

## Brief Communication



# CCAAT/enhancer binding protein $\beta$ Induces Post-Switched B Cells to Produce Blimp1 and Differentiate into Plasma Cells

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Received: Apr 14, 2020  
Revised: Aug 20, 2020  
Accepted: Aug 26, 2020

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### Conflict of Interest

The authors declare no potential conflicts of interest.

### Abbreviations

ASC, Ab-secreting cell; AU, arbitrary unit; BCL6, B cell lymphoma 6; Blimp1, B lymphocyte-induced maturation protein 1; BM, bone marrow; C/EBP $\beta$ , CCAAT/enhancer binding protein  $\beta$ ; cKO, conditional knockout;

## ABSTRACT

Long-lasting post-switched plasma cells (PCs) arise mainly from germinal center (GC) reactions, but little is known about the mechanism by which GC B cells differentiate into PCs. Based on our observation that the expression of the transcription factor CCAAT/enhancer binding protein  $\beta$  (C/EBP $\beta$ ) is associated with the emergence of post-switched PCs, we enquired whether a cell-autonomous function of C/EBP $\beta$  is involved in the program for PC development. To address this, we generated C/EBP $\beta$ -deficient mice in which the *Cebpb* locus was specifically deleted in B cells after transcription of the Ig  $\gamma$ 1 constant gene segment (*C $\gamma$ 1*). In response to *in vitro* stimulation, B cells from these *Cebpb*<sup>fl/fl</sup>*C $\gamma$ 1*<sup>Cre/+</sup> mice had defects in the induction of B lymphocyte-induced maturation protein 1 (Blimp1) and the formation of IgG1<sup>+</sup> PCs, but not in proliferation and survival. At steady state, the *Cebpb*<sup>fl/fl</sup>*C $\gamma$ 1*<sup>Cre/+</sup> mice had reduced serum IgG1 titers but normal IgG2c and IgM titers. Moreover, upon immunization with T-dependent Ag, the mice produced reduced levels of Ag-specific IgG1 Ab, and were defective in the production of Ag-specific IgG1 Ab-secreting cells. These results suggest that a cell-autonomous function of C/EBP $\beta$  is crucial for differentiation of post-switched GC B cells into PCs through a Blimp1-dependent pathway.

**Keywords:** Plasma cells; Immunoglobulin class switching; C/EBP $\beta$

## INTRODUCTION

Plasma cells (PCs) are terminally differentiated effector cells responsible for the production of Abs. Mature PCs that secrete class-switched high affinity Abs are central to establishing long-lasting Ab responses (1). The mature PCs mainly arise through germinal center (GC) reactions, although in a few mouse models such cells are generated via an extrafollicular pathway (2-4). In the GC—the transient structure formed in lymphoid follicles upon exposure to T-dependent (TD) Ags—primed CD4<sup>+</sup> T cells provide co-stimulation and cytokine signals to their cognate B cells (5,6). As a result, the latter undergo somatic hypermutation and class-switch recombination (CSR) of Ig genes. The post-switched cells ultimately give rise to either PCs or memory cells.

C $\gamma$ 1, Ig  $\gamma$ 1 constant gene segment; CSR, class-switch recombination; GC, germinal center; IRF4, interferon regulatory factor; KLH, keyhole limpet hemocyanin; LAP, liver-enriched activator protein; NP, 4-hydroxy-3-nitrophenylacetyl; Pax5, paired box gene 5; PC, plasma cell; Prdm1, PR domain containing 1, with ZNF domain; TD, T-dependent.

#### Author Contributions

Conceptualization: Youn J; Data curation: Jang E, Youn J; Formal analysis: Lee G; Funding acquisition: Youn J; Investigation: Lee G, Jang E; Methodology: Lee G; Supervision: Youn J; Validation: Jang E; Visualization: Lee G; Writing - original draft: Lee G; Writing - review & editing: Youn J.

Conversion of GC B cells into PCs requires silencing of the GC B cell transcriptional program, so allowing induction of the PC transcriptome. Transcription factors participating in this transition include paired box gene 5 (Pax5) and B cell lymphoma 6 (BCL6) as GC B cell identity factors, and B lymphocyte-induced maturation protein 1 (Blimp1) and interferon regulatory factor 4 (IRF4) as the main inducers of the PC transcriptional program (7,8). Unlike GC B cells, PCs lack both Pax5 and BCL6 but contain large amounts of IRF4 and Blimp1. Mutual exclusion between the genetic programs of GC B cells and PCs is evident: BCL6 inhibits the transcription of PR domain containing 1, with ZNF domain (*Prdm1*), the gene that encodes Blimp1, and Blimp1 represses transcription of the genes that encode Pax5 and BCL6 (9,10). Although the presence of this double negative feedback loop is relatively well reported, less is known about the signaling events that control the expression of the relevant transcription factors. In particular, it is not clear what other factors are needed to achieve PC differentiation after maturation of GC B cells.

