1 Microbial regulation of hexokinase 2 links mitochondrial metabolism and 2 cell death in colitis

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56 **Summary**

57 Hexokinases (HK) catalyze the first step of glycolysis and thereby limit its pace.
 58 HK2 is highly expressed in the gut epithelium, plays a role in immune responses
 59 and is upregulated in inflammation and ulcerative colitis ¹⁻³. Here, we examined
 60 the microbial regulation of HK2 and its impact on intestinal inflammation by
 61 generating mice lacking HK2 specifically in intestinal epithelial cells (*Hk2^{ΔIEC}*).
 62 *Hk2^{ΔIEC}* mice were less susceptible to acute intestinal inflammation upon
 63 challenge with dextran sodium sulfate (DSS). Analyzing the epithelial
 64 transcriptome from *Hk2^{ΔIEC}* mice during acute colitis revealed downregulation
 65 of cell death signaling and mitochondrial dysfunction dependent on loss of HK2.
 66 Using intestinal organoids derived from *Hk2^{ΔIEC}* mice and Caco-2 cells lacking
 67 HK2, we identified peptidyl-prolyl cis-trans isomerase (PPIF) as a key target of
 68 HK2-mediated regulation of mitochondrial permeability and repression of cell-
 69 death during intestinal inflammation. The microbiota strongly regulated HK2
 70 expression and activity. The microbially-derived short-chain fatty acid (SCFA)
 71 butyrate repressed HK2 expression and oral supplementation protected
 72 wildtype but not *Hk2^{ΔIEC}* mice from DSS colitis. Our findings define a novel
 73 mechanism how butyrate may act as a protective factor for intestinal barrier
 74 homeostasis and suggest targeted HK2 inhibition as a promising therapeutic
 75 avenue in intestinal inflammation.

76 Hexokinases (HK) catalyze the first step of glycolysis and thereby limit the rate
77 of this fundamental biological process. HK2 is considered the prototypic
78 inducible isoform of all HK family members as it can be upregulated by various
79 environmental factors and signaling pathways, e.g. during inflammation and in
80 ulcerative colitis ¹⁻³. In addition to its metabolic function, HK2 acts as a receptor
81 for bacterial cell wall components ⁴ and has been suggested to counteract
82 mitochondria-mediated cell death ⁵. Chemical inhibition of HK impairs immune
83 cell activation and promotes infection by *Listeria monocytogenes* in immune
84 cells ⁶, whereas hyperglycemia and high glycolytic flux have been associated
85 with an increased risk of enteric infection in intestinal epithelial cells ⁷. However,
86 complete ablation of HK2 is embryonically lethal ⁸. In the intestine, *Hk2* is
87 predominantly expressed by intestinal epithelial cells (IECs) ^{9,10}. Therefore, we
88 aimed to investigate, whether selective ablation of HK2 in IECs alters epithelial
89 function during intestinal inflammation. Here we show that (i) loss of HK2 in
90 IECs protects from DSS-induced colitis by decreasing inflammation-induced
91 epithelial cell death and that (ii) specific bacterial species and the microbial
92 metabolite butyrate ameliorate colitis by repressing *Hk2* expression.

93 To determine the role of epithelial HK2 for intestinal inflammation, we generated
94 *Hk2^{fl/fl}-Villin::Cre⁺* mice lacking HK2 specifically in IECs, hereinafter referred to
95 as *Hk2^{ΔIEC}* mice. We used littermate *Hk2^{fl/fl}* mice, hereinafter referred to as WT
96 mice, as controls. Unchallenged *Hk2^{ΔIEC}* mice did not display any major inflam-
97 matory or metabolic phenotype except for an improved glucose tolerance (Ex-
98 tended data Figure 1), compared to littermate controls. However, when *Hk2^{ΔIEC}*
99 mice and their WT littermates were challenged with dextran sodium sulfate
100 (DSS) to induce intestinal inflammation, *Hk2^{ΔIEC}* mice lost significantly less

