

Supplemental information

Biglycan regulated colorectal cancer progress

by modulating enteric neuron-derived

IL-10 and abundance of *Bacteroides thetaiotaomicron*

Yuyu Xu, Fei Wang, Kai Mi, Xinyuan Wang, Danlei Wang, Qing Zhao, Jingjing Wang, Zhi Liu, Qingqing Zhang, Yang Liu, Xuemei Zhang, and Xingyin Liu

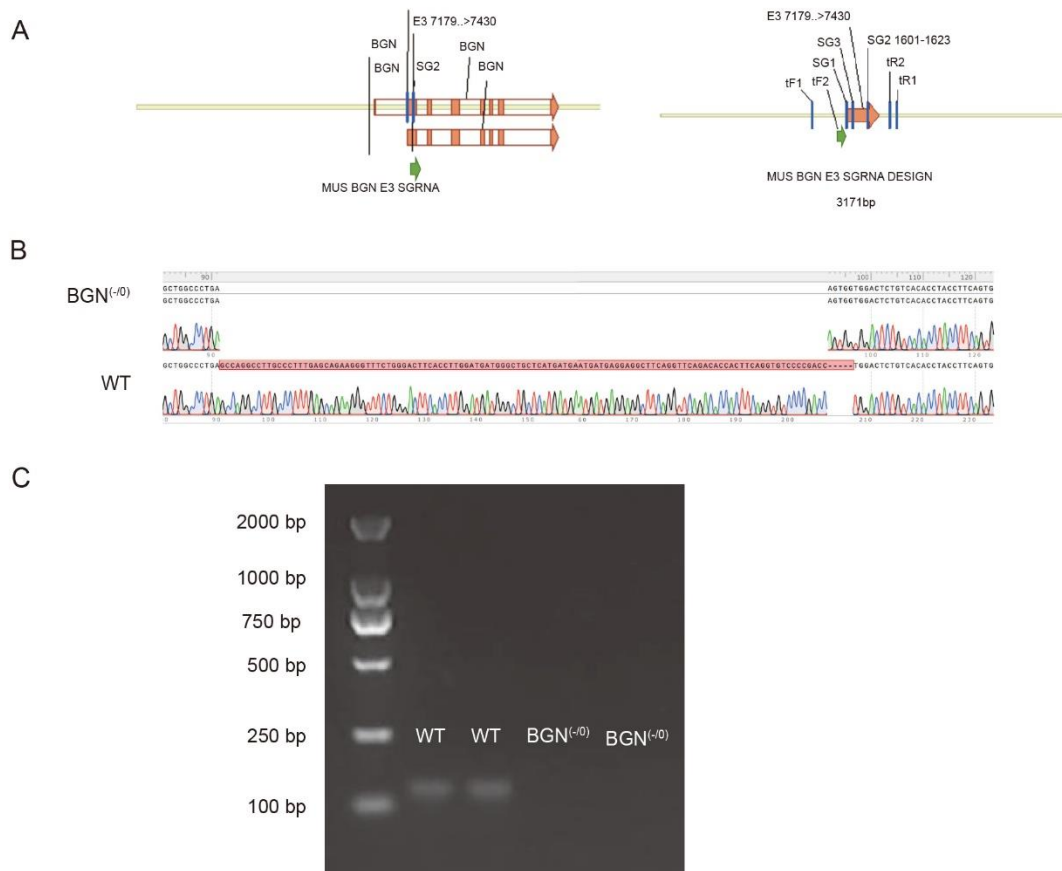


Figure S1 The establishment of BGN deficiency mouse, related to Figure 1 and Figure 2.

