Generation of AID conditional knockout mice

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Activation-induced cytidine deaminase (AID) initiates class-switch recombination and somatic hypermutation of immunoglobulin genes. In addition to this function, AID is also implicated in epigenetic regulation in pluripotent stem cells and in oncogenesis of lymphoid and non-lymphoid origins. To examine role of AID in specific cell types, we developed conditional knockout of AID in mice and serve it freely available to the community. We took so-called "three-loxP strategy". Aicda gene consistis of 5 exons and catalytic domain is encoded by exons 2 and 3. The targeting vector contains loxP-RFP-frt-Neo-frt-loxP before exon 2 and single loxP after exon 3. Heterozygously targeted mice were crossed with Flp mice to obtain loxP-RFP-frt-loxP-exon 2-exon 3-loxP configuration [Aicda-RFP]. We confirmed expression of RFP in B cells of geminal centers of spleen and intestine-associated lymphoid tissue. After crossing Aicda-RFP mice with Cre mice driven by tissue-nonspecific alkaline phosphate promoter, we could obtain partial and complete deletion, namely: loxP-exon 2-exon 3-loxP [Aicda-FL] and deletion of exons 2 and 3 [Aicda-KO]. Homozygous mice were obtained for each genotype and were checked for AID activity by serum IgG ELISA and in vitro class-switch assay. AID activity was normal for Aicda-FL but partially and completely absent for Aicda-RFP and Aicda-KO, respectively. Aicda-FL mice would be useful for the studies of AID function in subpopulation of B cells and in non-lymphoid cells.

1. Conditional knockout strategy

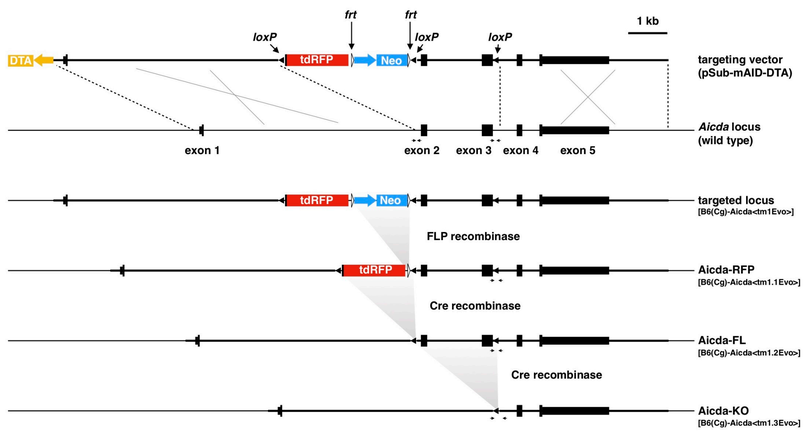

Figure 1. Generation of AID conditional knockout mice. In the targeted locus, loxP-franking element containing tandem-dimer RFP (tdRFP) fused with splice acceptor of exon 2 and frt-franking Neo cassette was inserted before exon 2. The third loxP was introduced after exon 3. ES cells with C57BL/6J background (BRUCE-4) were electroporated with the targeting vector. Targeted mice were crossed with FLP-transgenic mice (C57BL/6J background) to obtain **Aicda-RFP** mice. **Aicda-RFP** mice (3 loxPs) were crossed with male TNAP-Cre mice (C57BL/6J background) that express Cre in primordial germ cells to obtain 2-loxP partial recombination products **Aicda-FL** and **Aicda-RFP-KO** (deletion between the 2nd and the 3rd loxPs; not shown) and single-loxP product **Aicda-KO** allele. Homozygous **Aicda-RFP**, **Aicda-FL** and **Aicda-KO** mice were viable. Horizontal arrows represent positions of primers for genotyping.
DTA, diphtheria toxin A; RFP, red fluorescent protein. Neo, neomycin resistance. TNAP, tissue-nonspecific alkaline phosphatase.

