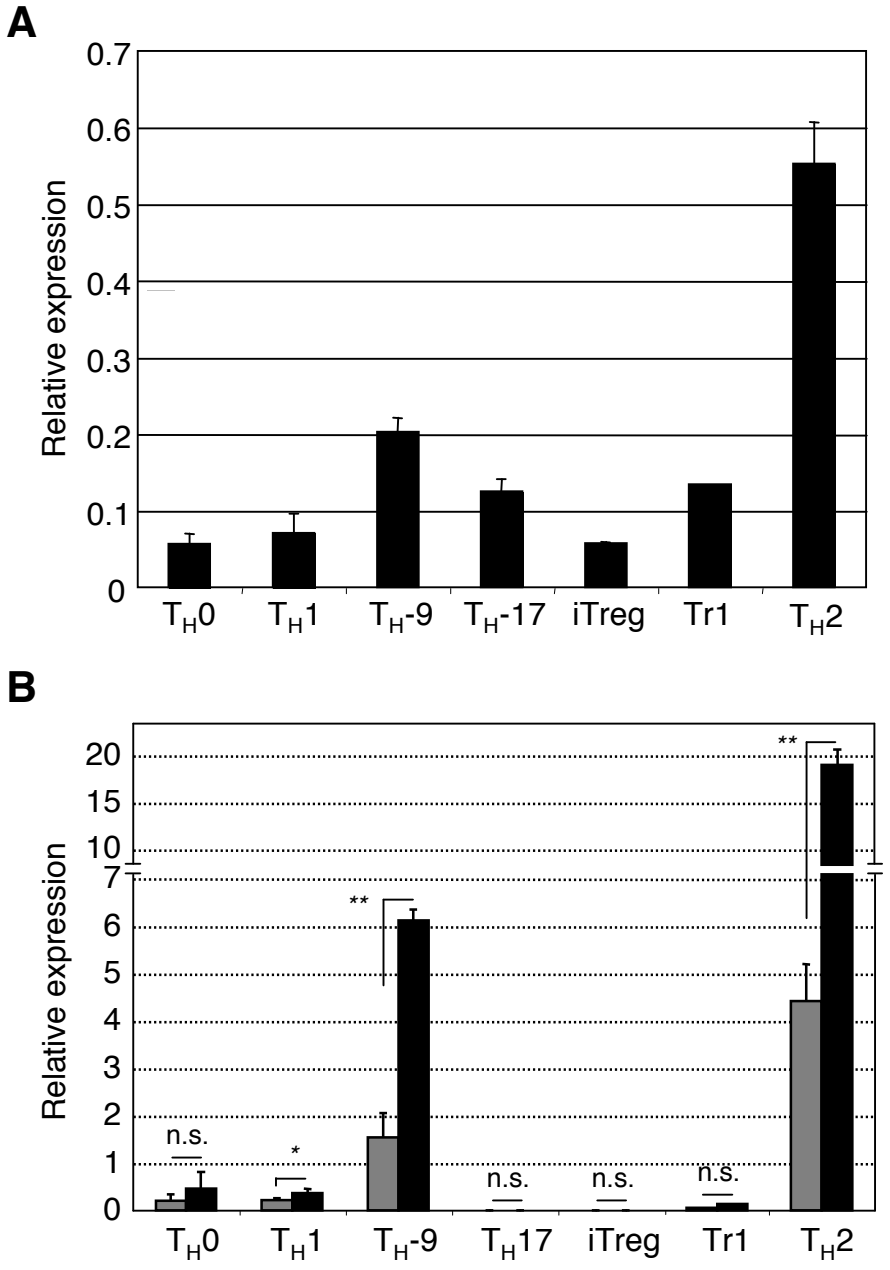
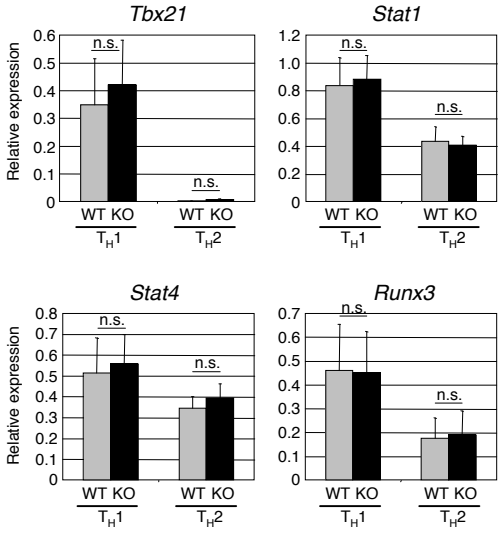
**Online supplemental materials**

