Supporting Information

Schwartz et al. 10.1073/pnas.1412663111

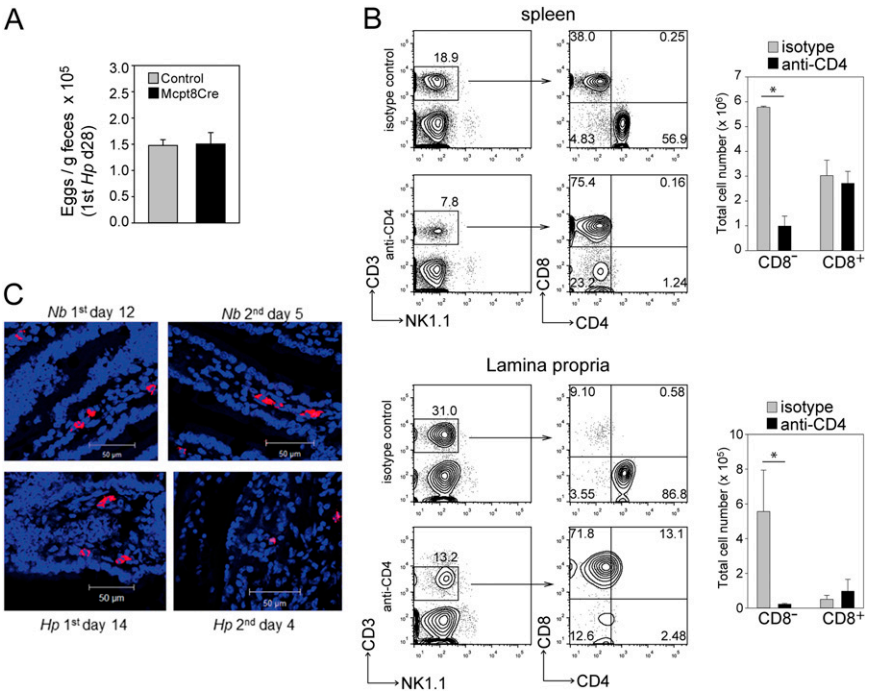

Fig. S1. Immunofluorescence staining of basophils after helminth infection and egg burden during primary infection with *Hp* (also see Fig. 1). (*A*) Number of *Hp* eggs in the feces of infected Mcpt8Cre mice (black bar) and control mice (gray bar) 28 d after the primary infection. The bar graph shows the mean + SEM from two pooled experiments with 10 mice per group. (*B*, *Left*) Contour plots show the frequency of total T cells ($CD3^+NK1.1^-$) and expression of CD4 and CD8 on gated $CD3^+NK1.1^-$ cells in the spleen (*Upper*) and lamina propria (*Lower*) of CD4-depleted and isotype control-injected mice 4 d after the second infection with *Hp*. (*Right*) Bar graphs show the mean + SD of total $CD8^-$ T cells (including $CD4^+$ T cells that were not depleted) and $CD8^+$ T cells from two mice per group. (*C*) Cryosections from fixed small intestinal tissue were stained with anti-mMCP-8 (red) and DAPI (blue). The pictures show enlarged views of the immunofluorescence staining depicted in Fig. 1*H*.

