

which should be cited to refer to this work.

# Supporting Information

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### 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/secretory 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<sup>+</sup> cells were depleted by magnetic cell sorting via Nk1.1 biotin and streptavidin microbeads (MACS) (Milteny Biotech). For intracellular cytokine staining of stimu-

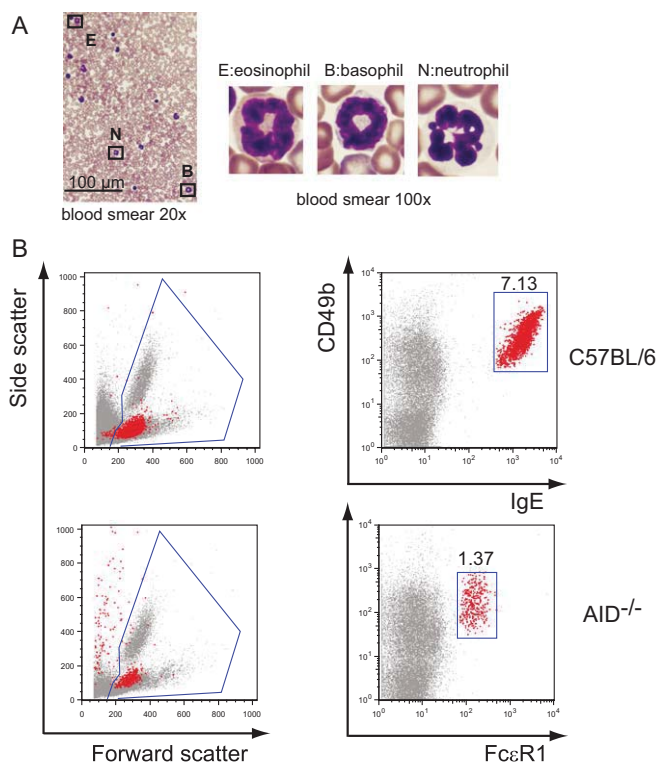
lated bone marrow or spleen cells, cells were additionally incubated with monensin, permeabilized, and stained with anti-IL-3 PE (M12-SF8) (Biolegend).

**Quantification of IL-3 mRNA Expression.** Basophils were purified from in vitro bone marrow cultures by MACs sorting of CD49b<sup>+</sup> (DX5 Microbeads; Milteny 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'.

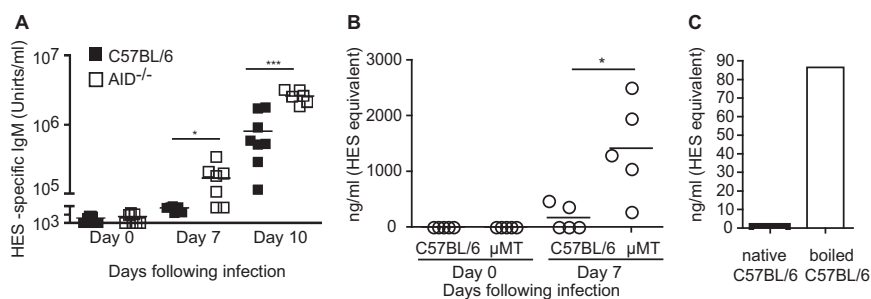
**Statistical Analysis.** For all data, significant differences were determined between gene-deficient or treatment groups and wild-type mice by a one-tailed Student *t* test with a confidence interval of 95%. Significant *P* values are shown at \**P* < 0.05, \*\**P* < 0.01, or \*\*\**P* < 0.001.