	♀wild type x ♂Aicda-RFP+TNAP-Cre	♀Aicda-RFP/RFP x ♂TNAP-Cre
wild type	9 (3)	0 (0)
Aicda-RFP	0 (0)	3 (1)
Aicda-RFP-KO	0 (0)	2 (2)
Aicda-FL	2 (1)	0 (0)
Aicda-KO	8 (5)	4 (4)

Table 1. Partial deletion by Cre
The number of pups with indicated genotype is shown after two kinds of crossing: female wild type with male Aicda-RFP heterozygote with TNAP-Cre transgene; and female Aicda-RFP homozygote with male TNAP-Cre transgenic mouse. Both types of partial recombination products Aicda-FL and Aicda-RFP-KO were obtained. Aicda-RFP was chosen for subsequent analyses because it maintains all the sequence including exon 2 and exon 3 which potentially contains regulatory elements. Number in parenthesis is the number of pups possessing TNAP-Cre transgene.
TNAP, tissue-nonspecific alkaline phosphatase.

2. RFP expression reflects endogenous AID expression in Aicda-RFP mice

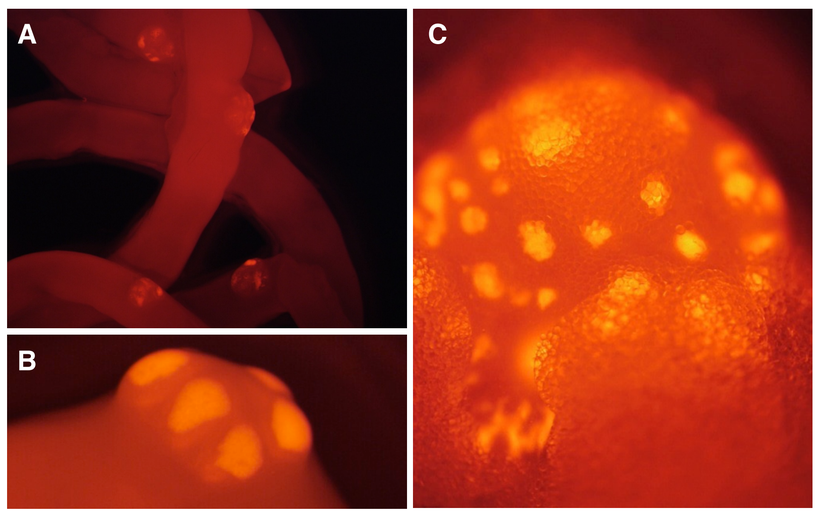

Figure 2. Red fluorescence images of small intestine from Aicda-RFP mice. RFP fluorescence was observed in germinal centers. A. small intestine. B. Peyer's patch. C. Mesenteric lymph node. Germinal centers in spleen were also RFP-positive (not shown). Other organs did not show overt fluorescence.

3. Aicda-RFP mice show leaky AID expression

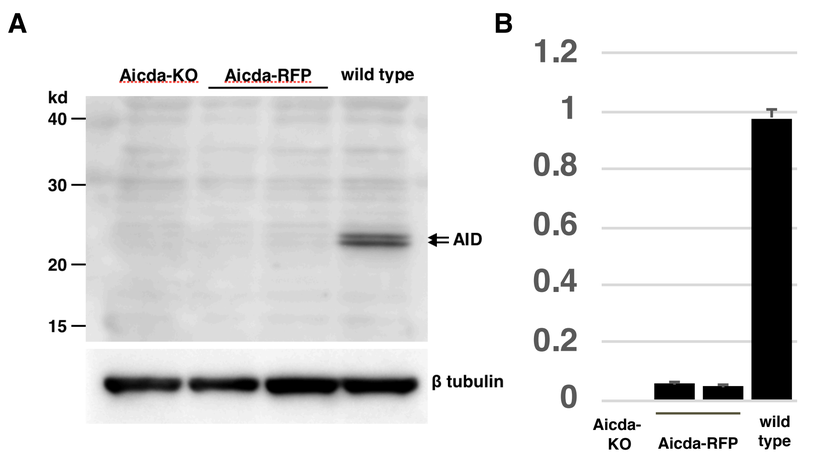

Figure 3. AID expression by western blot (**A**) and quantitative RT-PCR (**B**). Two individuals were analyzed for Aicda-RFP homozygous mice. The PCR primers for (B) span intron between exon 2 and exon 3. The result was calibrated with ribosomal 18S RNA amount.

