SUPPLEMENTAL INFORMATION

RT-PCR primers:

IL-1β	Fw: 5'-CAACCAACAAGTGATATTCTCCATG-3'	Rev: 5'-GATCCACACTCTCCAGCTGCA-3'
IL-6	Fw: 5'-CCACTTCACAAGTCGGAGGC-3'	Rev: 5'-TGCAAGTGCATCATCGTTGTTC-3'
IL-17A	Fw: 5'-GGACTCTCCACCGCAATGA-3'	Rev: 5'-GGCACTGAGCTTCCCAGATC-3'
IL-21	Fw: 5'-AAGATTCCTGAGGATCCGAGAAG-3'	Rev: 5'-TGCATTCGTGAGCGTCTATAGTG-3'
IL-23 p19	Fw: 5'-CTGGAACGCACATGCACCAG-3'	Rev: 5'-TGTTGTCCTTGAGTCCTTGTGG-3'
TGF-β	Fw: 5'-CACTGATACGCCTGAGTGGC-3'	Rev: 5'-TGCTGTCACAAGAGCAGTGAG-3'
SAA1	Fw: 5'-CATTTGTTCACGAGGCTTTCC-3'	Rev: 5'-GTTTTTCCAGTTAGCTTCCTTCATGT-3'
SAA3	Fw: 5'-CGCAGCACGAGCAGGAT-3'	Rev: 5'-CCAGGATCAAGATGCAAAGAATG-3'
NOS2	Fw: 5'-ATGCTGCCACCTTGGAGTTCAC-3'	Rev: 5'-GGCCACCCACCTCCAGTAGC-3'
Reg3γ	Fw: 5'-CCTGATGCTCCTTTCTCAGG-3'	Rev: 5'-ATGTCCTGAGGGCCTCTTTT-3'

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. SFB-induced intestinal Th17 cells require MHCII expression in the periphery (related to Figure 1)

A. Cytokine expression in SI LP TCRβ$^+$CD4$^+$ cells in 10-week old SFB-monocolonized mice (SFB-mono) and germ-free (GF) controls or in SFB-negative conventionally-raised mice (SPF) before and 2 weeks after colonization with SFB

B. Foxp3 expression in CD4T cells isolated from the corresponding tissues of SFB-positive WT and *H2-Ab1*$^{-/-}$ mice. Plots gated on TCRβ$^+$CD4$^+$ cells

C-D. 5x10^6 sorted naïve CD4 T cells were transferred into WT and *H2-Ab1*$^{-/-}$ mice before or after colonization with SFB. IL-17 expression was examined in transferred cells in the SI LP 2 weeks after transfer. Plots gated on donor TCRβ$^+$CD4$^+$ cells

Figure S2. SFB do not induce Th17 cell differentiation of non-SFB Tg T cells (related to Figure 2)

A. IL-17 expression in SI LP CD4 T cells from SFB-positive OTII.B6 TCR Tg mice before or after

stimulation with cognate antigen. OVA stimulation was performed by supplying 1% OVA protein in the drinking water *ad libitum* for 10-14 days. Left, $V\alpha2^{hi}V\beta5^{hi}$ plots are gated through the gate shown in (B). Right, IL-17 expression in CD4 T cells outside the $V\alpha2^{hi}V\beta5^{hi}$ gate

B. Expression of the Tg Vα2 and Vβ5 in CD4 T cells isolated from the corresponding tissues of SFB-positive OTII.B6 TCR Tg mice in the absence of OVA stimulation. Plots are gated on $TCR\beta^{+}CD4^{+}$ cells. $V\alpha2^{hi}V\beta5^{hi}$ CD4 T cells include cells expressing the Tg TCR

C. SI LP Th17 cells express alternative TCRs. Vα2 and Vβ5 expression in $IL\text{-}17^{+}$ vs $IL\text{-}17^{-}$ CD4 T cells in the SI LP of SFB-positive OVA.B6 Tg mice. All plots initially gated on $TCR\beta^{+}CD4^{+}$ cells

D. SI LP Th17 cells and lymph node CD4 T cells from OTII.B6 *Il17*^{GFP} mice were labeled with CellTrace Violet (SI) or CFSE (LN) and stimulated *in vitro* with TCRα-deficient splenocytes and OVA peptide or SFB lysates for 3 days. Plots gated on $TCR\beta^{+}CD4^{+}$ cells. Top, GFP^{+} ($IL\text{-}17^{+}$) CD4 T cells purified by FACS from SI LP. Middle, CD4 T cells were purified from MLNs by MACS using anti-CD4 magnetic beads. Bottom, Splenic CD4 T cells from TRP-1.RAG Tg mice were purified by MACS using anti-CD4 magnetic beads, labeled with CFSE, and stimulated *in vitro* with TRP-1 peptide or SFB antigens for 3 days in the presence TCRα-deficient splenocytes

E-F. SFB-free OTII.RAG or TRP-1.RAG TCR Tg mice were colonized with SFB-containing microbiota from Taconic B6 mice. Colonization levels were confirmed by Q-PCR. 2-4 weeks later SFB-negative and SFB-positive mice were stimulated *in vivo* with the corresponding antigen

E. OTII.RAG mice were stimulated with OVA by oral gavage at Day 1, 3 and 5 and by providing 1% OVA in the drinking water *ad libitum*. SI LP cells were isolated on Day 12-14. RAG-sufficient SFB-positive control mouse is shown for comparison

F. TRP-1.RAG mice were immunized i.p. with 50 μg TRP-1 peptide and 10 μg LPS on Day 1 and 7. Control mice received 10 μg LPS only. SI LP cells were isolated on Day 12-14

Figure S3. SFB do not induce Th17 cell differentiation of non-SFB Tg T cells (related to Figure 2)

