**Cell Metabolism, Volume *31***

## Supplemental Information

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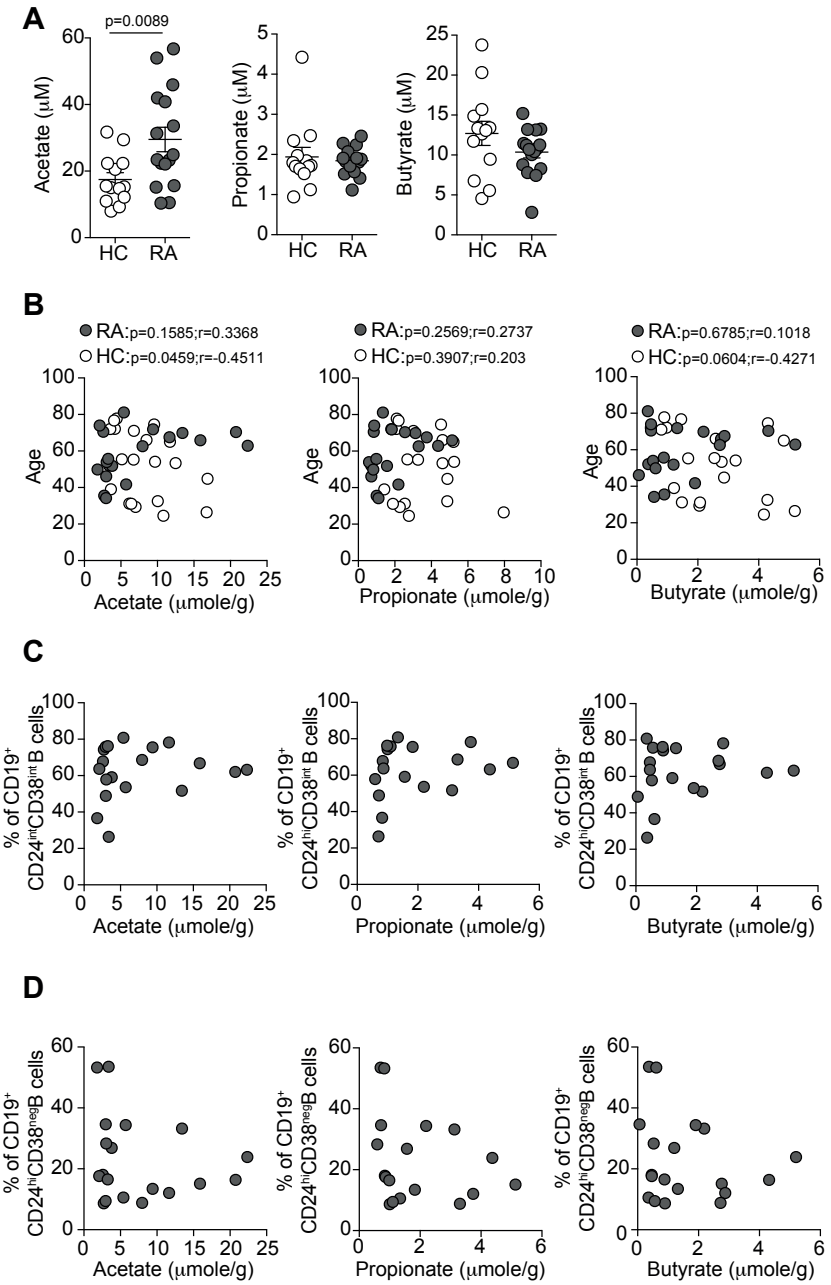
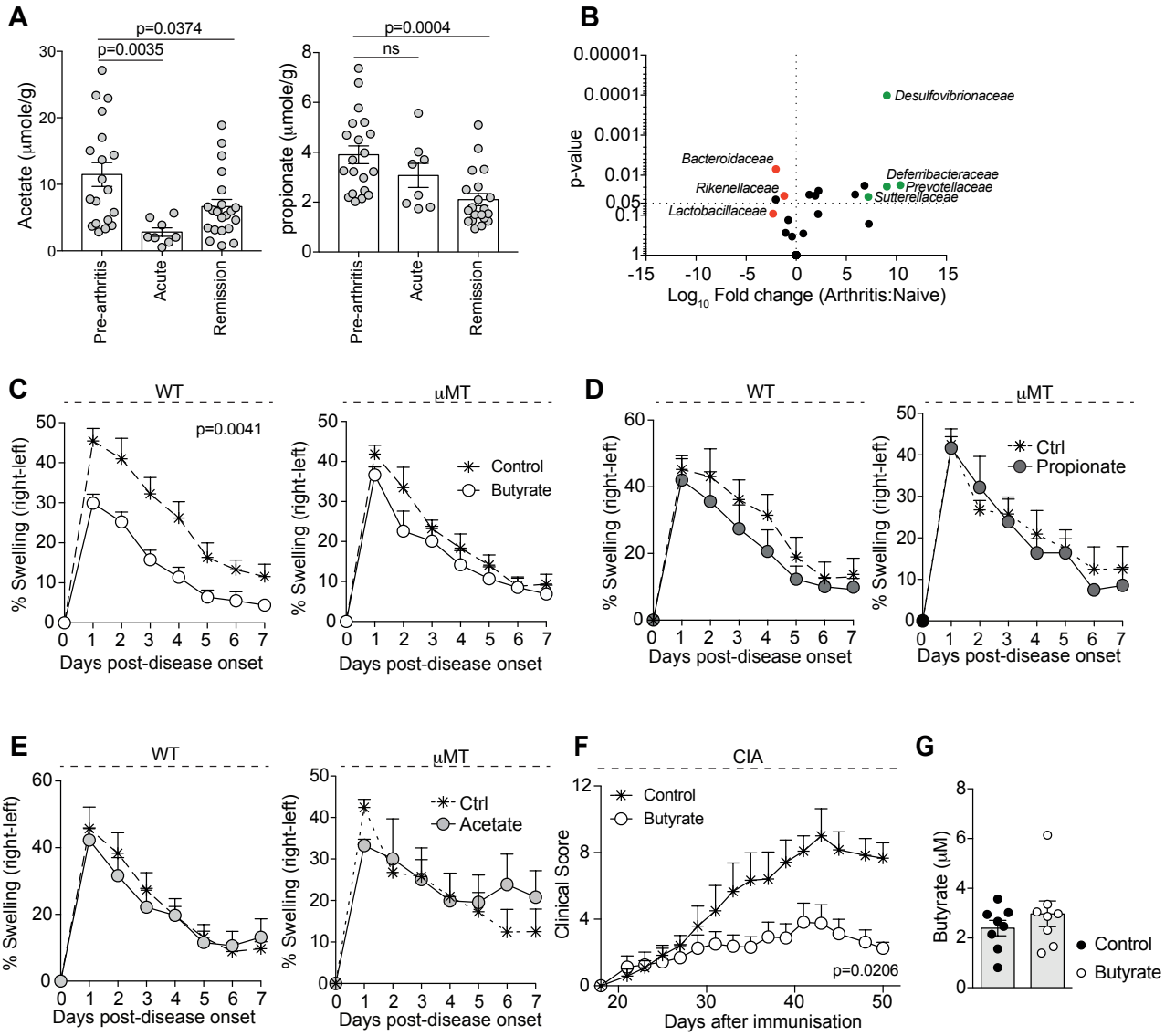
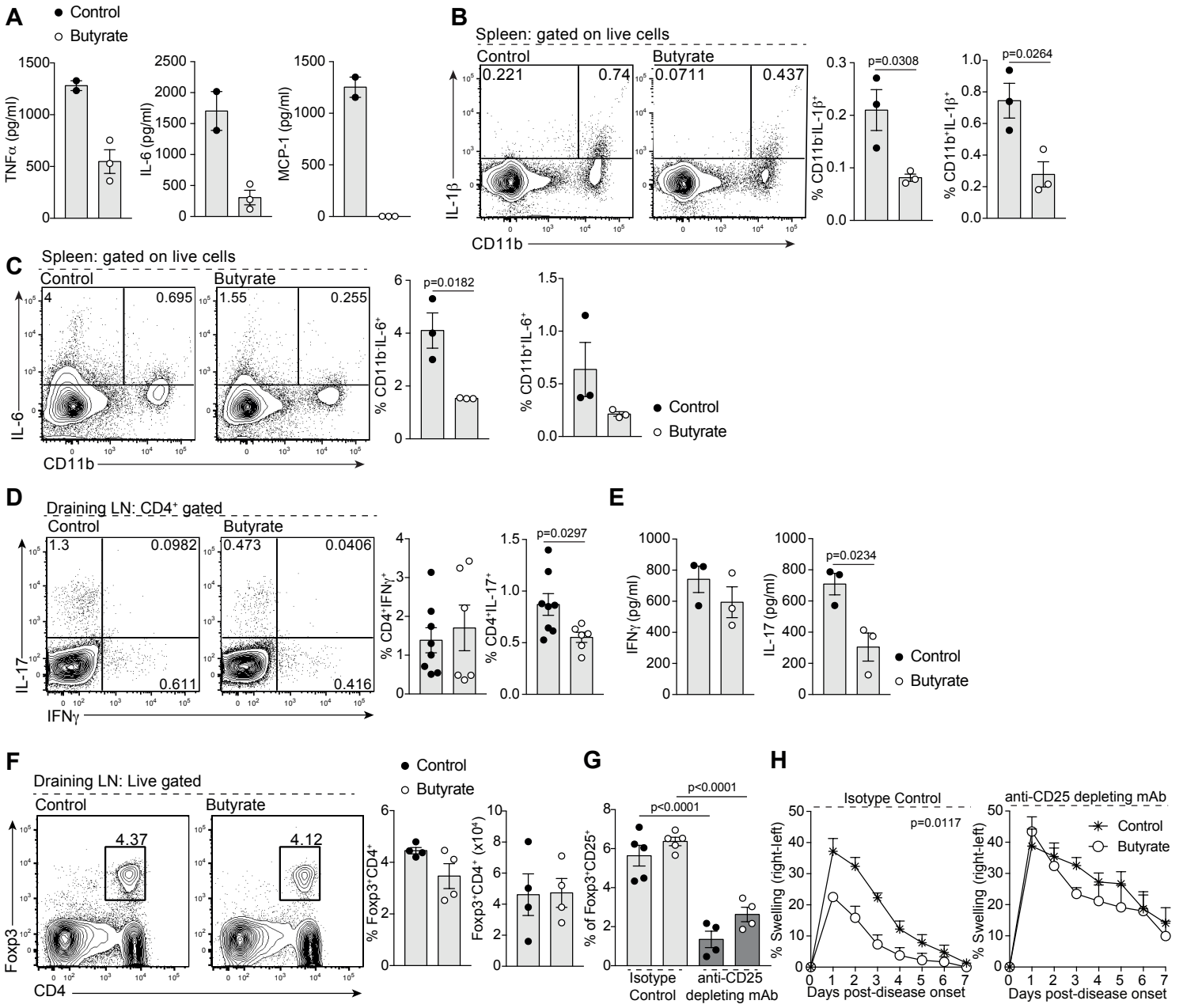


