RESEARCH ARTICLE

Differential Modulation by *Akkermansia muciniphila* and *Faecalibacterium prausnitzii* of Host Peripheral Lipid Metabolism and Histone Acetylation in Mouse Gut Organoids

Sabina Lukovac,[a] Clara Belzer,[b] Linette Pellis,[a] Bart J. Keijser,[a] Willem M. de Vos,[b,c] Roy C. Montijn,[a] Guus Roeselers[a]

Microbiology & Systems Biology, TNO, Zeist, The Netherlands[a]; Laboratory of Microbiology, Wageningen University, Wageningen, The Netherlands[b]; Departments of Bacteriology & Immunology and Veterinary Biosciences, University of Helsinki, Helsinki, Finland[c]

S.L. and C.B. contributed equally to this article.

ABSTRACT **The gut microbiota is essential for numerous aspects of human health. However, the underlying mechanisms of many host-microbiota interactions remain unclear. The aim of this study was to characterize effects of the microbiota on host epithelium using a novel *ex vivo* model based on mouse ileal organoids. We have explored the transcriptional response of organoids upon exposure to short-chain fatty acids (SCFAs) and products generated by two abundant microbiota constituents, *Akkermansia muciniphila* and *Faecalibacterium prausnitzii*. We observed that *A. muciniphila* metabolites affect various transcription factors and genes involved in cellular lipid metabolism and growth, supporting previous *in vivo* findings. Contrastingly, *F. prausnitzii* products exerted only weak effects on host transcription. Additionally, *A. muciniphila* and its metabolite propionate modulated expression of Fiaf, Gpr43, histone deacetylases (HDACs), and peroxisome proliferator-activated receptor gamma (Pparγ), important regulators of transcription factor regulation, cell cycle control, lipolysis, and satiety. This work illustrates that specific bacteria and their metabolites differentially modulate epithelial transcription in mouse organoids. We demonstrate that intestinal organoids provide a novel and powerful *ex vivo* model for host-microbiome interaction studies.**

IMPORTANCE **We investigated the influence of the gut microbiota and microbially produced short-chain fatty acids (SCFAs) on gut functioning. Many commensal bacteria in the gut produce SCFAs, particularly butyrate, acetate, and propionate, which have been demonstrated to reduce the risk of gastrointestinal disorders. Organoids—small crypt-villus structures grown from ileal intestinal stem cells—were exposed to SCFAs and two specific gut bacteria. *Akkermansia muciniphila*, found in the intestinal mucus, was recently shown to have a favorable effect on the disrupted metabolism associated with obesity. *Faecalibacterium prausnitzii* is a commensal gut bacterium, the absence of which may be associated with Crohn's disease. We showed that in our model, *A. muciniphila* induces stronger effects on the host than *F. prausnitzii*. We observed that *A. muciniphila* and propionate affect the expression of genes involved in host lipid metabolism and epigenetic activation or silencing of gene expression. We demonstrated that organoids provide a powerful tool for host-microbe interaction studies.**

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Address correspondence to Guus Roeselers, guus@roeselers.com.

The gut microbiota plays an important role in the regulation of human health and pathogenesis of disease, such as obesity, type 2 diabetes, and inflammatory bowel disease (IBD) (1–4).

Common microbial metabolites, such as butyrate, acetate, propionate, and other short-chain fatty acids (SCFAs), have been shown to affect multiple intestinal cell signaling pathways. Besides serving as an energy source, SCFAs can signal through several G protein-coupled receptors to elicit a wide range of cellular responses (5). Butyrate has been shown to inhibit histone deacetylases (HDACs), thereby inducing histone hypermethylation, leading to changes in gene transcription (6). In addition, butyrate was found to induce a specific transcriptional response in the human colon, affecting genes and pathways involved in fatty acid oxidation, epithelial integrity, and apoptosis (7). Elucidation of the functional and mechanistic *in vivo* implications of these host-microbiota interactions holds great potential for the development of therapeutic targets for many metabolic (and possibly other) diseases (8).

The gut microbiota has been shown to be involved in regulation of intestinal barrier function and nutrient absorption, as well as fat metabolism and storage in mice (9–11). However, effects of the microbiota on the host metabolism are highly diet, species, and strain dependent, making it difficult to draw definitive conclusions regarding underlying mechanisms (12, 13). To circumvent issues of diet and species discrepancy and to study direct effects of specific bacteria on intestinal epithelial function at the molecular and cellular levels, several *in vitro* studies with both human and rodent intestinal epithelial cell lines exposed to bacterial monocultures or SCFAs have been performed. Grootaert et al. (14) studied the effects of bacterial monocultures (*Clostridium*

perfringens, *Enterococcus faecalis*, *Bacteroides thetaiotaomicron*, and *Escherichia coli*) and SCFAs on human intestinal epithelial cell lines, where both bacterial monocultures and SCFAs were shown to modulate fasting-induced adipose factor/angiopoietin-like protein (Fiaf/Angptl4) expression. The thoroughly investigated Fiaf protein is a multifunctional signal protein expressed in several tissues (15) and is upregulated during fasting, hypoxia, and adipocyte differentiation (16, 17) and has been found to link the gut microbiota and obesity (18). In addition, Fiaf has been suggested to modulate lipid metabolism in different tissues (19). These studies extended our understanding of the role of microbial communities in regulation of host cellular function, but the effects analyzed were only representative for one epithelial cell type. Moreover, the cell lines used, which have been maintained for decades in the laboratory, may not show the dynamic responses found in primary and differentiated intestinal cells. Hence, the effect of the gut microbiota on other epithelial cell types and mucosal function remains to be elucidated.

To study and understand the dynamic interactions between the intestinal epithelium and the microbiota, a need for robust, physiologically representative, and reproducible model systems has emerged (20). Organoids, cultured from crypts or single $Lgr5^+$ stem cells, hold great potential as a sophisticated intestinal model (21). These stem-cell-based gut organoids make an attractive *ex vivo* model system in two ways. First, organoids show self-renewing capacity. Second, they recapitulate the *in vivo* tissue architecture, consisting of both stem cells and differentiated functional epithelial cells, namely, enterocytes, goblet cells, enteroendocrine cells, and Paneth cells, the latter previously impossible to culture (21, 22).

