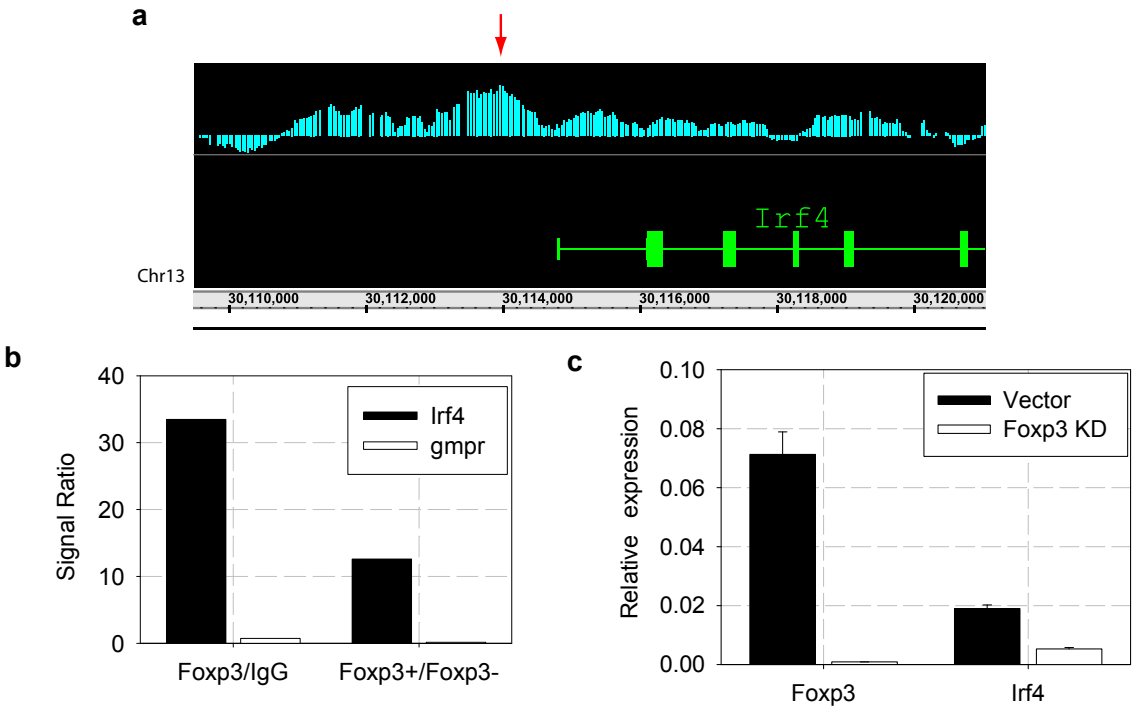
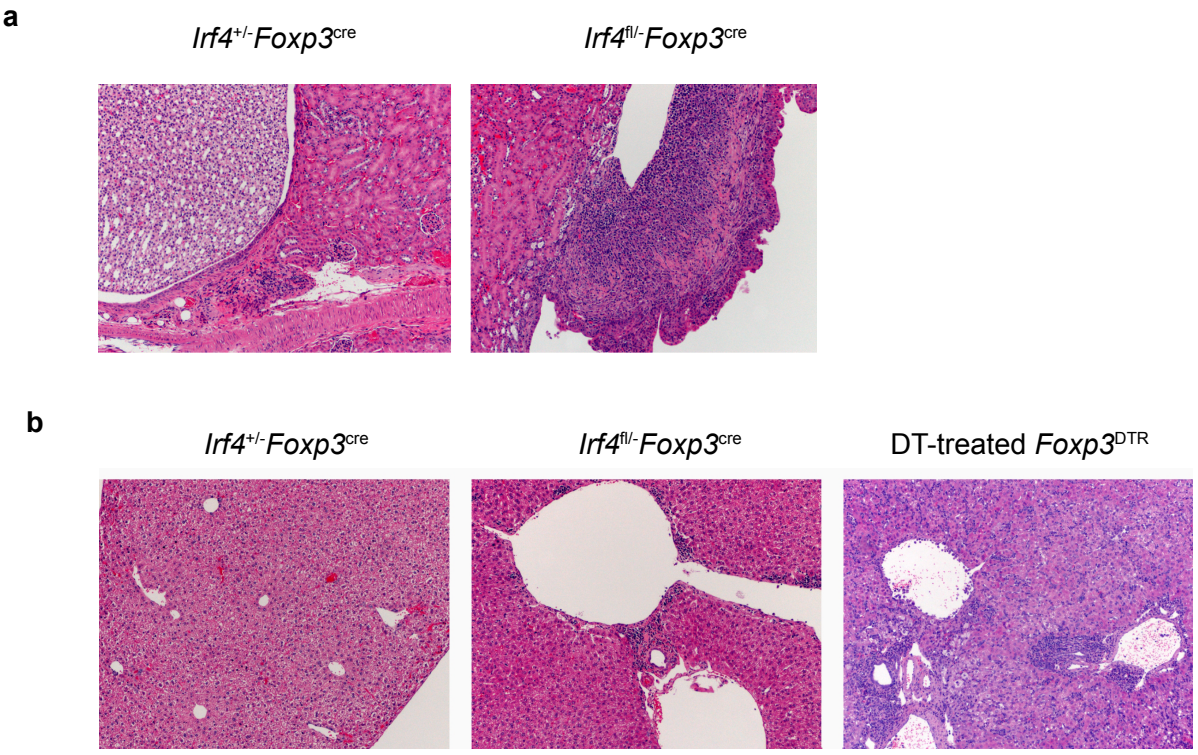
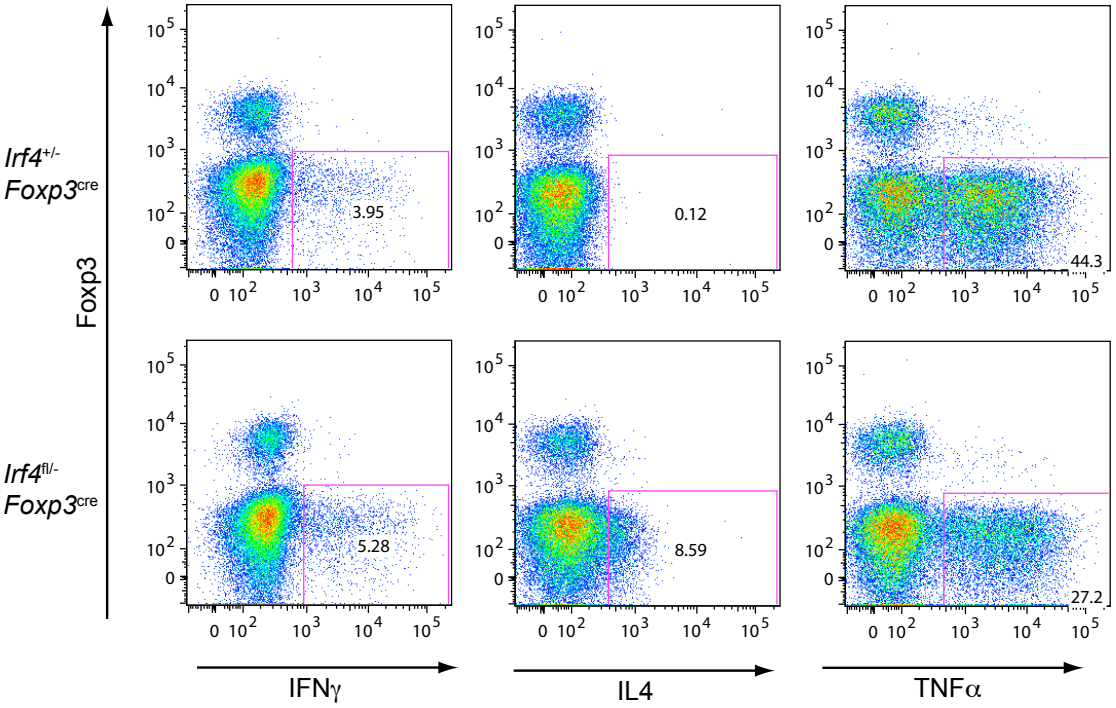


Supplementary Figure 1. IRF4 expression is directly regulated by Foxp3. **a**, Foxp3 binds to the promoter region of IRF4 gene. Foxp3 ChIP-on-chip experiment and data analysis were previously described[1]. Each bar represents the signal intensity of an individual oligonucleotide probe on Affymetrix Mouse Tiling 2.0R Array. Red arrow indicates the location of Foxp3 binding peak. **b**, Confirmation of Foxp3 binding to the IRF4 promoter by ChIP-qPCR. Quantitative PCR was performed using a primer set corresponding to the *Irf4* promoter region and Foxp3 antibody or control IgG precipitated chromatin isolated from wild-type Treg cells and *Foxp3*$^{-}$ CD4^{+} T cells. Signal ratios of anti-Foxp3/IgG (both from Foxp3^{+} Treg) and Foxp3^{+}/Foxp3^{-} (both from anti-Foxp3 ChIP) were calculated. *Gmpr* locus was used as a negative control. Primer sequences are listed in Supplementary Table 1. **c**, Foxp3 regulates high level of IRF4 expression in Treg cells. For Foxp3 shRNA knockdown, shRNA sequences were designed using commonly used algorithms. The sequences targeted by two Foxp3 specific shRNAs are A: CACTATCACACATAGGTGT; B: CAGACACCATCCTAATATTT. These sequences were cloned into a retroviral vector pLUMPIG, packaged using Phoenix-E cells and used for transduction of MACS purified CD4^{+}CD25^{+} cells stimulated overnight with plate bound anti-CD3 (1μg/ml), anti-CD28 (1μg/ml), and IL-2 (200U/ml). Cells were maintained in IL2 for 60-72h post-transduction, and transduced GFP^{+} cells were sorted by FACS Aria. Expression of Foxp3 and IRF4 was analyzed by quantitative PCR. Primer sequences are listed in Supplementary Table 1.

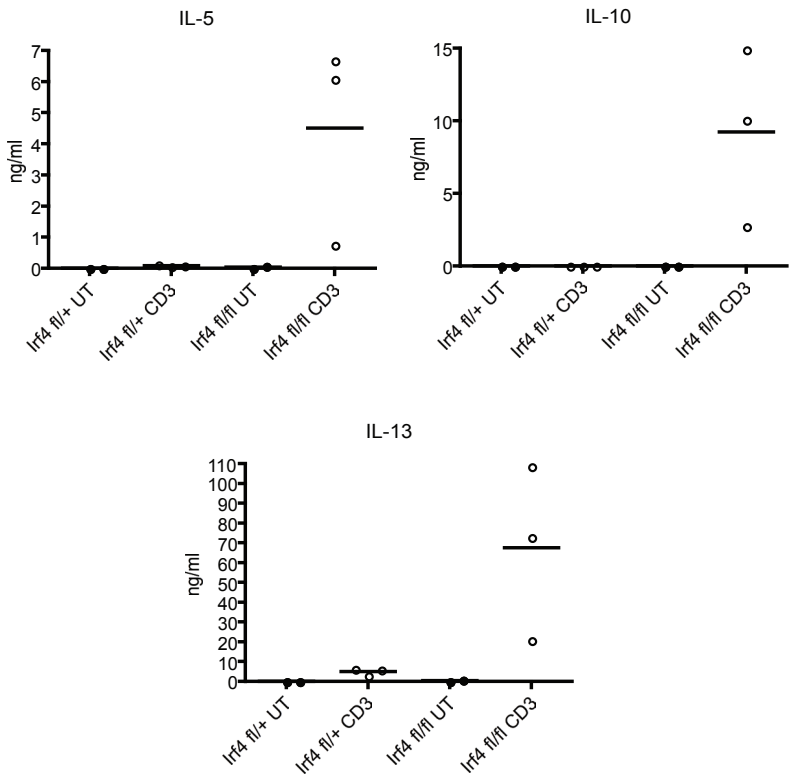
1. Zheng, Y. et al. Genome-wide analysis of Foxp3 target genes in developing and mature regulatory T cells. Nature 445, 936-40 (2007).



