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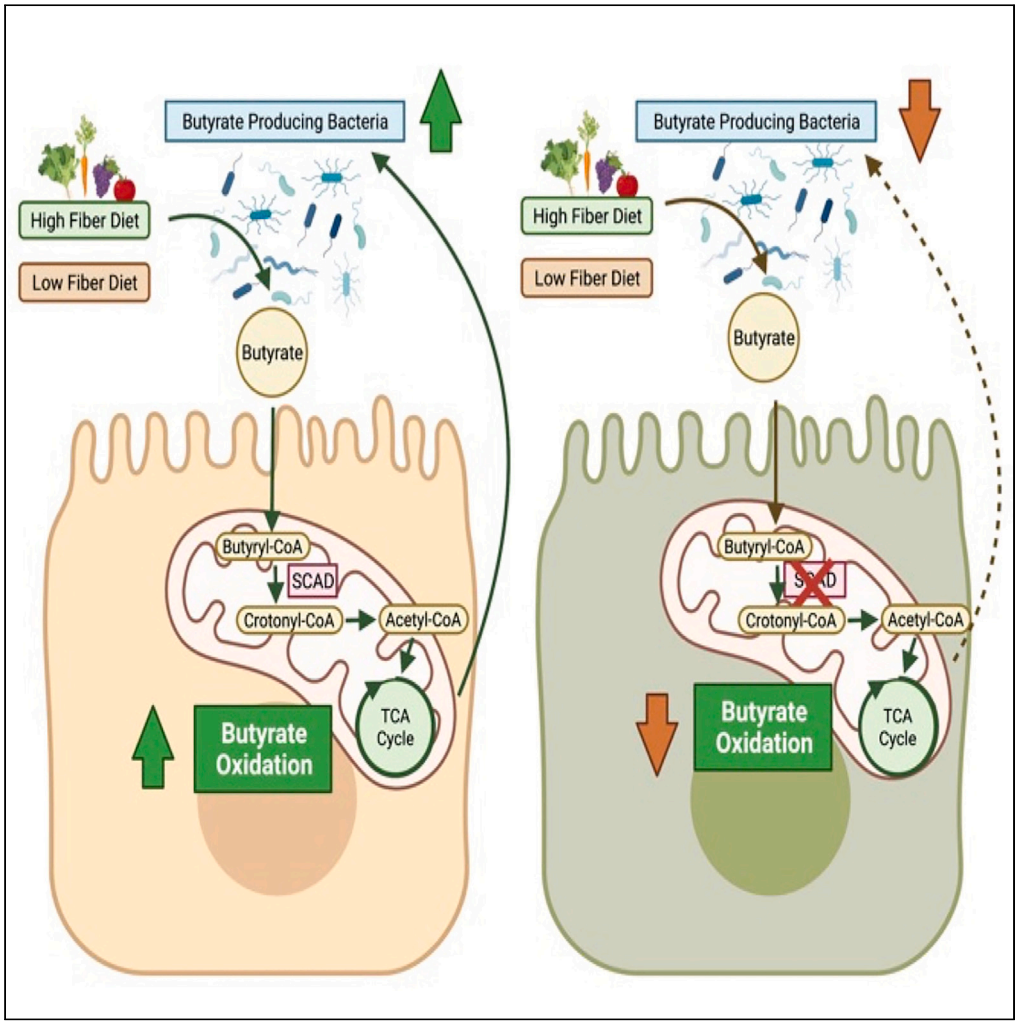

Bohye Park, Ji Yeon Kim, Olivia F. Riffey, Triston J. Walsh, Jeremiah Johnson, Dallas R. Donohoe

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Highlights

Prebiotic effect of a high-fiber diet is dependent on host cell metabolism

Loss of SCFA oxidation by the host diminishes bacteria producing butyrate

Location of microbiome sample collection impacts the detection of butyrate producers

Colonocytes increase glycolysis when butyrate oxidation is diminished

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Crosstalk between butyrate oxidation in colonocyte and butyrate-producing bacteria

Bohye Park,[1,3] Ji Yeon Kim,[1] Olivia F. Riffey,[2] Triston J. Walsh,[2] Jeremiah Johnson,[2] and Dallas R. Donohoe[1,2,4,*]

SUMMARY

The composition of gut microbiota, including butyrate-producing bacteria (BPB), is influenced by diet and physiological conditions. As such, given the importance of butyrate as an energetic substrate in colonocytes, it is unclear whether utilization of this substrate by the host would enhance BPB levels, thus defining a host-microbiome mutualistic relationship based on cellular metabolism. Here, it is shown through using a mouse model that lacks short-chain acyl dehydrogenase (SCAD), which is the first enzyme in the beta-oxidation pathway for short-chain fatty acids (SCFAs), that there is a significant diminishment in BPB at the phylum, class, species, and genus level compared to mice that have SCAD. Furthermore, SCAD-deficient mice do not show a prebiotic response from dietary fiber. Thus, oxidation of SCFAs by the host, which includes butyrate, is important in promoting BPB. These data help define the functional importance of diet-microbiome-host interactions toward microbiome composition, as it relates to function.

INTRODUCTION

Trillions of microbes inhabit the gastrointestinal tract (GI) comprising a diverse and complex ecosystem, fluctuating based on environmental factors, including diet and its interaction with the host.[1] Evidence indicates that the host-microbiome commensal relationships and the functions of microbial-derived metabolites, including short-chain fatty acid (SCFA) coming from the fermentation of dietary fiber by specific bacteria, play a key role in colonic health.[2] Metagenomic studies have demonstrated variability and individual differences in the bacteria species in healthy and diseased populations.[3–5] However, despite the differences, many of these bacteria species share similar functions, such as the ability to ferment complex polysaccharides or dietary fiber.[6,7] Microbiota with the capacity to ferment fiber into SCFAs are sometimes referred to as butyrate-producing bacteria (BPB), and variation in these bacteria between individuals provides a source of nutritional diversity. There is an important interrelationship between BPB and host physiology in regulating metabolic and immunological functions.[8–10] How diet impacts this relationship is not entirely understood.