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

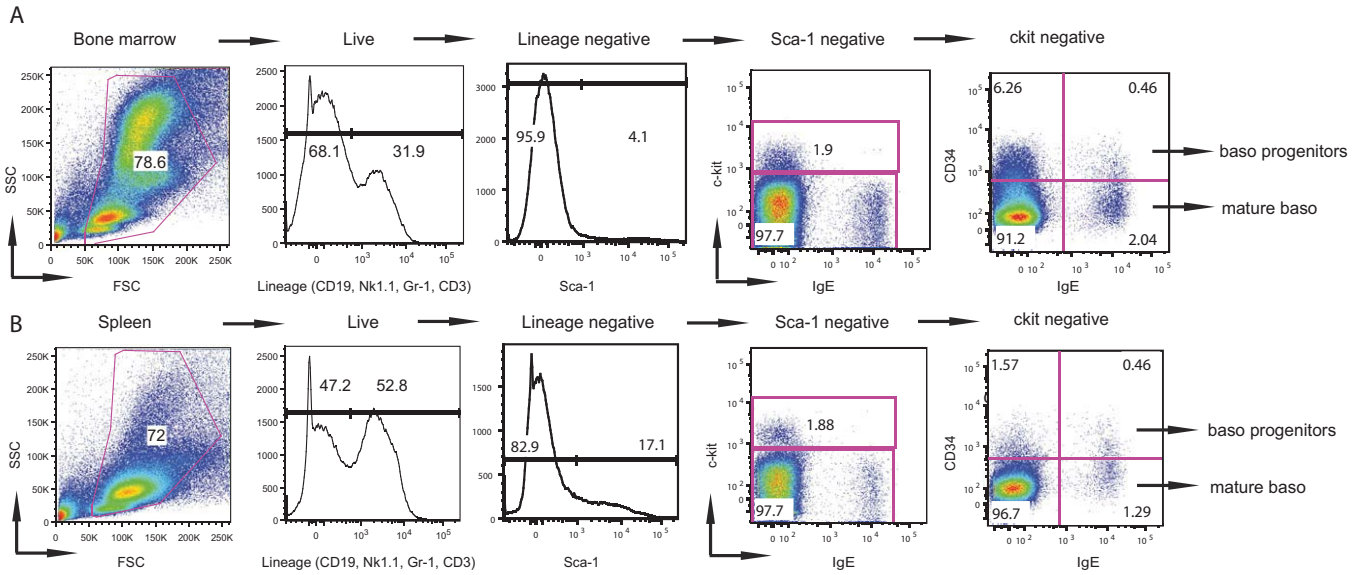
2. Hewitson JP, et al. (2011) *Heligmosomoides polygyrus* elicits a dominant nonprotective antibody response directed against restricted glycan and peptide epitopes. *J Immunol* 187:4764–4777.



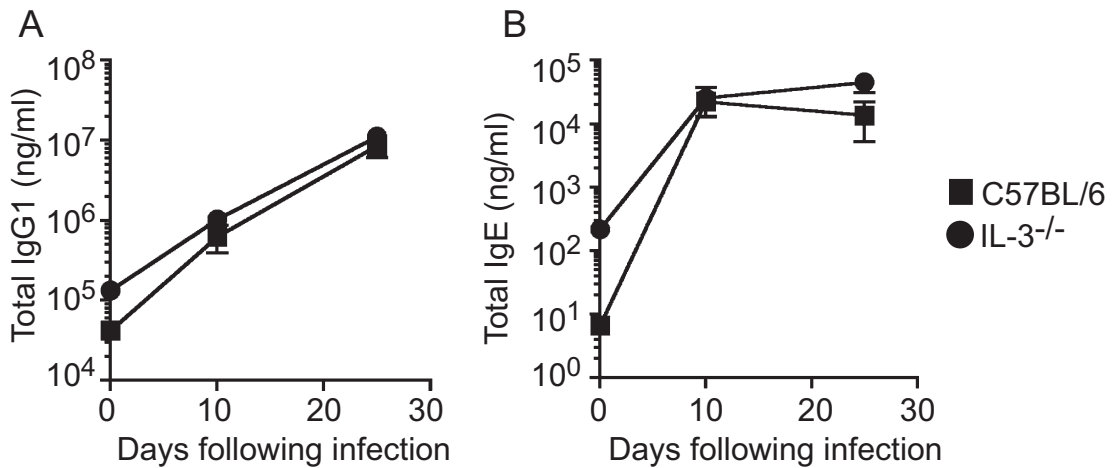
**Fig. S1.** Identification of basophils in C57BL/6 and antibody-deficient mice. (A) Pictures represent blood smears from *H. polygyrus bakeri*-infected mice stained with Diff-Quik. The eosinophils, basophils, and neutrophils were determined on the basis of morphology and staining as illustrated. (B) Representative FACS plots of basophils using CD49b and IgE or Fc $\epsilon$ R1 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 Fc $\epsilon$ R1. However, as a high degree of IgE binding in infected mice was observed to interfere with the efficiency of anti-Fc $\epsilon$ R1 staining, surface IgE was generally determined as a more reliable marker of Fc $\epsilon$ R1 expression in these mice.