CCAAT/enhancer binding protein  $\beta$  (C/EBP $\beta$ ), also known as NF-IL6, is a transcription factor containing a basic leucine zipper domain (11). C/EBP $\beta$  is expressed in many types of cell, including hepatocytes, macrophages and B cells (12). By targeting a variety of genes associated with the acute phase response, inflammation, and hematopoiesis, C/EBP $\beta$  is able to exert diverse effects on fundamental cellular processes including proliferation, survival and differentiation in a cell type-specific manner (13-15). The functional versatility of C/EBP $\beta$  is in part attributable to the presence of 3 isoforms differing in their N-terminal domains: liver-enriched activator protein (LAP), LAP\* (both are transcriptional activators), and liver-enriched inhibitory protein (a trans-dominant repressor), each having unique roles in various cellular processes (16). Previous studies suggest that C/EBP $\beta$  is also implicated in the development, survival and/or tumorigenesis of B-lineage cells. For example, mice deficient in C/EBP $\beta$  exhibit defects in the development and homeostatic expansion of B cells early in the pre-B cell stage in a cell-extrinsic manner (17). An *in vitro* study revealed that C/EBP $\beta$  regulates both proliferation and survival of multiple myeloma cells, which are neoplastic PCs, by regulating the expression of IRF4, Blimp1, and BCL2 (18). This result suggests that C/EBP $\beta$  is involved in the network of transcription factors critical for PC differentiation and survival. However, this idea has not been directly addressed.

In the present study, we aimed to determine whether C/EBP $\beta$  plays a role in the differentiation of activated GC B cells into PCs during humoral immune responses. C/EBP $\beta$  knockout mice would not be useful for this purpose, because they have an early B-cell lymphopenia. Since the program of PC development follows closely on the execution of CSR in the GC, we generated C/EBP $\beta$  conditional knockout (cKO) mice in which the gene encoding C/EBP $\beta$  could be specifically deleted in B cells after transcription of the Ig  $\gamma$ 1 constant gene segment (*C $\gamma$ 1*). We found that the emergence of PCs at the post-switched stage was impaired in the C/EBP $\beta$ -deficient B cells, and this was associated with reduced induction of Blimp1 but not of IRF4. Our data provide evidence that C/EBP $\beta$  is involved in the genetic regulatory network controlling the differentiation of post-switched B cells into PCs.

## MATERIALS AND METHODS

### Mice

B6.129P2(Cg)-*Ighg1<sup>tm1(IRES-cre)Cgn</sup>/J* (*C $\gamma$ 1<sup>Cre/+</sup>*) and B6.129S1-*Cebpb<sup>tm1Es</sup>/Mmnc* (*Cebpb<sup>fl/+</sup>*) mice (14,19) were purchased from Jackson Laboratory (Bar Harbor, ME, USA) and the Mutant Mouse

Regional Resource Center at the University of North Carolina (Chapel Hill, NC, USA), respectively, and their inbred descendants were crossed to generate *Cebpb<sup>fl/fl</sup>Cy1<sup>Cre/+</sup>* and *Cebpb<sup>fl/+</sup>Cy1<sup>Cre/+</sup>* mice. Mice were maintained in a specific pathogen-free barrier facility at Hanyang University. Sex-matched mice at 8–12-wk of age were used for experiments. This study was approved by the Institutional Animal Care and Use Committee (HY-IACUC-12-003). All animal experiments were carried out in strict accordance with guidelines and regulations.

### Primary mouse cell culture, FACS and immunoblotting assays

Single-cell suspensions were prepared from spleen as described previously (20). B220<sup>+</sup> B cells were sorted by positive selection using MACS columns (Miltenyi Biotec, Bergisch Gladbach, Germany). The purity of B cells routinely exceeded 97%. Cells were cultured in RPMI 1640 supplemented with 10% heat-inactivated FBS, 2 mM L-glutamine, 100 U/ml penicillin, 100 mg/ml streptomycin, and  $5 \times 10^{-5}$  M 2-mercaptoethanol (all from Gibco-Life Technologies Corporation, Grand Island, NY, USA) under the stimulation with 10  $\mu$ g/ml LPS (Sigma-Aldrich, St. Louis, MO, USA) and 10 ng/ml IL-4 (Peprotech, Cranbury, NJ, USA) in the presence or absence of 10 ng/ml IL-21 (Peprotech). In some experiments they were stained with 3  $\mu$ M cell proliferation dye eFluor 670 (eBioscience, San Diego, CA, USA) before culture. After growth for 96 h, cells were assayed by FACS and immunoblotting, as described previously (21,22). For FACS, cells were treated with FcR block reagent and surface or intracellular stained with anti-IgG1-PE (A85-1), anti-B220-PerCP (RA3-6B2), and/or anti-CD138-APC (281-2) mAbs (all from BD Biosciences, San Jose, CA, USA) in PBS containing 0.1% sodium azide and 0.5% BSA. For intracellular staining, cells were fixed and permeabilized using a Cytofix/Cytoperm solution kit (BD Biosciences) according to manufacturer's instructions. Data were acquired by FACS canto II, with more than 200,000 events per sample, and analyzed using Flowjo v10.6.2 (all from BD Biosciences). For immunoblotting, Abs to C/EBP $\beta$  (1H7; Biolegend, San Diego, CA, USA), Blimp1 (6D3; eBioscience), IRF4 (D9P5H; Cell Signaling, Danvers, MA, USA), GAPDH (0411; Santa Cruz, Dallas, TX, USA) and  $\beta$ -actin (C4; Santa Cruz) were used. The intensities of protein bands were quantitated using ImageJ software (NIH, Bethesda, MD, USA).