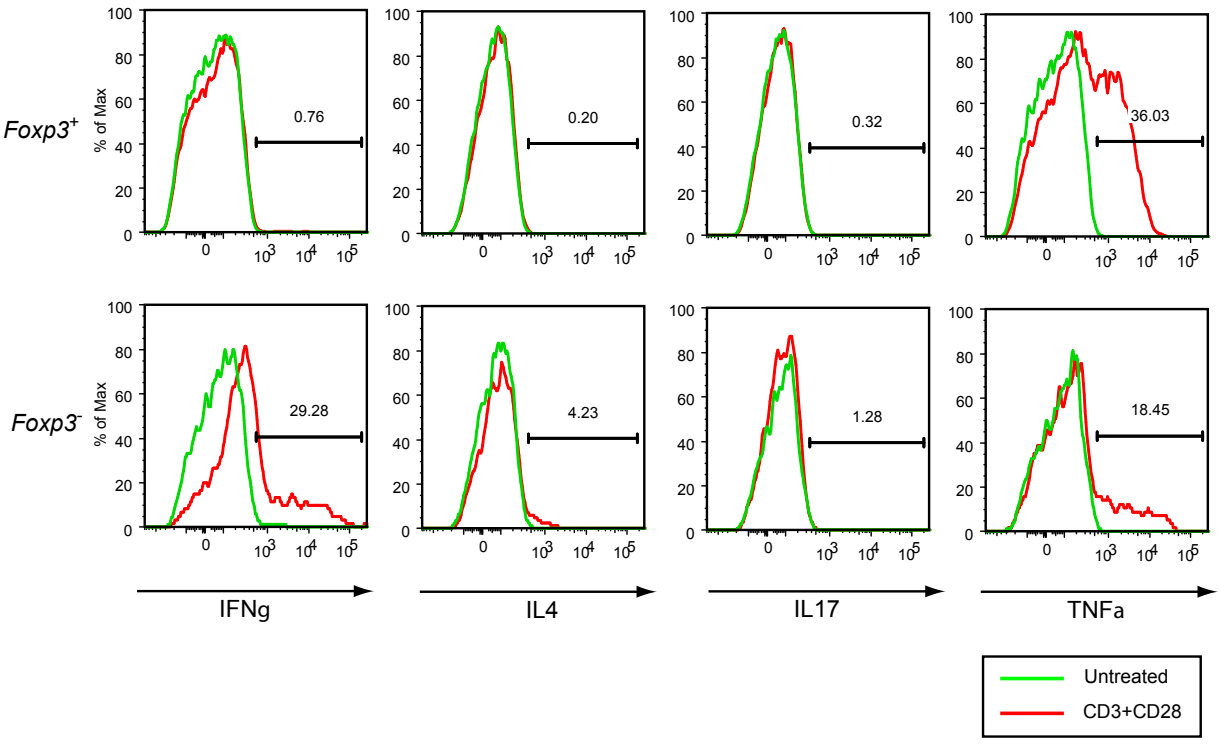
Supplementary Figure 2. Hematoxylin and eosin-stained tissue sections from *Irf4*$^{+/-}$*Foxp3*^{Cre}, *Irf4*$^{fl/-}$*Foxp3*^{Cre} and DT-treated *Foxp3*^{DTR} mice. **a**, The kidneys of the *Irf4*$^{fl/-}$*Foxp3*^{Cre} mice showed severe inflammatory infiltrates. The kidneys of *Irf4*$^{+/-}$*Foxp3*^{Cre} and DT treated *Foxp3*^{DTR} mice (data not shown) did not exhibit noticeable lesions. **b**, Minor infiltrates were observed in the liver of *Irf4*$^{fl/-}$*Foxp3*^{Cre} mice as compared to control *Irf4*$^{+/-}$*Foxp3*^{Cre} mice. This was in contrast to massive infiltrates and lesions observed in the liver of DT-treated *Foxp3*^{DTR} mice. Representative sections are shown (n=3-5 per group). Original magnification for all panels 10x.