Firmicutes and Bacteroidetes account for more than 90% of the microbiome in the colon.[11,12] In regard to the production of SCFAs, the majority of BPB belong to the Firmicutes phylum (gram positive) and mainly generate butyrate, while the Bacteroidetes phylum (gram negative) is characterized by producing the other SCFAs acetate and propionate.[13,14] Bacteroidetes species such as *Bacteroides ovatus*, although containing the enzyme capable of producing butyrate, mainly generates isobutyrate and 2-methyl-butyric acid.[15] In contrast, *Clostridium* cluster IV and XIVa, which belong to the Firmicutes phylum, are generally accepted to be butyrate producers and are highly oxygen-sensitive anaerobic bacteria.[16] The two dominant BPB species in humans, *Faecalibacterium prausnitzii* within *Clostridium* cluster IV and *Eubacterium rectale* within *Clostridium* cluster XIVa, are two major producers of SCFAs including butyrate.[13,14] *F. prausnitzii* and *E. rectale* are not the dominant BPB in mice. The family S24-7 bacteria make up the greatest abundance of butyrate producers in the mouse gut.[17] For butyrate production in these bacteria, two acetyl-CoA molecules form butyryl-CoA in a process resembling reversed butyrate oxidation. After that, two final enzymes, butyryl-CoA: acetate CoA-transferase (*but*) and butyrate kinase (*buk*), are alternatively utilized to convert butyryl-CoA to butyrate.[18]

Butyrate itself has multiple physiological functions to the host including being the primary energy source of the colon.[19,20] In addition, butyrate suppresses inflammation by stimulating the production and release of anti-inflammatory cytokines, regulates tight junctions between colonocytes, and slows cell proliferation in cancer cells by inhibiting histone deacetylases.[20–26] Thus, the colonocyte directly and indirectly benefits from butyrate creating a relationship between the host and BPB. The importance of butyrate as a primary energy source for the colonocyte and whether this impacts the microbiome, specifically BPB, provides an interesting host-microbe selection scenario and question. Does the colonocyte, through utilizing butyrate as a primary energetic substrate, select for, or promote BPB? GI diseases such as ulcerative colitis and colorectal cancer (CRC), which have both been shown to have diminished butyrate oxidation, also have decreased BPB.[27–30]

The mouse BALB/cBy substrain diverged from the parental BALB/c strain approximately 75 years ago.[31] BALB/cBy mice have been reported to lack short-chain acyl-CoA dehydrogenase (SCAD), an enzyme used in the oxidation of SCFAs.[32] Thus, this mouse strain provides

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a disease-free model to test the impact of reduced oxidation of SCFAs on the microbiome and BPB. Here, we demonstrate an overall diminishment in the relative abundance of BPB in SCAD-deficient BALB/cBy mice when compared to BALB/c mice. Moreover, we find that a high-fiber diet (HFD) elicits a prebiotic response in regard to BPB in BALB/c, whereas BALB/cBy mice fail to show this same prebiotic response. Therefore, the oxidation of SCFAs by the host, especially butyrate, positively feeds back to increase the relative abundance of BPB and alter the composition of the microbiome.

RESULTS

Characterization of BALB/cBy colonocytes and their reduced butyrate oxidation

Colonocytes were isolated from BALB/c and BALB/cBy mice, and the expression of SCAD was analyzed by western blot. As shown in Figure 1A, the levels of SCAD were significantly diminished, but they were not completely abolished as we still could detect the protein. Since BALB/cBy mice are deficient in SCAD, the relative levels of monocarboxylate transporter 1 (MCT1), a major butyrate transporter, were measured in comparison to BALB/c to assess whether SCAD-deficient colonocytes increased MCT1 as a compensatory mechanism (Figure 1A). However, the levels of MCT1 did not show a significant change in BALB/cBy colonocytes. AMP kinase (AMPK) is a proxy for cellular energetics, as phosphorylation of this protein at Thr172 occurs under energetic stress or deprivation.[33] Phosphorylation of AMPK (Thr172) was significantly higher in SCAD-deficient (BALB/cBy) compared to non-deficient (BALB/c) colonocytes (Figure 1B). Thus, it is likely that the diminishment in SCAD results in decreased butyrate oxidation and energetic stress in the colonocyte. Furthermore, SCAD-deficient colonocytes showed increased phosphorylation and thus inactivation of acetyl-CoA carboxylase (ACC), which is also a downstream target of AMPK (Figure 1C). Inactivation of ACC would increase fatty acid transport via carnitine palmityl transferase as a result of reduced malonyl-CoA levels.[34] However, given the diminishment in SCAD, which is located in the mitochondria, and is the first step in the beta-oxidation process for SCFAs, it is unlikely that inactivation of ACC would have a significant impact on the oxidation of butyrate.

The Seahorse XFe24 Extracellular Flux Analyzer, which measures oxidative (oxygen consumption) and non-oxidative (lactate production or glycolysis) metabolism in cells, was utilized to test whether reduced levels of SCAD in the colonocytes resulted in decreased butyrate oxidation.[35,36] Isolated colonocytes derived from SCAD-deficient mice did not respond to butyrate as judged by the change in their oxygen consumption rate (OCR) after injection of butyrate or 2-deoxy-glucose (2DG), which is used to block glucose oxidation leaving butyrate as the only exogenous energy source for the colonocyte (Figure 1D). In fact, the OCR in the butyrate 5 mM-treated BALB/cBy group was almost identical to the control group that had no butyrate. If butyrate, which is the primary energy source of colonocytes, is abolished, then the colonocyte may compensate through increasing glycolysis. This was indeed the case as SCAD-deficient colonocytes displayed elevated glycolysis as judged by the increased extracellular acidification rate (ECAR) after the addition of glucose (Figure 1E). The ECAR measurement represents an indirect measurement of lactate secretion and glycolysis. Taken together, these data demonstrate that diminishment in SCAD impacts cellular metabolism by reducing butyrate oxidation and increasing glycolysis in the primary isolated colonocytes.

16S bacterial DNA sequencing in BALB/c and BALB/cBy

The major hypothesis or model to be tested was that a diminishment in butyrate and SCFA oxidation in the colonocytes through decreased SCAD would result in a reduction in the levels of BPB. To get an initial assessment of the microbial difference between two mouse strains, we performed a microbiome analysis using 16S sequencing on DNA isolated from BALB/c and BALB/cBy fecal pellets. We identified 35 and 50 different OTUs in BALB/c and BALB/cBy, respectively (Figure 2A). The percent abundance of several classes of known butyrate producers, *Butyricicoccus*, *Porphyromonadaceae*, and *Clostridium XIVa*, was all decreased in BALB/cBy as compared to BALB/c (Figure 2B). This 16S sequencing data analysis is included in Table S4. This is suggestive of a decrease in BPB in BALB/cBy mice that lack SCAD. However, based on these findings, a more targeted and focused approach was developed to quantify the relative levels of BPB.

