

Supplementary Information

**Changes in bursal B cells in chicken during embryonic
development and early life after hatching**

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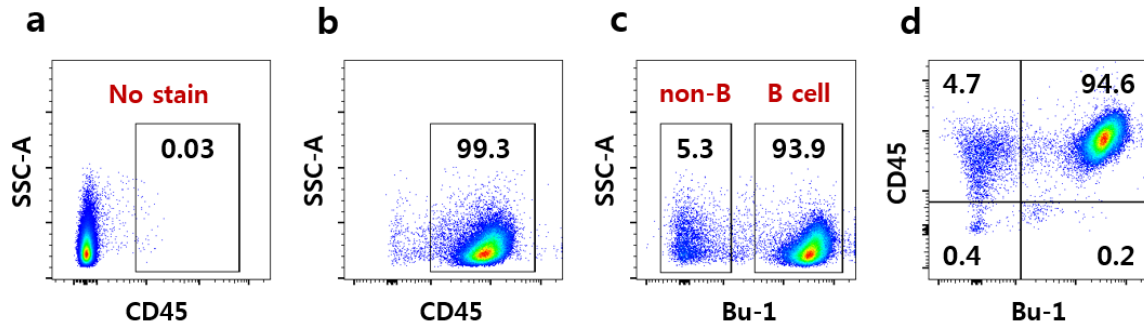
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21 **Supplementary Figures**

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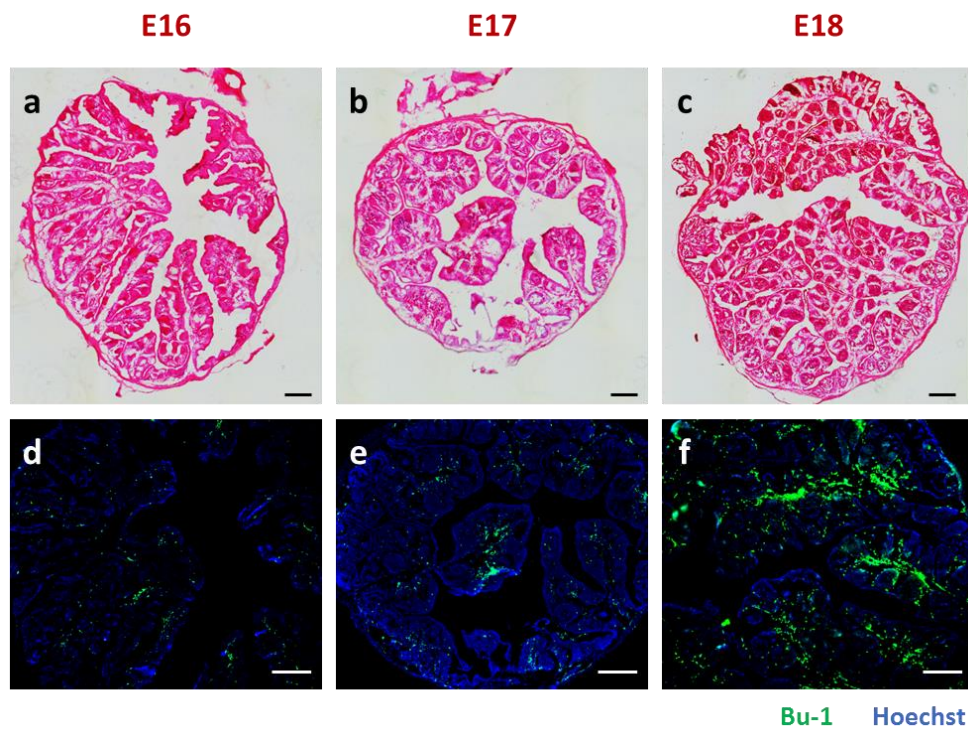


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24 **Supplementary Figure 1. Flow cytometric gating strategy for chicken B cells.** To gate and
25 analyze chicken B cells, CD45 and bu-1 antibodies were used. Single cells from bursa of
26 Fabricius were shown (a) without stain, and stained with (b) anti-CD45, (c) anti-bu-1 or (d)
27 both anti-CD45 and anti-bu-1 antibodies. The number in each area indicates the percentage of
28 the cells.

