SUPPLEMENTAL MATERIAL

Steinfelder et al., http://www.jem.org/cgi/content/full/jem.20082462/DC1

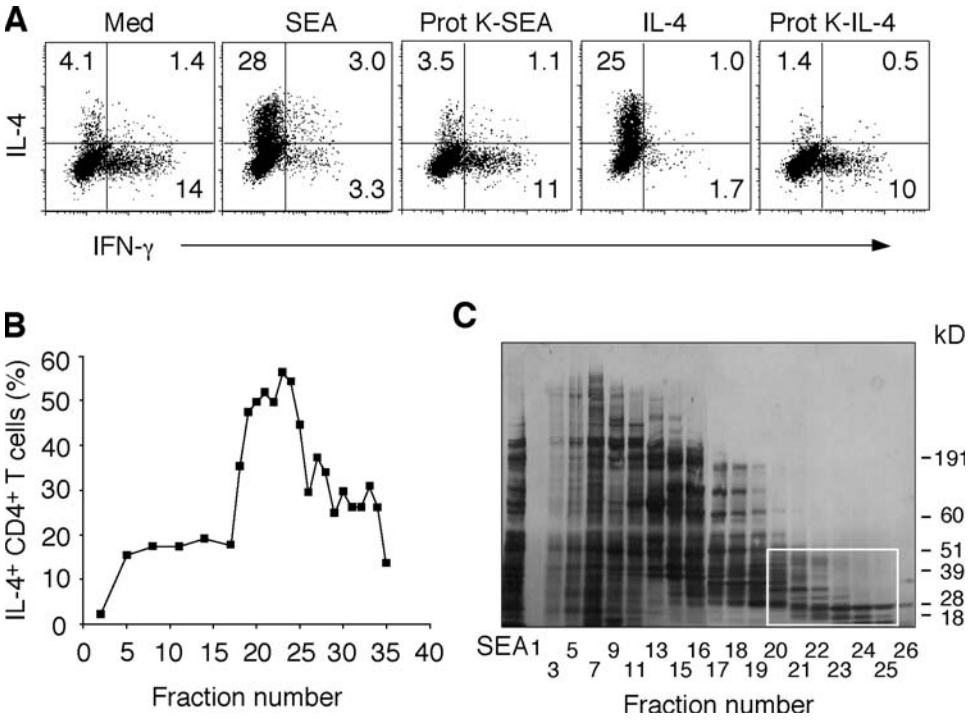

Figure S1. Biochemical characterization of Th2-polarizing activity in SEA. (A) Components required for SEA-induced DC-dependent Th2 polarization are protease sensitive. Before adding to the Th2 polarization assay, SEA or rIL-4, as a control, was treated or mock treated with proteinase K–coupled acrylic beads. The frequency of IL-4+ and IFN-γ+ DO.11.10 CD4+ lymphocytes was examined by ICS on day 6 after restimulation with anti-CD3 mAb (percentages are shown). (B) Th2-inducing activity of fractions from gel filtration of SEA on a Superdex 75 column. Flow cytometry of IL-4+ Tg CD4+ T cells after ICS in response to one fixed dilution for each fraction determined is shown. Results represent two independent experiments. (C) Silver stained SDS-PAGE of a SEA starting sample and SEA fractions obtained after the gel filtration shown in B. The fractions with the highest Th2-polarizing activity are indicated within the white box.

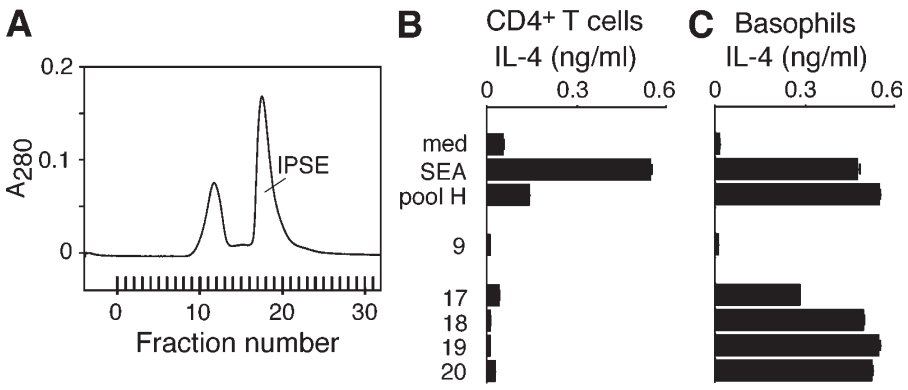

Figure S2. Purified IPSE triggers IL-4 secretion from basophils but not DC-dependent Th2 polarization. (A) The Superdex 75 elution profile of IPSE-enriched pool H obtained after the initial ion exchange fractionation of SEA shown in Fig. 2 A. Purified material in fraction 19 was identified as IPSE based on the N-terminal sequence DSXKYXLQLYDETYE. (B and C) The indicated fractions were tested in Th2 polarization (B) and basophil (C) assay by measuring IL-4 secretion. 40 μg/ml SEA and 20 μg/ml of pool H were included as a positive control. Bars represent mean IL-4 concentrations ± SD determined by ELISA. The data are representative of two independent experiments.