### Quantitative RT-PCR

B cells stimulated with LPS and IL-4 for 96 h were sorted into surface IgG1<sup>+</sup> and IgG1<sup>-</sup> cells with a FACS<sub>AriaIII</sub> flow cytometer (BD Biosciences). Total RNA was purified from the sorted cells and assayed by quantitative RT-PCR, as described previously (20). Primer sequences used were as follows: C/EBP $\beta$  (5' CAA GCT GAG CGA CGA GTA CA 3' and 5' GAC AGC TGC TCC ACC TTC TT 3') and  $\beta$ 2 microglobulin (5' TGA CCG GCC TGT ATG CTA TC 3' and 5' CAG TGT GAG CCA GGA TAT AG 3'). Relative amounts of C/EBP $\beta$  transcripts were normalized to the amounts of  $\beta$ 2 microglobulin transcripts.

### Mouse immunization

*Cebpb<sup>fl/fl</sup>Cy1<sup>Cre/+</sup>* and *Cebpb<sup>fl/+</sup>Cy1<sup>Cre/+</sup>* mice were injected intraperitoneally with 100  $\mu$ g of 4-hydroxy-3-nitrophenylacetyl (NP) conjugated with keyhole limpet hemocyanin (KLH) (Biosearch Technologies, Novato, CA, USA) adsorbed on 100  $\mu$ l alum (Thermo Scientific, Waltham, MA, USA). Serum, spleen and bone marrow (BM) were collected *post mortem* at days 14 and 28 post-immunization and assayed by ELISA and ELISPOT methods.

### ELISA and ELISPOT assay

Serum titers of total and NP-specific Abs were determined by standard sandwich ELISA (4). In brief, immunosorbent plates were coated with 10  $\mu$ g/ml goat anti-mouse IgG Fc fragment Ab (Bethyl Laboratories, Montgomery, TX, USA) or 10  $\mu$ g/ml NP<sub>8</sub>-BSA (Biosearch

Technologies). Sera were diluted 1:10,000-50,000 in PBS. Biotinylated Abs to mouse IgG1 (A85-1; BD Biosciences), IgG2b (R12-3; BD Biosciences), IgG2c (Abcam, Cambridge, UK), and IgM (Southern Biotech, Birmingham, AL, USA) were used as detection Abs. A serum sample containing high titer of each Ab was serially diluted and used as the reference measurement of the Ab. The amount of Ab in each serum sample was quantified as an arbitrary unit (AU) that defined the ratio of amount of the Ab to a reference measurement. The data are presented as the means $\pm$ SEMs (AU/ml). Ab-secreting cells (ASCs) were enumerated by standard ELISPOT assay (4).

## RESULTS AND DISCUSSION

### Production of cKO mice with C/EBP $\beta$ selectively deleted in C $\gamma$ 1-expressing B cells

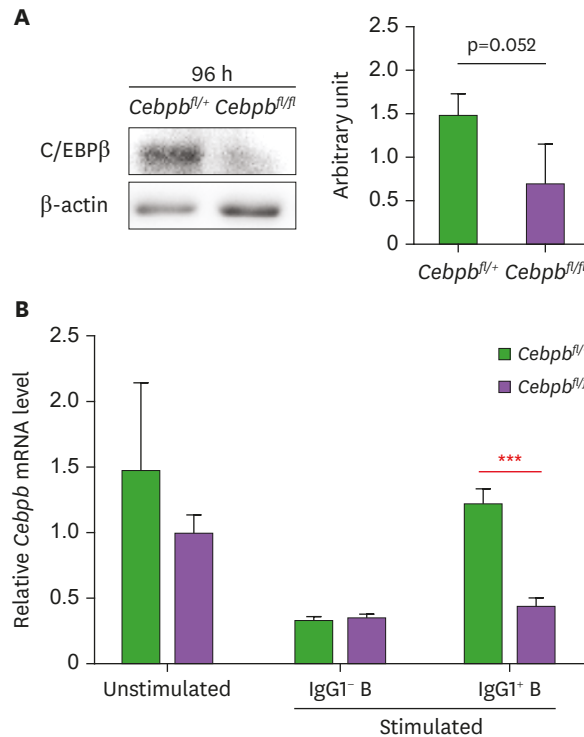
To determine if C/EBP $\beta$  expressed in post-switched B cells plays a cell-autonomous role in the differentiation into PCs, we used the C $\gamma$ 1<sup>Cre/+</sup> knock-in system to specifically delete the floxed *Cebpb* locus in B cells after C $\gamma$ 1 transcription, because C $\gamma$ 1<sup>Cre/+</sup> mice were previously proven to execute Cre-mediated recombination only in IgG1-switched GC B cells (19,23).

