Supporting Information

Hung et al. 10.1073/pnas.1206587110

SI Materials and Methods

Worm Infections. Mice were inoculated s.c. with 750 or 500 infectious third stage larvae (L_3) for primary and secondary infections, respectively. For secondary challenge, mice were treated with 100 μL of the anthelminthic pyrantel pamoate (Phoenix Pharmaceuticals) via oral gavage on d 14, allowed to recover for 14 d and reinoculated s.c. on d 28. Secondary responses were evaluated on d 3 and d 6 after reinfection. Worm burdens within the intestinal lumen and fecal egg counts were determined as previously described (1). Photomicrographs of worms recovered from the intestinal lumen were traced and measured using Motic Images Plus (2.0ML) software. *Escherichia coli* lipopolysaccharide was purchased from Sigma.

IVCCA and RT-PCR. RNA was DNase I-treated and cDNA prepared using SuperScript II Reverse Transcriptase (Invitrogen). Real-time PCR was carried out on a Gene Amp 7500 instrument (PE Biosystems) with the Syber Green detection reagent. CT values for genes evaluated were determined and expressed using the $1/\Delta\Delta^{ct}$ method, as described previously (2). Relative amounts of in vivo IL-4 and IL-13 secretion were determined by the IVCCA (3). Serum levels of MCPT-1 (eBioscience) and total IgE (Biolegend) were determined by sandwich ELISA. Mouse MCPT-8 primer assay was purchased from Qiagen and used according to the manufacturer's protocol. Mouse primers specific for IL-4, IL-13, and RELMβ have been described previously (4).

To obtain cells within the bronchoalveolar lavage (BAL) fluid, lungs were lavaged three times with a 1.0 mL aliquot of cold Hanks' balanced salt solution (Invitrogen). Recovered lavage fluid (70–80%) was centrifuged (300 g for 10 min) and the cell pellet resuspended in 1.0 mL of 10% (vol/vol) 1× PBS, followed by concentration with Amicon Ultra centrifugal filter units with 3K cutoff (Millipore). Total cells were counted with a hemocytometer. Slides were prepared by cytocentrifugation (Cytospin 2; Shandon Instruments) and stained with Diff-Quik (Dade Behring). BAL cell differential counts were determined using morphologic criteria under a light microscope with evaluation of ~300 cells/slide.

Flow Cytometry. Before intracellular cytokine staining, cells were stimulated overnight with phorbol myristate acetate (PMA)/Ionomycin. To block cytokine secretion, Brefeldin and Monensin were added for the last 4 h before staining. Acquisition was performed with BD LSRII (lasers tuned to 355 nm, 405 nm, 488 nm, 640 nm, and 541 nm) or BD LSR Fortessa (lasers tuned to 405 nm, 640 nm, and 488 nm) flow cytometers, and data were analyzed with Flojo software (v8.8, Tree Star). For sorting, single cell suspensions from lymph nodes and lung digests were further processed with lineage depletion Microbeads (Miltenyi Biotec) before staining. Cell sorting was performed on a BD Aria within the Division of Experimental Medicine (DEM) at University of California, San Francisco, San Francisco General Hospital.

1. Herbert DR, et al. (2009) Intestinal epithelial cell secretion of RELM-beta protects against gastrointestinal worm infection. *J Exp Med* 206(13):2947–2957.
2. Herbert DR, et al. (2010) Arginase I suppresses IL-12/IL-23p40-driven intestinal inflammation during acute schistosomiasis. *J Immunol* 184(11):6438–6446.
3. Finkelman FD, Morris SC (1999) Development of an assay to measure in vivo cytokine production in the mouse. *Int Immunol* 11(11):1811–1818.
4. Wills-Karp M, et al. (2012) Trefoil factor 2 rapidly induces interleukin 33 to promote type 2 immunity during allergic asthma and hookworm infection. *J Exp Med* 209(3):607–622.

