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Supporting Information

Herbst et al.

SI Materials and Methods

ELISAs. Total and *Heligmosomoides polygyrus bakeri* excretory–secretory (HES)-specific antibody ELISAs were performed as previously described (1). IL-3 ELISAs were performed using the BioLegend antibodies MP2-8F8 and MP2-43D11 according to manufacturer instructions. Circulating HES was detected in the serum of infected mice by sandwich ELISA using a previously described mAb (clone 13.1 at 2 μg/mL) (2) labeled with EZ-link NHS–LC–biotin (Thermo Scientific); the nominal concentration of circulating HES was calculated by reference to standard curve using a stock concentration of culture-derived HES.

Analysis of Helminth-Induced Cytokine Production. MesLN from infected mice were cultured in medium Iscove's modified Dulbecco's medium (Lonza) plus 7% FCS (Lonza) and 5 μg/mL HES (excretory/secretary products collected from adult L5 *H. polygyrus bakeri*) for 72 h then restimulated with phorbol–12-myristat–13-acetate (PMA) (Sigma-Aldrich) and ionomycin (Sigma-Aldrich,) for 4 h with Brefeldin A (10 μg/mL) added for the final 2 h. Permeabilized cells were stained with CD4–PercP, anti–IL-4–APC (11B11), IFN–γ-FITC (XMG1.2), or anti–IL-3–PE (M12-SF8) (Biolegend). Alternatively, bone marrow or spleen cells were incubated with PMA/ionomycin for 24 h and IL-3 levels in the supernatant was determined by ELISA. For some samples, $NK1.1^+$ cells were depleted by magnetic cell sorting via Nk1.1 biotin and streptavidin microbeads (MACS) (Milteny Biotech). For intracellular cytokine staining of stimulated bone marrow or spleen cells, cells were additionally incubated with monensin, permeabilzed, and stained with anti–IL-3 PE (M12-SF8) (Biolegend).

Quantification of IL-3 mRNA Expression. Basophils were purified from in vitro bone marrow cultures by MACs sorting of $CD49b^+$ (DX5 Microbeads; Miltenyi Biotech) cells and incubated with 1 μg/mL IgE (TIB141) or 1 μg/mL anti-FcγRIII (24.G2, rat IgG2a) for 60 min at 37 °C. Antibodies were cross-linked with 1 μg/mL antimouse IgE (6HD5) or 1 μg/mL antirat IgG2a (2A 8F4; Southern Biotech) for 60 min. Total RNA was isolated from the indicated cells using TRI reagent (Molecular Research Center) and reverse transcribed using Fast Lane kit (Qiagen). Real-time RT-PCR was performed using Brilliant SYBR Green (Stratagene) and an iCycler (Bio-Rad Laboratories). Expression was normalized according to expression of the housekeeping gene β-actin. Sequences of primers used were IL-3, 5′-TTA GCA CTG TCT CCA GAT C-3′ and 5′-ACT GAT GAT GAA GGA CC-3′; and β-actin, 5′-CTT TTC ACG GTT GGC CTT AG-3 and 5′-CCC TGA AGT ACC CCA TTG AAC-3′.

Statistical Analysis. For all data, significant differences were determined between gene-deficient or treatment groups and wild-type mice by a one-tailed Student *t* test with a confidence interval of 95%. Significant *P* values are shown at $^*P < 0.05$, $^{**}P < 0.01$, or $^{***}P < 0.001$.

1. McCoy KD, et al. (2008) Polyclonal and specific antibodies mediate protective immunity against enteric helminth infection. *Cell Host Microbe* 4:362–373.

2. Hewitson JP, et al. (2011) Heligmosomoides polygyrus elicits a dominant nonprotective antibody response directed against restricted glycan and peptide epitopes. *J Immunol* 187:4764–4777.