Analyzing the butyrate synthesis enzyme as a biomarker of BPB in BALB/c and BALB/cBy

A gene-targeted quantitative polymerase chain reaction (qPCR) was utilized to analyze BPB in BALB/c and BALB/cBy mice. The two final enzymes in the bacterial butyrate synthesis pathways, *but* and *buk*, were targeted for amplification and each represented a biomarker for relative BPB amounts. A qPCR for total 16S was used to analyze *but* or *buk* relative to total bacteria. Initially, degenerative primers designed by Louis and Flint[37,38] were utilized for *but* and *buk* genes, but these PCR reactions yielded several unexpected bands, which precluded their use in qPCR. From the *but* and *buk* PCR reactions, the expected amplicons were sequenced, and several new primers were designed and tested. Importantly, PCR reactions with these new primers gave the expected amplicon, while having no non-specific bands. The process to get the specific *buk* primer from the degenerative primers is explained in Figure S1A.

Comparison of *but* and *buk* between BALB/c and SCAD-deficient BALB/cBy mice from collected individual fecal pellets yielded significant variability, even following the same mouse day-to-day (Figures S1B and S1C). The pH conditions in the colon can have a significant impact on microbial survival, and there is an increase in pH moving from the proximal to the distal part of the colon.[39,40] Dietary fibers are fermented by intestinal microbes to produce SCFAs, which lower the pH in the proximal colon.[41] The more acidic conditions in the proximal colon favor the growth of BPB. Thus, it was not clear whether the variability was an inherent part of the BPB or was due to where and how we collected samples. Taking a more location-centric approach, such as taking feces directly from the colon, would maybe decrease variability in our samples. Thus, a new experimental approach was used to collect bacterial DNA. The bacterial DNA was isolated from the dissected cecum, colon, and feces from the sacrificed mice. Cecum was also used because it is the primary site of fermentation in mice.[42] With this experimental approach,

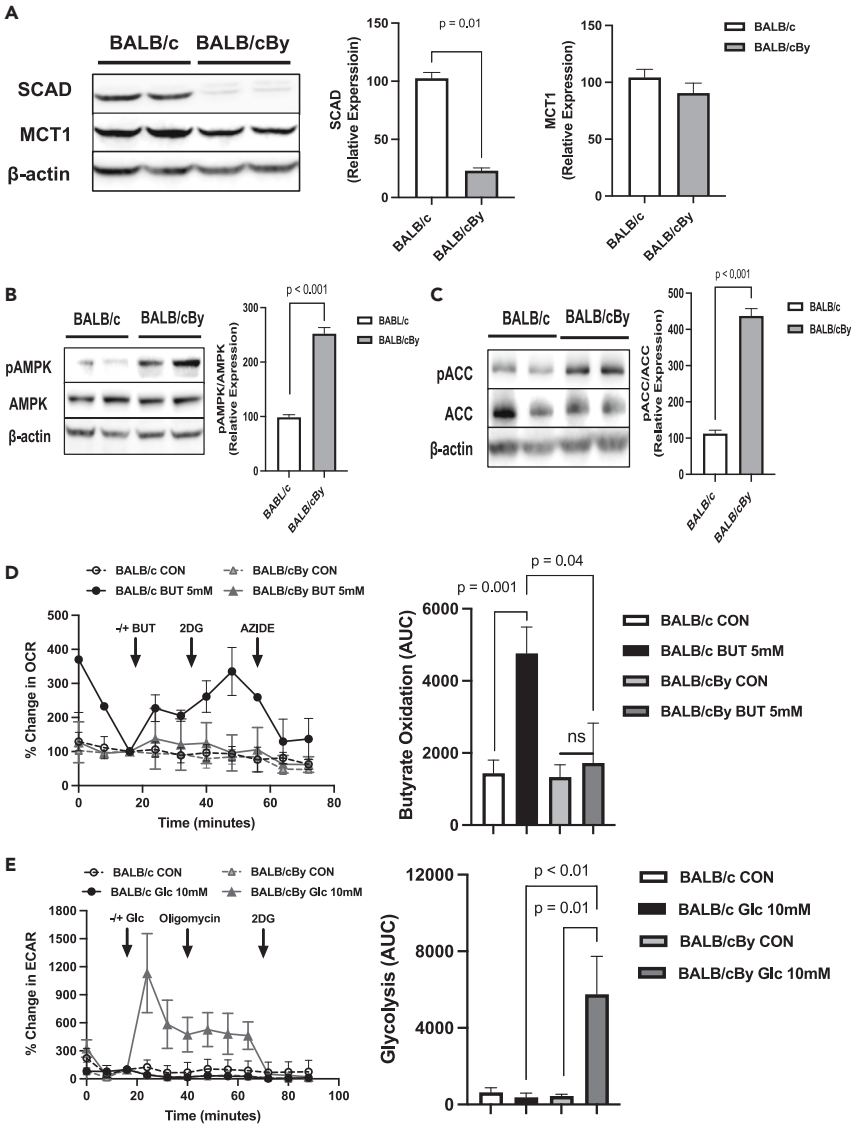

Figure 1. Molecular and metabolic consequences in SCAD mutant mouse colonocytes

(A) Western blot analysis of SCAD and MCT1 levels in the isolated colonocytes from BALB/c and BALB/cBy mice with β-actin as a loading control.

(B) Western blot analysis of phospho-AMPK (Thr172) and total AMPK levels in the isolated colonocytes from BALB/c and BALB/cBy mice with β-actin as a loading control.

(C) Western blot analysis of phospho-ACC (Ser 79) and total ACC levels in the isolated colonocytes from BALB/c and BALB/cBy mice with β-actin as a loading control.

(D) Percent change in OCR relative to baseline in which isolated colonocytes from BALB/c and BALB/cBy mice treated with or without butyrate (5 mM). Total contribution of butyrate toward OCR (%) is observed after injection of 2DG. Right: shows the area under the curve (AUC) analysis from OCR measurements taken after 2DG injection but before azide injection. These measurements represent butyrate oxidation (arbitrary units). Data points are the average OCR (%) over 3–5 replicates per condition for butyrate oxidation measurements. Error bars are ±SEM.

