Supporting Information

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SI Methods

Phospho-STAT6 Staining. Splenocytes were harvested from naive OT-I WT or OT-I IFN regulatory factor 4 $(Irf4)^{fl/fl}CD4\text{-}Cre^{+}$ [hereafter referred to as $Irf4^{-/-}(T)$] mice and rested for 30 min at 37 °C; for 10n (a small-molecule inhibitor of Tec family tyrosine kinase) experiments, cells were preincubated with dilutions of the inhibitor in RPMI-10. After incubation, cells were incubated in the presence or absence of IL-4 (10 ng/mL) in RPMI-10 or diluted into the same concentrations of 10n or DMSO for 30 min. Cells were fixed with BD Biosciences Cytofix for 15 min on ice, washed, and surface-stained. Cells were then permeabilized with BD Sciences Phosphoflow-Perm Buffer III for 30 min on ice, washed, stained with α-phospho-STAT6 at room temperature for 1 h in the dark, and analyzed immediately by flow cytometry.

Bone Marrow Chimeras. Bone marrow was harvested from the femurs and tibia of congenically marked WT or $Irf4^{-/-}(T)$ mice. Lineage-specific cells were depleted using CD4, CD8, and CD90.1/CD90.2 magnetic affinity cell sorting beads. WT and $Irf4^{-/-}(T)$ bone marrow cells were mixed in various ratios, and 6×10^6 cells were adoptively transferred into sublethally (600-rad) irradiated congenic $Rag2^{-/-}$ ($CD45.1^{+}$) hosts. Seven and one-half weeks after reconstitution, splenocytes or peripheral blood cells were stained with antibodies to CD8, CD44, CD45.1, CD45.2, CD90.1, and CD90.2.

Fig. S1. Characterization of cell subsets in the thymus of *Irf4*$^{-/-}$*(T)* mice. (*A*) (*Left*) Thymocytes from WT (red lines) and *Irf4*$^{-/-}$*(T)* (blue lines) mice were analyzed for GFP expression in gated CD4^{+}8^{+} (DP), CD4^{+}T-cell receptor (TCR)-β^{+}, and CD8^{+}TCR-β^{+} cells to assess the extent of Cre-mediated deletion at the *Irf4* locus. (*Right*) Immunoblot analysis of IRF4 protein levels in CD4^{-}CD8^{+} (CD8SP) thymocytes and CD8^{+} peripheral T cells from WT and *Irf4*$^{-/-}$*(T)* mice stimulated for 20 h with αCD3/αCD28. Data are representative of two independent experiments. (*B*) (First row) CD4^{+}8^{-} (CD4SP) thymocytes from WT, Tec family tyrosine kinase IL-2 inducible T-cell kinase (*Itk*)$^{-/-}$, and *Irf4*$^{-/-}$*(T)* mice were stimulated with phorbol 12-myristate 13-acetone and ionomycin for 4 h and analyzed for IL-4 and IFN-γ production by intracellular staining. Numbers indicate the percentages of cytokine-producing CD4SP cells in each quadrant. (Second row) Thymocytes from WT, *Itk*$^{-/-}$, and *Irf4*$^{-/-}$*(T)* mice were analyzed for invariant natural killer T (iNKT) cells by staining with αTCR-β antibodies and CD1d/αgalcer tetramer. Numbers indicate the percentage of iNKT cells in each sample. (*Right*) Compilation of the data is shown; statistical significance was determined by the Mann–Whitney test. (Third row) Thymocytes from WT, *Itk*$^{-/-}$, and *Irf4*$^{-/-}$ *(T)* mice were analyzed for γδ NKT cells by staining with antibodies to CD4 vs. TCR-δ. Numbers indicate the percentage of γδ T cells in each sample. (Fourth row) TCRγδ^{+} thymocytes from WT, *Itk*$^{-/-}$, and *Irf4*$^{-/-}$*(T)* mice were stimulated with PMA and ionomycin for 4 h and analyzed for IL-4 and IFN-γ production by intracellular staining. Numbers indicate the percentages of cytokine-producing γδ T cells in each quadrant. (Fifth row) CD4^{+}CD8^{-} single positive (CD4SP) thymocytes from WT, *Itk*$^{-/-}$, and *Irf4*$^{-/-}$*(T)* mice were analyzed for promyelocytic leukemia zinc finger (PLZF) vs. CD44 staining. Data are representative of five independent experiments.

Fig. S2. *Irf4*$^{-/-}$*(T)* mice have normal numbers of invariant natural killer T (iNKT) cells in liver and spleen. T cells were isolated from spleens (*Top*) and livers (*Middle*) of WT, Tec family tyrosine kinase IL-2 inducible T-cell kinase (*Itk*)$^{-/-}$, and *Irf4*$^{-/-}$*(T)* mice and were analyzed for iNKT cells by staining with αT-cell receptor (TCR)-β antibody and CD1d/αgalcer tetramer. Numbers indicate the percentage of iNKT cells in each sample. (*Right*) Compilations of data indicating percentages of CD1d-Tet^{+} cells are shown. Data are representative of three independent experiments with total of $n \geq 6$ mice per group. Statistical significance was determined by the Mann–Whitney test. (*Bottom*) Splenic CD4^{+} T cells from WT, *Itk*$^{-/-}$, and *Irf4*$^{-/-}$*(T)* mice were stimulated with phorbol 12-myristate 13-acetone and ionomycin for 4 h and analyzed for IL-4 and IFN-γ production by intracellular staining. Numbers indicate the percentages of cytokine-producing CD4^{+} cells in each quadrant. (*Right*) Compilation of data is shown. No statistically significant differences were seen by the Mann–Whitney test.