A. The establishment of BGN^(-/-) mouse, by CRISPR/Cas9 technology.

B. The verification of BGN^(-/-) mouse, by Sanger sequence

C. The verification of BGN^(-/-) mouse, by RT-PCR.

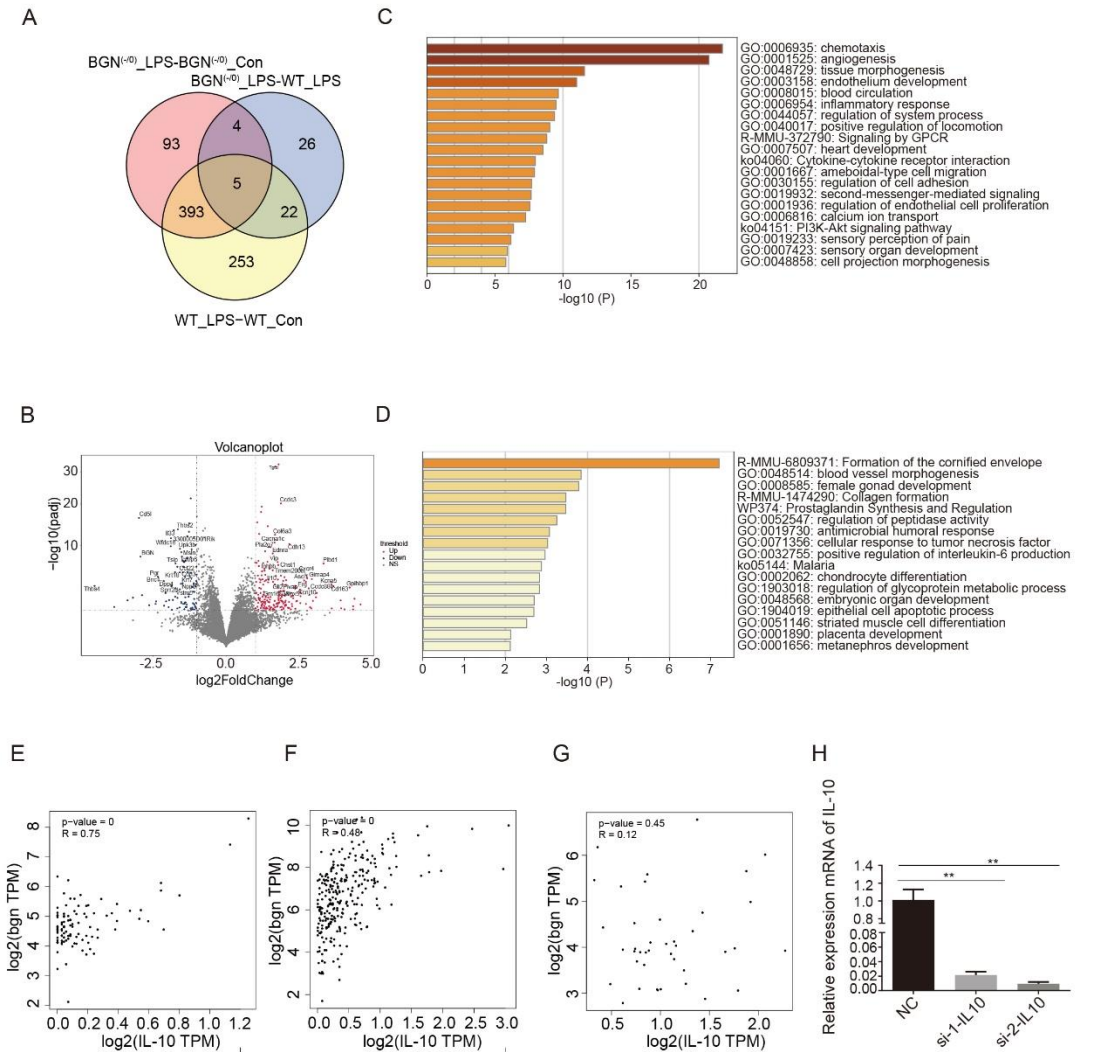


Figure S2 RNA-seq analysis revealed that BGN regulated IL-10 expression in enteric neurons, related to Figure 3.