Unlike C/EBP $\beta$  whole KO mice that have B lymphopenia (17), the *Cebpb*<sup>fl/fl</sup>C $\gamma$ 1<sup>Cre/+</sup> mice had normal frequencies of B cells as well as T cells in central and peripheral organs at steady state (**Supplementary Fig. 1**). To evaluate if the cKO system was functional, we sorted splenic B cells from *Cebpb*<sup>fl/fl</sup>C $\gamma$ 1<sup>Cre/+</sup> and *Cebpb*<sup>fl/+</sup>C $\gamma$ 1<sup>Cre/+</sup> mice and stimulated with LPS and IL-4 for 96 h to polarize them mainly into IgG1 class-switched cells. We found that the *Cebpb*<sup>fl/fl</sup>C $\gamma$ 1<sup>Cre/+</sup> B cells expressed significantly lower amounts of C/EBP $\beta$  protein than the *Cebpb*<sup>fl/+</sup>C $\gamma$ 1<sup>Cre/+</sup> B cells (**Fig. 1A**).

To confirm the selective ablation of C/EBP $\beta$  in the C $\gamma$ 1-expressing cells, we sorted surface IgG1<sup>+</sup> and IgG1<sup>-</sup> cells after 96 h and conducted quantitative RT-PCR. We found that the level of C/EBP $\beta$  mRNA was reduced by approximately 60% in the IgG1 class-switched cells from the *Cebpb*<sup>fl/fl</sup>C $\gamma$ 1<sup>Cre/+</sup> mice, but not in the IgG1<sup>-</sup> and unstimulated B cells (**Fig. 1B**). This result clearly demonstrates specific ablation of C/EBP $\beta$  in those B cells that underwent CSR to IgG1. Despite its specificity, the reduction of C/EBP $\beta$  expression was not 100%, presumably due to allelic exclusion in heterozygotes for wild-type C $\gamma$ 1 and mutant C $\gamma$ 1-Cre (24).

### C/EBP $\beta$ deficiency impairs development of PCs into post-switched B cells

To see whether the absence of C/EBP $\beta$  affected the activation and differentiation of B cells at the post-switched stage, we cultured B cells from *Cebpb*<sup>fl/fl</sup>C $\gamma$ 1<sup>Cre/+</sup> mice and their control littermates in the presence of LPS, IL-4, and IL-21 for 96 h, and assayed the phenotypes of the resultant cells. Unlike the situation in multiple myeloma cells (18), the B cells from *Cebpb*<sup>fl/fl</sup>C $\gamma$ 1<sup>Cre/+</sup> mice proliferated and survived in response to stimulation just like those from *Cebpb*<sup>fl/+</sup>C $\gamma$ 1<sup>Cre/+</sup> mice (**Fig. 2A and B**). In contrast, the intracellular IgG1<sup>+</sup> *Cebpb*<sup>fl/fl</sup>C $\gamma$ 1<sup>Cre/+</sup> B cells gave rise to significantly fewer cells expressing CD138, a marker of PCs, than the intracellular IgG1<sup>+</sup> *Cebpb*<sup>fl/+</sup>C $\gamma$ 1<sup>Cre/+</sup> B cells, and this phenomenon was restricted to IgG1<sup>+</sup> cells only (**Supplementary Fig. 2 and Fig. 2C**). Consistent with this, whereas Blimp1 expression was induced in the *Cebpb*<sup>fl/+</sup>C $\gamma$ 1<sup>Cre/+</sup> B cells in response to the 96-h stimulation, it failed to be induced in the *Cebpb*<sup>fl/fl</sup>C $\gamma$ 1<sup>Cre/+</sup> B cells (**Fig. 2D**). However, the presence or absence of C/EBP $\beta$  did not significantly affect the expression of IRF4. These results demonstrate that C/EBP $\beta$  expression in post-switched B cells plays a cell-autonomous role in the differentiation of PCs but not in their proliferation and survival. This effect is associated with the induction



**Figure 1.** Selective deficiency of C/EBPβ in IgG1<sup>+</sup> B cells of *Cebpb*<sup>fl/fl</sup>*Cy1*<sup>Cre/+</sup> mice. (A) B220<sup>+</sup> B cells sorted from *Cebpb*<sup>fl/fl</sup>*Cy1*<sup>Cre/+</sup> and *Cebpb*<sup>fl/+</sup>*Cy1*<sup>Cre/+</sup> mice were cultured with LPS and IL-4 for 96 h and assayed by immunoblotting. A representative blot and quantitated results of 3 independent experiments are shown. (B) The cultured cells were fractionated into surface IgG1<sup>+</sup> and IgG1<sup>-</sup> cells by FACS, and each fraction was assayed by quantitative RT-PCR. Unstimulated cells were used as a control. The data are representative of 3 independent experiments. \*\*\**p*<0.001 by unpaired 2-tailed Student's *t*-test.