(E) Percent change in ECAR relative to baseline in which isolated colonocytes from BALB/c or BALB/cBy mice as a response to glucose, 2DG, and azide. Right: shows AUC from ECAR measurements taken after glucose injection but before 2DG injection. These measurements represent glycolysis (arbitrary units). Data points are the average ECAR (%) over 3–5 replicates per condition for glycolysis measurements. For statistical analysis, all western blots were conducted 5 times. Quantification of the western blots is shown in the right. Data are represented as +/− SEM.

the variability was diminished, and the amount of both butyrate synthesis enzymes (*but* and *buk*) was significantly decreased in bacterial DNA samples of the cecum, colon, and feces in SCAD-deficient BALB/cBy mice compared to BALB/c mice (Figures 2C and 2D). *Coprococcus comes* is a BPB that contains and utilizes butyrate kinase and not butyrate transferase.[13,43,44] Consistent with diminished butyrate kinase in BALB/cBy mice, we also find that *Coprococcus comes* is diminished in these mice (Figure S4). These data demonstrate a diminishment in

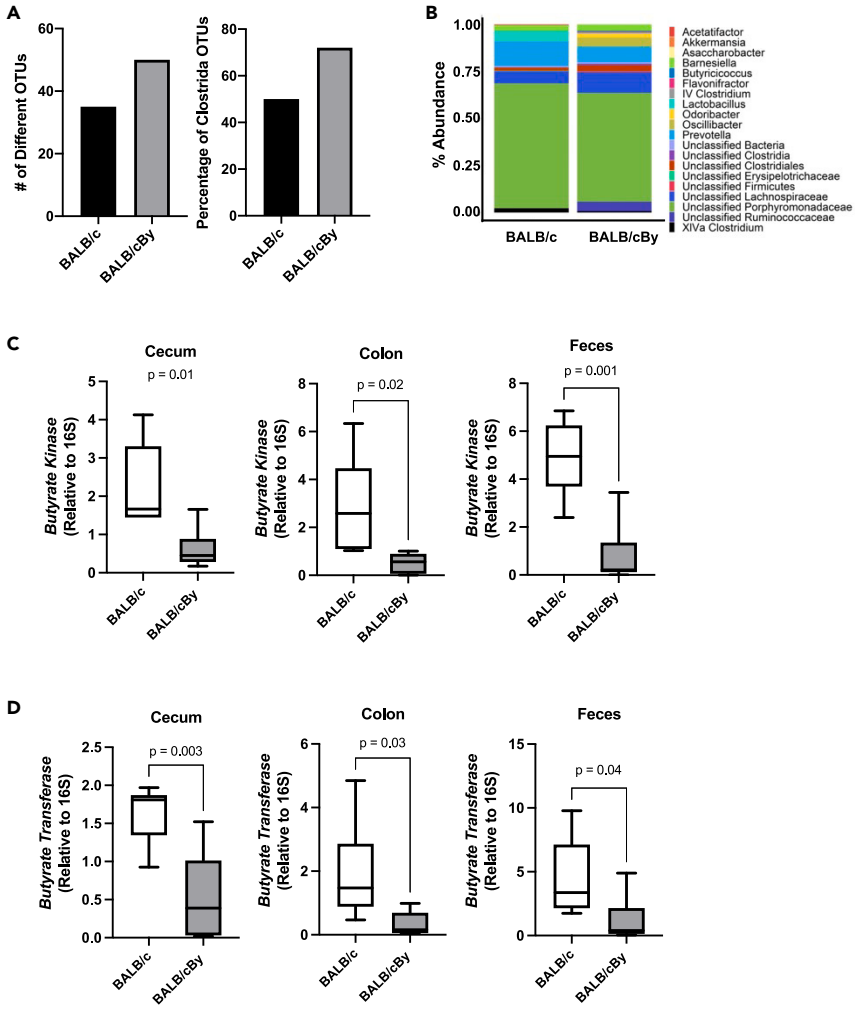

Figure 2. 16S rRNA and qPCR analysis from BALB/c and BALB/cBy fecal pellets

(A) The total number of different OTUs (left) and the percentage of those OTUs that belong to the Clostridia family (right) are shown in histographs.

(B) Relative abundance of bacteria families/genus in BALB/c and BALB/cBy mice. Quantification of *buk* (C) and the level of butyrate synthesis enzyme *but* (D) in different gut locations and feces of BALB/c and BALB/cBy mice relative to 16S rRNA. Data are represented as +/− SEM.

the relative BPB population in BALB/cBy mice, which lack SCAD, as compared to BALB/c mice. To confirm whether lower BPB in BALB/cBy mice also represent a decrease in the SCFAs, acetate, propionate, and butyrate were measured in collected fecal contents. Acetate and propionate levels were not found to be significantly different; however, butyrate was decreased in BALB/cBy fecal contents (Figure S2).

Diminished BPB in SCAD mutant BALB/cBy mice

To compare the abundance of specific BPB populations, we investigated BPB from phylum to species level with collected bacterial DNA samples isolated from the dissected cecum, colon, and feces of BALB/c and BALB/cBy mice using qPCR. Sequence information for the primers of specific bacterial groups is given in Table S1. At the phylum level, the amount of Firmicutes in the cecum, colon, and feces of SCAD-deficient BALB/cBy mice was significantly decreased compared to that in BALB/c mice (Figure 3A). However, the relative amount of Bacteroidetes, which are mainly non-butyrate-producing bacteria (non-BPB), showed an opposite trend of being slightly increased in BALB/cBy, although only reaching a significant difference in the colon (Figure 3B). Looking at the class cluster level, the amount of *Clostridium* IV and XIVa, known butyrate producers,[13,14] was significantly diminished in BALB/cBy compared to BALB/c mice when analyzing the cecum, colon, and feces (Figures 3C and 3D). At the species level, the amount of *F. prausnitzii* and *E. rectale* at each location decreased in BALB/cBy mice compared to BALB/c mice (Figures 3E and 3F). However, *F. prausnitzii* was not detected at all in the fecal samples, which suggests that location and collection method is particularly important for detecting this specific bacterial species. These data demonstrate, in this non-disease model, that decreased butyrate and SCFA oxidation of SCAD-deficient mice is associated with a significant diminishment in BPB, thereby defining an important relationship between host cellular metabolism and the gut microbiota as it pertains to BPB.