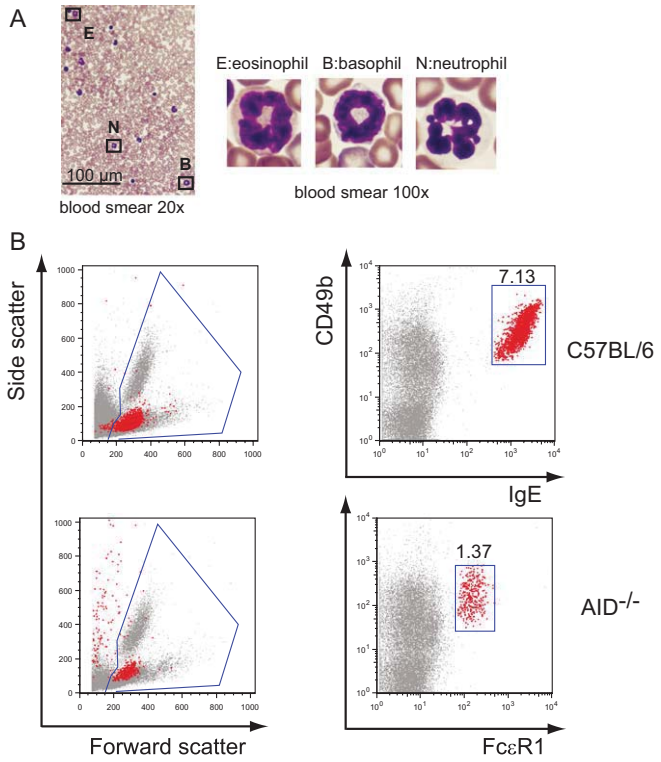

Fig. S1. Identification of basophils in C57BL/6 and antibody-deficient mice. (*A*) Pictures represent blood smears from *H. polygyrus bakeri*-infected mice stained with Diff-Quik. The eosinophils, basophils, and neutrophils were determined on the basis of morphology and staining as illustrated. (*B*) Representative FACS plots of basophils using CD49b and IgE or FcεR1 as markers for C57BL/6 or antibody-deficient mice, respectively. Backgated basophils are shown in red to indicate their forward versus side scatter properties. In all experiments, basophils from C57BL/6 mice were additionally stained for FcεR1. However, as a high degree of IgE binding in infected mice was observed to interfere with the efficiency of anti-FcεR1 staining, surface IgE was generally determined as a more reliable marker of FcεR1 expression in these mice.

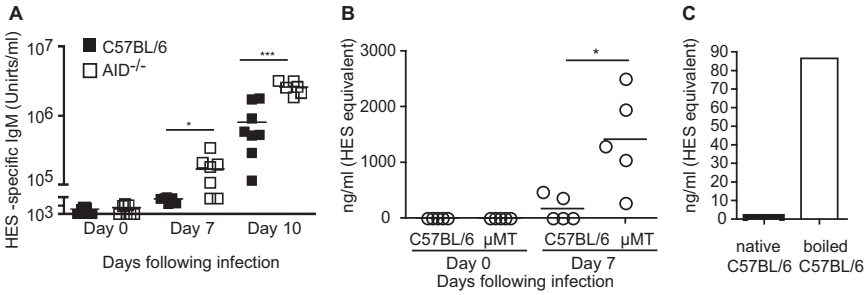

Fig. S2. B-cell–deficient mice exhibit increased levels of circulating helminth antigens. (*A*) *H. polygyrus bakeri*-specific IgM was quantified for C57BL/6 and AID$^{-/-}$ mice following primary infection. (*B*) Presence of *H. polygyrus bakeri*-derived antigens (HES) in the serum of C57BL/6 or B-cell–deficient (µMT$^{-/-}$) mice was determined by ELISA as described in *Materials and Methods*. (*C*) In a separate experiment, the presence of HES in the pooled serum of C57BL/6 (n = 5) was determined before and after boiling.