Supplementary Figure 1



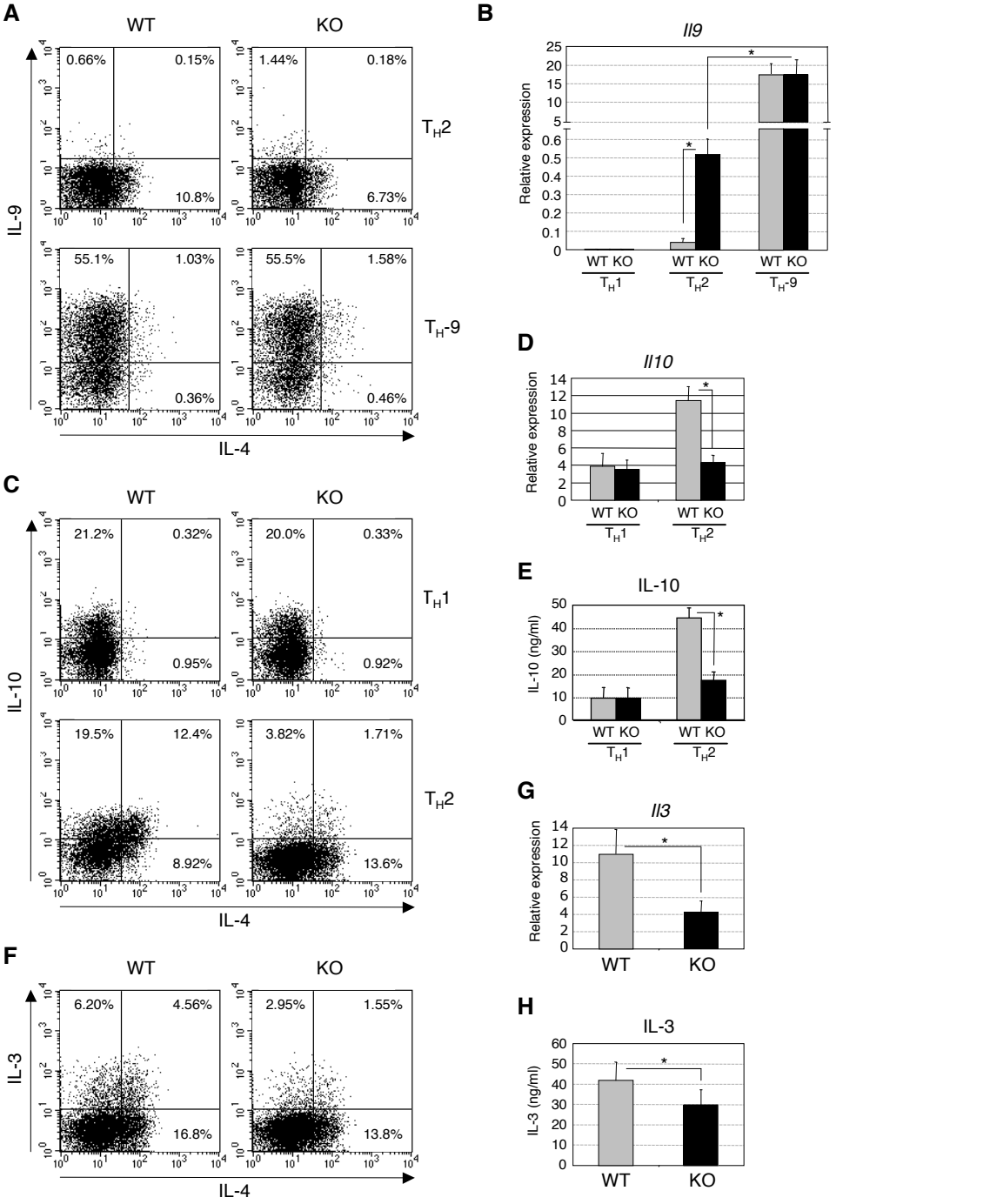

**Supplementary Figure 1 Expression of *Nfil3* and *Il13* genes in helper T cell lineages.** (**A**) Expression of *Nfil3*in helper T cells differentiated *in vitro*. Quantitative real-time RT-PCR was performed to determine the level of expression of *Nfil3* gene in each helper T cell lineages. (**B**) Enhanced expression of the *Il13* gene in helper T cells in the absence of NFIL3. Quantitative real-time RT-PCR was performed to determine the level of expression of the *Il13* gene in WT and *Nfil3*$^{-/-}$ helper T cells. Data show the mean and s.d. from at least three experiments. *$p < 0.05$, **$p < 0.0001$, n.s.: not significant.

