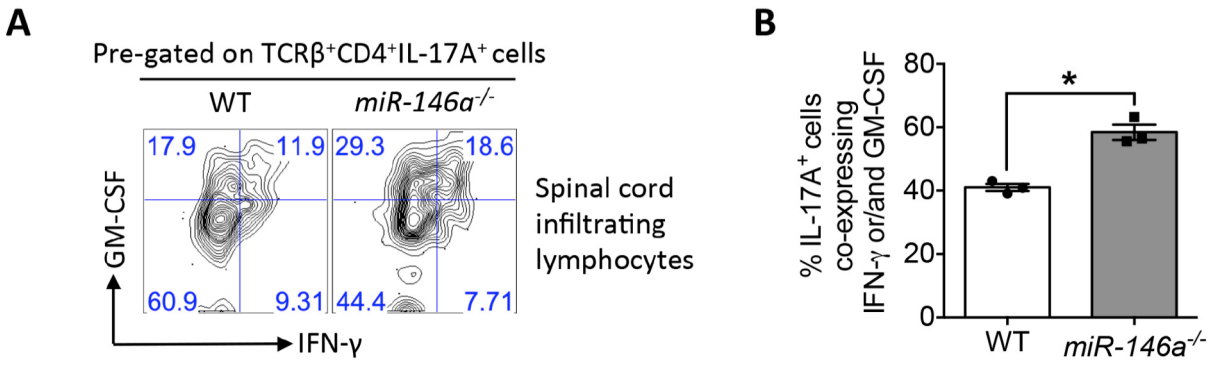
Supplemental Material

Supplemental Data

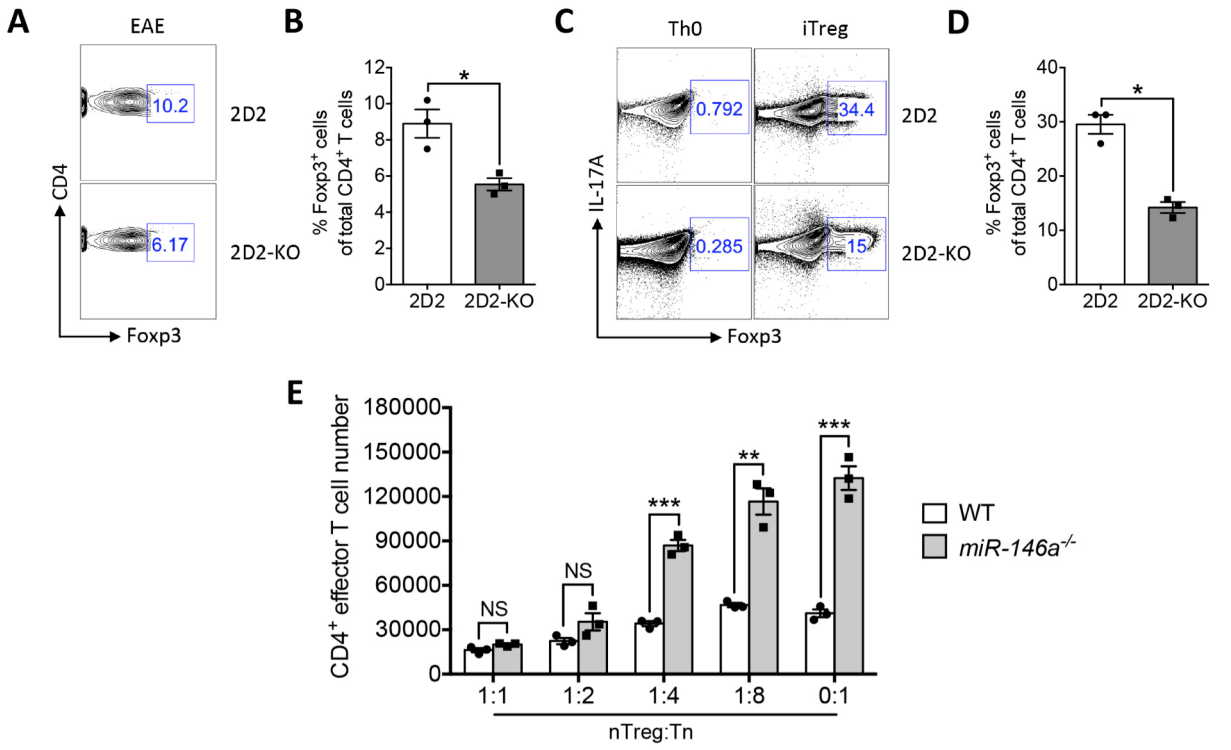


Supplemental Figure 1. miR-146a deficient mice develop more severe experimental autoimmune encephalomyelitis (EAE) featuring exaggerated Th17 responses against autoantigen.

