

ORIGINAL ARTICLE

EXPERIMENTAL ALLERGY AND IMMUNOLOGY

Plant homeodomain finger protein 11 promotes class switch recombination to IgE in murine activated B cells

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allergy; class switch recombination; IgE; NF- κ B; plant homeodomain finger protein 11.

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Abstract

Background: Polymorphisms of the *Plant homeodomain finger protein 11 (PHF11)* are strongly associated with high serum IgE levels and clinical severity of atopic patients. However, the precise mechanism has not been fully elucidated. We investigated the role of Phf11 in class switch recombination (CSR) to IgE by activated B cells.

Methods: We generated Phf11 transgenic (Lckd-Phf11-Tg) mice that express the exogenous murine Phf11 in lymphocytes under the control of distal Lck promoter. We examined IL-4-induced CSR to IgE in activated Lckd-Phf11-Tg B cells *in vitro*. We analyzed production of ovalbumin (OVA)-specific IgE and nose-scratching symptoms in Lckd-Phf11-Tg mice using an OVA-induced allergic rhinitis model.

Results: The exogenous Phf11 promoted CSR to IgG1 and IgE in activated B cells with an increase in germ line transcript (*GLT*) γ_1 and *GLT* ϵ expression. The exogenous Phf11 augmented transcriptional activity of the *GLT* γ_1 and *GLT* ϵ promoters through permissive histone modifications and binding of NF- κ B and STAT6. Furthermore, the exogenous Phf11 bound to the *GLT* ϵ promoter with increased binding of NF- κ B. Silencing of the endogenous Phf11 reduced the frequency of CSR to IgE and *GLT* ϵ expression, but not to IgG1 or *GLT* γ_1 expression, in activated B cells. In an allergic rhinitis model, Lckd-Phf11-Tg mice showed a significant increase in the production of OVA-specific IgE and the frequency of nose scratching.

Conclusion: Phf11 accelerates CSR to IgE in activated B cells by increasing the transcriptional activity of *GLT* ϵ promoter and contributes to the exacerbation of allergic responses. These findings provide a novel therapeutic target for allergic diseases.

The spreading epidemic of allergies has heightened interest in IgE as the central player in the response (1). Treatment for patients suffering from asthma and other allergic diseases with antibodies (Abs) specific for IgE has been very successful (2). Thus, understanding of the regulatory mechanism of IgE production is important for the treatment (3). Class switch recombination (CSR) is the process by which B cells rearrange the immunoglobulin (Ig) heavy chain locus and thus alter the constant region of their Ig molecules. CSR requires the induction of activation-induced cytidine deaminase (AID) (4) and the corresponding germ line transcript (*GLT*) upstream of the new constant region (5, 6).

The Th2-type cytokine IL-4 and CD40 ligand from helper T cell synergistically induce *GLT* γ_1 and *GLT* ϵ transcription through activation of STAT6 and NF- κ B and thus promote CSR to IgG1 and IgE (3).

Recently, the *Plant homeodomain finger protein 11 (PHF11)* has been identified as an asthma susceptibility gene by positional cloning (7). Single nucleotide polymorphisms of *PHF11* are strongly associated with high serum IgE levels and clinical severity of asthma and atopy (7, 8). PHF11 protein contains two plant homeodomain (PHD) fingers and thus is a member of the PHD family (7). The PHD finger regulates gene expression through interaction

with trimethylated lysine 4 on histone H3 (H3K4me3), a universal chromatin modification at the beginning of gene activation (9, 10). PHF11 is highly expressed in immune-competent cells, including B and T cells (7, 11). Recent studies demonstrated interaction of PHF11 with p65 of NF- κ B (11, 12). NF- κ B plays a critical role in IgE CSR in terms of induction of *GLT* and AID expression (3). Furthermore, production of IgE is preferentially impaired in NF- κ B-deficient mice (13). However, a physiological role of PHF11 in B-cell CSR and allergic response *in vivo* has never been reported.

In our study, we have generated Phf11 transgenic (Lckd-Phf11-Tg) mice that express the exogenous murine *Phf11* gene in lymphocytes under the control of distal Lck (Lckd) promoter (14). We demonstrated that the exogenous Phf11 enhanced the frequency of CSR to IgE in activated B cells accompanied by augmented expression of *GLT* ϵ mRNA. Furthermore, the exogenous Phf11 exaggerated antigen-specific IgE production and allergic responses *in vivo*. These results provide a novel mechanism that may contribute to the development of a novel treatment for allergic diseases.

Materials and methods

Additional information is provided in the Supporting Information.

Animals

BALB/c mice were purchased from Japan SLC Co. Ltd (Shizuoka, Japan). The murine *Phf11* cDNA (Source: MGI, ID: MGI: 1918441) was subcloned into the *Bam*H1 site of the Lckd promoter cassette to generate pLckd-Phf11-hGH (14). Transgenic mice were produced by the method described by Hogan et al. (15). The transgenic mice were backcrossed with BALB/c mice for more than ten generations. The transgenic mice were kept as hemizygotes (Tg/+) and were compared with sex-matched 6- to 8-week-old wild-type littermate mice in experiments. All procedures conformed to the Chiba University Resolution on Use of Animals in Research and were approved by the Institutional Animal Care and Use Committee at Chiba University School of Medicine. These mice were maintained under specific pathogen-free conditions.

Preparation of splenic B cells

Spleen cells were incubated with biotin-anti-CD43 Abs (BD Pharmingen, San Jose, CA, USA). These cells were subsequently reacted with immunomagnetic beads coated with anti-streptavidin Abs (Miltenyi Biotec, Gladbach, Germany). Labeled cells were removed by a MACS system (Miltenyi Biotec).

Cell culture and stimulation of lymphocytes

Purified B cells (1×10^6 cells/ml) were cultured in RPMI 1640 medium (Sigma-Aldrich, St. Louis, MO, USA) supplemented

with 10% FCS (Intergen, New York, NY, USA), 2-ME (50 μ M), streptomycin (100 μ g/ml, Wako Chemical, Osaka, Japan), and penicillin G/potassium (100 U/ml, Banyu Pharmaceutical, Tokyo, Japan) in a humidified atmosphere at 37°C with 5% CO₂. Purified B cells were stimulated with F(ab)₂ fragments of anti-mouse IgM Abs (0.1 g/ml, Alpha Diagnostic Co., San Antonio, TX, USA) and anti-mouse CD40 mAbs (1.0 μ g/ml, BD Pharmingen, San Jose, CA, USA) plus rIL-4 (200 U/ml, Wako Chemical). For CD4 T-cell activation in an murine allergic rhinitis model, purified CD4 T cells (1.5×10^5 cells/well) from the cervical lymph nodes (LNs) were stimulated with OVA (1 mg/ml) in the presence of irradiated splenic feeder cells (5×10^5 cells/well) for 48 h. Before each experiment, viable cell numbers were assessed by trypan blue staining (Invitrogen Corporation, Auckland, New Zealand).

