Supplemental Figures

Group 2 Innate Lymphoid Cells Promote An Early Antibody Response To A Respiratory Antigen In Mice

Running Title: B cell regulation by ILC2

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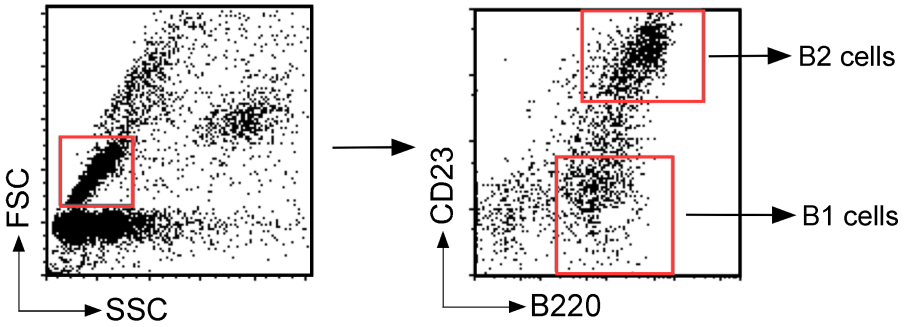
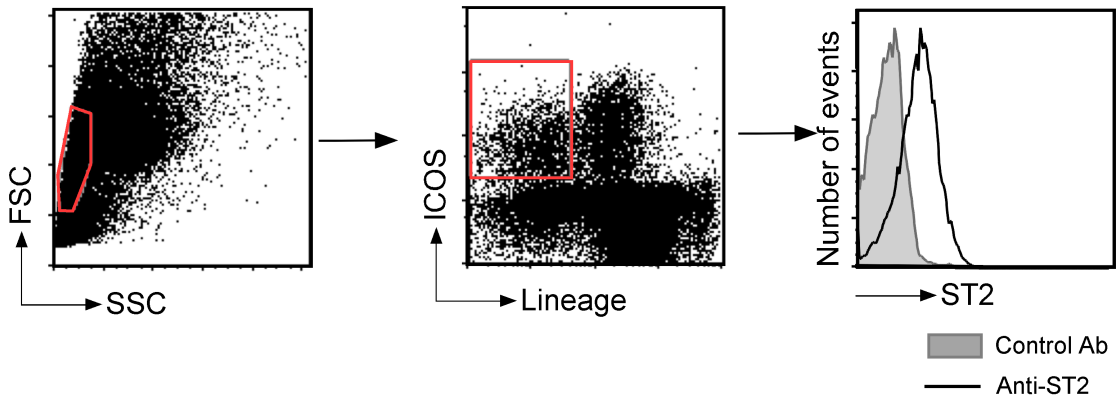