A. $5x10^6$ $CD45.2^+$ CD4 T cells were purified from spleens and peripheral LNs of TRP-1.RAG mice by MACS using anti-CD4 magnetic beads and transferred into $CD45.1^+$ recipients before or 12-14 days after colonization with SFB (Day 0). Recipient animas were immunized i.p. with 50 μg TRP-1 peptide and 10 μg LPS on Day 1 and 7. Control mice received transferred CD4 T cells and 10 μg LPS only. Th17 cell induction was examined in transferred Tg ($CD45.2^+$) and endogenous WT ($CD45.1^+$) cells 12-14 days after transfer

B. $5x10^6$ $CD45.2^+CD90.1^+$ WT CD4 T cells and $5x10^6$ $CD45.2^+CD90.2^+$ TRP-1 Tg CD4 T cells were purified from spleens and peripheral LNs of WT and TRP-1.RAG mice respectively, combined and co-transferred into WT $CD45.1^+CD90.2^+$ recipients before or 12-14 days after colonization with SFB (Day 0). Recipient animas were immunized i.p. with 50 μg TRP-1 peptide and 10 μg LPS on Day 1 and 7. Th17 cell induction was examined in three different types of cells in the same animal 12 days after transfer. H - endogenous host WT cells ($CD45.1^+CD90.2^+$); W – transferred WT cells ($CD45.2^+CD90.1^+$); T - transferred Tg cells ($CD45.2^+CD90.2^+$)

C. GFP and IL-17 expression in total SI LP cells (left and middle panel) and in CD4 T cells (right panel) isolated from the SI LP of heterozygous *Il17*^{GFP} reporter mice

D. Sorting scheme for isolation of purified SFB filaments. Feces from SFB-monocolonized mice were processed as described in Methods and stained with the SYTO9 component of the Live/Dead^R*Bac*Light^{TM} Bacterial Viability Kit (Life Technologies). SFB filaments were identified by SYTO9 staining and large size (SSC-W) and sorted on FACSAriaII (BD) to high purity. Representative FACS plots and photos show the presence of mostly large SFB filaments and absence of small bacterial cells or host/fecal debris after sorting. PBS only, negative control of sterile PBS used to resuspend the SFB sample after sorting. Arrowheads, SFB filaments of variable size in the sample used for sorting

E. GFP^+ (Th17) and GFP^- (non-Th17) $TCR\beta^+CD4^+$ cells were purified by FACS from SI LP of SFB-positive *Il17*^{GFP} reporter mice and stimulated *in vitro* with SFB lysates prepared after density gradient

(SFB Gradient) or from SFB filaments purified by cell sorting on panel C (SFB Sorted)

F. GFP^+ (Th17) and GFP^- (non-Th17) $TCR\beta^+CD4^+$ cells were purified by FACS from SI LP of SFB-positive (SFB+) or SFB-negative (No SFB) *Rorc*^{GFP} reporter mice and stimulated *in vitro* with SFB antigens

G. IL-17 expression in proliferated ($CellTrace^{lo}$) GFP^+ and GFP^- SI LP CD4 T cells from Figure 2F stimulated *in vitro* with SFB antigens for 3 days. Plots are gated on live, proliferated T cells

Figure S4. SFB induce Th17 cells with diverse Vβ utilization (related to Figure 3)

A. SFB-negative Jackson B6 mice (Jax) were colonized with SFB by oral gavage with SFB-containing Taconic feces (Jax + SFB) and IL-17 induction in different Vβ families was examined by flow cytometry 14 days after colonization. Plots represent percentage of $IL\text{-}17^+$ cells in the $TCR\beta^+CD4^+V\beta^+$ subset for the corresponding Vβ.

B. Vβ and IL-17 expression in SI LP CD4 T cells of germ-free and SFB-monocolonized C57BL/6 mice. Plots represent percentage of $IL\text{-}17^+$ cells in the $TCR\beta^+CD4^+V\beta^+$ subset for the corresponding Vβ. ND, not determined

Figure S5. Effects of SFB colonization in $DC^{\Delta MHCII}$ mice (related to Figure 4)

A. $CD11c^+$ cell subsets in the SI LP of $DC^{\Delta MHCII}$ and control littermates. Bar plots represent total numbers or percentage of the corresponding subset in the $CD11c^+$ gate

B. MHCII expression in APC subsets from SI LP of WT and $DC^{\Delta MHCII}$ mice

C. Relative expression of SFB-induced genes in terminal ileum of $DC^{\Delta MHCII}$ and control littermates (WT) before and after colonization with SFB determined by RT-PCR. ns, not significant

D. Th1 cells in the SI LP of $DC^{\Delta MHCII}$ and control littermates before and after SFB colonization

E. Foxp3 expression in CD4 T cells from the indicated organs. SFB-negative $DC^{\Delta MHCII}$ mice and control littermates were colonized with SFB by oral gavage and $Foxp3^+$ Tregs analyzed 12-14 days later. Plots

represent the percentage of $Foxp3^+$ cells in the $TCR\beta^+CD4^+$ population. p values compare $DC^{\Delta MHCII}$ mice to corresponding WT littermate group

F. SFB levels in fecal pellets from $DC^{\Delta MHCII}$ and control littermates 3 weeks after SFB colonization

G. SFB attachment and MHCII expression in terminal ileum epithelial cells in WT and $DC^{\Delta MHCII}$ mice colonized with SFB. Note lack of MHCII expression in LP of the $DC^{\Delta MHCII}$ littermate. Blue, DNA; Green, MHCII; Red, Actin

H. SFB-negative Cμ-deficient and WT control mice were obtained from Jackson Laboratory. The mice were co-housed for a week and colonized with SFB by gavage with fecal homogenates from SFB-monocolonized mice. Control animals were gavaged with fecal homogenates from SFB-negative mice. SFB absence or colonization was confirmed by RT-PCR. Th17 cell induction was examined 12 days after gavage

Figure S6. SFB-mediated Th17 cell responses in $IEC^{\Delta MHCII}$ and $ILC3^{\Delta MHCII}$ mice (related to Figure 5)