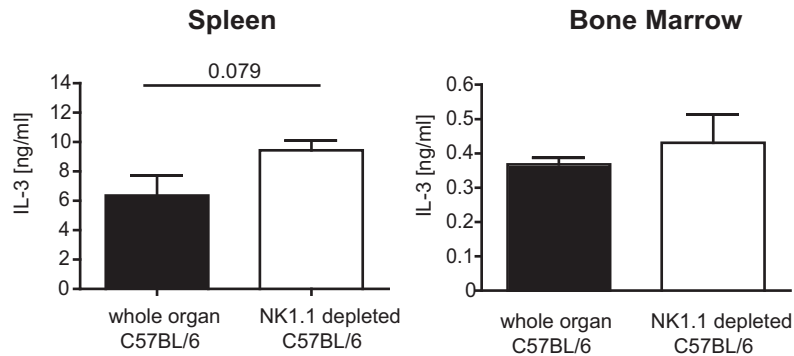
**Fig. S2.** B-cell-deficient mice exhibit increased levels of circulating helminth antigens. (A) *H. polygyrus bakeri*-specific IgM was quantified for C57BL/6 and AID<sup>-/-</sup> mice following primary infection. (B) Presence of *H. polygyrus bakeri*-derived antigens (HES) in the serum of C57BL/6 or B-cell-deficient ( $\mu$ MT<sup>-/-</sup>) 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.



