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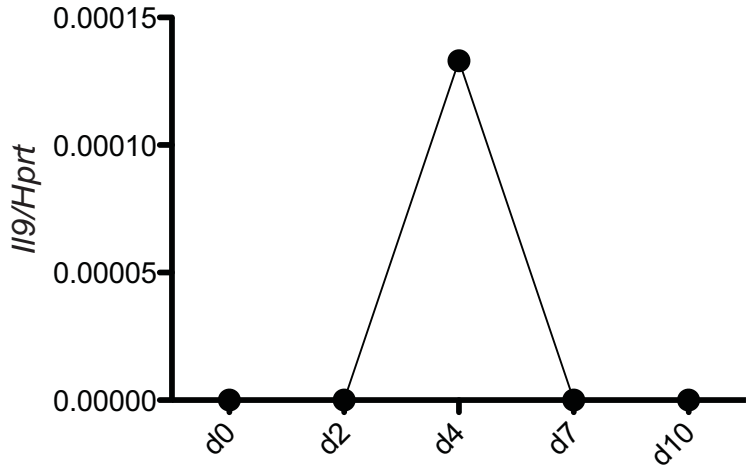
Supplemental Information

Th9 Cells Drive Host Immunity against Gastrointestinal Worm Infection

Paula Licona-Limón, Jorge Henao-Mejia, Angela U. Temann, Nicola Gagliani, Ileana Licona-Limón, Harumichi Ishigame, Liming Hao, De'Broski R. Herbert, and Richard A. Flavell

A


0.00015
0.00010
0.00005
0.00000
Il9/Hprt
d0
d2
d4
d7
d10

B

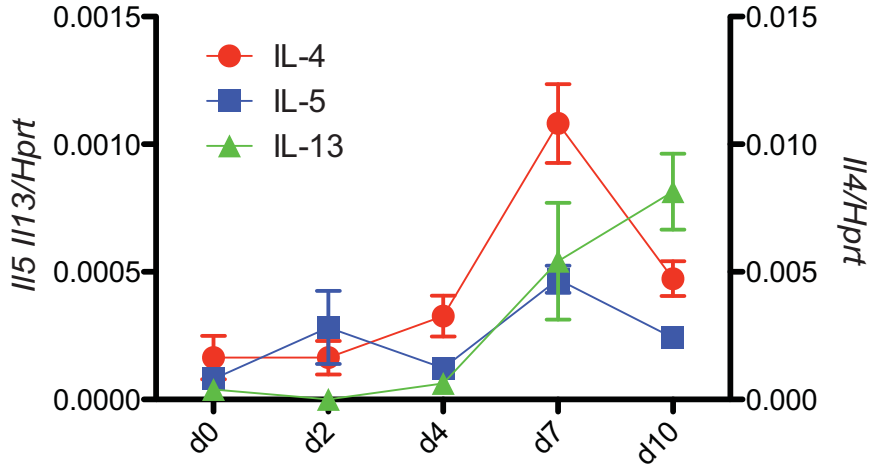

0.0015
0.0010
0.0005
0.0000
Il5 Il13/Hprt
IL-4
IL-5
IL-13
0.015
0.010
0.005
0.000
Il4/Hprt
d0
d2
d4
d7
d10

Figure S1. IL-9 expression precedes IL-4, IL-5 and IL-13 in spleen of *N. brasiliensis* infected mice

(A) C57BL/6 mice were subcutaneously infected with 625 L3 *N. brasiliensis* larvae. Spleen was collected and homogenized at different days p.i. for assessment of *Il9* mRNA expression by real time RT-PCR. The experiment was perfomed two times with similar results with 2-3 mice per day p.i. Statistically significant p values were determined by one-way ANOVA when comparing basal expression (d0) with at least one other time point for each gene.

(B) Same samples as above analyzed for *Il4, Il5* and *Il13* mRNA expression.