A. MHCII expression on cell subsets from SI LP of WT and $IEC^{\Delta MHCII}$ mice

B. SFB levels in feces of $IEC^{\Delta MHCII}$ and control littermates assessed by 16S rRNA gene RT-PCR

C. MHCII expression in terminal ileum of $IEC^{\Delta MHCII}$ mice and WT littermates 2 weeks after colonization with SFB. MHCII expression is observed only in the LP in $IEC^{\Delta MHCII}$ mice. Arrows point to SFB filaments, attaching to IECs

D. MHCII expression on $ROR\gamma t^+c\text{-}kit^+NKp46^+$ (R2) and $ROR\gamma t^+c\text{-}kit^-NKp46^-$ (R3) ILCs in WT and $ILC3^{\Delta MHCII}$ mice. R2 and R3 gates as shown on Figure 5D

E. MHCII expression on cell subsets from SI LP of WT and $ILC3^{\Delta MHCII}$ mice

F. Normal colonic histology and lack of intestinal inflammation in $ILC3^{\Delta MHCII}$ mice

G. $Foxp3^+$ Tregs in SI LP of $ILC3^{\Delta MHCII}$ mice and control littermates before and after colonization with SFB

H. WT SFB-negative mice were gavaged twice with fecal homogenates from SFB-negative ILC3$^{\Delta MHCII}$ mice (with high levels of SI LP Th17 cells). IFNγ and IL-17 expression in SI LP CD4 T cells was examined 3 weeks after gavage

I. CD4 T cells were purified by cell sorting from SI LP of SFB-positive and SFB-negative WT and ILC3$^{\Delta MHCII}$ mice and incubated *in vitro* with SFB or other bacterial lysates as in Figure 2. T cell proliferation was examined on Day 3 of culture

J-K. SFB colonization in feces and attachment to terminal ileum villi in ILC3$^{\Delta MHCII}$ mice and control littermates

L. Total numbers of Vβ14^{+}IL-17^{+} Th17 cells in ILC3$^{\Delta MHCII}$ mice and control littermates before and after colonization with SFB

Figure S7. SFB priming of CD4 T cells in gut mucosa (related to Figure 6)

10^7 MACS-purified CD4 T cells from spleens and LNs of CD45.2^{+} *Il17*^{GFP} mice were labeled with CellTrace Violet proliferation dye and adoptively transferred into WT CD45.1^{+} mice before or 12 days after SFB colonization. T cell proliferation (dye dilution) and Th17 cell induction (GFP expression) was examined at different time points in small intestinal lamina propria (SI LP) and Peyer's Patches (PP). Very low proliferation and no Th17 cell induction was detected in SFB-negative animals (not shown)

Table S1. Diverse TCR repertoire of SFB-recognizing hybridomas (related to Figure 3)

CDR3 regions and Vβ utilization in TCRβ chains, sequenced from 15 SFB-recognizing hybridomas. Clone numbers correspond to clone numbers on Figure 3B. The sequences are arranged by Vβ usage (right-most column). The two identical sequences are highlighted in red

