Capicua deficiency induces autoimmunity and promotes follicular helper T cell differentiation via derepression of ETV5

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High-affinity antibody production through the germinal centre (GC) response is a pivotal process in adaptive immunity. Abnormal development of follicular helper T (TFH) cells can induce the GC response to self-antigens, subsequently leading to autoimmunity. Here we show the transcriptional repressor Capicua/CIC maintains peripheral immune tolerance by suppressing aberrant activation of adaptive immunity. CIC deficiency induces excessive development of TFH cells and GC responses in a T-cell-intrinsic manner. ETV5 expression is derepressed in Cic null TFH cells and knockdown of Etv5 suppresses the enhanced TFH cell differentiation in Cic-deficient CD4+ T cells, suggesting that Etv5 is a critical CIC target gene in TFH cell differentiation. Furthermore, we identify Maf as a downstream target of the CIC-ETV5 axis in this process. These data demonstrate that CIC maintains T-cell homeostasis and negatively regulates TFH cell development and autoimmunity.
The germinal centre (GC) response is one of the most elegant processes in adaptive immunity and produce antibodies that have high affinity to antigens. In follicles, B cells that recognize antigens proliferate and form GCs. GCs are expanded by the proliferation of GC B cells and polarize into two microenvironments, the dark zone and the light zone. GC B cells cycle between these two zones. In the dark zone, GC B cells rapidly proliferate and undergo somatic hypermutation, which enables antibody diversification and affinity maturation. In the light zone, GC B cells are selected on the basis of antigen affinity, undergo immunoglobulin class-switch recombination, and eventually give rise to antibody-secreting plasma cells or memory B cells. During the GC response, several types of immune cell collaborate with B cells in the follicles, where follicular helper T (TFH) cells have an instrumental function. TFH cells facilitate the selection and maturation of high-affinity GC B cells by multiple rounds of cognate interaction with B cells in the light zone; these interactions provide the selected B cells with crucial signals for survival and re-entry into the dark zone. Because TFH cells have an important function in the generation of isotype-switched B cells, affinity-matured antibodies, deregulation of TFH cell development and function is closely associated with immunodeficiency-related pathogenesis or antibody-mediated autoimmune diseases including systemic lupus erythematosus.

Differentiation of TFH cells is initiated by the interaction of naïve T cells with dendritic cells (DCs), which, together with environmental factors, including cytokines, triggers expression of the chemokine receptor CXCR5 on DC-primed T cells. The surface expression of CXCR5 enables T cells to migrate into B-cell follicles. TFH cells that are targeted to enter B-cell follicles upregulate expression of the transcriptional repressor BCL6 and express an intermediate level of typical TFH molecules (for example, CXCR5, PD-1, ICOS and SAP) at the junction between T-cell and B-cell zone. At this stage, developing TFH cells interact with cognate B cells and differentiate into GC TFH cells that express high levels of TFH molecules, such as PD-1 and CXCR5 (ref. 9). BCL6 as a master transcription factor for TFH cell differentiation and BLIMP1 as an antagonist of BCL6 (ref. 11), plus several other transcription factors, help orchestrate TFH cell differentiation by exerting either a positive or negative effect, depending on the cellular context. Among these factors, MAF (also known as c-MAF) was identified as a positive regulator of TFH cell differentiation in mice and humans. MAF deficiency decreases the frequency of CD4+ CXCR5+ T cells in mice. MAF regulates expression of IL21 in mouse TFH cells and, in conjunction with BCL6, MAF induces expression of CXCR4, CXCR5, PD-1, ICOS and IL21 in human TFH cells. Moreover, MAF expression is induced in CD4+ T cells by ICOS co-stimulation or by IL-6, an important cytokine for initiating TFH cell differentiation.

Capicua/CIC is a transcriptional repressor that is evolutionarily conserved from cnidarians to mammals, and it exists in short (CIC-S) and long (CIC-L) isoforms. In mammals, CIC interacts with Ataxin-1/ATXN1 (ref. 18), of which polyglutamine (polyQ)-expanded form causes spinocerebellar ataxia type-1 (SCA1) neuropathogenesis, and its haploinsufficiency alleviates SCA1 progression. Loss of the ATXN1–CIC complex results in spinocerebellar ataxia type-1 (SCA1) mice.

In vivo functions of CIC have been reported in studies of Cic hypomorphic (Cic-L−/−) mice. These mice die within 4 weeks after birth and have defects in lung alveolarization and bile acid homeostasis. However, CIC is expressed in most mouse tissues, and it may also have other important physiological functions. CIC levels are relatively high in the thymus and in immune cells including T and NK cells. Moreover, our previous study shows that genes associated with immune activation and autoimmune diseases, such as SLE, asthma and thyroiditis, are significantly upregulated in the liver of Cic-L−/− mice. These findings suggest that CIC may regulate immune responses and immune disorders.

Here we investigate CIC functions in immune system using various immune cell-specific Cic null mice. Our study finds previously unrecognised functions of CIC in regulation of T-cell activation and GC responses and suggests CIC as a key transcription factor in the suppression of autoimmunity.

Results

Autoimmunity in immune-cell-specific Cic null mice. To investigate in vivo requirement of CIC in homeostasis and functions of immune cells, we generated haematopoietic lineage cell-specific Cic null mice (Cicf/f Vav1-Cre) by crossing mice carrying floxed Cic alleles (Cicf/f) with Vav1-Cre mice (Supplementary Fig. 1a), and characterized them at 9–12 weeks of age. Development of lymphocytes in primary lymphoid organs was largely normal (Supplementary Fig. 1b,c), although an increase in proportion of thymic CD4+ FOXP3+ regulatory T (Treg) cells was observed (Supplementary Fig. 2).

Strikingly, all Cicf/f Vav1-Cre mice developed enlarged secondary lymphoid organs, including spleens (Fig. 1a), mainly due to proliferation of B220+ B cells (Fig. 1b). In addition, the number of CD8+ T cells was significantly decreased, whereas that of macrophages and DCs was increased in spleen of Cicf/f Vav1-Cre mice (Fig. 1b). The expression levels of several T-cell co-stimulatory ligands, such as CD80, CD86, CD40 and ICOSL, on the surface of DCs were comparable between Cicf/f (WT) and Cicf/f Vav1-Cre mice (Supplementary Fig. 3). Cicf/f Vav1-Cre mice had a higher frequency of effector/memory-phenotype (CD44hiCD62Llo) cells for both CD4+ and CD8+ subsets, compared with wild-type (WT) mice (Fig. 1c). Cic-deficient CD4+ CD44+ T cells showed elevated expression of cell surface co-stimulatory molecules such as ICOS, PD-1, OX40 and GITR (Supplementary Fig. 4). Flow cytometry analysis on cytokine expression profiles revealed that the frequency of IFNγ+, TNF+, IL-21+, IL-13+ and IL-22+ CD4+ T cells was significantly increased in spleen of Cicf/f Vav1-Cre mice (Supplementary Fig. 5a). Cicf/f Vav1-Cre mice also had a higher frequency of GCRA4– CCR3+ (TH1-related) and CCR4+ CCR6– (TH2-related) CD4+ T cells (Supplementary Fig. 5b). Thus, loss of CIC spontaneously activates T cells, resulting in accumulation of effector/memory cells with mixed T helper (TH1) phenotypes in mice. On the other hand, Cic null naïve CD4+ T cells differentiated into each TH subset with similar efficiency to WT cells under in vitro polarizing conditions (Supplementary Fig. 6), suggesting that CIC might not be a crucial factor for each TH subset differentiation.

