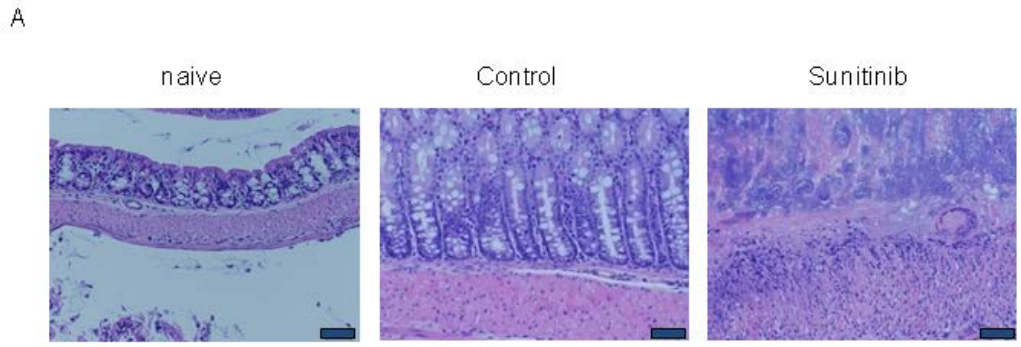


Figure S1. Sunitinib impairs the host defense against intestinal *C. rodentium* infection.

(**A**) 8 weeks old wild type mice were orally inoculated with 2×10^9 CFU of *C. rodentium*. Sunitinib (4 mg/Kg, n=6 or 40 mg/Kg, n=8) or control (n=5) was administered orally from day 0 to day 7, once daily. Histological analysis of representative colons from naïve, control or Sunitinib (40 mg/Kg) treated mice at day 11 after infection were shown. H&E staining shows severely disruption of the colonic epithelial layer and massive cell infiltration in Sunitinib treated mice. Scale bars, 50 μm. Data are representative of two independent experiments. Figure S1, related to Figure 1.

