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Supplementary Materials for

Intestinal butyrate-metabolizing species contribute to autoantibody production and bone erosion in rheumatoid arthritis

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The PDF file includes:

Figs. S1 to S11
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Other Supplementary Material for this manuscript includes the following:

Excel file S1 to S7

Fig S1

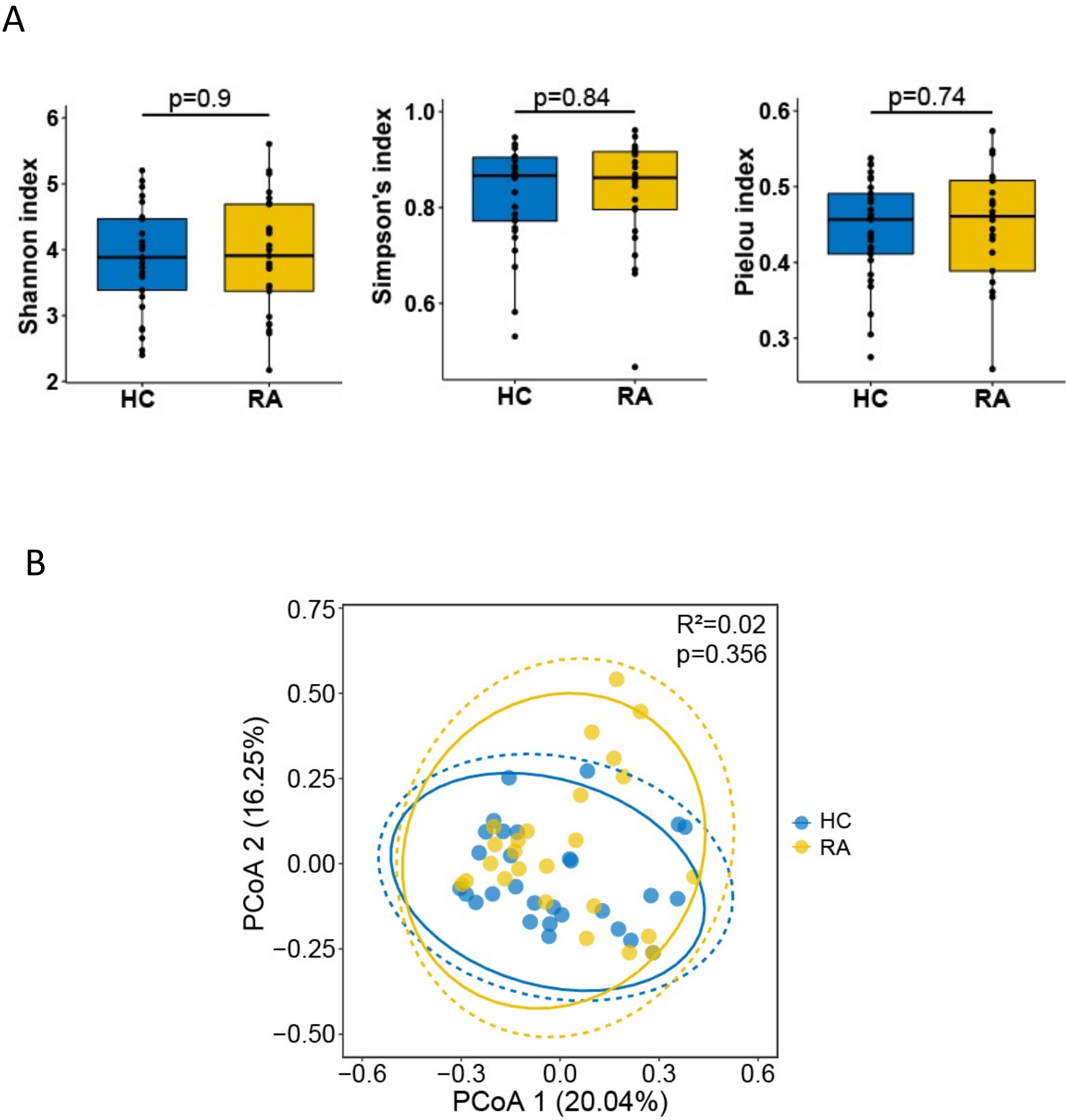
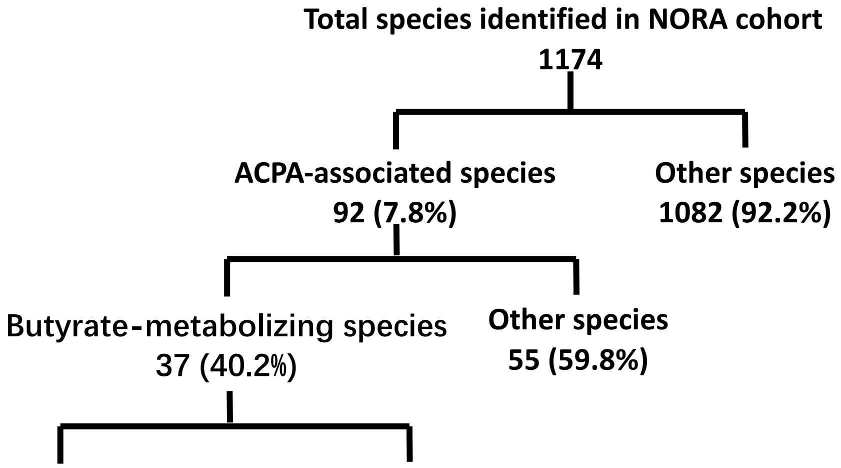


Fig S1. Biodiversity in RA vs control subjects of the NORA cohort. A. The alpha-diversity of the microbiome in RA patients and controls of the NORA cohort**.** HC, healthy control group, n=29; RA, rheumatoid arthritis group, n=25. Mann- Whitney U test. **B**. The beta-diversity of the microbiome in RA patients and controls by principal coordinate analysis (PCoA) of Bray–Curtis dissimilarity (PERMANOVA, R2 = 0.02, *p* = 0.356).

Fig S2



Enriched in	Butyrate-producers	Butyrate-consumers
ACPA(-)	12 (32.4%)	0 (0%)
ACPA(+)	1 (2.7%)	24 (64.9%)

Fig.S2 Distribution of butyrate-metabolizing species among 92 ACPA-associated species

In NORA cohort, we identified 92 ACPA-associated species, 37/92 (40.2%) were butyrate-metabolizing species. Among them, 64.9% (24/37) butyrate-consumers and only 2.7% (1/37) butyrate-producers were enriched in ACPA (+) group, whereas 32.4% (12/37) butyrate-producers were enriched in ACPA (-) group with no butyrate-consumers.

Fig. S3

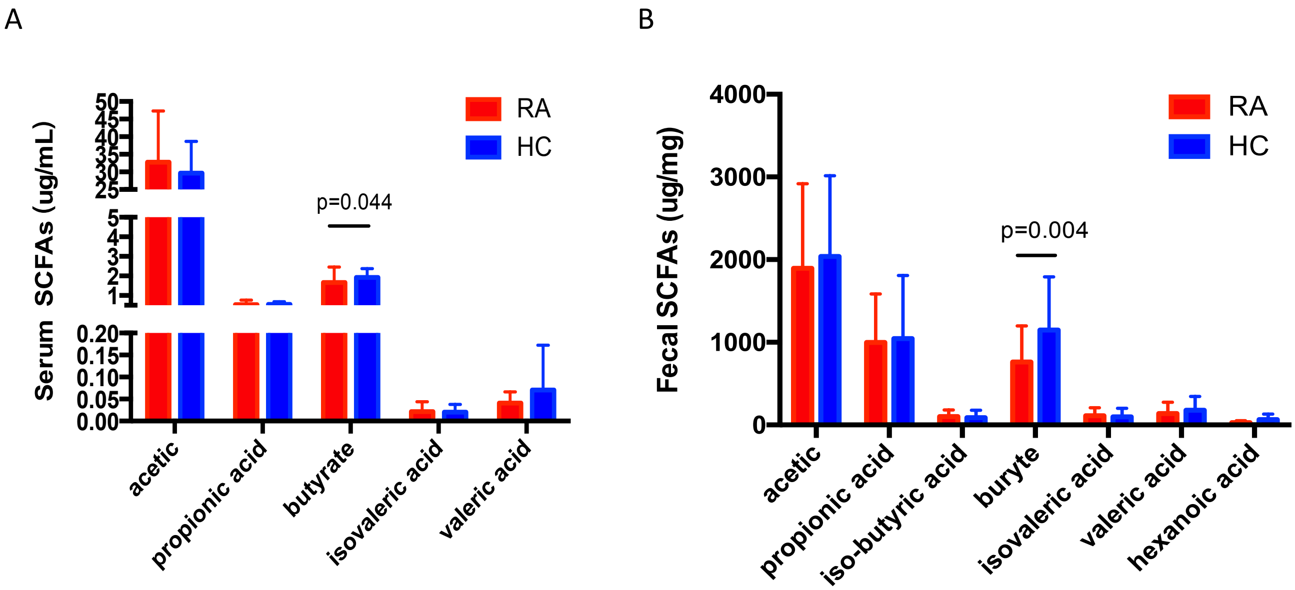


Fig. S3 Serum and fecal concentration of SCFA in subject of the treated cohort.

The levels of SCFAs in patients of cohort I were tested. **A.** Serum levels of SCFA in patients vs. controls. **B.** Fecal levels of SCFA in patients vs. controls.

Fig.S4.