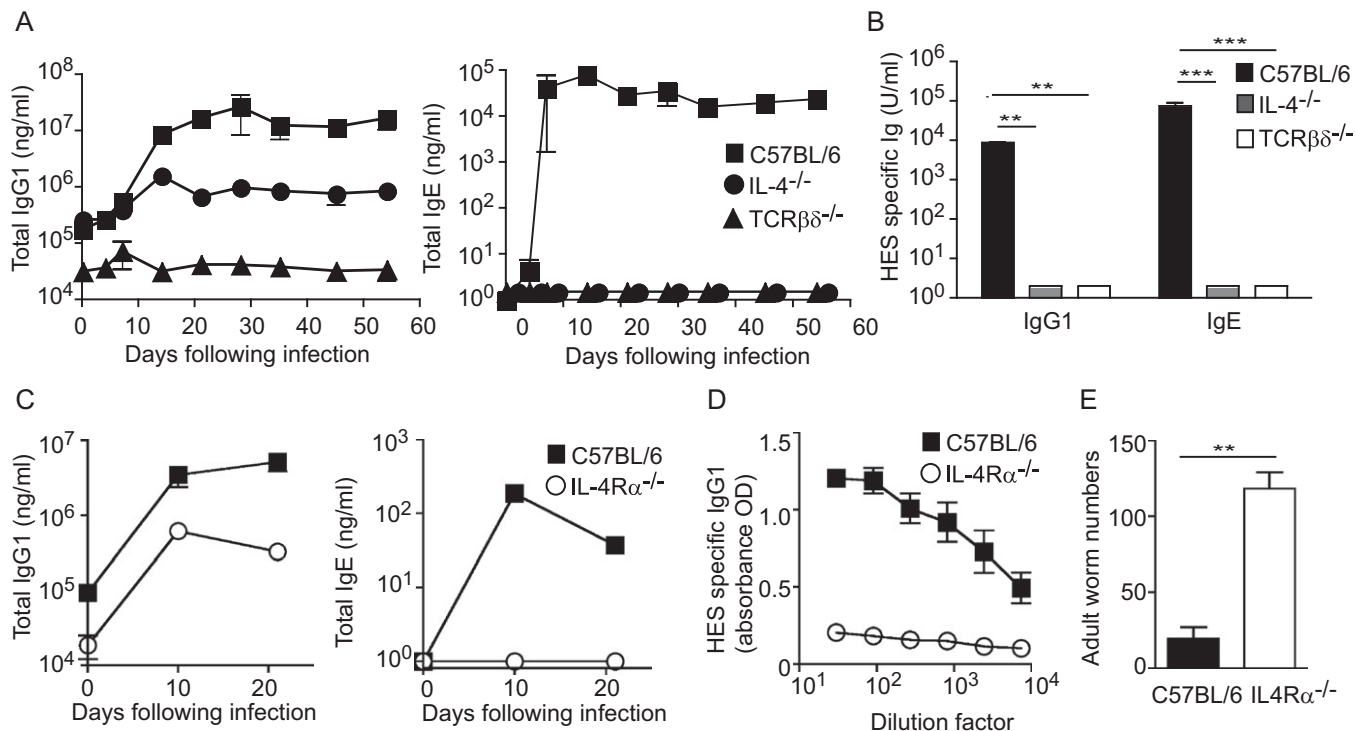
**Fig. S3.** Gating strategy of mature and progenitor basophils. Basophils from the bone marrow (A) or spleen (B) were defined by a lack of expression of the markers CD3, CD19, NK1.1, Ly6G, Sca-1, and c-kit and a positive expression for CD16/CD32<sup>+</sup> and FcεRI or IgE. Basophils were then further 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.



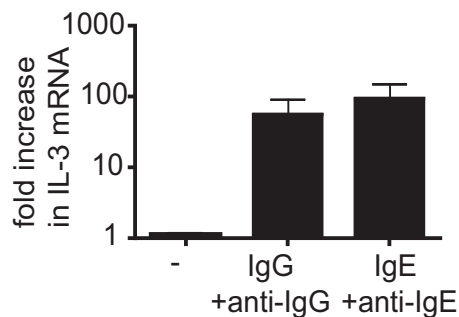
**Fig. S4.** IL-3 is not necessary for antibody production following helminth infection. (A) Total IgG1 and (B) IgE levels present in the serum of C57BL/6 or IL-3<sup>-/-</sup> mice are shown for the indicated time points following primary infection with *H. polygyrus bakeri*.



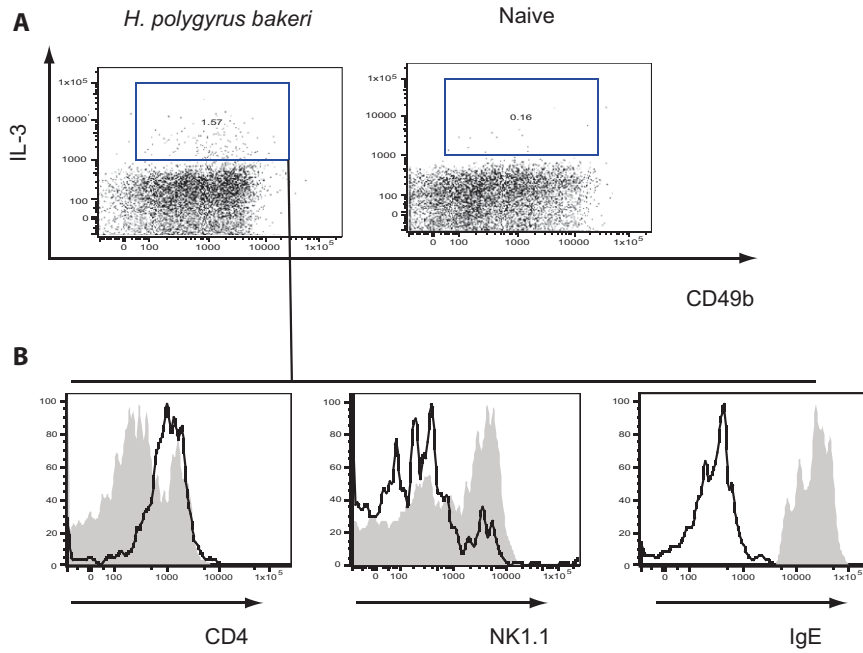
**Fig. S5.** NK1.1<sup>+</sup> cells do not contribute to ex vivo IL-3 production following helminth infection. Bone marrow and spleen cells were isolated from *H. polygyrus bakeri* infected C57BL/6 mice at day 10 postinfection and restimulated with PMA/ionomycin for 24 h. Levels of IL-3 in supernatant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