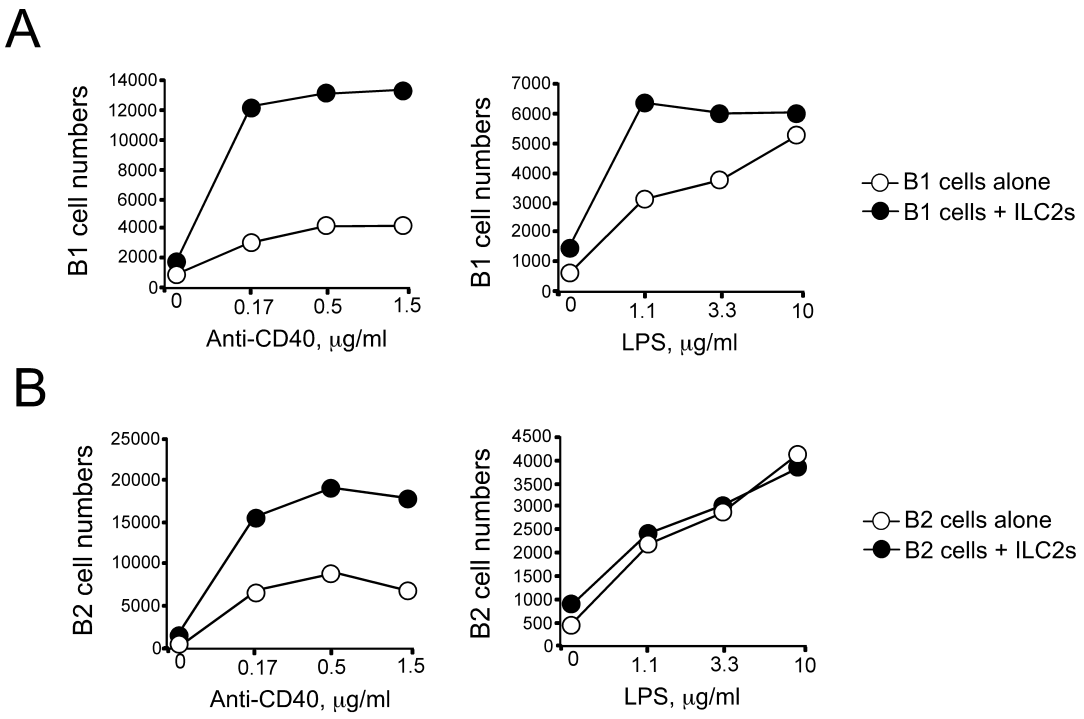
Supplemental Figure 1

SUPPLEMENTAL FIGURE 1. Gating strategy for B1 and B2 cell sorting. Peritoneal lavage cells were collected from naïve BALB/c mice and stained with anti-CD23 and anti-B220 antibodies. Lymphocytes were gated on forward scatter and side scatter as shown in the left panel. B1 cells ($B220^{low}CD23^{-}$) and B2 cells ($B220^{high}CD23^{high}$) were gated as shown in the right panel.



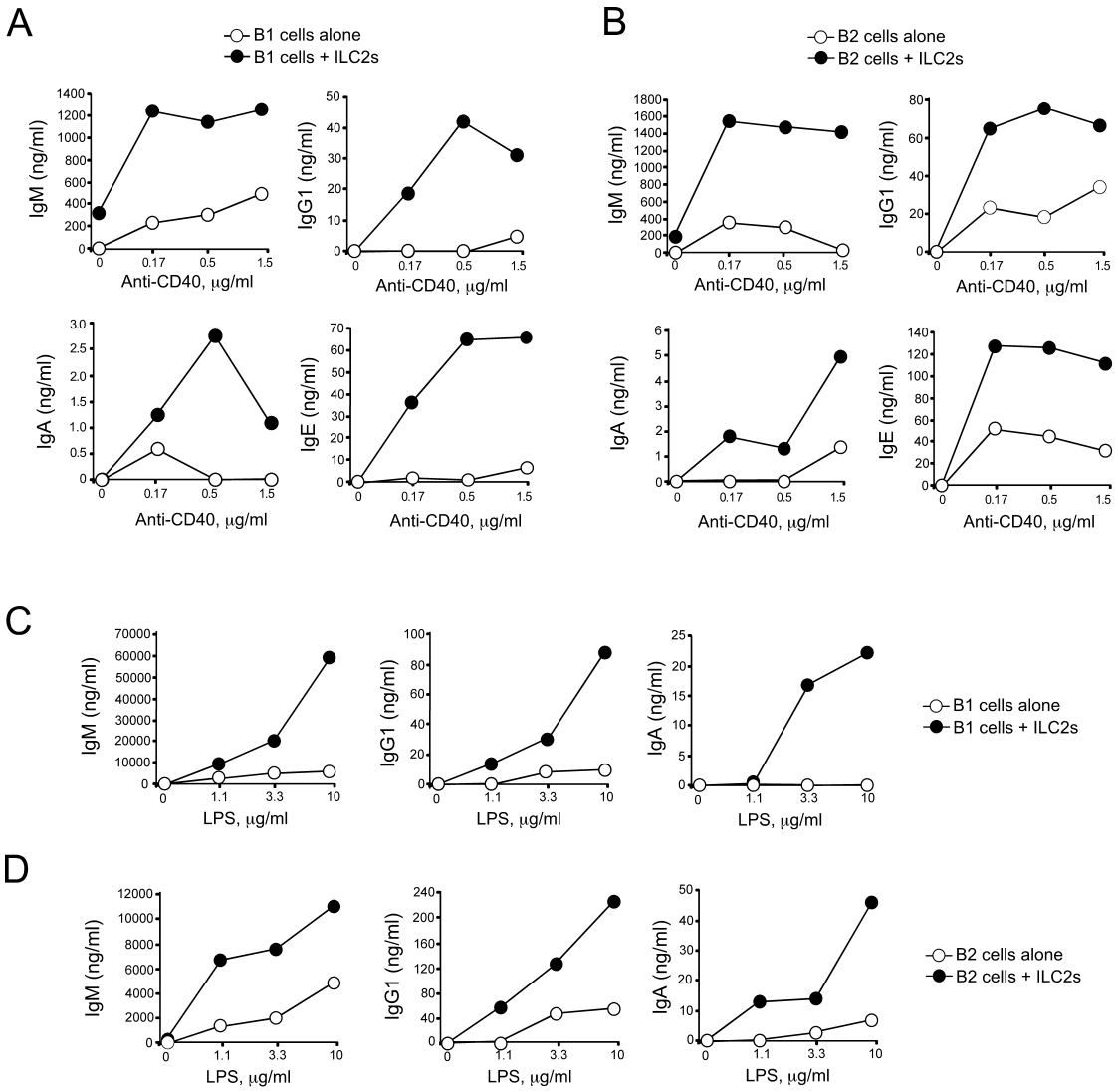
Supplemental Figure 2

SUPPLEMENTAL FIGURE 2. Gating strategy and ST2 staining of lung ILC2s. C57BL/6 mice were i.p. injected with IL-33 and IL-25 (400 ng/mouse) daily for 4 days to expand ILC2s *in vivo*. Lungs were collected, and single cell suspensions were made as described in the Materials and Methods. Cells were stained with fluorescence-labeled antibodies to lineage markers (CD3, CD14, CD11b, CD16/CD32 and B220), CD278 (ICOS) and ST2. Lymphocytes were gated as shown in the left panel. Lung ILC2s were identified as the $Lin^{-}ICOS^{+}$ cell population as shown in the middle panel. ST2 staining in the $Lin^{-}ICOS^{+}$ cell population is shown in the right panel. Gray-filled area, control Ab; black line, anti-ST2 Ab.