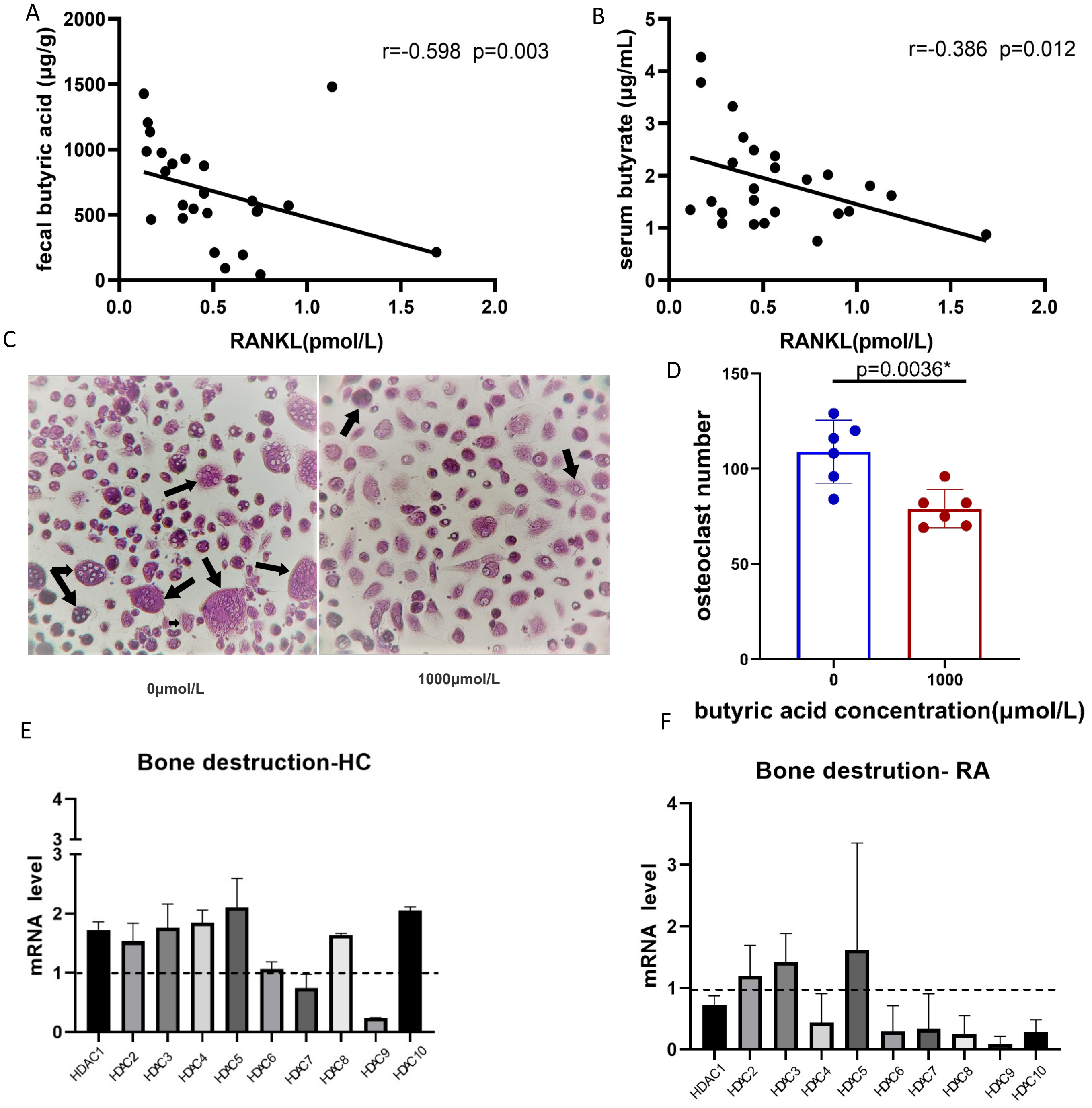


Fig. S4. Butyrate treatment suppressed osteoclast differentiation.

A. Correlation between fecal butyric acid and the level of serum RANKL in RA patients.

B. Correlation between serum butyric acid and the level of serum RANKL in RA patients.

C. Tartrate-resistant acid phosphatase (TRAP) staining of mature osteoclasts (OCs) obtained from peripheral blood mononuclear cells (PBMCs) of RA and cultured in the presence of either non-butyrate or butyrate at a concentration of 500 μmol/L (original magnification 200×). The graph represents the comparison of OC (TRAP-positive cells with ≥3 nuclei) numbers between those cultured with non-butyrate and with 500 μmol/L butyrate.

D. Tartrate-resistant acid phosphatase (TRAP) staining of mature osteoclasts (OCs) obtained from peripheral blood mononuclear cells (PBMCs) of RA and cultured in the presence of either no butyrate or butyrate at a concentration of 500 μmol/L.

E. The level of messenger RNA (mRNA) of each histone acetylase subtype in osteoclasts of HC patients.

F. The level of messenger RNA (mRNA) of each histone acetylase subtype in osteoclasts of RA patients.

Significance between groups tested by Mann-Whitney U test, *, $p < 0.05$. **, $p < 0.01$; ***, $p < 0.001$.

Fig. S5

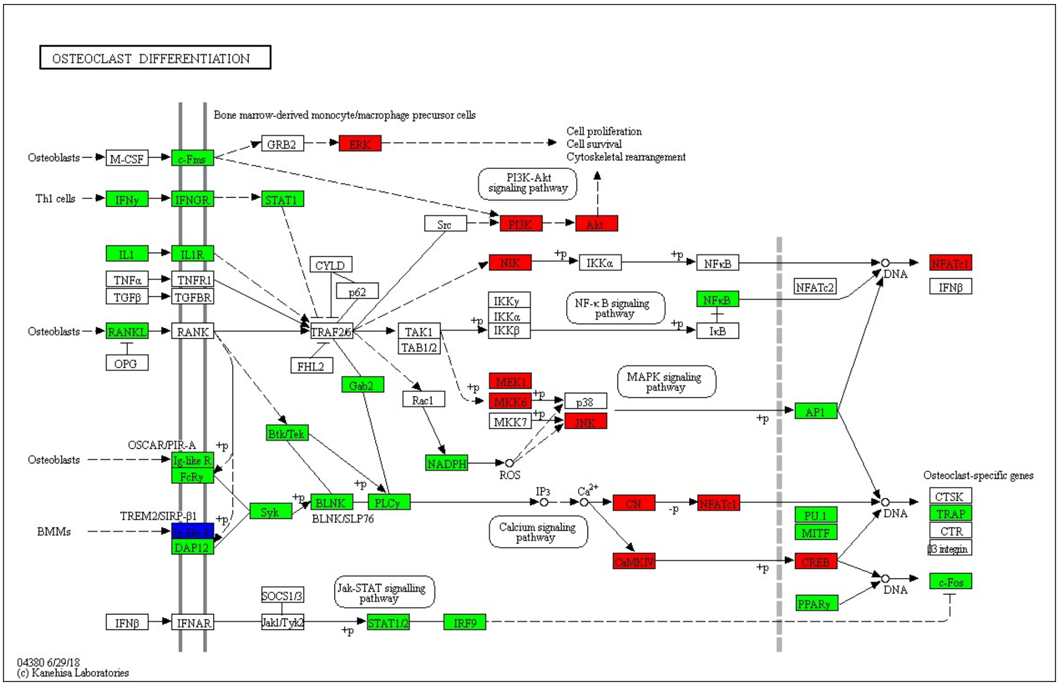


Fig. S5 Butyrate administration altered the KEGG pathway of osteoclast differentiation.

The altered genes within the pathway is shown. The coloring scheme is as follows: red are upper-regulated in osteoclast differentiation, green are down-regulated in osteoclast differentiation, blue are unidentified changed gene in osteoclast differentiation.

Fig. S6

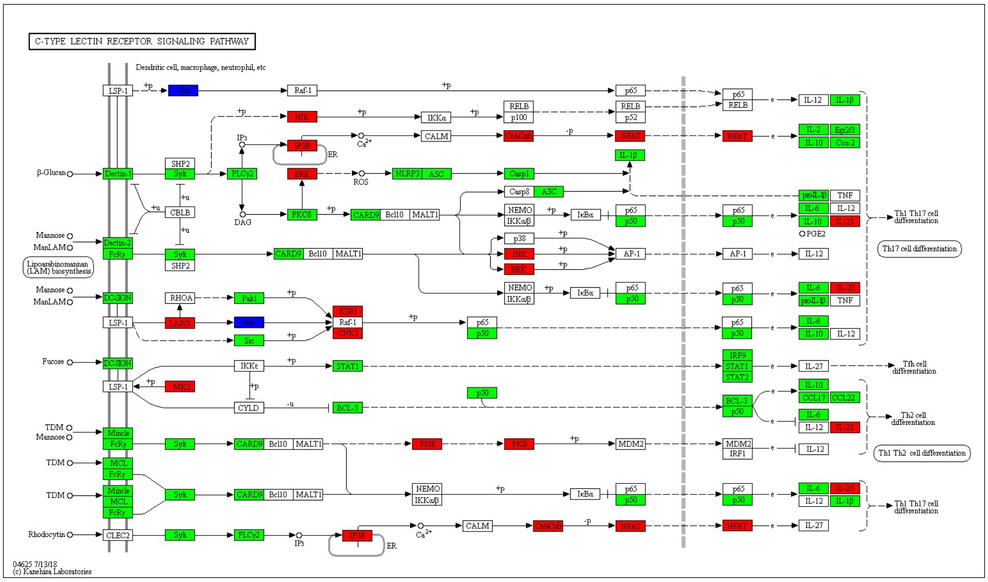


Fig. S6 Butyrate administration altered the differentiation of Tconv cells. The altered KEGG genes within the pathway is shown. The coloring scheme is as follows: red are upper-regulated in Tconv differentiation, green are down-regulated in Tconv differentiation.

Fig. S7

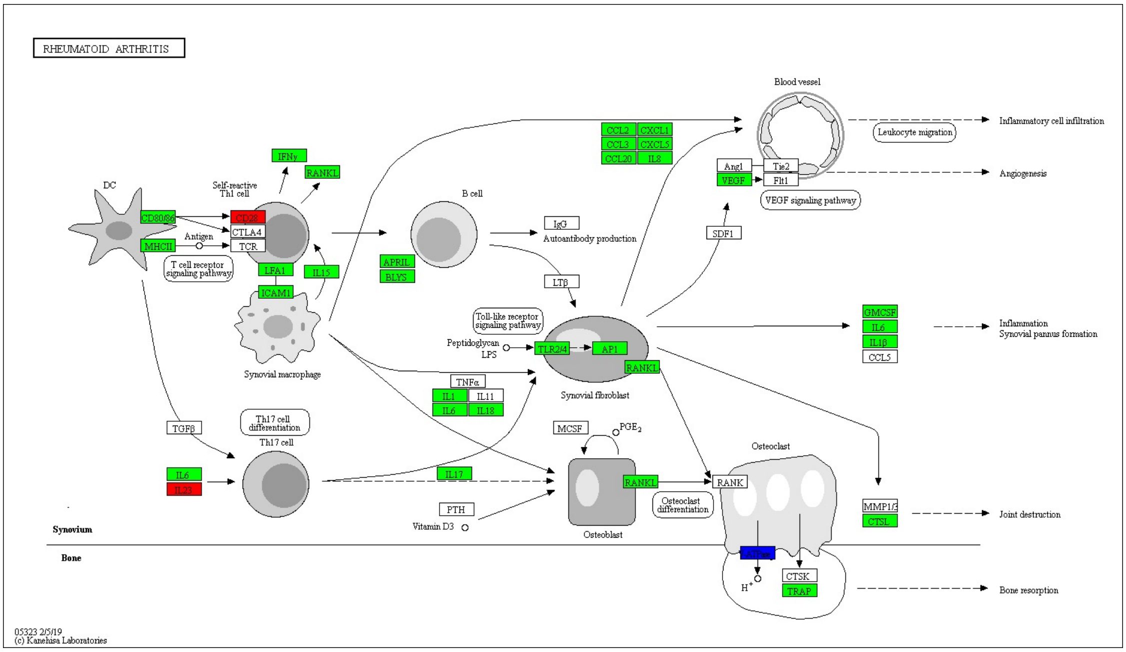


Fig. S7 Butyrate administration altered the KEGG RA pathway.
The altered genes within the pathway is shown. The coloring scheme is as follows: red are upper-regulated in RA, green are down-regulated in RA, blue are unidentified changed gene in RA.

Fig. S8

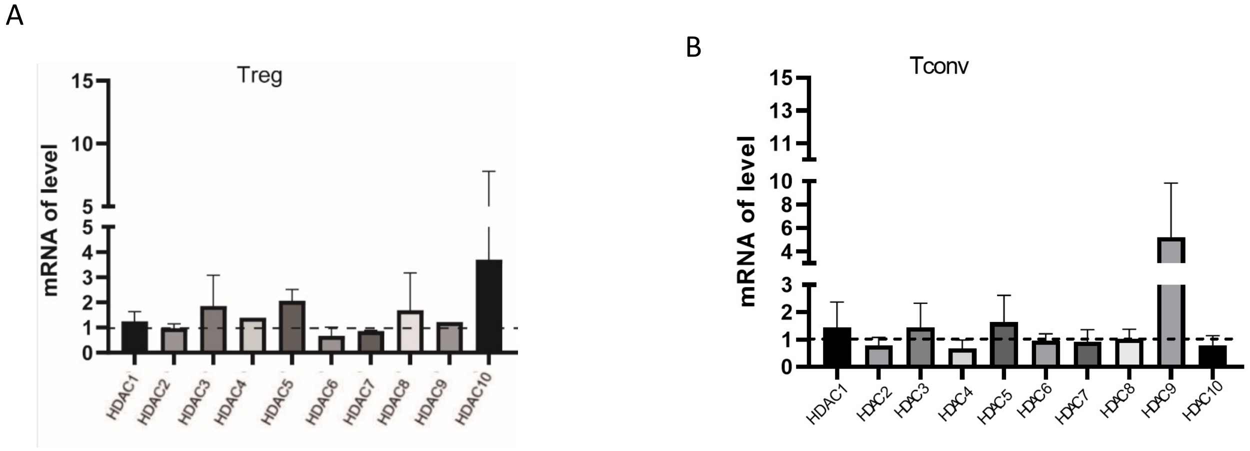


Fig. S8 Butyrate administration inhibited HDACs in Tregs and Tconv cells.