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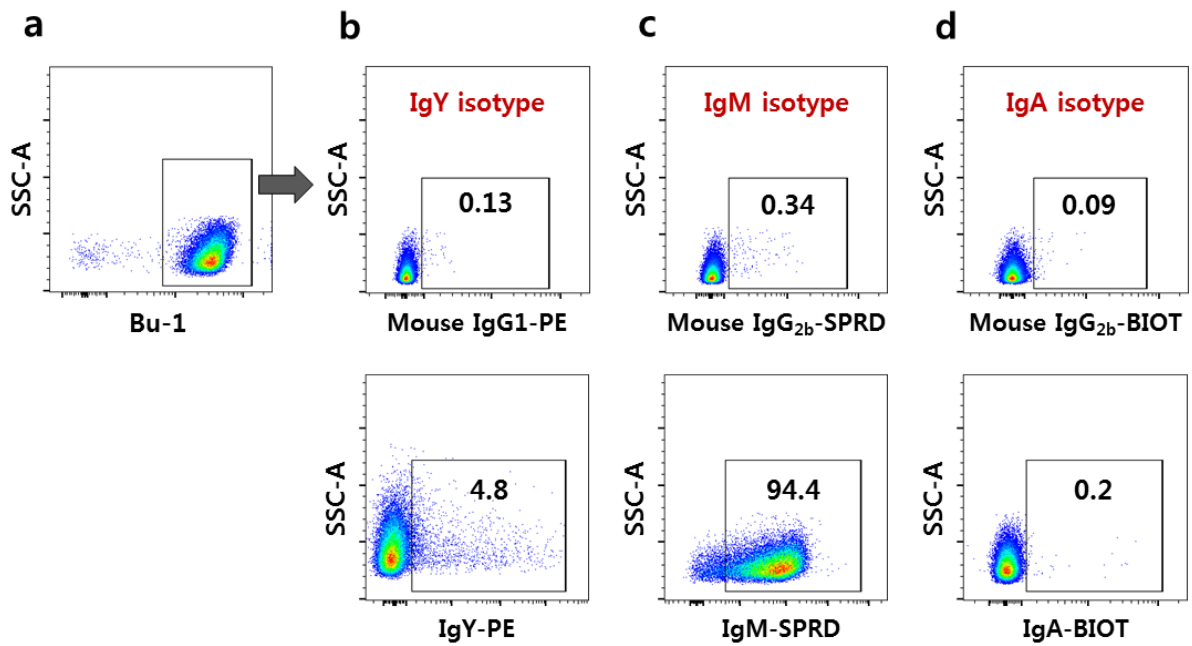


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32 **Supplementary Figure 2. Histological analysis and immunohistochemical staining of bu-**
 33 **1 in embryonic bursa.** Bursal tissues from chicks at E16 to E18 were fixed and
 34 cryosectioned. The images for H&E staining and IHC were obtained from adjacent sections.
 35 (a-c) bursal cryosections were stained with hematoxylin and eosin (H&E) for histological
 36 analysis. (d-f) the cryosections were stained with bu-1 antibody (green) and hoechst (blue),
 37 and visualized by digital inverted fluorescence microscope. One of the representative pictures
 38 with similar result from at least 5 samples per embryonic stage is shown. Scale bars
 39 correspond to 100 μ m.

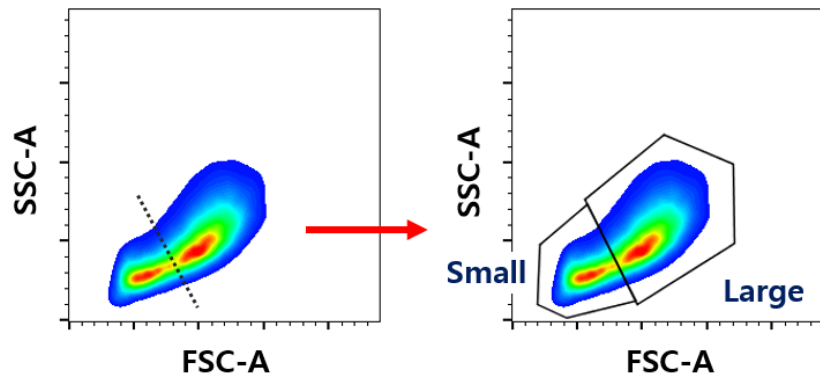
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 43 **Supplementary Figure 3. Gating strategy for Ig expression on bursal B cells.** Single cells
 44 produced from the bursa of 1-week-old chicks were stained with anti-bu-1 antibody or
 45 together with anti-IgY, -IgM or -IgA antibody followed by streptavidin-conjugated BV605.
 46 Then, background level was examined by using isotype control for the IgY, IgM, and IgA
 47 antibodies, respectively. (a) Bursal B cells were first identified by bu-1 gating, and then (b)
 48 IgY-, (c) IgM-, and (d) IgA-producing B cells were examined by flow cytometry. The number
 49 in each area of the panel indicates the percentage of the cells.