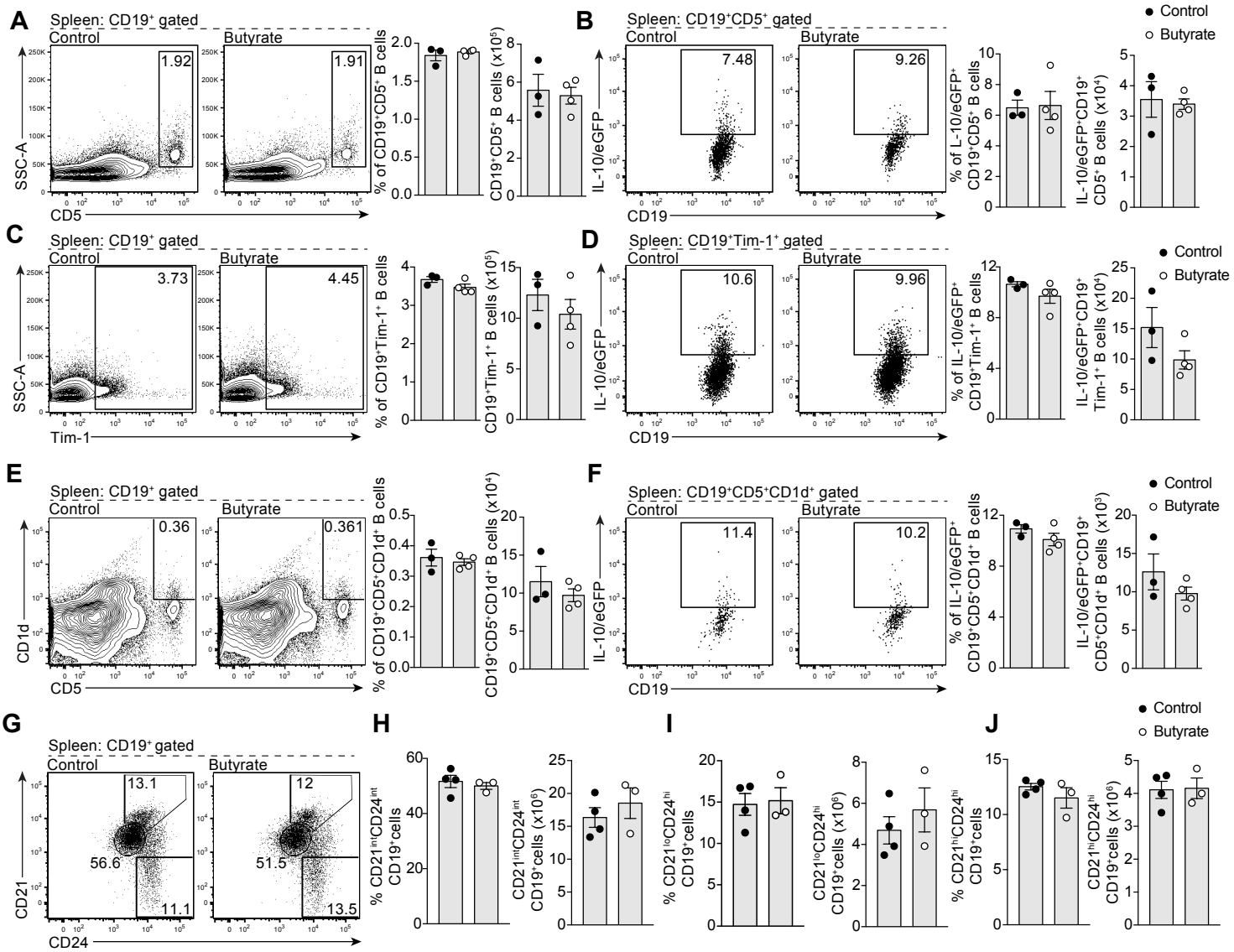

**Supplementary Figure 1. There is no association between SCFA levels and mature B cells or memory B cells (Related to Figure 1).** (A) Representative histograms show level of acetate, propionate and butyrate in sera of healthy individ-uals (n=13) and individuals with rheumatoid arthritis (RA) (n=14). (B) Scatter plots show correlation between the levels of acetate, propionate and butyrate in faeces and age at time of sample (HC, n=20; RA, n=19). (C) Scatter plots show correlation between the levels of acetate, propionate and butyrate in the faeces and the frequency of $CD19^{+}CD24^{int}CD38^{int}$ (mature naive B cells) in the peripheral blood of patients with RA (n=19). (D) Scatter plots show correlation between the levels of acetate, propionate and butyrate in the faeces and the frequency of $CD19^{+}CD24^{hi}CD38^{neg}$ (memory B cells) in the peripheral blood of patients with RA (n=19). A, Data represent mean ± SE; (A, Student's t test; B-D, Pearson's correlation).