Cicf/f Vav1-Cre mice had a higher proportion of CD4+ CD25+ FOXP3+ T cells in spleen, but a comparable proportion of CD4+ CD25+ FOXP3+ T cells, compared with WT mice (Supplementary Fig. 7a). Expression levels of surface molecules that are critical for controlling Treg cell homeostasis and function, such as GITR, CTLA-4, CD103 and GARP (refs 31,32), were comparable between WT and Cic null CD4+ FOXP3+ T cells regardless of CD25 expression (Supplementary Fig. 7b). Consistent with this result, WT and Cic-deficient CD4+ CD25+...
Figure 1 | Lymphoproliferative autoimmunity in immune cell-specific Cic null mice. (a,b) Spleen weights (a) and the numbers of total splenocytes, B220+ B, CD3+ T, CD4+ T, CD8+ T, macrophages (CD11b+ CD1c− F4/80+) and dendritic cells (CD11b+ CD11c+ MHCII+) (b) in 10-week-old Cicf/WT and Cicf/Vav1-Cre (cKO) mice. (c) FACS analysis showing the proportion of CD44loCD62Lhi naïve T cells and CD44hiCD62Llo effector/memory T cells in spleen of Cicf/ and Cicf/Vav1-Cre mice. All data are representative of two independent experiments. Data are presented as box-and-whisker plots. n = 4–5 per each genotype. (d) Serum levels of total IgG, IgG1, IgG2a and IgM in 12-week-old Cicf/ and Cicf/Vav1-Cre mice were measured by ELISA. The graphs in (a,b,d) show data as mean ± s.e.m. and each dot in graphs represents an individual mouse. *P<0.05, **P<0.01 and ***P<0.001 (two-tailed two-sample unequal variance Student t-test). (e) Images of dissected spleens (SPL) and draining lymph nodes (LN) from Cicf/ and Cicf/Vav1-Cre mice at the age of 12 months. (f) Spleen weights (a) and the numbers of total splenocytes, B220+ B, CD3+ T, CD4+ T, CD8+ T, macrophages (CD11b+ CD1c− F4/80+) and dendritic cells (CD11b+ CD11c+ MHCII+) (b) in 10-week-old Cicf/WT and Cicf/Vav1-Cre (cKO) mice. (c) FACS analysis showing the proportion of CD44loCD62Lhi naïve T cells and CD44hiCD62Llo effector/memory T cells in spleen of Cicf/ and Cicf/Vav1-Cre mice. All data are representative of two independent experiments. Data are presented as box-and-whisker plots. n = 4–5 per each genotype. (d) Serum levels of total IgG, IgG1, IgG2a and IgM in 12-week-old Cicf/ and Cicf/Vav1-Cre mice were measured by ELISA. The graphs in (a,b,d) show data as mean ± s.e.m. and each dot in graphs represents an individual mouse. *P<0.05, **P<0.01 and ***P<0.001 (two-tailed two-sample unequal variance Student t-test). (e) Images of dissected spleens (SPL) and draining lymph nodes (LN) from Cicf/ and Cicf/Vav1-Cre mice at the age of 12 months. (f) Spleen weights (a) and the numbers of total splenocytes, B220+ B, CD3+ T, CD4+ T, CD8+ T, macrophages (CD11b+ CD1c− F4/80+) and dendritic cells (CD11b+ CD11c+ MHCII+) (b) in 10-week-old Cicf/WT and Cicf/Vav1-Cre (cKO) mice. (c) FACS analysis showing the proportion of CD44loCD62Lhi naïve T cells and CD44hiCD62Llo effector/memory T cells in spleen of Cicf/ and Cicf/Vav1-Cre mice. All data are representative of two independent experiments. Data are presented as box-and-whisker plots. n = 4–5 per each genotype. (d) Serum levels of total IgG, IgG1, IgG2a and IgM in 12-week-old Cicf/ and Cicf/Vav1-Cre mice were measured by ELISA. The graphs in (a,b,d) show data as mean ± s.e.m. and each dot in graphs represents an individual mouse. *P<0.05, **P<0.01 and ***P<0.001 (two-tailed two-sample unequal variance Student t-test). (g) Immunostaining for IgG deposition in kidney glomeruli of the 12-month-old Cicf/Vav1-Cre mice. Representative images from two independent experiments are shown. (g–i) Scale bars, 100 μm.
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Treg cells had a comparable suppressive activity on IL-2 secretion and proliferation of responding CD4+ CD25− T cells (Supplementary Fig. 7c,d). The numbers of CD8+ CD25− FOXP3+ and CD8+ ICOSL+ CXCR5+ (Qa-1-restricted CD8+ Treg cells)33,34. T cells were also comparable between WT and Cicf/fVav1-Cre mice (Supplementary Fig. 7e,f). These results, together with the increased frequency of thymic Treg cells (Supplementary Fig. 2), indicate that the T cell hyperactivation in Cicf/Vav1-Cre mice was not due to defects in Treg cell compartment.

Because the abnormal activation of T cells and the increased frequency of CD4+ CD25− FOXP3+ T cells are often associated with autoimmunity35,36. we assessed whether Cicf/Vav1-Cre mice display autoimmune phenotypes with age. The Cicf/Vav1-Cre mice developed hyperglobulinemia at 12 weeks of age (Fig. 1d), and exhibited several typical autoimmune attributes at around 1 year of age, including enlarged secondary lymphoid organs (Fig. 1e), increased serum levels of anti-dsDNA antibody (Fig. 1f), infiltration of immune cells into tissues (Fig. 1g), glomerulonephritis (Fig. 1h) and IgG deposition in kidney glomeruli (Fig. 1i). Together, these findings demonstrate that CIC is indispensable for suppression of lymphoproliferative autoimmunity.

**Spontaneous induction of GC responses by CIC deficiency.** Increased frequencies of TFH-like cells are often observed in patients with autoimmune diseases including SLE6, and studies of a co-stimulatory molecule highly expressed in T FH cells37,38, Consistent with these results, the proportion of CD4+ T cells when stimulated with anti-CD3 (Fig. 3c,d), suggesting that CIC negatively regulates an activation signal that is mediated by T-cell receptors (TCRs). However, this difference was largely abrogated by addition of anti-CD28 (Fig. 3c,d), indicating that a strong co-stimulatory signal through CD28 overrules the enhanced TCR response in Cic-deficient T cells.

To determine the in vivo consequences of CIC deficiency in T cells, we generated mice with Cic deletion specific to T cells (Cicf/Cd4-Cre) (Fig. 4a). Development of T cells in thymus was largely normal in Cicf/Cd4-Cre mice (Supplementary Fig. 8a). Given that Cic deficiency induced autoimmunity and that Cic null CD4+ T cells more robustly responded to TCR stimulation than WT T cells in vitro (Fig. 3c,d), we assessed thymic negative selection, which is a critical process that removes autoreactive T cells in thymus63, in WT and Cicf/Cd4-Cre mice. TCR-induced apoptosis comparably occurred in WT and Cic null double positive (DP) thymocytes (Supplementary Fig. 8b), suggesting that TCR-induced negative selection might not be affected by Cic deficiency. Consistent with this finding, WT and Cic null CD4+ thymocytes exhibited a comparable induction of Nur77, which has been implicated in negative selection through its ability to convert BCL2 into a proapoptotic molecule44,45, in response to TCR stimulation (Supplementary Fig. 8c).

On the other hand, as observed in the Cicf/Vav1-Cre mice, hyperglobulinemia, T-cell hyperactivation and increased populations of CD25− FOXP3− T1H1-type and T1H2-type CD4+ T cells in spleen occurred in Cicf/Cd4-Cre mice at 12 weeks of age (Fig. 4b, Supplementary Fig. 9). These mice also showed several systemic autoimmune phenotypes with age, including increased serum levels of anti-dsDNA antibody (Fig. 4c), infiltration of immune cells into tissues (Fig. 4d) and glomerulonephritis (Fig. 4e). Moreover, the proportions of Tfh, GC Tfh, CD4+ ICOS+ T and GC B cells were significantly higher in Cicf/Cd4-Cre mice than in WT mice (Fig. 4f–h). The frequency of IFNγ-expressing Tfh cells was increased in the spleen of Cicf/Cd4-Cre mice (Supplementary Fig. 10a). Because IFNγ drives IgG2a class-switching in B cells during GC reactions46,47, we further assessed IgG2a+ GC B cells. As expected, the frequency and the number of IgG2a+ GC B cells were also increased in Cicf/Cd4-Cre mice (Supplementary Fig. 10b). Taken together, these findings demonstrate that the spontaneous inductions of T-cell activation, GC response and systemic autoimmunity were due to loss of CIC in T cells.