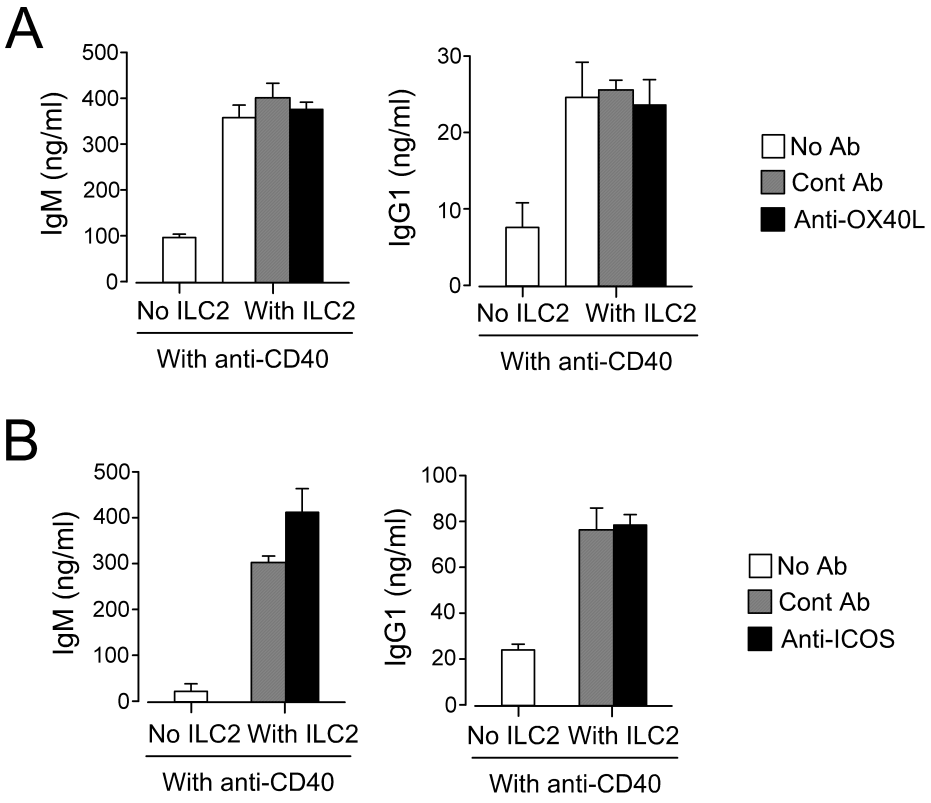
Supplemental Figure 3

SUPPLEMENTAL FIGURE 3. Lung ILC2s enhance the proliferation of B lymphocytes. Lung ILC2s and peritoneal B cells were isolated from naïve BALB/c mice. Peritoneal B1 cells and B2 cells were cultured at 2×10^4 cells/well with or without ILC2s (10^4 cells/well) in the presence of serial dilutions of anti-CD40 antibody or LPS plus IL-4 (10 ng/ml) for 4 days. The number of $B220^+$ cells was determined by FACS. Each data point represents the cell numbers from one well. Data are representative of two independent experiments.



Supplemental Figure 4

SUPPLEMENTAL FIGURE 4. Lung ILC2s enhance Ig production by B1 and B2 cells. Lung ILC2s and peritoneal B1 and B2 cells were isolated from BALB/c mice. B1 cells (Panels A and C) and B2 cells (Panels B and D) were cultured at 2×10^4 cells/well with or without ILC2s (10^4 cells/well) in the presence of serial dilutions of anti-CD40 or LPS plus IL-4 for 4 days. Ig levels in the supernatants were analyzed by ELISA. Each data point represents the Ig concentration from one well. Data are representative of three independent experiments



Supplemental Figure 5

SUPPLEMENTAL FIGURE 5. ILC2s enhance B cell antibody production independent of OX40/OX40L and ICOS/ICOSL interaction. Lung ILC2s and splenic B cells were isolated from BALB/c mice. B cells were stimulated with anti-CD40 (1.5 μg/ml) plus IL-4 with or without ILC2s for 4 days. Anti-OX40L (2 μg/ml) (Panel A), anti-ICOS (2 μg/ml) (Panel B) or corresponding control Abs were added to some groups. Data (mean±SEM, n=3) are representative of three independent experiments.