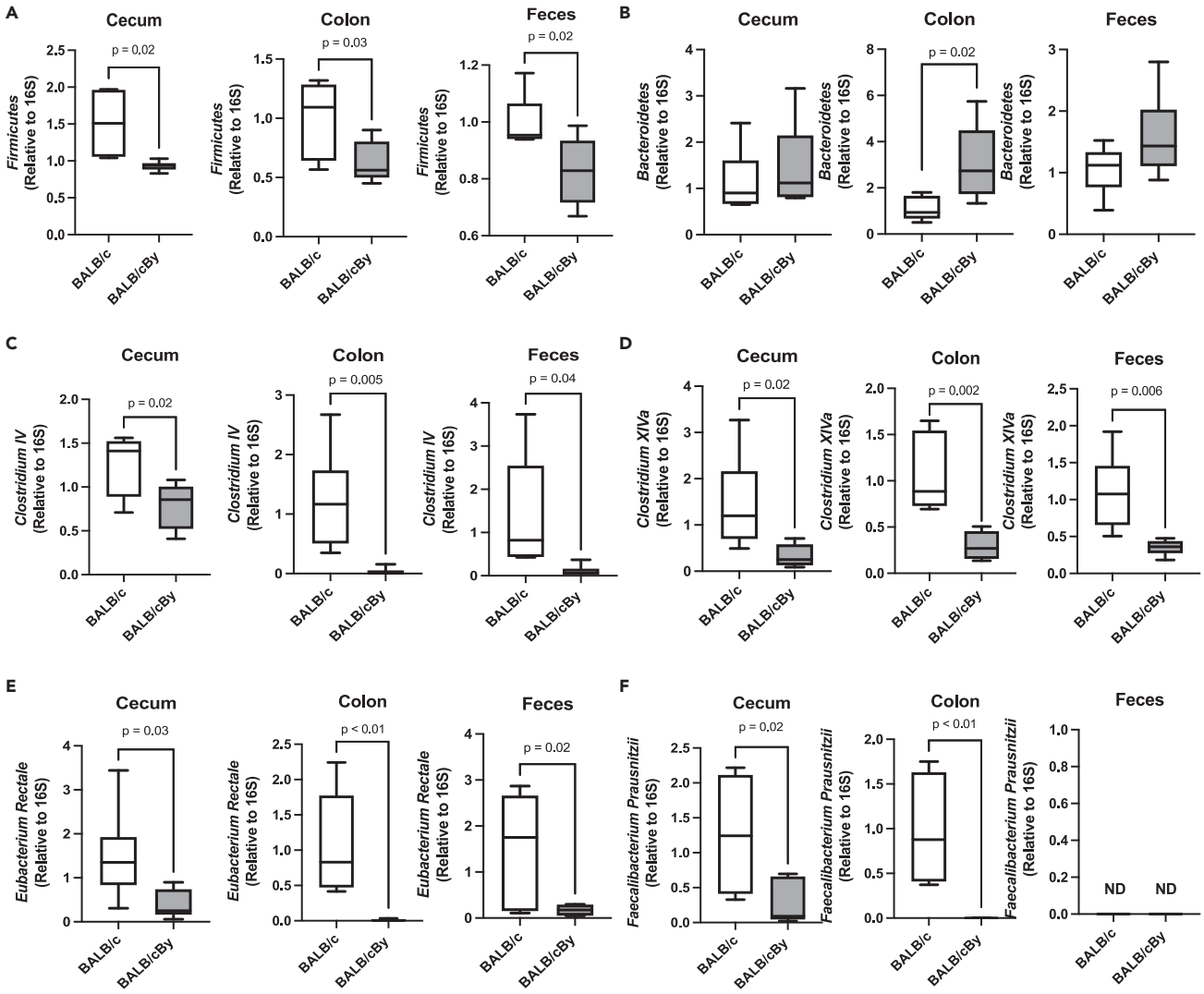

Figure 3. Exploring the BPB community based on qPCR analysis

(A) Quantification of the level of Firmicutes in different gut locations and feces of BALB/c and BALB/cBy mice relative to 16S.

(B) Quantification of the level of Bacteroidetes in different gut locations and feces of BALB/c and BALB/cBy mice relative to 16S.

(C) Quantification of the level of *Clostridium* XIVa in different gut locations and feces of BALB/c and BALB/cBy mice relative to 16S.

(D) Quantification of the level of *Clostridium* IV in different gut locations and feces of BALB/c and BALB/cBy mice relative to 16S.

(E) Quantification of the level of *Eubacterium rectale* in different gut locations and feces of BALB/c and BALB/cBy mice relative to 16S.

(F) Quantification of the level of *Faecalibacterium prausnitzii* in different gut locations and feces of BALB/c and BALB/cBy mice relative to 16S rRNA. For statistical analysis, qPCR was conducted three times per condition. Data are represented as +/− SEM.

BPB responded in HFD in wild-type mice

Fermentable dietary fiber contributes to an increased BPB population, associated with less inflammation and inflammatory disease development.[45] Differences in the prebiotic effect derived from fiber have been observed, where selective individuals do not increase BPB.[46–49]

Since SCAD-deficient BALB/cBy mice showed a diminishment in BPB as compared to BALB/c mice on a standard chow diet, a defined low-fiber diet (LFD) and HFD were used to test whether BALB/cBy mice would show a prebiotic effect represented by an increase in BPB from LFD to HFD. To study how dietary fibers affect BPB, BALB/c and BALB/cBy mice were randomly divided into four groups based on diet. They were fed a calorically matched LFD with cellulose and HFD with inulin to analyze the effects of diet on relative BPB levels.

The level of *but* and *buk* in BALB/c mice increased from LFD to HFD conditions; however, SCAD-deficient BALB/cBy mice showed no difference (Figures 4A and 4B). A similar result was found in analyzing *E. rectale* and *F. prausnitzii* where both were increased in the HFD condition of BALB/c mice, but there was no response in BALB/cBy mice in HFD compared to LFD group (Figures 4C and 4D). Furthermore, in conditions of low butyrate production and levels, there were no differences in the LFD groups between BALB/c and BALB/cBy when analyzing