Follicular regulatory T (TFR) cells, defined as CD4+ FOXP3+ PD-1+ CXCR5+ cells, are a specialized subset of regulatory T cells that inhibit antibody production48–50. Because the proportion of PD-1+ CXCR5+ T cells more robustly responded to TCR stimulation (Supplementary Fig. 8b), we set out to determine whether the TFR subset was elevated in Cicf/Vav1-Cre mice. The frequencies of Tfh (PD-1+CXCR5+) and GC Tfh (PD-1+CXCR5) cells were significantly increased in the spleen of 9-week-old Cicf/Vav1-Cre mice at the expense of non-Tfh cells (Fig. 2a). The increased Tfh cell frequency in Cicf/Vav1-Cre mice was confirmed by flow cytometry analysis of CD4+ BCL6+ CXCR5+ T cells (Fig. 2b). Consistent with these results, the proportion of CD4+ ICOS+ T cells was also markedly increased (Fig. 2c). On the other hand, levels of BCL6 in PD-1+ CXCR5+ Tfh cells were comparable between WT and Cicf/Vav1-Cre mice (Fig. 2d). The frequency of IFNγ+ Tfh cells was substantially increased in Cicf/Vav1-Cre mice, whereas that of IL-21+ and IL-4+ Tfh cells was comparable between WT and Cicf/Vav1-Cre mice (Fig. 2e). Because the primary function of Tfh cells is to help B cells form GC reactions, we further analysed GC B (B220+ Fas+ GL−7+) cells. The proportion of GC B cells was significantly higher in Cicf/Vav1-Cre mice than in WT mice (Fig. 2f). Taken together, these data indicate that CIC is required for suppression of spontaneous induction of GC reactions.

**T-cell-intrinsic functions of CIC.** Because several abnormalities in T cells were observed in Cicf/Vav1-Cre mice, we next examined CIC in T cells. CIC protein levels gradually increased along with the activation of CD4+ T cells by anti-CD3 and anti-CD28 antibodies (Fig. 3a), and were higher in effector/memory CD4+ T cells than in naive CD4+ T cells (Fig. 3b). Cic-deficient CD4+ T cells secreted IL-2 and proliferated more efficiently than WT T cells when stimulated with anti-CD3 (Fig. 3c,d), suggesting that Cic negatively regulates an activation signal that is mediated by T-cell receptors (TCRs). However, this difference was largely abrogated by addition of anti-CD28 (Fig. 3c,d), indicating that a strong co-stimulatory signal through CD28 overrules the enhanced TCR response in Cic-deficient T cells.

Normal T-cell homeostasis in Treg-specific Cic null mice. Although the suppressive activity toward conventional T cells and expression profiles of Treg cell–associated surface molecules were comparable between WT and Cic-deficient CD4+ Treg cells (Supplementary Fig. 7b–d), the frequency of CD4+ CD25− FOXP3+ T cells was substantially increased in Cic-deficient mice (Supplementary Figs 7a and 9b). To directly address the function of CIC in development and function of FOXP3+ Treg cells,
we generated FOXP3^{+} T_{reg} cell-specific Cic null (Cic^{f/f}/Foxp3-YFP-Cre) mice by crossing Cic^{f/f} mice with Foxp3-YFP-Cre mice\footnote{A1}. The ablation of Cic alleles in Foxp3-expressing (YFP\textsuperscript{+}) cells did not affect formation of CD25^{+}FOXP3\textsuperscript{+} and CD25\textsuperscript{-}FOXP3\textsuperscript{+} CD4\textsuperscript{+} T cells in both thymus and spleen (Supplementary Fig. 11a–c). The proportion of naïve and effector/memory cells for both CD4\textsuperscript{+} and CD8\textsuperscript{+} subsets, T_{Fr}, T_{Fr} and GC B cells was also comparable between Cic\textsuperscript{+/+}/Foxp3-YFP-Cre (WT) and Cic\textsuperscript{f/f}/Foxp3-YFP-Cre mice (Supplementary Fig. 11d–f). These results suggest that Cic deficiency in FOXP3\textsuperscript{+} T_{reg} cells does not cause defects in development and function of FOXP3\textsuperscript{+} T_{reg} cells.

Since the FOXP3\textsuperscript{+} T_{reg} cell-specific Cic deletion did not promote formation of CD4\textsuperscript{+}CD25\textsuperscript{-} FOXP3\textsuperscript{+} T-cell population in spleen (Supplementary Fig. 11c), we examined expression of FOXP3 in conventional CD4\textsuperscript{+} T cells from WT and Cic\textsuperscript{f/f}/Cd4-Cre mice. Interestingly, the proportion of FOXP3-expressing naïve (CD45RB\textsuperscript{hi}CD44\textsuperscript{lo}CD62L\textsuperscript{hi}) CD4\textsuperscript{+} T cells was markedly increased in the spleen of Cic\textsuperscript{f/f}/Cd4-Cre mice, compared with WT mice (Supplementary Fig. 12a). Consistent with this result, CD4\textsuperscript{+}CD25\textsuperscript{-}FOXP3\textsuperscript{+} T cells in Cic\textsuperscript{f/f}/Cd4-Cre mice were composed of both CD44\textsuperscript{hi}CD62L\textsuperscript{hi} and CD44\textsuperscript{hi}CD62L\textsuperscript{lo} populations, while those in WT mice were mainly CD44\textsuperscript{hi}CD62L\textsuperscript{lo} cells (Supplementary Fig. 12b). We also determined the origin of CD4\textsuperscript{+}CD25\textsuperscript{-} FOXP3\textsuperscript{+} T cells in Cic\textsuperscript{f/f}/Cd4-Cre mice by flow cytometry analysis for expression of Helios and Neurophilin-1/NRP1, both of which are markers for thymus-derived T_{reg} (tT_{reg}) cells\textsuperscript{52-54}. The frequency of Helios\textsuperscript{-}NRP1\textsuperscript{-} cells for both CD25\textsuperscript{+}FOXP3\textsuperscript{+} and CD25\textsuperscript{-}FOXP3\textsuperscript{+} T-cell subsets was significantly increased in the spleen of Cic\textsuperscript{f/f}/Cd4-Cre mice (Supplementary Fig. 12c), demonstrating that the increased frequency of CD4\textsuperscript{+}CD25\textsuperscript{-} FOXP3\textsuperscript{+} T cells in Cic\textsuperscript{f/f}/Cd4-Cre mice was due to the expansion of peripherally-induced T_{reg} (pT_{reg}) cells. Taken together, our data suggest that Cic deficiency induces FOXP3 expression in naïve CD4\textsuperscript{+} T cells, thereby increasing the population of CD4\textsuperscript{+}CD25\textsuperscript{-} FOXP3\textsuperscript{+} T cells in the periphery.

**Regulation of T_{Fr} cell differentiation by the CIC-ETV5 axis.** To understand the molecular mechanism mediating the Cic deficiency-induced increase in T_{Fr} cells, we set out to identify CIC target genes in CD4\textsuperscript{+} T cells. We analysed gene expression profiles in naïve and anti-CD3/CD28 antibody-activated CD4\textsuperscript{+} T cells from WT and Cic\textsuperscript{f/f}/Cd4-Cre mice (Supplementary Data 1). Both Etv4 and Etv5 were well-characterized as CIC target genes in various tissues\textsuperscript{19,22,24,27}. qRT-PCR analysis for mRNA levels of Pea3 group genes (Etv1, Etv4 and Etv5) (Fig. 5a) and quantitative PCR (qPCR) analysis for Pea3 group gene promoter regions in total CIC-associated DNA fragments (Fig. 5b) verified that Etv4 and Etv5 are direct targets of Cic in CD4\textsuperscript{+} T cells.