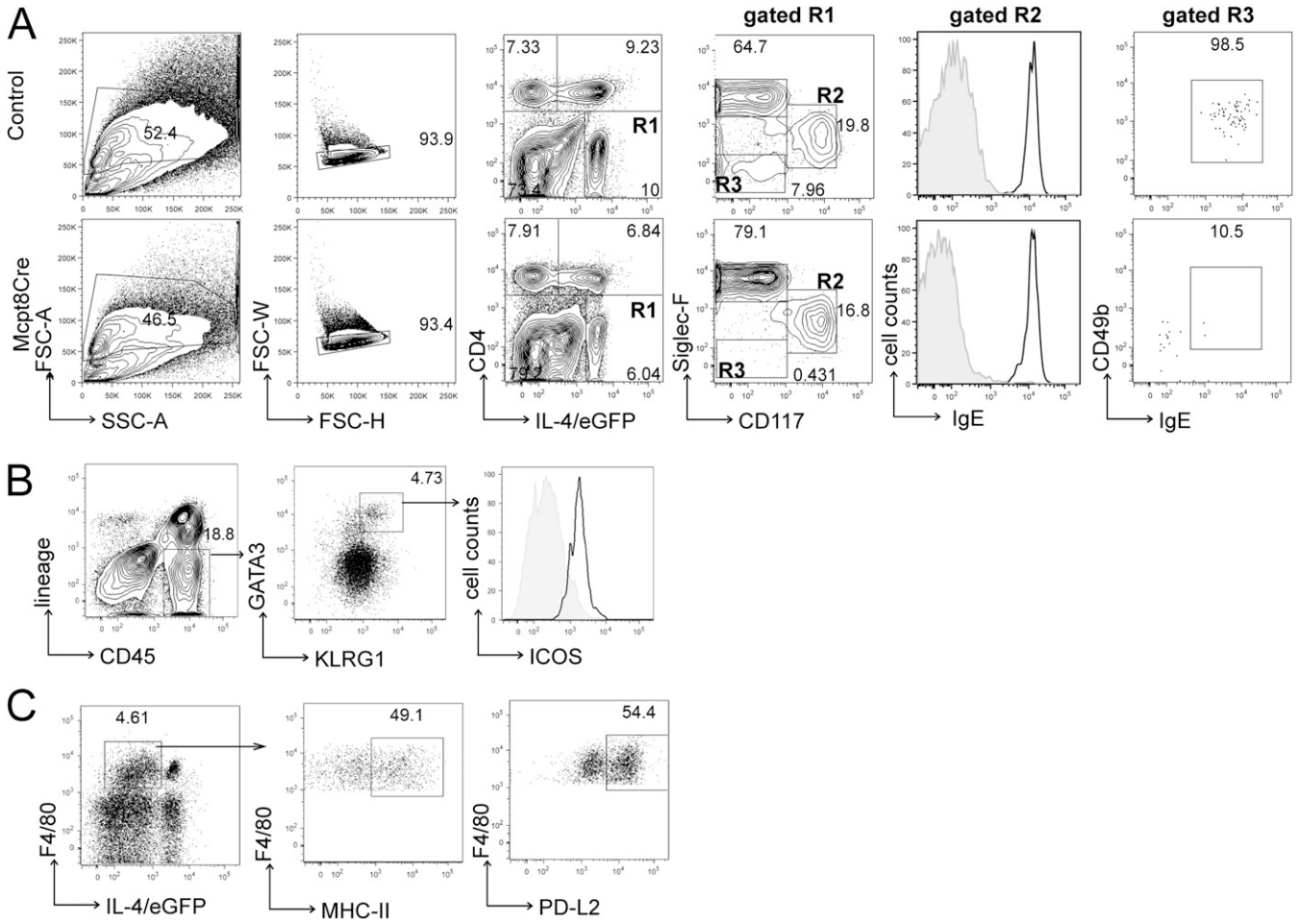

Fig. S2. Flow cytometric gating strategy used to identify cell populations shown in Fig. 2*A*. (*A*) Contour plots and histograms outline the gating strategy used to identify Th2 cells (CD4$^+$IL-4/eGFP$^+$), eosinophils (CD4$^-$IL-4/eGFP$^+$Siglec-F$^+$), mast cells (CD4$^-$IL-4/eGFP$^+$Siglec-F^{lo}CD117$^+$IgE$^+$), and basophils (CD4$^-$IL-4/eGFP$^+$ Siglec-F$^-$CD117$^-$CD49b$^+$IgE$^+$) isolated from the lamina propria of control (*Upper*) and Mcpt8Cre (*Lower*) mice infected with *Hp*. The histogram shows IgE staining on mast cells (black line) compared with eosinophils (shaded gray area). (*B*) Contour, dot plots, and histograms outline the gating strategy used to identify type 2 innate lymphoid cells (lineage$^-$CD45$^+$GATA3$^+$KLRG1$^+$ICOS$^+$) in the lamina propria 4 d after the second infection with *Hp*. The shaded histogram shows isotype control staining. (*C*) AAMs were identified as F4/80$^+$IL-4/eGFP$^-$MHCII^{hi}PD-L2$^+$. Numbers adjacent to the gates indicate the frequency of the parental gate. Leukocyte singlets always were pregated based on forward and side scatter characteristics as depicted in *A*.