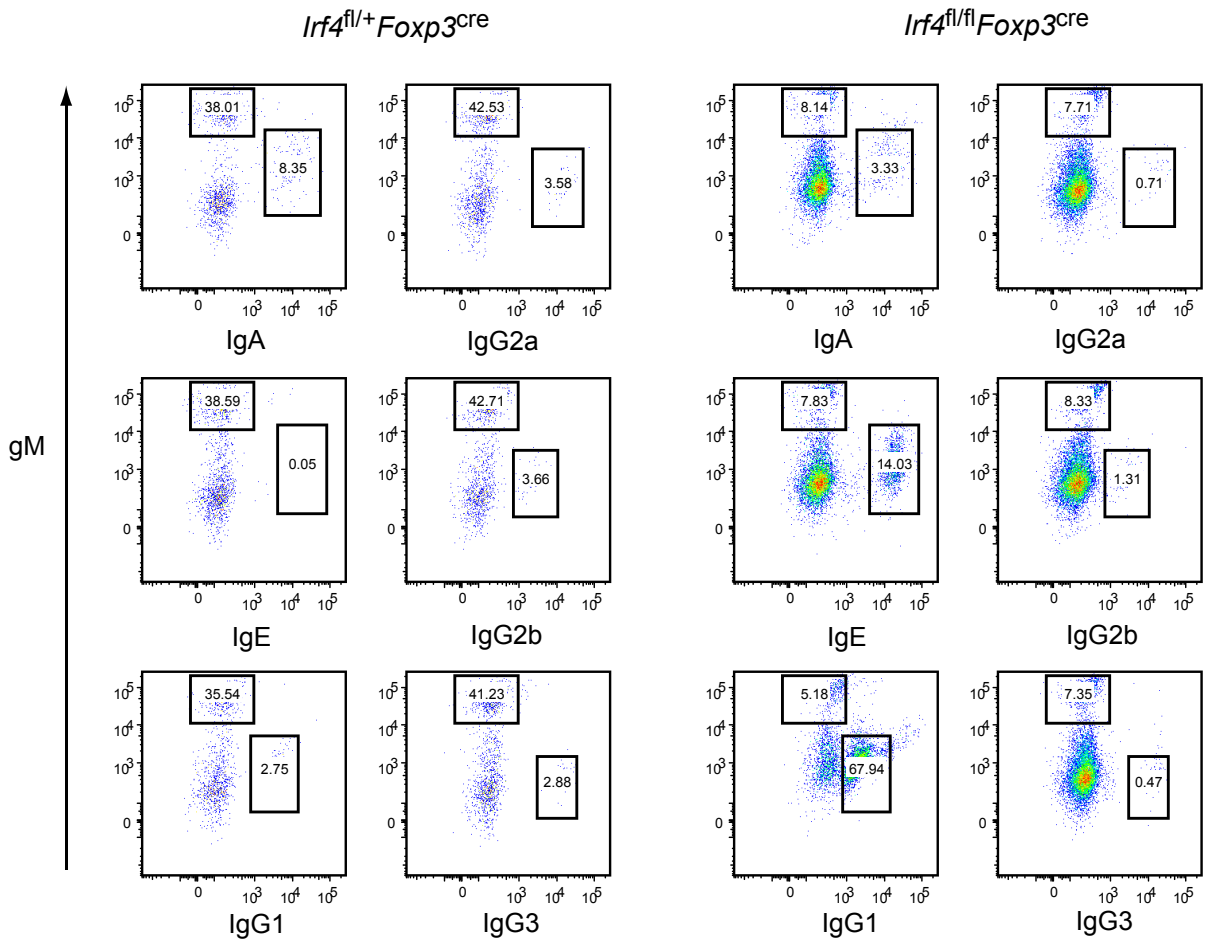
Supplementary Figure 3. Foxp3$^+$ Treg cells do not produce cytokines in the absence of IRF4. Splenocytes from *Irf4*$^{fl/-}$*Foxp3*^{Cre} and *Irf4*$^{+/-}$*Foxp3*^{Cre} mice were stimulated with CD3 (5μg/ml) and CD28 (5μg/ml) antibodies in the presence of Golgi-Plug (1μg/ml) for 5 hrs, and stained for CD4, CD8, Foxp3, and the indicated cytokines. FACS plots were gated on CD4$^+$ cells. A representative of two independent experiments is shown.