Flow cytometry

Cultured B cells were fixed and permeabilized with FACS Permeabilizing Solution 2 (BD Biosciences, San Jose, CA, USA) before intracellular Ig staining. Abs used were fluorescein isothiocyanate (FITC)-anti-IgG1 (BD Pharmingen), phycoerythrin (PE)-anti-B220 (eBioscience, San Diego, CA, USA) and biotinylated anti-IgE (BD Pharmingen). Biotinylated Abs were detected by allophycocyanin-conjugated streptavidin (BD Pharmingen). Splenic T cells were stained with FITC-anti-CD4 (BD Pharmingen) and PE-anti-CD8 (BD Pharmingen). Cells were analyzed on a FACS Calibur (BD, Franklin Lakes, NJ, USA) using CellQuest software (BD).

Allergic rhinitis model

Mice were immunized intraperitoneally with ovalbumin (OVA) (100 μ g/mouse, Sigma-Aldrich) adsorbed on alum (4 mg/mouse, Sigma-Aldrich) on days 0, 7, and 14 and then rechallenged intranasally with 1% OVA from days 21 to 28 after immunization.

Statistical analysis

Values are expressed as mean $\pm$ SEM. Data were analyzed for statistical significance using the Student's *t* test (StatView 5.0, Abacus Concepts, Berkeley, CA, USA). Data were considered to be significantly different when the *P* value was <0.05 .

Results

Mice carrying the murine *Phf11* gene under the control of Lck distal promoter (Lckd-Phf11-Tg) were created (Fig. 1A). *Phf11* mRNA expressions of Lckd-Phf11-Tg mice in CD4 and CD8 T cells and B cells were augmented three- to fivefold compared with those of wild-type mice (Wt), as determined by quantitative real-time PCR (qPCR) (Fig. 1B). FACS analysis showed similar populations and numbers of CD4 and CD8 T cells and mature B cells

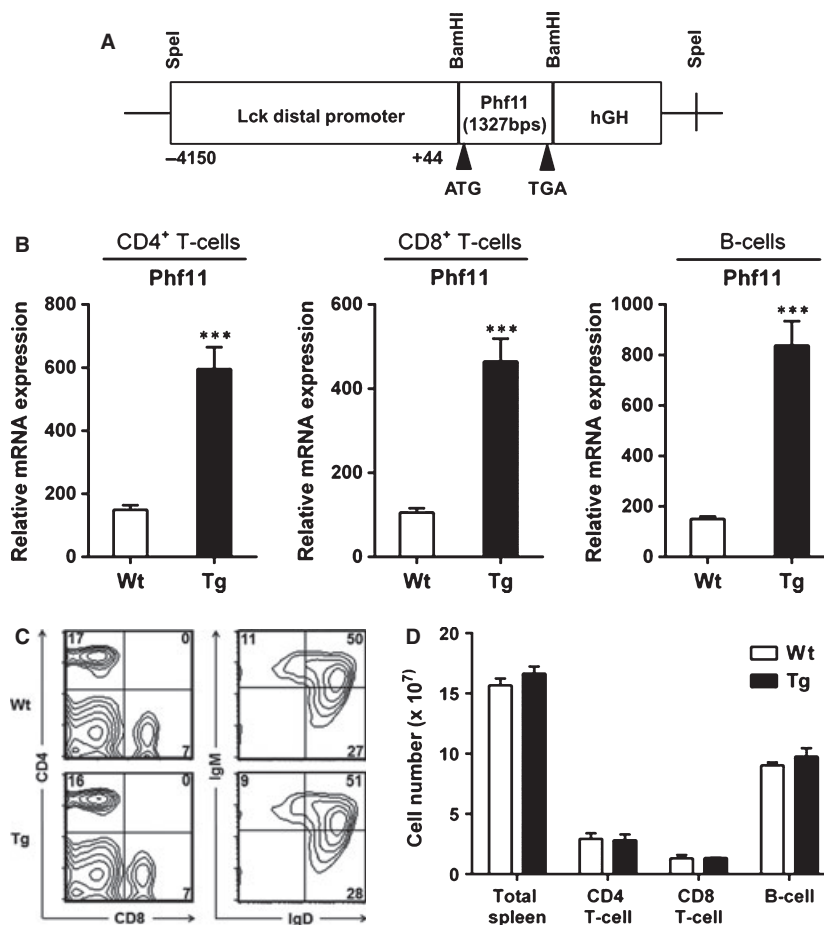


Figure 1 Generation of Phf11 Tg mice. (A) Schematic diagram of a construct used for peripheral lymphocyte-specific expression of the murine *Phf11* gene. (B) Naive splenic CD4 and CD8 T cells and B cells were purified from Wt (open bars) and Lckd-Phf11-Tg (filled bars) mice, and *Phf11* mRNA expression was measured by real-time qRT-PCR. (C) Naive splenic T cells and B cells from Wt and Lckd-Phf11-Tg mice were purified. Percentages of CD4 and CD8 T

cells (C, left) and mature B cells (IgM⁺, IgD⁺, B220⁺) (C, right) were analyzed by FACS. Representative FACS profiles from three independent experiments are shown. (D) Numbers of CD4 T cells, CD8 T cells and B cells in the spleen of Wt (open bars) and Lckd-Phf11-Tg (filled bars) mice were calculated by FACS profiles. Data represent means $\pm$ SEM from three independent experiments (****P* < 0.001).

(B220⁺IgM⁺IgD⁺) in the spleen, between Wt and Lckd-Phf11-Tg mice (Fig. 1C,D).

Phf11 increases frequency of CSR to IgG1 and IgE

To investigate the effect of Phf11 on CSR to IgG1 and IgE by activated B cells, splenic naive B cells were stimulated with anti-IgM Abs and anti-CD40 Abs plus IL-4 for 5 days. Percentages and numbers of IgG1⁺ B cells and IgE⁺ B cells in the Lckd-Phf11-Tg B-cell culture were significantly larger than those in the Wt B-cell culture, although total cell numbers of both Wt and Lckd-Phf11-Tg B cells were comparable (Fig. 2A–C). In addition, the Lckd-Phf11-Tg B cells produced larger amounts of IgG1 and IgE Abs in culture supernatants than the Wt B cells (Fig. 2D). Thus, the exogenous Phf11 in activated B cells accelerates frequency of CSR to IgG1 and IgE.