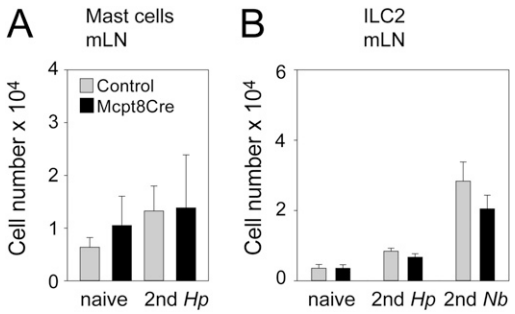

Fig. S3. Numbers of mast cells and ILC2s after the second infection with *Hp* (also see Fig. 2). (*A*) Total number of mast cells in mLNs from naive control mice (gray bars) and Mcpt8Cre mice (black bars) and 4 d after the second infection with *Hp*. (*B*) Total number of ILC2s in mLNs from control mice (gray bars) and Mcpt8Cre mice (black bars) before infection, 4 d after the second infection with *Hp*, or 5 d after the second infection with *Nb*. Bar graphs show the mean + SD from one experiment with four mice per group (*A*) and the mean + SEM from pooled experiments with four to six mice per group (*B*).

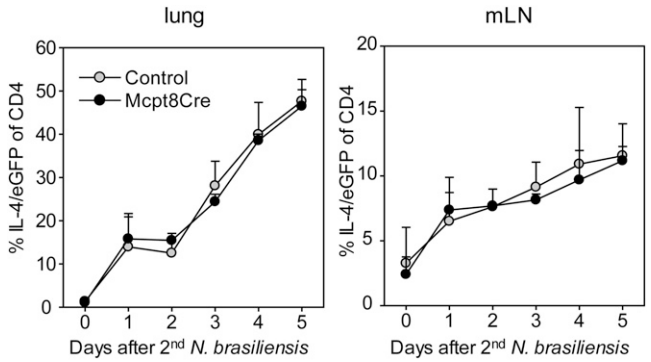

Fig. S4. Basophils are dispensable for Th2 polarization during the second infection with *Nb* (also see Fig. 3). Frequency of IL-4/eGFP^{+} cells among CD4 T cells in the lung and mLN from control mice (gray symbols) and Mcpt8Cre mice (black symbols) at the indicated time points after the second infection with *Nb*. Plots show the mean + SD from one experiment with two mice per group per time point.

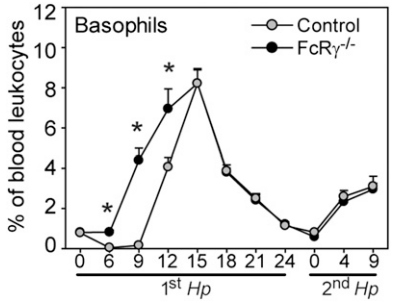

Fig. S5. Basophils in FcR$\gamma^{-/-}$ mice expand during the first and second *Hp* infection (also see Fig. 4). Frequency of basophils in the blood of FcR$\gamma^{-/-}$ mice (black symbols) and control mice (gray symbols) at the indicated time points after *Hp* infection. Plots show the mean + SEM from two pooled experiments with 10 mice per group. *$P < 0.05$.