Data represent the mean +/- SEM ratio of cytokine gene to *Hprt* expression as determined by the relative quantification method ($\Delta\Delta$Ct). The experiment was perfomed two times with similar results with 2-3 mice per day p.i. Statistically significant p values were determined by one-way ANOVA when comparing basal expression (d0) with at least one other time point for each gene.

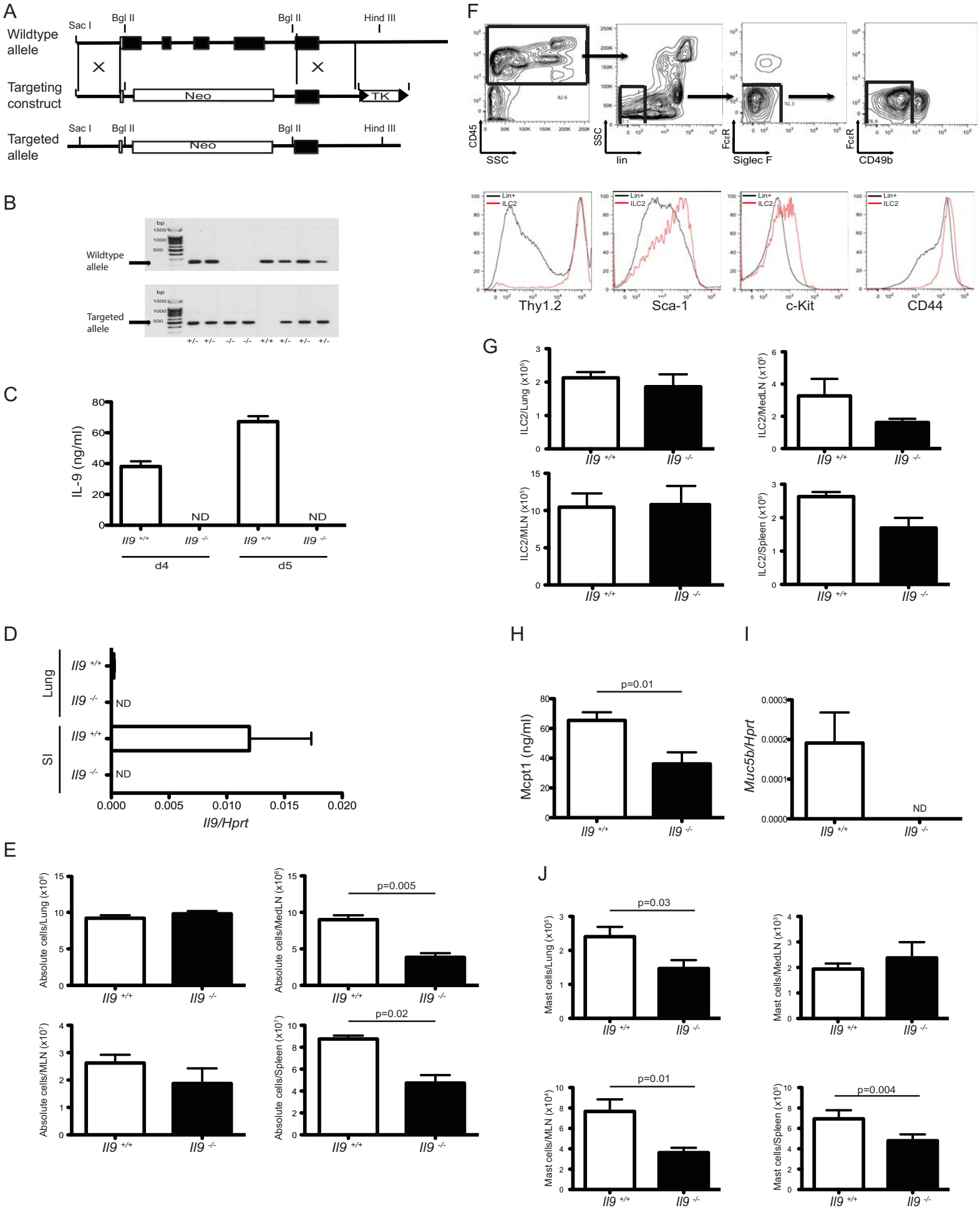

A
Wildtype allele
Targeting construct
Targeted allele
Sac I
Bgl II
Bgl II
Hind III
Neo
TK
B
Wildtype allele
Targeted allele
bp
1500
1000
500
+/- +/- -/- -/- +/+ +/- +/- +/-
C
IL-9 (ng/ml)
ND
ND
Il9 +/+
Il9 -/-
Il9 +/+
Il9 -/-
d4
d5
D
Lung
SI
Il9 +/+
Il9 -/-
ND
ND
Il9/Hprt
E
Absolute cells/Lung (x10⁶)
Absolute cells/MedLN (x10⁶)
p=0.005
Absolute cells/MLN (x10⁷)
Absolute cells/Spleen (x10⁷)
p=0.02
F
CD45
SSC
SSC
lin
FcεR
Siglec F
FcεR
CD49b
Lin+
ILC2
Thy1.2
Sca-1
c-Kit
CD44
G
ILC2/Lung (x10⁵)
ILC2/MedLN (x10⁵)
ILC2/MLN (x10⁵)
ILC2/Spleen (x10⁵)
H
Mcpt1 (ng/ml)
p=0.01
I
Muc5b/Hprt
ND
J
Mast cells/Lung (x10⁵)
p=0.03
Mast cells/MedLN (x10³)
Mast cells/MLN (x10⁴)
p=0.01
Mast cells/Spleen (x10⁵)
p=0.004

Figure S2. Generation and characterization of IL-9 deficient mice

(A) Schematic representation of IL-9 wild-type allele, targeting construct and targeted allele. The targeting construct (middle) had a neomycin resistance gene substituting exons 1-4, followed by a loxp-flanked thymidine kinase (TK) downstream of exon 5. Deletion of TK by Cre recombinase resulted in the targeted allele (bottom). Filled boxes represent exons 1-5. Loxp sites are shown as arrow heads. Restriction sites are depicted.