Supplementary Figure 2



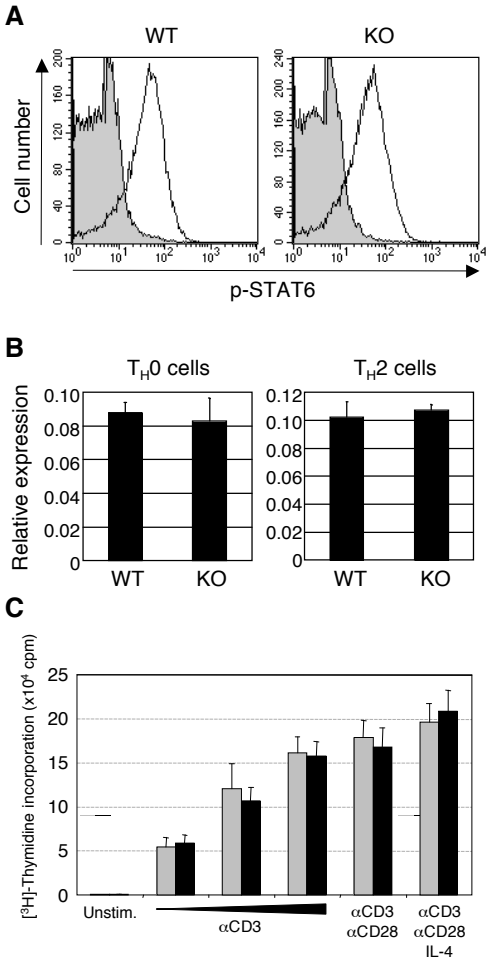

**Supplementary Figure 2 Expression of transcription factors in *Nfil3*$^{-/-}$ $T_H1$ cells.** Quantitative real-time RT-PCR was performed to determine the level of expression of $T_H1$ transcription factors. Data show the mean and s.d. from three experiments. n.s.: not significant.

# Supplementary Figure 3



**Supplementary Figure 3 Altered IL-9, IL-10, and IL-3 production in *Nfil3*$^{-/-}$ $T_H2$ cells** (**A**) Intracellular staining for IL-9 of $T_H2$ and $T_H$-9 cells. Restimulated cells were stained and analyzed by flow cytometry. Data are representative of three experiments with similar results. (**B**) Increased expression of *Il9* mRNA in *Nfil3*$^{-/-}$ $T_H2$ cells. Expression of mRNA was determined by quantitative real-time RT-PCR. Data show the mean and s.d. from three experiments (*$p < 0.001$). (**C**) Intracellular staining for IL-10 of $T_H1$ and $T_H2$ cells. Restimulated cells were stained and analyzed by flow cytometry. Data are representative of four experiments with similar results. (**D**) Decreased expression of *Il10* mRNA in *Nfil3*$^{-/-}$ $T_H2$ cells. Expression of mRNA was determined by quantitative real-time RT-PCR. Data show the mean and s.d. from four experiments (*$p < 0.001$). (**E**) Decreased secretion of IL-10 by $T_H1$ and $T_H2$ cells restimulated with anti-CD3/CD28 for 24 hours. Cytokine concentration was determined by ELISA and data show the mean and s.d. from four experiments (*$p < 0.01$). (**F**) Intracellular staining for IL-3 of $T_H2$ cells. Restimulated cells were stained and analyzed by flow cytometry. Data are representative of four experiments with similar results. (**G**) Decreased expression of *Il3* mRNA in *Nfil3*$^{-/-}$ $T_H2$ cells. Quantitative real-time RT-PCR was performed to determine the level of expression of *Il3* mRNA. Data show the mean and s.d. from four experiments (*$p < 0.1$). (**H**) Decreased secretion of IL-3 by *Nfil3*$^{-/-}$ $T_H2$ cells restimulated with anti-CD3/CD28 for 24 hours. Cytokine concentration was determined by ELISA and data show the mean and s.d. from four experiments (*$p < 0.1$).