Phf11 augments expression of *GLT* γ_1 and *GLT* ϵ mRNA

Next we explored the molecular mechanism of the augmented CSR to IgG1 and IgE. Expression of mature γ_1 and mature ϵ mRNA, which are transcribed as a consequence of CSR to IgG1 and IgE, was significantly higher in Lckd-Phf11-Tg B cells than in Wt B cells (Fig. 3A). We did not find any differences in *Bcl6*, *Blimp1*, *IRF4*, *Xbp1*, and *Pax5* expressions, which are crucial for the development of plasma cells (PCs) and Ig CSR in germinal centers (16), between Wt and Lckd-Phf11-Tg B cells (Fig. 3B). Next, we examined the expression of IgM-, IgG1- and IgE-specific *GLTs* and AID, which are essential for CSR. The amounts of *GLT* γ_1 and *GLT* ϵ mRNA, but not those of *GLT* μ and AID mRNA, were significantly higher in Lckd-Phf11-Tg B cells than in Wt B cells (Fig. 3C). Notably, *GLT* ϵ mRNA in Lckd-Phf11-Tg B cells showed a sixfold increase compared with Wt B cells, while *GLT* γ_1 mRNA increased by

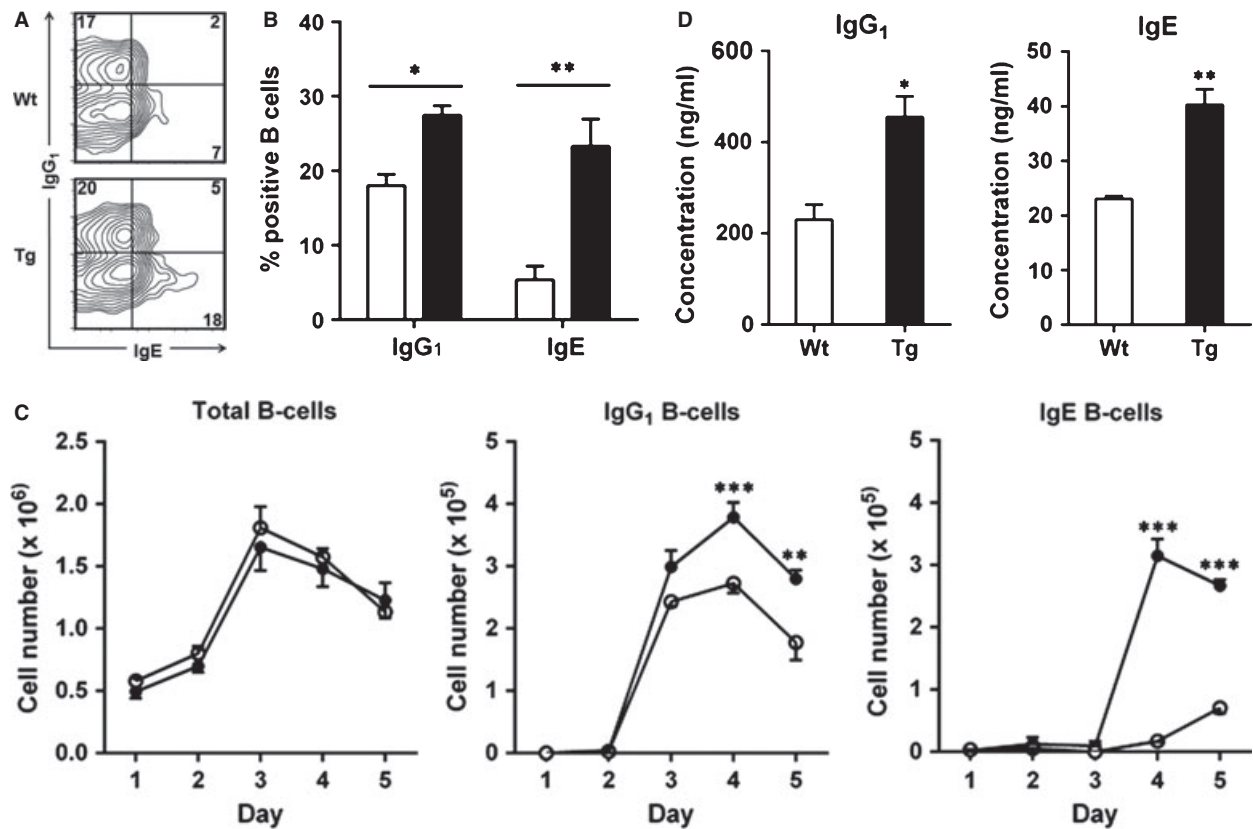


Figure 2 Phf11 increases frequency of class switch recombination to IgG1 and IgE in activated B cells. Splenic B cells from Wt and Lckd-Phf11-Tg mice were stimulated with anti-IgM Abs and anti-CD40 Abs plus rIL-4 for 5 days. (A) Percentages of IgG1⁺ B cells and IgE⁺ B cells in activated B cells were analyzed by FACS. Representative FACS profiles from three independent experiments are shown. (B) Percentages of IgG1⁺ B cells and IgE⁺ B cells on day 5 of culture were shown. Open bars: Wt; filled bars:

Lckd-Phf11-Tg. (C) Numbers of total B cells, IgG1⁺ B cells, and IgE⁺ B cells were shown. Open circles: Wt; closed circles: Lckd-Phf11-Tg. (D) Amounts of IgG1 and IgE Abs in culture supernatants on 5 days after stimulation were measured by ELISA. Open bars: Wt; filled bars: Lckd-Phf11-Tg. Data represent means $\pm$ SEM from three independent experiments (* P < 0.05, ** P < 0.01, *** P < 0.001).