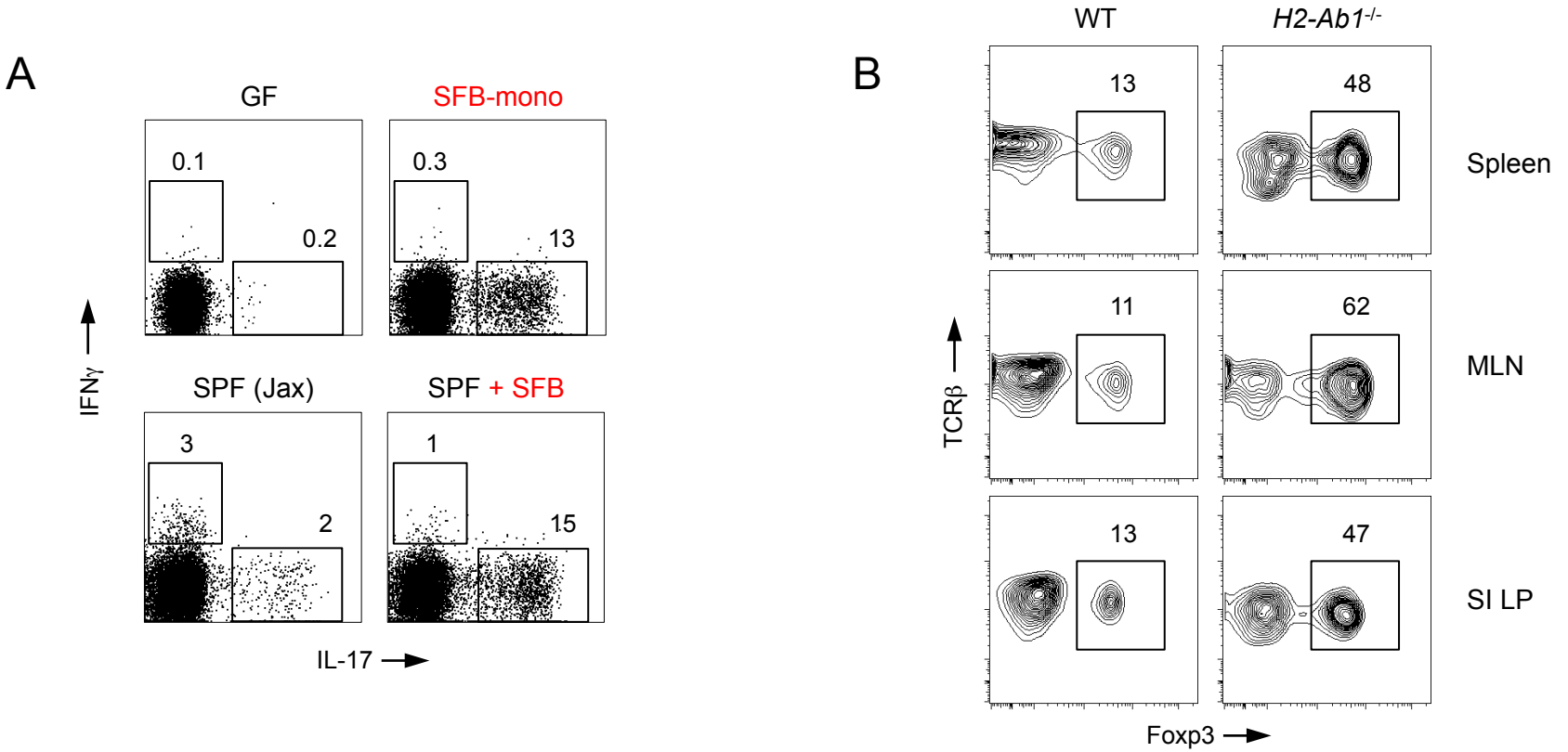

A
GF
SFB-mono
0.1
0.2
0.3
13
IFNγ
SPF (Jax)
SPF + SFB
3
2
1
15
IL-17
B
WT
H2-Ab1-/-
13
48
Spleen
11
62
MLN
13
47
SI LP
TCRβ
Foxp3
C
Naïve CD4 > WT
No SFB
Naïve CD4 > WT
+ SFB
Naïve CD4 > H2-Ab1-/-
+ SFB
6
0
4
40
8
0
IFNγ
IL-17
D
Th17 in donor cells
Th17 cell (%)
50
40
30
20
10
0
***
***
WT
No SFB
WT
+SFB
H2-Ab1-/-
+SFB

Figure S1

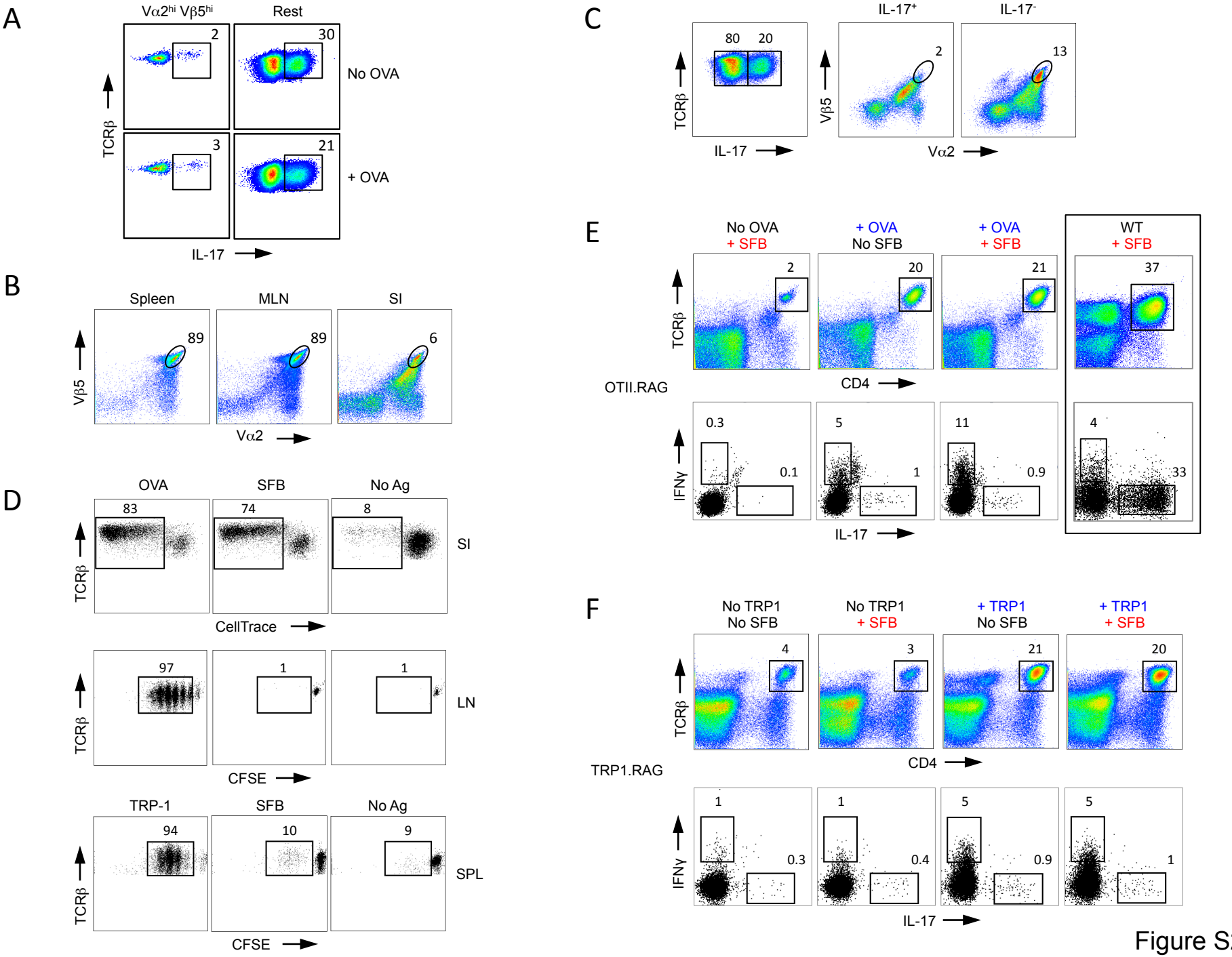

A
Vα2hi Vβ5hi
Rest
2
30
No OVA
3
21
+ OVA
TCRβ
IL-17
B
Spleen
MLN
SI
89
89
6
Vβ5
Vα2
C
IL-17+
IL-17-
80
20
2
13
TCRβ
IL-17
Vβ5
Vα2
D
OVA
SFB
No Ag
83
74
8
SI
TCRβ
CellTrace
97
1
1
LN
CFSE
TRP-1
SFB
No Ag
94
10
9
SPL
E
No OVA
+ SFB
+ OVA
No SFB
+ OVA
+ SFB
WT
+ SFB
2
20
21
37
TCRβ
CD4
OTII.RAG
0.3
0.1
5
1
11
0.9
4
33
IFNγ
IL-17
F
No TRP1
No SFB
No TRP1
+ SFB
+ TRP1
No SFB
+ TRP1
+ SFB
4
3
21
20
TCRβ
CD4
TRP1.RAG
1
0.3
1
0.4
5
0.9
5
1
IFNγ
IL-17