The aim of this study was to determine the effects of important intestinal bacteria and their products on host ileal epithelium gene expression.

For this purpose, we have selected two symbiotic and numerically abundant members of the human gut microbiota, *Akkermansia muciniphila* and *Faecalibacterium prausnitzii*. *A. muciniphila* is a mucus-colonizing member of the microbiota and may constitute up to 3% of the gut microbiota (23). Its mucin degradation activity leads to the production of propionate and acetate (24). An extensive study regarding the effects of *A. muciniphila* on host intestinal function revealed modulation of host intestinal epithelial genes involved in basal metabolism (25). *F. prausnitzii* is also an abundant intestinal anaerobe that can make up approximately 4% of the mainly luminal microbiota (26). It induces an anti-inflammatory immune response in a mouse model of inflammation, while butyrate, the main SCFA produced by *F. prausnitzii*, was indicated to be unresponsive in the disease suppression (27). Both bacteria are considered promising next generation probiotics (28).

The effects of bacterial species and SCFA on host gene expression were previously assessed in mouse ileal intestinal tissue and Caco-2 cells (25, 29, 30) The latter phenotypically resemble differentiated ileal intestinal enterocytes, both morphologically and physiologically, rather than colonic epithelial cells (31).

In addition to the colon, the microbiota of the distal ileum, which consists of up to 10^8 bacterial cells/g, including *A. muciniphila* and *F. prausnitzii* (32), is another source of SCFA production. For these reasons, we applied distal ileal organoids *t*o elucidate the effect of these microbiota members and their products on intestinal epithelial cells.

We exposed mature ileal organoids to supernatant collected from *A. muciniphila* and *F. prausnitzii* cultures, as well as to individual butyrate, propionate, and acetate solutions.

Subsequently, an extensive analysis of gene transcription modulations and pathway analysis was performed by means of a microarray analysis.

We focused on 5 genes coding for the following products that have been shown to be involved in cell cycle control, adipocyte function, and peripheral lipid metabolism: (i) Fiaf, involved in the deposition of triglycerides in adipocytes (19); (ii) G protein-coupled receptor 43 (Gpr43), which binds SCFAs and is involved in several pathological conditions, such as obesity and inflammatory diseases (33); (iii) histone deacetylase 3 (Hdac3) and (iv) Hdac5, epigenome-modifying enzymes responsible primarily for deacetylation of lysine residues within histones (6); and (v) peroxisome proliferator-activated receptor gamma (Pparγ), previously shown to be involved in microbiota-induced expression of Fiaf (34).

The results revealed distinct transcriptional responses elicited by the intestinal symbionts and indicate that *A. muciniphila* has the strongest impact, notably on the lipid metabolism, as may be expected from its location within the intestinal mucus layer.

RESULTS

Growth of *A. muciniphila* and *F. prausnitzii* and SCFA analysis of the conditioned media. *A. muciniphila* and *F. prausnitzii* were grown anaerobically, harvested at the end of their exponential phase, and used for later incubation studies. To further understand the role of the conditioned medium, its SCFA and residual sugar compositions were determined. The medium conditioned by *A. muciniphila* contained residual concentrations of 0.71 mM glucose and 0.77 mM L-fucose, while the SCFAs included 3.65 mM acetate and 7.14 mM propionate. The medium conditioned by *F. prausnitzii* contained a residual amount of 0.16 mM glucose, while the SCFAs included 1.51 mM acetate, 5.51 mM formate, 7.06 mM propionate, and 8.03 mM butyrate. It should be noted that the small amount of propionate in the conditioned growth medium of *F. prausnitzii* is due to the addition of this SCFA to stimulate growth, mimicking the rumen conditions (35). At 7 days after splitting (Fig. 1), mature intestinal organoids were exposed to either the cultured cells or the conditioned medium from both strains for 3 h, followed by the gene expression analysis as described below.

Exposure to *A. muciniphila* and *F. prausnitzii* results in strain-specific effects on gene expression of intestinal organoids. Organoids exposed to either of the bacterial monoculture-conditioned media showed distinct transcriptomic responses, as indicated by the hierarchical clustering of genes of replicates exposed to same conditions (Fig. 2A). Analysis of the data through an unsupervised clustering method (principal component analysis [PCA]) further supported the specific effects of the conditioned media on the clustering of expressed genes of the intestinal organoid genes (Fig. 2B). On top of this, organoids exposed to *A. muciniphila*-conditioned medium (Am+ in Fig. 2B) showed a distinct pattern of gene expression compared to its control consisting of only growth medium (Am− in Fig. 2B). Hence, it is evident that *A. muciniphila* compounds induce a different cluster of genes than just its culture medium as these are well separated in the PCA plot. In contrast, an overlapping set of clusters of genes are found in organoids that have been exposed to

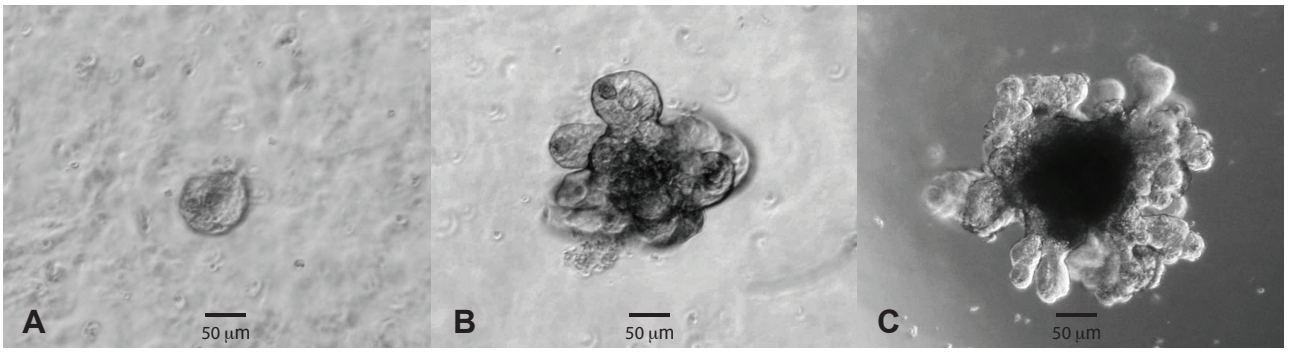

FIG 1 Bright-field images of mouse small-intestinal organoids cultured in Matrigel for 1 (A), 3 (B), and 7 (C) days after splitting.