(B) Genotyping of IL-9 deficient mice showing PCR amplification of a 250 bp and 480 bp product corresponding to the IL-9 wild-type (+/+) and targeted (-/-) allele, respectively.

(C) Sorted naïve $CD4^+$ T cells from *Il9*$^{+/+}$ or *Il9*$^{-/-}$ mice were polarized *in vitro* in Th9 cultures. IL-9 secretion was determined by ELISA at day 4 and day 5. Data represents mean +/- SEM. The experiment was repeated three times with similar results. ND; not detected.

(D) *Il9*$^{+/+}$ or *Il9*$^{-/-}$ mice were subcutaneously infected with 625 L3 *N. brasiliensis* larvae. Lung and small intestine was collected and homogenized at day 7 p.i. for assessment of *Il9* mRNA expression by real time RT-PCR. Data represent the mean +/- SEM expression of the cytokine gene to *Hprt* using the $\Delta\Delta$Ct method.

(E) Total numbers of cells in lung, MedLN, MLN and spleen of *Il9*$^{+/+}$ or *Il9*$^{-/-}$ mice at day 7 p.i.

(F) Gating strategy for analysis if ILC2 cells by flow cytometry in *N. brasiliensis* infected mice. Example shown is lung at day 7 p.i. Numbers represent percentage of boxed cells. The lower panels are representative flow cytometry analysis for surface

expression of Thy1.2, Sca-1, c-kit and CD44 in ILC2 cells (red line) and lin^+ cells (black line) from lung infected mice.

(G) Total numbers of ILC2 cells as determined by flow cytometry in lung, medLN, MLN and spleen of *Il9*$^{+/+}$ or *Il9*$^{-/-}$ mice at day 7 p.i.