- A.** Venn diagram showing the overlap of DEGs in three comparisons.
- B.** Volcano Plot showed the genes in BGN^(-/0)_LPS vs WT_LPS enteric neurons.
- C.** GO enrichment analysis of upregulated genes in BGN^(-/0)_LPS vs WT_LPS comparison.
- D.** GO enrichment analysis of downregulated genes in BGN^(-/0)_LPS vs WT_LPS comparison.
- E.** IL-10 expression is highly correlated with BGN in brain cortex ($p=0$, $R=0.75$).
- F.** IL-10 expression has correlation with BGN in COAD tumor tissues ($p=0$, $R=0.48$).
- G.** IL-10 expression has no correlation with BGN in COAD normal tissue ($p=0.45$,

R=0.12).

H. Knockdown of IL-10 using siRNA in enteric neurons from BGN^(-/0) mice, detected by RT-qPCR.

Data are representative or cumulative results of at least two independent experiments (A-D and H). Data are presented as mean \pm S.E.M. *p < 0.05, **p < 0.01, ***p < 0.001, and n.s. indicates not significant (p > 0.05).

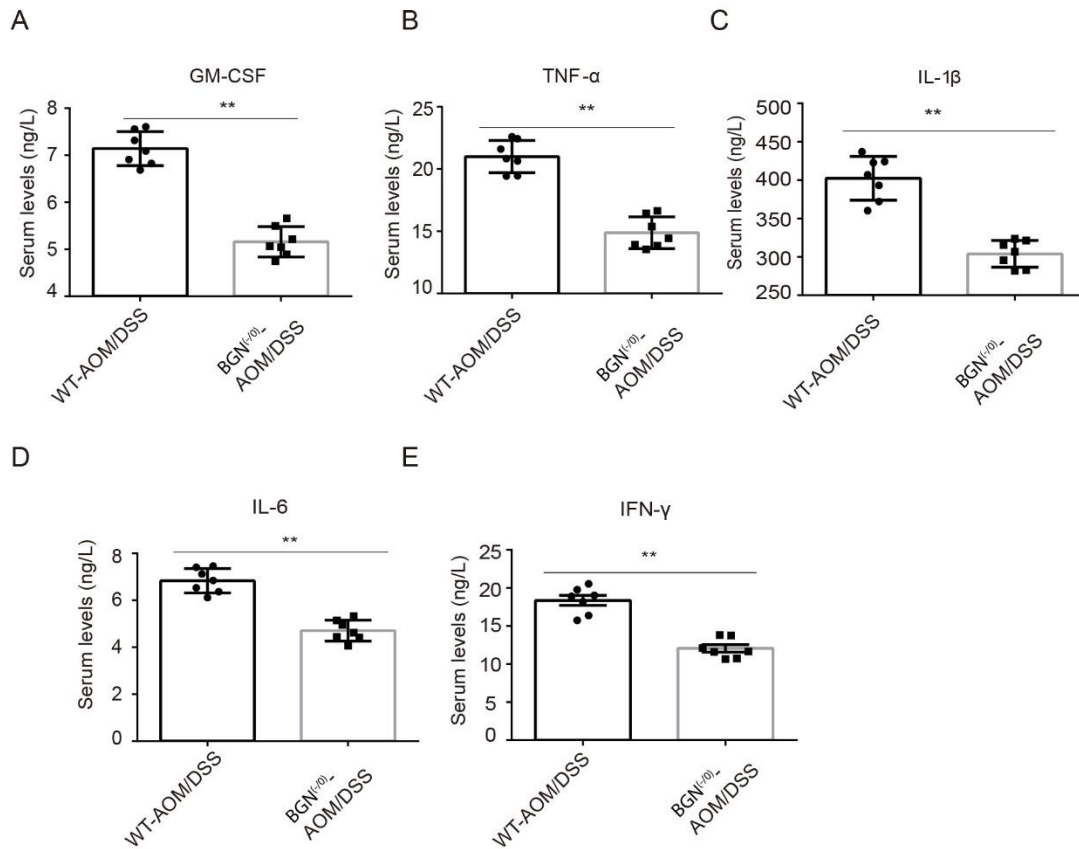


Figure S3 BGN deficiency reduced cytokine expression in CAC mice, related to Figure 3.