F. prausnitzii-conditioned medium (Fp+ in Fig. 2B) and its control medium (Fp− in Fig. 2B), suggesting that metabolites from *F. prausnitzii* do not have a major effect on the gene expression profile. Interestingly, the number of overlapping genes affected by both *A. muciniphila*- and *F. prausnitzii*-conditioned media was very low, namely 7 in total (see Fig. S1 in the supplemental material).

Regarding the effects of exposure to SCFA, our results demonstrate that each SCFA elicits a specific response on gene expression in the intestinal epithelium (Fig. 2C and D; see Fig. S1C and S1D). However, propionate and butyrate share the largest number of host epithelial genes that were affected by exposure. The greatest effect on gene expression was observed after exposure to butyrate (Fig. 2B). Acetate did not induce major gene expression changes relative to the control treatment, and the cluster of genes affected by acetate was relatively close to genes exposed to the control condition (Fig. 2B). The intrinsic biological variation within groups was relatively small, amounting to <5% (calculated as Pearson's coefficient), and no extreme outliers were detected.

***A. muciniphila* and its metabolite propionate regulate expression of transcription factors and genes involved in host metabolic pathways.** *A. muciniphila*-conditioned medium affected the expression of 1,005 genes in intestinal organoids by fold changes of 1.5 and higher ($P = 0.01$), of which 503 were upregulated, and 502 were downregulated (Fig. 2C). The number of genes affected by exposure to *F. prausnitzii*-conditioned medium was only 190, of which 86 genes were upregulated and 104 genes were downregulated (Fig. 2D). The differences and similarity in genes affected by the SCFA were also compared (see Fig. S1C and S1D in the supplemental material); however, the main focus was the analysis of genes affected by the two intestinal bacteria, and the comparison of the effects of individual SCFAs produced (butyrate, propionate, and acetate). Genes affected by *A. muciniphila*-conditioned medium showed the greatest overlap with organoid genes affected by exposure to propionate or butyrate (204 genes upregulated and 202 genes downregulated by all three conditions) (Fig. 2C). Genes with a specific response to individual SCFAs were analyzed and compared to genes affected by *A. muciniphila*. The *A. muciniphila*-conditioned medium induced the expression of genes with the greatest overlap with those induced upon exposure to butyrate (67 genes upregulated and 68 genes downregulated) (Fig. 2C). Compared to propionate, the *A. muciniphila*-conditioned medium showed overlap of 12 upregulated genes and 23 downregulated genes specifically affected by propionate (Fig. 2C). Very little overlap was observed between genes affected in organoids exposed to *A. muciniphila*-conditioned medium and acetate (with 1 gene upregulated and 0 downregulated under both conditions) (Fig. 2C). Overall, 33 genes were upregulated in organoids treated under any of the four conditions of *A. muciniphila*, butyrate, propionate, and acetate (Fig. 2C), whereas 10 genes were affected in organoids exposed to any of these four conditions (Fig. 2C).

Organoids exposed to *F. prausnitzii*-conditioned medium showed a very limited number of genes that were similarly affected by exposure to SCFAs (Fig. 2D)—namely less than 10 genes were affected by exposure to any of the four conditions.

To analyze the effect of microbiota and SCFA on the main regulators of gene expression in the intestinal epithelium, we focused on the effects of these conditions on transcription factor expression (Fig. 3A). We observed that major overlap of effects on transcription factor expression was evident after treatment with *A. muciniphila* and propionate and butyrate. However, butyrate additionally affected expression of specific transcription factors in the intestinal epithelium of organoids not affected by other conditions (Fig. 3A). The expression of a very small number of transcription factors was affected specifically by exposure of organoids to acetate or propionate (Fig. 3A). Subsequently, the overlapping transcription factors affected by both *A. muciniphila* and its main metabolite, propionate, were analyzed. Many transcription factors regulating lipid metabolism and proliferation were affected by both conditions, such as Hnf4α and members of p53 family of proteins (Tp53 and Tp73), respectively (Fig. 3B) (36). Supporting these effects of *A. muciniphila* and propionate on transcription factor expression, Ingenuity pathway analysis (IPA) demonstrated lipid metabolism as one of the top 10 altered associated networks affected by exposure to *A. muciniphila*-conditioned medium (Fig. 3C). The main regulators of lipid metabolism, such as nuclear receptor Lxr (Nr1h3), Cpt1, and 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) synthase (37), were all altered in their expression in response to *A. muciniphila* exposure, supporting previous findings in mice where cholesterol homeostasis and lipoprotein metabolic processes were affected after *A. muciniphila* colonization (25). More detailed information and the effects of the other three conditions on molecular, cellular, and biological pathways are shown in Table S1 and Table S2 in the supplemental material. Database for Annotation, Visualization and Integrated Discovery (DAVID) analyses of gene ontologies in intestinal organoids exposed to *A. muciniphila*, *F. prausnitzii*, propionate, butyrate, and acetate confirmed our findings by IPA and demonstrated large overlap between the effects of *A. muciniphila*