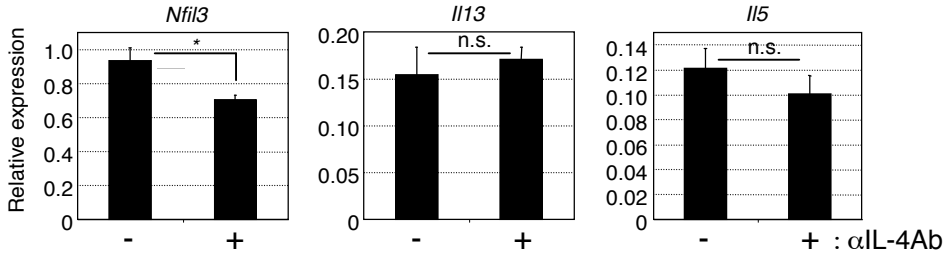
Supplementary Figure 4




**Supplementary Figure 4 Normal IL-4 signaling, *Il4ra* expression, and proliferation of *Nfil3*$^{-/-}$ T cells**.

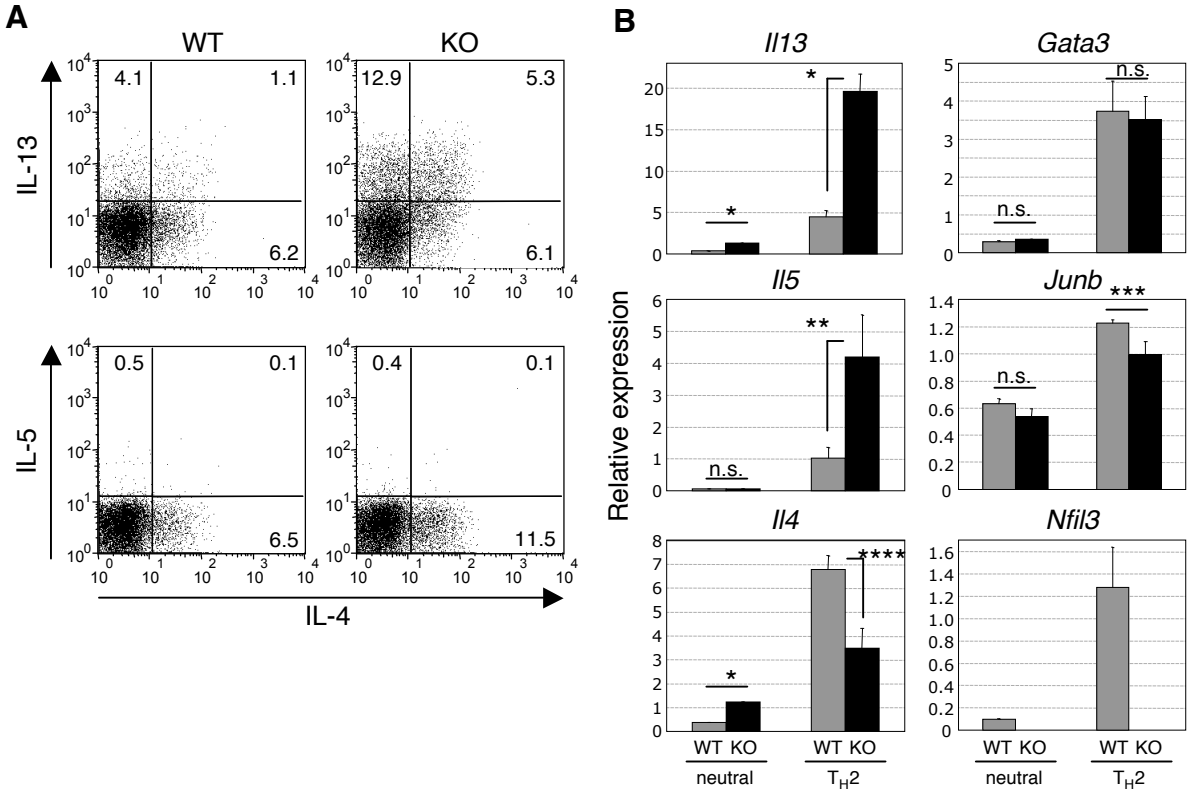
(**A**) Purified splenic CD4$^+$ T cells from WT and *Nfil3*$^{-/-}$ mice were unstimulated (shaded histogram) or stimulated (open histogram) with IL-4 for 20 min and analyzed for intracellular expression of phospho-STAT6 by flow cytometry. Three experiments were performed with similar results. (**B**) Expression of *Il4ra* gene in $T_H0$ and $T_H2$ cells was determined by quantitative real-time RT-PCR. Data show the mean and s.d. from three experiments. (**C**) Thymidine incorporation by CD4$^+$ T cells from WT (gray bars) and *Nfil3*$^{-/-}$ mice (black bar) in response to stimulation indicated. Mean values of [$^3$H]-thymidine incorporation are indicated. Two experiments were performed with similar results.