A. BGN deficiency reduced the level of GM-CSF in serum of CAC mice, as detected by ELISA assay (n=7 mice/group).

B. BGN deficiency reduced the level of TNF-α in serum of CAC mice, as detected by ELISA assay (n=7 mice/group).

C. BGN deficiency reduced the level of IL-1β in serum of CAC mice, as detected by ELISA assay (n=7 mice/group).

D. BGN deficiency reduced the level of IL-6 in serum of CAC mice, as detected by ELISA assay (n=7 mice/group).

E. BGN deficiency reduced the level of IFN-γ in serum of CAC mice, as detected by ELISA assay (n=7 mice/group).

Data are representative or cumulative results of at least two independent experiments (A-E). Data are presented as mean ± S.E.M. **p < 0.01.

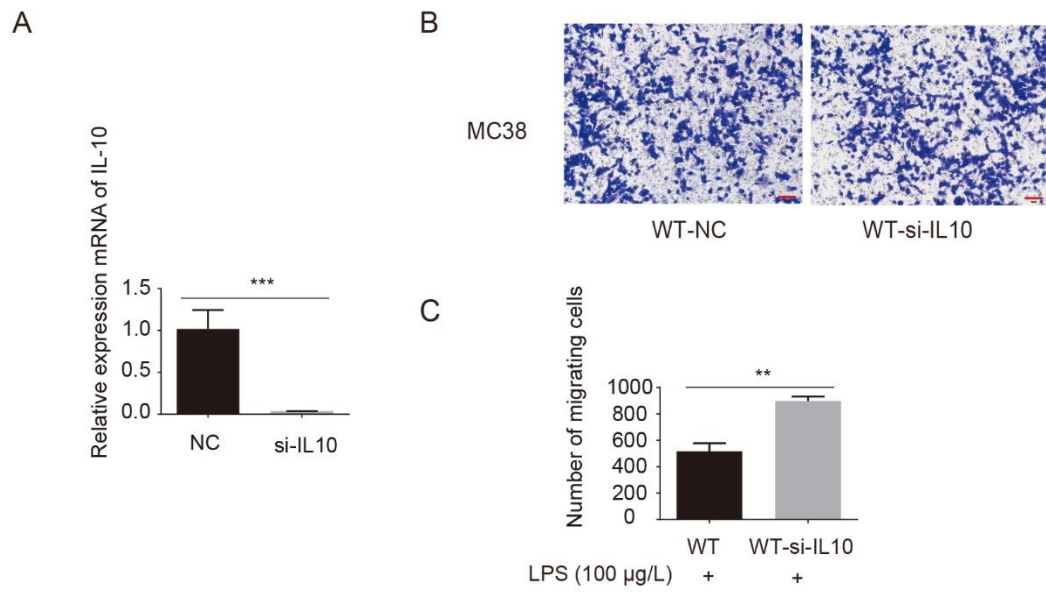


Figure S4 Knockdown of IL-10 increased the migration of cancer cells, related to Figure 3.

A. Knockdown of IL-10 using siRNA in enteric neurons from WT mice, detected by RT-qPCR (n=3 biological replicates/ group).

B. The number of MC38 cells migration was increased in co-culture with IL-10 knockdown of WT mice enteric neurons with LPS (100 µg/L) (n=3 biological replicates/group).