We then examined the levels of Etv4 and Etv5 in CD4\textsuperscript{+} PD-1\textsuperscript{-}/CXCR5\textsuperscript{+} T_{Fr} cells from WT and Cic\textsuperscript{f/f}/Cd4-Cre mice by...
qRT-PCR analysis. Consistent with the previous result (Fig. 5a), levels of both genes were markedly increased in Cic-deficient T_{FH} cells (Fig. 5c), indicating that their expression was derepressed in T_{FH} cells in the absence of Cic. Interestingly, however, only ETV5 expression, but not ETV4 expression, was substantially increased in Cic-deficient T_{FH} cells at the protein level (Fig. 5d), suggesting that ETV4 expression is tightly controlled in T_{FH} cells at the posttranscriptional level. We also found that ETV5 levels were higher in T_{FH} cells than in non-T_{FH} cells (Fig. 5c,e), whereas Cic levels in the two T-cell populations were comparable (Fig. 5e), implying that ETV5 might be critical for differentiation or function, or both, of T_{FH} cells. To further explore this possibility, we performed adoptive transfer experiments using OT-II cells, which express ovalbumin (OVA)-specific TCRs. Thy1.2^+ OT-II cells were infected with control or ETV5-expressing retrovirus (Supplementary Fig. 13a) and adoptively transferred to Thy1.2^+ recipient mice. Immunization of the host mice with 4-hydroxy-3-nitrophenyl (NP)-OVA in alum resulted in generation of a higher percentage of T_{FH} cells (Fig. 5f), demonstrating that ETV5 promotes T_{FH} cell differentiation. Consistent with this result, knockdown of ETV5 in OT-II cells by shRNA against Etv5 (shETV5) suppressed T_{FH} cell differentiation (Fig. 5g). Lastly, we conducted the adoptive transfer experiment to examine whether the increased T_{FH} cells in Cic mutant mice was due to derepression of ETV5. WT and Cic-deficient OT-II cells infected with control or shETV5-expressing retrovirus were adoptively transferred to Thy1.2^+ recipient mice. Seven days after immunization with NP-OVA in alum, the donor OT-II cells were analysed for T_{FH} cell differentiation by flow cytometry. As expected from previous results (Figs 2a,b and 4f), T_{FH} cell differentiation was more prominent with Cic-deficient donor OT-II cells than with WT OT-II cells (Figs 5h and 6f). Strikingly, such enhanced T_{FH} cell differentiation of Cic-deficient OT-II cells was blunted when ETV5 expression was knocked-down with shETV5 (Fig. 5h, Supplementary Fig. 13b). Altogether, these data demonstrate that the Cic-ETV5 axis is crucial for regulation of T_{FH} cell differentiation.

**Maf as a critical ETV5 target in T_{FH} cell differentiation.** ETV5 function in T_{FH} cell development has not been investigated. To comprehensively understand how the Cic–ETV5 axis regulates differentiation of T_{FH} cells, we sought to identify ETV5 target genes that could be involved in this process. We initially analysed expression profiles of several T_{FH}-related genes in non-T_{FH} and T_{FH} cells from WT and Cic^floxed/Cd4-Cre mice. Bcl6, Batf, Maf, Cxcr5 and Ifng were significantly increased, whereas Lef1 and Foxp1 were decreased in WT T_{FH} cells compared with WT non-T_{FH} cells (Fig. 6a). Importantly, Batf, Maf, Icos and Ifng were significantly upregulated in Cic-deficient T_{FH} cells compared with WT T_{FH} cells; the increase was greatest in Maf, which promotes T_{FH} cell differentiation and GC reactions (Fig. 6a). We confirmed the overexpression of MAF in Cic-deficient T_{FH} cells at the protein level (Fig. 6b).

We also examined which T_{FH}-related genes are regulated by ETV5 in CD4^+ T cells. Anti-CD3/CD28 antibody-activated CD4^+ T cells were infected with control or ETV5-expressing retrovirus and cultured in the presence of IL-6 and IL-21 (T_{FH}-like condition). Among the genes tested, only Maf levels were substantially increased by ETV5 overexpression (Fig. 6c).
Consistent with this, it is known that \( \text{Maf} \) is a direct target gene of ETV5 in ocular lens cells\(^5\). We confirmed that ETV5 directly bound to \( \text{Maf} \) promoter in IL-6-treated CD4\(^+\) T cells by chromatin immunoprecipitation (ChIP)-qPCR analysis (Supplementary Fig. 14). In contrast, in the absence of IL-6 and IL-21, ETV5 overexpression did not significantly increase levels of \( \text{Maf} \) (Fig. 6d), suggesting that ETV5 might induce expression of \( \text{Maf} \) during T\(_{\text{FH}}\) cell development. These results were also confirmed by western blot analysis (Fig. 6e). Because IL-6 and IL-21 can activate STAT3 pathway, we examined whether...
activation of STAT3 is required for ETV5-mediated induction of Maf expression. Treatment with AG490 (inhibitor of JAK2-STAT3 pathway) and Statc (inhibitor for STAT3 dimerization) completely suppressed the induction of Maf expression by ETV5 in CD4+ T cells incubated under Tfh-like condition (Supplementary Fig. 15a,b). However, treatment

**Figure 5 | De-repression of ETV5 promotes Tfh cell differentiation in CIC-deficient CD4+ T cells.** (a) qRT-PCR analysis for levels of Pea3 group genes in splenic CD4+ T cells from Cicf/f WD and Cicf/fCd4-Cre (cKO) mice. n = 3 per each genotype. (b) Chromatin immunoprecipitation (ChIP)-qPCR analysis showing CIC promoter occupancy of ETV5 in DT and ETV5 in CD4+ T cells purified from spleen of Cicf/f and Cicf/fCd4-Cre mice were used. n = 3 per each genotype. (c) qRT-PCR analysis for levels of ETV4 and ETV5 in sorted CD4+ PD-1 CXCR5 - non-Tfh and CD4+ PD-1 CXCR5 + Tfh cells. Splenocytes from 5 to 6 Cicf/f mice or 3 to 4 Cicf/fCd4-Cre mice were pooled and subjected to cell sorting. Three independent experiments were conducted. (d) Western blot analysis for levels of ETV4 and ETV5 in sorted splenic CD4+ PD-1 CXCR5+ Tfh cells. The dash lines on blot images indicate cropping without image manipulation to either side. Three independent experiments were conducted. The graphs in a-d show data as mean ± s.e.m. *P<0.005 and **P<0.01 (two-tailed two-sample unequal variance Student t-test). (e) Western blot analysis for levels of CIC and ETV5 in sorted non-Tfh and Tfh cells from spleen of WT C57BL/6 (B6) mice. The images are representative of two independent experiments. (f) Thy1.1+ OT-II cells infected with control or ETV5-expressing retrovirus were adoptively transferred to Thy1.2- B6 recipient mice. Eight days after immunization with NP-OVA in alum, donor cells were analysed for Tfh cell differentiation using flow cytometry. Control-RV, n = 12; ETV5-RV, n = 9. ***P<0.001 (two-tailed two-sample unequal variance Student t-test). (g) Thy1.1+ OT-II cells infected with control (shNC) or ETV5 shRNA (shETV5) expressing retrovirus were subjected to adoptive transfer experiment. Eight days after immunization, donor cells were analysed for Tfh cell differentiation. shNC, n = 6; shETV5, n = 8. **P<0.01 (two-tailed two-sample unequal variance Student t-test). (h) WT and Cic KO OT-II cells were prepared from spleens of Thy1.1+ OT-II Cicf/f and Thy1.1+ OT-II Cicf/fCd4-Cre mice, respectively. WT OT-II cells infected with shNC retrovirus and Cic cKO OT-II cells infected with shNC or shETV5-expressing retrovirus were transferred into Tfh+ B6 recipient mice. Seven days after immunization with NP-OVA in alum, donor cells were analysed for Tfh cell differentiation. WT:shNC, n = 9; cKO:shNC, n = 6; cKO:shETV5, n = 6. **P<0.001 (two-tailed two-sample unequal variance Student t-test).