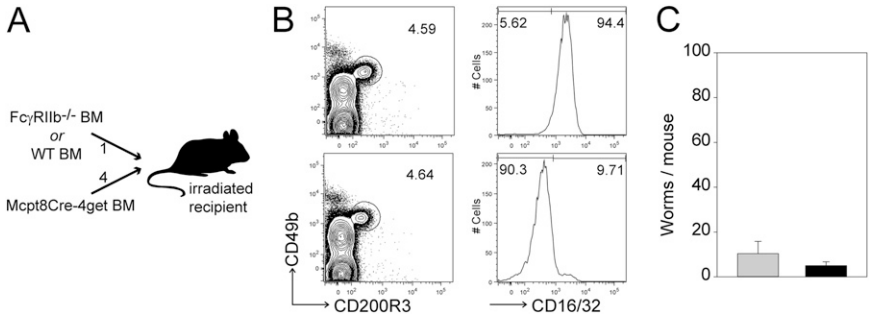

Fig. S6. FcγRIIb on basophils is dispensable for protective immunity against *Hp* (also see Fig. 5). (*A*) Schematic of the generation of MBMCs. *Mcpt8Cre*-4get BM was mixed at a ratio of 4:1 with BM from either WT mice (CD45.1) or FcγRIIb$^{-/-}$ mice and was injected i.v. into lethally irradiated recipient mice to create mice with normal (BasoWT) or FcγRIIb-deficient (BasoFcγRIIb$^{-/-}$) basophils. (*B*) Dot plots show basophils in the blood of chimeric mice described in *A* 4 d after second infection. Numbers indicate the frequency of gated cells. Histograms were gated on basophils and show staining with anti-CD16/32-antibody. (*C*) Number of adult worms in the small intestines of MBMCs 12 d after the second infection (grey bar: BasoWT; black bar: Baso-4-13ko). The graph shows the mean + SD; $n = 3$ mice per group.

Table S1. Antibodies used for flow cytometry

Antigen	Conjugate	Clone	Source
CD4	PerCP-Cy5.5	RM4-5	eBioscience
CD4	APC-eFluor 780	RM4-5	eBioscience
CD49b	Alexa Fluor 647	HMa2	BioLegend
CD200R3	PE	Ba13	BioLegend
IgE	FITC	R35-72	BD
Siglec-F	PE	E50-2440	BD
IL-4	PE	11B11	eBioscience
CD16/32	PE-Cy7	93	eBioscience
CD45.1	FITC	A20	Miltenyi
CD45.2	PerCP-Cy5.5	104	eBioscience
PD-L2	PE	TY25	eBioscience
MHCII	APC-Cy7	M5/ 114.15.2	BioLegend
F4/80	APC	BM8	eBioscience
CD3	Biotin, APC	145–2C11	eBioscience
CD8a	Biotin, APC	53–6.7	eBioscience
CD45R	Biotin, A647	RA3-6B2	BioLegend
Ter119	Biotin	TER-119	eBioscience
CD3	Alexa Fluor 647	17A2	eBioscience
FcεRIα	APC	MAR-1	eBioscience
CD11b	APC	M1/70	eBioscience
CD117	PE-Cy7	2B8	eBioscience
GATA3	PE	TWAJ	eBioscience
KLRG1	eFluor 450	2F1	eBioscience
ICOS	Biotin	7E.17G9	eBioscience
NK1.1	Biotin	PK136	eBioscience
Streptavidin	V500		BD
CD16/32 (Fc block)	Pure	2.4G2	BioXCell
RapidSphere beads	Streptavidin		Stem Cell Technologies

Table S2. Primer sequences

Porphobilinogen Deaminase	Fwd: 5′-TGGTTGTTCACTCCCTGAAGG-3′
	Rev: 5′-AAAGACAACAGCATCACAAGGGT-3′
Relm-α	Fwd: 5′-CCATAGAGAGATTATCGTGGA-3′
	Rev: 5′-TGGTCGAGTCAACGAGTAAG-3′
Muc5ac	Fwd: 5′-CTGTGACATTATCCCATAAGCCC-3′
	Rev: 5′-AAGGGGTATAGCTGGCCTGA-3′