Wild-type and miR-146a-deficient mice (denoted as WT or *miR-146a*$^{-/-}$, respectively) were induced to develop EAE, as presented in **Figure 1A**. The experiments were repeated for three times, and representative results are presented.

(**A**) Representative FACS plots showing the intracellular cytokine staining of spinal cord infiltrating lymphocytes harvested from day 18 EAE mice (n=3).

(**B**) Quantification of the FACS plots presented in **A**. Data are presented as the mean ± SEM (n=3). *$P < 0.05$, by Student's *t* test.



Supplemental Figure 2. miR-146a deficiency inhibits autoreactive CD4 T cells to differentiate into induced regulatory T (iTreg) cells, and inhibits autoreactive CD4 T cells to respond to natural regulatory T (nTreg) cell-mediated suppression.

The experiments were repeated for three times. Representative results are presented.

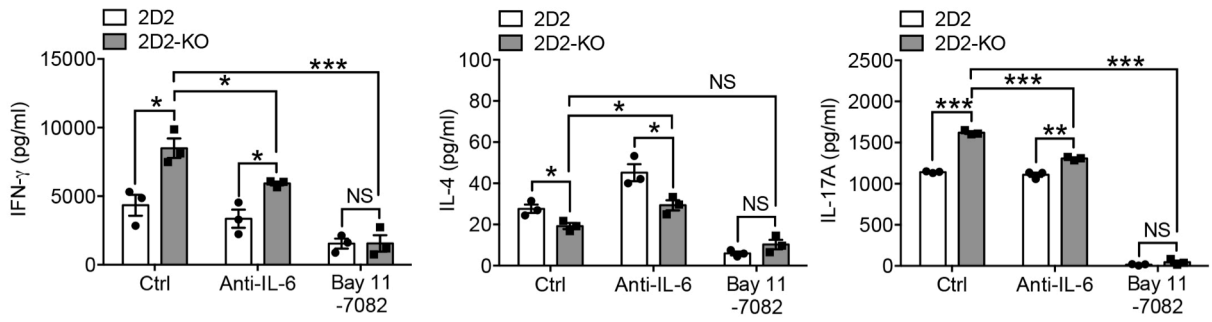
(**A**) Representative FACS plots showing the Foxp3 intracellular staining of splenocytes harvested from day 14 *RAG1*$^{-/-}$ EAE mice that have received adoptive transfer of either wild-type or miR-146a-deficient 2D2 autoreactive CD4 T cells (denoted as 2D2 or 2D2-KO, respectively) (n=3).

(**B**) Quantification of the FACS plots presented in **A**. Data are presented as the mean ± SEM (n=3). **P* < 0.05, by Student's *t* test.

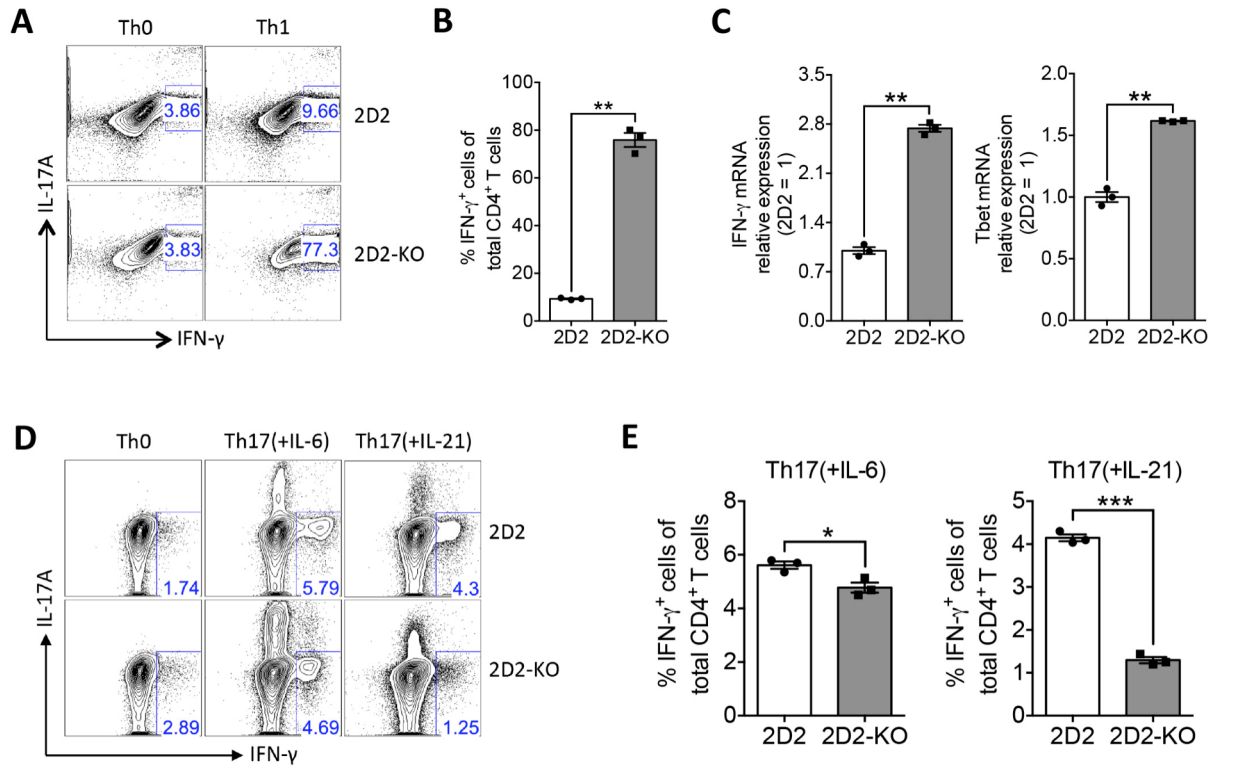
(**C**) Representative FACS plots showing the Foxp3 and IL-17A intracellular staining of wild-type or miR-146a-deficient 2D2 autoreactive CD4 T cells (denoted as 2D2 or 2D2-KO, respectively) that were cultured under the Th0 or iTreg conditions for 4 days (n=3).