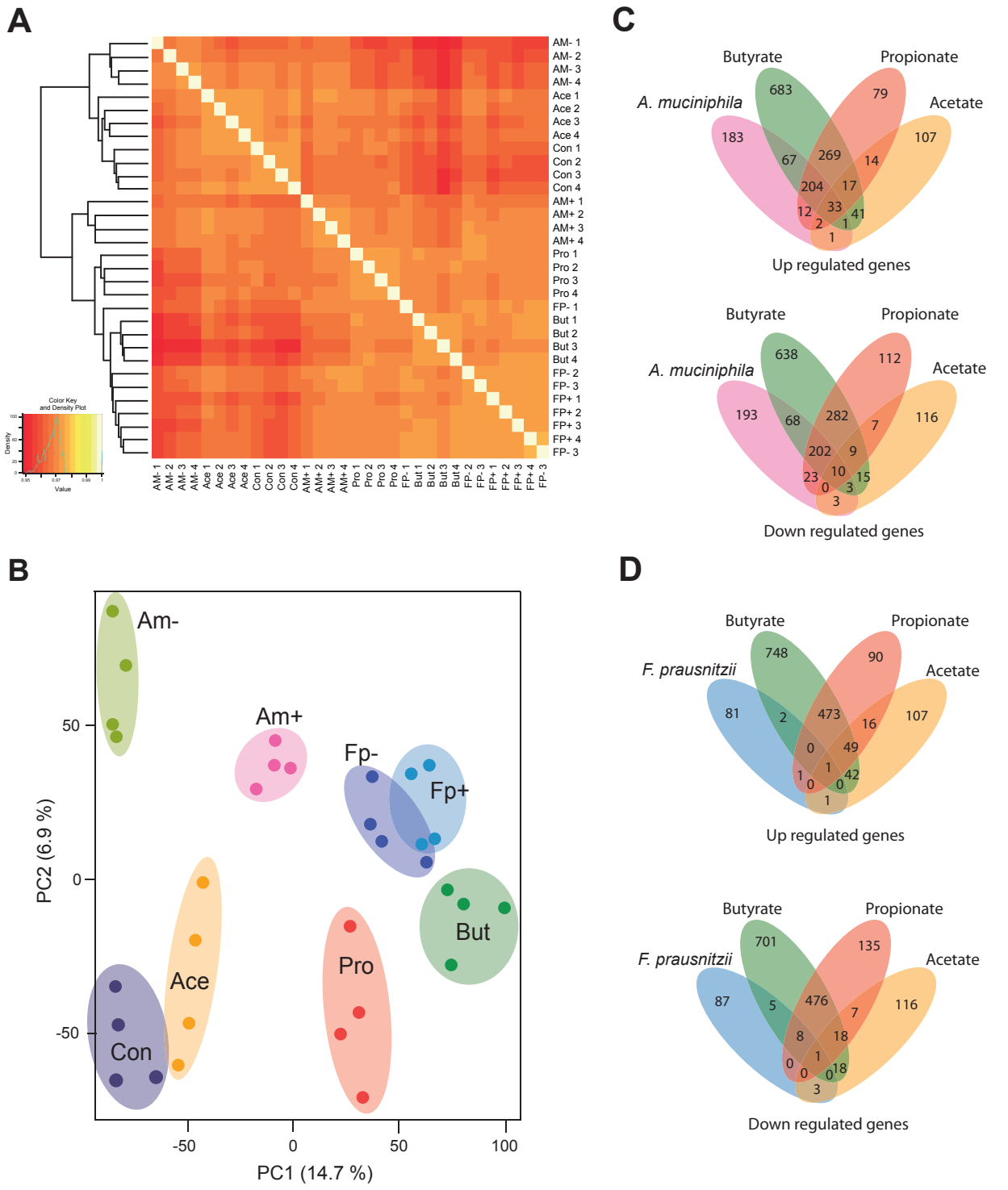

FIG 2 Distinct transcriptome signatures in intestinal organoids upon stimulation with bacterial monocultures and SCFAs. (A) Heat map and hierarchical clustering representing the array correlation plot of microarray data from all replicates. (B) PCA plot of the microarray data showing the distribution of different experimental treatment groups. Clusters represent samples exposed to *A. muciniphila* culturing medium (Am−), *A. muciniphila*-conditioned medium (Am+), *F. prausnitzii* culturing medium (Fp−), *F. prausnitzii*-conditioned medium (Fp+), acetate (Ace), butyrate (But), propionate (Pro), and standard organoid culturing medium (Con). (C) Venn diagrams representing the number of genes up- and downregulated by exposure of organoids to *A. muciniphila*-conditioned medium relative to their respective controls (unconditioned microbial culture medium) compared to exposure to the SCFAs butyrate, propionate, and acetate. (D) Venn diagrams representing the number of genes up- and downregulated by exposure to *F. prausnitzii*-conditioned medium relative to the respective controls compared to exposure to butyrate, propionate, and acetate.