**Figure 4 | Spontaneous induction of Tfh cell differentiation and GC response in T-cell-specific CIC null mice.** (a) Western blot analysis showing T-cell-specific ablation of CIC expression in Cicf/fCd4-Cre mice. B220+ B and CD4+ T cells were purified from spleen of 12-week-old Cicf/f WD and Cicf/fCd4-Cre (cKO) mice. (b) Levels of total IgG, IgG1, IgG2a and IgM in sera from 12-week-old Cicf/f and Cicf/fCd4-Cre mice. (c) Serum levels of anti-dsDNA antibody in 14.5-month-old Cicf/f and Cicf/fCd4-Cre mice were measured by ELISA. (d) The graphs show data as mean ± s.e.m. and each dot in graphs represent an individual mouse. *P<0.05 and **P<0.01 (two-tailed two-sample unequal variance Student t-test). (d) Immune cell infiltration in liver and lung from 14.5-month-old Cicf/f and Cicf/fCd4-Cre mice. Tissue sections were stained with H&E. (e) H&E-stained kidney sections showing glomerulonephritis in 14.5-month-old Cicf/fCd4-Cre mice. (f) Representative images from two Cicf/fCd4-Cre mice (cKO-1 and cKO-2) are shown. Scale bars, 100 μm. (f-i) Flow cytometry analyses of non-Tfh, Tfh and GC Tfh cells (f), CD4+ ICOS+ T cells (g), GC B cells (h) and CD4+ FOXP3+ PD-1+ CXCR5+ Tfh cells (i) in spleen from 12-week-old Cicf/f and Cicf/fCd4-Cre mice. All data are representative of three independent experiments with n = 4–5 mice per group in each experiment. Numbers adjacent to outlined areas indicate percent of each cell population among splenic CD4+ or B220+ cells. *P<0.05, **P<0.01 and ***P<0.001 (two-tailed two-sample unequal variance Student t-test). (j) Quantification of the ratio Tfh/Tfh from the experiments as in Fig. 3i. **P<0.01 (two-tailed two-sample unequal variance Student t-test).
with Static did not affect ETV5 promoter occupancy of Maf (Supplementary Fig. 15c). These results indicate that STAT3 activity is required for ETV5-mediated induction of Maf expression, but dispensable for ETV5 binding to Maf promoter.

Finally, we examined whether the upregulation of Maf expression contributed to the enhanced TFFH cell differentiation in Cic-deficient T cells by the adoptive transfer experiment. WT and Cic null OT-II cells transduced with control or Maf shRNA (shMaf)-expressing retrovirus were adoptively transferred into Thy1.2

R mice. Seven days after immunization with NP-OVA in alum, the Thy1.2

R OT-II cells were analysed for TFFH cell differentiation using flow cytometry. WT:shNC, n = 11; cKO:shNC, n = 9; cKO:shMaf, n = 8. **P < 0.01 (two-tailed two-sample unequal variance Student t-test). (g) Schematic illustration on how Cic deficiency induces T-cells, TFFH cell differentiation and autoimmunity. Cic deficiency in T cells makes the TCR response hypersensitive, thereby inducing T-cell activation. During TFFH cell differentiation, de-repression of ETV5 induces Maf expression in Cic null TFFH cells, subsequently promoting TFFH cell differentiation. These T-cell abnormalities could contribute to autoimmunity in the Cic-deficient mice.

Discussion

In this study, we examined a previously unrecognised function of Cic in the immune system. Our findings demonstrate that Cic is a key transcriptional repressor that maintains peripheral immune homeostasis and restrains TFFH cell differentiation, thereby suppressing autoimmunity (Fig. 6g). Cic deficiency causes lymphoproliferative autoimmunity in mice, which is accompanied by augmented T-cell responses and increased frequencies of TFFH and GC B cells in secondary lymphoid organs. Given that excessive formation of TFFH cells is often observed in patients with autoimmune diseases and that autoimmunity in Roquin

mice is reduced when TFFH cell development is suppressed either by removing one allele of Bcl6, by deleting SAP (Sh2d1a), or by dampening IFNγ signalling

0.06, spontaneous induction of the RC response might be a primary cause of the onset of autoimmune-like symptoms in Cic mutant mice.

The immune cell-specific Cic null mice shared many phenotypes including hyperglobulinemia, T-cell hyperactivation, accumulation of effector/memory cells with TFFH1 and TFFH2 phenotypes, systemic autoimmunity and increased proportions of CD4

FCOP3 CD25

T, TFFH and GC B cells with the T-cell-specific Cic null mice, demonstrating that T-cell-intrinsic functions of Cic are crucial for maintenance of T-cell homeostasis and suppression of spontaneous induction of the GC response and autoimmunity. A specific loss of Cic in B cells, from which antigen presentation and co-stimulatory signals are

Figure 6 | Maf is a critical downstream target of the Cic-ETV5 axis in the process of TFFH cell differentiation. (a) qRT-PCR analysis for levels of TFFH-related genes in sorted non-TFFH and TFFH cells from Cic

and Cic/Cd4-Cre mice. Splenocytes from 5 to 6 Cic

mice or 3 to 4 Cic/Cd4-Cre mice were pooled and subjected to cell sorting. Three independent experiments were performed. (b) FACS-sorted splenic TFFH cells from Cic

and Cic/Cd4-Cre mice were subjected to western blot analysis for MAF expression. The images are representative of two independent experiments. (c) qRT-PCR analysis of TFFH-related genes in CD4

T cells infected with control or ETV5-expressing retrovirus. The cells were incubated in the presence of IL-6 and IL-21, and re-stimulated with anti-CD3 for 2 h prior to RNA extraction. Four independent experiments were performed. (d) qRT-PCR analysis for Maf levels in CD4

T cells infected with control or ETV5-expressing retrovirus in the absence of IL-6 and IL-21. Before RNA extraction, the cells were activated with anti-CD3 for 2 h. Four independent experiments were performed. (a,c,d) Error bars indicate s.e.m. *P < 0.05, **P < 0.01 and ***P < 0.001 (two-tailed two-sample unequal variance student t-test). (e) Western blot analysis for MAF levels in CD4

T cells infected with control or ETV5-expressing retrovirus. The cells were cultured in the presence or absence of IL-6 and IL-21, and then re-stimulated with anti-CD3 for 12 h. The images are representative of two independent experiments. (f) WT Thy1

OT-II cells infected with control retrovirus and Cic null Thy1

OT-II cells infected with control or Maf shRNA (shMaf) expressing retrovirus were transferred into Thy1

B6 recipient mice. Seven days after immunization with NP-OVA in alum, the Thy1

OT-II cells were analysed for TFFH cell differentiation using flow cytometry. WT:shNC, n = 11; cKO:shNC, n = 9; cKO:shMaf, n = 8. **P < 0.01 (two-tailed two-sample unequal variance Student t-test). (g) Schematic illustration on how Cic deficiency induces T-cell activation, TFFH cell differentiation and autoimmunity. Cic deficiency in T cells makes the TCR response hypersensitive, thereby inducing T-cell activation. During TFFH cell differentiation, de-repression of ETV5 induces Maf expression in Cic null TFFH cells, subsequently promoting TFFH cell differentiation.
required for differentiation and maintenance of T<sub>FH</sub> cells<sup>8</sup>, did not promote T<sub>FH</sub> cell differentiation in mice (Supplementary Fig. 16), accentuating the T-cell-intrinsic requirement of CIC in regulation of T<sub>FH</sub> cell development. However, most phenotypes were more prominent in the immune cell-specific Cic null mice than in the T-cell-specific Cic-deficient mice; this difference implies that T-cell-extrinsic mechanisms also contributed in part to those phenotypes. To comprehensively understand the function of CIC in maintenance of peripheral immune tolerance, functions of CIC in other types of immune cell, such as DCs and B cells, need to be further determined.