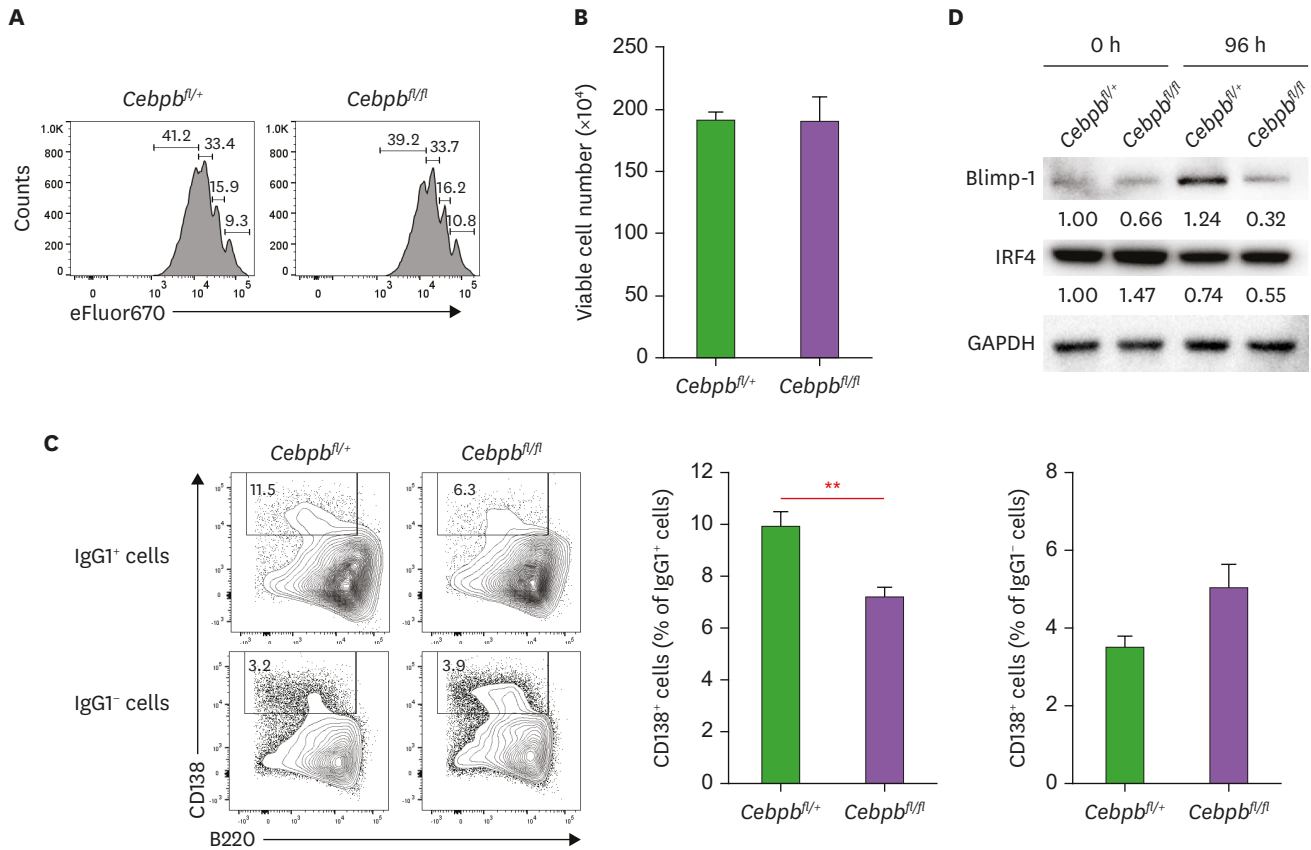
of Blimp1 but not IRF4, suggesting that C/EBPβ act as an upstream regulator of Blimp-1 but IRF4 is not.

Blimp1 is a master regulator that promotes the terminal differentiation of B cells. Indeed, within the B cell lineage, Blimp1 is exclusively expressed in ASCs, with its concentration dependent on the maturation state (25). The proposed functions of Blimp1 include repression of key regulators of the B cell program such as Pax5 and BCL6. Consistent with a previous study used multiple myeloma cell lines (18), our results provide evidence that the C/EBPβ–Blimp1 axis does indeed operate in post-GC B cells. However, our results differ from that study in terms of the effect of C/EBPβ on IRF4 induction. The discrepancy may stem from differences in the effect of IRF4 depending on its concentration. At low levels IRF4 promotes the GC fate through activation of *Aicda*, *Pou2af1* and *Bcl6*, while at high levels it promotes the PC fate through repression of *Bcl6* and activation of *Prdm1* and *Zbtb20* (26). Therefore, the expression level of IRF4 is critical for determining cell fate at the GC B cell stage, and our results suggest that C/EBPβ does not influence the level of IRF4 at that stage.