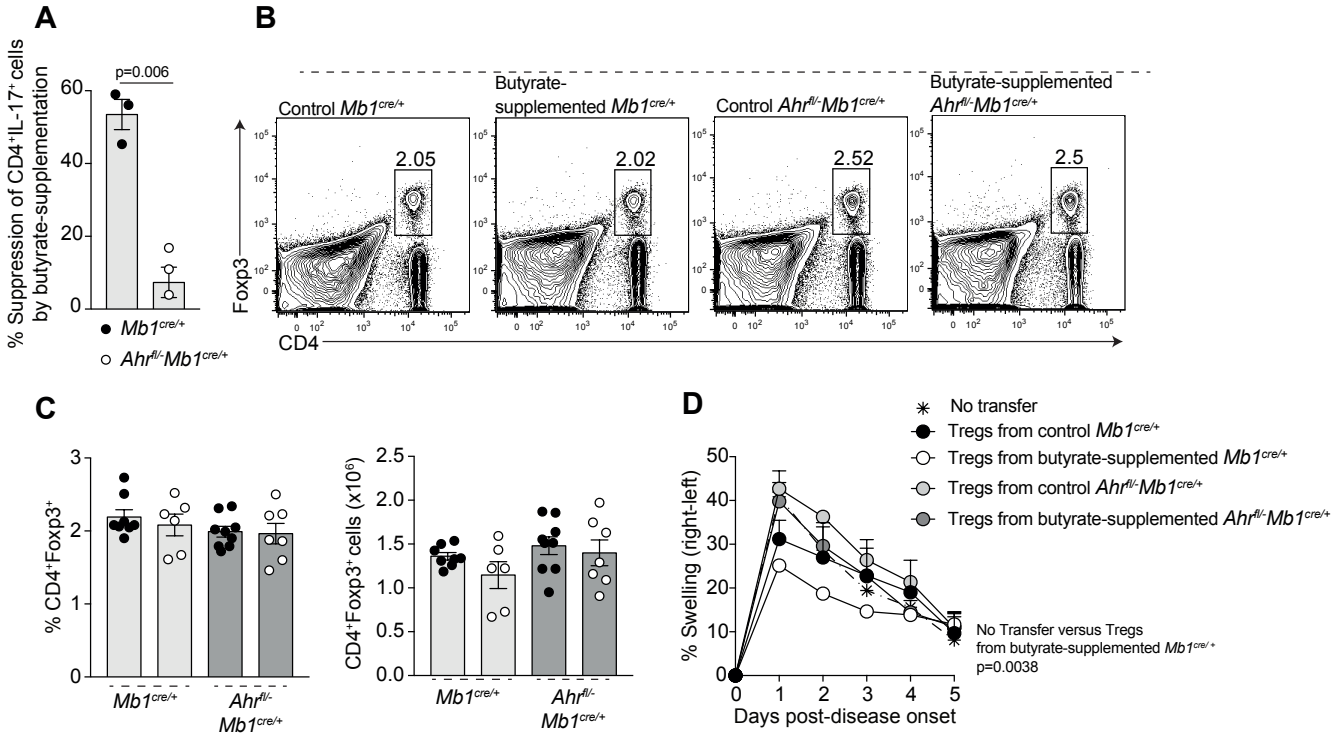

**Supplementary Figure 2. Supplementation with Acetate or Propionate does not suppress the development of antigen induced arthritis (Relative to Figure 2)**. (A) Concentration of acetate and propionate in faeces from WT mice pre-arthritis (n=23), with acute arthritis (n=8) and in remission from arthritis (n=18) as measured by high-performance liquid chromatography (cumulative data are shown). (B) Volcano plot shows fold change between bacterial families in the faeces of naïve mice compared to arthritic mice at day 3 post-disease onset (n=4 per group). (C) Mean clinical score of control (cumulative n=25) and butyrate-supplemented WT mice (cumulative n=24) (one representative experiment of six experiments is shown) or μMT mice (control, cumulative n=7; butyrate, cumulative n=9). (D) Mean clinical score of control and propionate-supplemented WT mice (left) or μMT mice (right) (WT: n =8, μMT: n=6 per group); y axis shows percent-age swelling in antigen-injected knee compared to control knee (one of two experiments is shown). (E) Mean clinical score of control and acetate-supplemented WT mice (left) or μMT mice (right) (cumulative n=10 per group); y axis shows percentage swelling in antigen-injected knee compared to control knee (one representative experiment of two experiments is shown. (F) Mean clinical score following induction of collagen-induced arthritis in control of butyrate-supplemented WT mice (cumulative n=8 per group). (G) Butyrate levels in the sera butyrate-supplemented and control mice (n=8 per group, cumulative data are shown). Data represent mean ± SE. (A, Student's t test; B, multiple unpaired t tests; C-F, two-way ANOVA; G, Student's t test).