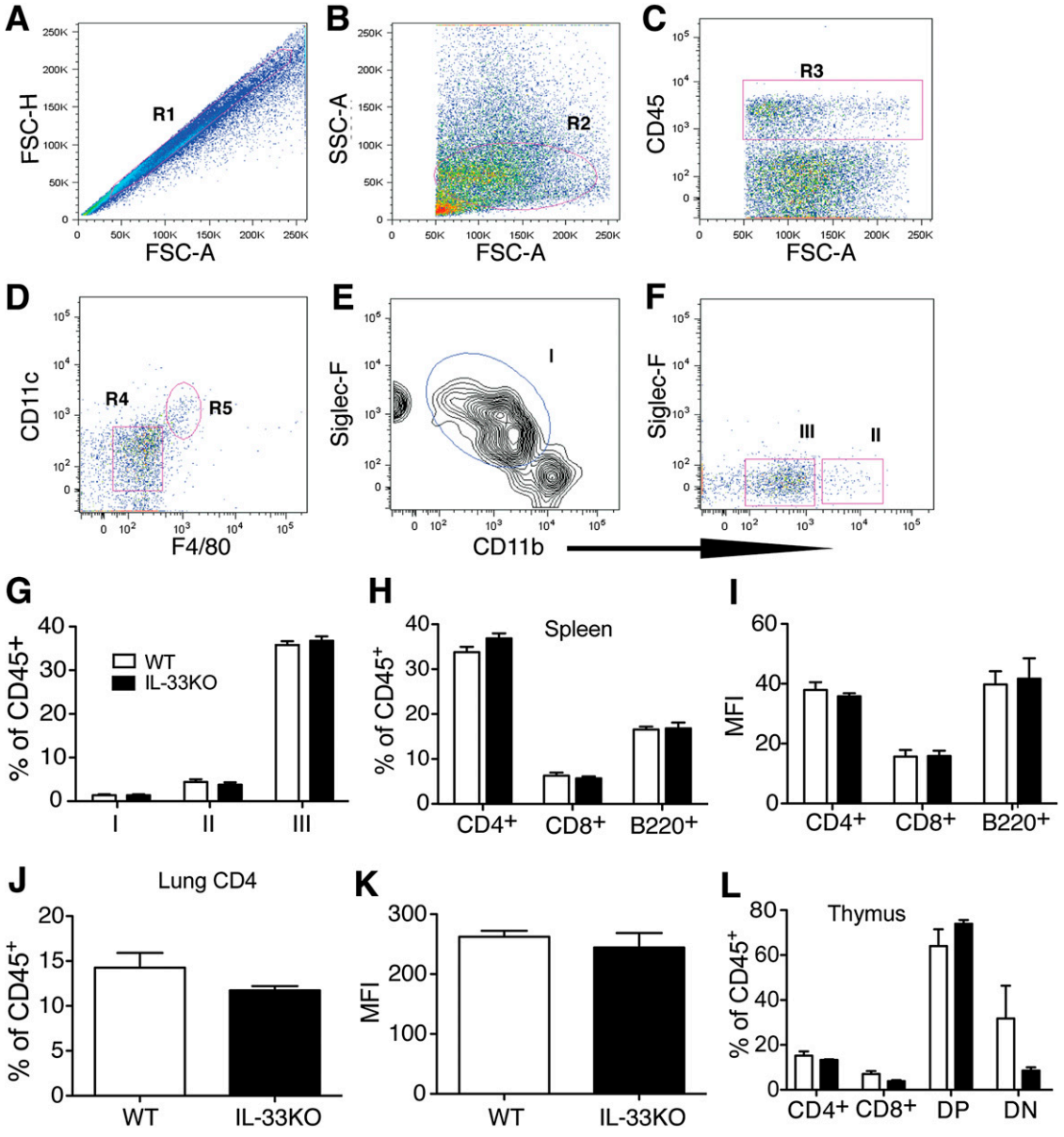

Fig. S1. Characterization of naïve WT and IL-33KO mice. (*A–F*) Gating strategies for myeloid cell subpopulations within the lungs. (*G*) Comparison of group I ($CD11c^{+}F4/80^{+}CD11b^{med}SiglecF^{+}$), group II ($CD11c^{lo}F4/80^{lo}CD11b^{+}SiglecF^{-}$), and group III ($CD11c^{lo}F4/80^{lo}CD11b^{lo}SiglecF^{-}$) as gated in *E* and *F*. (*H*) Comparisons of lymphocyte subsets in the spleen. (*I*) mean fluorescence intensity (MFI) for CD25 levels within splenocyte subsets gated in *H*. (*J*) CD4 T-cell abundance in WT and IL-33KO lung tissue. (*K*) MFI