### The presence of C/EBPβ in post-switched B cells is required to maintain the natural Ab pool at steady state

Sera from normal mice contain a substantial level of Abs, which arise naturally. The size of the natural Ab pool is important as are the isotypes that are present, because the Abs play isotype-specific roles, such as protection against infectious agents (27). To determine whether a





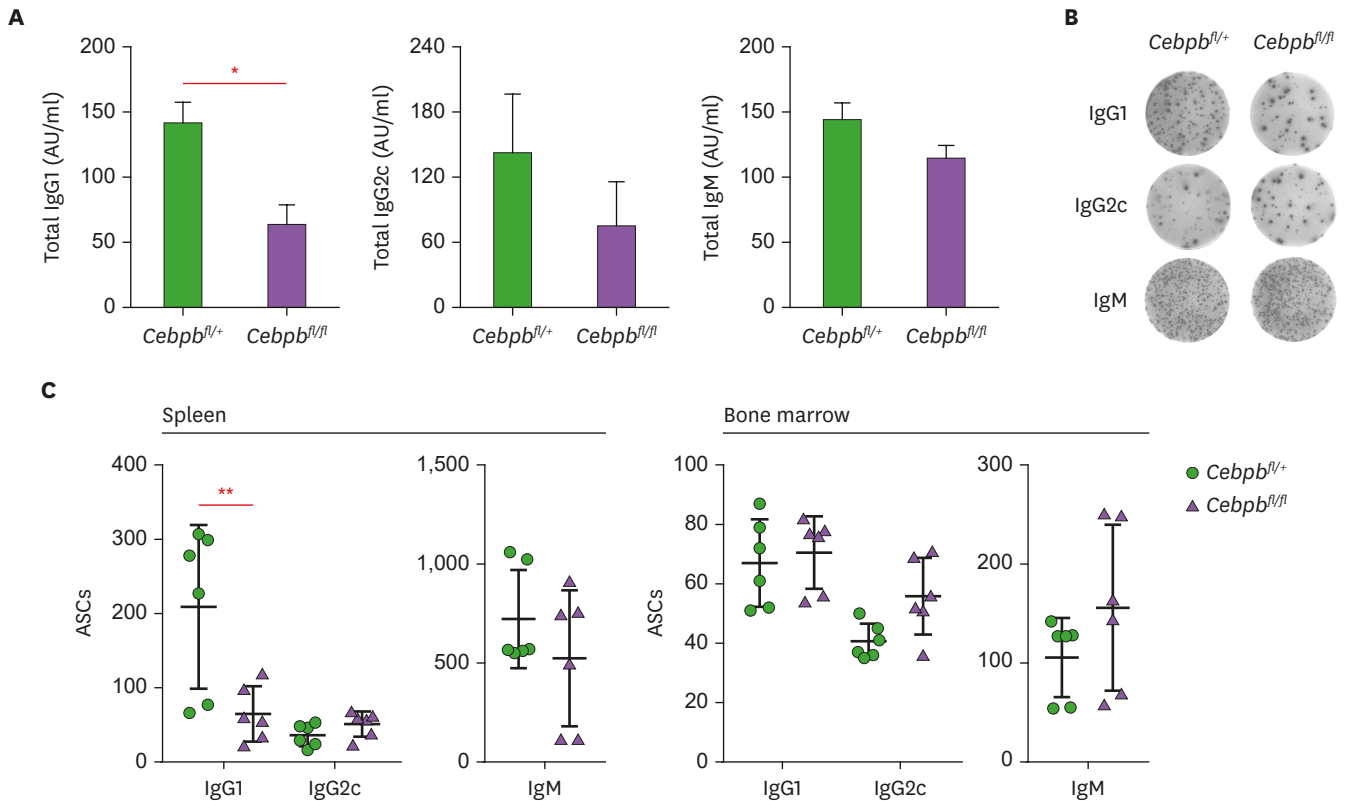
**Figure 2.** The effect of C/EBP $\beta$  ablation on post-switched B cells *in vitro*. B220<sup>+</sup> B cells sorted from *Cebpb<sup>fl/fl</sup>Cy1<sup>Cre/+</sup>* and *Cebpb<sup>fl/fl</sup>Cy1<sup>Cre/+</sup>* mice were stained with eFluor 670 (A) or left unstained (B-D) and stimulated with LPS, IL-4, and IL-21 for 3 (A) or 4 (B-D) days. They were then assayed by FACS (A, C), trypan blue exclusion (B) and immunoblotting (D) methods. Representative FACS profiles gated on lymphocytes (A) and either intracellular IgG1<sup>+</sup> or IgG1<sup>-</sup> cells (C), with percentages of cells in the indicated areas, are shown. A representative immunoblot with quantitated results is shown (D). The data are representative of 2 independent experiments.

