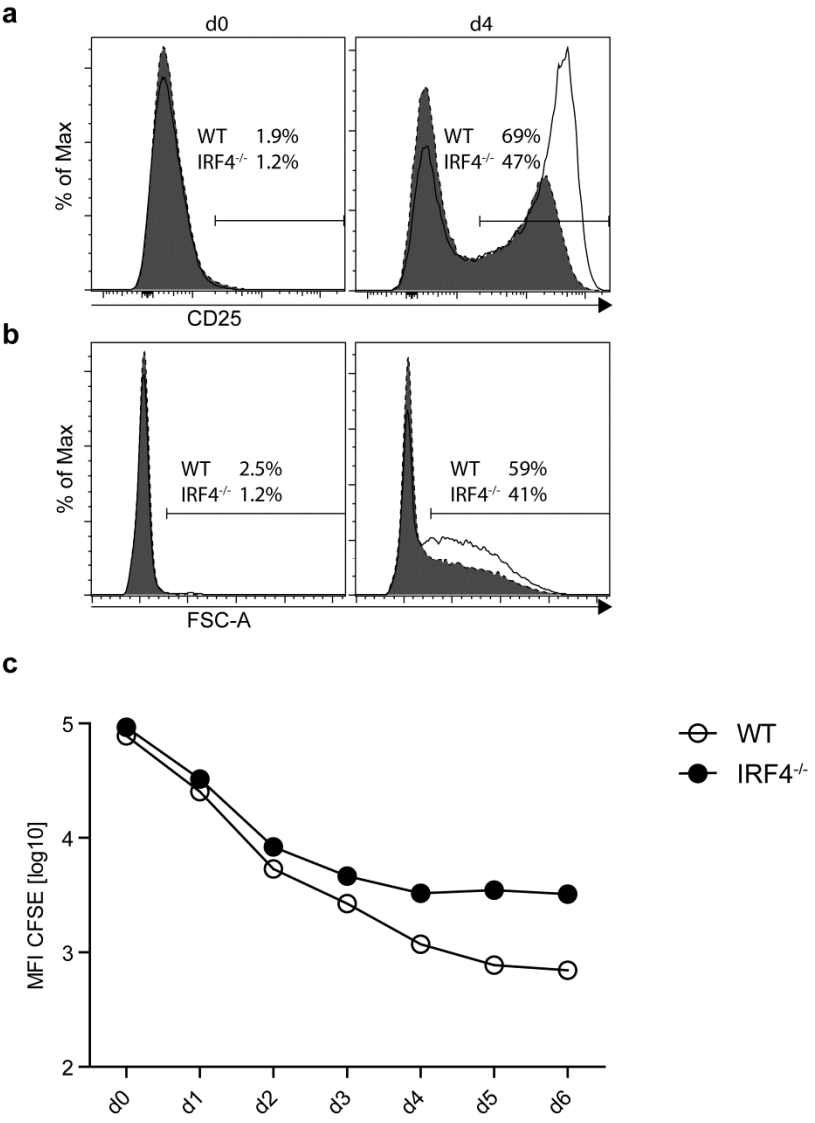
Figure S6



Expression of *Runx3* and *Prdm1* in activated WT and IRF4$^{-/-}$ CD4^{+} T cells.

CD4^{+} T cells were purified and stimulated in individual cultures for 2d as described in Figure 5. Expression of *Runx3* and *Prdm1* was measured by RT PCR. Bars represent the mean ± SEM of 4 individual samples from 2 independent experiments.

