SUPPLEMENTAL MATERIAL

Nair et al., http://www.jem.org/cgi/content/full/jem.20082048/DC1

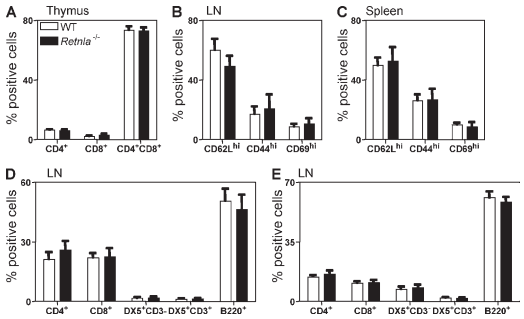

Figure S1. Characterization of the immune cell compartments of WT and *Retnla*$^{-/-}$ mice. Results are presented as means ± SEM (n = 4) and are representative of two independent experiments.

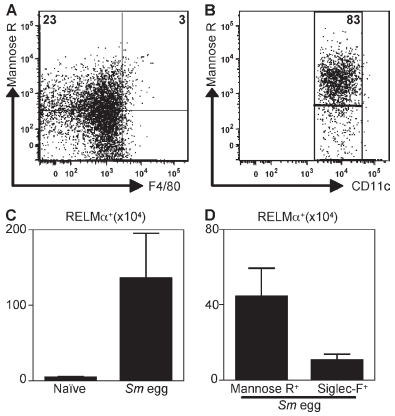

Figure S2. CD11c^{+}mannose receptor^{+} lung macrophages are a dominant cellular source of RELM-α. Dissociated lung cells from *Sm* egg-challenged WT mice were stained for the mannose receptor, F4/80 (A), and CD11c (B). Flow cytometric plots are gated on the large cells (A) or CD11c^{+} cells (B). (C) Total numbers of RELM-α^{+} cells within dissociated lung. (D) Total number of RELM-α^{+}mannose receptor^{+} or RELM-α^{+}siglec-F^{+} cells within the lung. Data (±SEM) are representative of two independent experiments (naive, n = 3; *Sm* egg-challenged, n = 5).

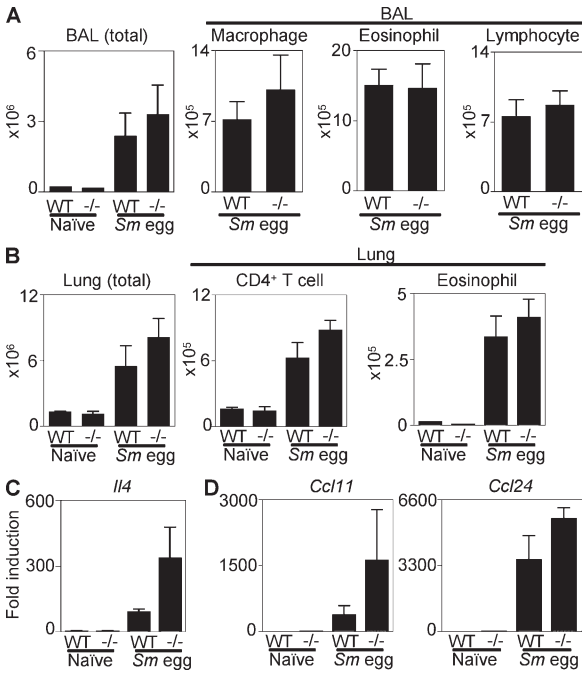

Figure S3. Characterization of the inflammation in the lung and BAL of naive and *Sm* egg-challenged WT and *Retnla*$^{-/-}$ mice. (A) Number of BAL cells recovered and characterization of cell types as determined by microscopic examination of cytocentrifuge preparations. (B) Total number of lung cells and frequency of the CD4$^+$ T cells and siglec-F$^+$ eosinophils as determined by flow cytometry. (C and D) Real-time PCR analysis of the lung RNA for expression levels of *Il4* (C) and *Ccl11* and *Ccl24* (D), measured as fold induction over naive WT mice. Results (±SEM) are representative of three independent experiments (naive, $n = 3$; *Sm* egg-challenged, $n = 10$).