\*\* $p < 0.01$  by unpaired 2-tailed Student's *t*-test.

cell-autonomous role of C/EBP $\beta$  in post-switched B cells contributes to Ab production in mice at steady state, we measured the serum titers of Abs and numbers of ASCs in untreated mice. Compared with their control littermates, *Cebpb<sup>fl/fl</sup>Cy1<sup>Cre/+</sup>* mice contained normal titers of serum IgM and IgG2c, but a significantly reduced titer of serum IgG1 (Fig. 3A). IgG1 ASCs were also significantly less numerous in the spleens of *Cebpb<sup>fl/fl</sup>Cy1<sup>Cre/+</sup>* mice, but numbers of IgG2c- and IgM ASCs were unaltered (Fig. 3B and C), as were numbers of ASCs in the BM, regardless of isotypes. These data demonstrate that deficiency of C/EBP $\beta$  at the post-switched stage impairs the emergence of ASCs in the spleen, thereby leading to reduced serum titers of class-switched Abs. This result thus suggests that C/EBP $\beta$  is crucial for maintenance of the class-switched natural Ab pool.

### A cell-autonomous role of C/EBP $\beta$ in post-switched B cells is necessary for long-lasting TD Ab responses

Affinity-matured class-switched Abs are mostly produced as a result of the TD GC reaction. To determine whether a cell-autonomous function of C/EBP $\beta$  in post-switched GC B cells is critical for eliciting TD Ab responses, we immunized mice with a mixture of NP-KLH and alum, and measured Ab titers and ASCs specific for NP. On day 14 post-immunization, sera from *Cebpb<sup>fl/fl</sup>Cy1<sup>Cre/+</sup>* mice contained a significantly lower level of NP-specific IgG1 Ab than