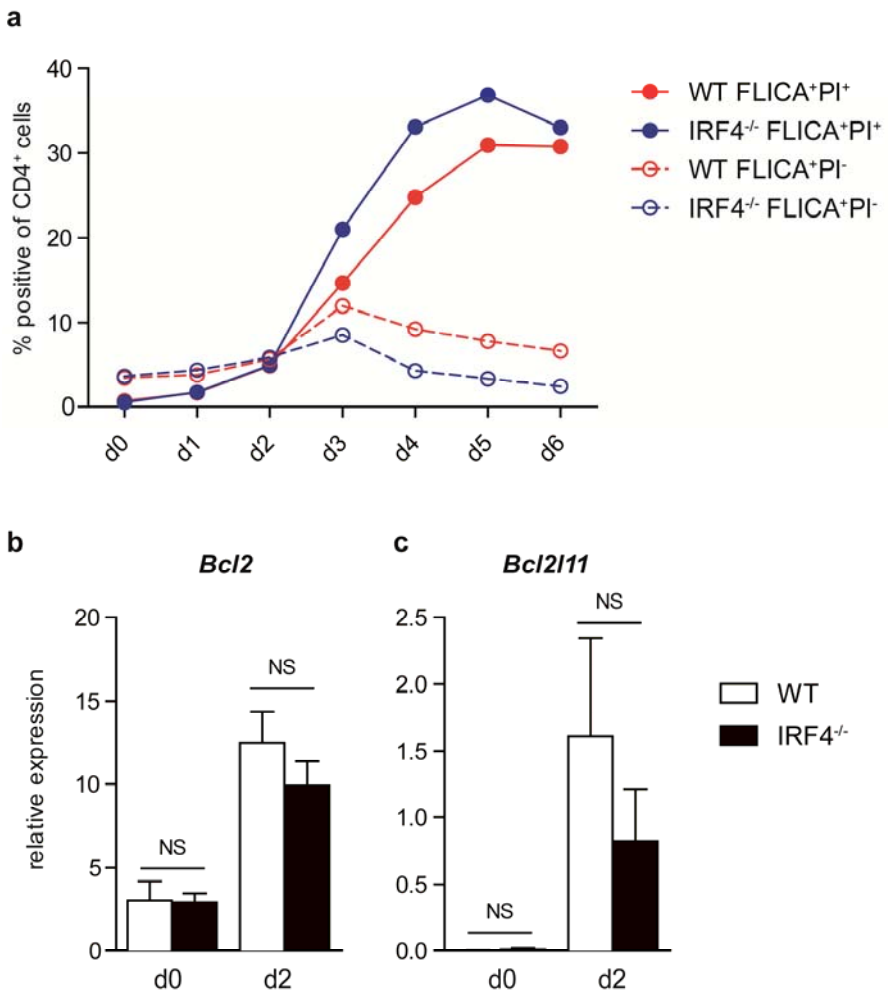
Figure S7



Phenotype and proliferation of activated WT and IRF4$^{-/-}$ CD4 T cells

CD4^{+} T cells from WT (CD90.1^{+}) and IRF4$^{-/-}$ mice (CD90.2^{+}) were mixed with a 1:1 ratio and stimulated *in vitro* with anti-CD3 mAb, anti-CD28 mAb and IL-2 in the presence of IL-12 and anti-IL-4 mAb. (A) CD25 expression and (B) size (FSC-A) of WT and IRF4$^{-/-}$ CD4^{+} T cells at d0 and d4 of stimulation. Histograms in (A) and (B) show representative results for CD4-gated cells. (C) CD4^{+} T cells from WT and IRF4$^{-/-}$ mice were CFSE labelled and stimulated *in vitro* with anti-CD3 mAb, anti-CD28 mAb and IL-2 in the presence of IL-12 and anti-IL-4 mAb. CFSE staining intensity of viable cells was determined at the indicated days. Results are representative for 3 independent experiments (A, B) or one experiment (C).

Figure S8



Apoptosis of WT and IRF4$^{-/-}$ CD4 T cells

CD4^{+} T cells from WT and IRF4$^{-/-}$ mice were stimulated in individual cultures with immobilized anti-CD3 mAb, soluble anti-CD28 mAb, IL-2 and IL-12. Frequencies of early (FLICA^{+}PI^{-}) and late apoptotic (FLICA^{+}PI^{+}) CD4^{+} T cells were determined at indicated days of culture. (B) Expression of *Bcl2* and *Bcl2l11* was measured by RT PCR. Result in (A) is representative for 2 independent experiments. Bars in (B) represent the mean ± SEM of 4 individual samples from 2 independent experiments.