A. Expression of mRNA of each histone acetylase subtype measured by real-time PCR in Tregs.

B. Expression of mRNA of each histone acetylase subtype measured by real-time PCR in non-Tregs (Tconvs).

Fig. S9

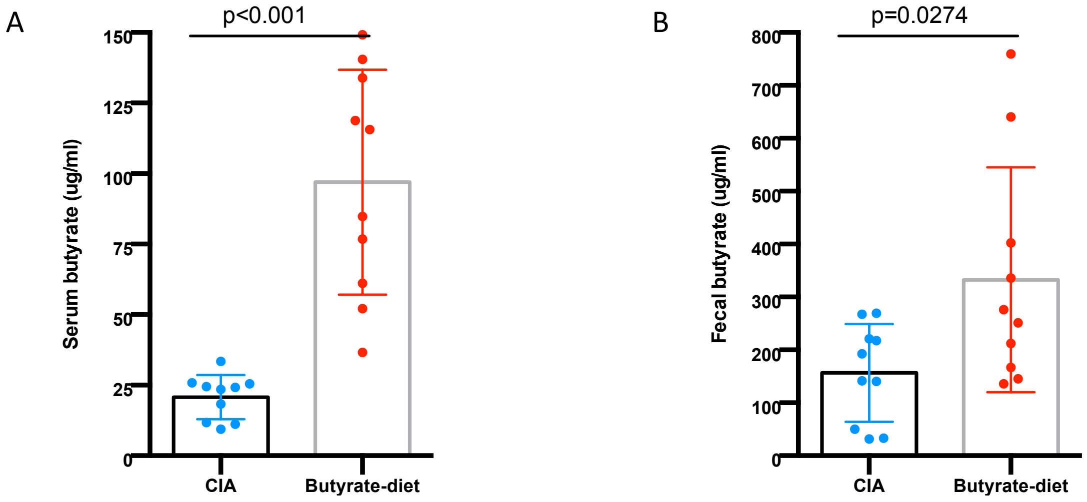


Fig. S9 Serum and fecal butyrate level after butyrate treatment in CIA mice.

A. Serum levels of butyrate in CIA and butyrate-diet mice. **B.** Fecal butyrate levels of butyrate in CIA and butyrate-diet mice.

Fig. S10

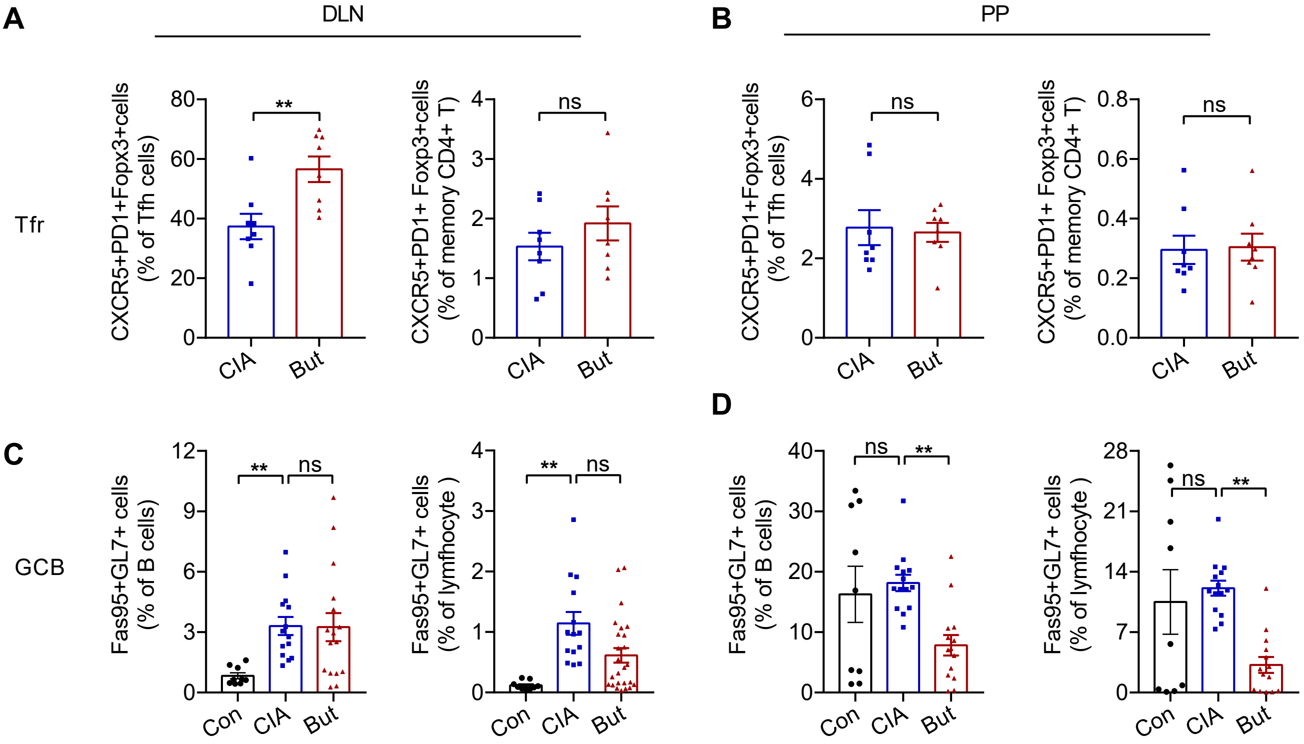


Fig. S10 Butyrate suppressesed immune responses in CIA mice.

Representative flow cytometry plots with graphs showing the frequency of CD4$^+$CD44$^+$CXCR5$^+$PD1$^+$FoxP3$^+$Tfr cells (**A** and **B**) and B220$^+$CD4$^-$Fas95$^+$GL7$^+$GCB cells (**C** and **D**) in draining lymph modes (DLN) and peyer's patches (PP) of the indicated groups.

Con, n=9; CIA, n=8-14; But, n=8-14. Data were pooled from two independent experiments and are here expressed as the mean ± SEM. Significance determined using Mann-Whitney U test (e-n). *P < 0.05, **P < 0.01.

Fig. S11

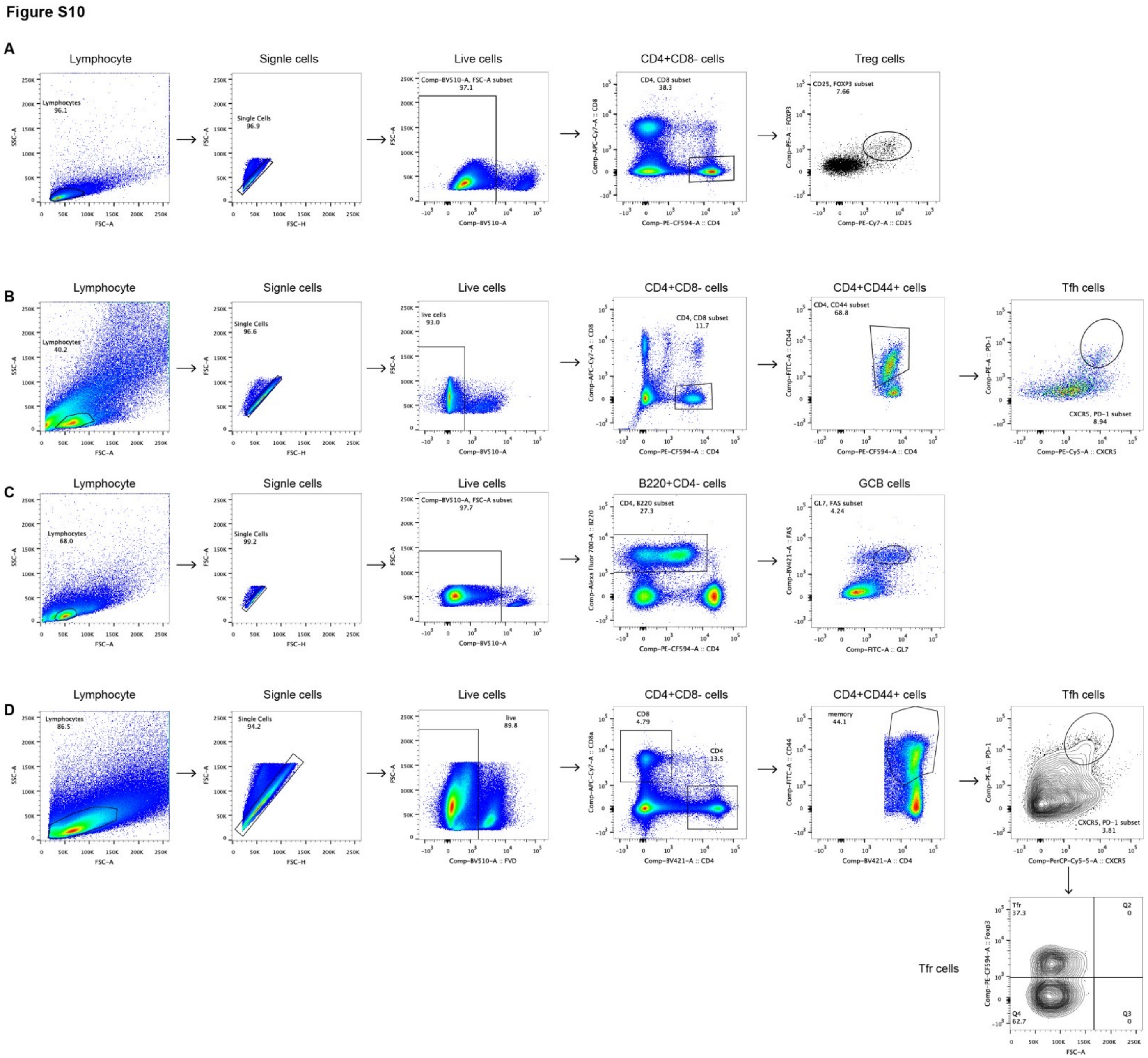


Fig. S11 Gating strategies of T cells subsets and GC B cells by flow cytometry.

Panel A, Tregs is identified as $CD4^+CD25^+Foxp3^+$ subset. **Panel B**, Tfh cells were determined as $CD4^+CD44^+CXCR5^+PD\text{-}1^+Bcl6^+$ cells. **Panel C**, Germinal center B was identified as $B220^+CD4^-CD95^+GL\text{-}7^+$. **Panel D**, Tfr cells were determined as $CD4^+CD44^+CXCR5^+PD\text{-}1^+Foxp3^+$.

Th, helper T cells; Tfh, follicular helper T cells; PD-1, programmed cell death 1; CXCR5, C-X-C chemokine receptor type 5.