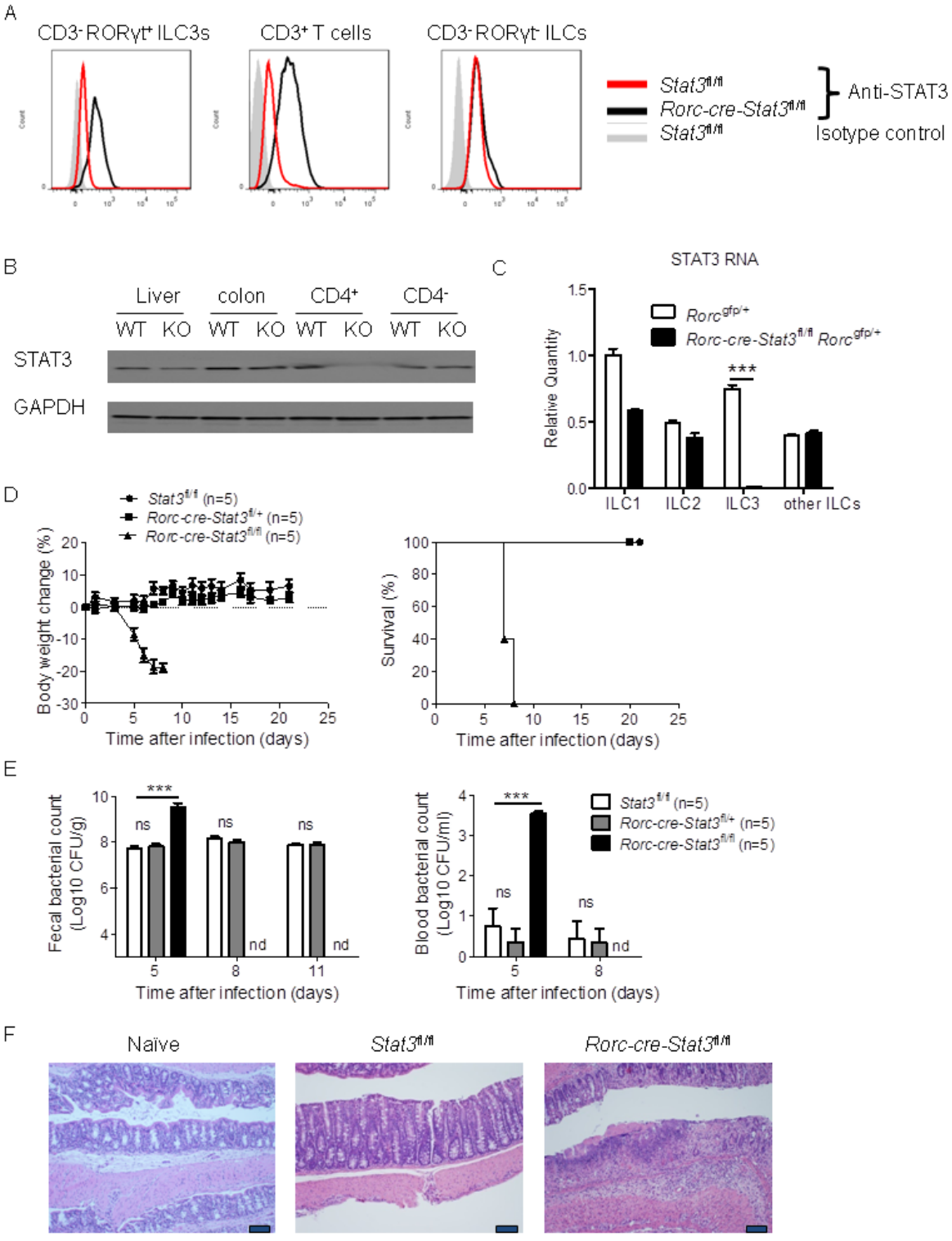

A
CD3-RORγt+ ILC3s
CD3+ T cells
CD3-RORγt- ILCs
Stat3fl/fl
Rorc-cre-Stat3fl/fl
Stat3fl/fl
Anti-STAT3
Isotype control
B
Liver
colon
CD4+
CD4-
WT KO WT KO WT KO WT KO
STAT3
GAPDH
C
STAT3 RNA
Rorcgfp/+
Rorc-cre-Stat3fl/fl Rorcgfp/+
Relative Quantity
***
ILC1
ILC2
ILC3
other ILCs
D
Stat3fl/fl (n=5)
Rorc-cre-Stat3fl/+ (n=5)
Rorc-cre-Stat3fl/fl (n=5)
Body weight change (%)
Time after infection (days)
Survival (%)
Time after infection (days)
E
Fecal bacterial count (Log10 CFU/g)
***
ns
nd
Time after infection (days)
Blood bacterial count (Log10 CFU/ml)
Stat3fl/fl (n=5)
Rorc-cre-Stat3fl/+ (n=5)
Rorc-cre-Stat3fl/fl (n=5)
Time after infection (days)
F
Naïve
Stat3fl/fl
Rorc-cre-Stat3fl/fl

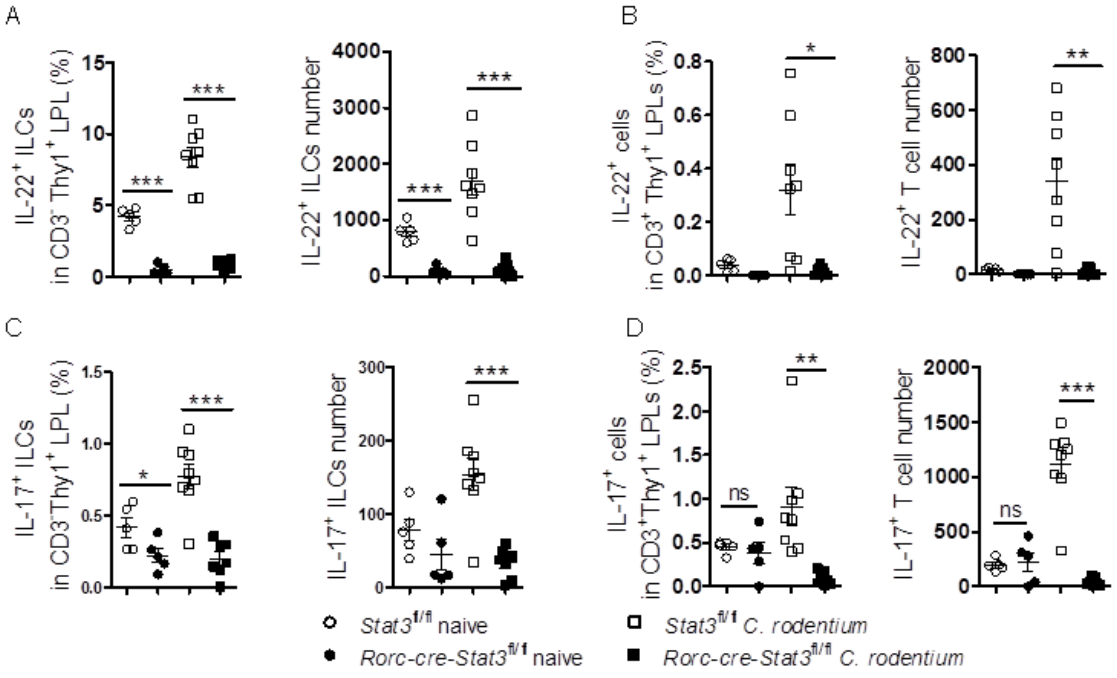
Figure S2. STAT3 signaling from RORγt$^+$ lymphocytes controls *C. rodentium* infection.