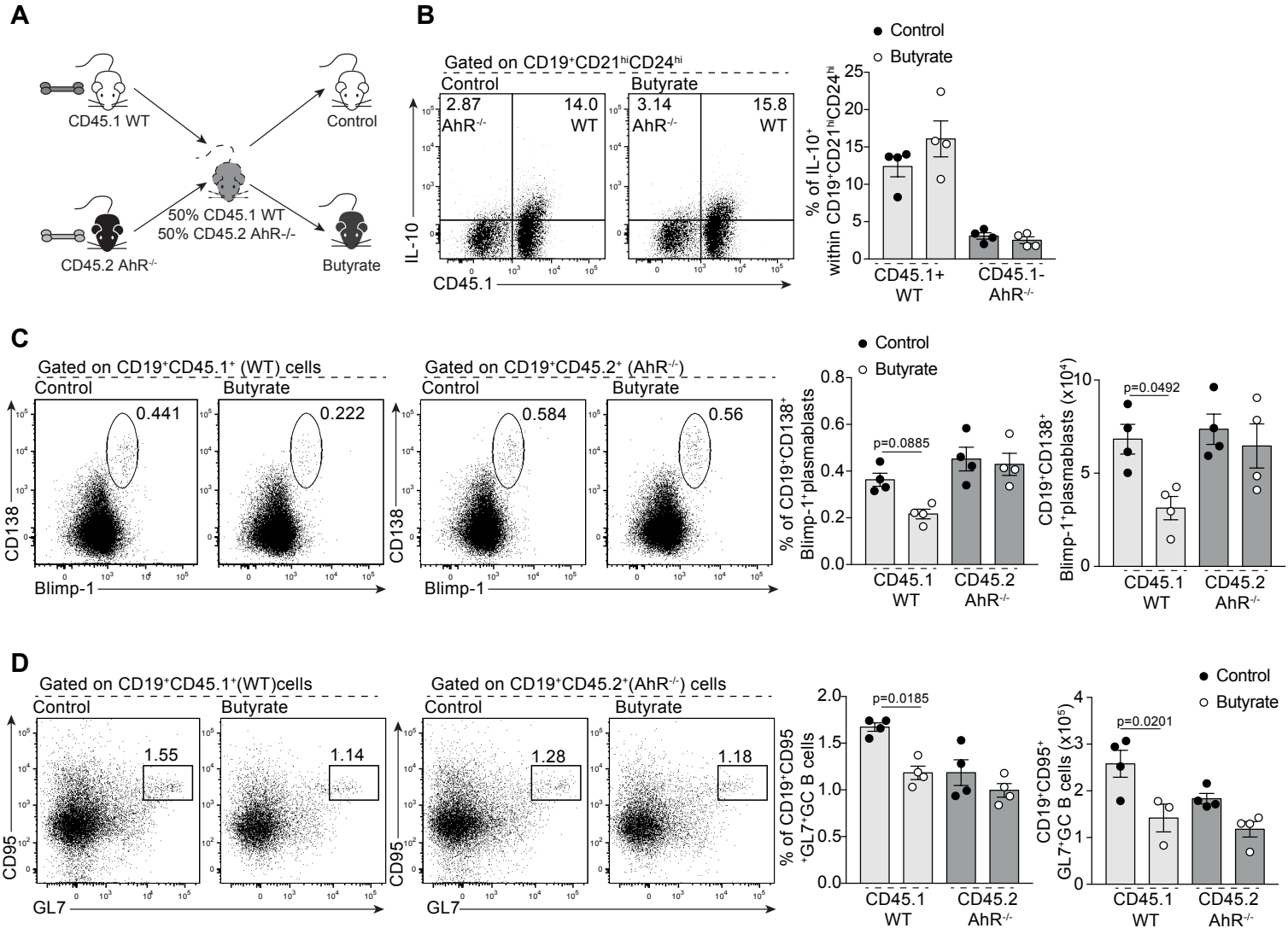

**Supplementary Figure 3. Suppression of arthritis by butyrate-supplementation is associated with a reduction in pro-arthritogenic stimuli (Related to Figure 2).** (A) Production of TNFα, IL-6, MCP-1 by total lymphocytes as measured by cytokine bead array (cumulative n=5 per group, one representative experiment of two experiments is shown). (B) Representative plots (left) and bar chart (right) showing the percentage of IL-1β$^+$CD11b$^-$ and IL-1β$^+$CD11b$^+$ in the spleen at day 7 post-disease onset. (C) Representative plots (left) and bar chart (right) showing the percentage of IL-6$^+$CD11b$^-$ and IL-6$^+$CD11b$^+$ and in the spleen at day 7 post-disease onset (cumulative n=8 per group, one representative experiment of two experiments is shown). (D) Representative plots (left) and bar chart (right) showing the percentage and absolute number of CD4$^+$IL-17$^+$ cells and CD4$^+$IFNγ$^+$ cells in the draining LN at day 7 post-disease onset (cumulative n=10 per group, one representative experiment of three experiments is shown). (E) Production of IL-17 and IFNγ by total lymphocytes as measured by cytokine bead array (cumulative n=5 per group, one of two repre-sentative experiments is shown). (F) Representative plots (left) and bar chart (right) showing the percentage and absolute number of CD4$^+$Foxp3$^+$ cells in the draining LN at day 7 post-disease onset (cumulative n=10 per group, one representative experiment of three experiments is shown). (G) Frequency of CD4$^+$CD25$^+$Foxp3$^+$ in the spleen of control and butyrate-supplemented mice treated with anti-CD25 depleting mAb compared to isotype control (control mice treated with anti-CD25 depleting mAb compared to isotype control: n=5 per group; butyrate-supplemented mice treated with anti-CD25 depleting mAb compared to isotype control: n=4 per group (cumulative data are shown). (H) Mean clinical score of control and butyrate-supplemented anti-CD25 depleting mAb-treated mice or isotype control mice; y axis shows percentage swelling in antigen-injected knee compared to control knee (cumulative data is shown). Data represent mean ± SE. (A-F, Student's t test; G, one-way ANOVA; H, two-way ANOVA).