Table S1. Demographic and clinical information of the naïve cohort

Characteristic	HC (n=29)	ACPA (+) (n=25)	*P* value	ACPA (+) (n=25)	ACPA (-) (n=15)	*P* ACPA（+） vs ACPA（-）
Age, year, median (range)	52 (25, 66)	48 (29-68)	0.5561	48 (29-68)	59 (25-79)	0.532
Female/Male	20/9	15/10	0.501	15/10	12/3	0.201
BMI, median (range)	22.5 (19.5,32.7)	23.7 (19.5, 32.1)	0.1285	23.7 (19.5, 32.1)	22.1 (18.6, 32.0)	0.199
Duration, Months, median (range)	N/A	5 (1-20)	N/A	5 (1-20)	7 (1-22)	0.532
Clinical parameters						
ESR, mm/h, median (range)	N/A	49 (15, 134)	N/A	49 (15, 134)	36 (7, 25)	0.016
CRP, mg/l, median (range)	N/A	36.2 (1.06, 200)	N/A	36.2 (1.06, 200)	5.88 (1.32, 67.5)	0.082
DAS28-ESR, median (range)	N/A	6.3 (3.7, 8.8)	N/A	6.3 (3.7, 8.8)	5.2 (1.9, 8.4)	0.001
DAS28-CRP, median (range)	N/A	5.6 (3.1, 8.3)	N/A	5.6 (3.1, 8.3)	4.7 (1.5, 7.8)	0.001
TJC-28, median (range)	N/A	11 (4, 39)	N/A	11 (4, 39)	11 (0, 44)	0.034
SJC-28, median (range)	N/A	8 (0, 25)	N/A	8 (0, 25)	6 (0, 24)	0.616
DJC-28, n (%)	N/A	4 (16)	N/A	4 (16)	2 (13.3)	0.852
Treatment	N/A	0	N/A	0	0	N/A

Table S2. RA-associated species identified from NORA cohort (Wilcoxon signed-rank test)

species	Pvalue	HCmean	meanRA	HCmedian	RAmedian	nrichGroup	Qvalue	butyrate-metabolizing
Citrobacter_braakii	6.71E-18	0.0000706	3.57E-06	0.0000181	0	HC	2.68116E-15	
Bifidobacterium_adolescentis	1.12E-12	0.0158298	0.000767	0.0011529	0.0001812	HC	7.45876E-11	
Lactobacillus_ruminis	3.35E-10	0.0001344	0.000021	0.0000473	0	HC	6.08446E-09	
Citrobacter_freundii	5.95E-10	0.0022316	0.0000756	0.0002123	0.0000232	HC	1.00895E-08	
Clostridium_argentinense	4.32E-09	6.43E-06	7.25E-07	0	0	HC	5.56829E-08	producing
Bifidobacterium_catenulatum	2.97E-08	0.0003639	0.0001552	0.0002182	0.0000561	HC	3.14771E-07	
Clostridium_beijerinckii	1.38E-07	6.12E-06	8.31E-07	0	0	HC	1.07585E-06	producing
Aggregatibacter_actinomycetemcomitans	4.36E-07	5.83E-06	5.5E-07	0	0	HC	2.71084E-06	
Bifidobacterium_kashiwanohense	4.97E-07	0.0016031	0.0006142	0.0007044	0.0001325	HC	3.00429E-06	
Desulfosporosinus_orientis	6.27E-07	5.26E-06	7.96E-07	0	0	HC	3.57835E-06	consuming
Myroides_odoratimimus	6.61E-07	0.0000179	3.12E-06	0	0	HC	3.71806E-06	
Staphylococcus_aureus	6.73E-07	9.32E-06	1.16E-06	0	0	HC	3.76641E-06	
Lactobacillus_agilis	8.02E-07	0.0000111	4.51E-06	0.0000118	0	HC	4.28452E-06	
Bacteroides_cellulosilyticus	1.08E-06	0.0275732	0.0162939	0.0190555	0.0116699	HC	5.39271E-06	
Clostridium._cellulosi	1.19E-06	0.0275714	0.0163143	0.0191012	0.0116815	HC	5.7938E-06	
Citrobacter_werkmanii	1.87E-06	0.0000329	9.98E-06	0.0000106	0	HC	8.31458E-06	
Campylobacter_jejuni	2.53E-06	0.0007703	0.000351	0.00047	0.0002273	HC	1.05813E-05	
Pseudomonas_viridiflava	2.69E-06	0.0000392	1.62E-06	0	0	HC	1.10909E-05	
Oceanisphaera_profunda	4.77E-06	0.0000528	2.97E-06	0	0	HC	1.80126E-05	
Brevibacillus_laterosporus	4.83E-06	8.55E-06	1.27E-06	0	0	HC	1.81984E-05	
Serratia_liquefaciens	0.0000064	9.26E-06	2.96E-06	0	0	HC	2.31159E-05	
Flammeovirgaceae_bacterium_311	0.0000106	0.0000279	5.83E-06	0	0	HC	3.46921E-05	
Thermodesulfovibrio_yellowstonii	0.0000133	0.0000084	0.0000037	0	0	HC	4.10693E-05	
Niastella_koreensis	0.0000139	0.0000514	4.48E-06	0	0	HC	4.23978E-05	
Pasteurellaceae_bacterium_NI1060	0.000015	2.01E-06	0	0	0	HC	4.52317E-05	
Pseudonocardia_sp.	0.0000161	0.0000116	3.73E-06	0	0	HC	4.80018E-05	consuming
Bifidobacterium_animalis	0.0000166	0.0001131	0.0000527	0.00006	0.0000267	HC	4.92404E-05	
Seonamhaeicola_sp.	0.0000185	0.0000111	1.96E-06	0	0	HC	5.38341E-05	consuming
Raoultella_ornithinolytica	0.0000258	0.0003608	0.0000347	0.0000307	0.0000187	HC	7.02253E-05	
Desulfurella_acetivorans	0.0000268	0.0000354	0.0000175	0.0000236	0.0000124	HC	7.23097E-05	
Aerococcus_urinae	0.0000303	0.0000121	2.99E-06	0	0	HC	8.0016E-05	

Rufibacter_sp.	0.0000372	4.79E-06	6.06E-07	0	0	HC	9.46767E-05	
Pasteurella_multocida	0.0000585	6.51E-06	1.92E-06	0	0	HC	0.000136733	
Pseudorhodoplanes_sinuspersici	0.0000613	7.24E-06	0.0000036	0	0	HC	0.000141756	
Sphingomonas_panacis	0.0000639	3.63E-06	8.01E-07	0	0	HC	0.000146326	consuming
Deinococcus_deserti	0.0000656	0.000016	5.41E-06	0	0	HC	0.000149266	consuming
endosymbiont_TC1_of_Trimyema_compress	0.0000825	0.0000134	5.33E-06	0	0	HC	0.000180137	
Pseudomonas_stutzeri	1.88E-17	0.0000834	0.0003963	0.0000439	0.0001953	RA	3.75602E-15	consuming
Dehalococcoides_mccartyi	2.87E-15	3.99E-06	0.0002621	0	0.0000139	RA	3.82261E-13	
Cutibacterium_acnes	1.46E-14	0.0001208	0.0004039	0.0001166	0.0002236	RA	1.45845E-12	
Desulfitobacterium_dehalogenans	1.1E-12	4.96E-06	0.0000192	0	0.0000141	RA	7.37001E-11	consuming
Pontibacter_akesuensis	1.87E-12	0.0000188	0.0000415	0.0000114	0.0000307	RA	1.06744E-10	
Vibrio_furnissii	5.18E-12	4.69E-07	8.91E-06	0	0	RA	2.35752E-10	
Methylobacterium_sp.	7.14E-12	6.02E-06	0.000023	0	0.000015	RA	2.90133E-10	consuming
Bordetella_sp.	7.45E-12	0.0000136	0.0000576	0.0000114	0.0000297	RA	2.97684E-10	consuming
Delftia_tsuruhatensis	1.38E-11	0	0.0000103	0	0	RA	5.01287E-10	consuming
Cupriavidus_necator	3.27E-11	0.0000088	0.0000316	0.00001	0.0000185	RA	1.03796E-09	consuming
Microlunatus_phosphovorus	3.76E-11	2.35E-06	0.0000308	0	0	RA	1.1557E-09	
Mesorhizobium_loti	6.69E-11	7.92E-07	0.0000102	0	0	RA	1.74705E-09	consuming
Sulfuricaulis_limicola	7.09E-11	1.18E-06	0.0000164	0	0	RA	1.81426E-09	
Thalassolituus_oleivorans	7.58E-11	8.85E-07	0.0000076	0	0	RA	1.89299E-09	consuming
Cupriavidus_taiwanensis	1.04E-10	2.62E-06	0.0000207	0	0	RA	2.4236E-09	consuming
Klebsiella_variicola	1.25E-10	2.24E-06	0.0000354	0	0	RA	2.77483E-09	
Bartonella_apis	1.74E-10	1.53E-06	0.000014	0	0	RA	3.53455E-09	
Pseudomonas_poae	1.98E-10	1.58E-06	0.0000232	0	0	RA	3.86146E-09	consuming
Clostridium_estertheticum	2.13E-10	0.0000065	0.0000281	0	0.0000142	RA	4.05284E-09	producing
Mycobacterium_kansasii	7.06E-10	5.16E-07	0.0000161	0	0	RA	1.16419E-08	consuming
Paenibacillus_naphthalenovorans	9.24E-10	3.41E-06	0.0000299	0	0	RA	1.44545E-08	consuming
Desulfotomaculum_gibsoniae	1E-09	1.13E-06	0.0000112	0	0	RA	1.53683E-08	
Gloeomargarita_lithophora	1.48E-09	2.19E-06	0.0000285	0	0	RA	2.19027E-08	
Streptomyces_davawensis	4E-09	2.11E-06	0.0000305	0	0	RA	5.23189E-08	
Thermincola_potens	4.12E-09	8.67E-06	0.0000331	0	0.0000114	RA	5.35919E-08	
Erwinia_gerundensis	4.24E-09	0	7.53E-06	0	0	RA	5.48511E-08	
Bifidobacterium_dentium	6.59E-09	0.0000687	0.000173	0.0000518	0.0001348	RA	8.07952E-08	
Peptoniphilus_sp.	8.16E-09	5.72E-07	0.0000255	0	0	RA	9.67761E-08	producing