Figure S2

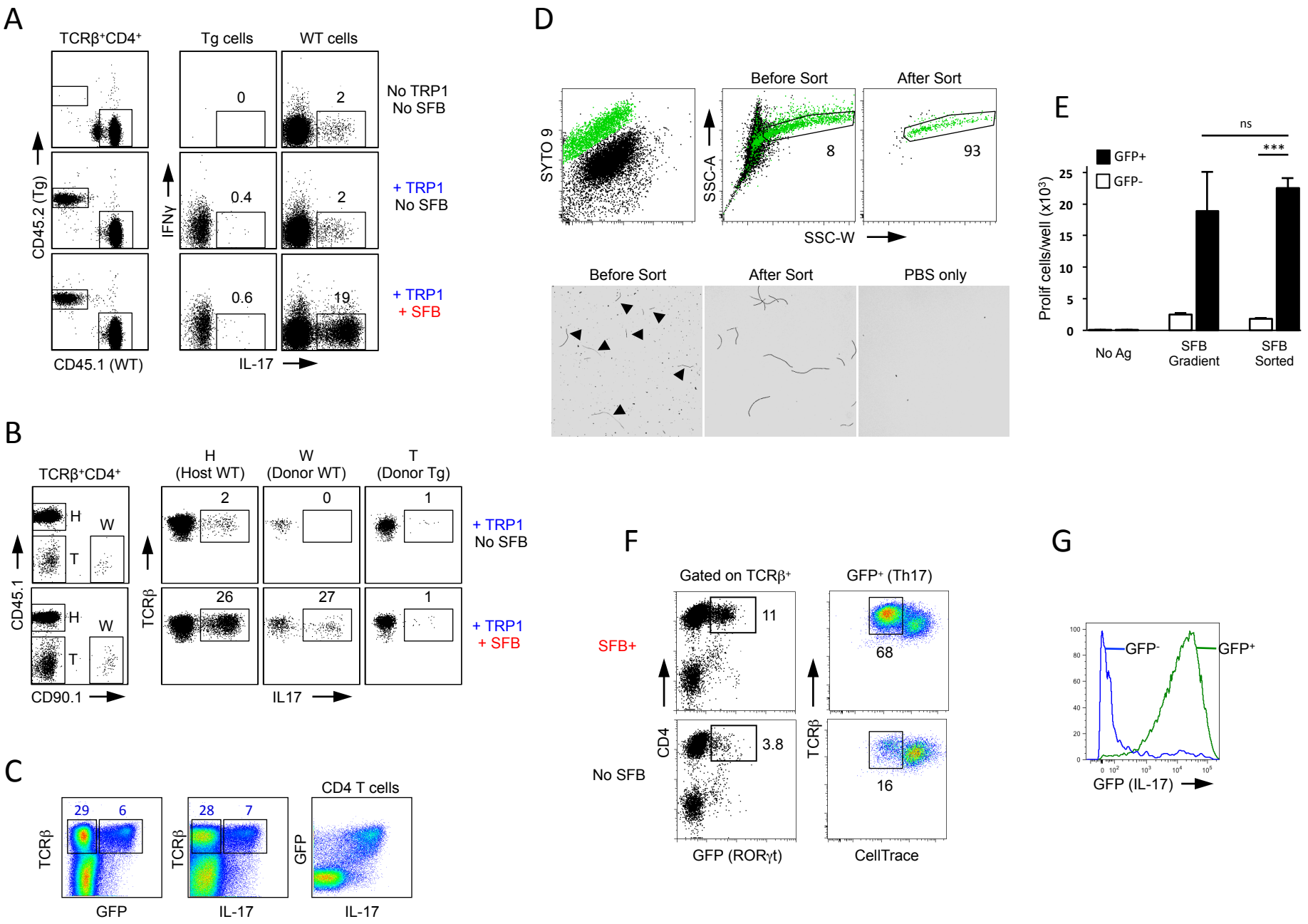

A
TCRβ+CD4+
Tg cells
WT cells
No TRP1
No SFB
+ TRP1
No SFB
+ TRP1
+ SFB
CD45.2 (Tg)
CD45.1 (WT)
IFNγ
IL-17
B
TCRβ+CD4+
H
(Host WT)
W
(Donor WT)
T
(Donor Tg)
CD45.1
CD90.1
TCRβ
IL17
C
CD4 T cells
TCRβ
GFP
IL-17
GFP
D
Before Sort
After Sort
SYTO 9
SSC-A
SSC-W
PBS only
E
GFP+
GFP-
ns
***
Prolif cells/well (x10³)
No Ag
SFB
Gradient
SFB
Sorted
F
Gated on TCRβ+
GFP+ (Th17)
SFB+
No SFB
CD4
TCRβ
GFP (RORγt)
CellTrace
G
GFP-
GFP+
GFP (IL-17)