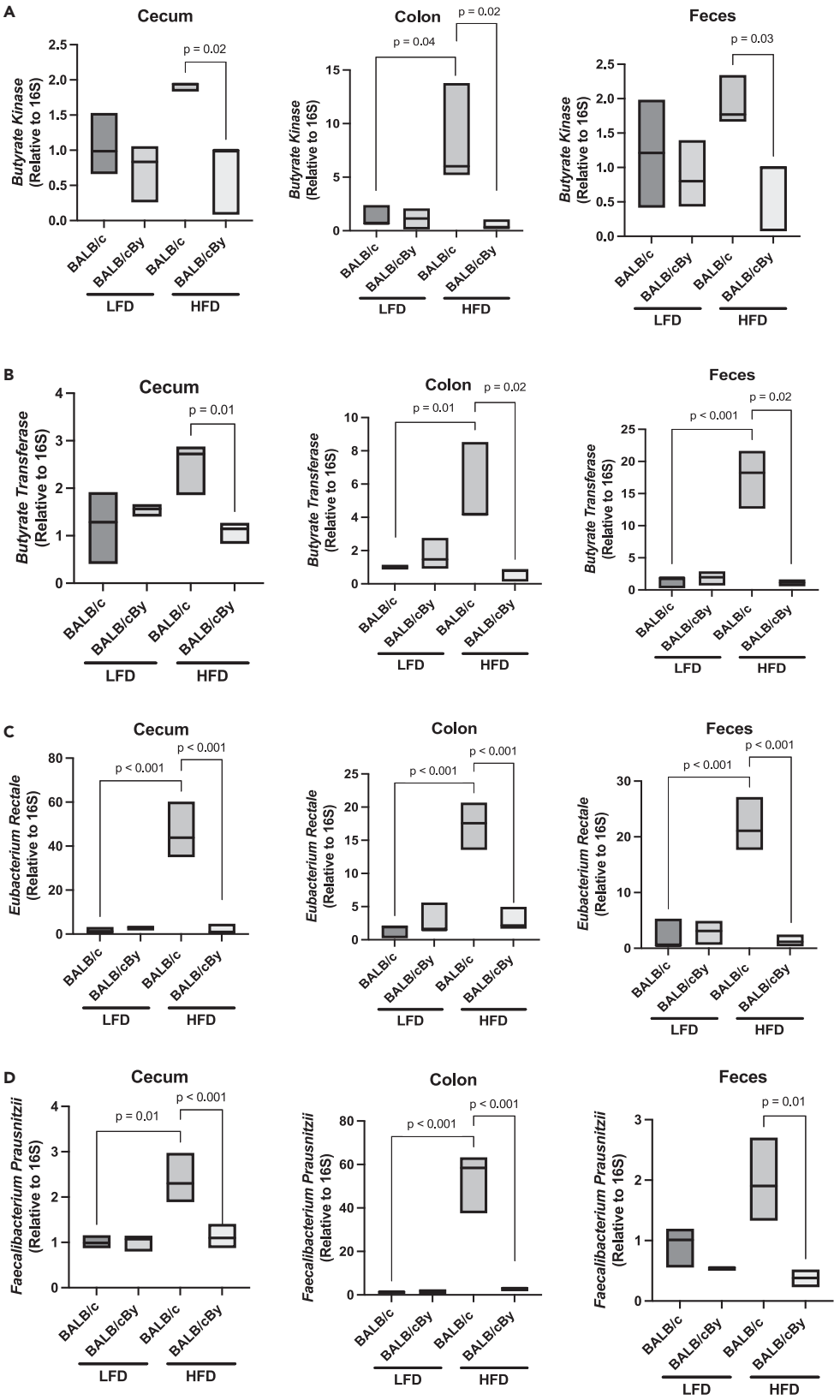
A
Cecum
Butyrate Kinase (Relative to 16S)
p = 0.02
Colon
p = 0.04
p = 0.02
Feces
p = 0.03
B
Cecum
Butyrate Transferase (Relative to 16S)
p = 0.01
Colon
p = 0.01
p = 0.02
Feces
p < 0.001
p = 0.02
C
Cecum
Eubacterium Rectale (Relative to 16S)
p < 0.001
p < 0.001
Colon
p < 0.001
p < 0.001
Feces
p < 0.001
p < 0.001
D
Cecum
Faecalibacterium Prausnitzii (Relative to 16S)
p = 0.01
p < 0.001
Colon
p < 0.001
p < 0.001
Feces
p = 0.01
BALB/c
BALB/cBy
LFD
HFD

Figure 4. Impact of a low-fiber (LFD) or high-fiber (HFD) diet on BPB levels in BALB/c and BALB/cBy mice

Mice were fed LFD containing 2% cellulose, or HFD containing 8% inulin for 2 weeks.

(A) Quantification of the level of *buk* in different gut locations and feces of BALB/c and BALB/cBy mice in each diet group relative to 16S.

(B) Quantification of the level of *but* in different gut locations and feces of BALB/c and BALB/cBy mice in each diet group relative to 16S.

(C) Quantification of the level of *E. rectale* in different gut locations and feces of BALB/c and BALB/cBy mice in each diet group relative to 16S.

(D) Quantification of the level of *F. prausnitzii* in different gut locations and feces of BALB/c and BALB/cBy mice in each diet group relative to 16S rRNA. For statistical analysis, qPCR was conducted three times per condition. Data are represented as +/− SEM.

but and *buk* or the species *E. rectale* and *F. prausnitzii* (Figure 4). These data are consistent with the idea that reduced butyrate oxidation in SCAD mutant colonocytes causes a decrease in the amount of BPB even with enough fermentable substrates.

DISCUSSION

The GI tract is a complex ecosystem in which the host intestinal epithelium, immune cells, and gut microbiota all coincide and interact to impact the overall host's health.[50] GI diseases such as inflammatory bowel disease or CRC are associated with changes in the gut microbiota community, which is influenced by general host physiology and numerous environmental factors including diet, hygiene, and the use of antibiotics.[51–53] The loss of gut microbiota diversity and decreased butyrate oxidation are major characteristics of patients with CRC and ulcerative colitis.[27,29] However, whether the diminished utilization of butyrate as an energetic source is promoting the gut microbial dysbiosis in these colon diseases is not known. It is especially relevant since this microbial dysbiosis is further defined by a diminishment in BPB. To test this relationship between oxidation of SCFAs and microbial composition, a non-disease SCAD-deficient mouse model was utilized and lowered SCFA oxidation resulted in decreased BPB at all taxonomic levels.

A limitation of this study and the use of the BALB/cBy mouse strain is that there are likely other genetic differences between BALB/c and BALB/cBy besides SCAD. Previous studies have identified changes in organic acids and intermediate metabolites (Table S3). Thus, although the direct result is an allele produces a non-functional protein SCAD in these mice, secondary effects toward changes in glycolysis cannot be discounted in mediating the effects toward BPB. Additionally, BALB/cBy mice harbor a whole-body diminishment in SCAD as opposed to a specific targeted deletion to the colonic epithelium. Nevertheless, it was demonstrated that SCAD-deficient mouse colonocytes have a significant decrease in butyrate oxidation and an increase in glycolysis, most likely through the loss of being able to use butyrate for ATP production or as an energetic substrate. The SCAD-deficient colonocytes have adapted to this energetic stress by increasing glucose utilization and glycolysis. Similarly, cancerous colonocytes display this same metabolic shift toward glycolysis, which is sometimes referred to as the Warburg effect.[27,35] Although these metabolic patterns are similar between SCAD-deficient colonocytes and cancerous colonocytes, it is likely that the reason for the increase in glycolysis is not because of the Warburg effect in SCAD-deficient colonocytes, but rather because they can obtain energy from glycolysis as a compensatory mechanism. This is supported through the fact that AMPK is activated in the SCAD-deficient colonocytes. In addition, loss of SCAD would diminish the oxidation of acetate, thus a contribution from decreased oxidation of acetate in the colonocyte cannot be ruled out in regards to regulating BPB.