Our study provided a molecular basis of how CIC deficiency promotes T<sub>FH</sub> cell differentiation. We determined that ETV5 is a critical CIC target that promotes T<sub>FH</sub> cell differentiation. Among PEA3 group genes, Etv4 and Etv5 are major CIC target genes in CD4<sup>+</sup> T cells. Although mRNA levels of both genes increased greatly in the Cic null T<sub>FH</sub> cells, only ETV5 expression increased significantly at the protein level, indicating that ETV4 levels are tightly controlled in T<sub>FH</sub> cells by posttranscriptional regulation. ETV5 expression also seems to be regulated at the posttranscriptional level in the Cic null T<sub>FH</sub> cells, because the increase in ETV5 expression by CIC deficiency was greater at the mRNA level (~5.5-fold) than at the protein level (~3.4 fold). One possible mechanism of posttranscriptional control of PEA3 group transcription factor levels in T<sub>FH</sub> cells is proteasomal degradation mediated by E3 ubiquitin ligase COP1 (also known as RFWD2). COP1-mediated degradation of PEA3 group transcription factors is involved in regulation of prostate cancer progression<sup>61</sup>, β-cell insulin secretion<sup>62</sup> and lung-banchong morphogenesis<sup>63</sup>, but the function of COP1 in T<sub>FH</sub> cell development has not been determined. Because Rfwd2 is expressed in T lymphocytes<sup>64</sup>, one interesting follow-up would be to examine whether COP1 regulates T<sub>FH</sub> cell differentiation through degradation of ETV5 or other PEA3 group transcription factors in T cells.

We identified Maf as an important downstream target gene of the CIC–ETV5 axis in the process of T<sub>FH</sub> cell differentiation. To identify genes that may be regulated by ETV5 during T<sub>FH</sub> cell differentiation, we examined 14 genes that encode transcription factors, cytokines or cell surface molecules, all of which are critically involved in regulation of T<sub>FH</sub> cell development. Strikingly, ETV5 overexpression induced only Maf expression in CD4<sup>+</sup> T cells. The finding that T<sub>FH</sub> cell differentiation of the Cic null OT-II cells was suppressed by knockdown of Maf further demonstrates that the CIC–ETV5 axis regulates T<sub>FH</sub> cell differentiation via MAF. However, in the Cic-deficient OT-II cells, Maf RNAi suppressed T<sub>FH</sub> cell differentiation less efficiently than did Etv5 RNAi; this comparison suggests that, in addition to Maf, other ETV5 target genes may contribute to promotion of T<sub>FH</sub> cell differentiation. Moreover, Cic-deficient T<sub>FH</sub> cells exhibited the enhanced expression of IFNγ, which is critical for T<sub>FH</sub> cell differentiation<sup>40</sup>, but not regulated by MAF<sup>41</sup>. This result also implies that Maf may not be the only ETV5 target gene that contributes to promotion of T<sub>FH</sub> cell differentiation. To improve our understanding of how ETV5 promotes T<sub>FH</sub> cell development, studies of the function of ETV5 in T<sub>FH</sub> cells, such as a genome-wide identification of ETV5 targets and their function in T<sub>FH</sub> cells, should be conducted.

Given that ETV5 upregulated Maf expression only in the presence of IL-6 and IL-21, which are cytokines that induce T<sub>FH</sub> cell development<sup>58</sup>, and that Maf levels were increased in Cic null T<sub>FH</sub> cells, but in neither naïve nor anti-CD3/CD28-activated Cic-deficient CD4<sup>+</sup> T cells (Supplementary Data 1), ETV5 might specifically regulate Maf expression during T<sub>FH</sub> cell development. Consistent with this inference, Maf levels were comparable between WT and Etv5 deficient T<sub>FH</sub>17 cells<sup>65</sup>. However, ETV5 facilitates differentiation of T<sub>FH</sub>17 cells<sup>65</sup> and of IL-17-producing γδ effector cells<sup>66</sup> by activating IL17 expression. ETV5 also promotes IL-9 production in T<sub>FH</sub> cells by binding and recruiting histone acetyltransferases to the Il9 locus<sup>67</sup>. These findings, together with our data, suggest that ETV5 participates in differentiation processes of several T<sub>H</sub> subtypes by regulating a different set of genes in each T<sub>H</sub> subset.

Our study is the first demonstration that the CIC–ETV5 axis is critical for regulation of T<sub>FH</sub> cell development. This axis also contributes to cancer progression<sup>22–24</sup>, therefore, dysregulation of this regulatory axis could contribute to pathogenesis of both cancer and antibody-mediated autoimmune diseases. In this regard, CIC and ETV5 themselves or their target genes that commonly mediate pathogenesis of both diseases could be evaluated as molecular targets for treatment of both autoimmune diseases and cancers.

**Methods**

All mice were maintained on a C57BL/6 background. Varil-Cre (ref. 68), Cd44<sup>Cre</sup> (ref. 69), Foxp3-TFP-Cre (ref. 51), Cd19-Cre (ref. 70) and Ot-II (ref. 55) mice have been described previously. The mouse with a loxP-flanked Cic allele was generated by mating Cic<sup>fl/fl</sup>/KOMP<sup>2</sup> with Actin-FLP mice (Stock No: 003800). The Cic<sup>fl/fl</sup>/KOMP<sup>2</sup> allele had been obtained as ES cells (Clone No. EPOD083_3_F07) from EUCOMM and injected into C57BL/6 blastocysts. Animals were maintained in a specific pathogen-free animal facility under standard 12 h light/12 h dark cycle. Mice were fed standard rodent chow and water ad libitum. Three-to-thirteen mice that were used in individual experiments were assigned randomly to the experimental groups. Male and female mice were also randomly assigned to the experimental groups. Blinding was not possible in most animal experiments. All procedures were approved by the Pohang University of Science and Technology Institutional Animal Care and Use Committee.

**Enzyme-linked immunosorbent assay.** Ninety-six well ELISA plates were pre-coated with 2 μg/ml<sup>-1</sup> anti-mouse IgG (1010-01, Southern Biotechnology) at 4°C overnight. On the next day, the plates were washed with washing buffer (phosphate-buffered saline (PBS) with 0.05% Tween 20) and blocked with washing buffer (washing buffer with 2% bovine serum albumin (BSA)) for 1–2 h at room temperature (RT); then 50 μl of diluted serum and standards were added and incubated for 2 h at RT. The plates were washed and incubated for 1 h at RT with secondary antibodies conjugated with horseradish peroxidase. For total IgG, goat anti-mouse IgG (H + L) (pooled antisera from goats hyperimmunized with mouse IgG, 1:3,000 diluted, 1070-05) was used; for IgG2a, goat anti-mouse IgG2a (pooled antisera from goats hyperimmunized with mouse IgG2a, 1:10,000 diluted, 1070-05) was used; and for IgM, goat anti-mouse IgM (pooled antisera from mice hyperimmunized with mouse IgM, 1:2,000 diluted, 1020-05) was used. The plates were washed at least five times, then 50 μl of TMB substrate (SubModics, TMBM-1000-01) was added and incubated for 20 min in the dark. Finally, 50 μl of stop solution (1 M H<sub>2</sub>SO<sub>4</sub>) was added and the plate was read at 450 nm. The serum concentration was calculated according to the standard curve generated. Serum anti-dsDNA concentration was measured using mouse anti-dsDNA ELISA Kit (Shibayagi, AKRDD-061) according to the manufacturer’s instruction. For IL-2 ELISA, naïve CD4<sup>+</sup> T cells were activated with plate-bound anti-CD3 (1.0 μg/ml<sup>-1</sup>, 145-211, BD) in the presence or absence of plate-bound anti-CD28 (2.0 μg/ml<sup>-1</sup>, 37,51, BD), then cell supernatants were collected and 72 h after stimulation. Briefly, plates pre-coated with 2 μg/ml<sup>-1</sup> anti-IL-2 (14-7022, ebioscience) were washed and blocked as described above, then 50 μl of diluted samples and standards were added and incubated for 2 h at RT. The plates were washed, then incubated sequentially with biotin-conjugated anti-IL-2 (13-7021) for 1.5 h, avidin-HRP (18-4100) for 30 min, TMB substrate for 15 min, and 1 M H<sub>2</sub>SO<sub>4</sub> as a stop solution.