Supplementary Figure 5



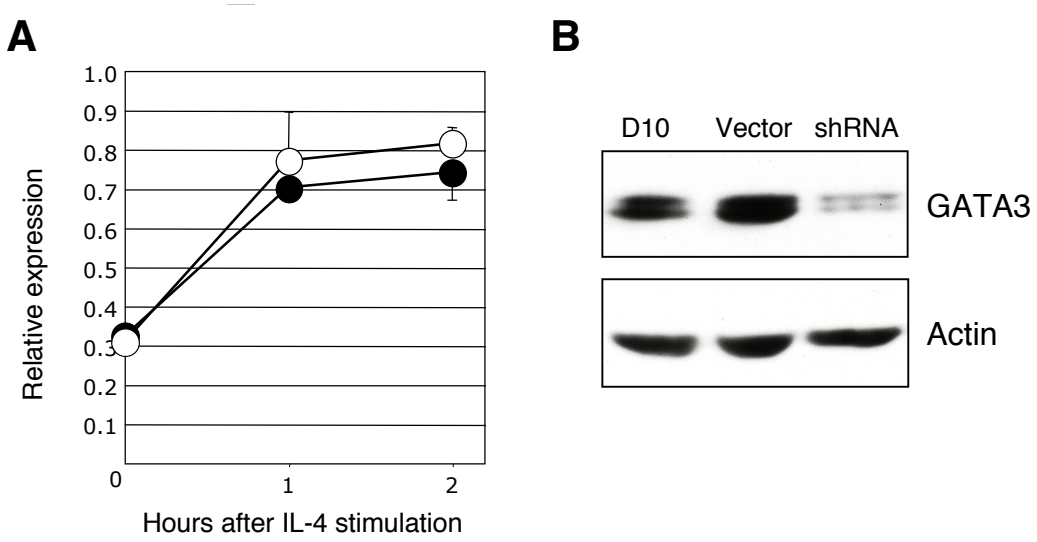

**Supplementary Figure 5 IL-4 does not negatively regulate *Il13* and *Il5* expression in differentiated $T_H2$ cells.** Quantitative real-time RT-PCR was performed to determine the level of expression of *Nfil3*, *Il13*, *Il5* genes after neutralization of IL-4 by αIL-4 antibody in $T_H2$ T cells. Data show the mean and s.d. from three experiments. **p*<0.01, n.s. not significant.

Supplementary Figure 6

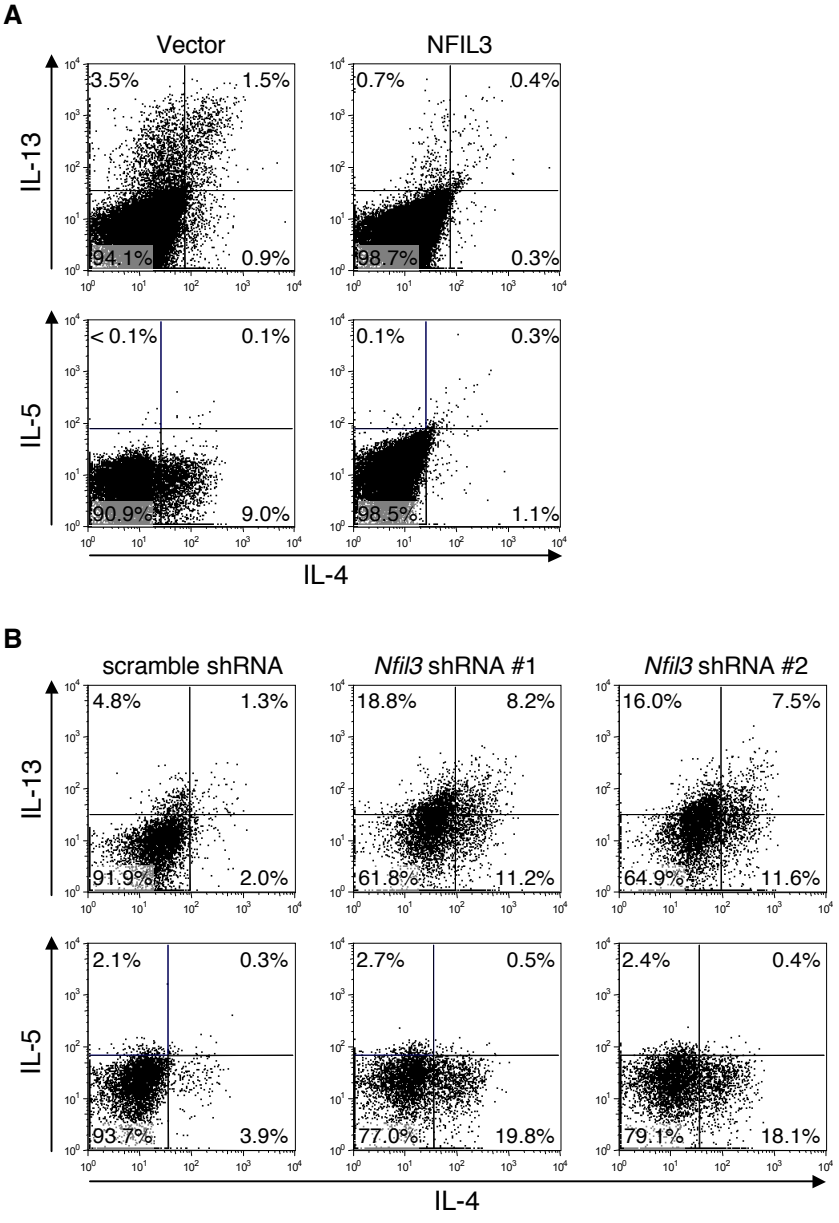



**Supplementary Figure 6 Altered $T_H2$ cytokine production in *Nfil3*$^{-/-}$ T cells cultured under neutral conditions**. (**A**) Increased IL-13 and IL-4 production in *Nfil3*$^{-/-}$ T cells. $CD4^+$ T cells cultured under neutral conditions were restimulated with PMA/ionomycin, and cytokine production was determined by intracellular staining. Data are representative of three experiments with similar results. (**B**) Increased expression of *Il13* and *Il4* mRNA in *Nfil3*$^{-/-}$ T cells. Expression of mRNA was determined by quantitative real-time RT-PCR. Data show the mean and s.d. from three experiments (*$p < 0.0001$, **$p < 0.005$, ***$p < 0.02$, ****$p < 0.002$).