Figure S3

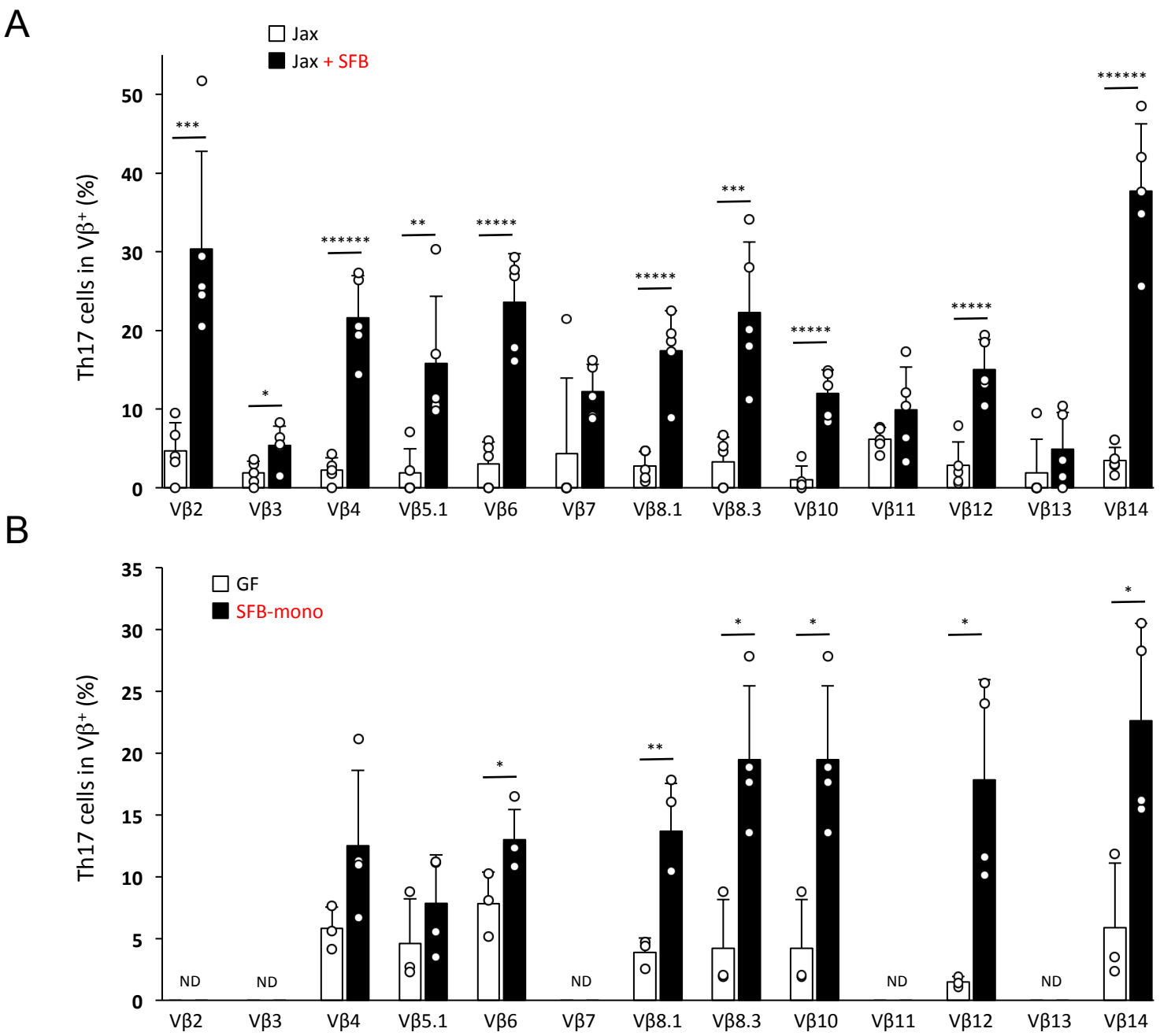
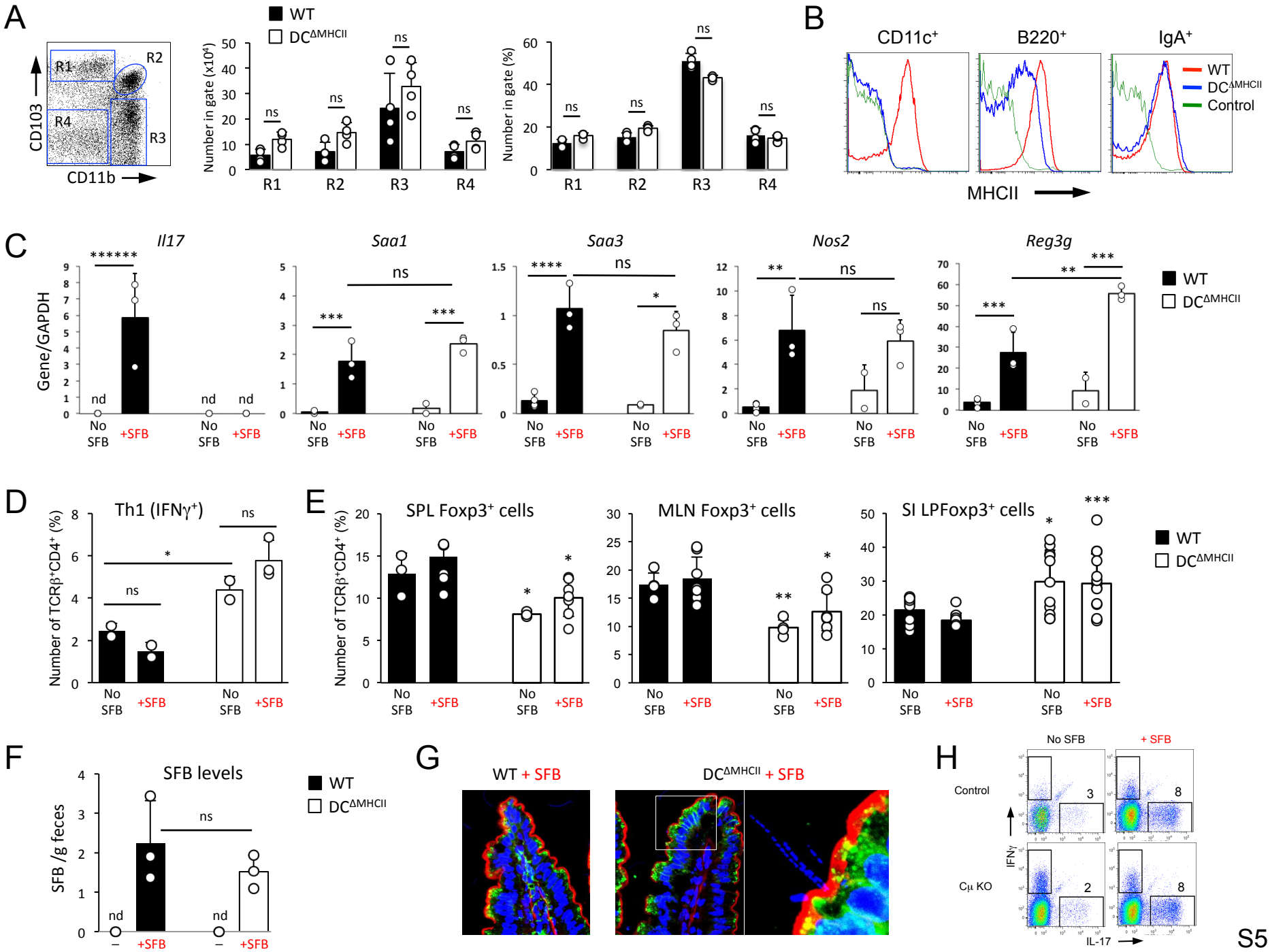