**Tissue histology.** Liver, lung and kidney tissues obtained from 12-month-old Cic<sup>−/−</sup> and Cic<sup>−/−</sup>Vav1-Cre mice or 14.5-month-old Cic<sup>−/−</sup> and Cic<sup>−/−</sup>Cd4-Cre mice were fixed in 10% formalin, then embedded in paraffin before sectioning. The tissues were cut into 5-μm sections (Leica RM2245), then sections were deparaffinized and dehydrated by using xylene, 100% ethanol and 95% ethanol sequentially. They were washed in distilled water, then stained with haematoxylin Sigma, HHS32) and eosin (Sigma, H110132). After H&E staining, × 200 images of specimens were obtained using an Olympus CX31 light microscope.

**Immunofluorescence staining of IgG deposition.** Kidney tissues from 12-month-old Cic<sup>−/−</sup> and Cic<sup>−/−</sup>Vav1-Cre mice were snap-frozen in OCT medium (Sakura Finetek, 4583), then cut into 10-μm sections (Leica CM3050S). The kidney sections were air-dried for 24 h, then washed in PBS (pH 7.4) to remove fixation.
compound. Diluted anti-mouse CD16/CD32 antibodies (eBioscience, 1:500 diluted, Sigma, F0257) and 4′,6-diamidino-2-phenylindole to reveal the IgG complexes and the nuclei, respectively. The specimens were washed in PBS, then covered in mounting medium (Dako, Citifluor), and × 400 images of specimens were obtained using an Olympus IX82-ZDC2 fluorescence microscope.

Plasmids and retrovirus production. The amino-terminal FLAG-tagged coding sequence of mouse Envs was amplified by PCR, initially cloned into T blunt vector (SolGent), and then verified by sequencing. A confirmed EnvS CDS fragment digested by Xhol/HpaI was sub-cloned into MigR1 retroviral vector (MigR1-ETV5-GFP). The rDNA expression vectors for knockdown of mouse Envs and Maf were generated using MCV-LTRmR3-Pgk (LMP) vector (Open Biosystems) according to the manufacturer’s instruction. The target sequences were as follows. For shET5: 5'-AACCAGCAAGCTGAGGACCAGG-3' and for shMAF: 5′-AAG ATATAACCTGGAACCATAT-3'.

Viruses were generated by transient co-transfection of Platinum-E (Plat-E) retroviral packaging cell line (293 T Cells) with the cloned retroviral vectors and pCL-Eco vector using FUGENE HD transfection reagent (Promega). Retrovirus-containing supernatants were collected 48 h later and frozen in 6-well plates. On the next day, the cells were transfected with 1.2 μg of retroviral vector and 0.8 μg of pC-Eco using FuGENE HD transfection reagent (E2311, Promega). Retrovirus-containing supernatants were collected 48 h later and frozen at −80 °C.

Flow cytometry and cell sorting. Single-cell suspensions of spleens and thymuses were prepared and surface-stained in FACs buffer (PBS + 1.5% fetal bovine serum (FBS)) with control antibodies. The following antibodies were obtained from eBioscience, BD PhaMingen or BioLegend: anti-CD4 (1:100 diluted, GK.1), anti-CD8 (1:100 diluted, 53-67), anti-CD3 (1:100 diluted, 17A2), anti-CD11b (1:100 diluted, M1/70), anti-CD11c (1:100 diluted, N418), anti-MHCII (1:100 diluted, AF5-120.1), anti-F4/80 (1:100 diluted, BM8), anti-CD4 (1:100 diluted, IM7), anti-CD26 (1:100 diluted, MEL-14), anti-CD25 (1:50 diluted, PC61), anti-CD16 (1:50 diluted, J43), anti-GITR (1:100 diluted, DTA-1), anti-OX-40 (1:100 diluted, OX-86), anti-ICOS (1:100 diluted, 7E.17G9), anti-B220 (1:200 diluted, RA3-6B2), anti-IgM (1:100 diluted, II/41), anti-CD4 (1:100 diluted, R2/60), anti-CD3 (1:100 diluted, R3/104), anti-GL-7 (1:250 diluted, GL7), biotinylated anti-mouse FAS (1:200 diluted, Jo2), anti-CD8 (1:100 diluted, 2C7), anti-GATA3 (1:100 diluted, TC11-18H10) and anti-CD89 (1:100 diluted, 806-927), anti-CCR4 (1:100 diluted, 2G12), anti-CCR6 (1:100 diluted, 140706), anti-CXCR4 (1:150 diluted, CXCR3-173), anti-CTLA-4 (1:100 diluted, UC10-4B9), anti-ID3 (1:500 diluted, 10E8), anti-CD103 (1:100 diluted, TC11-18H10), TNF (1:25 diluted in 6-well plates). On the next day, the cells were transfected with 1.2 μg of retroviral vector and 0.8 μg of pC-Eco using FuGENE HD transfection reagent (E2311, Promega). Retrovirus-containing supernatants were collected 48 h later and frozen at −80 °C.

Thymic negative selection assay. In vitro thymic negative selection assay was performed as described previously. Briefly, DP thymocytes were prepared from Cicf/f and Cicf/fCd4-Cre mice by the MoFlo-XDP (Beckman Coulter). Tresp cells were mixed with an equal number of irradiated splenocytes (3000 Rad) from RAG1−/− mice and different numbers of CD4+CD25+ Tresp cells and incubated in round bottom 96-well tissue culture plates with anti-CD3 (1 μg ml−1). Seventy-two hours later, CD4+ CD4 T cells were gated and proliferation was analysed based on CTV dilution. For detection of IL-2 secretion, CD4+ CD4 T resp cells from Cicf/f and Cicf/fCd4-Cre mice were used at a fixed concentration of 1 × 106 cells per well and co-cultured with CD4 + CD25 + Tresp cells in 96-well plates. The cells were stimulated with plate-bound anti-CD3 (1 μg ml−1) and anti-CD28 (1 μg ml−1), then supernatants were collected 72 h after stimulation. The samples were subjected to ELISA for IL-2 concentration.

Western blot analysis. Single-cell suspensions of spleen, lymph nodes and thymus from Cicf/f and Cicf/fVav1-Cre mice were used as total immune cells in lymphoid organs. Naïve CD4+ T cells were activated by anti-CD3 and anti-CD28 and collected on days 0 (unstimulated), 1, 2 and 3. B220+ B and CD4+ T cells from Cicf/f and Cicf/fCd4-Cre mice were prepared using MACS (130-049-501 and 130-104-454, Miltenyi Biotech) according to the manufacturer’s protocol. Naïve CD4+ T effector/memory CD4+ T, Tresp and non-Tresp cells were purified from total CD4+ T cells by FACS sorting using the MoFlo-XDP. Total lysates from 0.2 to 1.0 × 106 cells of each cell population were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE). The primary antibodies used are as follows. anti-CIC (1:500 diluted, Santa Cruz Biotechnology) and anti-ETV5 (1:1000 diluted, BD, 535649). To investigate the induction of Nur77 expression after TCR cross-linking, thymocytes from Cicf/f and Cicf/fCd4-Cre mice were stimulated with anti-CD3 (5 μg ml−1) and anti-CD28 (10 μg ml−1) for 6 h. The samples were analysed using the LSRFortessa flow cytometer, and data were analysed using FlowJo software.