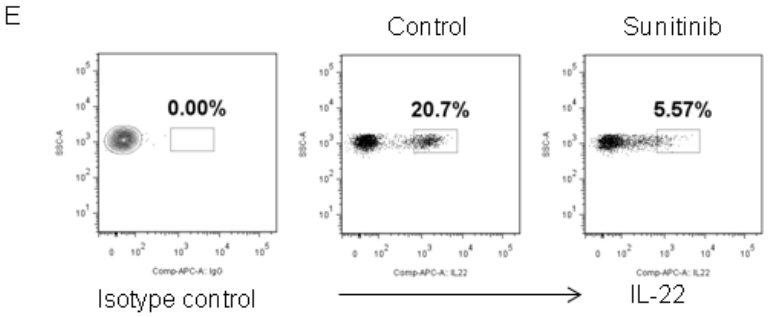
(**A-C**) Conditional deletion of STAT3 in RORγt$^+$ lymphocytes. (**A**) Colonic LPLs were isolated from 6 to 8 week-old *Stat3*$^{fl/fl}$ and *Rorc-cre-Stat3*$^{fl/fl}$ littermate mice and intracellular stained with STAT3 and RORγt. STAT3 expression was analyzed by flow cytometry after gating on CD3$^-$ RORγt$^+$ CD45$^+$, CD3$^-$ RORγt$^-$ CD45$^+$ and CD3$^+$ CD45$^+$ cells. Data are representative of two experiments with 2-3 mice for each genotype. (**B**) The STAT3 protein from the colon, liver, CD4$^+$ and CD4$^-$ splenocytes of WT (*Stat3*$^{fl/fl}$) or KO (*Rorc-cre-Stat3*$^{fl/fl}$) were examined by western blot. GAPDH was as internal control. Data are representative of two independent experiments. (**C**) Different ILCs were sorted from the intestine LPLs of *Rorc-cre-Stat3*$^{fl/fl}$ *Rorc*$^{gfp/+}$ and *Rorc*$^{gfp/+}$ mice. ILC1, CD90$^+$CD3$^-$NK1.1$^+$RORγt$^-$; ILC2, CD90$^+$CD3$^-$NK1.1$^-$RORγt$^-$ KLRG1$^+$; ILC3, CD90$^+$CD3$^-$RORγt$^+$; other ILCs, CD90$^+$CD3$^-$NK1.1$^-$RORγt$^-$ KLRG1$^-$. The mRNA levels of STAT3 were examined by realtime PCR. Data are representative of two independent experiments.

(**D, E**) 7 weeks old *Rorc-cre-Stat3*$^{fl/fl}$, wild type *Stat3*$^{fl/fl}$ and *Rorc-cre-Stat3*$^{fl/+}$ heterozygous mice (n = 5 per group) were orally inoculated with *C. rodentium* (2×10^9 CFU) and body weight was measured at the indicated time points. (**D**) Body weight change and survival rates are shown. (**E**) Bacterial titers from fecal homogenate cultures and blood at indicated day post infection in the mice in **D**. *P<0.05, **P<0.01, ***P<0.001; ns, no significant difference (Student's *t*-test); nd, nondetectable. Data are representative of two independent experiments (mean ± s.e.m.).

(**F**) Histological analysis of representative colons from *Stat3*$^{fl/fl}$ and *Rorc-cre-Stat3*$^{fl/fl}$ mice at day 8 after infection. *Rorc-cre-Stat3*$^{fl/fl}$ and their littermate wild type *Stat3*$^{fl/fl}$ mice were orally inoculated with high dose (2×10^9 CFU) of *C. rodentium*. Naïve *Stat3*$^{fl/fl}$ was as a control. H&E

staining shows severely disruption of the colonic epithelial layer and massive cell infiltration in *Rorc-cre-Stat3* $^{fl/fl}$ mice. Scale bars, 50 μm. Figure S2, related to Figure 2.


A
IL-22+ ILCs in CD3- Thy1+ LPL (%)
IL-22+ ILCs number
B
IL-22+ cells in CD3+ Thy1+ LPLs (%)
IL-22+ T cell number
C
IL-17+ ILCs in CD3-Thy1+ LPL (%)
IL-17+ ILCs number
D
IL-17+ cells in CD3+Thy1+ LPLs (%)
IL-17+ T cell number
ns
Stat3fl/fl naive
Rorc-cre-Stat3fl/fl naive
Stat3fl/fl C. rodentium
Rorc-cre-Stat3fl/fl C. rodentium


