Supplementary Figures

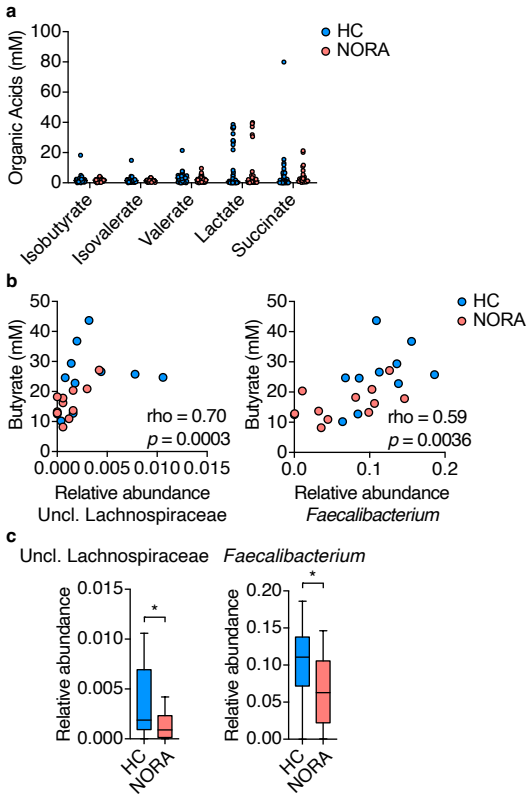

Figure S1. Butyrate-producing bacteria are reduced in patients with NORA

(a) Stool concentration of organic acids in healthy control (HC) and new-onset untreated rheumatoid arthritis (NORA) subjects (n = 41, 31).

(b) Correlation between Lachnospiraceae and *Faecalibacterium* and stool concentration of butyrate in HC and NORA patient subjects.

(c) The relative abundance of unclassified Lachnospiraceae and *Faecalibacterium* in faeces from HC and NORA patient subjects.

*P < 0.05 (a, Mann-Whitney test; b, Spearman's nonparametric correlation; c, Wilcoxon rank-sum test).

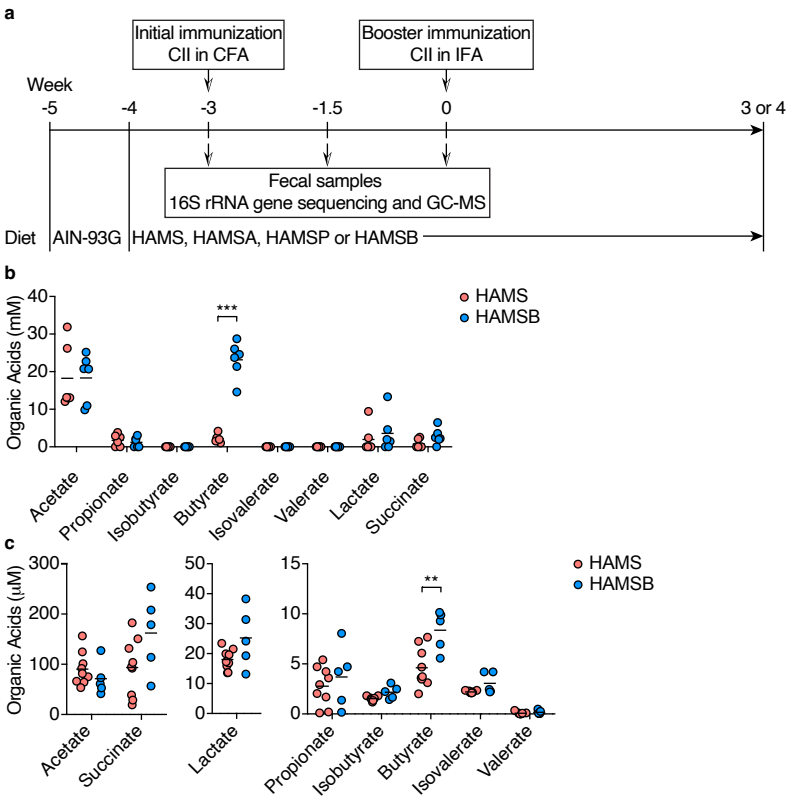

Figure S2. Faecal and serum concentration of organic acids in mice fed the control HAMS or HAMSB diet

(a) Schematic of CIA experiment.