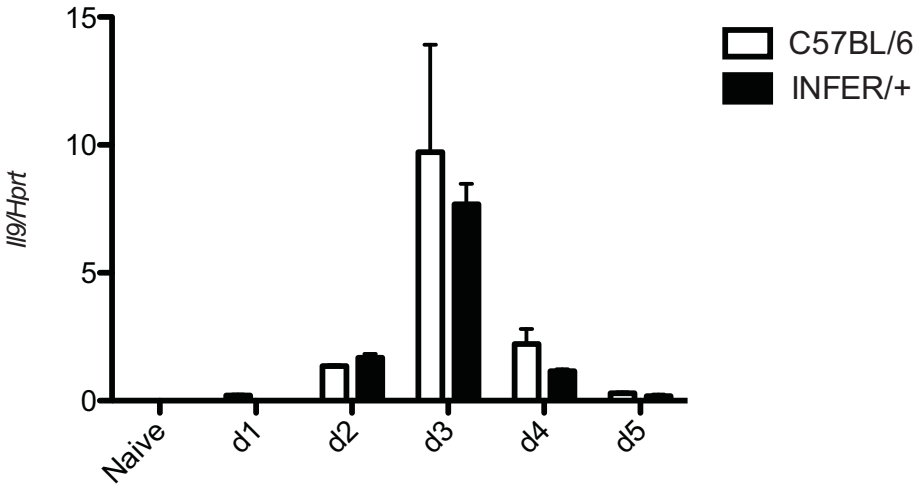
(H) Serum concentration of Mcpt1 as determined by ELISA in *Il9*$^{+/+}$ (white bars) or *Il9*$^{-/-}$ (black bars) mice at day 7 p.i.

(I) Small intestine from *Il9*$^{+/+}$ (white bars) or *Il9*$^{-/-}$ (black bars) mice was collected and homogenized at day 7 p.i for assessment of *Muc5b* mRNA expression by real time RT-PCR. Data represent the mean +/- SEM expression of mucin to *Hprt* using the $\Delta\Delta$Ct method.

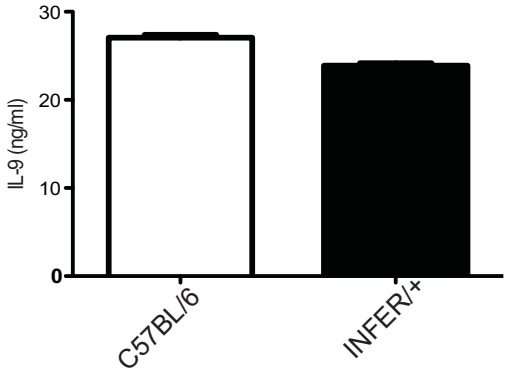
(J) Total mast cell ($FceRI^+$ $c\text{-}Kit^+$) numbers in lung, medLN, MLN and spleen of *Il9*$^{+/+}$ (white bars) or *Il9*$^{-/-}$ (black bars) at day 7 p.i. Mean +/- SEM of experiments with 6 mice per group.

Density plots and histograms are representative results of six mice per group analyzed. Data are represented as mean +/- SEM. All the experiments were performed twice with similar results. n= 6 mice per group. p value determined by unpaired two-tailed Student's t test is shown. ND; not detected.