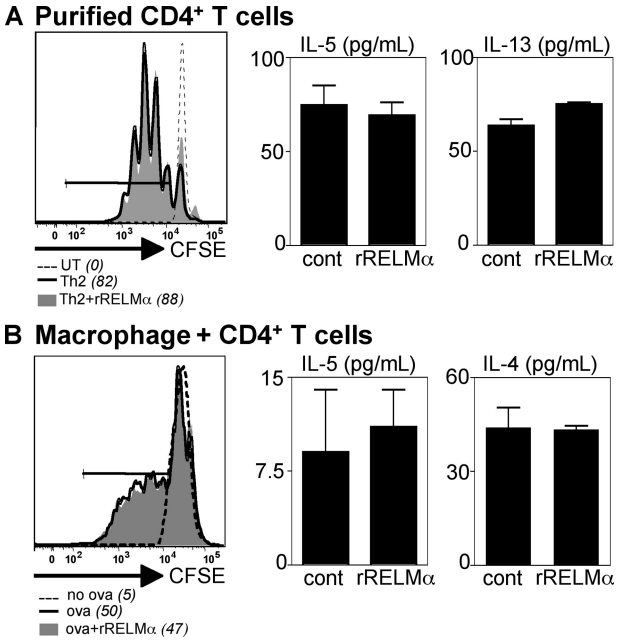

Figure S4. Effect of rRELM-α on purified CD4$^+$ T cells and BMMacs. (A) Purified WT CD4$^+$ T cells were CFSE labeled and left untreated or stimulated with plate-bound α-CD3/α-CD28 under Th2-permissive conditions, with or without 5 µg/ml rRELM-α. At day 4, cells were recovered for measurement of proliferation, and supernatants were recovered for measurement of IL-5 and IL-13 secretion by ELISA. (B) WT BMMacs were left untreated or pulsed with OVA and IL-4 with or without 5 µg/ml rRELM-α overnight, followed by washing in medium and coculture with OVA-specific CD4$^+$ T cells. 4 d later, cells were recovered for measurement of CD4$^+$ T cell proliferation and secretion of IL-5 and IL-4 by ELISA. Results (±SEM of duplicate wells) are representative of two independent experiments.

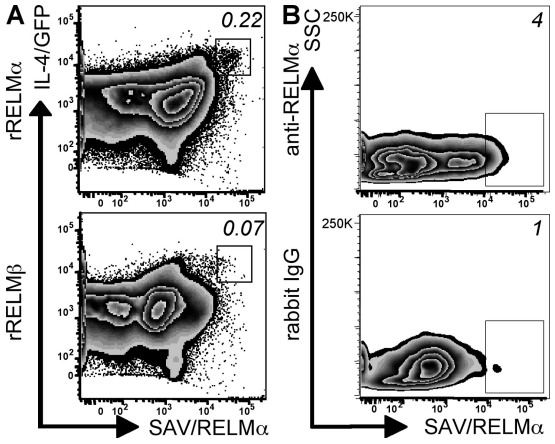

Figure S5. RELM-α binding assay is specific. (A) The rRELM-α capture assay was performed as described in Fig. 7, with an additional control involving incubation with rRELM-β. (B) A further control involved incubation with rabbit IgG instead of anti–RELM-α.

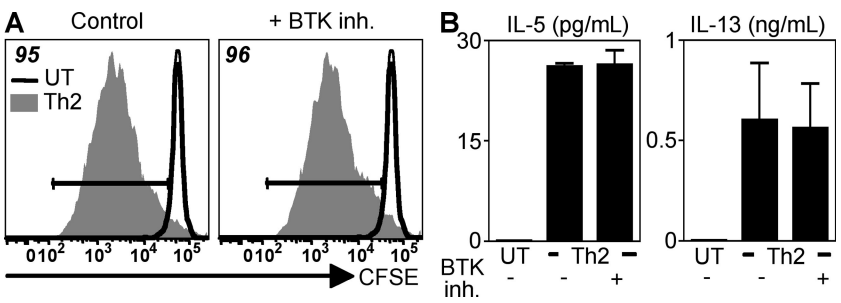

Figure S6. Effect of BTK inhibitor on WT splenocytes. CFSE-labeled splenocytes were left untreated or activated with α-CD3/α-CD28 under Th2-permissive conditions, with or without treatment with a BTK inhibitor (BTK inh.), and assayed 4 d later for $CD4^+$ T cell proliferation (A) and secretion of IL-5 and IL-13 (B). Results (±SEM of triplicate wells) are representative of two independent experiments.