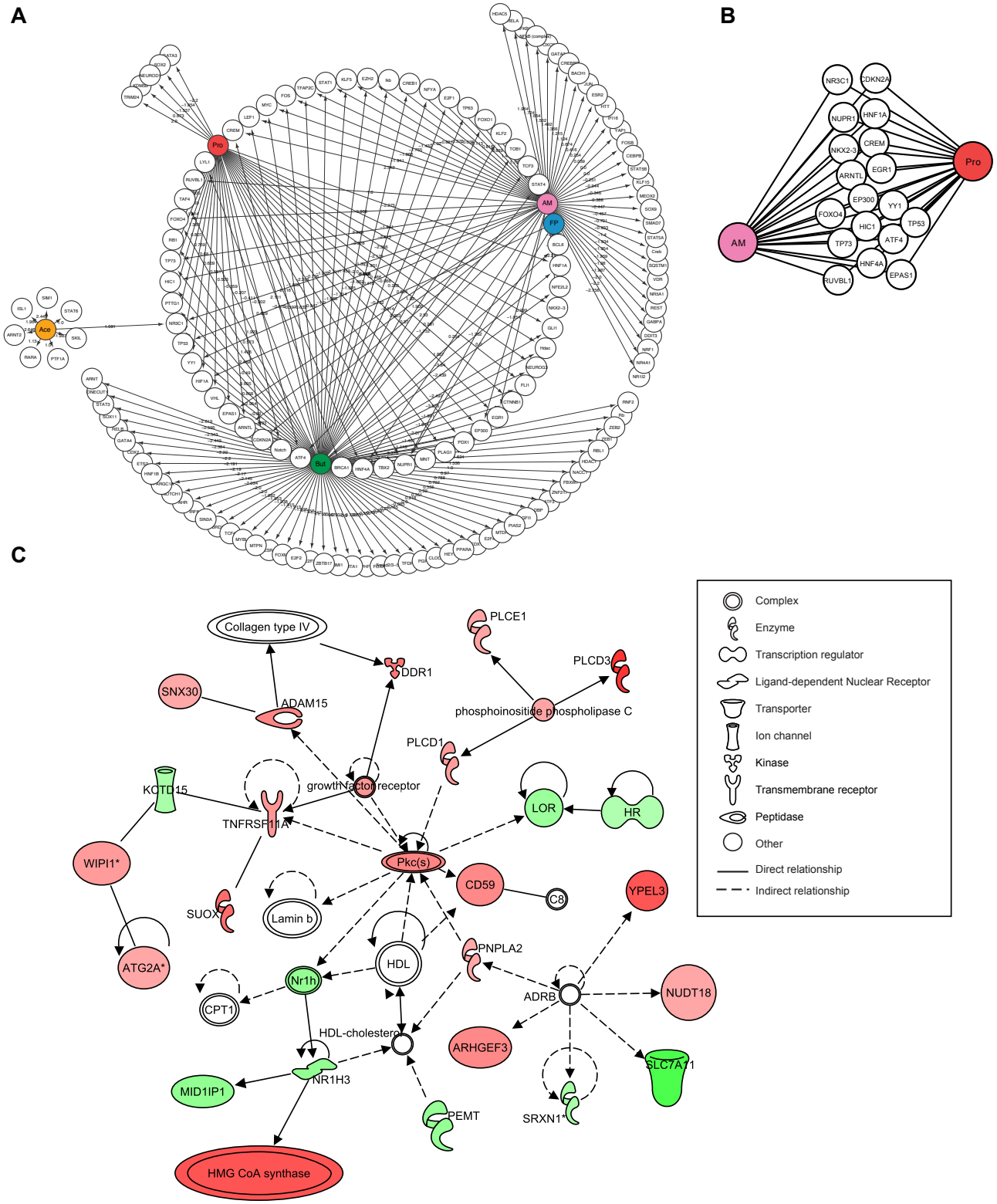

FIG 3 *A. muciniphila* regulates the expression of many host transcription factors and genes involved in cellular metabolic function. (A) Network representation of transcription factors affected by *A. muciniphila*, *F. prausnitzii*, and SCFAs. (B) Transcription factors affected in organoids after exposure to both *A. muciniphila* and its metabolite propionate. (C) IPA network demonstrating the effect of *A. muciniphila* on expression of genes involved in lipid metabolism. Nodes in green and red correspond to down- and upregulated genes, respectively. Noncolored nodes are proposed by IPA and suggest potential targets functionally coordinated with the differential genes.

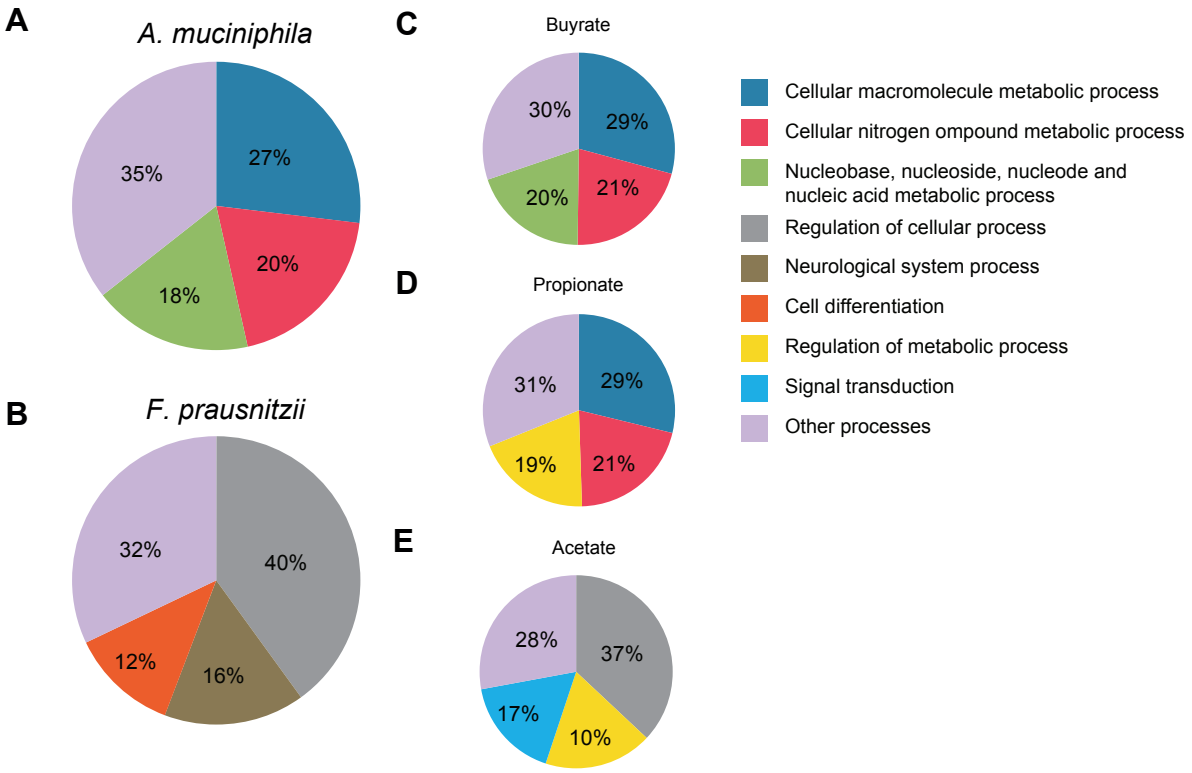

FIG 4 Many metabolic processes are affected in organoids after exposure to *A. muciniphila* and propionate. Pie charts show the distribution of affected genes in relation to *A. muciniphila* (A), *F. prausnitzii* (B), butyrate (C), propionate (D), and acetate (E) based on their annotations in biological functions (Gene Ontology).

with propionate and butyrate on genes specifically involved in metabolic processes (Fig. 4). Acetate and *F. prausnitzii* affected different biological pathways than the other three conditions, both mainly affecting general cellular processes, including cellular differentiation (*F. prausnitzii*) and signal transduction (acetate) (Fig. 4B and 3E).