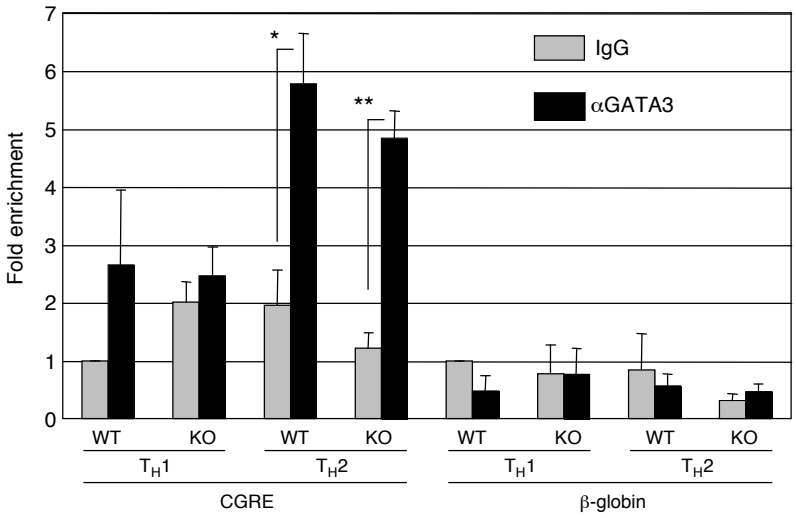
Supplementary Figure 7




**Supplementary Figure 7 GATA3 is not required for *Nfil3* expression. (A)** Quantitative real-time RT-PCR was performed to determine the level of expression of *Nfil3* in GATA3-knocked down $T_H2$ cells. Data show the mean and s.d. from three experiments. (**B**) Western blot analysis of GATA3 protein in GATA3-knocked down D10.G4.1 cells. Three experiments were performed with similar results.

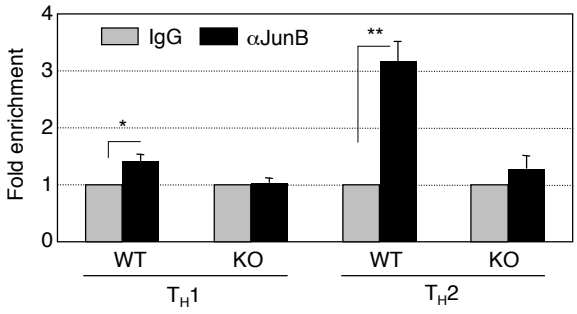



**Supplementary Figure 8 Altered $T_H2$ cytokine production by NFIL3 overexpression and Nfil3 knockdown during the late stage of $T_H2$ differentiation.**
**(A)** Splenic $CD4^+$ T cells from *Nfil3*$^{-/-}$ mice cultured under $T_H2$ conditions for 6 days were infected with retroviruses carrying NFIL3 or empty vector. Infected cells were cultured for an additional 2 days under $T_H2$ conditions and then restimulated with PMA/ionomycin in the presence of Brefeldin A to examine cytokine production by intracellular staining. Data are representative of two experiments with similar results. **(B)** Splenic $CD4^+$ T cells from *Nfil3*$^{+/+}$ mice cultured under $T_H2$ conditions for 6 days were infected with lentivirus carrying *Nfil3* shRNA or scramble shRNA. Infected cells were cultured for an additional 2 days under $T_H2$ conditions and then restimulated with PMA/ionomycin in the presence of Brefeldin A to examine cytokine production by intracellular staining. Data are representative of two experiments with similar results.