(b, c) Fecal (b) and serum (c) concentrations of organic acids in CIA mice fed the control HAMS or HAMSB diet. Data were obtained two weeks after the initial immunization (n = 5–9).

Results show one representative experiment of at least two experiments. $^{**}P < 0.01$, $^{***}P < 0.001$ (a–c, Welch's t-test or unpaired two-tailed Student's t-test).

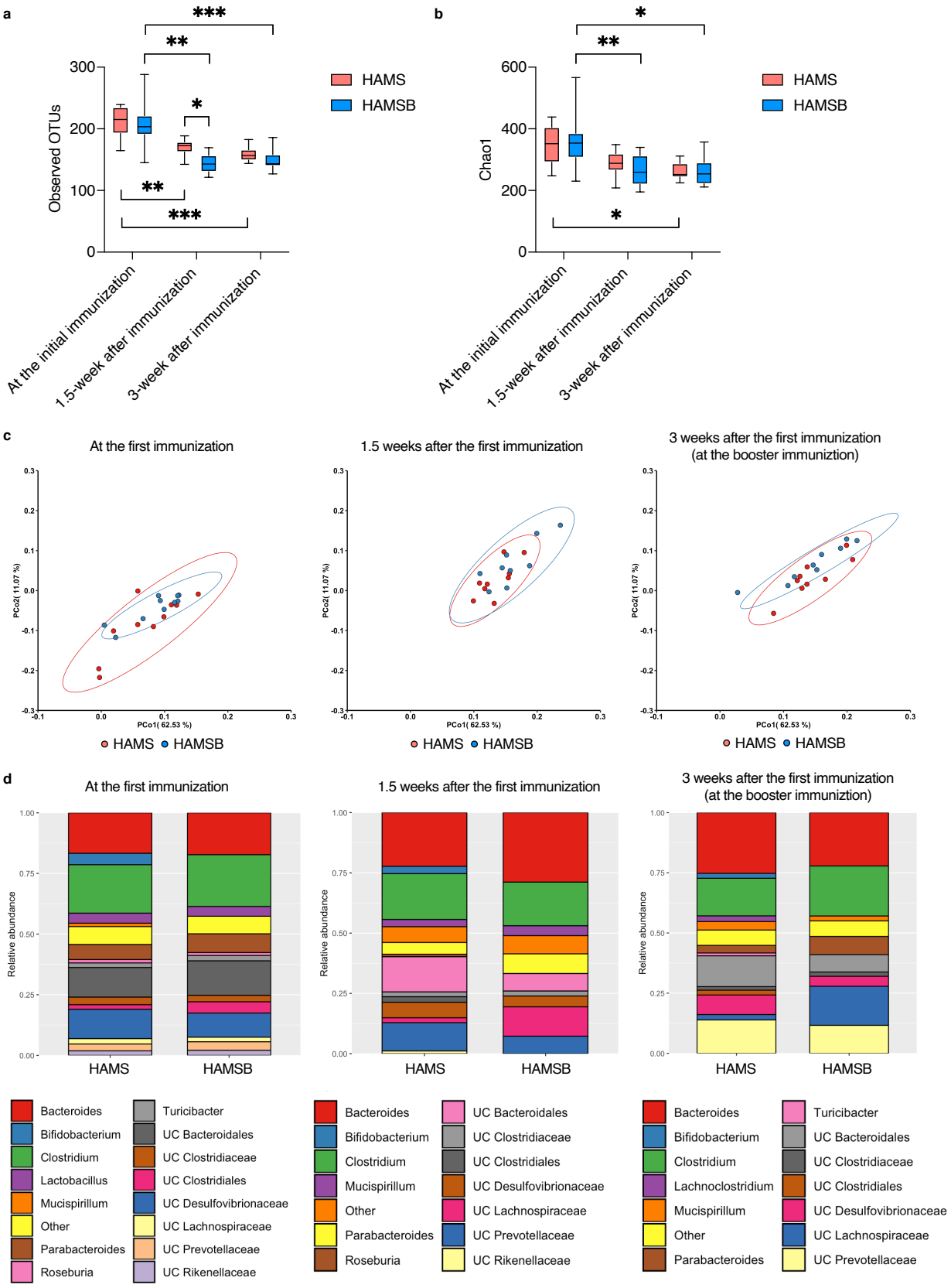
a
Observed OTUs
300
200
100
0
***
**
*
**
***
HAMS
HAMSB
At the initial immunization
1.5-week after immunization
3-week after immunization
b
Chao1
600
400
200
0
*
**
*
HAMS
HAMSB
At the initial immunization
1.5-week after immunization
3-week after immunization
c
At the first immunization
1.5 weeks after the first immunization
3 weeks after the first immunization
(at the booster immuniztion)
PCo2(11.07 %)
PCo1(62.53 %)
HAMS
HAMSB
d
At the first immunization
1.5 weeks after the first immunization
3 weeks after the first immunization
(at the booster immuniztion)
Relative abundance
HAMS
HAMSB
Bacteroides
Bifidobacterium
Clostridium
Lactobacillus
Mucispirillum
Other
Parabacteroides
Roseburia
Turicibacter
UC Bacteroidales
UC Clostridiaceae
UC Clostridiales
UC Desulfovibrionaceae
UC Lachnospiraceae
UC Prevotellaceae
UC Rikenellaceae
Bacteroides
Bifidobacterium
Clostridium
Mucispirillum
Other
Parabacteroides
Roseburia
UC Bacteroidales
UC Clostridiaceae
UC Clostridiales
UC Desulfovibrionaceae
UC Lachnospiraceae
UC Prevotellaceae
UC Rikenellaceae
Bacteroides
Bifidobacterium
Clostridium
Lachnoclostridium
Mucispirillum
Other
Parabacteroides
Turicibacter
UC Bacteroidales
UC Clostridiaceae
UC Clostridiales
UC Desulfovibrionaceae
UC Lachnospiraceae
UC Prevotellaceae