***A. muciniphila*, propionate, and butyrate, but not *F. prausnitzii* and acetate, regulate expression of Fiaf, Gpr43, and HDACs in intestinal epithelial organoids.** Previous studies suggested that SCFAs, mainly butyrate and propionate, modulate adiposity regulation via direct effect on Fiaf, Gpr, and Pparγ gene expression (34, 38). Furthermore, transcription of a broad range of genes through HDAC modulation was shown after exposure of intestinal cell lines to butyrate (6). Therefore, we decided to analyze the expression of these specific genes in organoids in response to exposure to SCFAs (Fig. 5). Our data show that, *F. prausnitzii* has no significant effect on expression of Fiaf, Gpr43, HDACs, or Pparγ (Fig. 5). *A. muciniphila* leads to significantly decreased expression of Gpr43 and Pparγ and increased expression of Hdac3 and Hdac5 (Fig. 5). It should be noted that the observed *A. muciniphila*-induced decrease of Fiaf is not significant. These results are partially supported by the effects of both butyrate and propionate stimulation, leading to significant increase in Fiaf expression, while the expression of Gpr43 and Pparγ is significantly decreased (Fig. 5). In addition, the expression of Hdac3 is significantly increased by all three SCFAs (Fig. 5). Acetate had no significant effect on the expression of Fiaf, Gpr43, Hdac5, or Pparγ, while the expression of Hdac3 was significantly increased by acetate (Fig. 5). Expression profiles of organoids exposed to *F. prausnitzii* did not show any overlap with the changes observed after exposure to individual SCFAs (Fig. 5).

DISCUSSION

The present study demonstrates the applicability of small intestinal epithelial organoids as a novel model system to study host-microbiota interactions, as well as high similarity to *in vivo* host epithelial response to microbiota. Interestingly, we observed that different members of the human gut microbiota elicit different gene responses in the organoids. We observed a strong regulation of host epithelial genes involved in lipid metabolism and lipolysis by *A. muciniphila* and the microbial metabolites propionate and butyrate. These results are well in agreement with previous *in vitro* and *in vivo* studies describing the effects of microbial monocultures and SCFAs on mouse host intestinal epithelium (9, 11, 25, 19).

Notably, we observed very little overlap between the genes up- or downregulated upon exposure to individual SCFAs and genes affected by exposure to conditioned media that also contained SCFAs (Fig. 2C and D). This may be caused by the fact that the conditioned media contained a complex mixture of compounds that might induce a different transcriptional response. Also, those genes similarly affected by culture media alone were subtracted and are therefore excluded from this comparison.

Although the functional relevance of some of the observed up- and downregulation of genes is difficult to reconcile with known cellular pathways, altogether, the observations with the mouse organoids align with those from other mouse studies, illustrating the usefulness of the organoid model.

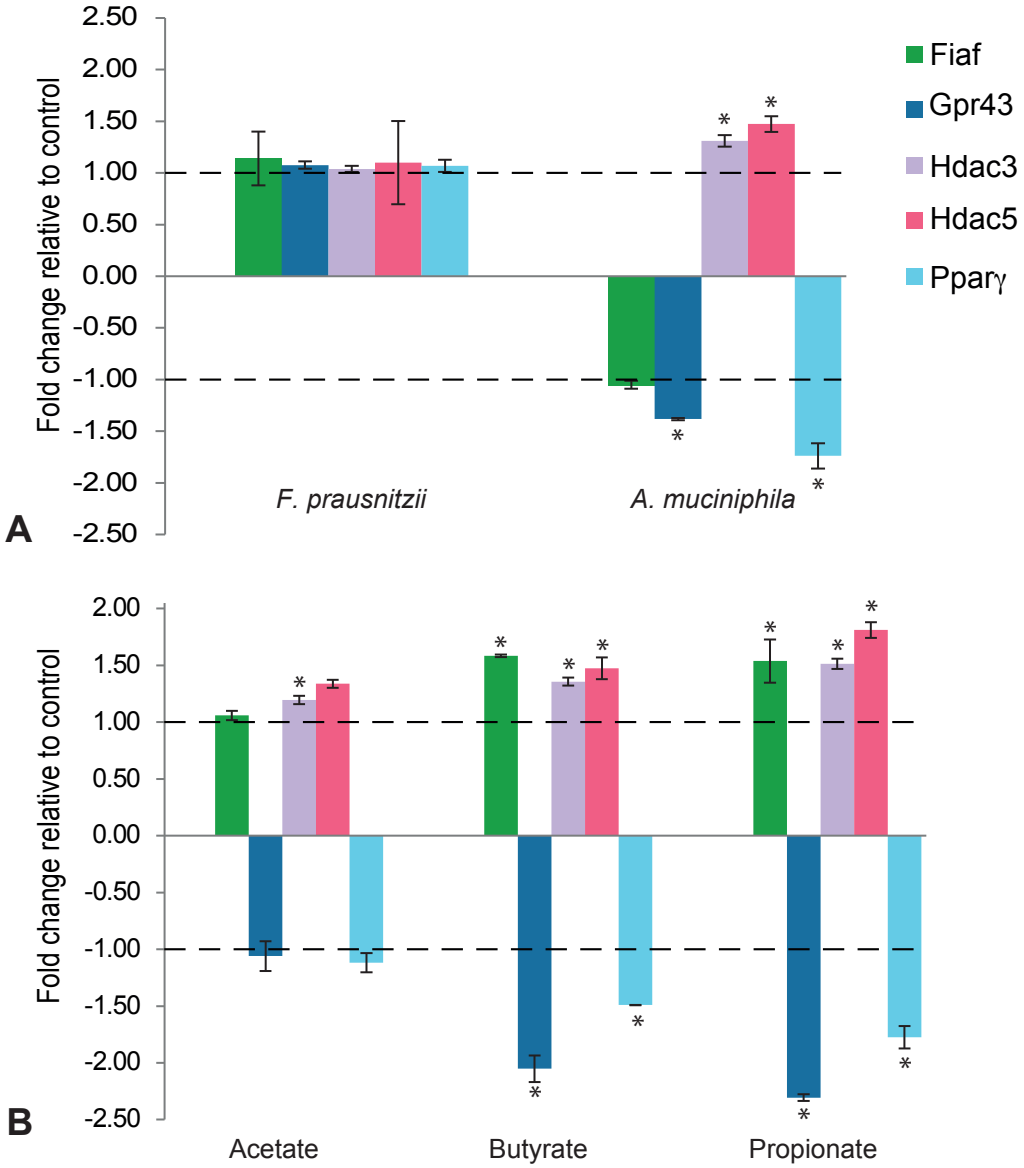

FIG 5 Effects of *A. muciniphila*, propionate, and butyrate on expression of metabolic genes coding for Fiaf, Gpr43, Hdac3, and Hdac4 in intestinal organoids. (A) Log fold change of gene expression after exposure to *F. prausnitzii* and *A. muciniphila* relative to control conditions (A) and after exposure to butyrate, propionate, and acetate relative to control conditions (B) (mean fold change ± standard deviation [SD]; $P < 0.01$; $n = 4$).