An important feature of BPB is the strict anaerobic conditions these bacteria require.[25] The oxidation of butyrate by the colonocyte consumes a considerable amount of oxygen, thereby creating hypoxic conditions (<1% O_2 or <7.6 mmHg) in the colonic lumen.[54,55] A plausible mechanism is that the reduction of SCAD and butyrate oxidation in the BALB/cBy colonocyte increased the oxygen level within the colonic lumen, which subsequently resulted in the diminishment of BPB. Moreover, since oxygen diffuses freely across epithelial membranes, the elevated oxygen concentration in the colonocytes would drive an increased oxygen availability in the intestinal lumen. Proteobacteria, which are generally facultative anaerobes and can tolerate an aerobic environment, are increased in fecal contents of BALB/cBy mice compared to BALB/c mice (Figure S3). In contrast to Proteobacteria, an aerobic environment in the GI tract of BALB/cBy would most likely deplete BPB and help drive potential pathogen expansion.[56,57] Moreover, since there is an inherent deficit in the colonocyte's ability to oxidize butyrate, attempting to increase butyrate levels and/or utilization via a HFD to stimulate BPB was ineffective. This suggests that reductions in SCAD or reduced butyrate oxidation may be an aspect of lack of prebiotic response. Patients with CRC where SCAD reduction is observed show a decreased level of BPB.[58–61] Based on these results, patients with CRC may not respond very well to prebiotics used to increase BPB.

The composition of the gut microbiome directly impacts the production of microbial-derived metabolites, including butyrate.[10,62,63] Fermentable dietary fiber contributes to an increase in the BPB population, which is associated with less inflammation and inhibition in the development of inflammatory bowel disease.[45] These results provide additional support toward the importance of diet-microbiome-host interactions in determining microbial composition and function. These data also highlight the importance of host metabolism in achieving a prebiotic effect from diet.

Limitations of the study

This study provides experimental evidence toward the process involving host metabolism of SCFAs, which includes butyrate, in regulating the composition of the GI microbiome, and specifically BPB. The study is limited by two major factors that include the use of non-isogenic mouse strains, BALB/c and BALB/cBy, and the general loss of SCAD in all cells as opposed to targeted deletion to specific tissue or cell type limits the interpretation that effects observed in BALB/cBy mice are selective to SCAD loss in the colonic epithelium.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Dallas R. Donohoe (ddonohoe@utk.edu).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- 16S sequencing data used in Figure 2 have been deposited at SRA database and are publicly available with BioProject accession number PRJNA1148109.
- This manuscript does not report original code.
- Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Dallas Donohoe (ddonohoe@utk.edu).

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AUTHOR CONTRIBUTIONS

B.P. helped design the experiments, performed the experiments, analyzed the data, and wrote part of the manuscript. J.Y.K. helped perform the experiments and analyzed the data. O.F.R. helped perform the experiments and analyzed the data. T.J.W. helped analyze the data. J.J. helped in the design of the experiments and analyzed the data. D.R.D. designed the experiments, analyzed the data, wrote part of the manuscript, and oversaw the completion of the study.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2024.110853.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Anti-SCAD	Abcam	Cat# ab154823; RRID: AB_2716802
Anti-MCT1	Abcam	Cat# ab179832
Anti-AMPKα	Cell Signaling	Cat# 5831; RRID: AB_10622186
Anti-pAMPKα (Thr172)	Cell Signaling	Cat# 2535; RRID: AB_331250
Anti-ACC	Cell Signaling	Cat# 3676; RRID: AB_2219397
Anti-pACC (Ser79)	Cell Signaling	Cat# 11818; RRID: AB_2687505
Anti-β-actin	Cell Signaling	Cat# 3700; RRID: AB_2242334
Chemicals, peptides, and recombinant proteins		
EDTA	Fisher Scientific	#S311-500
Feta bovine serum	Cytivia	#SH3091003
Glucose	Sigma	#G8270
Carnitine	Acros	#541-15-1
Sodium butyrate	Sigma	#B5887
2-Deoxy-glucose	Thermo Scientific Chemicals	#AAL0733806
Sodium azide	Sigma	#S8032
Oligomycin	Alfa Aesar	#AAJ61898MA
RIPA buffer	Cell Signaling	#9806s
PMSF	Cell Signaling	#8553
0.5M EDTA solution	Thermo Scientific	#R1021
Halt Phosphatase Inhibitor Cocktail	Thermo Scientific	#PI87786
Power Up SYBR master mix	Life technologies	#A25742
Critical commercial assays		
Pierce BCA Protein Assay Kit	Thermo Fisher	#PI23228
DNeasy PowerSoil Pro Kit	QIAGEN	#47014
Deposited data		
16s rRNA sequencing	Sequence Read Archive (SRA)	[SRA]: BioProject [PRJNA1148109]
Experimental models: Organisms/strains		
BALB/c	The Jackson Laboratory	RRID:IMSR_JAX:000651
BALB/cBy	The Jackson Laboratory	RRID:IMSR_JAX:001026
Oligonucleotides		
All primers used for qPCR, See Table S1	This study	N/A
Software and algorithms		
GraphPad Prism9	GraphPad Software	https://www.graphpad.com/
Image Studio Software	LO-COR Biosciences	https://www.licor.com/bio/image-studio/
Applied Biosystems 7300 Real-Time PCR System	Applied Biosystems	https://www.thermofisher.com/us/en/home/technical-resources/software-downloads/applied-biosystems-7300-real-time-pcr-system.html
R	CRAN	https://r-project.org
Mothur	1.48.0	https://mothur.org/wiki/download_mothur/

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Other		
Low-fiber diet	Research Diet	D11112201, Formula1
High-fiber diet	Research Diet	D11112201, Formula3
XF24 cell culture microplates	Agilent Technologies	#100850

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Animals

All mice (BALB/c *n* = 66, BALB/cBy *n* = 66) in this study were obtained from The Jackson Laboratories (Bar Harbor, ME) (BALB/c RRID:IMSR_JAX:000651; BALB/cBy RRID:ISMR_JAX:001026) or were bred in-house from these stocks. All mouse experiments were performed on mice 8–10 weeks of age at 22°C–23°C with 12h light/dark shift. The International Animal Care and Use Committee (IACUC) reviewed and approved animal research at the University of Tennessee, Knoxville (Protocol #2673). All experiments were performed according to relevant guidelines and regulations.