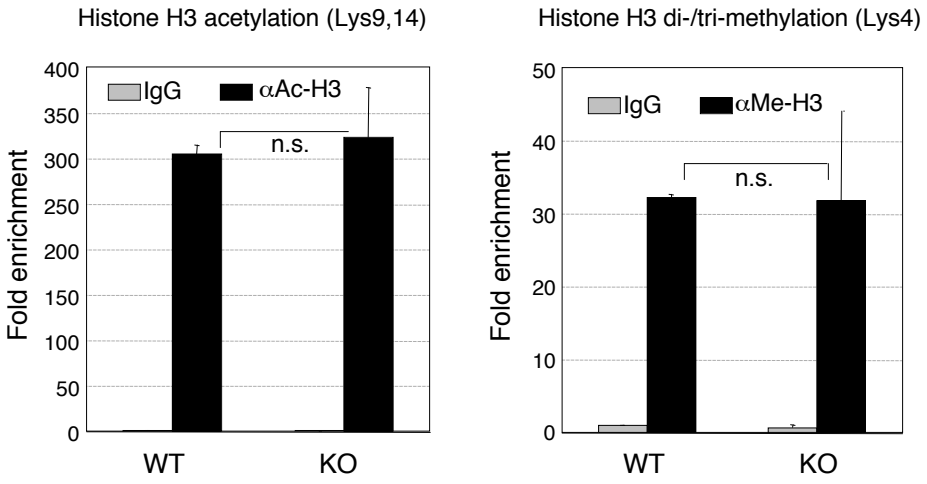



**Supplementary Figure 9 GATA3 binding activity to CGRE is not affected by NFIL3.** Splenic $CD4^+$ T cells from WT and *Nfil3*$^{-/-}$ mice cultured under $T_H1$ and $T_H2$ conditions for 7 days were crosslinked and soluble chromatin complexes were immunoprecipitated by anti-GATA3 antibody or control IgG. The region including CGRE in the co-precipitated DNA was amplified by PCR. The β-globin gene was used as a negative control. The average and s.d. of enrichment from three experiments were indicated (* $p < 0.05$, ** $p < 0.005$).

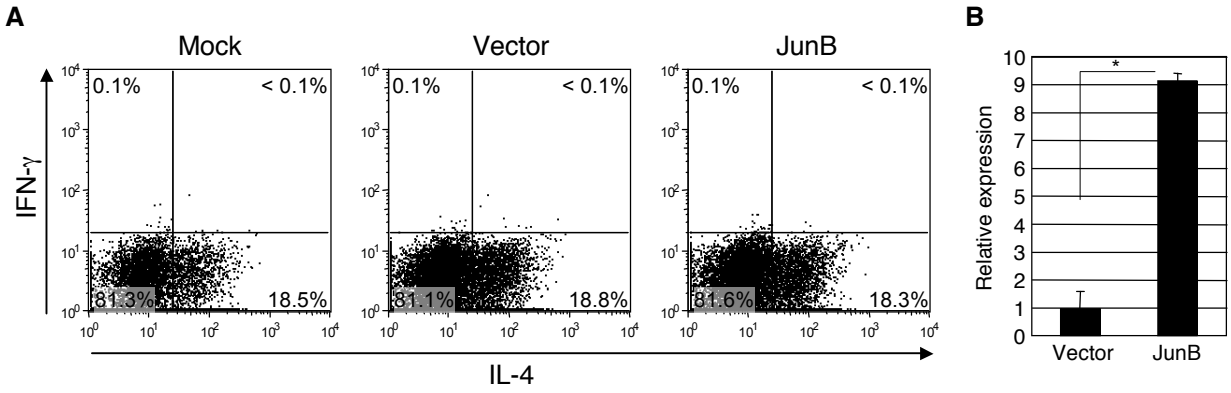
Supplementary Figure 10




**Supplementary Figure 10 JunB binding to the *Il4* promoter in $T_H1$ and $T_H2$ cells.** Splenic $CD4^+$ T cells from WT and *Nfil3*$^{-/-}$ mice cultured under $T_H1$ and $T_H2$ conditions for 7 days were crosslinked and soluble chromatin complexes were immunoprecipitated by anti-JunB antibody or control IgG. The *Il4* promoter region in the co-precipitated DNA was amplified by PCR. The average and s.d. of enrichment from three experiments were indicated (* $p < 0.005$, ** $p < 0.0005$).




**Supplementary Figure 11 Chromatin modification in the *Il4* promoter region.**
Splenic CD4 T cells from WT and *Nfil3*$^{-/-}$ mice cultured under $T_H2$ conditions for 7 days were crosslinked and soluble chromatin complexes were immunoprecipitated by anti-acetyl Histone H3, anti-di + tri methyl Histone H3, or control IgG. The promoter region of the *Il4* gene in the coprecipitated DNA was amplified by PCR. The average and s.d. of enrichment from three experiments were indicated (n.s.: not significant).




**Supplementary Figure 12 JunB transduction into *Nfil3*$^{-/-}$ $T_H2$ cells does not restore IL-4 production.**

(**A**) Splenic CD4$^+$ T cells from *Nfil3*$^{-/-}$ mice cultured under $T_H2$ conditions for 6 days were infected with lentiviruses carrying JunB or empty vector. Infected cells were cultured for an additional 2 days under $T_H2$ conditions and then restimulated with PMA/ionomycin in the presence of Brefeldin A to examine cytokine production by intracellular staining. Data are representative of two experiments with similar results. (**B**) Relative expression of *Junb* in *Nfil3*$^{-/-}$ $T_H2$ cells after transduction of JunB-expressing virus. The average and s.d. from two experiments were indicated (*$p < 0.001$).