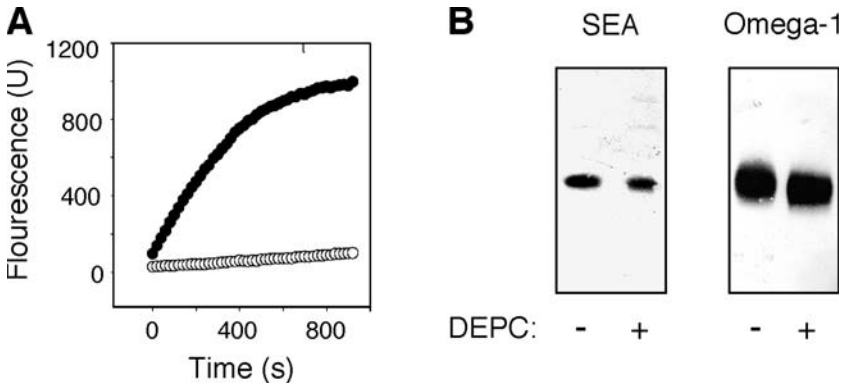

Figure S3. Ribonuclease activity of purified omega-1 preparation and its inhibition by DEPC treatment. (A) Ribonuclease activity of omega-1 before and after DEPC treatment measured by fluorometric RNase detection assay. At the substrate concentration tested (0.2 µM), the initial velocity for the untreated omega-1 (closed circles) was 0.23 nmol, whereas the velocity for the DEPC-treated enzyme (open circles) was 0.006 nmol of product formed/nmol of enzyme/min, indicating a 97.4% loss in activity. (B) Western blot of SEA and purified omega-1 before and after DEPC treatment developed with mAb 140-3E11, confirming that the treatment does not result in the degradation or loss of the protein. An equal amount of each sample before and after treatment were loaded on the gels.

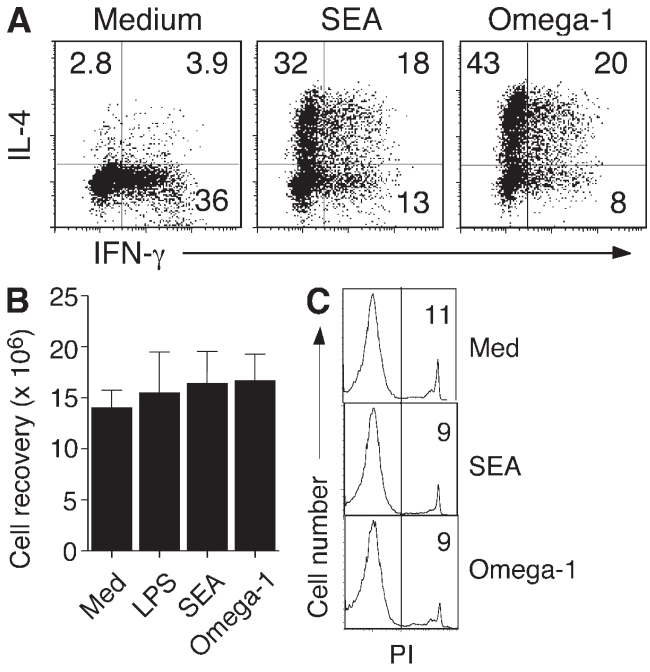

Figure S4. Omega-1 does not require either MyD88 or TRIF signaling for Th2 polarization and does not affect DC viability. (A) Splenic CD11c$^+$ DCs isolated from MyD88$^{-/-}$ × TRIF$^{-/-}$ double-deficient mice were co-cultured with OT-II Tg CD4$^+$ T cells in the presence of OVA and 40 µg/ml or 0.5 µg/ml omega-1. The ICS was performed on day 6 after restimulation with anti-CD3 antibody. The dot plots shown are gated on CD4$^+$ T lymphocytes (percentages are shown). The experiment shown is representative of three performed with similar results. (B) Numbers of BMDCs recovered after overnight culture in nontissue-treated plates in the presence of medium, 40 ng/ml LPS, 40 µg/ml SEA, or 1 µg/ml omega-1 was determined by counting viable adherent and nonadherent cell. Bars indicate total numbers ± SD from two independent experiments. (C) BMDCs cultured in medium, SEA, or omega-1 were stained with PI and analyzed by FACS. The histogram plots shown are gated on CD11c$^+$ cells, and percentages of cells are indicated. The vertical line indicates the gate between PI$^-$ (live) and PI$^+$ (dead) cells. The data shown are representative of three experiments performed.