for CD25 levels in lung CD4 cells gated in *J*. (*L*) T-cell subsets in the thymus of WT and IL-33KO mice.

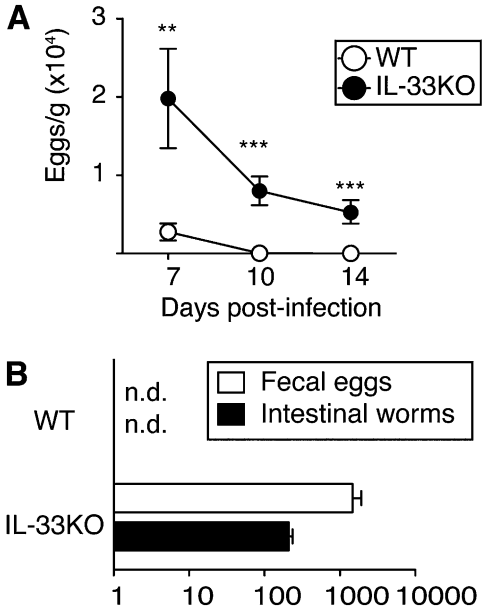

Fig. S2. IL-33KO mice are highly susceptible to primary infection with *N. brasiliensis*. (*A*) Numbers of *N. brasiliensis* ova in feces from WT and IL-33KO mice at the indicated time points following s.c. inoculation with 750 L_3. (*B*) Numbers of fecal egg and intestinal worms recovered from WT and IL-33KO mice at d 21 postinfection. Data show mean ± SE of 6–8 mice/group of three independent experiments. $^{**}P < 0.01$ and $^{***}P < 0.001$.