Overall, modifications in gene expression in organoids upon exposure to *A. muciniphila*, *F. prausnitzii*, or SCFAs involved mostly changes in expression of genes that contribute to regulation of metabolic pathways and genes involved in cellular growth and survival networks. This implies that the microbiota strongly affects the molecular and physiological function of the intestinal epithelium and underscores the importance of studying the interaction of both systems in addressing relevant (patho)physiological research questions, rather than studying isolated host epithelium and intestinal microbial systems. As previously demonstrated for *A. muciniphila* and *Lactobacillus plantarum*, different microbial inhabitants of the host may induce expression-specific mouse host genes at different locations (25). We confirmed this finding using the organoid system, in which we demonstrate very strong gene expression modifications upon *A. muciniphila* product exposure, while *F. prausnitzii* products only alter the expression of a limited number of genes in organoids after hours of exposure. The specificities of *A. muciniphila* and *F. prausnitzii* could be explained by the different physiologies of the two bacterial species, the metabolites they produce, and the different segments of the intestine they colonize.

Genes affected by *A. muciniphila* were involved in cellular metabolism, cell growth, and survival. The effect of *A. muciniphila* on cell survival was previously demonstrated *in vivo* in germfree mice by (25). In agreement with our findings, it was also demonstrated in germfree mice colonized with *A. muciniphilla* that a large number of genes involved in cell survival, more specifically in cell death receptor signaling, were affected in ileal tissue (25). The same study demonstrated the effect of *A. muciniphila* on lipid homeostasis and metabolism in cecum samples from germfree mice col-

onized with *A. muciniphila*, confirming our results regarding transcriptomic changes in organoids exposed to *A. muciniphila*-conditioned medium. We observed several key transcription factors and genes involved in fatty acid, cholesterol, and bile acid metabolism, such as those coding for Lxr, Cpt1, and HMG-CoA synthase, which were affected by *A. muciniphila*. This reveals a possible molecular mechanism underlying the effects of *A. muciniphila* on lipid metabolism in intestinal epithelium of the host (25). Similar conclusions were evident from *in vitro* findings in the human enterocyte cell line Caco-2, where cholesterol biosynthesis was downregulated upon SCFA administration (39).

SCFAs, mostly butyrate, have previously been identified as potent regulators of HDACs (6), associated with various biological processes, including transcriptional regulation of interleukin-8 (IL-8) and monocyte chemoattractant protein 1 (MCP-1) expression (40). Alenghat et al. (41) recently showed that Hdac3 expression is reduced in tissues from mice and humans with inflammatory bowel disease and that mice with gut epithelial cell-specific deletion of Hdac3 show loss of Paneth cells and an altered gut microbiota composition. This suggests a critical role for Hdac3 in maintaining the symbiotic balance between host and gut microbiota. Here, both *Hdac3* expression and *Hdac5* expression were significantly increased after exposure to physiological concentrations of both butyrate and propionate, supporting the hypothesis that these SCFAs regulate transcriptional response of the host. Notably, we show that in addition to butyrate and propionate, *A. muciniphila* products also affected the expression of HDACs, suggesting regulation of host transcriptional response by *A. muciniphila* via histone acetylation modifications.

Fiaf is postulated to play a role in the protection from obesity in germfree mice by selectively being suppressed in the intestinal epithelium of normal mice by conventionalization and by being fasting responsive (18, 42). However, a direct role of Fiaf as a mediator in the relationship between gut colonization and adiposity has been disputed as well (12, 13). Hepatic Fiaf has been shown to directly modulate lipid biosynthesis in liver tissue (19). Here, we analyzed the expression of Fiaf in response to both bacteria and SCFAs. We demonstrate that physiologically relevant SCFA concentrations (5 mM) lead to increased intestinal Fiaf expression in organoids. Furthermore, we show that the increase in Fiaf is accompanied by increased expression of genes regulating cholesterol metabolism. This is in agreement with previous rat studies that show that SCFAs regulate cholesterol synthesis in both liver and intestinal tissue (43) and with human intestinal cell line studies, where SCFAs were shown to regulate cholesterol biosynthesis (39) and to induce Fiaf expression (34).

In conclusion, our data illustrate that different symbiotic microbial species trigger specific transcriptome responses in the intestinal epithelium, most likely depending on the metabolic products produced. Further studies are required in order to translate these transcription dynamics into physiologically relevant responses. We propose that organoids provide a novel, physiologically relevant, gut model system to further explore microbiota-epithelial interactions underlying nutrition- and microbiota-induced health effects.

MATERIALS AND METHODS

Mouse ileal crypt isolation. Ileal tissue from an adult male wild-type (WT) C57BL/6 mouse (Charles River) was excised longitudinally and flushed with cold phosphate-buffered saline (PBS). The upper epithelium (villi) was scraped, and remaining tissue was washed again with cold PBS. Subsequently, the tissue was cut into 5-mm pieces and incubated in 5 mM EDTA for 30 min in order to release the crypts from the underlying muscle layer. After incubation, the released crypts were removed from the muscle layer and used for the organoid culture protocol, as described previously (21).

Organoid culture. In short, murine (WT C57BL/6) small intestinal crypts were isolated and embedded in 250 μl Matrigel (BD Biosciences) and submerged in 500 μl basal culture medium supplemented with penicillin-streptomycin, HEPES, Glutamax, N2, B27, *N*-acetylcysteine, and the murine growth factors epidermal growth factor (EGF), noggin, and R-spondin-1 (ENR), as previously described (21). Organoids were passaged every 7 days with a 1:4 splitting ratio (Fig. 1).