4. Aicda-RFP and Aicda-KO mice have partial and complete class switch defect, respectively

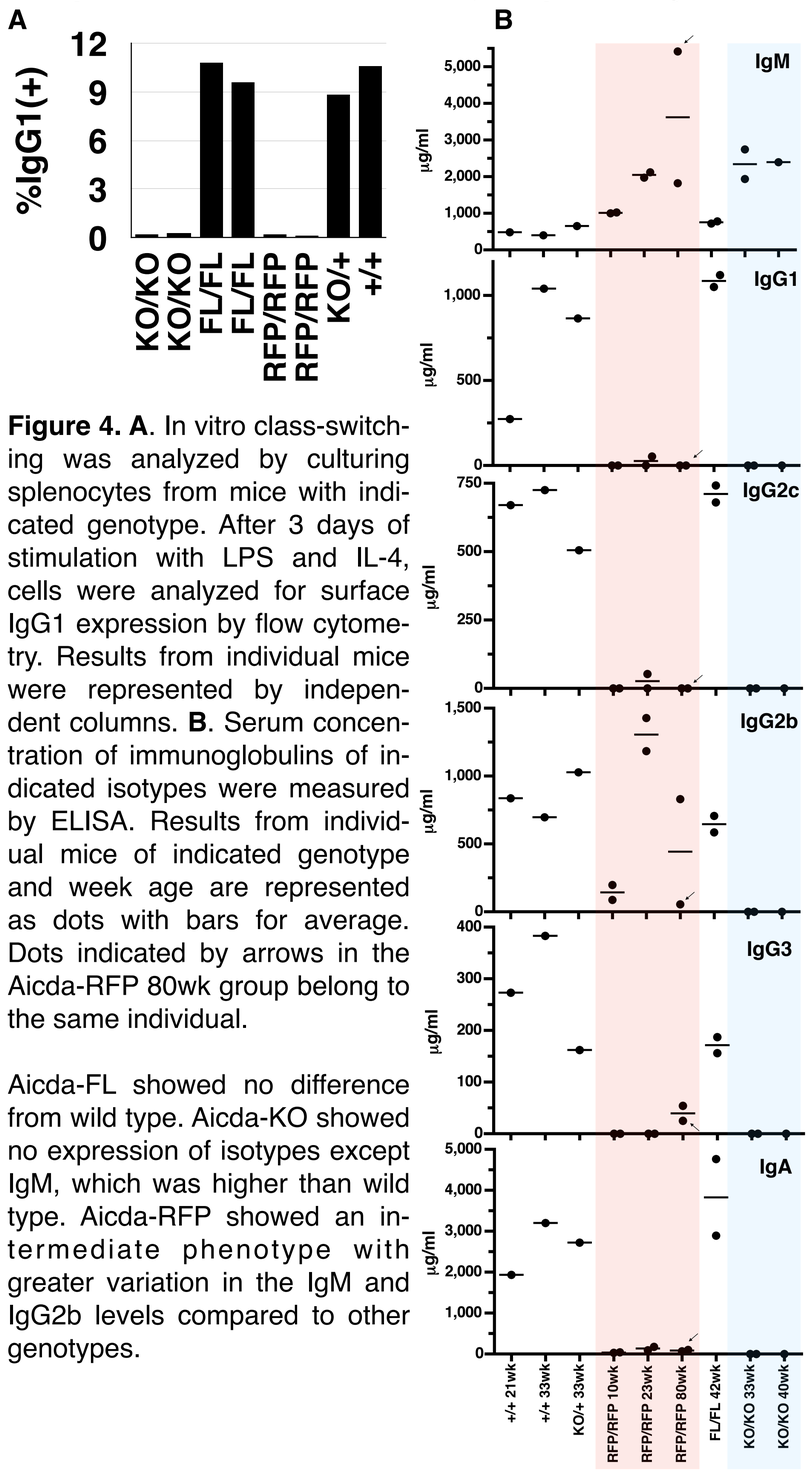

Figure 4. A. In vitro class-switching was analyzed by culturing splenocytes from mice with indicated genotype. After 3 days of stimulation with LPS and IL-4, cells were analyzed for surface IgG1 expression by flow cytometry. Results from individual mice were represented by independent columns. **B**. Serum concentration of immunoglobulins of indicated isotypes were measured by ELISA. Results from individual mice of indicated genotype and week age are represented as dots with bars for average. Dots indicated by arrows in the Aicda-RFP 80wk group belong to the same individual.