Nocardioides_sp.	8.6E-09	6.52E-06	0.000027	0	0.000011	RA	1.01069E-07	
Stenotrophomonas_maltophilia	1.6E-08	0.0003297	0.0010163	0.0002111	0.0004046	RA	1.8112E-07	
Limnohabitans_sp.	3.04E-08	1.74E-06	0.0000199	0	0	RA	3.21144E-07	consuming
Paenibacillus_stellifer	3.14E-08	0.0000234	0.0000534	0.0000191	0.0000275	RA	3.30176E-07	
Lactococcus_raffinolactis	4.74E-08	7.87E-06	0.0000263	0	0.0000131	RA	4.73624E-07	
Actinomyces_gaoshouyii	7.02E-08	8.98E-07	5.33E-06	0	0	RA	6.5501E-07	
Pectobacterium_carotovorum	7.4E-08	7.65E-06	0.0000173	0	0.0000169	RA	6.82932E-07	
Actinomyces_oris	8.27E-08	0.0000502	0.0000951	0.0000319	0.0000815	RA	7.44619E-07	
Cronobacter_sakazakii	8.47E-08	7.36E-06	0.0001553	0	0.0000144	RA	7.58377E-07	
Geobacter_daltonii	8.6E-08	6.18E-06	0.0000251	0	0.0000117	RA	7.67238E-07	consuming
Lactobacillus_mucosae	9.28E-08	0.0000178	0.0002593	0	0	RA	8.12565E-07	
Arcanobacterium_phocae	9.9E-08	3.18E-07	3.31E-06	0	0	RA	8.52453E-07	
Mesorhizobium_ciceri	1.2E-07	3.87E-06	0.0000104	0	0	RA	9.78235E-07	
Curvibacter_sp.	1.35E-07	4.63E-06	0.0000173	0	0	RA	1.06018E-06	
Candidatus_Desulfovibrio_trichonymphae	1.42E-07	7.13E-06	0.0000229	0	0.0000107	RA	1.09639E-06	
Bifidobacterium_breve	1.43E-07	0.0003725	0.0006528	0.000302	0.0005477	RA	1.10147E-06	
Bordetella_bronchialis	1.44E-07	4.31E-06	0.0000117	0	0	RA	1.10652E-06	consuming
Nocardiopsis_alba	1.66E-07	2.91E-06	0.0000136	0	0	RA	1.24189E-06	
Actinomyces_hongkongensis	1.88E-07	0.0000274	0.000063	0.0000226	0.0000472	RA	1.3703E-06	
Pseudarthrobacter_equi	2.06E-07	8.54E-06	0.0000274	0.0000106	0.0000121	RA	1.47054E-06	
Candidatus_Desulforudis_audaxviator	2.08E-07	1.05E-06	5.98E-06	0	0	RA	1.48143E-06	
Paenibacillus_sabinae	2.22E-07	4.81E-06	0.0000183	0	0.0000107	RA	1.55624E-06	
Cyanothece_sp.	2.44E-07	6.26E-06	0.0000166	0	0.0000109	RA	1.67727E-06	
Neisseria_zoodegmatis	2.61E-07	5.17E-06	0.0000144	0	0.0000127	RA	1.76762E-06	
Gordonibacter_urolithinfaciens	3.85E-07	0.0001416	0.0002818	0.0001119	0.0002003	RA	2.45231E-06	
Pantoea_sp.	4.27E-07	9.28E-06	0.0000221	0	0.0000144	RA	2.66612E-06	
Pseudomonas_extremaustralis	4.61E-07	0.0000023	7.81E-06	0	0	RA	2.83311E-06	
Deinococcus_puniceus	6.1E-07	2.56E-06	0.0000123	0	0	RA	3.50696E-06	consuming
Pseudomonas_cremoricolorata	6.34E-07	1.13E-06	6.36E-06	0	0	RA	3.60745E-06	consuming
Halobiforma_lacisalsi	6.44E-07	0	6.91E-06	0	0	RA	3.64871E-06	
Azospirillum_brasilense	6.9E-07	0.000044	0.000089	0.0000463	0.0000704	RA	3.83408E-06	consuming
Sphingobium_sp.	6.93E-07	0.0000279	0.0000574	0.0000161	0.0000377	RA	3.84592E-06	consuming
Pseudomonas_azotoformans	7.44E-07	4.95E-06	0.0000211	0	0	RA	4.0553E-06	consuming
Pseudomonas_fluorescens	8.1E-07	0.0001325	0.000208	0.000082	0.0001985	RA	4.31542E-06	consuming

Stenotrophomonas_rhizophila	8.98E-07	9.22E-06	0.0000247	0	0.0000139	RA	4.68205E-06	
Mogibacterium_pumilum	1.01E-06	0.0000199	0.0000415	0.0000132	0.0000309	RA	5.12662E-06	
Moraxella_osloensis	1.13E-06	0.0000244	0.0000504	0.0000155	0.0000266	RA	5.57754E-06	consuming
Devriesea_agamarum	1.14E-06	1.32E-06	0.0000113	0	0	RA	5.61399E-06	
Verrucomicrobia_bacterium	1.19E-06	0.0000116	0.0000228	0	0.0000131	RA	5.7938E-06	
Sphingomonas_sp.	1.24E-06	0.0000256	0.0000405	0.0000157	0.0000342	RA	5.96957E-06	consuming
Pseudopropionibacterium_propionicum	1.36E-06	0.0000386	0.000075	0.0000281	0.0000344	RA	6.39322E-06	
Actinoplanes_derwentensis	1.38E-06	5.64E-06	0.0000218	0	0	RA	6.4619E-06	producing
Thermus_sp.	1.42E-06	5.74E-06	0.0000155	0	0.0000111	RA	6.59765E-06	consuming
Zymomonas_mobilis	1.54E-06	3.63E-06	0.0000151	0	0	RA	7.07042E-06	
Zobellia_galactanivorans	1.97E-06	0.0000107	0.000021	0	0.0000206	RA	8.67624E-06	
Phaeobacter_piscinae	2.26E-06	1.96E-06	7.67E-06	0	0	RA	9.68735E-06	consuming
Acidovorax_ebreus	2.31E-06	5.33E-07	4.02E-06	0	0	RA	9.85624E-06	
Streptomyces_sp.	2.34E-06	0.0000533	0.0001008	0.0000361	0.0000572	RA	9.95683E-06	
Methylobacterium_phyllosphaerae	2.36E-06	0	5.38E-06	0	0	RA	1.00236E-05	
Paenibacillus_donghaensis	2.56E-06	0.0000185	0.0000421	0.0000146	0.0000187	RA	1.0678E-05	
Pseudomonas_sp.	0.0000027	0.0001024	0.0001531	0.0000914	0.0001296	RA	1.11222E-05	consuming
Aeribacillus_pallidus	3.07E-06	0	4.47E-06	0	0	RA	1.24097E-05	
Candidatus_Aquiluna	3.29E-06	3.41E-06	8.92E-06	0	0	RA	1.31526E-05	
Geoalkalibacter_subterraneus	0.0000033	0.0000497	0.0000881	0.0000381	0.000057	RA	1.3186E-05	
Chryseobacterium_indologenes	0.0000039	8.57E-06	0.0000153	0.000011	0.0000147	RA	1.52223E-05	
Stenotrophomonas_sp.	4.11E-06	0.0000305	0.0000804	0.0000255	0.0000312	RA	1.59128E-05	
Pseudomonas_psychrophila	4.21E-06	0.0000122	0.0000223	0	0.0000181	RA	1.62378E-05	
Meiothermus_ruber	0.0000045	9.75E-06	0.0000395	0	0	RA	1.71663E-05	consuming
Nitrospira_defluvii	5.07E-06	0.0000066	0.0000275	0	0	RA	1.89332E-05	
Deinococcus_soli_Cha_et_al._2016	7.82E-06	6.44E-06	0.0000159	0	0	RA	2.7289E-05	
Janthinobacterium_svalbardensis	0.000008	7.23E-06	0.0000137	0	0.0000144	RA	2.77979E-05	
Mannheimia_haemolytica	8.08E-06	0	2.04E-06	0	0	RA	2.80227E-05	
Adlercreutzia_equolifaciens	8.39E-06	0.0020032	0.0038359	0.0004119	0.0015642	RA	2.88858E-05	
Vibrio_vulnificus	8.83E-06	4.24E-06	0.000015	0	0	RA	3.00894E-05	
Gemmata_sp.	9.09E-06	3.22E-06	8.51E-06	0	0	RA	3.07891E-05	
Atopobium_parvulum	9.45E-06	0.0000228	0.0000535	0.0000177	0.0000243	RA	3.17442E-05	
Microbacterium_chocolatum	9.56E-06	1.27E-06	6.26E-06	0	0	RA	3.20328E-05	
Pseudomonas_brenneri	9.74E-06	0.0000191	0.0000323	0.0000115	0.0000321	RA	3.25021E-05	

Corynebacterium_variabile	9.95E-06	8.25E-06	0.0000157	0	0.0000126	RA	3.30447E-05	consuming
Rhodoferax_sp.	0.0000101	8.21E-06	0.0000138	0	0.0000118	RA	3.34292E-05	consuming
Rhodococcus_hoagii	0.0000106	6.08E-06	0.0000145	0	0	RA	3.46921E-05	
Pseudomonas_chlororaphis	0.0000109	2.21E-06	7.73E-06	0	0	RA	3.54363E-05	consuming
Pseudomonas_antarctica	0.0000114	4.63E-06	9.05E-06	0	0.0000118	RA	3.6655E-05	consuming
Kitasatospora_aureofaciens	0.0000114	9.03E-06	0.0000208	0	0.0000133	RA	3.6655E-05	consuming
Denitrovibrio_acetiphilus	0.0000122	3.02E-06	0.0000129	0	0	RA	3.85501E-05	
Terriglobus_roseus	0.0000123	0.0000247	0.0000517	0.0000156	0.0000179	RA	3.87824E-05	
Pseudomonas_trivialis	0.0000124	1.85E-06	6.23E-06	0	0	RA	3.90137E-05	consuming
Spirosoma_aerolatum	0.0000131	4.22E-06	0.000011	0	0	RA	4.06191E-05	
Burkholderia_multivorans	0.0000139	4.92E-06	0.0000173	0	0	RA	4.23978E-05	consuming
Halomonas_sp.	0.000018	9.93E-06	0.0000308	0	0.0000107	RA	5.26422E-05	
Halomonas_aestuarii	0.000019	0.0000657	0.0001165	0.0000487	0.0000722	RA	5.50141E-05	
Stenotrophomonas_acidaminiphila	0.000019	1.75E-06	0.0000054	0	0	RA	5.50141E-05	
Fibrella_aestuarina	0.0000201	0.0000242	0.0000558	0.0000244	0.0000337	RA	5.76049E-05	
Corynebacterium_aurimucosum	0.0000232	0.0000199	0.0000494	0.0000163	0.0000152	RA	6.46296E-05	
Chloroherpeton_thalassium	0.0000242	5.45E-06	0.0000195	0	0	RA	6.68126E-05	
Magnetospirillum_gryphiswaldense	0.0000248	0.0000137	0.00003	0	0.000019	RA	6.81037E-05	consuming
Thauera_sp.	0.0000252	0.0000238	0.0000557	0.0000183	0.0000335	RA	6.89568E-05	
Alcaligenes_faecalis	0.0000253	0.0000157	0.0000243	0.0000117	0.0000247	RA	6.91692E-05	consuming
Actinomyces_meyeri	0.0000259	0.0000293	0.0000698	0.0000172	0.0000222	RA	7.04354E-05	
Rhodoferax_antarcticus	0.0000261	0.0000223	0.0000474	0.0000143	0.0000268	RA	7.08544E-05	consuming
Paenibacillaceae_bacterium_GAS479	0.0000275	8.62E-06	0.0000224	0	0.0000127	RA	7.37473E-05	
Cupriavidus_pinatubonensis	0.0000307	2.13E-06	5.71E-06	0	0	RA	8.08959E-05	consuming
Deinococcus_proteolyticus	0.0000321	0.0000246	0.0000515	0.0000224	0.0000269	RA	8.39458E-05	consuming
Rubrivivax_gelatinosus	0.0000332	9.67E-06	0.000025	0.0000101	0.0000156	RA	8.631E-05	consuming
Vitreoscilla_filiformis	0.0000337	9.34E-07	4.79E-06	0	0	RA	8.73754E-05	
Agarilytica_rhodophyticola	0.0000368	1.55E-06	7.06E-06	0	0	RA	9.38558E-05	
Verrucomicrobium_spinosum	0.0000371	0.0000219	0.000038	0.0000104	0.0000195	RA	9.44718E-05	
Cryptobacterium_curtum	0.0000423	4.37E-07	3.63E-06	0	0	RA	0.000105413	
Bacillus_thuringiensis	0.0000426	7.55E-07	2.61E-06	0	0	RA	0.000106031	
Streptomyces_puniciscabiei	0.0000438	9.66E-07	6.38E-06	0	0	RA	0.000108486	
Leuconostoc_citreum	0.0000448	1.98E-06	0.0000646	0	0	RA	0.000110514	
Pseudomonas_putida	0.000045	0.0000305	0.0000476	0.0000226	0.0000375	RA	0.000110918	consuming