weight compared to WT littermates (Fig. 1a). The disease activity index (DAI), a measure of intestinal inflammation comprised of weight loss, stool consistency and fecal blood occurrence, confirmed the ameliorated disease course in *Hk2^{ΔIEC}* mice (Fig. 1b). Additionally, *Hk2^{ΔIEC}* mice displayed lower serum levels of the pro-inflammatory cytokine KC/CXCL1 as measured by ELISA (Fig. 1c). Histological evaluation of Hematoxylin and Eosin (H&E)-stained colon sections demonstrated a reduced score consisting of transmural inflammation, crypt hyperplasia, epithelial injury, polymorphonuclear and mononuclear cell infiltrates in *Hk2^{ΔIEC}* mice (Fig. 1d). In WT mice, HK2 expression increased during the course of colitis (Fig. 1e, Extended data figure 2). We therefore investigated whether *Hk2* expression is dysregulated in patients suffering from intestinal inflammation by evaluating expression data from colon-biopsies of patients suffering from Crohn's Disease (CD), Ulcerative Colitis (UC) or Non-IBD-Colitis (NIC) ¹¹. Biopsies were taken both from the site of inflammation and adjacent non-inflamed tissue. We found that *Hk2* expression was significantly higher in the inflamed tissue of CD (p = 0.010) and NIC (p = 0.027) patients, while only a trend was observed in UC patients (p = 0.180) (Fig. 1f). This data is in agreement with a published report revealing upregulation of *Hk2* expression in UC patients suggesting inhibition of HK2 as a potential therapeutic approach ³. To decipher the molecular mechanisms protecting *Hk2^{ΔIEC}* mice from intestinal inflammation, we isolated IECs from WT and *Hk2^{ΔIEC}* mice on day 0 (baseline), 3 (early inflammation) and 7 (late inflammation) from an independent acute DSS experiment and performed RNA sequencing. While we did not find any differentially expressed genes on day 3, we identified 420 differentially expressed genes on day 7 in comparison between WT and *Hk2^{ΔIEC}* mice (Extended data

Figure 3 and Extended data Table 1). Gene Ontology (GO) analysis of these differentially expressed genes revealed a downregulation of genes involved in cell death-signaling and regulation of mitochondrial membrane permeability in *Hk2^{ΔIEC}* mice (Fig. 1g). We confirmed a reduction of cell death in *Hk2^{ΔIEC}* mice by TUNEL-staining of colon sections from day 3 and day 7 of the experiment. In accordance with our transcriptomics data, we found fewer TUNEL-positive cells in the tip compartment of colonic crypts in *Hk2^{ΔIEC}* mice at day 7 indicating less cell death (Fig. 1h). This finding coincides with the spatial expression profile of HK2, which is mainly restricted to the colonic epithelial tip (Extended data Figure 4).

To further study the molecular processes involved in HK2-dependent protection from inflammation, we generated intestinal organoids derived from *Hk2^{ΔIEC}* and WT mice and investigated their response to stimulation with tumor necrosis factor (TNF). Western blot analysis demonstrated higher HK2 levels upon TNF stimulation (Fig. 2a). Organoids derived from *Hk2^{ΔIEC}* mice exhibited lower levels of cleaved Caspase 3 and Poly(ADP-Ribose)-Polymerase 1 (PARP1), markers for mitochondria-related types of cell death, compared to WT organoids upon TNF stimulation. This data therefore supported reduced levels of cell death in the absence of HK2 under inflammatory conditions. Furthermore, our transcriptome data suggested dysregulated mitochondrial function as a consequence of loss of HK2. We therefore performed metabolic flux analysis using Seahorse technology. As the 3D structure of organoids limits their use in this assay, we generated a Caco-2 cell clone lacking HK2 using the CRISPR Cas9 system, hereinafter named Caco-2^{ΔHk2}. We assessed glycolytic flux by meas-

150 uring the extracellular acidification rate (ECAR) upon addition of glucose to in-
151 duce glycolytic flux, oligomycin to stress the glycolytic reserve and 2-deoxy-
152 glucose to inhibit glycolysis. Throughout the entire experiment, we did not ob-
153 serve any significant changes between Caco-2^{ΔHk2} and Caco-2^{WT} cells indicat-
154 ing that ablation of HK2 did not affect glycolytic function (Fig. 2b). We also
155 measured the basal oxygen consumption rate (OCR), which comprises both
156 mitochondrial and non-mitochondrial oxygen consumption. Interestingly, we
157 discovered significantly lower basal mitochondrial respiration as well as a dras-
158 tically lower maximal mitochondrial respiration in Caco-2^{ΔHk2} cells compared to
159 Caco-2^{WT} cells (Fig. 2c). Since FCCP (cyanide-4-(trifluoromethoxy)-phenylhy-
160 drazone) induces maximal respiration by depolarization of the mitochondrial
161 membrane, our data therefore pointed towards a decrease in mitochondrial per-
162 meability. These results support our findings from transcriptome sequencing
163 and GO analysis, which also indicated a decreased regulation of mitochondrial
164 permeability. We therefore screened our transcriptome data for differentially
165 expressed genes that are involved in regulation of mitochondrial membrane
166 permeability. mRNA levels of *Ppif* (peptidyl-prolyl cis-trans isomerase), encod-
167 ing for a main component of the mitochondrial permeability transition pore
168 (MPTP), were downregulated in IECs of Hk2^{ΔIEC} mice on day 7 of colitis (Fig.
169 2d). PPIF coordinates mitochondrial permeability and metabolism¹²⁻¹⁵ and has
170 been suggested to directly interact with HK2 to suppress cell death⁵. To vali-
171 date our *in vivo* findings and transcriptome data, we stimulated Caco-2^{ΔHk2} and
172 Caco-2^{WT} cells with TNF and IL17A¹⁶ as well as with IFN-β to induce inflam-
173 matory responses and cell death. Indeed, *Ppif* expression was downregulated
174 under both conditions (Fig. 2e). Based on our findings and since PPIF interacts