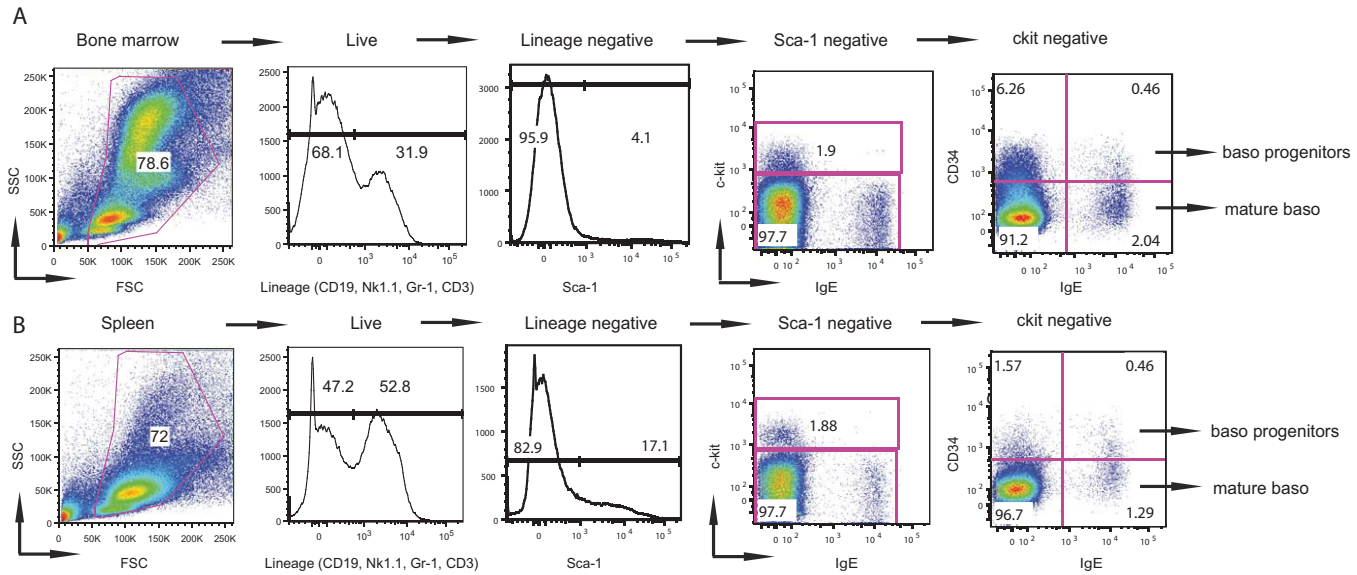

Fig. S3. Gating strategy of mature and progenitor basophils. Basophils from the bone marrow (*A*) or spleen (*B*) were defined by a lack of expression of the markers CD3, CD19, NK1.1, Ly6G, Sca-1, and c-kit and a positive expression for CD16/CD32^{+} and FcεRI or IgE. Basophils were then further defined as progenitors or mature cells by the presence or absence of CD34. Gating strategies are shown in representative plots from *H. polygyrus bakeri*-infected C57BL/6 mice.