E
Control
Sunitinib
0.00%
20.7%
5.57%
Isotype control
IL-22

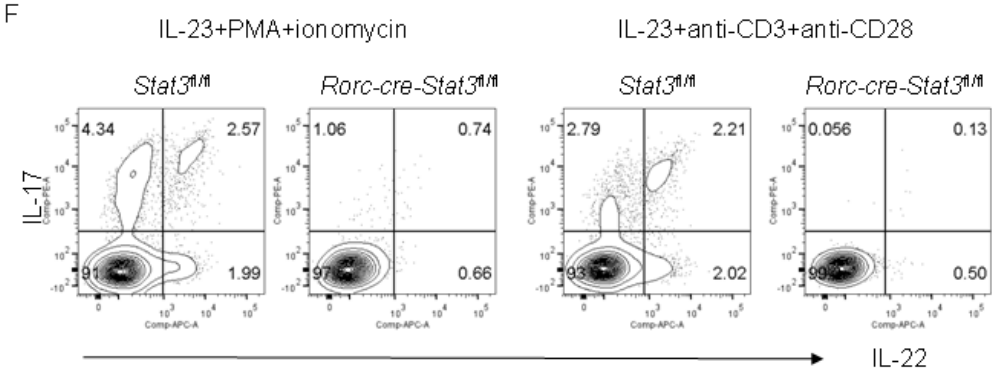

F
IL-23+PMA+ionomycin
IL-23+anti-CD3+anti-CD28
Stat3fl/fl
Rorc-cre-Stat3fl/fl
IL-17
4.34
2.57
91
1.99
1.06
0.74
97
0.66
2.79
2.21
93
2.02
0.056
0.13
99
0.50
IL-22

Figure S3. STAT3 signaling is required for IL-22 and IL-17 production from both innate and adaptive lymphocytes.

(**A-D**) IL-22 and IL-17 expression in $CD3^-$ and $CD3^+$ cells were analyzed by intracellular cytokine staining followed by flow cytometry. Intestinal lamina proprial lymphocytes (LPL) were isolated from the colons of *Stat3*$^{fl/fl}$ or *Rorc-cre-Stat3*$^{fl/fl}$ mice at day 5 post-infection and stimulated with IL-23 (25 ng/ml), PMA (50 ng/ml) and ionomycin (750 ng/ml) for 4 hours. Naïve mice were used as control.

(**A, C**) Percentage of IL-22^+ or IL-17^+ cells in the $CD3^-$ $Thy1^+$ ILCs population, as well as the absolute numbers of IL-22^+ or IL-17^+ ILCs in the colons of naïve or *C. rodentium* infected *Stat3*$^{fl/fl}$ and *Rorc-cre-Stat3*$^{fl/fl}$ mice are shown.

(**B, D**) Percentage of IL-22^+ or IL-17^+ cells in the $CD3^+$ $Thy1^+$ T cell population, as well as the absolute numbers of IL-22^+ or IL-17^+ T cells in the colons of naïve or *C. rodentium* infected *Stat3*$^{fl/fl}$ and *Rorc-cre-Stat3*$^{fl/fl}$ mice are shown. *P<0.05, **P<0.01, ***P<0.001 (Student's *t*-test). Each dot represents one individual mouse. Data are representative of two independent experiments (**A-D**; mean ± s.e.m.).

(**E**) Sunitinib suppresses IL-22 production in innate lymphocytes. Colonic LPLs were isolated from 6 to 8 week-old wild type mice were stimulated with IL-23 or IL-23 and Sunitinib (10μM) for 4 hours. IL-22 expression in $CD3^-$ $Thy1^+$ cells were analyzed by intracellular cytokine staining followed by flow cytometry. Data are representative of two independent experiments.

(**F**) LPLs were isolated from the colons of *Stat3*$^{fl/fl}$ or *Rorc-cre-Stat3*$^{fl/fl}$ mice at day 8 post-infection and stimulated with either IL-23 (25 ng/ml), PMA (50 ng/ml) and ionomycin (750 ng/ml) or IL-23 (25 ng/ml), anti-CD3 (plate coated, 1 μg/ml) and anti-CD28 (2 μg/ml) for 4

hours. IL-22 and IL-17 expression in $CD3^{+}CD4^{+}$ T cells were analyzed by intracellular cytokine staining. Data are representative of two independent experiments. Figure S3, related to Figure 3.