175 with HK2⁵ and since *Ppif*^{-/-} mice are less susceptible to colitis¹⁷, we propose
176 that mechanistically the HK2-dependent protection from intestinal inflammation
177 could be mediated by lower levels of PPIF and a subsequent decrease in MPTP
178 opening and mitochondrial membrane permeability.

179 Dysregulated host-microbiota interactions are a key element of intestinal in-
180 flammation¹⁸. By comparing the transcriptomes of intestinal epithelial cell frac-
181 tions isolated from germ-free (GF) and conventionally raised (CR) mice¹⁹, we
182 identified HK2 as significantly upregulated by the presence of a complex micro-
183 bial community. *Hk2* expression was specifically induced in the epithelial tips of
184 both ileum and colon by the microbiota (Fig. 3a). Upon colonization of GF mice
185 with a normal microbiota *Hk2* expression increased and normalized to that of
186 CR mice, demonstrating that the intestinal microbiota stimulates *Hk2* expres-
187 sion (Fig. 3b). However, although we were able to show a distinct effect of the
188 microbiota on *Hk2* expression, ablation of HK2 in IECs in turn did not impact
189 the composition of the intestinal microbiota as assessed by 16S rRNA amplicon
190 sequencing under basal unchallenged conditions (Extended data Fig. 5). Next,
191 we investigated how the microbiota regulates epithelial *Hk2* expression, specif-
192 ically whether only specific bacterial species modulate *Hk2* expression. To that
193 end, we colonized GF mice with either single bacterial species or minimal con-
194 sortia - namely the Altered Schaedler Flora (ASF)²⁰ and the Oligo Mouse Mi-
195 crobiota (OMM)²¹. Both minimal microbial consortia readily induced *Hk2* ex-
196 pression to a similar level as observed in CR mice (Fig. 3c). Mono-colonization
197 with the Gram-negative bacterium *Bacteroides thetaiotaomicron* was also able
198 to induce *Hk2* expression, whereas the Gram-negative bacterium *Escherichia*
199 *coli* and the Gram-positive bacterium *Bifidobacterium longum* did not alter *Hk2*

mRNA levels (Fig. 3c). Together, this data suggested a specific interaction between specific bacterial features and epithelial cells rather than general principles such as recognition of lipopolysaccharide or peptidoglycan as a mechanism regulating HK2 expression. We thus next aimed to disentangle the effects of individual OMM bacteria to identify potential candidate principles regulating *Hk2* expression. To that end, we stimulated Caco-2 cells with sterile-filtered culture supernatants of the individual species of the OMM consortium. We identified *Enterococcus faecalis* KB1 as the key inducer among this minimal microbiota (Fig. 3d). *Clostridium innocuum* I46 and *Flavonifractor plautii* YL31, both well-known producers of short-chain fatty acids (SCFA)²², significantly reduced *Hk2* expression (Fig. 3d). As other fatty acids such as palmitic acid can inhibit HK activity^{23,24}, we hypothesized that SCFAs could drive the regulation of *Hk2* expression. To test this hypothesis, we generated individual metabolic models predicting the SCFA synthesis potential for each OMM member (Extended data Figure 6a). A linear model of the predicted *Hk2* expression based on the butyrate and acetate levels significantly correlated with the experimental *Hk2* expression determined by qPCR (p value = 0.008, Pearson R = 0.75, R² = 0.56, AIC = -8.0; Extended data Figure 6b and Extended data Table 2). The predicted SCFA levels in the OMM culture supernatants were validated by targeted metabolomics (Extended data Figure 6c). We next stimulated Caco-2 cells with butyrate and acetate and found that acetate upregulated, whereas butyrate downregulated *Hk2* expression (Fig. 3e). We then tested whether stimulation of Caco-2 cells with butyrate also affected *Ppif* expression. Indeed, butyrate also downregulated *Ppif* expression (Fig. 3f). Together this data suggested that the microbial metabolite butyrate could potentially protect from inflammation via