(**D**) Quantification of the FACS plots presented in **C**. Data are presented as the mean ± SEM of triplicate cultures. **P* < 0.05, by Student's *t* test.

(**E**) Quantification of CD4^{+} effector T cells in a Treg in vitro suppression assay. Naïve CD4 T cells were isolated from wild-type or miR-146a-deficient mice (denoted as WT or *miR-146a*$^{-/-}$, respectively) using magnetic-activated cell sorting. Natural Treg cells (denoted as nTreg) were isolated from Foxp3-GFP reporter mice using flow cytometery (gated as CD4^{+}GFP^{+}). Splenocytes harvested from the BoyJ mice were irradiated with 20 Gy and were used as antigen presenting cells (APCs). Purified naïve CD4 T cells (denoted as Tn cells) were mixed with the purified nTregs at the indicated ratios and cultured with anti-CD3 (1 μg/ml) and anti-CD28 (1 μg/ml) in the presence of APCs for 3 days. On day 3, cell cultures were analyzed for the presence of CD4 effector T cells (gated as CD45.2^{+}TCRβ^{+}CD4^{+}) using flow cytometry. Data are presented as the mean ± SEM of triplicate cultures. NS, not significant; ***P* < 0.01, and ****P* < 0.001, by Student's *t* test.



Supplemental Figure 3. miR-146a-deficient autoreactive CD4 T cells show altered Th cytokine production, that is dependent on IL-6 and NF-κB signaling.
The experiments were repeated for three times, and representative results are presented.
WT or miR-146a-deficient 2D2 autoreactive CD4 T cells (denoted as 2D2 or 2D2-KO, respectively) were stimulated with plate-bound anti-CD3 (5 μg/ml) and soluble anti-CD28 (1 μg/ml) for 4 days, with or without the addition of anti-IL-6 neutralizing antibody (10 μg/ml) or the NF-κB inhibitor Bay 11-7082 (20 μM). On day 4, cell culture supernatants were collected and analyzed for cytokine production using ELISA. Bar graphs are presented showing the ELISA analysis of the indicated cytokines. Data are presented as the mean ± SEM of triplicate cultures. NS, not significant; **P* < 0.05, ***P* < 0.01, and ****P* < 0.001, by Student's *t* test for pairwise comparisons within each treatment group and by 2-way ANOVA for multiple comparisons between different treatment groups.