Achromobacter_xylosoxidans	0.0000478	0.0004217	0.0008517	0.0002662	0.000434	RA	0.000116501	consuming
Bordetella_genomosp._13	0.000057	5.09E-06	0.0000108	0	0	RA	0.000133997	
Cronobacter_turicensis	0.00006	9.88E-06	0.0000279	0	0	RA	0.000139437	
Brevundimonas_naejangsanensis	0.0000614	6.96E-06	0.0000154	0	0.0000106	RA	0.000141934	consuming
Paenibacillus_graminis	0.0000618	3.68E-07	2.27E-06	0	0	RA	0.000142642	
Lactococcus_piscium	0.0000629	0.0000414	0.0000978	0.0000239	0.0000393	RA	0.000144579	
Pandoraea_pulmonicola	0.0000633	2.71E-06	0.0000081	0	0	RA	0.00014528	consuming
Bradyrhizobium_sp.	0.0000635	0.000021	0.0000481	0	0.0000223	RA	0.000145629	
Brochothrix_thermosphacta	0.0000638	4.29E-06	0.0000103	0	0	RA	0.000146152	
Desulfovibrio_desulfuricans	0.0000662	0.0000195	0.0000427	0.0000124	0.0000224	RA	0.000150295	
Propionimicrobium_sp.	0.0000698	0.0000292	0.0000626	0.0000184	0.0000268	RA	0.000156802	
Streptococcus_anginosus	0.0000719	0.0013065	0.0022607	0.0011089	0.0013505	RA	0.000160535	
Variovorax_paradoxus	0.0000729	0.0000161	0.0000329	0.0000116	0.0000147	RA	0.000162297	consuming
Parascardovia_denticolens	0.0000739	0.0000105	0.0000283	0	0.0000134	RA	0.000164048	
Pseudomonas_synxantha	0.0000801	0.0000111	0.0000179	0	0.0000139	RA	0.0001757	consuming
Brevibacterium_sandarakinum	0.0000815	0.0000078	0.0000258	0	0	RA	0.000178293	
Serratia_sp.	0.0000864	0.0000555	0.0001204	0.0000357	0.0000491	RA	0.000187454	
Desulfobacterium_autotrophicum	0.0000892	2.17E-06	5.32E-06	0	0	RA	0.00019265	
Desulfococcus_oleovorans	0.0000925	0.0000122	0.0000303	0.0000114	0.0000126	RA	0.000198714	

Table S3. List of butyrate producing and consuming species in this study

Butyrate-producing species	Butyrate-consuming species
Acetobacterium_woodii	Achromobacter_denitrificans
Acetoanaerobium_sticklandii	Achromobacter_insolitus
Acidaminococcus_fermentans	Achromobacter_sp.
Acidaminococcus_intestini	Achromobacter_xylosoxidans
Actinoplanes_derwentensis	Alcaligenes_faecalis
Actinoplanes_missouriensis	Alicycliphilus_denitrificans
Actinoplanes_sp.	Bordetella_bronchialis
Alistipes_finegoldii	Bordetella_hinzii
Alistipes_shahii	Bordetella_holmesii
Alkaliphilus_metalliredigens	Bordetella_petrii
Alkaliphilus_oremlandii	Bordetella_pseudohinzii
Anaerococcus_mediterraneensis	Bordetella_sp.
Anaerococcus_prevotii	Brevundimonas_diminuta
Anaeromyxobacter_dehalogenans	Brevundimonas_naejangsanensis
Anaeromyxobacter_sp.	Brevundimonas_sp.
Anaerostipes_hadrus	Brevundimonas_subvibrioides
Anaerotignum_propionicum	Brevundimonas_vesicularis
Blautia_hansenii	Burkholderia_cenocepacia
Blautia_sp.	Burkholderia_gladioli
Brachyspira_pilosicoli	Burkholderia_lata
Butyrivibrio_hungatei	Burkholderia_metallica
Butyrivibrio_proteoclasticus	Burkholderia_multivorans
Clostridium_acetobutylicum	Burkholderia_oklahomensis
Clostridium_argentinense	Burkholderia_sp.
Clostridium_baratii	Castellaniella_defragrans
Clostridium_beijerinckii	Comamonas_kerstersii
Clostridium_bornimense	Comamonas_serinivorans
Clostridium_botulinum	Comamonas_testosteroni
Clostridium_butyricum	Cupriavidus_gilardii
Clostridium_carboxidivorans	Cupriavidus_metallidurans

Clostridium_cellulosi
Clostridium_cellulovorans
Clostridium_chauvoei
Clostridium_cochlearium
Clostridium_estertheticum
Clostridium_formicaceticum
Clostridium_kluyveri
Clostridium_pasteurianum
Clostridium_perfringens
Clostridium_saccharobutylicum
Clostridium_saccharoperbutylacetonicum
Clostridium_sp.
Clostridium_taeniosporum
Clostridium_tetani
Clostridium_tyrobutyricum
Eubacterium_limosum
Faecalibaculum_rodentium
Filifactor_alocis
Flavonifractor_plautii
Fusobacterium_nucleatum
Fusobacterium_varium
Megasphaera_elsdenii
Odoribacter_splanchnicus
Oscillibacter_valericigenes
Peptoniphilus_sp.
Porphyromonas_asaccharolytica
Porphyromonas_gingivalis
Roseburia_hominis
Ruminococcus_albus
Ruminococcus_bicirculans
Ruminococcus_champanellensis
Salinispora_tropica
Thermoanaerobacterium_thermosacchar
Thermotoga_profunda
Cupriavidus_necator
Cupriavidus_pinatubonensis
Cupriavidus_taiwanensis
Delftia_acidovorans
Delftia_sp.
Delftia_tsuruhatensis
Flavobacterium_columnare
Hydrogenophaga_sp.
Limnohabitans_sp.
Paraburkholderia_caribensis
Paraburkholderia_phymatum
Paraburkholderia_sp.
Paraburkholderia_sprentiae
Paraburkholderia_xenovorans
Polaribacter_sp.
Pseudomonas_aeruginosa
Pseudomonas_alcaligenes
Pseudomonas_antarctica
Pseudomonas_azotoformans
Pseudomonas_brassicacearum
Pseudomonas_chlororaphis
Pseudomonas_cichorii
Pseudomonas_citronellolis
Pseudomonas_cremoricolorata
Pseudomonas_fluorescens
Pseudomonas_fragi
Pseudomonas_knackmussii
Pseudomonas_koreensis
Pseudomonas_mendocina
Pseudomonas_parafulva
Pseudomonas_poae
Pseudomonas_psychrotolerans
Pseudomonas_putida
Pseudomonas_resinovorans

Intestinimonas_butyriciproducens
Clostridioides_difficile
Ruminococcaceae_bacterium_CPB6
Flavobacteriaceae_bacterium_UJ101
Peptoclostridium_acidaminophilum
Clostridium_novyi

Pseudomonas_sp.
Pseudomonas_stutzeri
Pseudomonas_synxantha
Pseudomonas_syringae
Pseudomonas_trivialis
Pseudomonas_veronii
Pseudomonas_versuta
Ralstonia_insidiosa
Ralstonia_mannitolilytica
Ralstonia_pickettii
Ralstonia_solanacearum
Ramlibacter_tataouinensis
Rhodoferax_antarcticus
Rhodoferax_ferrireducens
Rhodoferax_saidenbachensis
Rhodoferax_sp.
Roseomonas_gilardii
Sphingomonas_panacis
Sphingomonas_sp.
Sphingomonas_taxi
Tenacibaculum_dicentrarchi
Tenacibaculum_jejuense
Variovorax_boronicumulans
Variovorax_paradoxus
Variovorax_sp.
Deinococcus_puniceus
Desulfosporosinus_meridiei
Gramella_flava
Pseudomonas_pseudoalcaligenes
Sphingobium_sp.
Acidovorax_sp.
Acinetobacter_sp.
Altererythrobacter_epoxidivorans
Amycolatopsis_mediterranei

Aquaspirillum_sp.
Archangium_gephyra
Aromatoleum_aromaticum
Arthrobacter_alpinus
Azoarcus_sp.
Azospirillum_brasilense
Azospirillum_lipoferum
Bacillus_sp.
Bradyrhizobium_oligotrophicum
Brevibacterium_linens
Candidatus_Promineofilum_breve
Catenulispora_acidiphila
Celeribacter_indicus
Chloracidobacterium_thermophilum
Chondromyces_crocatus
Collimonas_pratensis
Corallococcus_coralloides
Corynebacterium_sphenisci
Corynebacterium_terpenotabidum
Corynebacterium_variabile
Croceibacter_atlanticus
Deinococcus_deserti
Deinococcus_geothermalis
Deinococcus_proteolyticus
Deinococcus_swuensis
Desulfarculus_baarsii
Desulfitobacterium_dehalogenans
Desulfitobacterium_dichloroeliminans
Desulfitobacterium_hafniense
Desulfobulbus_propionicus
Desulfococcus_multivorans
Desulfomonile_tiedjei
Desulfosporosinus_acidiphilus
Desulfosporosinus_orientis

Desulfurispirillum_indicum
Desulfuromonas_soudanensis
Dokdonia_donghaensis
Erythrobacter_flavus
Geobacter_daltonii
Geobacter_sp.
Gordonia_sp.
Heliobacterium_modesticaldum
Hyphomicrobium_denitrificans
Ilumatobacter_coccineus
Janthinobacterium_sp.
Kitasatospora_aureofaciens
Kocuria_palustris
Legionella_pneumophila
Magnetospirillum_gryphiswaldense
Marinobacter_salarius
Meiothermus_ruber
Mesorhizobium_loti
Methylobacterium_sp.
Microbacterium_aurum
Microbacterium_sp.
Moraxella_osloensis
Mycobacterium_kansasii
Myxococcus_fulvus
Nitrospirillum_amazonense
Nonomuraea_sp.
Novosphingobium_aromaticivorans
Paenibacillus_naphthalenovorans
Pandoraea_norimbergensis
Pandoraea_pulmonicola
Paracoccus_denitrificans
Pelosinus_fermentans
Phaeobacter_piscinae
Polaromonas_sp.