225 HK2- and PPIF-mediated changes in mitochondrial function and cell death. We
 226 therefore tested whether dietary supplementation of butyrate also functions *in*
 227 *vivo* to downregulate HK2 levels. We fed a butyrate-enriched diet to WT mice
 228 for 10 days and quantified HK2 levels in colon sections by immunohistochem-
 229 istry, which demonstrated a clear downregulation of HK2 (Fig. 3g). We then set
 230 out to test whether the SCFA-dependent modulation of HK2 levels also impacts
 231 colitis outcome. Therefore, we supplemented *Hk2^{ΔIEC}* mice and their WT litter-
 232 mates with three different diets – a control diet, a butyrate-enriched diet or an
 233 acetate-containing diet – and induced colitis by performing the DSS colitis
 234 model as before. Indeed, in WT mice dietary supplementation of butyrate ame-
 235 liorated colitis, which was evident by less weight loss (Fig. 3h), a lower DAI (Fig.
 236 3i), lower serum levels of KC/CXCL1 (Fig. 3j), fewer TUNEL-positive epithelial
 237 cells (Fig. 3k) and a lower histological score (Fig. 3l). In contrast, acetate sup-
 238 plementation worsened colitis outcome as evident by increased weight loss
 239 (Fig. 3h) and a higher DAI (Fig. 3i), which even led to premature termination of
 240 this experimental group for ethical reasons on day 8. Acetate supplementation
 241 significantly increased whereas butyrate lowered colonic HK2 levels in WT mice
 242 (Fig. 3m). In *Hk2^{ΔIEC}* mice, treatment with butyrate did not impact colitis outcome
 243 as measured by weight development, DAI, serum KC/CXCL1 levels, TUNEL-
 244 positive epithelial cells or histological score (Fig. 3 h-l and Extended data figure
 245 7). Together this data therefore demonstrates that ablation of HK2 in the intes-
 246 tinal epithelium completely blunted the butyrate-dependent effects on colitis.
 247 SCFAs are well-known for their pleiotropic effects on host physiology including
 248 intestinal motility, inflammation and carcinogenesis ²⁵. Regarding colitis, ace-

249 tate was shown to exacerbate ²⁶ whereas butyrate ameliorates ²⁷⁻²⁹ inflamma-
 250 tion. Butyrate is produced by the metabolic activity of the colonic microorgan-
 251 isms through fermentation of dietary fiber ³⁰. Mechanistically, butyrate is sensed
 252 by the G protein-coupled receptors GPR41 (FFAR3) ^{31,32}, GPR43 (FFAR2) ^{31,33-}
 253 ³⁵ and GPR109a (HCA2) ^{29,36}, which could trigger a signaling response leading
 254 to reduced *Hk2* expression. However, silencing these GPRs by siRNAs did not
 255 alter repression of *Hk2* expression by butyrate in Caco-2 cells (Extended data
 256 Figure 6d-e), which argues against a prominent role of these GPRs. Alterna-
 257 tively, butyrate also directly acts on histone deacetylases (HDACs) ²⁵, which
 258 function as epigenetic regulators and thereby could impact on *Hk2* expression.
 259 Using either a pan-HDAC inhibitor or those specific for single HDAC classes or
 260 enzymes, we found that class I HDACs, possibly HDAC2 or HDAC3 mediate
 261 the repression of *Hk2* expression by butyrate (Extended data Figure 6f).
 262 Taken together, our study revealed a novel regulatory circuit consisting of epi-
 263 thelial HK2 and the microbial metabolite butyrate. We found that ablation of
 264 HK2 protects from colitis by suppression of cell death that was linked to altered
 265 mitochondrial function, which could be due to PPIF-dependent opening of the
 266 MPTP. Moreover, we identified the intestinal microbiota and its metabolite bu-
 267 tyrate as potent regulators of HK2 and we demonstrated that the protective ef-
 268 fect of dietary butyrate supplementation is dependent on the functional pres-
 269 ence of HK2. Previous studies already pointed towards a beneficial function of
 270 butyrate-producing bacteria for a healthy intestine and prevention of gut inflam-
 271 mation ³⁷⁻⁴² and clinical trials using oral supplementation of germinated barley
 272 foods that are fermented into SCFA by the microbiota or rectal enema with bu-
 273 tyrate indeed demonstrated beneficial effects in ulcerative colitis patients ^{43,44}.