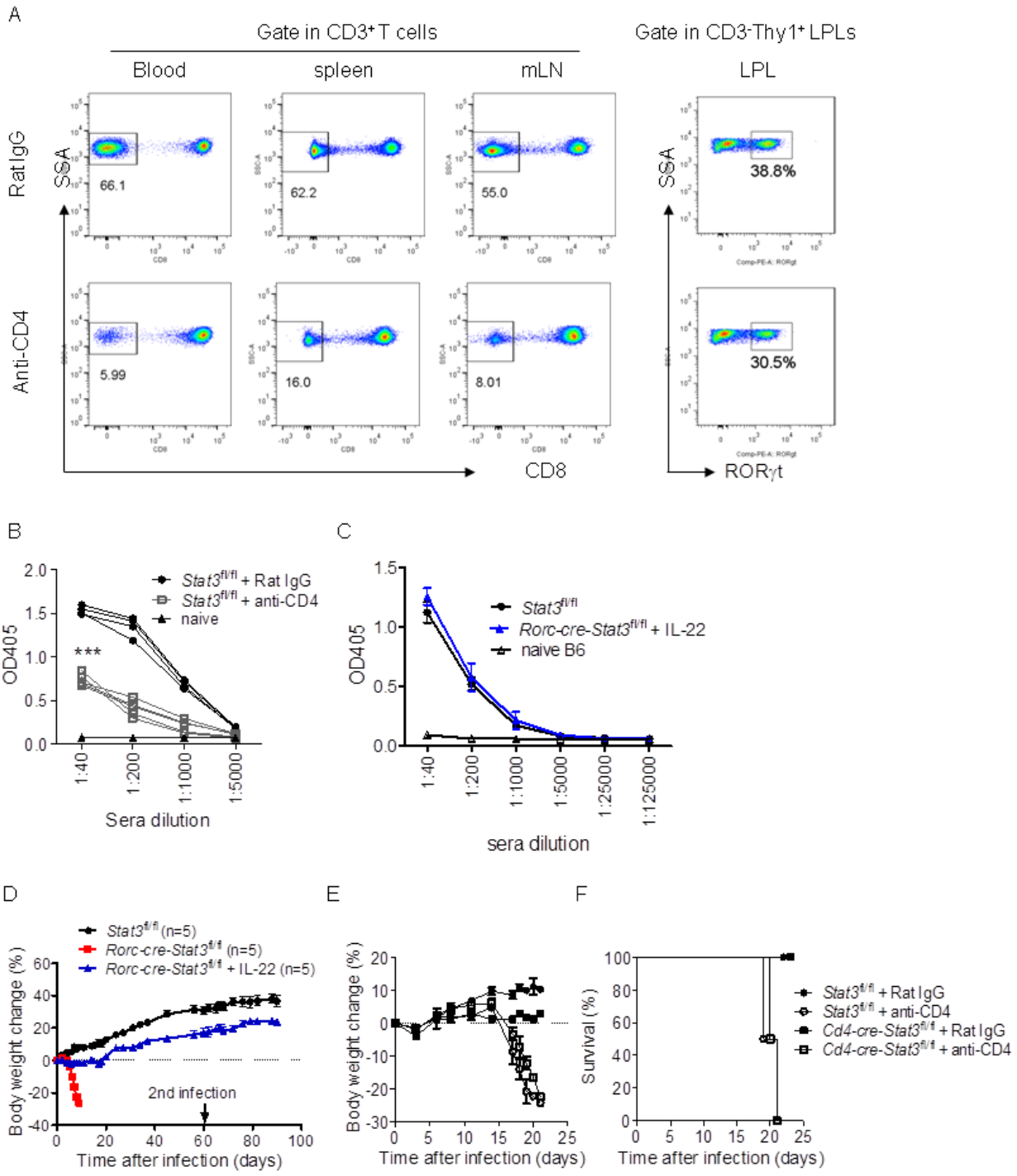

A
Gate in CD3+ T cells
Gate in CD3-Thy1+ LPLs
Blood
spleen
mLN
LPL
Rat IgG
Anti-CD4
SSA
66.1
62.2
55.0
38.8%
5.99
16.0
8.01
30.5%
CD8
RORγt
B
Stat3fl/fl + Rat IgG
Stat3fl/fl + anti-CD4
naive
OD405
***
1:40
1:200
1:1000
1:5000
Sera dilution
C
Stat3fl/fl
Rorc-cre-Stat3fl/fl + IL-22
naive B6
OD405
1:40
1:200
1:1000
1:5000
1:25000
1:125000
sera dilution
D
Stat3fl/fl (n=5)
Rorc-cre-Stat3fl/f (n=5)
Rorc-cre-Stat3fl/f + IL-22 (n=5)
Body weight change (%)
2nd infection
Time after infection (days)
E
Body weight change (%)
Time after infection (days)
F
Survival (%)
Stat3fl/fl + Rat IgG
Stat3fl/fl + anti-CD4
Cd4-cre-Stat3fl/f + Rat IgG
Cd4-cre-Stat3fl/f + anti-CD4
Time after infection (days)

Figure S4. CD4$^+$ T cells but not STAT3 signaling in T cells are required for the production of *C. rodentium* specific IgG.

(**A**, **B**) Wild type *Stat3*$^{fl/fl}$ mice were infected with 5 × 10^6 CFU of *C. rodentium* and injected intraperitoneally with mAb GK1.5 (n=5) or Rat IgG (n=4) (50 μg per mouse each time) at day -5, 0, 5, 10, 15 post infection for depletion of CD4$^+$ T cells. (**A**) The depletion efficiency was examined at day 8 post infection. (**B**) Blood samples were collected at day 12 post-infection and serum *C. rodentium* specific IgG was determined by ELISA.