Pseudonocardia_sp.
Rhizobium_sp.
Rhodopseudomonas_palustris
Rhodothermus_marinus
Rubrivivax_gelatinosus
Rubrobacter_xylanophilus
Seonamhaeicola_sp.
Sinorhizobium_fredii
Sphingopyxis_macrogoltabida
Stackebrandtia_nassauensis
Streptomyces_lincolnensis
Streptosporangium_roseum
Sulfitobacter_pseudonitzschiae
Thalassolituus_oleivorans
Thermaerobacter_marianensis
Thermus_sp.
Thiomonas_intermedia
Treponema_sp.
Vulgatibacter_incomptus

Table S4. RA-associated species identified from the NORA cohort (K-S test)

Differential_Species	KS_Pvalue	Enrich_Group	Butyrate-metabolizing
Aerococcus_urinae	8.52651E-05	HC	
Citrobacter_braakii	2.93729E-10	HC	
Citrobacter_werkmanii	5.12303E-05	HC	
Clostridium_argentinense	3.03962E-05	HC	producing
Clostridium_saccharobutylicum	3.03962E-05	HC	producing
Desulfovibrio_magneticus	8.52651E-05	HC	
Ensifer_adhaerens	5.53236E-07	HC	
Gluconobacter_oxydans	8.52651E-05	HC	
Hafnia_alvei	1.01826E-06	HC	
Lactobacillus_agilis	1.01826E-06	HC	
Lactobacillus_reuteri	3.32172E-06	HC	
Lactobacillus_ruminis	2.8844E-11	HC	
Melioribacter_roseus	5.53236E-07	HC	
Micromonospora_coxensis	5.53236E-07	HC	
Myroides_odoratimimus	1.57263E-07	HC	
Opitutaceae_bacterium_TAV5	3.03962E-05	HC	
Pasteurella_multocida	5.88737E-06	HC	
Pseudonocardia_sp.	5.53236E-07	HC	consuming
Saccharomonospora_viridis	1.03043E-05	HC	
Serratia_liquefaciens	1.78094E-05	HC	
uncultured_crAssphage	5.12303E-05	HC	
Bordetella_bronchialis	5.12303E-05	RA	consuming
Brevundimonas_naejangsanensis	1.78094E-05	RA	consuming
Burkholderia_multivorans	5.12303E-05	RA	consuming
Cronobacter_sakazakii	1.29587E-09	RA	
Cupriavidus_taiwanensis	5.88737E-06	RA	consuming
Dehalococcoides_mccartyi	1.09272E-08	RA	
Deinococcus_proteolyticus	4.25103E-08	RA	consuming
Delftia_tsuruhatensis	2.93729E-10	RA	consuming
Erwinia_gerundensis	5.88737E-06	RA	

Kitasatospora_aureofaciens	1.29587E-09	RA	consuming
Klebsiella_aerogenes	3.32172E-06	RA	
Klebsiella_variicola	8.52651E-05	RA	
Lactobacillus_mucosae	1.85072E-06	RA	
Mesorhizobium_loti	1.78094E-05	RA	consuming
Methylobacterium_populi	5.12303E-05	RA	
Methylobacterium_sp.	2.96825E-07	RA	consuming
Mycobacterium_kansasii	1.03043E-05	RA	consuming
Paenibacillus_naphthalenovorans	8.52651E-05	RA	consuming
Pantoea_sp.	5.88737E-06	RA	
Pantoea_vagans	1.03043E-05	RA	
Pectobacterium_carotovorum	6.33116E-11	RA	
Polaribacter_sp.	5.12303E-05	RA	consuming
Pontibacter_akesuensis	1.09272E-08	RA	
Pseudomonas_antarctica	2.67099E-09	RA	consuming
Pseudomonas_azotoformans	1.03043E-05	RA	consuming
Pseudomonas_cremoricolorata	8.52651E-05	RA	consuming
Pseudomonas_poae	1.85072E-06	RA	consuming
Pseudomonas_psychrophila	1.85072E-06	RA	
Pseudomonas_synxantha	8.52651E-05	RA	consuming
Spirosoma_aerolatum	8.22796E-08	RA	
Streptococcus_cristatus	3.03962E-05	RA	
Streptococcus_pneumoniae	3.32172E-06	RA	
Sulfuricaulis_limicola	1.01826E-06	RA	
Thalassolituus_oleivorans	2.96825E-07	RA	consuming
Thermus_sp.	3.03962E-05	RA	consuming
Vibrio_furnissii	6.20848E-10	RA	

Table S5 ACPA-associated species identified from NOAR cohort (Wilcoxon signed-rank test)

Species	Pvalue	ACPA(-)mean	meanACPA(+)	ACPA(-)median	ACPA(+)median	EnrichGroup	Qvalue	butyrate-metabolizing
Haemophilus_parainfluenzae	9.60E-05	0.007629523	0.000567766	0.000910384	2.16E-05	ACPA(-)	0.001195483	
Klebsiella_oxytoca	1.70E-06	0.006913014	2.65E-05	3.46E-05	1.09E-05	ACPA(-)	0.000114928	
Streptococcus_salivarius	2.62E-07	0.004515727	0.00051123	0.000933734	8.08E-05	ACPA(-)	3.01E-05	
Streptococcus_parasanguinis	8.01E-08	0.001443189	0.000196145	0.000636643	4.04E-05	ACPA(-)	1.88E-05	
Streptococcus_thermophilus	3.08E-08	0.000633825	6.26E-05	0.000241381	1.04E-05	ACPA(-)	1.67E-05	
Streptococcus_sp.	8.68E-05	0.000531329	0.000166513	0.000371601	0.000126529	ACPA(-)	0.00117981	
Haemophilus_influenzae	4.04E-05	0.000461455	3.23E-05	7.92E-05	0	ACPA(-)	0.00077181	
Haemophilus_pittmaniae	6.59E-05	0.00041062	3.21E-05	5.16E-05	0	ACPA(-)	0.001014919	
Aggregatibacter_aphrophilus	6.29E-05	0.000233708	2.68E-05	4.41E-05	0	ACPA(-)	0.001001482	
Lactobacillus_ruminis	4.03E-06	0.000212508	1.72E-05	2.57E-05	0	ACPA(-)	0.000160045	
Clostridium_butyricum	4.38E-05	6.87E-05	1.04E-05	2.40E-05	0	ACPA(-)	0.000772533	producing
Clostridium_botulinum	3.71E-06	4.95E-05	1.26E-05	2.63E-05	0	ACPA(-)	0.000160045	producing
Clostridium_cochlearium	2.06E-05	3.57E-05	9.02E-06	1.91E-05	1.12E-05	ACPA(-)	0.000491541	producing
Pasteurella_multocida	8.60E-05	2.95E-05	1.72E-06	0	0	ACPA(-)	0.00117981	
Clostridium_baratii	1.43E-05	2.86E-05	6.73E-06	1.43E-05	0	ACPA(-)	0.000390694	producing
Clostridium_kluyveri	6.49E-05	2.77E-05	1.27E-05	2.01E-05	1.09E-05	ACPA(-)	0.001014919	producing
Fusobacterium_nucleatum	1.13E-06	2.61E-05	4.63E-06	1.66E-05	0	ACPA(-)	9.09E-05	producing
Clostridium_bornimense	4.27E-05	2.10E-05	2.61E-05	1.97E-05	0	ACPA(-)	0.000772533	producing
Clostridium_argentinense	4.05E-06	1.99E-05	2.25E-07	0	0	ACPA(-)	0.000160045	producing
Pasteurellaceae_bacterium_NI1060	3.78E-05	1.79E-05	0	0	0	ACPA(-)	0.000733873	
Aggregatibacter_actinomycetemcomita	3.11E-06	1.71E-05	8.77E-07	0	0	ACPA(-)	0.000155169	
Bibersteinia_trehalosi	4.37E-08	1.64E-05	5.58E-07	1.81E-05	0	ACPA(-)	1.67E-05	
Streptococcus_intermedius	9.56E-05	1.38E-05	3.87E-06	1.32E-05	0	ACPA(-)	0.001195483	
Clostridium_taeniosporum	2.44E-06	1.20E-05	0	1.31E-05	0	ACPA(-)	0.000133979	producing
Clostridium_beijerinckii	2.36E-07	1.16E-05	4.23E-07	1.22E-05	0	ACPA(-)	3.01E-05	producing
Filifactor_alocis	4.20E-06	1.15E-05	1.61E-06	0	0	ACPA(-)	0.000160516	producing
Staphylococcus_aureus	7.23E-05	9.39E-06	9.12E-07	0	0	ACPA(-)	0.001088334	
Acetoanaerobium_sticklandii	9.55E-05	8.09E-06	1.70E-06	0	0	ACPA(-)	0.001195483	producing
Flavonifractor_plautii	2.45E-05	0.01703252	0.032391894	0.008040113	0.020366549	ACPA(+)	0.00053128	producing
Bacillus_cereus	5.93E-06	0.001226873	0.003167913	0.000150132	0.002015483	ACPA(+)	0.000219325	

Pseudomonas_stutzeri	3.07E-05	0.000158229	0.000368039	1.58E-05	0.000188325	ACPA(+)	0.000627834	consuming
Gordonibacter_pamelaeae	3.73E-05	0.000133418	0.000949042	7.42E-05	0.000190741	ACPA(+)	0.000733873	
Olsenella_sp.	2.46E-05	9.63E-05	0.000447651	4.17E-05	0.000126001	ACPA(+)	0.00053128	
Olsenella_umbonata	2.54E-06	8.11E-05	0.000286657	2.49E-05	0.000146333	ACPA(+)	0.000133979	
Synechococcus_sp.	1.57E-05	6.65E-05	0.000152775	3.55E-05	8.35E-05	ACPA(+)	0.000404007	
Pseudomonas_sp.	7.71E-06	6.32E-05	0.000134045	1.99E-05	9.45E-05	ACPA(+)	0.000252346	consuming
Desulfovibrio_vulgaris	9.45E-05	4.99E-05	0.000142877	2.82E-05	8.40E-05	ACPA(+)	0.001195483	
Denitrobacterium_detoxificans	6.16E-05	4.09E-05	0.000108998	1.24E-05	3.53E-05	ACPA(+)	0.00099505	
Halomonas_aestuarii	3.40E-06	3.51E-05	0.00010232	0	7.22E-05	ACPA(+)	0.000160045	
Hymenobacter_sp.	2.27E-05	3.29E-05	7.04E-05	2.85E-05	5.97E-05	ACPA(+)	0.000520945	
Desulfovibrio_gigas	6.54E-06	2.48E-05	6.95E-05	0	5.46E-05	ACPA(+)	0.00022702	
Geoalkalibacter_subterraneus	1.50E-07	2.32E-05	8.80E-05	1.37E-05	5.70E-05	ACPA(+)	2.74E-05	
Pelodictyon_luteolum	1.28E-05	2.03E-05	5.85E-05	0	4.04E-05	ACPA(+)	0.000366648	
Azospirillum_brasilense	7.25E-07	1.90E-05	8.25E-05	0	3.55E-05	ACPA(+)	6.39E-05	consuming
Corynebacterium_vitaeruminis	7.31E-05	1.87E-05	4.97E-05	0	1.90E-05	ACPA(+)	0.001088334	
Pseudomonas_putida	2.92E-05	1.64E-05	4.40E-05	0	3.72E-05	ACPA(+)	0.000607598	consuming
Sphingomonas_sp.	8.92E-06	1.40E-05	3.89E-05	0	3.42E-05	ACPA(+)	0.000276415	consuming
Pontibacter_akesuensis	1.67E-07	1.17E-05	4.36E-05	1.18E-05	3.07E-05	ACPA(+)	2.74E-05	
Bordetella_sp.	1.59E-05	1.17E-05	5.55E-05	0	2.97E-05	ACPA(+)	0.000404007	consuming
Terriglobus_roseus	6.64E-05	1.17E-05	4.24E-05	0	1.48E-05	ACPA(+)	0.001014919	
Rhodoferax_antarcticus	4.56E-05	1.07E-05	4.45E-05	0	1.87E-05	ACPA(+)	0.000791901	consuming
Pseudodesulfovibrio_aespoeensis	7.50E-05	1.03E-05	4.19E-05	0	2.41E-05	ACPA(+)	0.001093339	
Agrococcus_carbonis	8.55E-06	1.02E-05	4.01E-05	0	2.10E-05	ACPA(+)	0.000272138	
Deinococcus_proteolyticus	2.30E-08	9.85E-06	4.63E-05	0	2.69E-05	ACPA(+)	1.67E-05	consuming
Desulfovibrio_sp.	1.36E-05	9.02E-06	6.01E-05	0	2.58E-05	ACPA(+)	0.000381157	
Verrucomicrobium_spinosum	6.43E-06	8.66E-06	3.19E-05	0	1.76E-05	ACPA(+)	0.00022702	
Fibrella_aestuarina	2.59E-05	8.64E-06	5.14E-05	1.08E-05	2.09E-05	ACPA(+)	0.000550213	
Desulfurispirillum_indicum	1.20E-05	7.36E-06	2.99E-05	0	1.90E-05	ACPA(+)	0.000353295	consuming
Draconibacterium_orientale	5.60E-07	6.40E-06	5.53E-05	0	2.18E-05	ACPA(+)	5.35E-05	
Aeromonas_schubertii	4.34E-05	5.21E-06	2.57E-05	0	1.28E-05	ACPA(+)	0.000772533	
Methylobacterium_sp.	9.60E-05	5.19E-06	2.14E-05	0	1.50E-05	ACPA(+)	0.001195483	consuming
Lactococcus_raffinolactis	1.53E-05	4.93E-06	2.32E-05	0	1.31E-05	ACPA(+)	0.000404007	
Mucilaginibacter_paludis	2.00E-06	4.91E-06	3.75E-05	0	1.82E-05	ACPA(+)	0.000127089	
Microbacterium_aurum	1.36E-06	4.84E-06	2.81E-05	0	1.79E-05	ACPA(+)	9.76E-05	consuming
Magnetospirillum_gryphiswaldense	2.57E-06	4.59E-06	2.82E-05	0	1.90E-05	ACPA(+)	0.000133979	consuming