Figure S3. Faecal microbial diversity after the initial CII immunization in mice fed the control HAMS or HAMSB diet

(a, b) Operational taxonomic units (OTUs, a) and the Chao richness estimators (b) in mice fed the control HAMS or HAMSB diet (n = 9, 10). Faecal samples were obtained at the initial CII immunization, 1.5 weeks, or three weeks after immunization (see Figure. S2a for the schematic of CIA experiment). Bars on graphs indicate mean ± SEM; whiskers on box-and-whiskers plots represent min to max.

(c) The principal coordinate analysis (PCoA) based on weighted UniFrac distances between the control HAMS-fed and HAMSB–fed mice (n = 9, 10). Each symbol represents data from individual mice. Red symbols and circles indicate the HAMS–fed mice. Blue symbols and circles indicate HAMSB-fed mice.

(d) The composition of microbiota at the family and genus levels between the control HAMS-fed and HAMSB-fed mice (n = 9, 10)

Results show one representative experiment. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, (a-b, two-way ANOVA followed by Tukey's post-hoc test; b, Welch's t-test or unpaired two-tailed Student's t-test)**.**

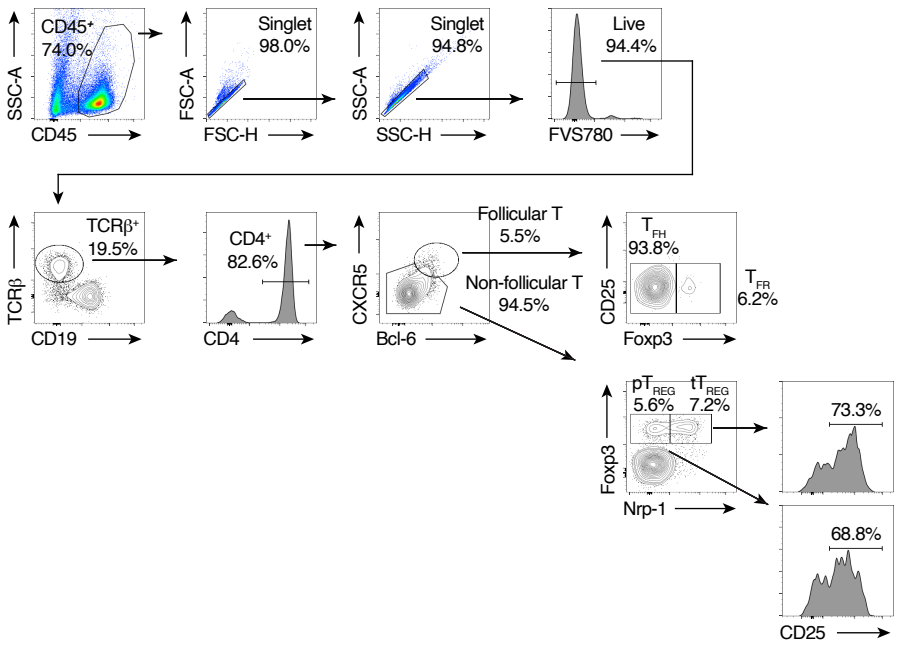

Figure S4. Flow cytometry gating strategy to identify T_{FH}, T_{FR}, tT_{REG} and pT_{REG} cells in CIA mice

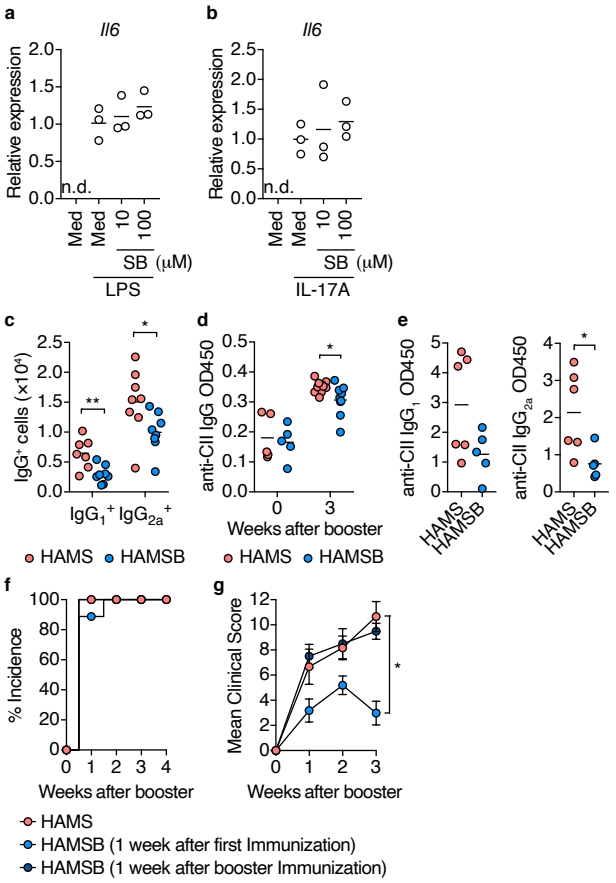

Figure S5. Feeding of HAMSB diet after booster immunization does not cure the CIA

(a,b) Relative *Il6* expression in primary synovial fibroblasts stimulated with 100 ng ml^{-1} LPS (a) or 100 ng ml^{-1} IL-17A (b) for two days. $Thy1^{+}CD11b^{-}$ synovial fibroblasts isolated from hind knee joints of unimmunized DBA/1J mice were used.

