Supplementary Figure S1.

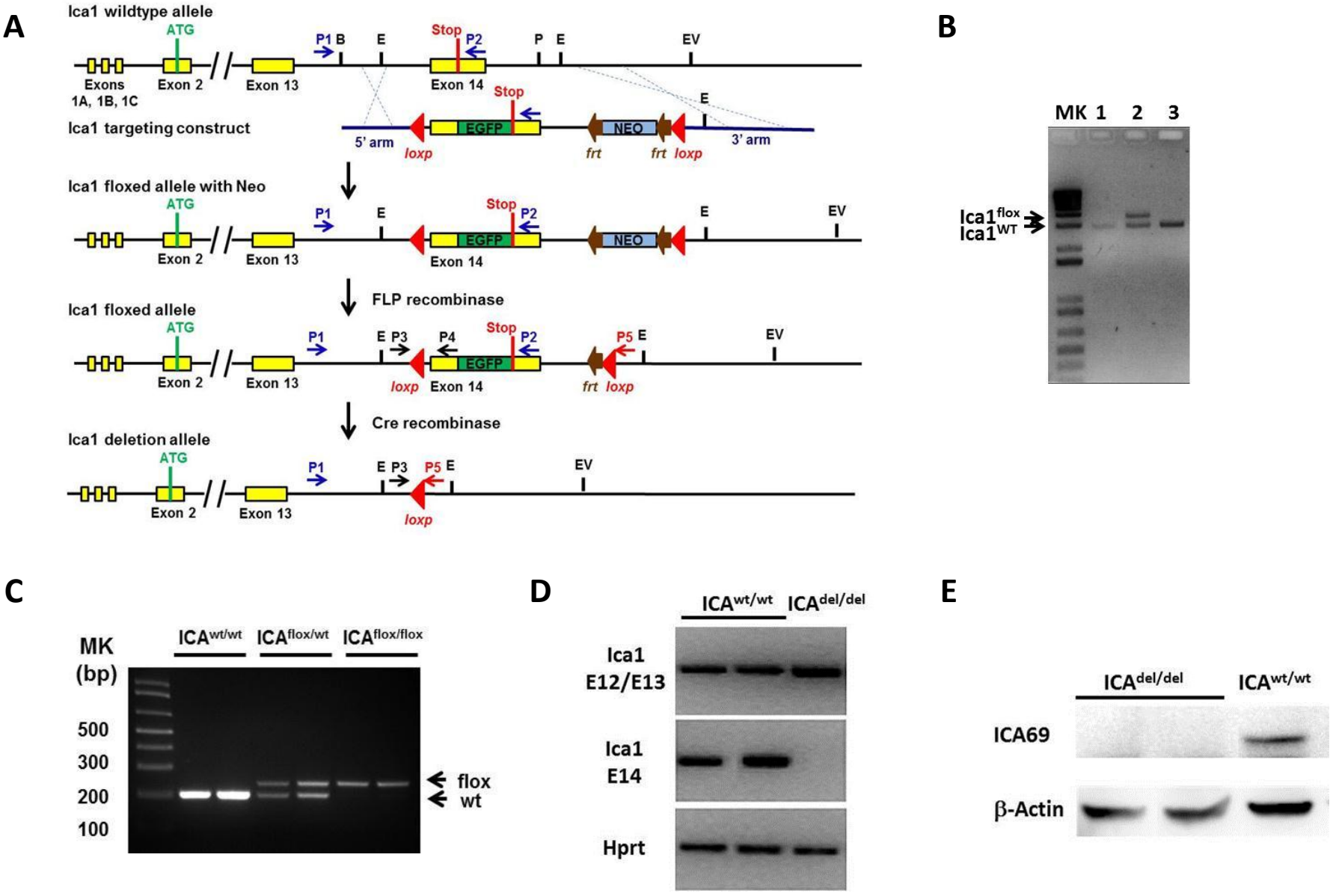

Figure S1. Generation of the Ica1 floxed mice and the Ica1 knockout mice

A. Schematic view of the experimental strategies to generate the Ica1 floxed and Ica1 global deletion mice.

The Ica1 targeting construct was assembled with traditional cloning method (details upon request). The sizes of the 5' and 3' homologous arms are 1.7 kb and 8 kb, respectively (drawing not to scale). In addition, the coding sequence of the enhanced green fluorescent protein (EGFP) gene was inserted in-frame at the 3' end of the *Ica1* gene, to encode an ICA69-EGFP fusion protein. Of the two *loxp* sites (red arrows) genetically engineered to flank the exon 14 of the *Ica1* gene, one was inserted at the 5' proximal location of the exon 14, and the second one was positioned at the 3' end of the *FRT*-Neo-*FRT* selection cassette.