274 Our findings therefore shed light on the molecular mechanism how butyrate
275 mediates its beneficial effects and may guide the development of more specific
276 therapeutic options by targeting HK2.

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423 Figure Legends

424 Figure 1: Loss of HK2 in the intestinal epithelium protects from colitis. a)

425 Body weight loss of WT and *Hk2*^{ΔIEC} mice lacking HK2 in intestinal epithelial
 426 cells during DSS-induced colitis. * p<0.01 and ** p<0.01 WT versus *Hk2*^{ΔIEC}
 427 mice using two-way ANOVA. b) Disease activity index (DAI) consisting of stool
 428 consistency, fecal blood occurrence and body weight loss. * p<0.01 and **
 429 p<0.01 WT versus *Hk2*^{ΔIEC} mice using two-way ANOVA. c) KC/CXCL1 (pro-
 430 inflammatory cytokine) levels in serum of WT and *Hk2*^{ΔIEC} mice as determined
 431 by ELISA. * p<0.01 using Mann-Whitney U-test. d) Histological score of H&E-
 432 stained sections from colon of WT and *Hk2*^{ΔIEC} mice including representative
 433 images of the experimental groups. The scale bar represents 500 μm. * p<0.01
 434 using Mann-Whitney U-test. e) Relative HK2 protein expression during the
 435 course of DSS-induced colitis in colon epithelium of WT mice as determined by
 436 immunohistochemistry. ** p<0.01 versus D0 using one-way ANOVA. f) *Hk2*
 437 expression is dysregulated in inflamed human intestinal mucosa. *Hk2*
 438 expression was determined in inflamed and non-inflamed intestinal mucosal
 439 biopsies from patients with Crohn's Disease (CD), Ulcerative Colitis (UC) or
 440 non-IBD Colitis (NIC) by RNA-Seq. n=4-6 per group. * p<0.05. g) Gene
 441 ontology terms enriched in up- and down-regulated genes in transcriptomes of
 442 colonic IEC isolated from *Hk2*^{ΔIEC} compared to WT mice sacrificed on day 7 of
 443 DSS colitis. h) Fewer apoptotic cells per colon crypt in *Hk2*^{ΔIEC} mice as
 444 determined by TUNEL assay including representative images. The scale bar
 445 represents 50 μm. * p<0.01 using two-way ANOVA.

446 Figure 2: Dysregulated mitochondrial function in response to loss of HK2.

447 a) Western blot analysis protein lysates of intestinal organoids raised from WT

448 and *Hk2*^{ΔIEC} mice after stimulation with 100 ng/ml TNF for 24h. **b,c)** Metabolic
 449 analysis of WT and *Hk2*-deficient *ΔHk2* Caco-2 cells using the Seahorse XF
 450 analyzer. * $p < 0.01$ and **** $p < 0.0001$ WT versus *ΔHk2* using two-way ANOVA.
 451 **b)** The extracellular acidification rate (ECAR) reflects the glycolytic flux (ns =
 452 non-significant). **c)** The oxygen consumption rate (OCR) indicates
 453 mitochondrial respiration, which was impaired due to loss of HK2. * $p < 0.05$, ****
 454 $p < 0.0001$. **d)** Epithelial *Ppif* expression was downregulated in *Hk2*^{ΔIEC}
 455 compared to WT mice during the course of DSS-induced colitis (days 0, 3 and
 456 7) as determined by RNA sequencing (normalized read counts). ** $p < 0.01$ WT
 457 versus *Hk2*^{ΔIEC} using two-way ANOVA. **e)** Reduced *Ppif* expression as
 458 measured by qPCR in *ΔHk2* Caco-2 compared to WT cells upon 24h stimulation
 459 with TNF (100 ng/ml) and IL17A (50 ng/ml) or with IFN- β (50 ng/ml) to induce
 460 inflammation. ** $p < 0.01$ WT versus *ΔHk2* using two-way ANOVA.