(c) Frequencies of IgG_1^{+} and IgG_{2a}^{+} B cells within $CD19^{+}$ B cells in the joint DLNs of CIA mice fed the control HAMS or HAMSB diet (n = 8). Data were obtained two weeks after the initial immunization.

(d) Serum levels of CII-specific total IgG in CIA mice fed HAMS or HAMSB (n = 5–10).

(e) The levels of CII-specific IgG_1 and IgG_{2a} in the hind knee joint extract from CIA mice fed the control HAMS or HAMSB. Data were obtained three weeks after booster immunization (n = 5, 6).

(f, g) Incidence of CIA (f) and arthritis score (g) in CIA mice after booster immunization. The feeding of the control HAMS or HAMSB diet was started one week after the initial immunization or one week after booster immunization (n = 6). AUC was calculated in each mice in g.

Results show one representative experiment of at least two experiments. n.d.: not detected, *P < 0.05, **P < 0.01 (a, b, g, one-way ANOVA followed by Dunnett's post-hoc test; c–e, Welch's t-test or unpaired two-tailed Student's t-test; F, Log-rank test).

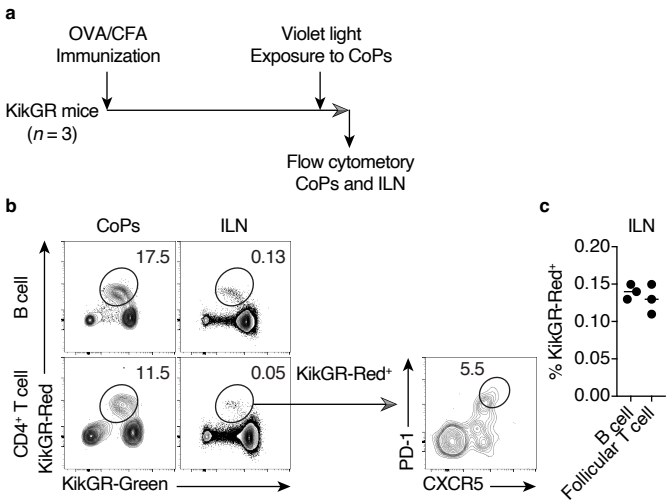

Figure S6. CoP B and follicular T cells migrate to ileac lymph nodes

(a) Schematic of KikGR photoconvertible transgenic mice experiment. KikGR mice were immunized with an OVA/CFA emulsion. Three weeks after immunization, CoPs in the middle colon were exposed to violet light for 2 min and resected after one day. Cells from ileac lymph node (ILN) and the photoconverted CoPs were subjected to flow cytometry to evaluate KikGR photoconversion.

(b) Frequency of KikGR-Green/Red-expressing cells within B cells and follicular T cell gate of CoPs and ILN (n = 3).

(c) Frequency of KikGR-Red-expressing cells within B cells and follicular T cells of ILN (n = 3).

Results show one representative experiment.