Data are presented as mean \pm S.E.M. **p < 0.01, ***p < 0.001

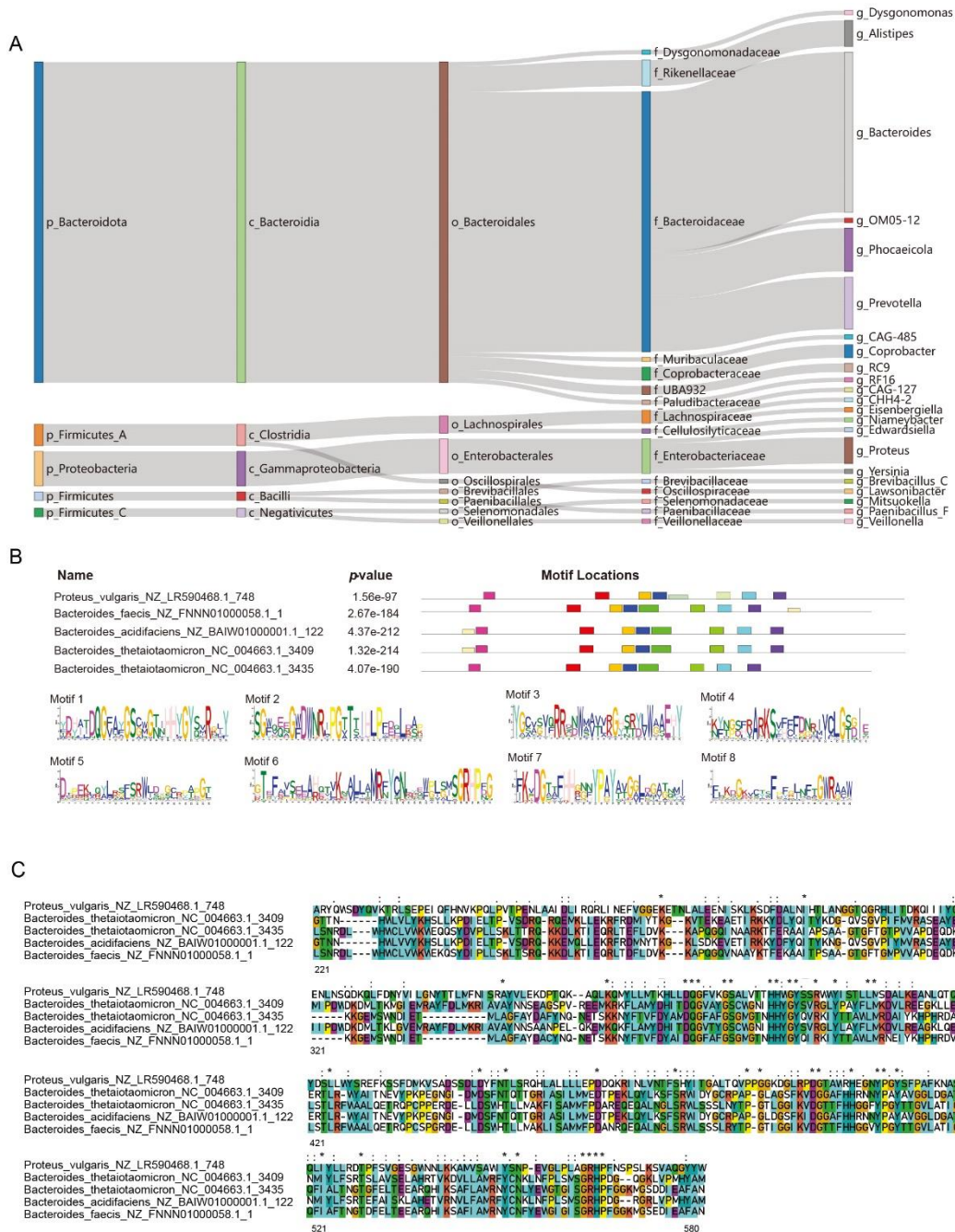


Figure S5 Bioinformatic analysis showed *B. thetaiotaomicron* encode conserved ChABC with that of *Proteus vulgaris*, related to Figure 4.