(**C**, **D**) 7 weeks old *Rorc-cre-Stat3*$^{fl/fl}$ and wild type *Stat3*$^{fl/fl}$ mice (n = 5 per group) were orally inoculated with *C. rodentium* (2 × 10^9 CFU). One group of *Rorc-cre-Stat3*$^{fl/fl}$ mice were rescued by IL-22-Ig fusion protein injection. At day 60 after first *C. rodentium* infection, IL-22-rescued *Rorc-cre-Stat3*$^{fl/fl}$ and wild type *Stat3*$^{fl/fl}$ mice were challenged with high dose of *C. rodentium* (2 × 10^9 CFU) again. (**A**) Blood samples were collected at day 14 post-infection and serum *C. rodentium* specific IgG was determined by ELISA. (**B**) Body weight was measured at the indicated time points. ***P<0.001 (Student's *t*-test). Data are representative of two independent experiments.

(**E**, **F**) *Cd4-cre-Stat3*$^{fl/fl}$ and wild type *Stat3*$^{fl/fl}$ mice were infected with 5 × 10^6 CFU of *C. rodentium* and injected intraperitoneally with mAb GK1.5 or Rat IgG (50 μg per mouse each time) at day -5, 0, 5, 10 and 15 post infection for depletion of CD4$^+$ T cells. Average body weight change (**E**) and survival rates (**F**) at the indicated time points are shown. Figure S4, related to Figure 4.

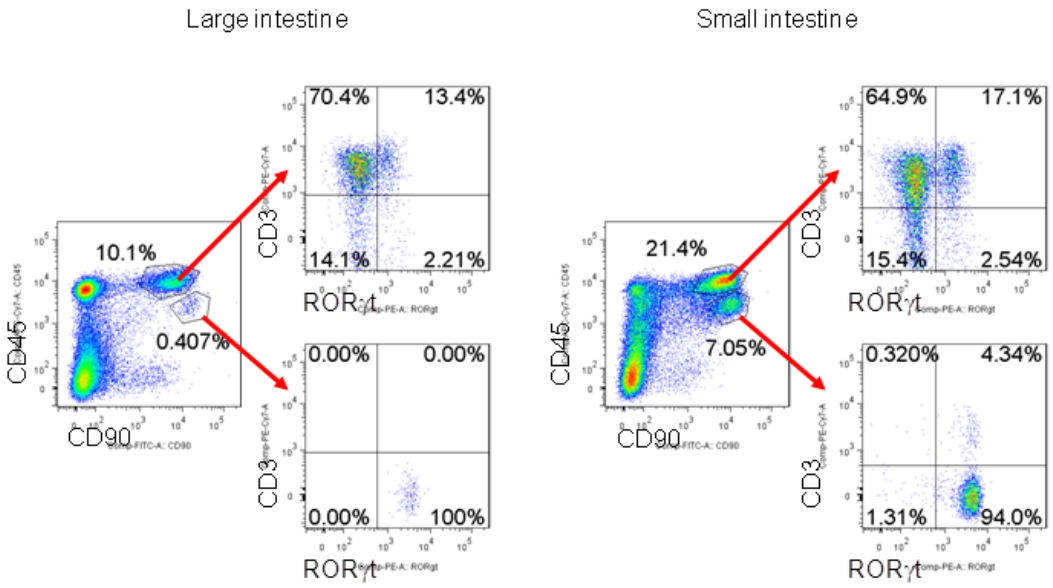


Figure S5. Intestinal CD90^{hi}CD45^{lo} LPLs are RORγt^{+} ILCs.

LPLs were isolated from both the large intestine and small intestine of wild type C57BL/6 mice and gated by CD45 and CD90 expression. Two separate populations, CD90^{hi}CD45^{lo} and CD90^{lo}CD45^{hi} were further analyzed with the expression of CD3 and RORγt. Data are representative of two independent experiments. Figure S5, related to Figure 5.