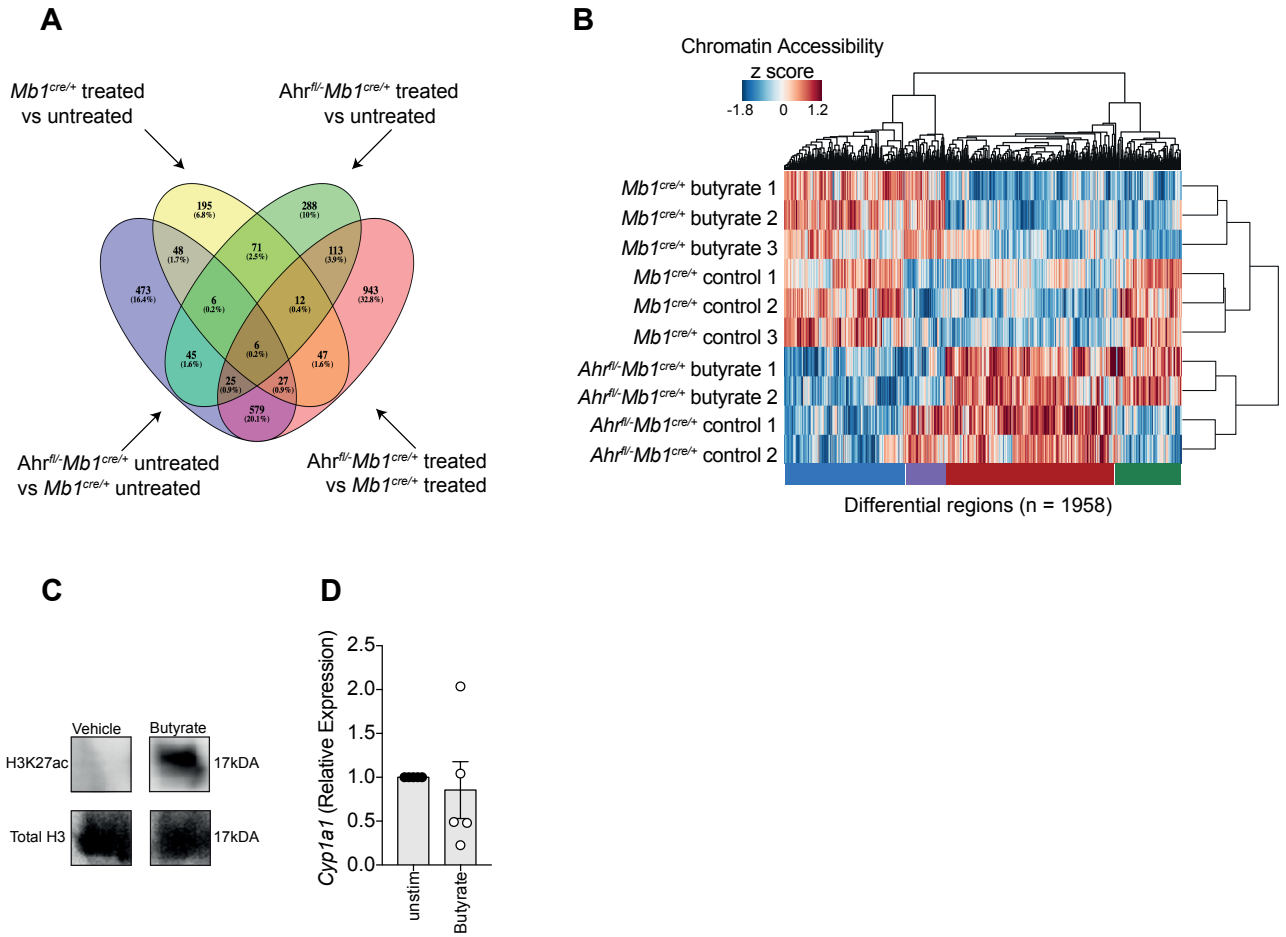

**Supplementary Figure 4. Suppression of arthritis by butyrate-supplementation does not alter the frequency or number of Breg subsets or naive splenic B cell subsets (Related to Figure 2).** (A) Representative plots (left) and bar chart (right) showing the percentage and number of CD5$^+$B cells in the spleen at day 7 post-disease onset. (B) Representative plots (left) and bar chart (right) showing the percentage and number of IL-10eGFP$^+$CD5$^+$B cells in the spleen at day 7 post-disease onset. (C) Representative plots (left) and bar chart (right) showing the percentage and number of Tim-1$^+$ B cells in the spleen at day 7 post-disease onset. (D) Representative plots (left) and bar chart (right) showing the percentage and number of IL-10eGFP$^+$Tim-1$^+$B cells in the spleen at day 7 post-disease onset. (E) Representative plots (left) and bar chart (right) showing the percentage and number of CD1d$^+$CD5$^+$B cells in the spleen at day 7 post-disease onset. (F) Representative plots (left) and bar chart (right) showing the percentage and number of IL-10eGFP$^+$CD1d$^+$CD5$^+$B cells in the spleen at day 7 post-disease onset (cumulative n=8, one representative experiment of two experiments is shown). (G) Representative plots showing the percentage of CD19$^+$CD21$^{int}$CD24$^{int}$B cells, CD19$^+$CD21$^{lo}$CD24$^{hi}$B cells, and CD19$^+$CD21$^{hi}$CD24$^{hi}$B cells in the spleen at day 7 post-disease onset. Bar chart showing the percentage and number of (H) CD19$^+$CD21$^{int}$CD24$^{int}$B cells, (I) CD19$^+$CD21$^{lo}$CD24$^{hi}$B cells, and (J) CD19$^+$CD21$^{hi}$CD24$^{hi}$B cells. (Control, cumulative n=15; Butyrate cumulative n=13; one representative experiment of three experiments is shown. Data represent mean ± SE. A-J, Student's t test.