Homologous recombination (blue dotted lines) between the targeting construct and the Ica1 wildtype allele result in the Ica1 floxed allele with Neo cassette (the third line drawing from the top). The action of the FLP flippase will mediate recombination of the two FRT sites and eliminate the Neo cassette from the genome to generate the Ica1 floxed allele ($Ica1^{flox}$, the fourth line drawing from the top). In cells expressing the Cre recombinase, recombination of the two loxp sites will delete the DNA element in between, including the exon 14 of the Ica1 gene, resulting the Ica1exon 14 deletion allele ($Ica1^{del}$). Brown arrows, the flippase recognition target (*FRT*) sites; Yellow boxes, Ica1 exons; Green box, EGFP, Blue box, Neo selection marker cassette. ATG, translational starting site; Stop, translational stop site. E, *Eco*RI; EV, *Eco*RV; B, *Bam*HI; P, *Pml*I. FLP, Flippase (recombinase).

PCR Primers

P1 and P2: PCR primers to screen homologous recombinant clones. PCR products for wildtype and the Ica1 floxed alleles are 2.9kb and 3.6kb, respectively.

P1	5' CAGTAAAAGGATAGAGTTTTAGGCATAA	3'
P2	5' CACTGAGCATATTAGACTTCTGCAAG	3'

P3 and P4: PCR primers for genotyping the wildtype and the Ica1 floxed alleles (200bp and 260bp, respectively).

P3	5' TCTTAAGAGTTCAGTGTCATTTAGA	3'
P4	5' TTCCCAACAGGTGGCGACGTCAGAA	3'

P3 and P5: PCR primers for genotyping the deletion allele (200bp).

P3	5' TCTTAAGAGTTCAGTGTCATTTAGA	3'
P5	5' GGAGACACTAGGATGTGCAAGGAT	3'

Figure S1. Generation of the Ica1 floxed mice and the Ica1 knockout mice

B. PCR analysis of genomic DNA isolated from clones of ES cells carrying heterozygous for the Ica1 floxed (Ica^{flox}) allele (lane #2), using PCR primer pairs (P1 and P2) shown in **A**. Lanes 1 and 3 were DNA from wild type ES cells. The sizes of the PCR products of the wildtype allele (Ica^{wt}) and the Ica^{flox} are about 2.9kb and 3.6kb, respectively. The PCR products were verified with sequencing.

C. Representative electrophoresis agarose gel of mouse tail DNA samples characterized with primers P3 and P4, showing wildtype, heterozygous and homozygous genotypes.

D. RT-PCR analysis of Ica1 mRNA transcripts in testes of $ICA^{del/del}$ mice. $Ica1^{flox/flox}$ mice were first crossed to B6.CMV-Cre mice (Jackson Lab, Maine) to obtain the $Ica1^{del/wt}$ mice, followed by heterozygous mating to generate the *Ica1* global deletion ($Ica1^{del/del}$) line. Top panel, primer pairs designed to amplify a cDNA fragment transcribed from both exon 12 and exon 13 of the Ica1 gene were used in the RT-PCR reaction, showing the presence of a 3' truncated Ica1 mRNA transcripts (Forward primer, 5' ACATGACACATGCTCAGGACCCAT 3'; Reverse primer, 5' TTGCAGAGAGGCCTGGAGATCTTT 3'. Middle panel, primer pairs specific to the targeted exon 14 of the Ica1 gene were used, showing the absence of exon 14-containing Ica1 mRNA transcript (Forward primer, 5' TGAGTCTGCAACCTTCAACAGGGA 3'; Reverse primer, 5' AAACAGGGCCTTGACCCTCTCATT 3').

E. Western blot analysis of ICA69 protein expression in testes of $ICA^{del/del}$ mice. Rabbit anti-mouse exon 13 antibodies were used to probe protein extracts isolated from testes of $ICA^{del/del}$ mice. These results showed that although a 3' truncated Ica1 mRNA transcript was transcribed, no detectable truncated ICA69 protein was produced in $ICA^{del/del}$ mice.