Exposure to bacterial cultures and SCFAs. *A. muciniphila* (ATTC BAA-835) (24) was grown anaerobically at 37°C in a basal liquid medium that contained the following (per liter of deionized water): 0.4 g KH_2PO_4, 0.669 g Na_2HPO_4 plus $2H_2O$, 0.3 g NH_4Cl, 0.3 g NaCl, 0.1 g $MgCl_2$ plus $6H_2O$, 10 g Casitone, 1 mM L-threonine, 1 ml trace mineral solution, 5 mM L-fucose, and 5 mM D-glucose.

F. prausnitzii strain A2-165 (DSM 17677) was grown anaerobically at 37°C in a liquid medium that contained the following (per liter of deionized water): 10 g Casitone, 2.5 g yeast extract, 4 g $NaHCO_3$, 1 ml resazurin (0.1% [wt/vol]), 0.45 g K_2HPO_4, 0.45 g KH_2PO_4, 0.9 g $(NH_4)_2SO_4$, 0.9 g NaCl, 0.09 g $MgSO_4$, 0.09 g $CaCl_2$, 2.5 mM acetate, 9 mM propionate, 1 mM *n*-valerate, 1 mM isovalerate, 1 mM isobutyrate, 0.28 g KOH, 2.5 ml ethanol (95%), 10 mg hemin, 10 μg biotin, 10 μg cobalamin, 30 μg *para*-aminobenzoic acid, 50 μg folic acid, 150 μg pyridoxamine, 1 g L-cysteine, and 25 mM glucose (35). The concentrations of the following organic acids and sugars were determined in the culture medium before and after growth using a high-performance liquid chromatography (HPLC) device equipped with a Shodex Sugar SH-G and Shodex SH1821 column as described previously (44): glucose, mannose, galactose, L-fucose, glucose-*N*-acetylglucosamine, lactate, formate, acetate, propionate, 1,2-propendiol, and butyrate. Overnight-grown cultures were pelleted by centrifugation at 20 000 × g for 10 min, and supernatant was collected. At 7 days after splitting, organoids were exposed to 250 μl *A. muciniphila* supernatant, 250 μl unconditioned *A. muciniphila* culture medium, 85 μl *F. prausnitzii* supernatant, or 85 μl *F. prausnitzii* culture medium for 3 h at 37°C, prior to RNA extraction. A 250-μl concentration of *A. muciniphila* supernatant and culture medium SCFAs sodium butyrate, sodium propionate, and sodium acetate (Sigma-Aldrich, Zwijndrecht, The Netherlands) was administered at a final concentration of 5 mM. At 7 days after splitting, mature organoids were exposed to individual SCFAs for 3 h at 37°C prior to RNA extraction.

RNA extraction. Cultured organoids were pooled (approximately 300 organoids per sample), and RNA was isolated using Trizol (Invitrogen, Breda, The Netherlands), followed by purification using the RNeasy kit (Qiagen) and DNase treatment. Samples were assayed for quantity and quality with the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA) and NanoDrop spectrophotometer (NanoDrop, Wilmington, DE).

Data are presented from $n = 4$ independent biological replicates from one line of organoids.

Microarray and statistical analysis. Total mRNA from the organoids was amplified, biotinylated, and randomly hybridized to MouseRef-8 v2 Expression bead arrays (Illumina, Inc., San Diego, CA), followed by scanning and feature extraction. Gene expression data from the Illumina beadchip microarrays proved to be highly reproducible, as indicated by low intersample variation among technical replicates.

Quality control, RNA labeling, hybridization, and data extraction were performed at ServiceXS (Leiden, The Netherlands). RNA concentrations were measured using the Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE). RNA quality and integrity were determined using Lab-on-Chip analysis on an Agilent 2100 Bioana-

lyzer (Agilent Technologies, Inc., Santa Clara, CA) and on Shimadzu MultiNA RNA analysis chips (Shimadzu Corporation, Kyoto, Japan). Biotinylated cRNA was prepared using the Illumina TotalPrep RNA amplification kit (Ambion, Inc., Austin, TX) according to the manufacturer's specifications with an input of 200 ng total RNA. Per sample, 750 ng of the obtained biotinylated cRNA samples was hybridized onto the Illumina MouseRef-8 v2 (Illumina, Inc., San Diego, CA). Each BeadChip contains eight arrays. Hybridization and washing were performed according to the instructions in the Illumina *Direct Hybridization Assay Guide* (Illumina, Inc. San Diego, CA). Scanning was performed on the Illumina iScan (Illumina, Inc., San Diego, CA). Image analysis and extraction of raw expression data were performed with the Illumina GenomeStudio v 2011.1 Gene Expression software with default settings (no background subtraction and no normalization).

Data were extracted using GenomeStudio. Quality control and normalization (quantile) of microarray data were performed using the lumi package of Bioconductor, available at www.bioconductor.org. Nonexpressed genes were removed by filtering on the detection value ($P \geq 0.99$ in at least one sample). This resulted in gene expression values for 25,697 probes. For differential expression analysis, Limma software was used. Subsequently, expression data of the transcriptomics were log transformed (base 2). Probes were used for biological interpretation if the P value threshold of <0.01 was passed and if the fold change between two conditions was $|1.5|$. In order to characterize those genes specifically affected by microbial compounds we normalized transcription profiles by subtracting the genes similarly affected by the unconditioned culture medium from genes affected by microbially conditioned medium. Further information on the function and biological role of the probes was derived from Ingenuity pathway analysis (IPA) (Ingenuity Systems, Redwood City, CA), the Database for Annotation, Visualization and Integrated Discovery (DAVID), and Cytoscape.

Microarray data accession number. Raw microarray data have been deposited in the GEO database (http://www.ncbi.nlm.nih.gov/geo/) under accession no. GSE59644.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at http://mbio.asm.org/lookup/suppl/doi:10.1128/mBio.01438-14/-/DCSupplemental.

Figure S1, JPG file, 0.1 MB.
Table S1, DOCX file, 0.1 MB.
Table S2, DOCX file, 0.1 MB.
Table S3, DOCX file, 0.1 MB.

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