A


15
10
5
0
Il9/Hprt
C57BL/6
INFER/+
Naive
d1
d2
d3
d4
d5

B


30
20
10
0
IL-9 (ng/ml)
C57BL/6
INFER/+

C

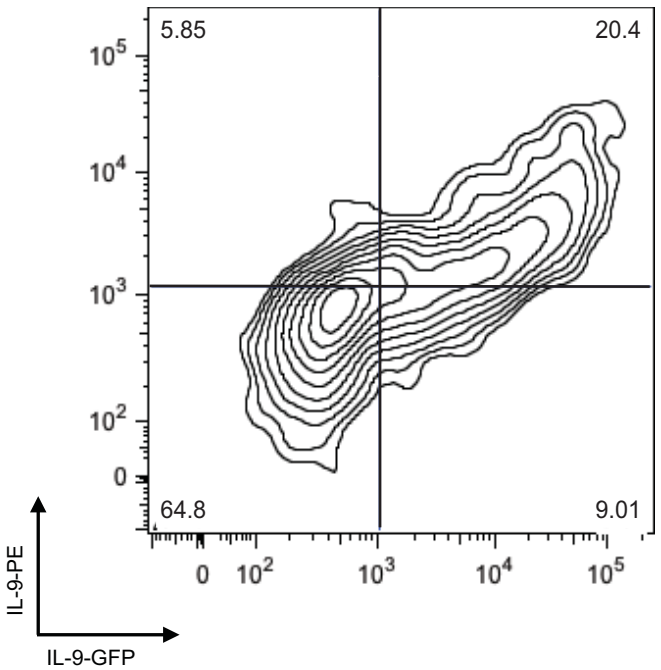

5.85
20.4
64.8
9.01
10^5
10^4
10^3
10^2
0
0
10^2
10^3
10^4
10^5
IL-9-PE
IL-9-GFP

Figure S3. Validation of INFER mice *in vitro*

(A) Sorted naïve $CD4^{+}CD62L^{+}CD25^{-}CD44^{-}$ T cells from wild-type C57BL/6 or INFER/+ mice were cultured in Th9 conditions for 5 days. *Il9* mRNA expression was assessed in samples collected daily by quantitative RT-PCR using the ΔΔCt method. Mean ± SEM normalized to *Hprt* from one out of three independent experiments is shown.

(B) Supernatants from cultures in (A) at day 5 were collected and analyzed by ELISA for IL-9 quantification. Mean ± SEM from one out of three independent experiments measured in triplicate is shown.

(C) Intracellular staining for IL-9 and GFP expression analyzed by flow cytometry in Th9 cultures from INFER/+ naïve T cells at day 5.