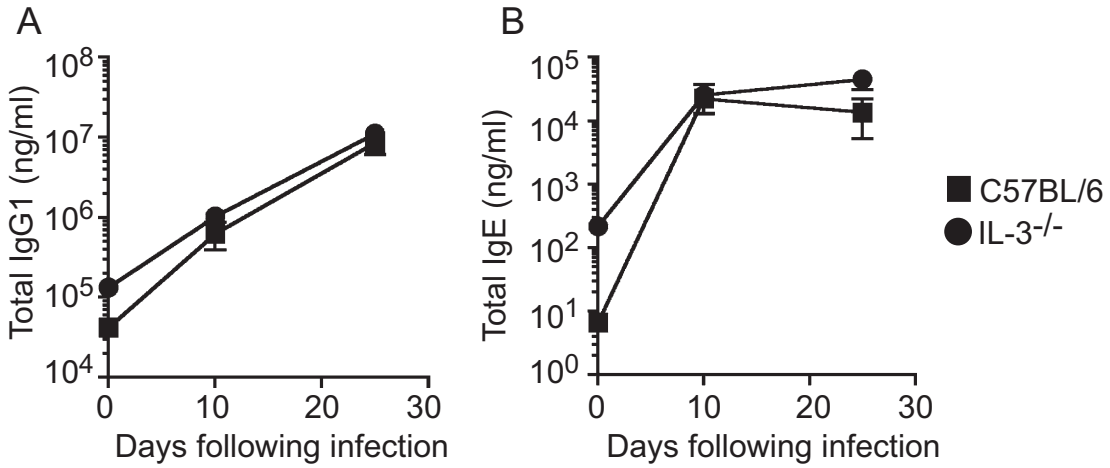

Fig. S4. IL-3 is not necessary for antibody production following helminth infection. (*A*) Total IgG1 and (*B*) IgE levels present in the serum of C57BL/6 or IL-3$^{-/-}$ mice are shown for the indicated time points following primary infection with *H. polygyrus backeri*.

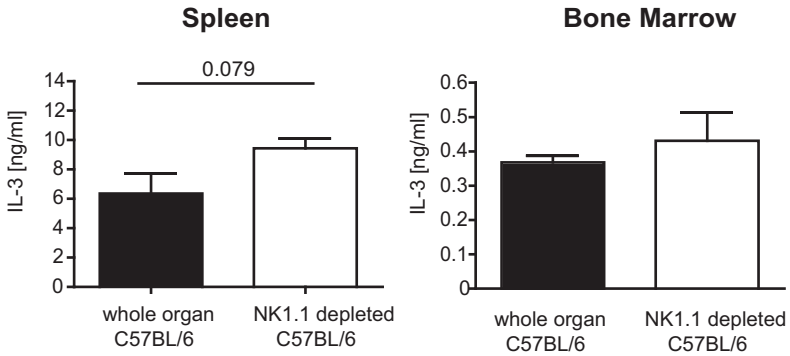

Fig. S5. NK1.1^{+} cells do not contribute to ex vivo IL-3 production following helminth infection. Bone marrow and spleen cells were isolated from *H. polygyrus bakeri* infected C57BL/6 mice at day 10 postinfection and restimulated with PMA/ionomycin for 24 h. Levels of IL-3 in supernatant of whole organ culture or NK1.1-depleted cell fractions were measured by ELISA.