**Supplementary Figure 5. Expression of AhR in B cells is fundamental for modulation of T cell function after butyrate-supplementation (Related to Figure 3).** (A) Bar chart showing the suppression of CD4$^+$IL-17$^+$T cells in *Mb1*$^{cre/+}$ or *Ahr*$^{fl/-}$*Mb1*$^{cre/+}$ mice that received butyrate-supplementation compared to control *Mb1*$^{cre/+}$ or *Ahr*$^{fl/-}$*Mb1*$^{cre/+}$ mice (cumulative n=6 per group, one representative experiment of two experiments is shown). Representative plots (B) and bar charts (C) showing the percentage and number of CD4$^+$Foxp3$^+$ T cells in *Mb1*$^{cre/+}$ or *Ahr*$^{fl/-}$*Mb1*$^{cre/+}$ mice that received butyrate-supplementation compared to control *Mb1*$^{cre/+}$ or *Ahr*$^{fl/-}$*Mb1*$^{cre/+}$ mice (cumulative data are shown). (D) Mean clinical score following transfer of Tregs from control or butyrate supplemented *Mb1*$^{cre/+}$ and control or butyrate supplemented *Ahr*$^{fl/-}$*Mb1*$^{cre/+}$, a control group that did not receive transfer; y axis shows percentage swelling in antigen-injected knee compared to control knee (n=3 per group). Data represent mean ± SE. (A, students *t* test; C, one-way ANOVA; D, two-way ANOVA).




**Supplementary Figure 6. Butyrate-supplementation suppresses B cell maturation through activation of AhR (Related to Figure 3).** (A) Schematic showing experimental design for competitive congenic bone marrow chimeric experiment. (B) Representative flow cytometry plots (left) and bar charts (right) showing the frequency and number of CD45.1$^+$IL-10$^+$CD21$^{hi}$CD24$^+$ B cells or CD45.1$^-$IL-10$^+$CD21$^+$CD24$^+$B cells in butyrate-supplemented or control chimeric mice (cumulative n=5 per group). (C) Representative flow cytometry plots (left) and bar charts showing the frequency and number of plasmablasts within CD45.1$^+$WT or CD45.2$^+$ AhR$^{-/-}$ derived cells in butyrate-supplemented or control chimeric mice (cumulative n=5 per group). (D) Representative flow cytometry plots (left) and bar charts (right) showing the frequency and number of GC B cells within CD45.1$^+$ WT or CD45.2$^+$ AhR$^{-/-}$ derived cells in butyrate-supplemented or control chimeric mice (cumulative n=5 per group). Data represent mean ± SE. (B-D, one-way ANOVA).




**Supplementary Figure 7. Butyrate does not directly activate AhR (Related to Figure 5 and Figure 6).** (A) Venn diagram indicating the number of significant ($p < 0.05$) DEG across all 4 comparisons and the number of overlapping genes between each comparison. (B) Heatmap shows differentially regulated regions of chromatin in $CD19^{+}CD21^{hi}CD24^{hi}B$ cells isolated from control *Mb1*$^{cre/+}$ mice, butyrate-supplemented *Mb1*$^{cre/+}$ mice, control *Ahr*$^{fl/-}$*Mb1*$^{cre/+}$ mice, and butyrate-supplemented *Ahr*$^{fl/-}$*Mb1*$^{cre/+}$ mice as measured by ATAC-seq. (C) Total splenic B cells were isolated from WT mice and treated either with a vehicle control or 500μM butyrate for 18h and analysed for H3K27ac by Western blot. Total H3 was used as a control. The numbers indicate the size of the protein bands in kDA. (D) Bar chart shows relative expression of *Cyp1a1* following 6 hours culture with butyrate (cumulative n=5).

| | Healthy control (n=20) | Rheumatoid arthritis (n=19) |
|---|---|---|
| **No. male/female** | 4/16 | 4/15 |
| **Mean age at sampling (years)** | 52.6 | 58.5 |
| **Treatment received within preceding 6 months of sample: None** | n/a | 10.5% |
| **Treatment received within preceding 6 months of sample: DMARD only (%)** | n/a | 26.3% |
| **Treatment received within preceding 6 months of sample: MTX (%)** | n/a | 36.8% |
| **Treatment received within preceding 6 months of sample: Biological therapy (%)** | n/a | 31.6% |
| **DAS (IQR)** | n/a | 2.13 (1.61-3.06) |
| **CRP (μg/mL)** | n/a | 1.7 (1.2-3.675) |
| **ESR mm/hr at time of sampling, median (IQR)** | n/a | 9 (5-13) |
| **RF + (%)** | n/a | 78.9% |

**Table S1: Patient demographics (Related to Figure 1).**

| **Gene set – AhR independent butyrate regulated genes** | | | | | |
|---|---|---|---|---|---|
| *Adamdec1* | *Casc4* | *Hip1* | *Mtfr1l* | *Pomt1* | *Tbxa2r* |
| *Ahdc1* | *Cbfa2t3* | *Hpse* | *Mtmr4* | *Ppcdc* | *Tecpr2* |
| *Anks1* | *Ddx11* | *Hsp90b1* | *Nfya* | *R3hdm1* | *Tmc4* |
| *Ano10* | *Ece1* | *Hyou1* | *Pafah2* | *Rpgrip1l* | *Tmcc3* |
| *Ano8* | *Fahd2a* | *L3mbtl3* | *Patz1* | *Sdf2l1* | *Tmem129* |
| *Asl* | *Fam173b* | *Lamc1* | *Pdia3* | *Slc16a6* | *Tnfrsf4* |
| *Bcl2* | *Fbxl5* | *Lman2l* | *Pdia6* | *Slc2a9* | *Unc119b* |
| *Bcl9* | *Fkbp2* | *Magt1* | *Piga* | *Slc37a2* | *Usp31* |
| *Bicd2* | *Flnb* | *Manf* | *Pik3r5* | *Smg7* | *Vti1a* |
| *Calr* | *Fuca1* | *Med16* | *Plod1* | *Sorbs3* | *Xbp1* |
| *Canx* | *Gprasp1* | *Mib2* | *Plxna1* | *St13* | *Znhit1* |
| *Capn5* | *Guca1b* | *Mrpl1* | *Poln* | *Tbc1d19* | |