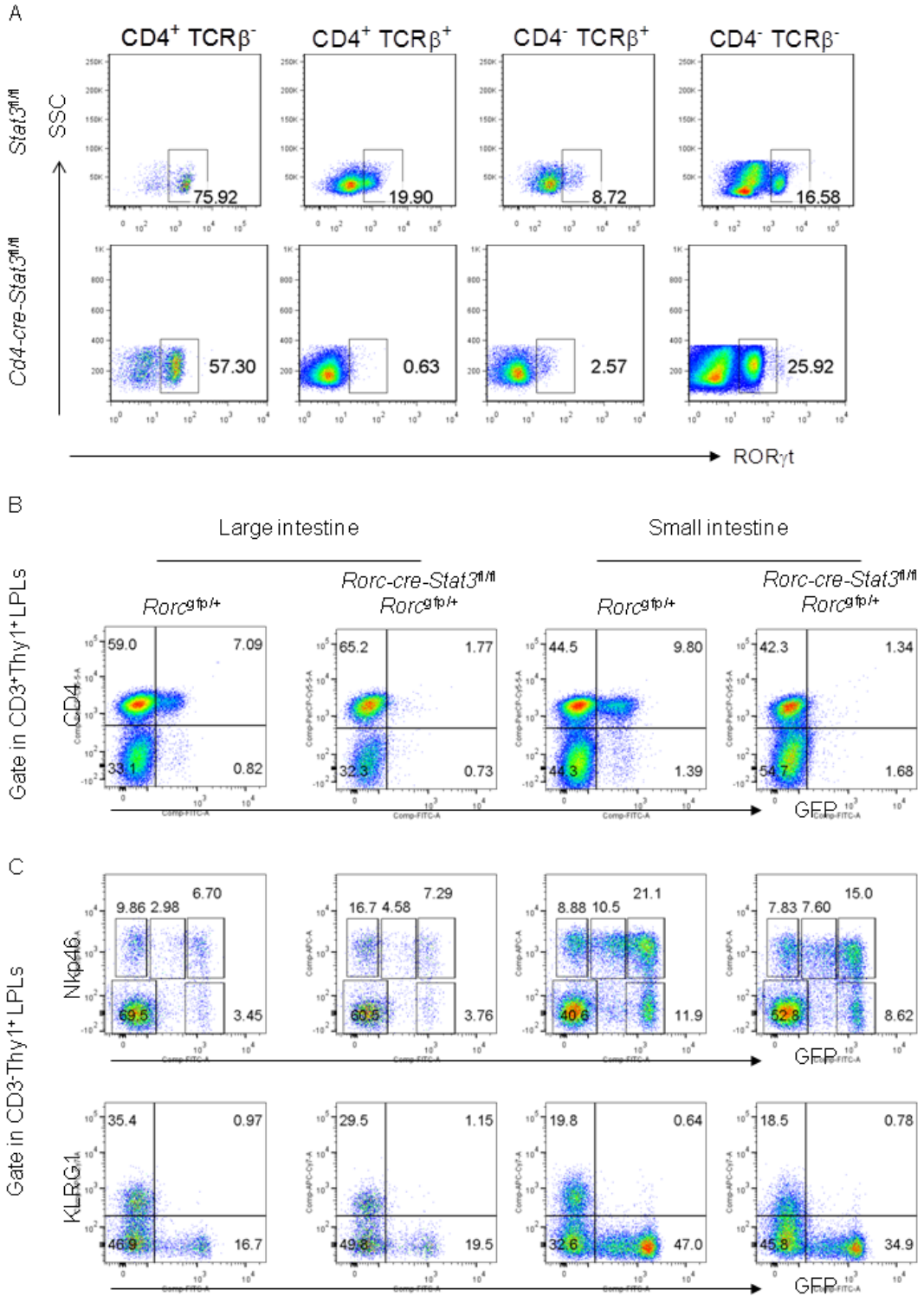

A
CD4+ TCRβ-
CD4+ TCRβ+
CD4- TCRβ+
CD4- TCRβ-
Stat3fl/fl
Cd4-cre-Stat3fl/fl
SSC
75.92
19.90
8.72
16.58
57.30
0.63
2.57
25.92
RORγt
B
Large intestine
Small intestine
Rorcgfp/+
Rorc-cre-Stat3fl/fl
Rorcgfp/+
Gate in CD3+Thy1+ LPLs
CD4
59.0
7.09
33.1
0.82
65.2
1.77
32.3
0.73
44.5
9.80
44.3
1.39
42.3
1.34
54.7
1.68
GFP
C
Gate in CD3-Thy1+ LPLs
Nkp46
9.86
2.98
6.70
69.5
3.45
16.7
4.58
7.29
60.5
3.76
8.88
10.5
21.1
40.6
11.9
7.83
7.60
15.0
52.8
8.62
KLRG1
35.4
0.97
46.9
16.7
29.5
1.15
49.8
19.5
19.8
0.64
32.6
47.0
18.5
0.78
45.8
34.9
GFP

Figure S6. The development of RORγt^{+} Th cells, but not RORγt^{+} ILCs, requires STAT3 signaling.

(**A**) Colonic LPLs were isolated from *Stat3*$^{fl/fl}$ and *Cd4-cre-Stat3*$^{fl/fl}$ littermate mice. RORγt expression were analyzed in colonic LPLs by flow cytometry after gating on CD4^{+} TCRβ^{+}, CD4$^{–}$ TCRβ^{+} adaptive lymphocytes and CD4^{+} TCRβ$^{–}$, CD4$^{–}$ TCRβ$^{–}$ innate lymphoid cells.