A
Jax
Jax + SFB
Th17 cells in Vβ+ (%)
50
40
30
20
10
0
***
*
******
**
*****
*****
***
*****
*****
******
Vβ2
Vβ3
Vβ4
Vβ5.1
Vβ6
Vβ7
Vβ8.1
Vβ8.3
Vβ10
Vβ11
Vβ12
Vβ13
Vβ14
B
GF
SFB-mono
Th17 cells in Vβ+ (%)
35
30
25
20
15
10
5
0
ND
ND
*
**
*
*
*
*
ND
ND
ND
Vβ2
Vβ3
Vβ4
Vβ5.1
Vβ6
Vβ7
Vβ8.1
Vβ8.3
Vβ10
Vβ11
Vβ12
Vβ13
Vβ14

Figure S4


A
R1
R2
R3
R4
CD103
CD11b
WT
DC^ΔMHCII
Number in gate (x10⁴)
Number in gate (%)
ns
R1
R2
R3
R4
B
CD11c⁺
B220⁺
IgA⁺
WT
DC^ΔMHCII
Control
MHCII
C
Il17
Saa1
Saa3
Nos2
Reg3g
Gene/GAPDH
nd
No SFB
+SFB
WT
DC^ΔMHCII
D
Th1 (IFNγ⁺)
Number of TCRβ⁺CD4⁺ (%)
E
SPL Foxp3⁺ cells
MLN Foxp3⁺ cells
SI LPFoxp3⁺ cells
F
SFB levels
SFB /g feces
G
WT + SFB
DC^ΔMHCII + SFB
H
No SFB
+ SFB
Control
Cμ KO
IFNγ
IL-17

