Supporting Information

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Fig. S1. ILC2 identification by flow cytometry. (*A*) Representative gating scheme for identification of ILC2s (CD45^{+/hi}Lin⁻IL-7Rα⁺ST2⁺) in the CNS. (*B*) Expression of additional canonical ILC2 markers CD25, VLA-4, and c-kit by ILC2s in the CNS.



Fig. 52. ILC2s derived from female and male SJL mice show similar responses to ILC2-activating factors. (A) ILC2 precursors (ILC2Ps: CD45⁺Lin⁻ $\alpha_4\beta_7^+$ CD25⁺) in the bone marrow of naïve mice. Numbers in flow cytometry plots represent percentage of CD45⁺Lin⁻ $\alpha_4\beta_7^+$ ns: *P* > 0.05 by Student's *t* test. Data from individual mice is graphed and expressed as the percentage CD45⁺Lin⁻ $\alpha_4\beta_7^+$ CD25⁺ cells of total cells. (*B*) Percentage of thymic ILC2Ps (Lin⁻CD4⁻CD8⁻CD44⁺c-kit⁺CD25⁺) in naïve mice. Data from individual mice is graphed and expressed as the percentage Lin⁻ CD4⁻ CD8⁻ CD44⁺ CD25⁺ cells of total cells. (*C*-*F*) Quantification of mature ILC2s (CD45^{+/hi} Lin⁻ IL-7R\alpha⁺ ST2⁺) in the indicated tissues of naïve mice. Numbers in flow cytometry plots represent the percentage of IL-7R\alpha⁺ ST2⁺ cells of the CD45⁺ Lin⁻ population. Data from individual mice is graphed and expressed as the percentage of the CD45⁺ population. (*G*-*J*) ILC2s (CD45^{+/hi} Lin⁻ IL-7R\alpha⁺ ST2⁺) in the indicated tissues of 300 ng IL-33. Numbers in flow cytometry plots represent the percentage of the CD45^{+/hi} Lin⁻ IL-7R\alpha⁺ ST2⁺) in the indicated and expressed as the percentage of CD45⁺ population. (*G*-*J*) ILC2s (CD45^{+/hi} Lin⁻ IL-7R\alpha⁺ ST2⁺) in the indicated tissues after three consecutive daily doses of 300 ng IL-33. Numbers in flow cytometry plots represent the percentage of the CD45^{+/hi} Lin⁻ IL-7R\alpha⁺ ST2^{+/hi} Lin⁻ IL-7R\alpha⁺ ST2^{+/hi} Lin⁻ Duplation. Data from individual mice is graphed and expressed as the percentage of CD45⁺ population. (*G*-*J*) ILC2s (CD45^{+/hi} Lin⁻ Lin⁻ Duplation. Data from individual mice is graphed and expressed as the percentage of CD45⁺ population. (*G*-*J*) ILC2s (CD45^{+/hi} Lin⁻ Duplation) controls. ns: *P* > 0.05 by Student's *t* test.



Fig. S3. Isolation of ILC2s by magnetic bead enrichment and FACS. (*A*) The gating scheme for the identification and sorting of ILC2s from the bone marrow. (*B*) Analyses of bone marrow-derived ILC2s after 6 d in culture as shown in Fig. 2K. Blue and red numbers represent relative purity of ILC2s (CD45⁺ Lin⁻ IL-7R α ⁺ ST2⁺ CD25⁺ Sca-1⁺). Numbers in black represent the percentage of c-kit⁺ ILC2s. (*C*) ILC2 numbers after 6 d in culture before restimulation.



Fig. 54. ILC2s are expanded and a Th2 response is established in the LNs of IL-33-treated and PLP₁₃₉₋₁₅₁-immunized female mice. (*A*) Representative flow cytometry plots of ILC2s (CD45^{hi}Lin⁻IL-7R α ⁺ST2⁺) in the LNs of immunized and IL-33-treated female mice as in Fig. 4A. Representative of three independent experiments. Numbers depict the percentage of ILC2s (IL-7R⁺ ST2⁺) of the CD45⁺Lin⁻ population. (*B*) The proportion of ILC2s of the CD45⁺Lin⁻ population in the LNs of immunized and IL-33 treated female mice as in Fig. 4A. (*C*) The percentage of IL-4-expressing CD3⁺CD4⁺ T cells in the LNs of immunized and IL-33-treated female mice as in Fig. 4A. ***P* < 0.01 and *****P* < 0.01 by Student's *t* test. Representative flow plots are shown. Gating is based on FMO controls.



Fig. S5. BMMC viability in the presence of flutamide and its solvent. The percentage of live BMMCs identified by Trypan blue negativity after stimulation under the indicated conditions for 6 h. Flutamide was dissolved in a 1:1,000 solution of ethanol (EtOH).

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