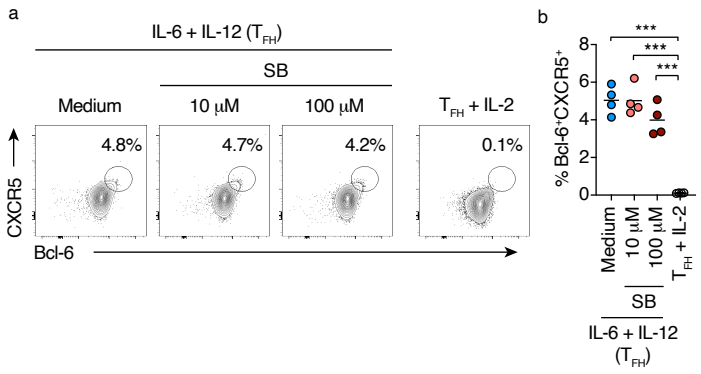

Figure S7. Butyrate has no impact on T_{FH} cell differentiation *in vitro*

(a, b) *In vitro* T_{FH} cell differentiation culture. Sort-purified naïve $CD4^+$ T cells from C57BL/6 mice were cultivated under T_{FH}-cell culture conditions in the absence or presence of sodium butyrate (SB) at 10 μM or 100 μM. Representative flow cytometry contour plots of Bcl-6 and CXCR5 staining (a), and the frequency of $Bcl\text{-}6^+CXCR5^+$ cells within $CD45^+CD4^+TCR\beta^+$ gate (b, n = 4). Results show one representative experiment of at least two experiments. ***$P < 0.001$ (b, one-way ANOVA followed by Tukey's post-hoc test).

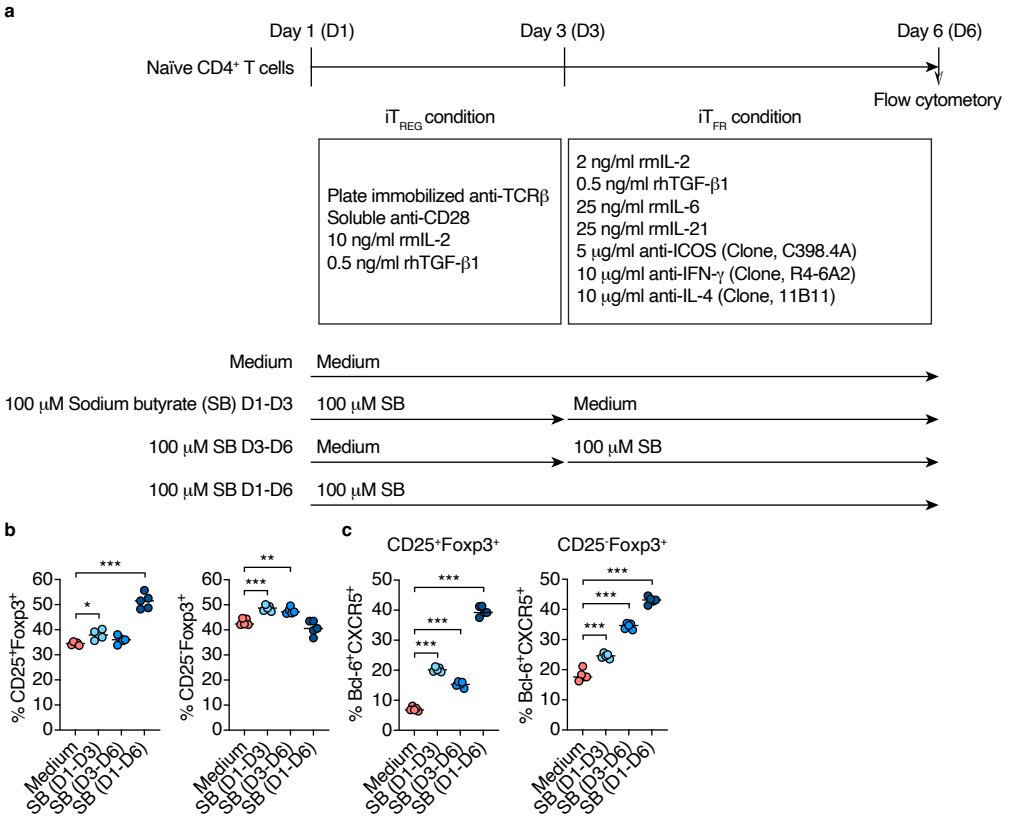

Figure S8. The later phase of SB treatment induces iT_{FR} cell differentiation

(a) Schematic of iT_{FR}-cell differentiation culture with a different period of SB treatment. Sort-purified naïve $CD4^+$ T cells from Bcl-6-tdTomato *Foxp3*^{hCD2} double reporter mice were cultivated under $iT_{REG/FR}$-cell common culture conditions followed by iT_{FR}-cell culture conditions in the presence or absence of 100 μM SB.