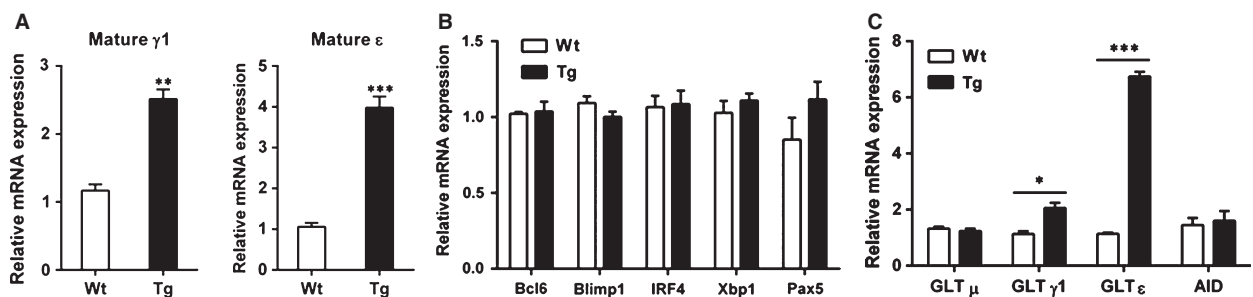


Figure 3 Phf11 augments expression of germ line transcript (GLT) γ 1 and GLT ϵ mRNA in activated B cells. (A–C) Splenic B cells from Wt (open bars) and Lckd-Phf11-Tg (filled bars) mice were stimulated with anti-IgM Abs and anti-CD40 Abs plus rIL-4 for 5 days *in vitro*. Expression of mature γ 1 and mature ϵ mRNA (A), *Bcl6*,

Blimp1, *IRF4*, *Xbp1* and *Pax5* mRNA (B) and GLT μ , GLT γ 1, GLT ϵ and activation-induced cytidine deaminase mRNA (C) in activated B cells were measured by real-time qRT-PCR. Data represent means $\pm$ SEM from three independent experiments (* P < 0.05, ** P < 0.01, *** P < 0.001).

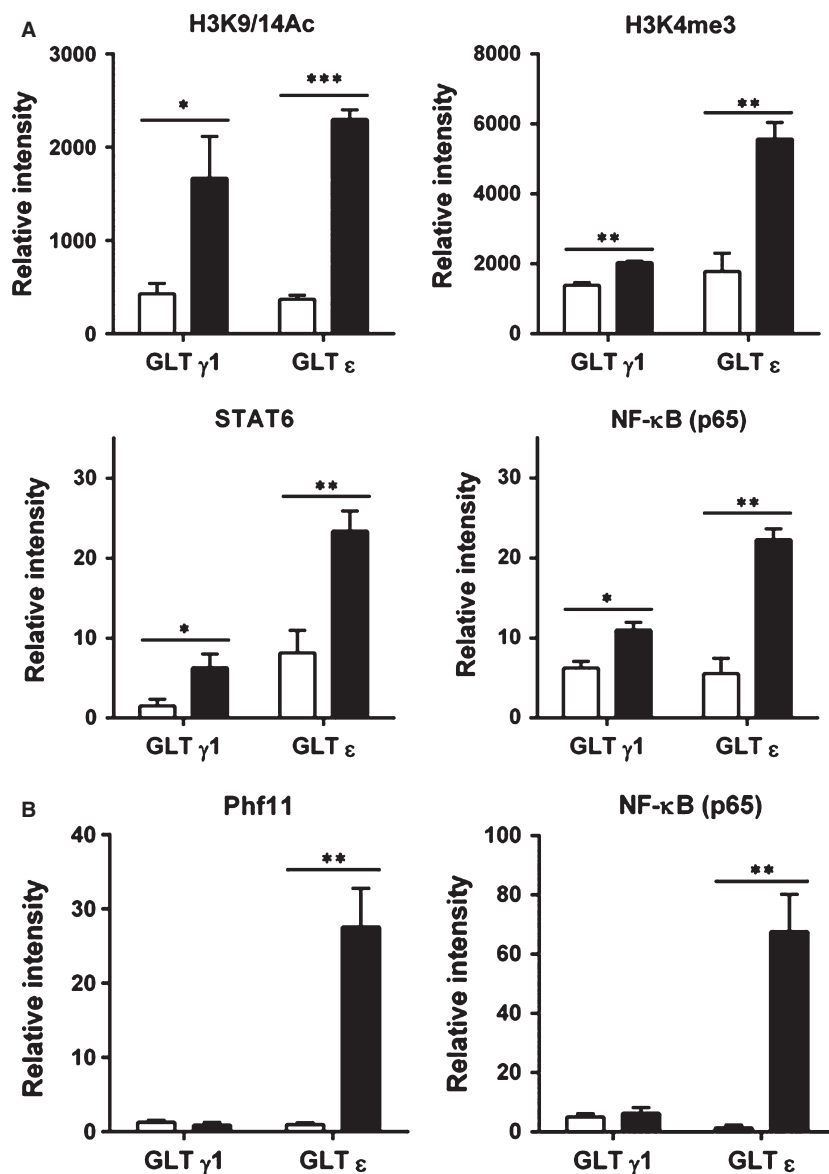


Figure 4 Phf11 augments transcriptional activity of the germ line transcript (*GLT*) γ_1 and *GLT* ϵ promoter regions. Splenic B cells from Wt and Lckd-Phf11-Tg mice were stimulated with anti-IgM Abs and anti-CD40 Abs plus rIL-4. (A) On day 5 of culture, levels of acetylated K9/14 on histone H3 (H3K9/14Ac) and methylated K4 on histone H3 (H3K4me3) of the *GLT* γ_1 and *GLT* ϵ promoter regions, and the amounts of STAT6 and NF- κ B (p65) binding to the same regions of activated Wt (open bars) and Lckd-Phf11-Tg (filled bars) B cells were all measured by ChIP assay. (B) The *myc-antisense*

twofold. Thus, the exogenous Phf11 in activated B cells augments the expression of *GLT* γ_1 and *GLT* ϵ mRNA.

Phf11 augments transcriptional activity of the *GLT* γ_1 and *GLT* ϵ promoter regions

To investigate the transcriptional activity of the *GLT* γ_1 and *GLT* ϵ promoter regions, the permissive histone modifications

(as) *Phf11* (open bar) or *myc-Phf11* (filled bars) construct was transfected to activated Wt B cells on day 2 of culture, and amounts of myc-Phf11 and NF- κ B (p65) binding to the *GLT* γ_1 and *GLT* ϵ promoter regions in those activated B cells on day 4 of culture were measured by ChIP assay. The vertical axis indicates relative intensity of PCR products normalized by the intensity with normal rabbit IgG Abs as 1. Data represent means $\pm$ SEM from three independent experiments (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

of both regions, as well as transcription factor binding to the same regions, were examined by ChIP assay. The levels of acetylated lysine 9/14 on histone H3 (H3K9/14Ac) and H3K4me3 of both regions were significantly higher in Lckd-Phf11-Tg B cells than in Wt B cells (Fig. 4A, upper left and upper right, respectively). The levels of STAT6 and NF- κ B binding to both regions were higher in the Lckd-Phf11-Tg B cells than in the Wt B cells (Fig. 4A, lower left and