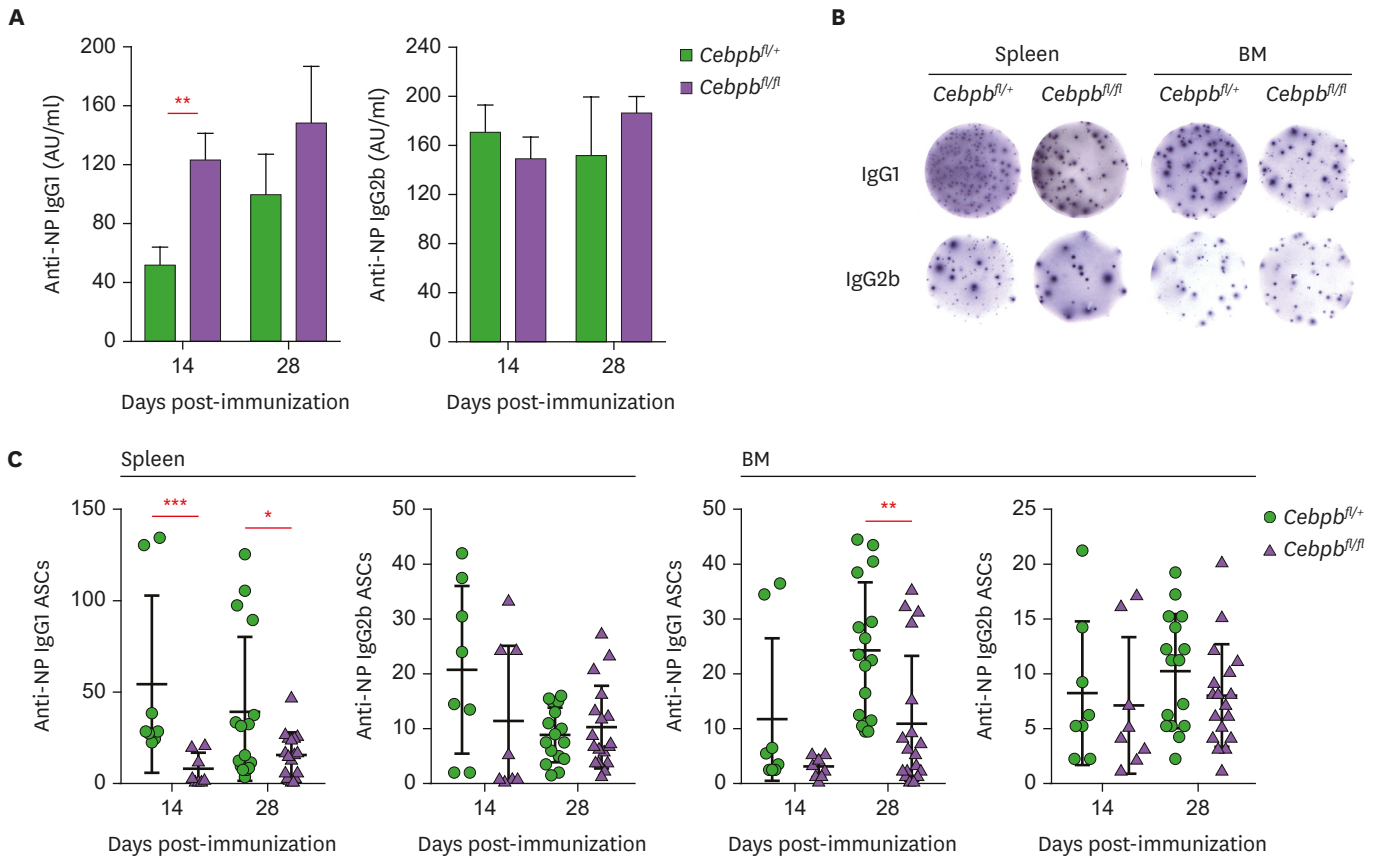


**Figure 3.** *Cebpb<sup>fl/fl</sup>Cγ1<sup>Cre/+</sup>* mice have lower IgG1 titers and less numerous IgG1-secreting cells at steady state. (A) Sera were collected from naive *Cebpb<sup>fl/+</sup>Cγ1<sup>Cre/+</sup>* and *Cebpb<sup>fl/fl</sup>Cγ1<sup>Cre/+</sup>* mice (n=12 per group) and assayed by ELISA to measure titers of total IgG1, IgG2c and IgM Abs. (B, C) Splens and BM were collected from naive *Cebpb<sup>fl/+</sup>Cγ1<sup>Cre/+</sup>* and *Cebpb<sup>fl/fl</sup>Cγ1<sup>Cre/+</sup>* mice and assayed by ELISPOT to enumerate ASCs. Representative ELISPOT wells with spleen cells are shown (B). The plots present the means±SEM of ASCs per 3×10<sup>5</sup> cells in splens and BM. Each symbol represents the value of an individual mouse. The data are representative of 3 independent experiments (B, C).

\*p<0.05, \*\*p<0.01 by unpaired 2-tailed Student's t-test.

those from their control littermates but a similar level of NP-specific IgG2b Ab (Fig. 4A). NP-specific IgG1 ASCs were less abundant in the splens of the *Cebpb<sup>fl/fl</sup>Cγ1<sup>Cre/+</sup>* mice on day 14 post-immunization and this difference was maintained to day 28 (Fig. 4B and C). Numbers of NP-specific IgG2b ASCs did not differ between the 2 strains at either time. This IgG1-specific decrease was also seen in the BM but only on day 28. These results demonstrate that *Cebpb<sup>fl/fl</sup>Cγ1<sup>Cre/+</sup>* mice have a defect in generating IgG1 class-switched Ab-secreting PCs in the spleen and in maintaining them in the BM. Thus, these results suggest that C/EBPβ plays a post-switched GC B cell-intrinsic role in differentiation of the B cells into PCs in the spleen and their maintenance in the BM as a long-lived PC compartment.

In summary, our current study demonstrates that C/EBPβ is crucial for execution of the PC program at the post-GC stage. By promoting the differentiation of post-switched B cells into PCs, it functions not only in maintaining the pool of natural Abs at steady state but also in eliciting effective humoral immunity involving the production of long-lasting Ag-specific isotype-switched Ab in response to immunization. Such a cell-autonomous function of C/EBPβ appears to be associated with its ability to induce Blimp1 expression through an IRF4-independent pathway. Thus, we have identified a novel function of C/EBPβ in the PC program operating in post-switched GC B cells.



**Figure 4.** The effect of C/EBPβ ablation in post-switched B cells on TD Ab responses. *Cebpb<sup>fl/fl</sup>Cγ1<sup>Cre/+</sup>* and *Cebpb<sup>fl/+</sup>Cγ1<sup>Cre/+</sup>* mice were immunized with NP-KLH in alum, and sera, spleens and BM were collected on days 14 and 28 after injection. (A) Sera were assayed by ELISA to determine levels of NP-specific IgG1 and IgG2b. (B, C) Spleens and BM were assayed by ELISPOT to enumerate NP-specific ASCs. Representative ELISPOT wells with spleen cells at day 28 post-immunization are shown (B). The plots show means±SEM of ASCs per 1×10<sup>6</sup> cells in spleens and per 5×10<sup>6</sup> cells in BM. Each symbol represents the value of an individual mouse. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001 by unpaired 2-tailed Student's t-test.

## ACKNOWLEDGMENTS

We thank Mr. Changyeon Kim for technical assistance and the Analytical Instrumentation Center (Seoul) at Hanyang University for technical support. This work was supported by a grant from National Research Foundation of Korea (NRF-2018R1A2B6004853).

## SUPPLEMENTARY MATERIALS

### Supplementary Figure 1

Normal development of B and T cells in *Cebpb<sup>fl/fl</sup>Cγ1<sup>Cre/+</sup>* mice. Cells from the indicated lymphoid organs of *Cebpb<sup>fl/fl</sup>Cγ1<sup>Cre/+</sup>* mice and their control littermates were analyzed by FACS. Representative FACS profiles gated on live lymphocytes are shown.

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**Supplementary Figure 2**

The emergence of IgG1<sup>+</sup> cells in *Cebpb<sup>fl/fl</sup>Cy1<sup>Cre/+</sup>* mice. B220<sup>+</sup> B cells sorted from *Cebpb<sup>fl/fl</sup>Cy1<sup>Cre/+</sup>* and *Cebpb<sup>fl/fl</sup>Cy1<sup>Cre/+</sup>* mice were stimulated with LPS, IL-4, and IL-21 for 4 days, stained to detect intracellular IgG1, and analyzed by FACS. Representative FACS profiles gated on live lymphocytes are shown.

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