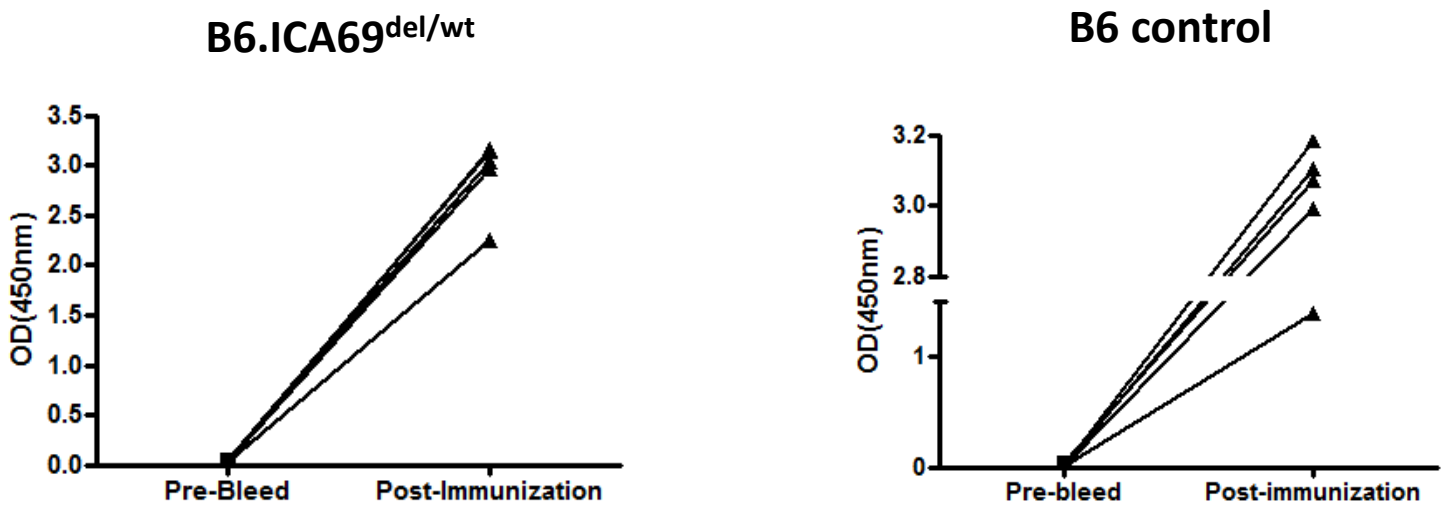

Figure S2. Development of anti-E14 humoral responses in B6.ICA69$^{del/wt}$ mice. Levels of IgG anti-E14 reactivity in sera of B6.ICA69 $^{del/wt}$ mice (*left* panel) and wildtype controls (right control), 4 months after immunization with E14.

Supplementary Figure S3.

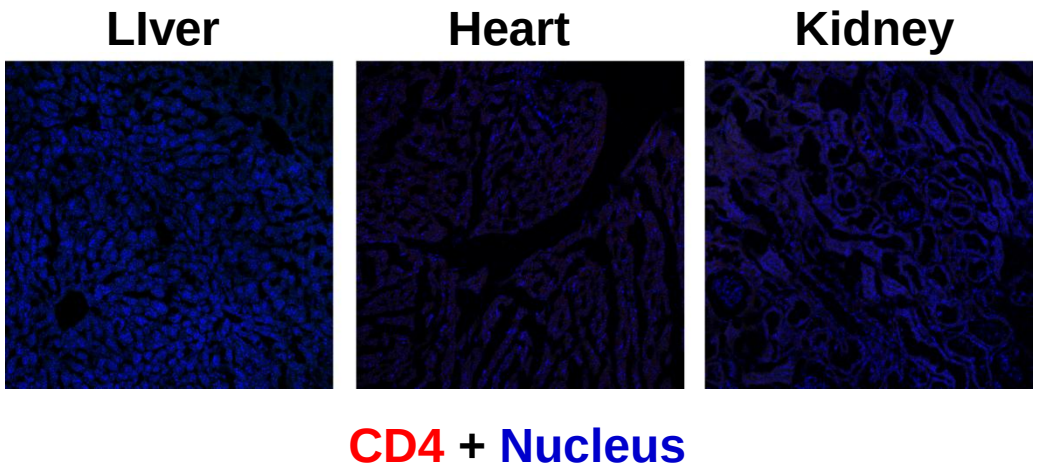

Figure S3. Absence of lymphocytic infiltration in organs with low levels of ICA69 expression in E14-immunized B6. ICA69$^{del/wt}$ mice. Immunohistochemistry showing lymphocytic infiltration into multiple tissues of 24-week old ICA69$^{del/wt}$ mice. Representative cryosections of liver, heart and kidney were stained with anti-CD4 antibody (red) and counter-stained with Hoechst 33342.

Supplementary Figure S4.