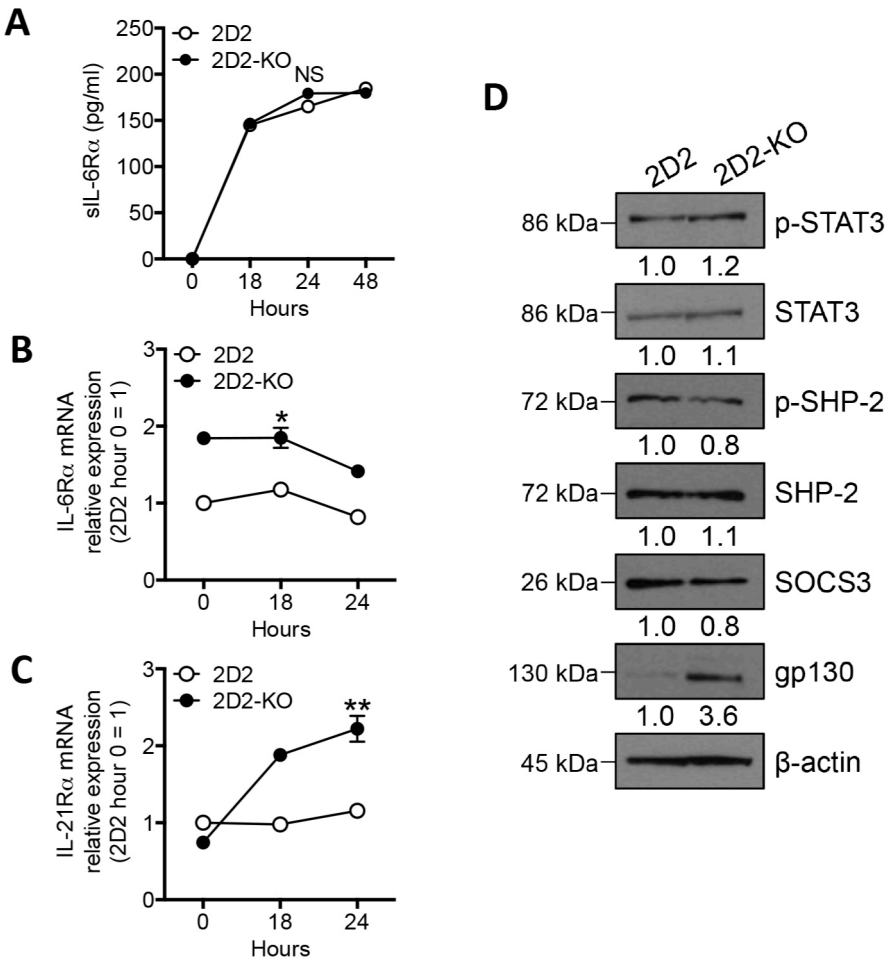


Supplemental Figure 4. miR-146a deficiency alters the Th1 differentiation of autoreactive CD4 T cells.

The experiments were repeated for three times, and representative results are presented.

(**A-C**) WT or miR-146a-deficient 2D2 autoreactive CD4 T cells (denoted as 2D2 or 2D2-KO, respectively) were cultured in vitro under the Th0 or Th1 differentiation conditions for 4 days. On day 4, cells were collected for analysis. (**A**) Representative FACS plots showing the intracellular IFN-γ and IL-17A cytokine staining of the cultured 2D2 and 2D2-KO T cells (n=3). (**B**) Quantification of the FACS plots presented in **A**. Data are presented as the mean ± SEM of triplicate cultures. $^{**}P < 0.01$, by Student's *t* test. (**C**) QPCR analysis of IFN-γ and Tbet mRNA expression in the cultured 2D2 and 2D2-KO T cells. Data are presented as the mean ± SEM of triplicate cultures. $^{**}P < 0.01$, by Student's *t* test.

(**D-E**) 2D2 and 2D2KO T cells were cultured under the Th0 or Th17(+IL-6) or Th17(+IL-21) differentiation condition for 4 days. On day 4, cells were collected for analysis. (**D**) Representative FACS plots showing the intracellular IFN-γ and IL-17A cytokine staining of cultured 2D2 and 2D2-KO T cells (n=3). (**E**) Quantification of the FACS plots presented in **D**. Data are presented as the mean ± SEM of triplicate cultures. $^{*}P < 0.05$, $^{***}P < 0.001$, by Student's *t* test.



Supplemental Figure 5. miR-146a deficiency enhances the autocrine IL-6 and IL-21 signaling pathways in autoreactive CD4 T cells.

WT or miR-146a-deficient 2D2 autoreactive CD4 T cells (denoted as 2D2 or 2D2-KO, respectively) were stimulated with plate-bound anti-CD3 (5 μg/ml) and soluble anti-CD28 (1 μg/ml). At the indicated time points, cells and cell culture supernatants were collected for analysis. The experiments were repeated for three times, and representative results are presented.

(**A**) ELISA analysis of soluble IL-6Rα (denoted as sIL-6Rα) in T cell culture supernatants collected at the indicated time points. Data are presented as the mean ± SEM of triplicate cultures. NS, not significant, by Student's *t* test.

(**B**) QPCR analysis of IL-6Rα mRNA expression in cultured T cells collected at the indicated time points. Data are presented as the mean ± SEM of triplicate cultures. **P* < 0.05, by Student's *t* test.

(**C**) QPCR analysis of IL-21Rα mRNA expression in cultured T cells collected at the indicated time points. Data are presented as the mean ± SEM of triplicate cultures. ***P* < 0.01, by Student's *t* test.

(**D**) Western blot analysis of the indicated protein levels in 2D2 and 2D2-KO T cells collected on day 3. Note the p-SHP-2 and SHP-2 blots show duplicate samples run on a parallel gel.