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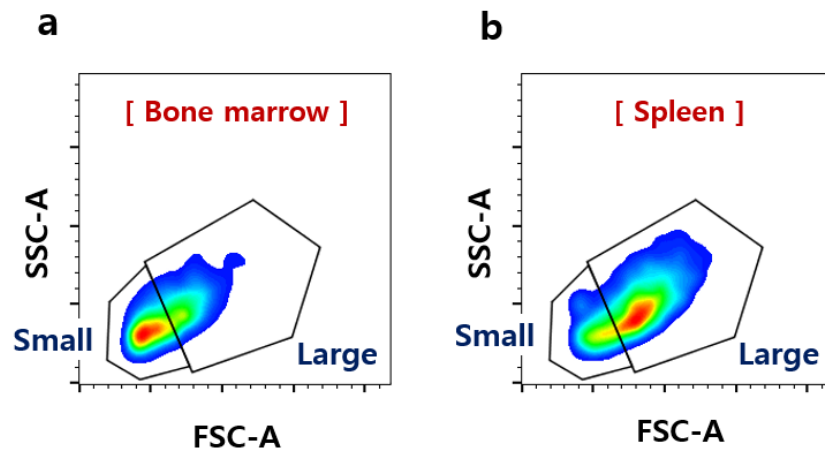


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52 **Supplementary Figure 4. Flow cytometric gating strategy for small and large B cells.**

53 Bursal cells were gated on bu-1⁺ cells and distinguished by the cell size by using flow
54 cytometry. The population gated at low forward scatter with low side scatter indicates small B
55 cells whereas high forward with high side scatter indicates large B cells.

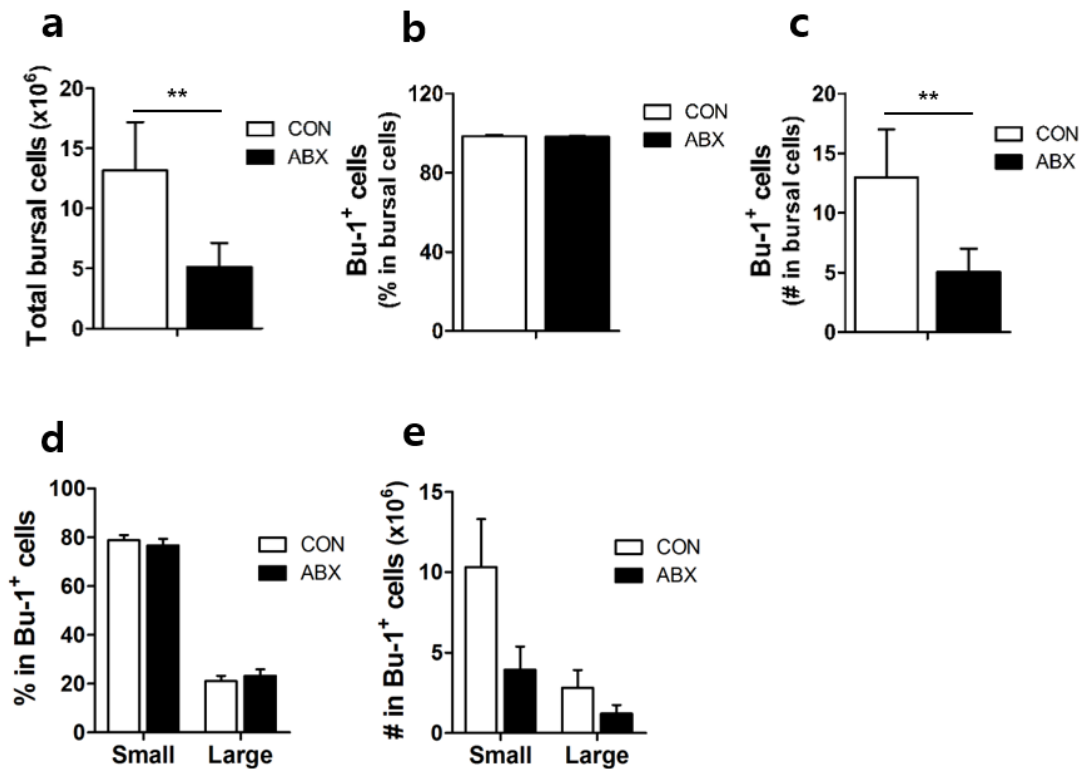
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58 **Supplementary Figure 5. Analysis of small and large B cells in the spleen and bone**
59 **marrow.** The single cells of (a) bone marrow and (b) spleen were gated on bu-1⁺ cells and
60 divided by the cell size and granularity by using flow cytometry, same as in **Supplementary**
61 **Fig. 4.**

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64 **Supplementary Figure 6. Commensal microbiota affects the development of bursal B**

65 **cells.** Newly hatched chicks were given the combination of different antibiotics consisting

66 ampicillin (100 mg/l), gentamycin (100 mg/l), metronidazole (100 mg/l), neomycin (100 mg/l)

67 and vancomycin (50 mg/l) in drinking water for 1 week. Bursal cells from control (CON) or

68 antibiotics (ABX)-treated chicks were analyzed for (a) absolute number of bursal cells, (b)

69 percentage and (c) absolute number of bursal B cells, (d) the percentage and (e) absolute

70 number of small and large B cells in bursa. Data represent the mean \pm SD. Similar results

71 were obtained from three separate experiments. **** $P < 0.01$.**