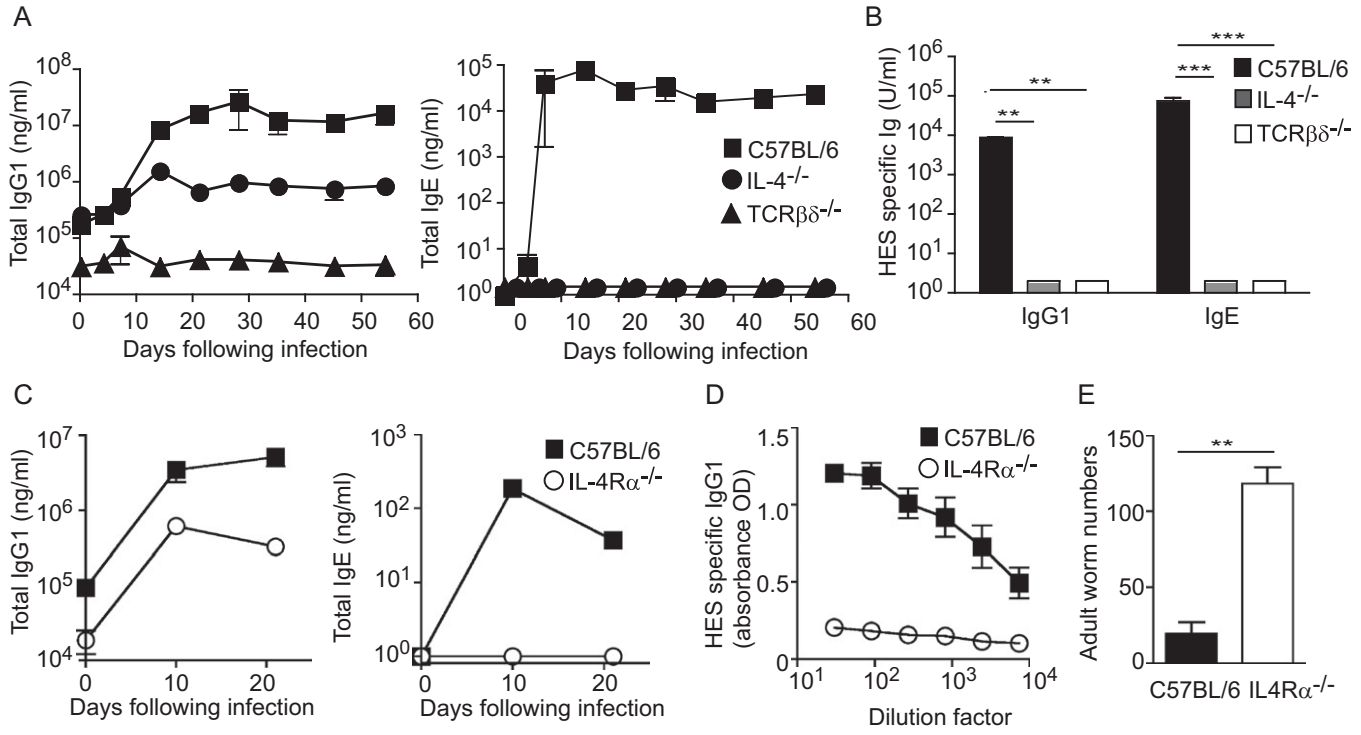

Fig. S6. IL-4–IL-4Rα interactions are required for helminth-induced antibodies and protective immunity. (*A*) Total IgG1 and IgE levels present in the serum of C57BL/6, IL-4$^{-/-}$, or TCRβδ$^{-/-}$ mice are shown for the indicated time points following primary infection with *H. polygyrus backeri*. (*B*) Antibodies exhibiting specificity for L5 HES products were determined for C57BL/6, IL-4$^{-/-}$, or TCRβδ$^{-/-}$ mice at day 13 following secondary infection with *H. polygyrus backeri*. (*C*) Total IgG1 and IgE levels present in the serum of C57BL/6 or IL-4Rα$^{-/-}$ mice are shown for the indicated time points following primary infection with *H. polygyrus backeri*. (*D*) Antibodies exhibiting specificity for L5 HES products and (*E*) numbers of adult worms were determined for C57BL/6 or IL-4Rα$^{-/-}$ mice at day 13 following secondary infection with *H. polygyrus backeri*.

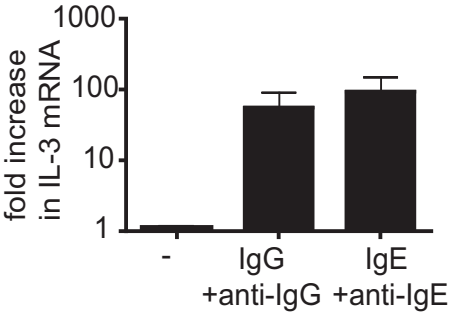

Fig. S7. IgG or IgE cross-linking can elicit IL-3 mRNA expression by bone-marrow–derived basophils in vitro. Relative expression of IL-3 mRNA was determined by quantitative real-time RT-PCR for bone-marrow–derived basophils stimulated by IgE or IgG cross-linking. Data are expressed as fold change of activated versus control basophils. All data are derived from one experiment and are representative of least two independent experiments.