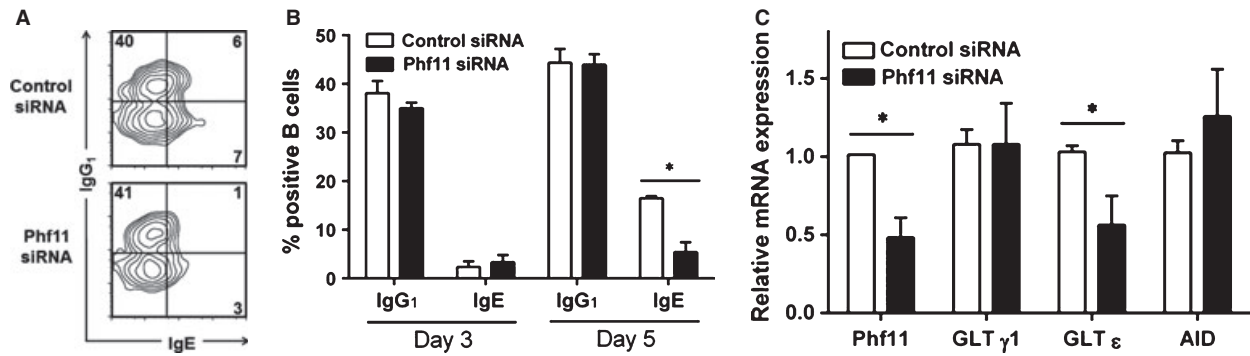


Figure 5 Phf11 silencing by short interfering RNA (siRNA) inhibits CSR to IgE in activated B cell. (A–C) Splenic B cells from Wt mice were stimulated with anti-IgM Abs and anti-CD40 Abs plus rIL-4 for 3 or 5 days, and *Phf11* siRNA or control siRNA was transfected on day 1 or day 3 of culture, respectively. (A) Percentages of IgG1⁺ B cells and IgE⁺ B cells in activated B cells were analyzed by FACS. Representative FACS profiles on day 5 of culture from three independent experiments are shown. (B) Percentages of IgG1⁺ B cells

and IgE⁺ B cells on day 3 and day 5 of culture were shown. Open bars: control siRNA; filled bars: *Phf11* siRNA. (C) Expression of *Phf11*, germ line transcript (*GLT*) γ_1 , *GLT* ϵ and activation-induced cytidine deaminase mRNA in the control siRNA (open bars)- or *Phf11* siRNA (filled bars)-transfected B cells on day 5 of culture was analyzed by real-time qRT-PCR. Data represent means $\pm$ SEM from three independent experiments (* P < 0.05).

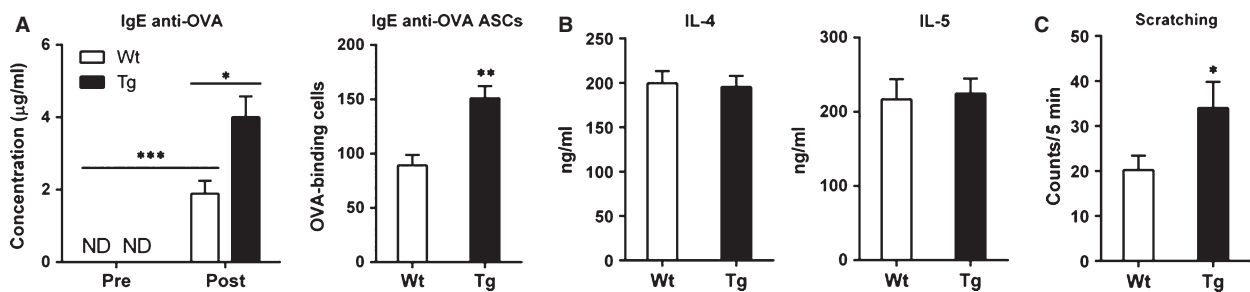


Figure 6 Allergic reactions are exacerbated in Lckd-Phf11-Tg mice. Wt (open bars) and Lckd-Phf11-Tg (filled bars) mice were immunized intraperitoneally with ovalbumin (OVA) in alum on days 0, 7, and 14 and then rechallenged intranasally with OVA from days 21 to 28 after immunization. (A, left) Serum titers of IgE anti-OVA Abs in immunized mice on day 21 (pre-intranasal challenging) and on day 28 (postintranasal challenging) were analyzed by ELISA. (A, right) Numbers of antibody-secreting cells (ASCs) producing IgE anti-OVA Abs in the cervical lymph node (LN)s of immunized mice

on day 28 were measured by ELISPOT assay. (B) Twenty-four hours after the last nasal administration of OVA, CD4 T cells isolated from cervical LNs were cultured in the presence of OVA for 48 h. Amounts of IL-4 and IL-5 in the supernatants were measured by ELISA. (C) Frequency of nose scratching for 5 min was measured after the last intranasal challenge with OVA. Data represent means $\pm$ SEM for each group (n = 5) (* P < 0.05, ** P < 0.01, *** P < 0.001).

lower right, respectively). Thus, the exogenous Phf11 increases transcriptional activity of the *GLT* γ_1 and *GLT* ϵ promoter regions. As recent studies have reported the interaction of PHF11 with p65 of NF- κ B (11, 12), we investigated the binding of Phf11 to the NF- κ B binding sites in the promoter regions. Significant enrichment of Phf11 binding to the NF- κ B binding site in the *GLT* ϵ region, but not that in the *GLT* γ_1 region, was detected in the *myc-Phf11*-transfected B cells (Fig. 4B, left). Notably, in the same transfected B cells, the binding of NF- κ B was augmented to the *GLT* ϵ region but not to the *GLT* γ_1 region (Fig. 4B, right). These data suggest that Phf11 intensively attracts NF- κ B to the promoter region of *GLT* ϵ , resulting in acceleration of CSR to IgE.

Silencing of Phf11 inhibits CSR to IgE

To examine a role of the endogenous Phf11 in CSR to IgG1 and IgE in activated B cells, we assessed the effect of Phf11 silencing using short interfering RNA (siRNA) in activated Wt B cells. The percentage of IgE⁺ B cells, but not IgG1⁺ B cells, was significantly reduced in *Phf11* siRNA-transfected B cells compared with those in control siRNA 5 days after stimulation (Fig. 5A,B). We confirmed that *Phf11* siRNA successfully inhibited the endogenous *Phf11* expression. Furthermore, the amount of *GLT* ϵ , but not *GLT* γ_1 or *AID* mRNA, was significantly reduced in activated B cells transfected with *Phf11* siRNA (Fig. 5C). Thus, the endogenous Phf11 plays an important role in CSR to IgE in activated B cells rather than IgG1.