A. BIO-Sankey Network for ChABC-encoding bacteria. The *Bacteroides* genus occupies a large proportion in the network.

B. Motif pattern on five ChABC sequences from *Proteus vulgaris* and *B. thetaiotaomicron*, etc.

C. Protein sequence alignment analysis on five ChABC sequences from *Proteus vulgaris* and *B. thetaiotaomicron*, etc.

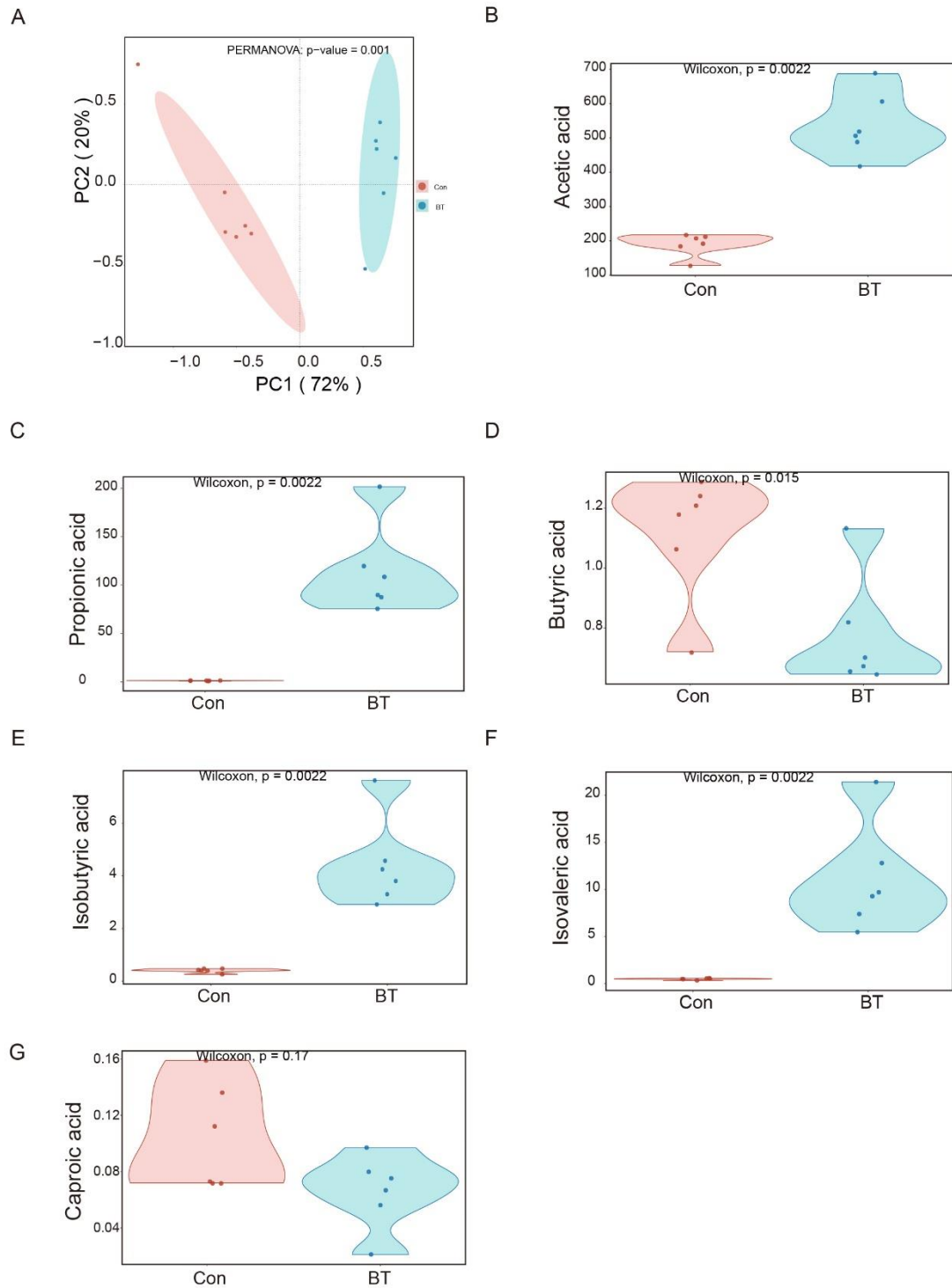


Figure S6 B. *thetaiotaomicron* supernatant enriched SCFAs, related to Figure 5.