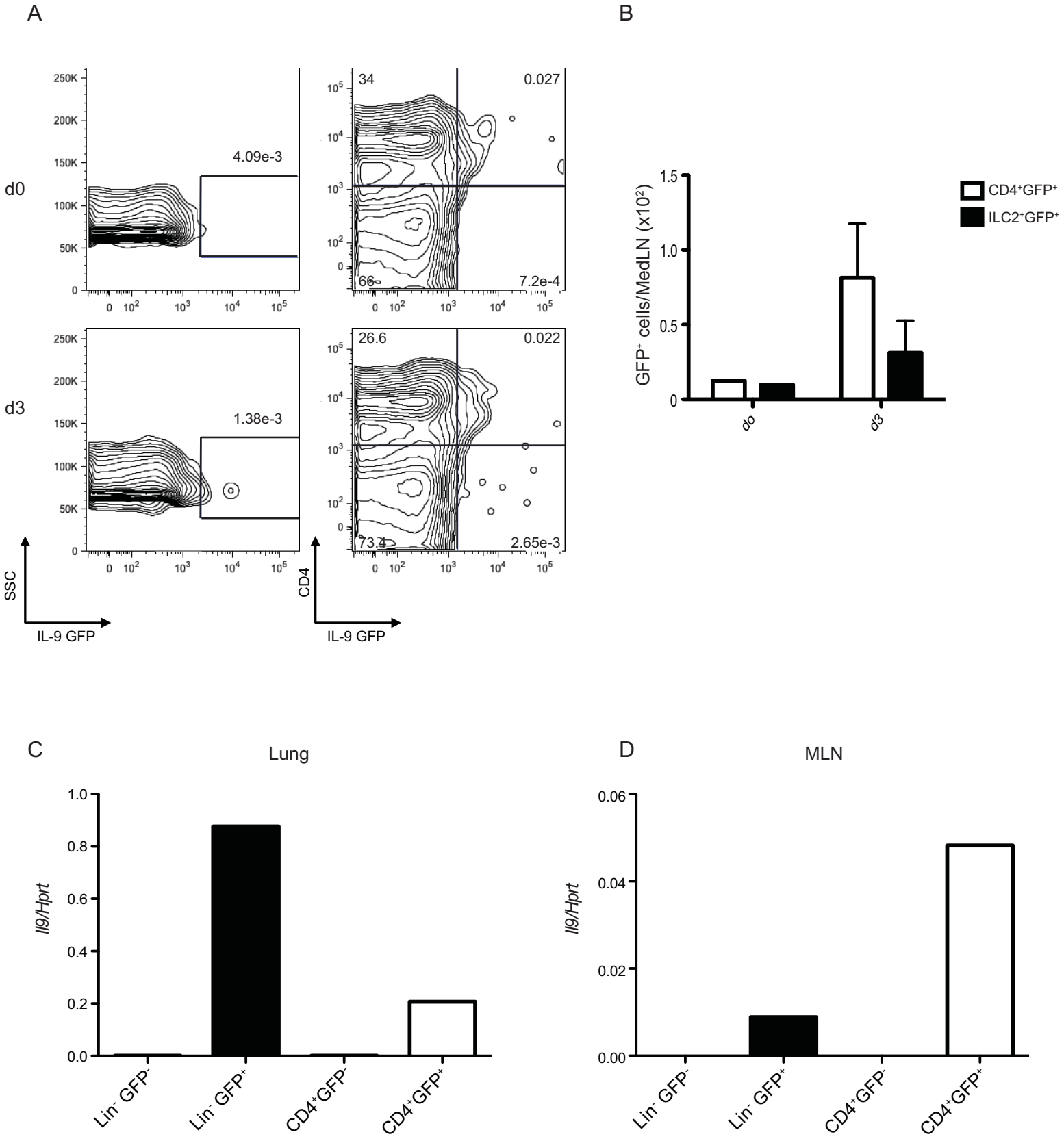

A
d0
d3
4.09e-3
1.38e-3
34
0.027
66
7.2e-4
26.6
0.022
73.4
2.65e-3
SSC
IL-9 GFP
CD4
IL-9 GFP
B
GFP+ cells/MedLN (x10²)
CD4+GFP+
ILC2+GFP+
d0
d3
C
Lung
Il9/Hprt
Lin- GFP-
Lin- GFP+
CD4+GFP-
CD4+GFP+
D
MLN
Il9/Hprt
Lin- GFP-
Lin- GFP+
CD4+GFP-
CD4+GFP+

Figure S4. IL-9 is expressed in GFP$^+$ cells *in vivo*

INFER mice were subcutaneously infected with 625 L3 *N. brasiliensis* larvae.

(A) MedLN were harvested and homogenized at days 0 and 3 p.i for assessment of IL-9 expression by flow cytometry. Numbers represent the frequencies of total GFP$^+$ cells (left column) or GFP$^+$ among CD4$^+$ T cells (right column).

(B) Total numbers of CD4$^+$ T cells (white bars) and ILC2 cells (black bars) expressing IL-9 (GFP$^+$) in medLN as determined by flow cytometry at days 0 and 3 p.i. Data are represented as mean ± SEM from one out of three independent experiments with 3-4 mice per day p.i.

(C-D) CD4$^+$ GFP$^-$, CD4$^+$ GFP$^+$, ILC2 GFP$^-$ and ILC2 GFP$^+$ cells, isolated from lung (C) or MLN (D) of INFER infected mice at day 10 p.i. were assessed for *Il9* mRNA expression by quantitative RT-PCR. Data represent ratio of IL-9 to *Hprt* expression by the relative quantification method ($\Delta\Delta$Ct). The experiment was repeated three times with similar results using 10 pulled INFER mice.