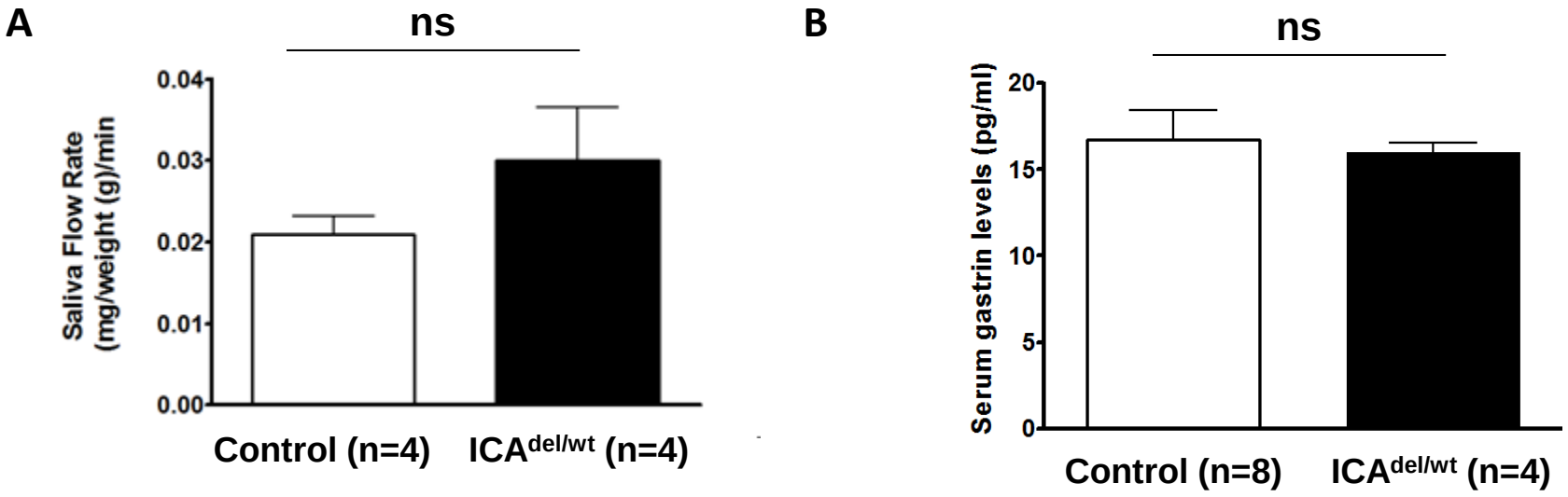

Figure S4. Normal saliva and gastrin secretion in E14-immunized B6. ICA69$^{del/wt}$ mice. A. No significant difference of saliva flow rates were detected between ICA$^{del/wt}$ mice and ICA$^{wt/wt}$ littermate controls. Whole saliva was collected for 20 minutes after anesthetization and pilocarpine stimulation. Salivary flow rate data are shown as mean ±SEM. ns, not significant. **B.** Serum gastrin levels of ICA$^{del/wt}$ (n=4) and control ICA$^{wt/wt}$ mice (n=8) were measured with ELISA kit. ns, not significant.

Supplementary Figure S5.