A. The PcoA analysis showed the level of SCFAs were clearly distinctive from each other in the two groups.

B and C. Acetic acid and propionic acid were in higher level in the supernatant of *B. thetaiotaomicron* medium than that in control medium.

D. The level of butyric acid was lower in the supernatant of *B. thetaiotaomicron* medium than that in control medium.

E and F. Isobutyric acid and Isovaleric acid were in higher level in the supernatant of *B. thetaiotaomicron* medium than that in control medium.

G. No significant difference in the level of Caproic acid between the two groups.

Data are presented as mean \pm SD. p-value $<$ 0.05 was considered statistically significant, p-value $>$ 0.05 was considered statistically significant.

Table S1 Primer used in RT-qPCR and RT-PCR

18S	Forward	TTGACGGAAGGGCACCACCAG
18S	Reverse	GCACCACCACCCACGGAATCG
IL-10	Forward	CTGGACAACATACTGCTAACCGAC
IL-10	Reverse	ATTCATTCATGGCCTTG TAGACACC
BGN	Forward	AGAGAGAACCCTAGCTACTACTGCTGG
BGN	Reverse	CCCATCTCACAGGGTAGCAAATGT

The list of primers for qPCR, related to Figure S1, Figure S2 and Figure S4.

Table S2 The list of taxa can encode the four enzymes (3.2.1.180, 4.2.2.20, 4.2.2.21, 4.2.2.5) and belongs to Bacteroides genus.

Genus	Species	Annotation
Bacteroides	Bacteroides ndongoniae	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides ndongoniae
Bacteroides	Bacteroides sp003545565	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides sp003545565
Bacteroides	Bacteroides cellulolyticus	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides cellulolyticus
Bacteroides	Bacteroides timonensis	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides timonensis
Bacteroides	Bacteroides sp900556625	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides sp900556625
Bacteroides	Bacteroides sp012113595	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides sp012113595
Bacteroides	Bacteroides congonensis	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides congonensis
Bacteroides	Bacteroides sp902362375	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides sp902362375
Bacteroides	Bacteroides salyersiae	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides salyersiae
Bacteroides	Bacteroides acidifaciens_A	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides acidifaciens_A
Bacteroides	Bacteroides sp900765785	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides sp900765785
Bacteroides	Bacteroides sp902388495	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides sp902388495
Bacteroides	Bacteroides sp900066265	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides sp900066265
Bacteroides	Bacteroides sp900556215	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides sp900556215
Bacteroides	Bacteroides sp900555635	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides sp900555635
Bacteroides	Bacteroides sp900761785	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides sp900761785
Bacteroides	Bacteroides sp900552405	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides sp900552405
Bacteroides	Bacteroides stercorisoris	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides stercorisoris
Bacteroides	Bacteroides sp007097645	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides sp007097645
Bacteroides	Bacteroides oleiciplenus	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides oleiciplenus
Bacteroides	Bacteroides intestinalis	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides intestinalis
Bacteroides	Bacteroides sp900755095	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides sp900755095
Bacteroides	Bacteroides rodentium	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides rodentium

Bacteroides	Bacteroides intestinalis_A	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides intestinalis_A
Bacteroides	Bacteroides sp900557355	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides sp900557355
Bacteroides	Bacteroides clarus	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides clarus
Bacteroides	Bacteroides thetaiotaomicron	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides thetaiotaomicron

The list of taxa can encode the four enzymes (3.2.1.180, 4.2.2.20, 4.2.2.21, 4.2.2.5) and belongs to Bacteroides genus, related to Figure 4.