Excess amounts of Phf11 exacerbated allergic reactions *in vivo*

To demonstrate a role of Phf11 in the pathogenesis of allergic diseases, we used an OVA-induced murine allergic rhinitis model. The level of IgE anti-OVA in serum and the number of IgE anti-OVA Abs secreting cells in the cervical LNs were increased in Lckd-Phf11-Tg mice compared with Wt mice (Fig. 6A). However, there was no significant difference in production of Th2 cytokines, IL4 and IL-5, by OVA-stimulated cervical LN T cells between the Wt and the Lckd-Phf11-Tg mice (Fig. 6B). Furthermore, the frequency of the nasal rubbing of Lckd-Phf11-Tg mice was higher than that of Wt mice after intranasal challenges (Fig. 6C). Thus, the exogenous Phf11 exacerbates allergic reactions *in vivo*.

Discussion

In our study, we generated and analyzed Lckd-Phf11-Tg mice. Despite normal T-cell and B-cell development, Phf11 promoted CSR to IgG1 and IgE in activated B cells through augmented transcription of *GLT* γ_1 and *GLT* ϵ . We elucidated the precise mechanism by examining the transcriptional activity of the *GLT* γ_1 and *GLT* ϵ promoters. Both H3K9/14Ac and H3K4me3 are considered to be permissive histone modifications that link to active gene transcription (17–20). Furthermore, recent studies showed that levels of H3-K9/14Ac and H3K4me3 were significantly augmented at *GLT* promoters during CSR and that loss of H3K4me3 at *GLT* promoters inhibits CSR (21, 22). Binding of STAT6 and NF- κ B to *GLT* γ_1 and *GLT* ϵ promoters is crucial for *GLT* γ_1 and *GLT* ϵ transcription (3). The augmented binding of these transcriptional factors in the present study may be advantageous for CSR to IgE as STAT6 and NF- κ B synergistically promote *GLT* ϵ expression (3, 23, 24). Thus, the results of the present study suggest that Phf11 augments transcriptional activity of the *GLT* γ_1 and *GLT* ϵ promoters through permissive histone modifications and binding of NF- κ B and STAT6. Furthermore, we demonstrated that binding of Phf11 and NF- κ B at *GLT* ϵ promoter was significantly augmented by transfection with the exogenous Phf11. Thus, it might be possible that Phf11 as a PHD family may recognize H3K4me3 at the *GLT* ϵ promoter and augment transcriptional signaling by recruiting NF- κ B, as recent studies reported the interaction of PHF11 with p65 of NF- κ B (11, 12). However, the precise molecular mechanism for the augmentation of *GLT* γ_1 expression remains to be elucidated in the future work.

We demonstrated that silencing of Phf11 reduced the frequency of CSR to IgE, but not to IgG1 in activated B cells. Thus, the endogenous Phf11 is required for CSR to IgE, but not to IgG1. Furthermore, exogenous Phf11 preferentially augmented *GLT* ϵ rather than *GLT* γ_1 . We consider that dependency of Phf11, such as STAT6 (25), on CSR to

IgE is higher than that to IgG1. In addition, there might be more redundant mechanisms for CSR to IgG1 compared with IgE. Thus, we believe Phf11 plays an important role for CSR to IgE. These results indicate that suppression of Phf11 could be a novel target for the treatment of allergic diseases.

We believe this is the first study on the physiological role of Phf11 in an animal allergy model. We demonstrated that exogenous Phf11 augmented OVA-specific IgE production and nasal-rubbing symptoms in the model. Furthermore, we confirmed that exogenous Phf11 augmented production of antigen-specific IgG1, but not IgM, in a humoral response (Figure S1). As both T cells and B cells express exogenous Phf11 in our model, we cannot exclude the effect of T cells in the exacerbated allergic response. Recently, Clarke et al. demonstrated that silencing of human PHF11 inhibited the expression of the Th1-type cytokine IFN- γ and IL-2 (11). However, IFN- γ and IL-4 productions by CD4 T cells were comparable between Wt and Lckd-Phf11-Tg mice *in vitro* (Figure S2). Furthermore, Th2 cytokine release from CD4 T cells induced by OVA was similar between Wt and Lckd-Phf11-Tg mice. However, numbers of OVA-specific IgE-producing PCs in Lckd-Phf11-Tg mice were larger than those in Wt mice. Thus, we believe that Phf11 in B cells may contribute importantly to the exaggerated IgE production and allergic responses.

In summary, murine Phf11 physiologically functions as a crucial molecule for CSR to IgE. The exogenous Phf11 in activated B cells augments frequencies of CSR to IgE, and it also contributes to the exacerbation of allergic responses. As human *PHF11* has been identified as the atopy-associated gene, these results may contribute to the development of a novel treatment for allergic diseases.

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Author contributions

JI performed initial experiments and participated in the writing of the paper; AI, TY, HT, NY, TT and LF analyzed the data; MA, AS, MH, KT and TT designed the study and wrote the paper. All authors read and approved this version of our manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Supplementary materials and methods.

Figure S1. The primary IgG1 production is augmented in Lck-Phf11-Tg mice.

Figure S2. Th1 or Th2 cytokine production from CD4 T cell *in vitro*.

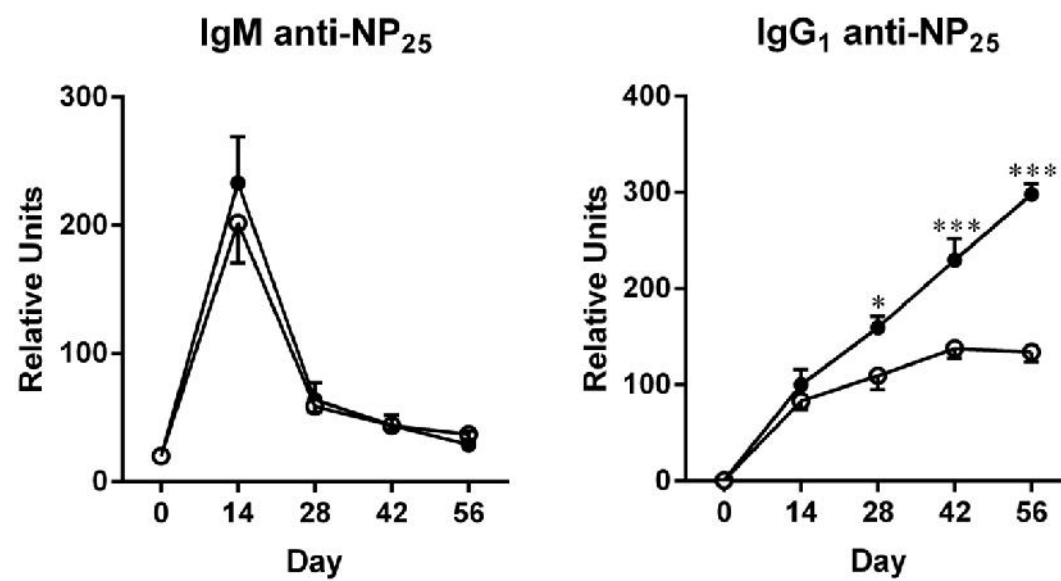
Table S1. Primer sequences for real time qPCR.