(b) The frequency of $CD25^+Foxp3^+$ and $CD25^-Foxp3^+$ cells within $CD4^+$ $TCR\beta^+$ gate ($n = 5$).

(c) The frequency of Bcl-6-$tdTomato^+CXCR5^+$ cells among $CD25^+Foxp3^+$ and $CD25^-Foxp3^+$ gates ($n = 5$). Results show one representative experiment of at least two experiments. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ (b, c, one-way ANOVA followed by Dunnett's post-hoc test).

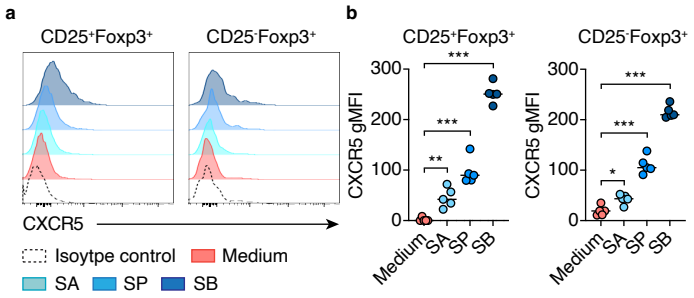

Figure S9. Butyrate upregulates CXCR5 expression in iT_{REG} cells.

(a, b) CXCR5 expression by iT_{REG} cells differentiated *in vitro* with 100 μM SCFAs treatment. Sort-purified naïve $CD4^+$ T cells from *Foxp3*^{hCD2} reporter mice were cultivated under iT_{REG}-cell culture conditions in absence or the presence of sodium acetate (SB), sodium propionate (SP) or sodium butyrate (SB) at 100 μM. Representative flow cytometry histograms of CXCR5 expression (a) and CXCR5 gMFI (b) among $CD25^+Foxp3^+$ and $CD25^-Foxp3^+$ gate ($n = 5$).

Results show one representative experiment of at least two experiments. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ (b, one-way ANOVA followed by Dunnett's post-hoc test).

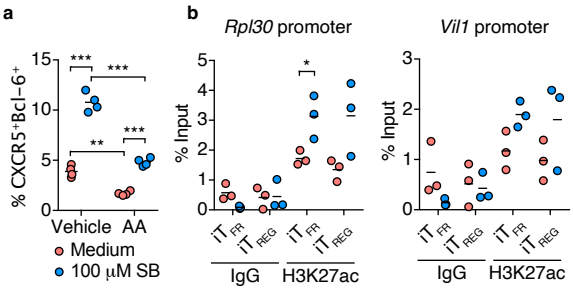

Figure S10. HATi nullified butyrate-induced iT_{FR} cell differentiation

(a) The frequency of Bcl-6$^+$CXCR5$^+$ iT_{FR} cells within CD25$^-$Foxp3$^+$CD4$^+$ T cells with or without 100 μM sodium butyrate (SB). Naïve CD4$^+$ T cells were cultured for five days under iT_{FR} cell polarizing condition (see Figure 5A) with the histone acetyltransferase inhibitor (HATi) anacardic acid (AA) at 25 nM or vehicle control 0.01% DMSO.

(b) Accumulation of H3K27 acetylation (ac) at the promoter region of *Rpl30* and *Vil1* in Foxp3$^+$ cells from iT_{FR} or iT_{REG} cultures. ChIP quantitative RT-PCR (qPCR) analysis of H3K27ac levels in the promoters in sort purified Foxp3$^+$ T cells cultured for total three days under iT_{FR} or iT_{REG}-cell polarizing conditions (see Figure 5a) with or without the treatment of 100 μM SB.

Results show one representative experiment of at least two experiments. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (b, Welch's *t*-test or unpaired two-tailed Student's *t*-test; a, two-way ANOVA followed by Tukey's post-hoc test)