Thermincola_potens	4.12E-05	4.22E-06	3.28E-05	0	0	ACPA(+)	0.000772533	
Cupriavidus_necator	8.19E-08	4.11E-06	3.69E-05	0	1.85E-05	ACPA(+)	1.88E-05	consuming
Rubrivivax_gelatinosus	1.88E-05	3.74E-06	2.51E-05	0	1.45E-05	ACPA(+)	0.000458575	consuming
Bacillus_amyloliquefaciens	5.16E-05	3.74E-06	1.48E-05	0	1.14E-05	ACPA(+)	0.000870453	
Stenotrophomonas_rhizophila	7.73E-05	3.70E-06	1.58E-05	0	1.02E-05	ACPA(+)	0.001093339	
Ilumatobacter_coccineus	2.34E-05	3.11E-06	1.91E-05	0	1.40E-05	ACPA(+)	0.000526632	consuming
Serratia_ficaria	7.68E-05	2.97E-06	1.98E-05	0	0	ACPA(+)	0.001093339	
Kyrpidia_tusciae	4.35E-05	2.68E-06	1.79E-05	0	1.02E-05	ACPA(+)	0.000772533	
Vibrio_fluvialis	1.70E-05	2.67E-06	1.32E-05	0	1.49E-05	ACPA(+)	0.000424186	
Rhodoferax_sp.	2.15E-05	2.49E-06	1.06E-05	0	0	ACPA(+)	0.000502958	consuming
Agrobacterium_tumefaciens	9.28E-05	2.47E-06	1.22E-05	0	0	ACPA(+)	0.001195483	
Neoasaia_chiangmaiensis	4.10E-07	2.37E-06	1.75E-05	0	1.04E-05	ACPA(+)	4.28E-05	
Thermus_sp.	7.09E-06	2.28E-06	1.40E-05	0	1.11E-05	ACPA(+)	0.000239043	consuming
Neisseria_zoodegmatis	2.05E-07	2.15E-06	2.04E-05	0	1.49E-05	ACPA(+)	2.94E-05	
Brevundimonas_naejangsanensis	8.69E-05	2.08E-06	1.50E-05	0	1.06E-05	ACPA(+)	0.00117981	consuming
Variovorax_paradoxus	3.89E-06	1.75E-06	3.08E-05	0	1.47E-05	ACPA(+)	0.000160045	consuming
Pseudomonas_cichorii	7.57E-05	1.64E-06	1.54E-05	0	0	ACPA(+)	0.001093339	consuming
Nakamurella_panacisegetis	4.66E-05	1.25E-06	1.57E-05	0	0	ACPA(+)	0.000797326	
Robiginitalea_biformata	3.33E-05	1.19E-06	1.12E-05	0	1.01E-05	ACPA(+)	0.000668763	
Bordetella_bronchialis	1.02E-05	1.14E-06	1.06E-05	0	0	ACPA(+)	0.000306645	consuming
Pseudomonas_poae	8.88E-05	1.09E-06	2.38E-05	0	0	ACPA(+)	0.001182713	consuming
Kitasatospora_aureofaciens	1.19E-06	1.04E-06	2.12E-05	0	1.02E-05	ACPA(+)	9.09E-05	consuming
Brevibacterium_linens	2.52E-06	0	2.24E-05	0	1.16E-05	ACPA(+)	0.000133979	consuming
Gardnerella_vaginalis	3.85E-06	0	3.77E-05	0	0	ACPA(+)	0.000160045	
Bacillus_horikoshii	5.79E-05	0	6.82E-06	0	0	ACPA(+)	0.000961739	
Rhodopirellula_baltica	5.96E-05	0	9.48E-06	0	0	ACPA(+)	0.000975513	
Sulfuriferula_sp.	8.75E-05	0	8.84E-06	0	0	ACPA(+)	0.00117981	

Table S6. Demographic and clinical information of the validation cohort

Characteristic	**RA (n=37)**	**HC (n=31)**	***p* value**
Age, year, median (range)	56 (49-64)	55 (51, 60)	0.8506
Female/Male	29/8	24/7	0.926
BMI, median (range)	23.4 (20.3, 25.9)	23.4 (22.5, 27.7)	0.1285
Duration, years, median (range)	8.0 (3.5-20.0)	N/A	N/A
Clinical parameters			
ESR, mm/h, median (range)	33 (11, 58)	N/A	N/A
CRP, mg/l, median (range)	12.1 (3.2, 33.3)	N/A	N/A
DAS28-ESR, median (range)	4.9 (3.8, 6.1)	N/A	N/A
DAS28-CRP, median (range)	4.4 (3.4, 5.5)	N/A	N/A
TJC-28, median (range)	4 (1, 15)	N/A	N/A
SJC-28, median (range)	3 (0, 12)	N/A	N/A
DJC-28, n (%)	23 (62.2)	N/A	N/A
Anti-CCP，IU/ml，median (range)	163.4 (38.5-213.1)	N/A	N/A
IgM-RF，IU/ml，median (range)	120 (20-682)	N/A	N/A
Medication use, n, (%)	31 (83.8)	N/A	N/A
Methotrexate or/and other DEMARDs, n, (%)	25 (67.6)	N/A	N/A
Prednisone, n, (%)	12 (32.4)	N/A	N/A
Biological agent, n, (%)	2 (5.4)	N/A	N/A

Characteristic	**DJ (n=13)**	**NDJ (n=24)**	***p* value**
Age, year, median (range)	52 (30, 69)	58 (39-70)	0.2938
Female/Male	11/2	18/6	0.511
BMI, median (range)	21.5 (20.2, 30.4)	23.4(17.7, 26.7)	0.3018
Duration, years, median (range)	12.9 (1.0-20.0)	9.0 (1-30.0)	0.087
Clinical parameters			
ESR, mm/h, median (range)	36 (5, 92)	25 (8, 102)	0.0503
CRP, mg/l, median (range)	19.1 (0.4, 80.4)	8.7 (1.1, 64.7)	0.1367
DAS28-ESR, median (range)	4.7 (1.3, 7.5)	4.8 (1.7, 7.6)	0.4453
DAS28-CRP, median (range)	4.0 (1.4, 6.2)	4.3 (2.0, 7.1)	0.6919
TJC-28, median (range)	3 (0, 28)	5 (0, 28)	0.8182
SJC-28, median (range)	4 (0, 20)	2 (0, 28)	0.1437
DJC-28, n (%)	6 (2, 20)	0 (0, 0)	<0.0001
Anti-CCP，IU/ml，median (range)	152.7 (4.7-268.9)	169.7 (2.7-236.2)	0.8692
IgM-RF，IU/ml，median (range)	121.0 (20-2400)	82.75 (20-5840)	0.7226
Medication use, n, (%)			
Methotrexate	6 (42.9)	12 (52.2)	0.829
other DEMARDs, n, (%)	7 (57.1)	12 (52.2)	0.486
Prednisone, n, (%)	5 (35.7)	11 (47.8)	0.676
Biological agent, n, (%)	3 (21.4)	7 (30.4)	0.7

Table S7. List of antibodies used in the study

Antibodies	source	Cat#
Alexa Fluor® 700 anti-human CD3	Biolegend	317340
FITC anti-human CD4	Biolegend	344604
PerCP anti-human CD8	Biolegend	344708
APC anti-human CD185 (CXCR5)	Biolegend	356908
Biotin anti-human CD279 (PD-1)	Biolegend	329934
PE/Cy7 Streptavidin	Biolegend	405206
Brilliant Violet 421™ anti-human CD197 (CCR7)	Biolegend	353208
PE/Dazzle™ 594 anti-human CD183 (CXCR3)	Biolegend	353736
Zombie NIR™ Fixable Viability Kit	Biolegend	423106
PE anti-human CD25	Biolegend	302606
Brilliant Violet 510™ anti-human CD45RA	Biolegend	304142
Brilliant Violet 605™ anti-human CD127 (IL-7Rα)	Biolegend	351334
Brilliant Violet 650™ anti-human CD196 (CCR6)	Biolegend	353426
FITC anti-mouse CD4	Biolegend	100406
PerCP/Cyanine5.5 anti-mouse CD4	Biolegend	100434
PE/Cy7 anti-mouse/human CD11b	Biolegend	101216
Alexa Fluor® 700 anti-mouse/human CD45R/B220	Biolegend	103232
PerCP/Cyanine5.5 anti-mouse CD1d (CD1.1, Ly-38)	Biolegend	123514
Anti-Human/Mouse GL7 (T and B Cell Activation Marker) eFluor® 660	eBioscience	50-5902-82
Brilliant Violet 650™ anti-mouse IgD	Biolegend	405721
Brilliant Violet 421™ anti-mouse CD5	Biolegend	100618
PE/Dazzle™ 594 anti-mouse CD138 (Syndecan-1)	Biolegend	142528
Anti-Mouse CD95 (APO-1/Fas) PE	eBioscience	12-0951-81
FITC anti-mouse CD3	Biolegend	100204
APC/Cy7 anti-mouse CD8a	Biolegend	100714
FITC anti-mouse/human CD44	Biolegend	103006
PE/Cy7 anti-mouse CD25	Biolegend	102016
Brilliant Violet 421™ anti-mouse CD4	Biolegend	100438
Biotin anti-mouse CD185 (CXCR5)	Biolegend	145510
APC anti-human/mouse Bcl-6	Biolegend	358506

Zombie Aqua™ Fixable Viability Kit	Biolegend	423102
Biotin anti-mouse CD185 (CXCR5)	Biolegend	145510
SA-PE-CY5.5	life	SA1018
Ms IL-17A PE TC11-18H10 100ug	BD	559502
Ms IFN-Gma APC XMG1.2 100ug	BD	554413
Anti-Mouse/Rat Foxp3 PE-eFluor® 610 100 ug	eBioscience	61-5773-82