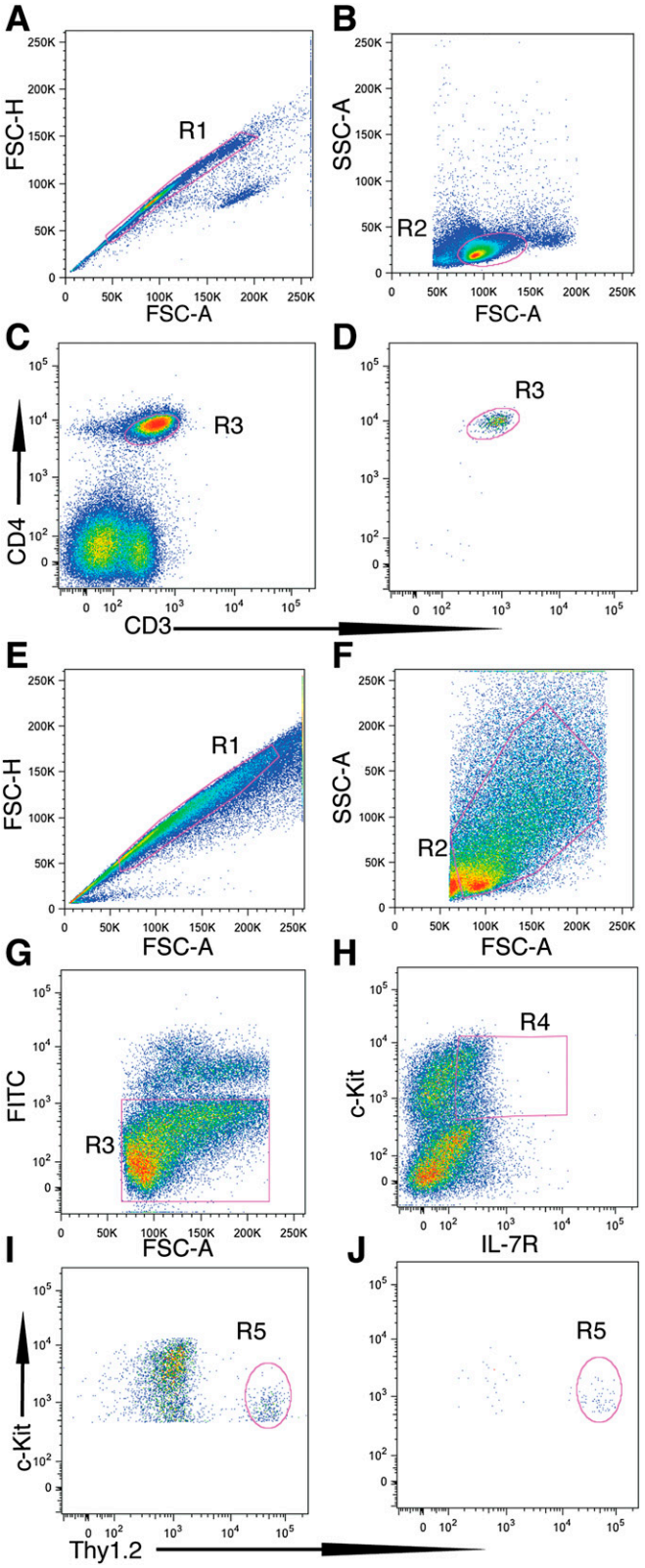

Fig. S3. Gating strategy for CD4$^+$T cells and ILC2. (*A–D*) Plots show the sorting strategy for CD4 $^+$T cells isolated from mesenteric lymph nodes (CD3$^+$CD4$^+$). Data showing the purity of the CD4 T population (*C*) before and (*D*) after cell sorting using the FACS Aria sorter. (*E–J*) Plots show the gating strategy for purification of ILC2s from lung homogenate. A lineage-negative depletion kit (Miltenyi) was used to enrich for ILC2s before the sort. ILC2s were defined as lin$^-$, c-kit$^+$, IL-7R$^+$, and Thy1.2$^+$ cells. Figures show the IL-7$^+$c-kit$^+$Thy1.2$^+$ population (*I*) before and (*J*) after sorting.

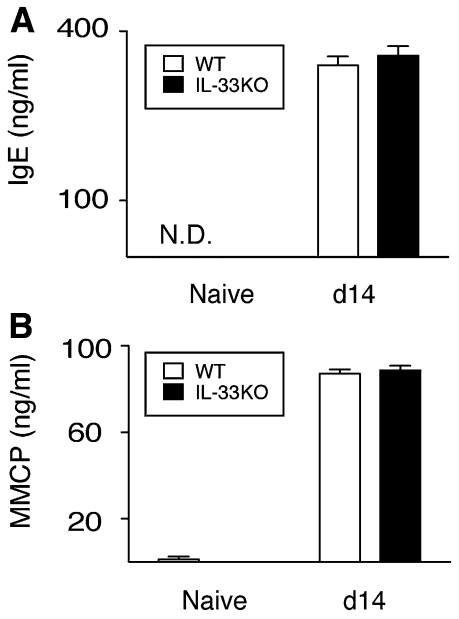

Fig. S4. IL-33KO mice generate canonical Type 2 immunity following primary infection with *N. brasiliensis*. Serum levels of (*A*) total IgE and (*B*) MCPT-1 in WT and IL-33KO mice at the indicated time points following infection with 750 *N. brasiliensis* L_3. Data shown represent the mean ± SE of 6–8 mice/group of three independent experiments.