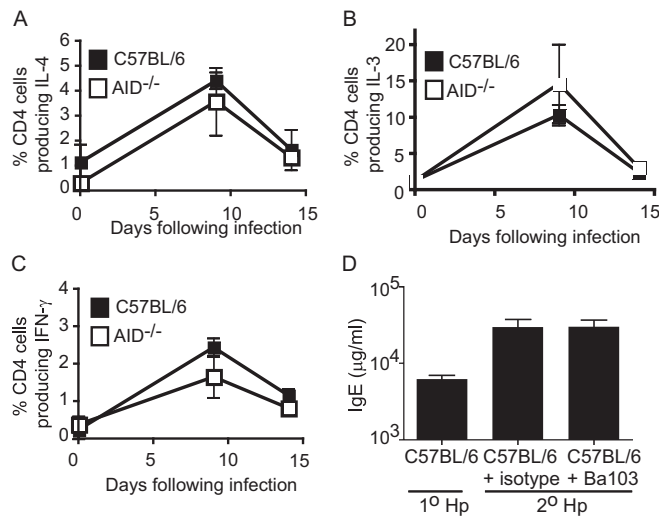
**Fig. 56.** IL-4-IL-4R $\alpha$  interactions are required for helminth-induced antibodies and protective immunity. (A) Total IgG1 and IgE levels present in the serum of C57BL/6, IL-4<sup>-/-</sup>, or TCR $\beta\delta$ <sup>-/-</sup> mice are shown for the indicated time points following primary infection with *H. polygyrus bakeri*. (B) Antibodies exhibiting specificity for L5 HES products were determined for C57BL/6, IL-4<sup>-/-</sup>, or TCR $\beta\delta$ <sup>-/-</sup> mice at day 13 following secondary infection with *H. polygyrus bakeri*. (C) Total IgG1 and IgE levels present in the serum of C57BL/6 or IL-4R $\alpha$ <sup>-/-</sup> mice are shown for the indicated time points following primary infection with *H. polygyrus bakeri*. (D) Antibodies exhibiting specificity for L5 HES products and (E) numbers of adult worms were determined for C57BL/6 or IL-4R $\alpha$ <sup>-/-</sup> mice at day 13 following secondary infection with *H. polygyrus bakeri*.



**Fig. 57.** 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. 58.** CD49<sup>+</sup>CD4<sup>+</sup>NK1.1<sup>-</sup> 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<sup>+</sup> 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<sup>+</sup> cells (all CD49b<sup>int-hi</sup>) 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<sup>-ve</sup> cells or for IgE in IgE<sup>+</sup>IL-3<sup>-ve</sup> basophils.



**Fig. 59.** AID<sup>-/-</sup> 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<sup>-/-</sup> mice were cultured with L5 excretory/secretory (HES) antigens and (A) IL-4, (B) IL-3, and (C) IFN $\gamma$  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  $\mu\text{g}$  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.