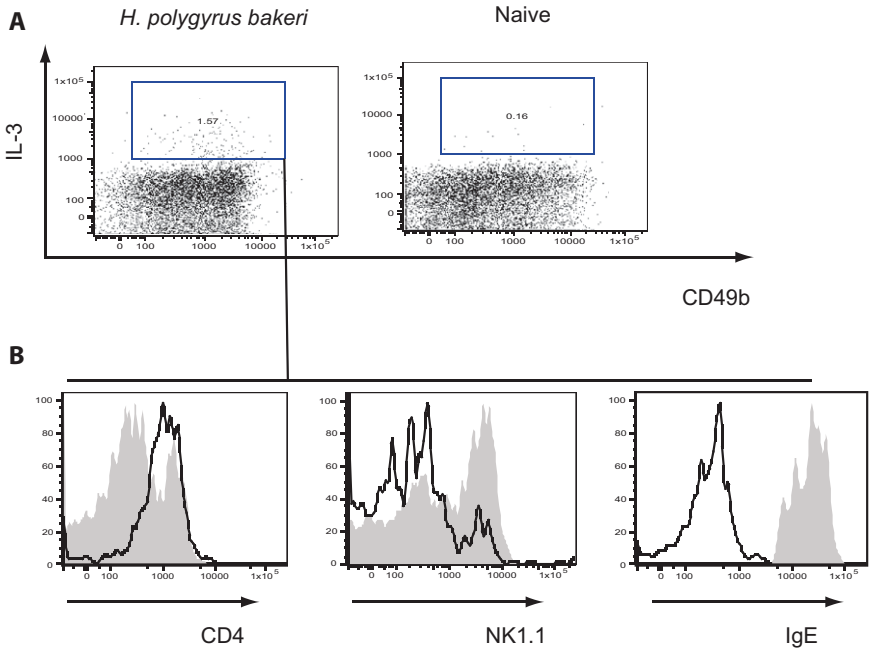

Fig. S8. CD49^{+}CD4^{+}NK1.1^{-} T cells are the main ex vivo producers of IL-3 following helminth infection. Spleen cells were isolated from *H. polygyrus bakeri*-infected C57BL/6 mice at day 10 postinfection, enriched for CD49^{+} cells by positive selection using DX5 MACs beads, and restimulated with PMA/ionomycin + monensin for 24 h. (*A*) IL-3 production was determined for cells isolated from infected or naïve mice by intracellular cytokine staining. (*B*) IL-3^{+} cells (all CD49b$^{int\text{-}hi}$) from infected mice were gated and their expression of CD4, NK1.1, and IgE was determined (solid lines). Shaded histograms indicate staining for CD4 and NK1.1 in IL-3^{-ve} cells or for IgE in IgE^{+ve}IL-3^{-ve} basophils.

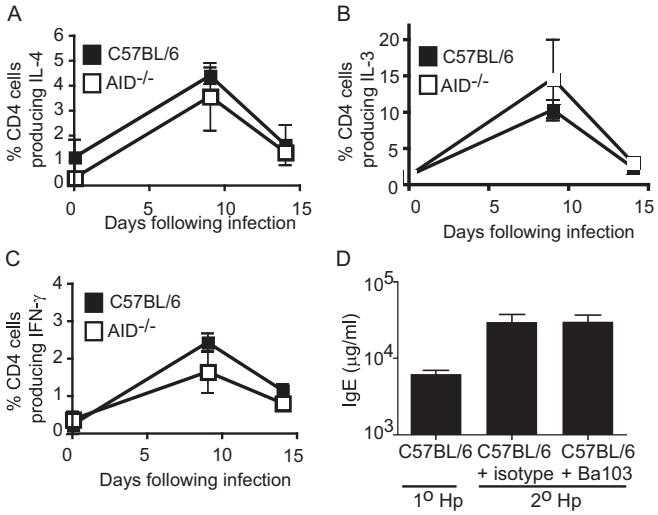

Fig. S9. AID$^{-/-}$ mice exhibit normal T-cell cytokine production following *H. polygyrus bakeri* infection. At the indicated time points following *H. polygyrus bakeri* infection, mesenteric lymph node cells from C57BL/6 or AID$^{-/-}$ mice were cultured with L5 excretory/secretory (HES) antigens and (*A*) IL-4, (*B*) IL-3, and (*C*) IFNγ production was determined by intracellular cytokine staining and flow cytometry. (*D*) C57BL/6 mice were subjected to primary or secondary infection with *H. polygyrus bakeri*. Secondary infected mice additionally received 10 μg of an isotype control or basophil-depleting antibody (Ba103) on days −2, 0, 5, and 8 postinfection and total IgE levels were determined by ELISA at day 12 postinfection.