Aicda-FL showed no difference from wild type. Aicda-KO showed no expression of isotypes except IgM, which was higher than wild type. Aicda-RFP showed an intermediate phenotype with greater variation in the IgM and IgG2b levels compared to other genotypes.

5. Aicda-RFP and Aicda-KO mice have partial and complete somatic hypermutation defect, respectively

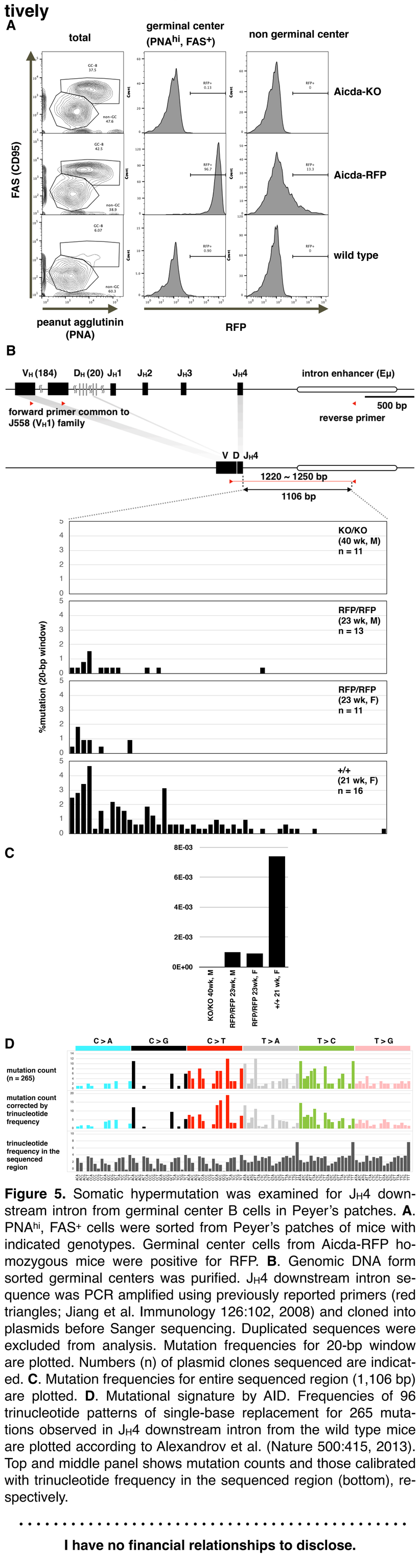

Figure 5. Somatic hypermutation was examined for J_H4 downstream intron from germinal center B cells in Peyer's patches. **A**. PNA^{hi}, FAS^{+} cells were sorted from Peyer's patches of mice with indicated genotypes. Germinal center cells from Aicda-RFP homozygous mice were positive for RFP. **B**. Genomic DNA form sorted germinal centers was purified. J_H4 downstream intron sequence was PCR amplified using previously reported primers (red triangles; Jiang et al. Immunology 126:102, 2008) and cloned into plasmids before Sanger sequencing. Duplicated sequences were excluded from analysis. Mutation frequencies for 20-bp window are plotted. Numbers (n) of plasmid clones sequenced are indicated. **C**. Mutation frequencies for entire sequenced region (1,106 bp) are plotted. **D**. Mutational signature by AID. Frequencies of 96 trinucleotide patterns of single-base replacement for 265 mutations observed in J_H4 downstream intron from the wild type mice are plotted according to Alexandrov et al. (Nature 500:415, 2013). Top and middle panel shows mutation counts and those calibrated with trinucleotide frequency in the sequenced region (bottom), respectively.

I have no financial relationships to disclose.