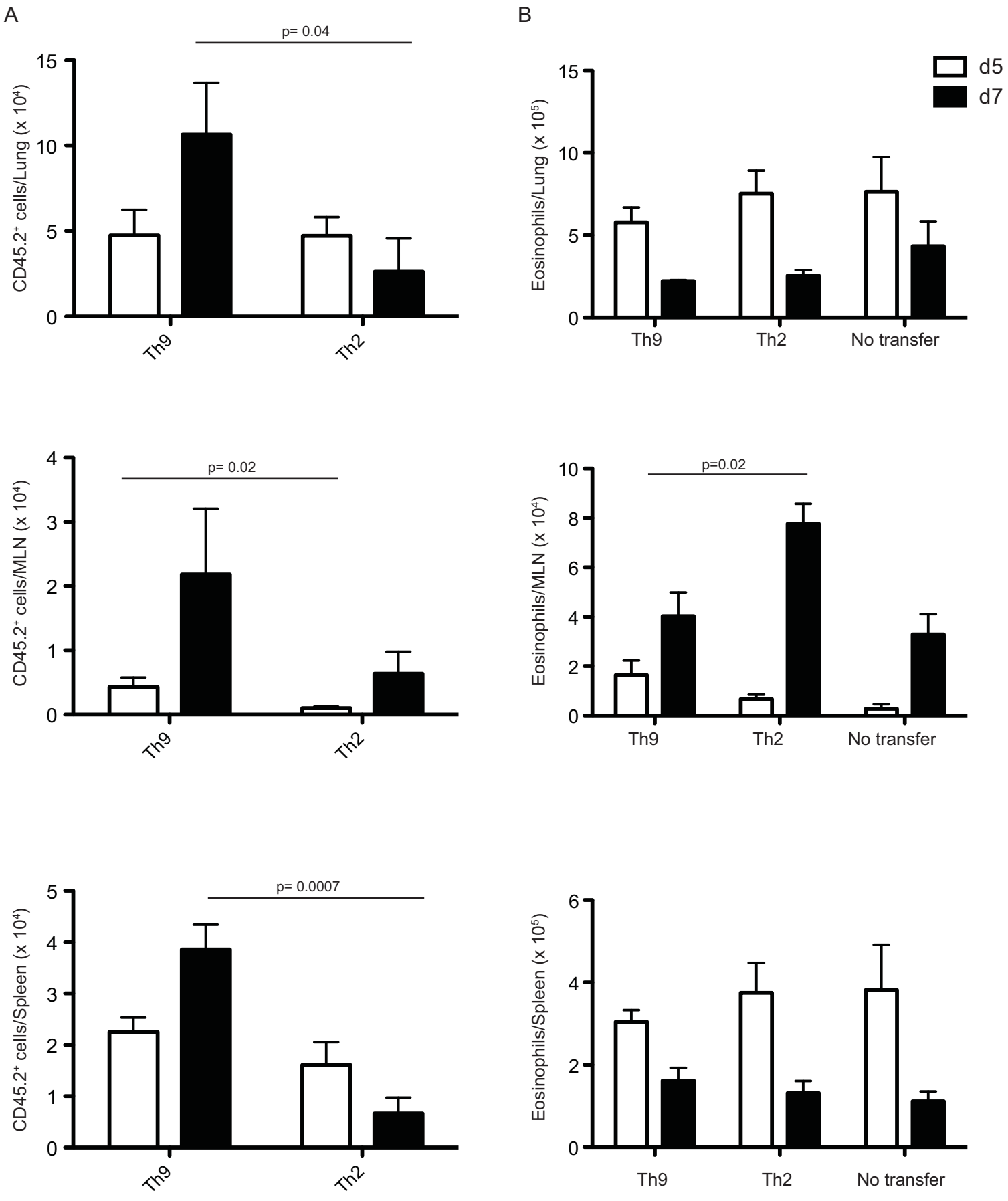

A
B
p= 0.04
CD45.2⁺ cells/Lung (x 10⁴)
15
10
5
0
Th9
Th2
d5
d7
Eosinophils/Lung (x 10⁵)
No transfer
p= 0.02
CD45.2⁺ cells/MLN (x 10⁴)
4
3
2
1
p=0.02
Eosinophils/MLN (x 10⁴)
10
8
6
4
2
p= 0.0007
CD45.2⁺ cells/Spleen (x 10⁴)
5
Eosinophils/Spleen (x 10⁵)
6