(**B, C**) LPLs were isolated from both large and small intestine of *Rorc-cre-Stat3*$^{fl/fl}$ *Rorc*$^{gfp/+}$ and *Rorc*$^{gfp/+}$ mice. RORγt^{+} T cells, NCR^{+}RORγt^{+} ILC3, NCR^{-}RORγt^{+} ILC3, NCR^{+}RORγt^{-} ILC1 and RORγt^{-}KLRG1^{+} ILC2 were analyzed by flow cytometry. Figure S6, related to Figure 6.

Table S1 Primers for realtime RT-PCR and ChIP assays.

Il22 FW	CATGCAGGAGGTGGTACCTT
Il22 RV	CAGACGCAAGCATTTCTCAG
Il23p19 FW	GGTGGCTCAGGGAAATGT
Il23p19 RV	GACAGAGCAGGCAGGTACAG
Il23p40 FW	ACATCTACCGAAGTCCAATGCA
Il23p40 RV	GGAATTGTAATAGCGATCCTGAGC
Il23r FW	TCAGTGCTACAATCTTCAGAGGACA
Il23r RV	GCCAAGAAGACCATTCCCGA
Rorc FW	TACCTTGGCCAAAACAGAGG
Rorc RV	ATGCCTGGTTTCCTCAAAA
Ahr FW	GGCTTTCAGCAGTCTGATGTC
Ahr RV	CATGAAAGAAGCGTTCTCTGG
Reg3g FW	CAAGGTGAAGTTGCCAAGAA
Reg3g RV	CCTCTGTTGGGTTCATAGCC
Reg3b FW	ATGGCTCCTACTGCTATGCC
Reg3b RV	GTGTCCTCCAGGCCTCTT
Hprt FW	TGAAGAGCTACTGTAATGATCAGTCA
Hprt RV	AGCAAGCTTGCAACCTTAACCA
Il17a FW	CTCCAGAAGGCCCTCAGACTAC
Il17a RV	AGCTTTCCCTCCGCATTGACACAG
Stat3 FW	GAAGACCAAGTTCATCTGTGTGAC
Stat3 RV	GGTTTCAGCTCCTCACATGG
IL22 pro FW	GGGAGATCAAAGGCTGCTCTA
Il22 pro RV	CCACCTTGAGAGATGGGAAGT
Il22 Intron FW	AGCTGCATCTCTTTCTCTCCA
Il22 Intron RV	TATCCTGAAGGCCAAAATAGGA
Il17 pro FW	GCCTTTGTGATTGTTTCTTGCAG
Il17pro RV	CCTTGCCCAAAGAAACCCACTC
Ltbr FW	CAGTGGCTCCAAGTGGCTTG
Ltbr RV	GCAAACCGTGTCTTGGCTGC

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

C. rodentium specific IgG

Blood samples were also collected and serum *C. rodentium* specific IgG was determined by ELISA with *C. rodentium* lysate and anti-mouse IgG (Jackson ImmunoResearch).

Plasmids and Retroviral Transduction of Cell Lines

STAT3C (constitutively active forms of STAT3) (Zhou et al., 2007) was cloned into a DFTC (double flag epitope tagged)-retroviral vector containing an IRES-puromycin resistant gene. Plasmid DNA was confirmed by sequencing. HEK293T cells were transfected with retroviral plasmids and the packaging plasmid 10A1 using Polyethylenimine (PEI). Viral supernatant was collected after transfection. EL4 cells were spun in 2 ml of virus supernatant containing 8 μg/ml polybrene at 2500 rpm for 1.5 hr at 30 °C. The retroviral transduction was repeated 24 h later. The cells were then selected in the presence of 7 μg/ml puromycin for 5 days (Qiu et al., 2011).

Western Blot

Colon and liver tissues were washed in PBS and homogenized in RIPA lysis buffer. Protein samples were resolved by SDS–PAGE and transferred to a nitrocellulose membrane. Western blotting was performed with anti-STAT3 rabbit mAb (D3Z2G) (Cell Signaling Technology) and a monoclonal anti-GAPDH antibody (Sigma).

SUPPLEMENTAL REFERENCE

Qiu, J., Heller, J.J., Guo, X., Chen, Z.M., Fish, K., Fu, Y.X., and Zhou, L. (2011). The aryl hydrocarbon receptor regulates gut immunity through modulation of innate lymphoid cells. Immunity *36*, 92-104.

Zhou, L., Ivanov, II, Spolski, R., Min, R., Shenderov, K., Egawa, T., Levy, D.E., Leonard, W.J., and Littman, D.R. (2007). IL-6 programs T(H)-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways. Nat Immunol *8*, 967-974.