**Supplementary Table 1 Primers for real-time PCR**

**RT-PCR**

| Primer | Sequence (5' to 3') |
|---|---|
| *Nfil3* forward | GAACTCTGCCTTAGCTGAGGT |
| *Nfil3* reverse | ATTCCCGTTTTCTCCGACACG |
| *Hprt1* forward | GTTGGATACAGGCCAGACTTTGTTG |
| *Hprt1* reverse | GATTCAACTTGCGCTCATCTTAGGC |
| *Il13* forward | CCTGGCTCTTGCTTGCCTT |
| *Il13* reverse | GGTCTTGTGTGATGTTGCTCA |
| *Il4* forward | GGTCTCAACCCCCAGCTAGT |
| *Il4* reverse | GCCGATGATCTCTCTCAAGTGAT |
| *Il5* forward | CTCTGTTGACAAGCAATGAGACG |
| *Il5* reverse | TCTTCAGTATGTCTAGCCCCTG |
| *Il9* forward | AAGGATGATCCACCGTCAAAATG |
| *Il9* reverse | ACCCGATGGAAAACAGGCAAG |
| *Il3* forward | GGGATACCCACCGTTTAACCA |
| *Il3* reverse | AGGTTTACTCTCCGAAAGCTCTT |
| *Il10* forward | GCTCTTACTGACTGGCATGAG |
| *Il10* reverse | CGCAGCTCTAGGAGCATGTG |
| *Ifng* forward | GAACTGGCAAAAGGATGGTGA |
| *Ifng* reverse | TGTGGGTTGTTGACCTCAAAC |
| *Gata3* forward | CTCGGCCATTCGTACATGGAA |
| *Gata3* reverse | GGATACCTCTGCACCGTAGC |
| *Junb* forward | TCACGACGACTCTTACGCAG |
| *Junb* reverse | CCTTGAGACCCCGATAGGGA |
| *Stat6* forward | CTCTGTGGGGCCTAATTTCCA |
| *Stat6* reverse | CATCTGAACCGACCAGGAACT |
| *Bhlhe41* forward | TGTGTAAACCCAAAAGGAGCTT |
| *Bhlhe41* reverse | TGTTCGGGCAGTAAATCTTTCAG |
| *Maf* forward | AGCAGTTGGTGACCATGTCG |
| *Maf* reverse | TGGAGATCTCCTGCTTGAGG |
| *Jund* forward | GGCGGGATTGAAACCAGGG |
| *Jund* reverse | AGCCCGTTGGACTGGATGA |
| *Fosl1* forward | ATGTACCGAGACTACGGGGAA |
| *Fosl1* reverse | CTGCTGCTGTCGATGCTTG |
| *Fosl2* forward | CCAGCAGAAGTTCCGGGTAG |
| *Fosl2* reverse | GTAGGGATGTGAGCGTGGATA |
| *Nfatc1* forward | TCTTCGAGTTCGATCAGAGCG |
| *Nfatc1* reverse | TGACACTAGGGGACACATAACTG |
| *Mina* forward | CAGTAGGGCCAGATAAGAATCCAT |
| *Mina* reverse | CATGTGCATCTGCCTCACATT |
| *Tbx21* forward | AGCAAGGACGGCGAATGTT |
| *Tbx21* reverse | GGGTGGACATATAAGCGGTTC |
| *Stat1* forward | TCACAGTGGTTCGAGCTTCAG |
| *Stat1* reverse | GCAAACGAGACATCATAGGCA |

| | |
|---|---|
| *Stat4* forward | TGGCAACAATTCTGCTTCAAAAC |
| *Stat4* reverse | GAGGTCCCTGGATAGGCATGT |
| *Runx3* forward | CAGGTTCAACGACCTTCGATT |
| *Runx3* reverse | GTGGTAGGTAGCCACTTGGG |
| *Il4ra* forward | TGGATCTGGGAGCATCAAGGT |
| *Il4ra* reverse | TGGAAGTGCGGATGTAGTCAG |
| *Irf4* forward | TCCGACAGTGGTTGATCGAC |
| *Irf4* reverse | CCTCACGATTGTAGTCCTGCTT |
| *Sfpi1* forward | ATGTTACAGGCGTGCAAAATGG |
| *Sfpi1* reverse | TGATCGCTATGGCTTTCTCCA |
| *Pias1* forward | GCGGACAGTGCGGAACTAAA |
| *Pias1* reverse | ATGCAGGGCTTTTGTAAGAAGT |

**ChIP**

| Primer | Sequence (5’ to 3’) |
|---|---|
| CGRE forward | TGGGACACTGATCCAGCGGTCCAG |
| CGRE reverse | CTCAAGACAAGCAGAAGGCATGCG |
| *Il13* promoter forward | GCTGGCTGCTCAGGAGCTT |
| *Il13* promoter reverse | GGACAGGGTTTCCAGGTTCTG |
| *Il13 intron* forward | GTGAGTAGCACACACAGCCCCTCC |
| *Il13 intron* reverse | TGATAAACAGTGGTCGCCACTCC |
| *Il4* promoter forward | GGGAGGGGTGTTTCATTTTC |
| *Il4* promoter reverse | CAATAGCTCTGTGCCGTCAG |
| β-globin forward | GCCTTGCCTGTTCCTGCTC |
| β-globin reverse | CAGACCATAAACTGTATTTTTCTTATTGAGCCC |