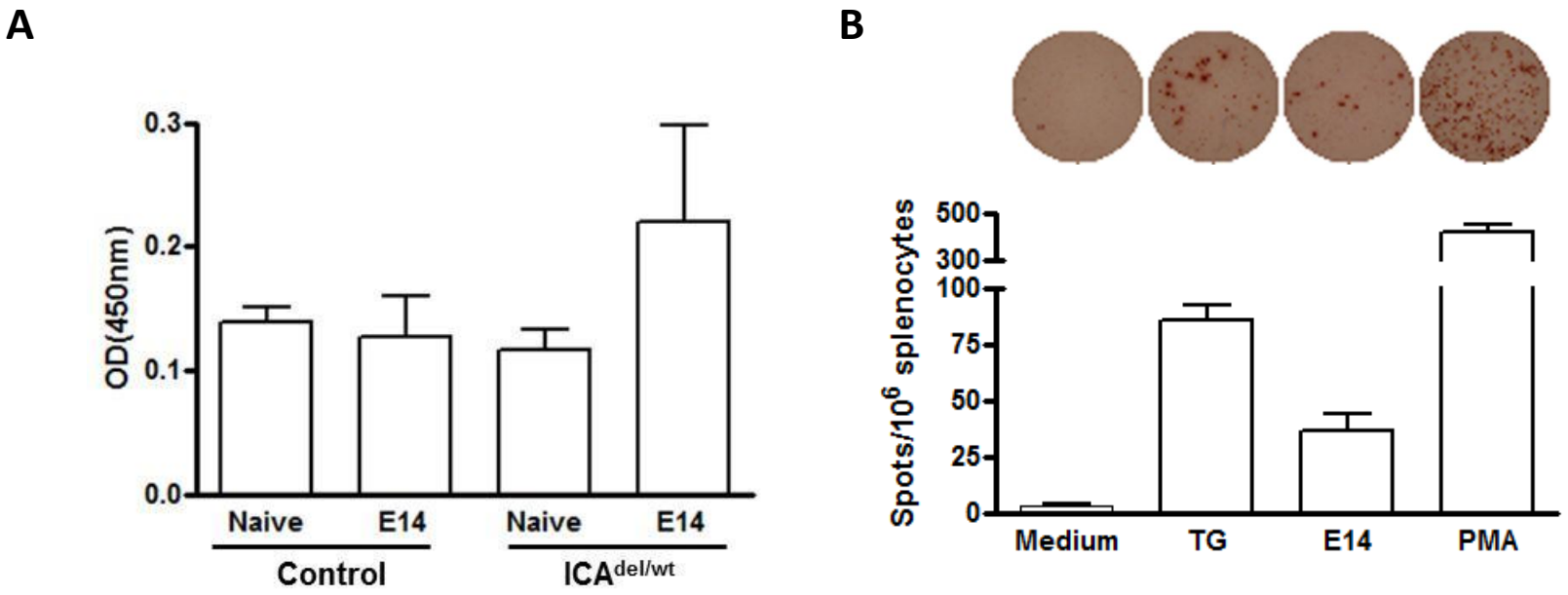

Figure S5 E14-immunized B6. ICA69$^{del/wt}$ mice develop autoimmunity against thyroglobulin (TG). A. Serum anti-TG IgG levels in B6.ICA69 $^{del/wt}$ mice 4 months after immunization with E14. Samples from wildtype littermates (ICA69$^{wt/wt}$) were used as controls. Naïve, prebleed serum samples. **B.** ELISPOT analysis of IFNg-producing cells in the spleens of E14 immunized ICA69$^{del/wt}$ mice. 1x10^6 splenocytes were cultured overnight *in vitro*, stimulated with medium (background), thyroglobulin (TG, 10ug/ml), exon 14 (E14, 10ug/ml) and PMA/ionomycin (PMA, 1x10^4 splenocytes as positive control). Numbers of spots similar to background levels were observed in splenocytes harvested from wild-type littermate controls stimulated with either TG or E14. Shown on the top are representative ELISPOT images.

Supplementary Figure S6.

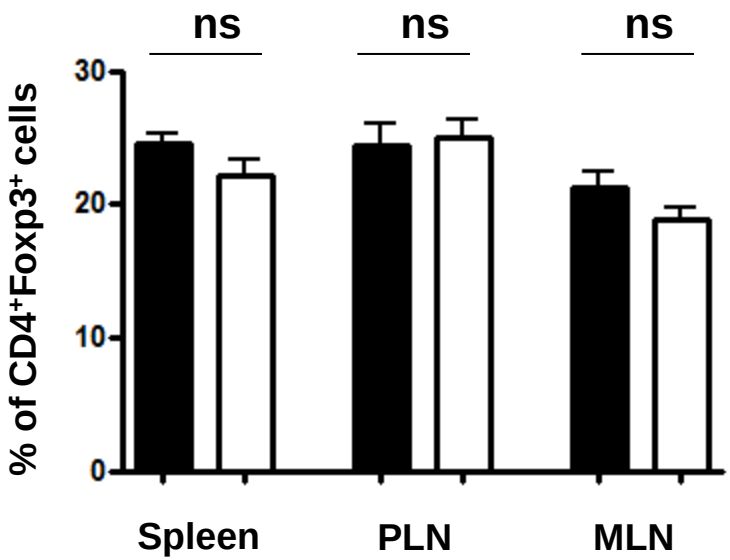

Figure S6. Normal Treg cell development in Aire-ΔICA69 mice. Cells were isolated from the spleen, pancreatic lymph nodes (PLN) and mesenteric lymph nodes (MLN) of 16-20 week old Aire-ΔICA (n=4, filled bar) and $ICA^{flox/flox}$ controls (n=4, open bar), stained intracellularly with anti-Foxp3 antibody, and analyzed with flow cytometry. The percentages of CD4+Foxp3+ Treg cells are presented as mean±SEM. ns, not significant.