**Table S2: AhR independent butyrate regulated genes (Related to Figure 4).** 71 identified genes which are significantly differentially expressed after butyrate-supplementation in both *Mb1*$^{cre/+}$ and *Mb1*$^{cre/+}$*Ahr*$^{fl/-}$ mice.

| Gene Set – AhR-dependent butyrate regulated genes | | | | | |
|---|---|---|---|---|---|
| *1110065P20Rik* | *Cchcr1* | *Gfod1* | *Nacc1* | *Rab26os* | *Top3a* |
| *1700048O20Rik* | *Cd180* | *Haghl* | *Nek1* | *Rala* | *Tpst1* |
| *1810014B01Rik* | *Cep104* | *Haus2* | *Nfe2l2* | *Recql* | *Trmt10b* |
| *1810024B03Rik* | *Cep162* | *Hdac11* | *Nfkbiz* | *Rnase12* | *Trmt2b* |
| *2010111I01Rik* | *Cep78* | *Hist1h4d* | *Noa1* | *Rpl12* | *Tsc22d1* |
| *2500004C02Rik* | *Cers4* | *Ift74* | *Nod1* | *Rpl37* | *Ttc13* |
| *3110009E18Rik* | *Cgrrf1* | *Ints3* | *Nt5c2* | *Rpn1* | *Unc119* |
| *4632415L05Rik* | *Chid1* | *Ints9* | *Oplah* | *Rps19-ps3* | *Urb1* |
| *4833418N02Rik* | *Creld1* | *Ipmk* | *Ovgp1* | *Rundc3b* | *Utp4* |
| *4930402H24Rik* | *Creld2* | *Ipp* | *Oxsm* | *Sel1l3* | *Vmac* |
| *6030419C18Rik* | *Cwc27* | *Itga10* | *P2rx7* | *Selenoi* | *Vps37b* |
| *A430033K04Rik* | *Cyp4v3* | *Kcnk13* | *Pacs2* | *Slamf1* | *Wdr62* |
| *A530072M11Rik* | *Dbp* | *Kctd1* | *Pcgf3* | *Slc12a3* | *Xpnpep3* |
| *Adam15* | *Dcxr* | *Kctd17* | *Pde6d* | *Slc12a5* | *Xrn2* |
| *AI504432* | *Dedd2* | *Kifc5b* | *Pde8a* | *Slc17a9* | *Zc3h12b* |
| *Aldh1l2* | *Dip2a* | *Ldhd* | *Pfkfb1* | *Slc25a1* | *Zdhhc20* |
| *Arid3b* | *Dirc2* | *Lrp11* | *Pfkfb4* | *Slc2a8* | *Zdhhc7* |
| *Arl6ip4* | *Dnajb11* | *Lrpap1* | *Pgp* | *Slc30a4* | *Zfp112* |
| *Asphd1* | *Dqx1* | *Lta* | *Pi4k2a* | *Spast* | *Zfp128* |
| *Atg10* | *Dscr3* | *Mapre3* | *Pik3ip1* | *Spata24* | *Zfp229* |
| *Atp9a* | *Eif2b4* | *Marf1* | *Plk2* | *Ssbp2* | *Zfp236* |
| *Atrn* | *Emsy* | *Mccc2* | *Ppard* | *St3gal1* | *Zfp280c* |
| *Atxn2* | *Ergic1* | *Med26* | *Ppm1d* | *Stxbp4* | *Zfp292* |
| *Baiap2* | *Evi5* | *Mettl22* | *Ppm1l* | *Susd2* | *Zfp39* |
| *BC051142* | *Fam120c* | *Mettl23* | *Ppp1r35* | *Suv39h2* | *Zfp438* |
| *Begain* | *Fam241a* | *Mfsd1* | *Ppp2r1b* | *Taf3* | *Zfp446* |
| *Bloc1s4* | *Fbf1* | *Mfsd2a* | *Praf2* | *Tbl1x* | *Zfp568* |
| *Bmt2* | *Fchsd1* | *Mlkl* | *Prelid3b* | *Tfcp2* | *Zfp729a* |
| *Btbd18* | *Flot1* | *Mrpl33* | *Prkar2a* | *Timd2* | *Zfp943* |
| *Camk2a* | *Fndc10* | *Mterf3* | *Prmt6* | *Tmem165* | *Zfyve21* |
| *Cars* | *Gabbr1* | *Mturn* | *Psph* | *Tmem71* | *Znrf1* |
| *Ccdc85b* | *Galnt7* | *Mzt2* | *Ptger1* | *Tomm6os* | *Zscan22* |
| | *Gfm2* | | *Pycr1* | | *Zscan26* |

**Table S3: AhR-dependent butyrate regulated genes (Related to Figure 4).** 195 identified genes which were significantly differentially expressed after butyrate-supplementation in *Mb1*$^{cre/+}$ mice, once baseline transcriptional changes between control *Mb1*$^{cre/+}$ mice versus *Mb1*$^{cre/+}$*Ahr*$^{fl/-}$ mice had been removed.