S5

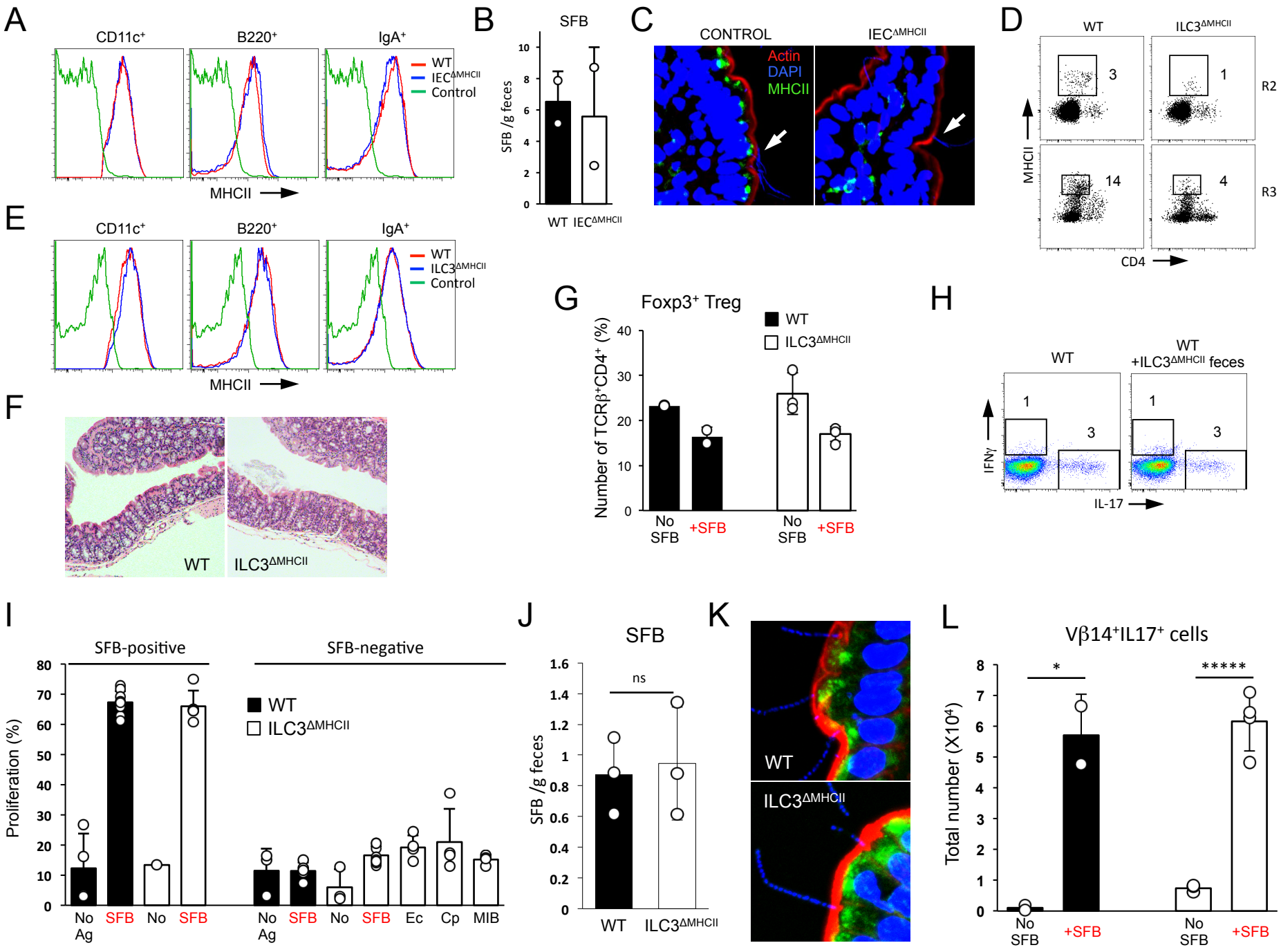

A
CD11c+
B220+
IgA+
WT
IEC ΔMHCII
Control
MHCII
B
SFB
SFB /g feces
WT IEC ΔMHCII
C
CONTROL
IEC ΔMHCII
Actin
DAPI
MHCII
D
WT
ILC3 ΔMHCII
MHCII
CD4
R2
R3
E
CD11c+
B220+
IgA+
WT
ILC3 ΔMHCII
Control
MHCII
F
WT
ILC3 ΔMHCII
G
Foxp3+ Treg
Number of TCRβ+CD4+ (%)
WT
ILC3 ΔMHCII
No SFB
+SFB
H
WT
WT +ILC3 ΔMHCII feces
IFNγ
IL-17
I
SFB-positive
SFB-negative
Proliferation (%)
WT
ILC3 ΔMHCII
No Ag
SFB
Ec
Cp
MIB
J
SFB
SFB /g feces
ns
WT
ILC3 ΔMHCII
K
WT
ILC3 ΔMHCII
L
Vβ14+IL17+ cells
Total number (X10⁴)
*
*****
No SFB
+SFB

Figure S6

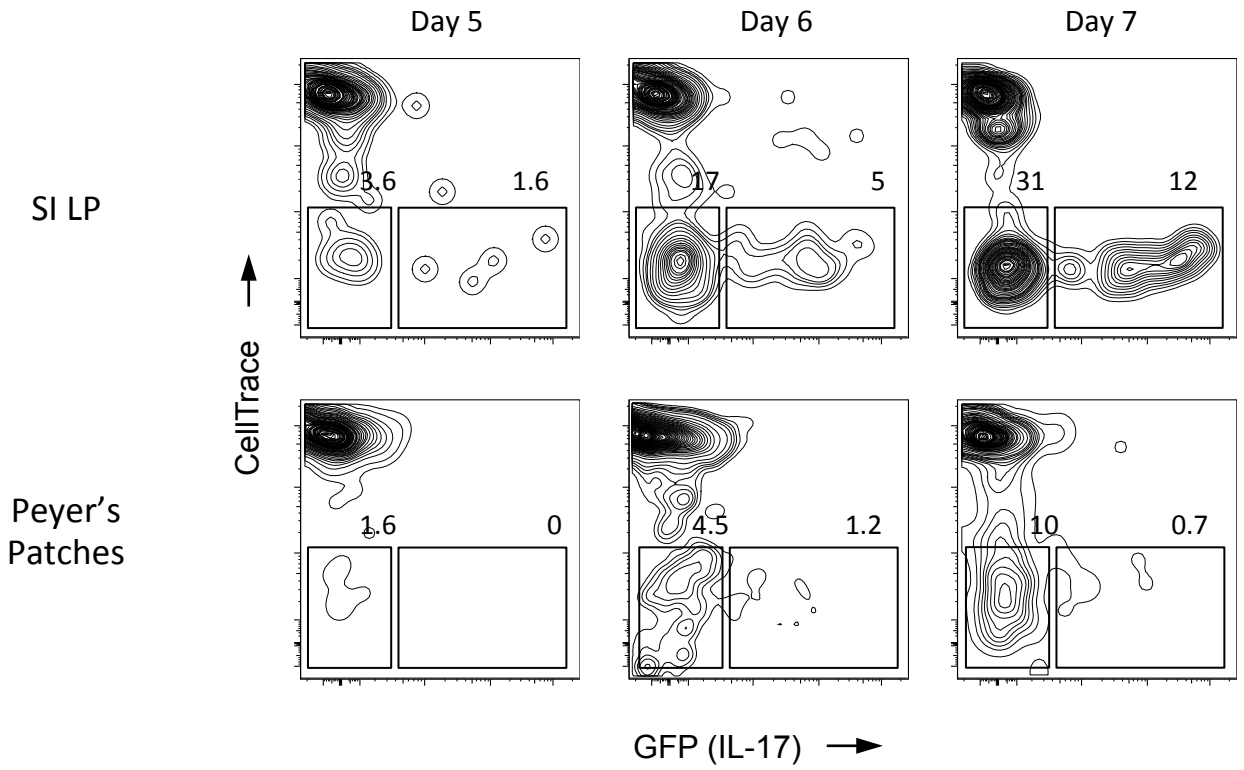

Day 5
Day 6
Day 7
SI LP
Peyer's Patches
CellTrace
GFP (IL-17)
3.6
1.6
17
5
31
12
1.6
0
4.5
1.2
10
0.7

Figure S7

Clone#	CDR3	CDR3 Length	Vβ
35	CTCSAVGGFGEQYF	12	2
5	CTCSAGLGGNTGQLYF	14	2
15	CASSLGDSAETLYF	12	5.1
4	CASSLSFFGGGLQNTLYF	16	7
20	CASSLSFFGGGLQNTLYF	16	7
22	CASSETISNERLFF	12	8.3
31	CASSATVSNERLFF	12	8.3
33	CASNDWGIEQYF	10	12
23	CAWSSRWGGARAEQFF	14	14
39	CAWSLGAEQFF	9	14
12	CAWSRGTGGNTEVFF	13	14
1	CAWSLANSDYTF	10	14
41	CGARGWGSNQDTQYF	13	15
21	CASSLRGGQNTLYF	12	16
32	CASSSQGAGNTLYF	12	16

Table S1