Table S2. Primer sequences for ChIP assay.

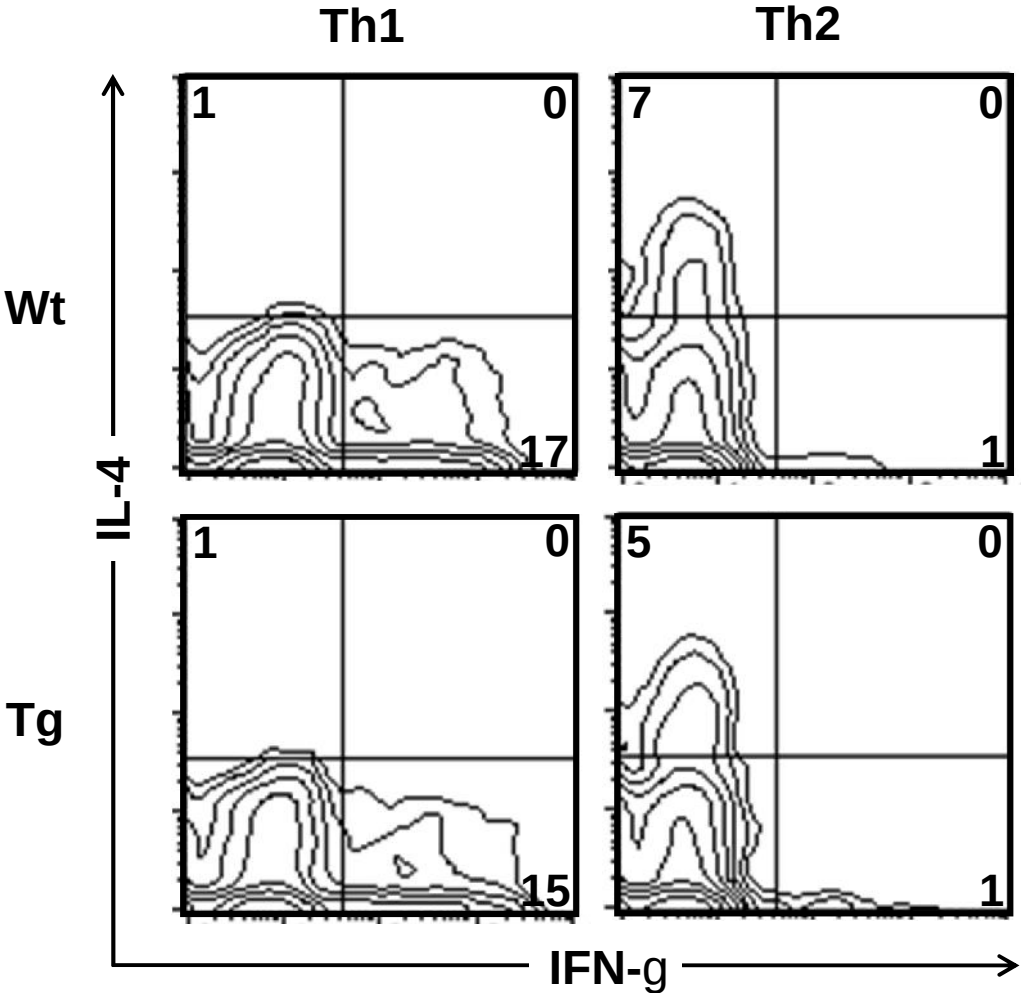
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Supplement Figure 1



Supplement Figure 2



Supplement Materials and methods

Preparation of splenic T cells

For isolation of T cells, single-cell suspensions from the spleen or the cervical lymph nodes were incubated with biotinylated anti-CD4 or -CD8 Abs (BioLegends) at 4°C for 30 min, followed by incubation with anti-biotin beads (Miltenyi Biotec). These cells were sorted using the MACS systems (Miltenyi Biotec).

Analysis of T cells *in vitro*

Splenic CD4 T cells were stimulated with a combination of plate-bound anti-CD3 mAbs (10 µg/ml, Cedarlane) and soluble anti-CD28 mAbs (2 µg/ml, Southern Biotechnology) in the presence of rIL-2 (25 U/ml, Peprotech).

Th1 or Th2 cells were generated from naive CD4 T cells cultured with rIL-12 (100 U/ml, Peprotech) / anti-IL-4 mAbs (5 µg/ml, BioLegend) or rIL-4 (100 U/ml, Peprotech) / anti-IL-12 mAbs (10 µg/ml, BD Biosciences) / anti-IFN-γ mAbs (10 µg/ml, eBioscience) for 7 days, respectively. For intracellular staining, cells were stimulated with PMA/ionomycin for 5 hours in the presence of permeabilization buffer 2 (BD). Cells were stained with anti-IFN-γ-PE (Caltag Laboratories) and anti-IL-4-APC (eBioscience).

Real-time quantitative (q) RT-PCR

Real-time PCR with cDNA was conducted using a SYBR Green PCR Master Mix (PE Applied Biosystems) and run on a CFX96 Real-Time PCR System (BIO RAD). Primers were shown in Supplement Table S1. Data were analyzed using a CFX Manager software (BIO RAD). The data were reported as the ratio of the calculated amount of candidate RNA in a given sample by the calculated amount of the housekeeping control (*GAPDH*) gene in the same sample.

ELISA

Amounts of IgM, IgG₁ and IgE Abs in sera and culture supernatants were analyzed by ELISA. Respective titers were detected with HRP conjugated anti-IgM, anti-IgG₁ or anti-IgE Abs using ELISA kit (BETHYL). Cytokines produced by activated CD4 T cells were analyzed by ELISA (R&D).

ELISPOT assay

ASCs producing IgE Abs were detected by ELISPOT assay as described elsewhere (1). Cervical lymph node cells were added to a 96-well cellulose ester membrane plate (Millipore) coated with OVA. Spots were developed with alkaline phosphatase-conjugated anti-IgE Abs (Southern Biotech).