Fig. S3. IRF4-deficient CD8^{+} T cells remain naive in mixed bone marrow chimeras. Bone marrow chimeras were generated by transferring 100% WT, 100% *Irf4*$^{-/-}$*(T)*, or mixtures of WT plus *Irf4*$^{-/-}$*(T)* bone marrow into congenic irradiated *Rag2*$^{-/-}$ recipients. WT plus *Irf4*$^{-/-}$*(T)* mixtures ranged from 30:70–70:30, and were all pooled for this analysis. The graph shows the percentages of CD8^{+}CD44^{hi} cells in the spleen or peripheral blood at 7.5 wk after reconstitution. WT (mixed) and *Irf4*$^{-/-}$*(T)* (mixed) indicate the data for each of these populations present in the set of chimeras generated from the bone marrow mixture.

Fig. S4. IRF4-deficient $CD4^{-}CD8^{+}$ (CD8SP) thymocytes do not up-regulate Eomesodermin (Eomes) in response to IL-4 alone. (*A*) WT CD8SP thymocytes were isolated and stimulated (stim) with 1 μg/mL CD3 plus 4 μg/mL CD28 ["high T-cell receptor (TCR)"] or 0.1 μg/mL CD3 plus 0.4 μg/mL CD28 ("low TCR") antibodies in the presence or absence of IL-4 (10 ng/mL) for 38 h. Cells were stained with antibodies to CD4, CD8, CD24, CD44, and intracellular IRF4. Histograms show IRF4 expression on gated $CD8^{+}CD24^{lo}CD44^{hi}$ cells; numbers on the right indicate the median florescence intensity of IRF4 for each population. Data are representative of four independent experiments. (*B*) WT and *Irf4*$^{-/-}$ *(T)* CD8SP thymocytes were isolated and stimulated with IL-4 (10 ng/mL) and IL-7 (10 ng/mL) in the absence ("No TCR") or presence of low TCR stimulation conditions for 38 h. Histograms show Eomes expression on gated $CD8^{+}CD24^{lo}TCR\beta^{hi}$ cells relative to their direct ex vivo levels (0 h); numbers on the right indicate the median florescence intensity of Eomes for each population. Data are representative of three independent experiments. (*C*) WT and *Irf4*$^{-/-}$*(T)* CD8SP thymocytes were isolated and stimulated with low TCR conditions in the presence vs. absence of IL-4 for 38 h. Histograms show Eomes (*Left*) and Tbet (*Right*) expression in gated $CD8^{+}CD24^{lo}TCR\beta^{hi}$ cells relative to their direct ex vivo levels (0 h); numbers on the right indicate the median florescence intensity of staining for the indicated transcription factor. Data are representative of three independent experiments.

Fig. S5. IL-4 signaling is not affected by the absence of IRF4 or by 10n treatment. (*A*) Naive OT-I WT peripheral CD8^{+} T cells were isolated and stimulated with 1 μg/mL CD3 plus 4 μg/mL CD28 ["high T-cell receptor (TCR)"] or 0.1 μg/mL CD3 plus 0.4 μg/mL CD28 ("low TCR") antibodies in the presence or absence of IL-4 (10 ng/mL) for 31 h. Cells were stained with a viability dye, and antibodies to CD8, CD69, CD44, and intracellular IRF4 or with an isotype control. Histograms show IRF4 expression relative to the isotype control; numbers on the right indicate the median florescence intensity of IRF4 staining for each sample. Data are representative of four independent experiments. (*B*) Peripheral naive OT-I WT and OT I *Irf4*$^{-/-}$*(T)* CD8^{+} T cells were rested for 30 min and then cultured in the presence or absence of IL-4 at 10 ng/mL for 30 min at 37 °C. (*Left*) Histograms show phospho-STAT6 (pSTAT6) staining in stimulated cells vs. unstimulated controls; numbers on the right indicate the median florescence intensities of pSTAT6 staining. (*Right*) Histograms show CD124 staining on unstimulated controls; numbers on the right indicate the median fluorescence intensities (MFIs) of CD124 staining. Data are representative of three independent experiments. (*C*) Peripheral naive OT-I WT CD8^{+} T cells were preincubated in 10n (0 nM or 300 nM) or DMSO alone for 30 min and then cultured in the presence or absence of IL-4 at 10 ng/mL for 30 min at 37 °C; IL-4 was diluted into medium containing 10n or DMSO, as for preincubation conditions. (*Left*) Histograms show pSTAT6 staining in stimulated cells vs. unstimulated controls; numbers on the right indicate the median florescence intensities of pSTAT6 staining. (*Right*) Histograms show CD124 staining on unstimulated controls; numbers on the right indicate the MFIs of CD124 staining. Data are representative of three independent experiments.