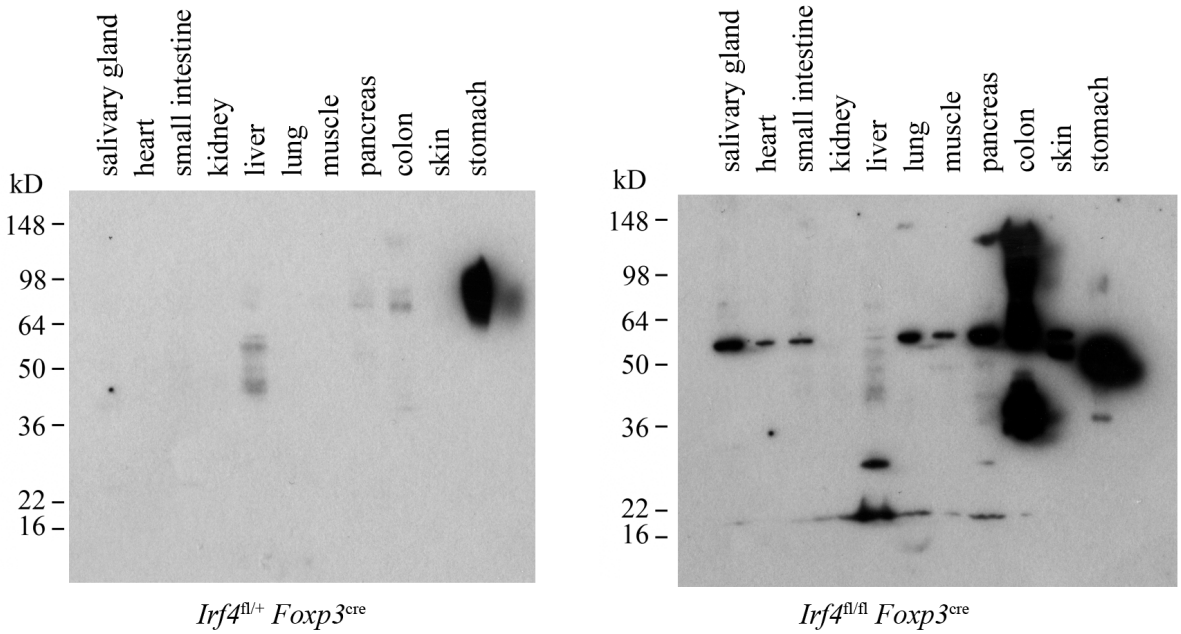
Supplementary Figure 4. Increased production of Th2 cytokines by splenic T cells from *Irf4*$^{fl/fl}$*Foxp3*^{Cre} mice. Splenic cells from *Irf4*$^{fl/+}$*Foxp3*^{Cre} and *Irf4*$^{fl/fl}$*Foxp3*^{Cre} mice were cultured in a 96-well plate (1x10^6 cells/well), either un-stimulated or stimulated with CD3 antibody (1μg/ml) for 4 days. Supernatants were collected, and IL-5, IL-10, and IL-13 concentrations were measured by ELISA.



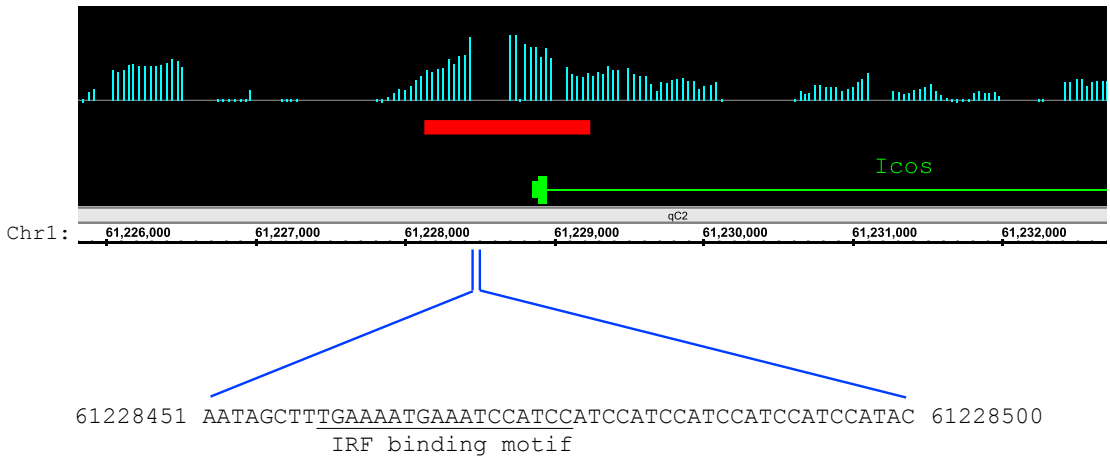
Supplementary Figure 5. $CD4^+$ T cells from *Foxp3*$^-$ mice produce both Th1 and Th2 cytokines. Splenocytes from 18 day-old *Foxp3*$^-$ and *Foxp3*$^+$ littermate control mice were stimulated with CD3 (5μg/ml) and CD28 (5μg/ml) antibodies in the presence of Golgi-Plug (1μg/ml) for 5 hrs, and stained for CD4, CD8, and the indicated cytokines. FACS plots were gated on $CD4^+$ cells. A representative of two independent experiments is shown.