Chromatin immunoprecipitation (ChIP) assay

ChIP assay was done by using the ChIP assay kit (UpState Biotechnology) according to the manufacturer's recommendations as described (2). The following Abs were used: anti-acetylated histone H3-K9/14 Abs (UpState Biotechnology), anti-mono/di/tri-methylated histone H3-K4 Abs (UpState Biotechnology), rabbit polyclonal anti-STAT6 Abs (Santa Cruz Biotechnology), rabbit polyclonal anti-NF-κB anti-p65 Abs (Abcam). Anti-Myc tag Abs (Abcam). Normal rabbit IgG Abs (Santa Cruz Biotechnology) was used as a control. Primer sequences for ChIP assay were shown in Supplement Table S2.

Phf11 silencing

For silencing of Phf11, we used a Mouse B cell Nucleofector Kit (Lonza). The small interfering RNAs (siRNAs) for knockdown of *phf11* (s10442) and the negative control (scrambled AM4635) were purchased from Applied Biosystems. Nucleofection of each siRNA was performed according to the manufacturer's recommendations.

Plasmid constructs

Myc-phf11 constructs. The *phf11* cDNA was amplified by PCR to introduce SalI sites on each side of the open reading frame and cloned as SalI fragments into pEF-BOS/Myc. The resulting myc-tagged constructs were ligated in sense or antisense orientation into the SalI site to generate pEF-BOS/Myc-phf11. These myc-tagged fragments were isolated from pEF-BOS/Myc-phf11 after XbaI digestion. The blunt ended XbaI fragment was inserted into a blunt ended XhoI site of pMXs-IRES-hNGFR. The PCR products were confirmed by the dideoxy chain termination method with a T7 Sequence 7-deaza-dGTP Sequencing Kit (Amersham Pharmacia Biotech).

Retrovirus transduction

Platinum-E packaging cells (3) (provided by Dr. T. Kitamura) were transfected with 1 µg of myc-phf11 retrovirus vector DNA mixed with 6 µl of Eugene (Boehringer Mannheim). Retrovirus-containing media were collected 48 hours after transfection. Retrovirus transduction was performed by using RetroNectin (Takara), according to the manufacturer's recommendations. Activated B cells were moved to a RetroNectin coated culture plate and then the retrovirus containing media was directly added to the culture.

Supplement References

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transient packaging of retroviruses. *Gene Ther* 2000;7:1063-1066.

Supplement Figure legends

Supplement Figure 1. The primary IgG1 production is augmented in Lck-Phf11-Tg mice. Wt (open circles) and Lckd-Phf11-Tg (filled circles) mice were immunized intraperitoneally with NP-CG in alum. Titers of IgM anti-NP₂₅ Abs (left), IgG₁ anti-NP₂₅ Abs (right) in sera from mice were measured by ELISA. Horizontal axis: Time after immunization (Days). Data represent means $\pm$ SEM for each group (n=5) (**P*<.05, ****P*<.001).

Supplement Figure 2. Th1 or Th2 cytokine production from CD4 T cell *in vitro*. Splenic CD4 T cells were purified from Wt and Lckd-Phf11-Tg mice. Th1 or Th2 cells were generated from naive CD4 T cells cultured with rIL-12 and anti-IL-4 mAbs or rIL-4, anti-IL-12 mAbs and anti-IFN- γ mAbs for 7 days respectively. Those cells were stimulated with PMA/ionomycin for 5 hours and intracellular IFN- γ and IL-4 expression were analyzed by FACS. Representative FACS profiles from three independent experiments are shown.

Supplement Tables

Table S1: Primer sequences for real time qPCR

Primer	Direction	sequence
<i>GLT</i> γ 1	Forward	5'-GGCCCTTCCAGATCTTTGAG-3'
	Reverse	5'-GGATCCAGAGTTCCAGGTCAC-3'
<i>GLT</i> ϵ	Forward	5'-CACAGGGGGCAGAAGATG-3'
	Reverse	5'-AGGGGTAGAGCTGAGGGTTC-3'

<i>AICDA (AID)</i>	Forward	5'-CTTTGCCCAACGAAATGTAGGAA-3'
	Reverse	5'-CTGGGTGATAATTGCACA-3'
<i>Phf11</i>	Forward	5'-TGGCTGTGGATTGTTTGGAGACAC-3'
	Reverse	5'-TGAGCCTGCTTGCACTTCAGGAGA-3'
<i>GAPDH</i>	Forward	5'-TGTGTCCGTCGTGGATCTGA-3'
	Reverse	5'-TTGCTGTTGAAGTCGCAGGAG-3'
<i>Cy1 mature transcript</i>	Forward	5'-TACGACGAGAACGGGTTTGCTTAC-3'
	Reverse	5'-GGATCCAGAGTTCCAGGTCAC-3'
<i>Cε mature transcript</i>	Forward	5'-TACGACGAGAACGGGTTTGCTTAC-3'
	Reverse	5'-AGTTCACAGTGCTCATGTTTCAG-3'
<i>Bcl6</i>	Forward	5'-CCGGCTCAATAATCTCGTGAA-3'
	Reverse	GGTGCATGTAGAGTGGTGAGTGA-3'
<i>Pax5</i>	Forward	5'-AACAGGTCTCCCCGCATCT-3'
	Reverse	AAACGCAAGAGGGATGAAGGT-3'
<i>Prdm1(Blimp1)</i>	Forward	5'-ACCAAAGCATGACCTGACATTGAC-3'
	Reverse	TGTTCAATTGTGGGCTCAACACTC-3'
<i>Xbp1</i>	Forward	5'-GGAGTGGAGTAAGGCTGGTG-3'
	Reverse	5'-ATGTTCTGGGGAGGTGACAA-3'
<i>IRF4</i>	Forward	5'-GTGGAAACACGCGGGCAAGC-3'
	Reverse	5'-GGCTCCTCTCGACCAATTCCTCA-3'

Table S2: Primer sequences for ChIP assay

Primer	Direction	sequence
<i>GLT</i> $\gamma 1$	Forward	5'-TCACCCTCACCCACACATTCACAT-3'
	Reverse	5'-AGAGAGGTGAATGCTACCCTCTTTCC-3'
<i>GLT</i> ε (STAT6 binding sequence)	Forward	5'-ACCTGGGATCAGACGATGGAGAAT-3'
	Reverse	5'-AGCAGCCTTGGAAGTTCCCTTT-3'
<i>GLT</i> ε (NF- κ B binding sequence)	Forward	5'-AAGGGAACTTCCAAGGCTGCTAAG-3'
	Reverse	5'-ACCATTAATTCATGCCCAGATGGTAGT-3'