METHOD DETAILS

Diets

Each diet was from Research Diet. The LFD (Research Diet, #D11112201, Formula 1) contained 2% cellulose, whereas the HFD (Research Diet, #D11112201, Formula 3) contained 8% inulin. The formulas used in these diets are listed in Table S2. BALB/c (*n* = 5) and BALB/cBy (*n* = 5) mice were fed each diet for 2 weeks.

Primary colonocytes isolation

Mouse colonic epithelial cells were isolated from excluding enteric neurons, immune cells, and smooth muscle. The colons were dissected from the euthanized mice, flushed with sterile phosphate-buffered saline (PBS), and placed in PBS solution containing 5 mM EDTA (Fisher Scientific, #S311-500) and 1% FBS (Cytivia, #SH3091003). The submerged colon was incubated for ~30 min at 37°C on a rotator. The colon tissues were removed, and the left isolated colonocytes were collected by centrifuge and used for experiments.

Flux experiments

Seahorse XFe24 Extracellular Flux Analyzer (Seahorse Bioscience, North Billerica, MA) was used to measure butyrate oxidation and glycolysis. BALB/c (*n* = 3) and BALB/cBy (*n* = 3) mice were used for the flux experiments. All Seahorse assay experiments were conducted following the manufacturer's instructions. The % change of the OCR after butyrate injection was measured. The collected mouse primary colonocytes were seeded in XF[24] cell culture microplates (Agilent Technologies, #100850). 1x Krebs-Henseleit Buffer (KHB) including 5 mM glucose (Sigma, #G8270) and 500 μM carnitine (Acros, #541-15-1) were used as the culture medium for the butyrate oxidation analysis. Briefly, for the butyrate oxidation analysis, 1x KHB media or sodium butyrate (Sigma, #B5887) at 5mM final concentration were injected, and the change in OCR was measured from baseline (%OCR). Next, 2DG 50mM (Thermo Scientific Chemicals, # AAL0733806) was injected to competitively inhibit glucose utilization and leave butyrate as the only exogenous energy substrate. Finally, 10% sodium azide (Sigma, #S8032) was injected to block mitochondrial respiration by inhibiting complex IV. Also, for the glycolysis stress test, 1x KHB media or glucose at 10 mM final concentration were injected, and the change in ECAR was measured from baseline (%ECAR). Then, Oligomycin 2uM (Alfa Aesar, #AAJ61898MA) was injected to inhibit ATP synthase (complex V), resulting in an increased dependence on glycolysis following basal measurement. At last, 2DG 50mM was injected to inhibit glucose utilization competitively and functioned to shut down glycolysis.

Western blot analysis

BALB/c (*n* = 4) and BALB/cBy (*n* = 4) mice were used for Western blot experiments. Proteins from the collected mouse primary colonocytes were extracted with 1x RIPA buffer (Cell Signaling, #9806s), 1mM PMSF (Cell Signaling, #8553), 0.5M EDTA Solution (Thermo Scientific, #R1021), and Halt Phosphatase Inhibitor Cocktail (Thermo Scientific, #PI87786). Protein samples were collected by centrifuge at 13,000 x g for 10 min at 4°C, and protein supernatant was transferred to a new 1.5 mL Eppendorf tube. Protein concentrations were determined by Pierce BCA Protein Assay Kit (Thermo Fisher, #PI23228). Proteins were separated on 10 and 12% SDS-polyacrylamide gels and transferred onto the PVDF membrane. Membranes were incubated on a rotator in 5% BSA in 1x TBST (0.1%) for 1 h at room temperature (RT). Blocked membranes were incubated overnight at 4°C with the respective primary antibodies on a rotator. Antibodies that were used included SCAD (Abcam, #ab154823), MCT1 (Abcam, #ab179832), AMPKα (Cell Signaling, #5831), pAMPKα (Cell Signaling, #2535s), ACC (Cell Signaling, #3676s), pACC (Cell Signaling, #11818s), and β-actin (Cell Signaling, #3700). Blots were washed 3 times for 10 min/wash in 1 x TBST (0.1%) at RT and incubated with secondary antibody for 2h on rotator at RT following 3 times for 10 min/wash in 1x TBST (0.1%) at RT. Fluorescent or

chemiluminescence detection was performed with the Odyssey Fc, and bands were quantified with Image Studio Software (LI-COR Biosciences, Lincoln, NE). The antibody used in this study for SCAD can detect both *scad 1* isoform and *scad 2* isoform.[61]

Microbial DNA preparation and quantitative real-time PCR analysis

BALB/c (n = 6) and BALB/cBy (n = 6) mice were used for the microbial DNA preparation and quantitative real-time PCR analysis. Microbial DNA was isolated from the collected feces, dissected cecum, colon, and flushed feces from the dissected colon. Total bacterial DNA was isolated using DNeasy PowerSoil Pro Kit (QIAGEN, #47014) according to the manufacturer's instructions. Total DNA was quantified using the NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). About 100ng of DNA from each sample was used to analyze the bacterial population. Primers for quantifying specific bacterial groups and *buk* and *but* gene are given in Table S1. Targeted DNA expression was measured quantitatively using the Power Up SYBR master mix (Life technologies, #A25742). Thermocycling was done as follows; 2 min at 95°C; 45 s at 95°C, 45 s at 54°C, 45 s at 72°C (x35); 10 min at 72°C.

16S rRNA sequencing

BALB/c (n = 5) and BALB/cBy (n = 6) mice were used for the 16S rRNA sequencing. Microbial DNA was isolated using DNeasy PowerSoil Pro Kit according to the manufacturer's instructions. Total DNA was quantified using the NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE) and 16S rRNA V4 libraries were amplified. Illumina adapters with Nextera indexed barcodes were added onto the amplicons. These were sequenced on an Illumina MiSeq using v3 chemistry. The reads were then analyzed using Mothur, and sequences were aligned to SILVA 16s reference alignment. The OTU analysis was performed in R.

QUANTIFICATION AND STATISTICAL ANALYSIS

For Figures 1, 2, 3, and 4, an ANOVA was used to test for differences between experimental groups followed by a Tukey post-hoc test, with the Prism 9 software. All data are expressed as mean ± SEM.