Retroviral transduction and cell transfers. OT-II Thy1.1 CD4+ T cells were purified from whole splenocytes by negative selection using EasySep (StemCell), then re-suspended in T-cell medium (RPMI-1640 + 10% FBS, supplemented with 2.05 mM l-Glutamine, 100 U ml−1 Penicillin/Streptomycin and 55 mM BME). The purified OT-II T cells were stimulated in 24-well plates pre-coated with 2 μg ml−1 anti-CD3 (145-2C11, BD) and anti-CD28 (37.51, BD) in the presence of rmIL-2 (50 U ml−1). The OT-II T cells were then co-transduced (MigR1-GETV5-GFP) or ETV5-expressing retrovirus (MigR1-ETV5-GFP) at 24 h and 48 h after in vitro stimulation. A total of 72 h stimulation, the cells were transferred into six well plates with rmIL-2 for 24 h, and then transferred again into new six-well plates in the presence of rmIL-7 for 24 h prior to cell sorting. For splenic RNA extraction, after in vitro stimulation and double transduction with shNC, ETV5 or shMAF expressing retrovirus for 72 h, the cells were transferred into six-well plates with rmIL-2 for 12 h and then transferred further into new six-well plates with rmIL-7 for 12 h.

For adoptive transfer experiments, 0.2–0.5 × 107 OT-II T cells infected with retroviruses were transferred into C57BL/6 Thy1.2 recipient mice by tail vein injection. Three days later, mice were intraperitoneally with 200 μl of NaF (406-59-1, Sigma) in 200 μl of saline. Seven or eight days after
immunization, the donor cells were analysed for T<sub>FH</sub> differentiation by flow cytometry.

**Cell culture.** Naïve (CD4<sup>+</sup>CD25<sup>-</sup>CD44<sup>+</sup>CD62L<sup>lo</sup>) CD<sup>4</sup><sup>+</sup> T cells were isolated by negative selection using EasySep (StemCell), then activated with plate-bound anti-CD3 (145-2C11, BD) and anti-CD28 (37.51, BD) under neutral (anti-IFNγ, anti-IL-4, anti-ATG-F) or Th1-like (anti-IFNγ, anti-IL-4, anti-ATG-F, rmIL-6, rmIL-21) condition. The cells were transduced with retrovirus (MiRgR-GFP or MiRgR-ETV5-GFP) at 24 h and at 36 h after in vitro stimulation. Two days after culture, the cells were rested for 24 h in the presence of rmL-7, then re-stimulated with pre-coated anti-CD3 for 2 h for RNA samples and for 12 h for protein samples.

For STAT3 inhibitor treatment experiments, naïve CD4<sup>+</sup> T cells were polarized under Th1-like condition as described above. The cells were transduced with retrovirus (MiRgR-1-GFP or MiRgR-ETV5-GFP) at 24 h after in vitro stimulation, then treated with AG490 (Tokyo Chemical, 50 µM) for 16 h or Stattic (Santa Cruz Biotechnology, 20 µM) for 12 h. Two days after culture, the cells were rested for 24 h. The cells were collected for RNA samples.

**ChIP and qPCR.** Chromatin immunoprecipitation was performed as previously described. Chromatin from 5 x 10<sup>6</sup> CD<sup>4</sup><sup>+</sup> T cells were crosslinked in 1.5% formaldehyde for 12 min with constant shaking, quenched cross-linking by adding 1.5 M glycine for 10 min, and then rinsed with cold PBS twice. After centrifugation for 5 min, the pellet was resuspended in Buffer A (100 mM Tris pH 9.4, 1 x protease inhibitor cocktail (1183617001, Roche)), Buffer 1 (10 mM HEPES, 10 mM EDTA, 0.5 mM EGTA, 0.25% Triton-X, 1 x protease inhibitor cocktail), and Buffer 2 (10 mM HEPES, 0.2 M NaCl, 1 mM EDTA, 0.5 mM EGTA, 1 x protease inhibitor cocktail), successively. T-cell lysate was resuspended in 200 µl of nuclei lysis buffer (5 mM Tris pH 8.1, 10 mM EDTA, 1% SDS, 1 x protease inhibitor cocktail) and sonicated. After microcentrifugation, the supernatant was pre-cleared with protein G agarose (16-266, Millipore) in dilution buffer (16.7 mM Tris pH 8.1, 167 mM NaCl, 1.2 mM EDTA, 0.01% SDS, 1.1% Triton-X, 1 x protease inhibitor) for 1–2 h. Six micrograms of anti-CIC antibody (homemade) were added to chromatin samples and incubated overnight at 4°C. The chromatin and antibody mixtures were further incubated with protein G agarose for 2–4 h at 4°C. After washing, bound chromatin was eluted twice by elution buffer (0.5% SDS and 0.1 M NaHCO<sub>3</sub>) and reverse-crosslinked with 200 µM NaCl for at least 4 h at 65°C. Proteins were digested by proteinase K and DNA was purified by AccuPrep Gel Purification Kit (K-3034, Bioneer). qPCR was performed to quantify the relative enrichment of promoter regions of Peak group genes in the immunoprecipitated DNA fragments.

For ETV5 ChIP experiment, CD4<sup>+</sup> T cells were activated with plate bound anti-CD3 (145-2C11, BD) and anti-CD28 (37.51, BD) in the presence of rmIL-6. Sixteen hours after in vitro stimulation, 1 x 10<sup>5</sup> cells and 2 µg of anti-ETV5 antibody (sc-22807, Santa Cruz Biotechnology) were used for each ChIP experiment. To inhibit STAT3 activity, CD4<sup>+</sup> T cells were activated with anti-CD3/CD28 in the presence of rmIL-6 and then Stattic (20 µM) for 12 h. The primer sequences used are listed in Supplementary Data 2.

**qRTP-PCR.** Total RNA was extracted using Trizol reagent (301-001, GeneAll), then 1–2 µg of the total RNA was subjected to cDNA synthesis using a GoScript Reverse Transcription System (A3000, Promega). Each gene expression level was normalized to Hprt levels and presented as relative to WT. The primers used in qRTP-PCR analyses are listed in Supplementary Data 3.

**RNA sequencing and data analysis.** Spleens were dissected from 10-week-old C57BL/6 and C57BL/6-Cre mice. CD4<sup>+</sup> T cells were pre-enriched using EasySep (19860 and 18001, StemCell), then further sorted on the basis of surface markers of naïve CD4<sup>+</sup> T cells (CD4<sup>+</sup>CD44<sup>+</sup>CD62L<sup>lo</sup>). Sorted naïve CD4<sup>+</sup> T cells were activated in 24-well plates pre-coated with 2 µg/ml anti-CD3 (145-2C11, BD) and anti-CD28 (37.51, BD) for 3 d. Total RNA was extracted from naïve and activated CD4<sup>+</sup> T cells using ReliaPrep RNA Cell Miniprep Kit (Z6011, Promega). The library for mRNA sequencing was generated using a Illuminia TrueSeq Preparation Kit (RS-122-2001) and sequenced on a NextSeq 500 sequencer. The library for mRNA sequencing was generated using an Illuminia TruSeq Preparation Kit (RS-122-2001) and sequenced on a NextSeq 500 sequencer (Illumina). TruSeq (v 2.0.10) was used to map sequencing reads to the mouse reference genome (mm10 RefSeq). Assembly of transcripts and identification of differentially expressed genes (DEGs, Fold change (log<sub>2</sub>) > 1.5; P < 0.05) were conducted by Cufflinks [v 2.1.1].

**Statistical analysis.** For statistical analysis, all experiments were performed more than three times independently. Statistical analyses were performed using the Student t-test (two-tailed, two-sample unequal variance). P < 0.05 was considered significant. In bar graphs, bars indicate means and error bars indicate SEM. In box-and-whisker plots, boxes represent median with upper and lower quantiles and whiskers represent values of maximum and minimum.

**Data availability.** The authors declare that the data supporting the findings of this study are available within the article and its Supplementary Information Files, or from the corresponding authors on reasonable request. The RNA-seq data were deposited in the Gene Expression Omnibus (NCBI) data repository under accession number GSE84125.
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Author contributions

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Additional information

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