Figure S5. Donor cells and eosinophil quantification in Rag2$^{-/-}$ mice adoptively transferred with Th2 or Th9 cells

(A) Sorted effector Th2 or Th9 (2 x 10^5) cells (CD45.2$^+$GFP$^+$) from day 5 *in vitro* cultures with naïve CD4$^+$ T cells isolated from 4get or INFER mice were intravenously transferred into CD45.1$^+$ *Rag2*$^{-/-}$ recipient mice. 24 hrs later, mice were subcutaneously infected with 625 L3 *N. brasiliensis* larvae. Total numbers of transferred Th9 or Th2 cells recovered from lung, MLN, and spleen at day 5 (white bars) and day 7 (black bars) p.i. were determined by flow cytometry (gated on CD45.2$^+$).

(B) Total numbers of eosinophils in lung, MLN and spleen of *Rag2*$^{-/-}$ mice adoptively transferred with effector Th2 or Th9 (2 x 10^5) cells (CD45.2$^+$GFP$^+$) at day 5 (white bars) and day 7 (black bars) p.i. As a control a third group of *Rag2*$^{-/-}$ mice that did not received T cells was also infected.

Data are represented as mean +/- SEM. The experiment was repeated three times with similar results. n= 4-5 mice per group. Statistics were calculated by unpaired two-tailed Student's t test. The p values between groups are shown.

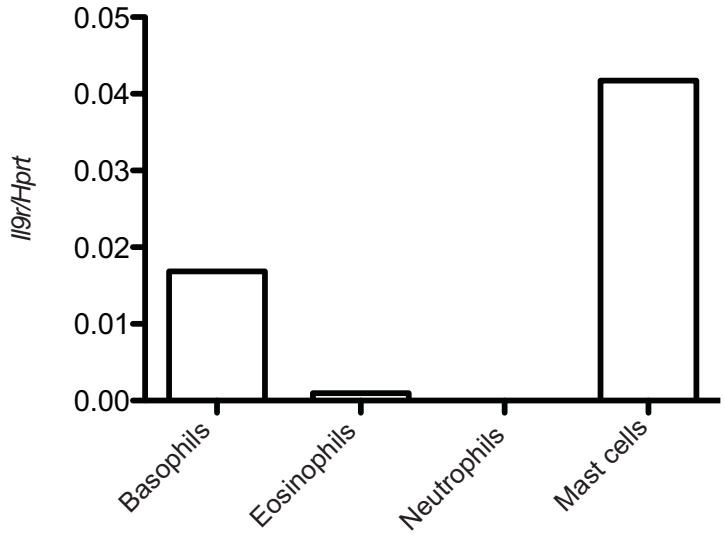

0.05
0.04
0.03
0.02
0.01
0.00
Il9r/Hprt
Basophils
Eosinophils
Neutrophils
Mast cells

Figure S6. IL-9R expression in sorted populations from *N. brasiliensis* infected lung

Sorted eosinophils ($CD11b^+$ $SiglecF^+$), basophils ($CD49b^+$ $FceR^+$ $c\text{-}kit^-$), neutrophils ($Ly6G^+CD11b^+$) and mast cells ($FceR^+$ $c\text{-}Kit^+$) from *N. brasiliensis* infected lung at day 7 p.i. were assessed for *Il9r* mRNA expression by real-time RT-PCR. Data represent ratio of *Il9r* to *Hprt* mRNA expression by the relative quantification method ($\Delta\Delta$Ct). The experiment was repeated twice with similar results using 5 pulled wildtype C57BL/6 mice.