Supplementary Figure 6. Flow cytometric analysis of Ig isotype production by plasma cells in *Irf4*$^{fl/fl}$ *Foxp3*^{Cre} and *Irf4*$^{fl/+}$ *Foxp3*^{Cre} mice. Splenocytes from 6 wk-old mice of the indicated genotypes were stained for CD138, B220, fixed, permeabilized, and stained for the indicated Ig isotypes. The plasma cell gate (CD138^{+}B220^{-}) is shown. A representative of two independent experiments is shown.



Supplementary Figure 7. Autoantibodies are present in the serum of *Irf4*$^{fl/fl}$ *Foxp3*^{Cre} mice. Proteins were extracted from the indicated tissues of *Rag1*$^{-/-}$ mice using 1% NP-40, PBS, pH 7.4, supplemented with a cocktail of protease inhibitors. After normalization for protein concentrations, lysates were separated by SDS-PAGE in 4-20% gradient gels and transferred to the nitrocellulose membrane. Sera from *Irf4*$^{fl/fl}$*Foxp3*^{Cre} and *Irf4*$^{fl/+}$*Foxp3*^{Cre} mice were diluted to final IgG1 concentration at 1μg/ml, and used to probe the tissue blots followed by HRP-conjugated mouse IgG1 specific secondary antibody.



Supplementary Figure 8. Co-localization of Foxp3 binding region and IRF binding motif at Icos promoter. Visualization of Foxp3 ChIP-on-Chip data at Icos promoter region is described in Supplementary Figure 1. Red block represents location of the Foxp3 binding region. Location and sequence of IRF binding motif relative to Icos gene is shown. Coordinates were derived from mouse genome build mm7.

	Gene	Forward	Reverse
RT-PCR Primers	Irf4	TCTTCAAGGCTTGGGCATTG	CACATCGTAATCTTGTCTTCCAAGTAG
	Ctla4	TGGATCCTTGTCGCAGTTAGCT	ACTTCTTTTCTTTAGCATCTTGCTCAA
	Entpd1	TCCTCTCTCTCCTGCAAGGCTAT	ACCCCGCGTTGCTGTCT
	Nt5e	GCACAGCGTGCATCGCTAT	CAGGGCTTTCGGTTAATATCGT
	Il10	GGTTGCCAAGCCTTATCGGA	ACCTGCTCCACTGCCTTGCT
	Ebi3	CGACTTTCACCCTCAGGAACTC	CCAGTCACTTGGTTTCCCATAATC
	Gzmb	CTCCACGTGCTTTCACCAAA	AGGATCCATGTTGCTTCTGTAGTTAG
	Fgl2	CCTGTCACGAAACGAGAAAGC	CCTCGCACACGAGCTCTGT
	Maf	CCCAAGGGACTCCTCTACATCTG	GCAACAAGGAGCGAATAAGCA
	Ccr8	AGAAGAAAGGCTCGCTCAGATAATT	TCAGGGTAGTAGTCGGTCATCGT
	Icos	CAGCTTTCGTTGTGGTACTCCTT	CTATTAGGGTCATGCACACTGGAT
	Il1rl1	ACTCTGCCCGACGTTCTTGA	CACGGTGGCTGCATCTTG
	Tbx21	TCAGGACTAGGCGAAGGAGA	TAGTGGGCACCTTCCAATTC
	Gata3	GTCATCCCTGAGCCACATCT	AGGGCTCTGCCTCTCTAACC
	Foxp3	GCATGTTCGCCTACTTCAGAAA	CCACTCGCACAAAGCACTTG
ChIP Primers	Irf4	CCAGAACCCAGGATGGAAGA	GGTCAACTTGGAGCGTTTGTAAA
	Icos	ACCACATCAACCTCCACAAACC	CCTCCAGTGCTCAAAAGTGTCA
	Gmpr	CAGCTGGAACAGCCTTGGAA	AAATGTCAAGGCCCCTGTGA

Supplementary Table 1. Sequences of quantitative PCR primers.