461 **Figure 3: The microbial butyrate ameliorates colitis via HK2.** *Hk2*
 462 expression in crypts and tips of ileum and colon of **a)** germ-free (GF) and
 463 conventionally raised (CR) mice and **b)** during colonization of GF mice with a
 464 normal microbiota. **** $p < 0.0001$ GF vs. CR using moderated t test with FDR
 465 correction. # $p < 0.001$ GF vs. d1 or d3 or d5 or d7 using one-way ANOVA. **c)**
 466 *Hk2* expression in GF mice and those mono-colonized with the single bacteria
 467 *Bacteroides thetaiotaomicron* (Gram-), *Escherichia coli* (Gram-) or
 468 *Bifidobacterium longum* (Gram+) or the minimal microbiomes ASF (Altered
 469 Schaedler Flora) and OMM (Oligo-Mouse-Microbiota). * $p < 0.01$, ** $p < 0.01$ and
 470 **** $p < 0.0001$ versus GF using one-way ANOVA. **d)** Relative *Hk2* expression
 471 in Caco-2 cells stimulated with sterile-filtered culture supernatants of the OMM
 472 species grown *in vitro*. The used strains were: *Acutalibacter muris* KB18,

473 *Akkermansia municipihila* YL44, *Bacteroides ceacimuris* I48, *Bifidobacterium*
474 *animalis* YL2, *Blautia coccoides* YL58, *Enterocloster clostridioforme* YL32,
475 *Clostridium innocuum* I46, *Enterococcus faecalis* KB1, *Flavonifractor plautii*
476 YL31, *Limosilactobacillus reuteri* I49, *Muribaculum intestinale* YL27. ** $p < 0.01$
477 and *** $p < 0.001$ versus Medium using one-way ANOVA. **e)** Relative *Hk2*
478 expression in Caco-2 cells stimulated with the microbial metabolites acetate
479 and butyrate (10 mM each for 24h). * $p < 0.01$ versus PBS using one-way
480 ANOVA. **f)** Relative *Ppif* expression in Caco-2 cells stimulated with butyrate.
481 **** $p < 0.0001$ versus PBS using one-way ANOVA. **g)** Feeding WT mice a
482 butyrate-enriched diet reduced HK2 protein expression in colonic epithelium as
483 measured by immunohistochemistry. * $p < 0.01$ versus CTRL diet using one-way
484 ANOVA. **h-m)** Dietary supplementation of the microbial metabolite butyrate
485 protected from colitis dependent on HK2. WT and *Hk2*^{ΔIEC} mice were fed either
486 an acetate-enriched, butyrate-enriched or control diet and were then orally
487 administered DSS to induce colitis. **h)** Body weight development, **i)** DAI, **j)**
488 serum KC/CXCL1 levels, **k)** TUNEL-positive cells per colon crypt, **l)** histological
489 score of WT (upper panel) and *Hk2*^{ΔIEC} (lower panel) mice. **m)** HK2 protein
490 levels in colon epithelium of treated WT mice as per immunohistochemistry.
491 Acetate stimulated whereas butyrate repressed HK2 expression. * / # indicate
492 statistical significance in Butyrate (*) or Acetate (#) versus CTRL diet using two-
493 way ANOVA in (**h-i**) and one-way ANOVA in (**j-m**).

494 **Methods**

495 **Animals.** All animal experiments were approved by the local animal safety
 496 review board of the federal ministry of Schleswig Holstein and conducted
 497 according to national and international laws and policies (V 312-72241.121-33
 498 (95-8/11) and V242-62324/2016 (97-8/16)). Specific-pathogen free (SPF)
 499 animals were housed in the Central Animal Facility (ZTH) of the University
 500 Hospital Schleswig Holstein (UKSH, Kiel, Germany). To create the Hk2^{ΔIEC}
 501 mouse line, we crossed commercially available mice carrying a floxed *Hk2*
 502 allele (EMMA #02074, ⁴⁵) with mice expressing the CRE recombinase under
 503 the control of the *Villin* promoter. As controls we used littermate Hk2^{fl/fl} mice,
 504 referred to as WT mice. All mice were kept under a 12-h light cycle and fed
 505 gamma-irradiated diet *ad libitum*. Mice were killed by cervical dislocation prior
 506 to removing tissues for histological and molecular analyses. For basal
 507 phenotyping we used 9 to 11 and 86 to 92 weeks old mice. For DSS-induced
 508 colitis we used 10 to 14 weeks old male mice. Both genotypes were co-housed
 509 throughout the entire experiment. To induce colitis, mice received 1,5% (w/v)
 510 dextran sodium sulfate in autoclaved tap water. For the SCFA intervention mice
 511 were fed either a control diet, a butyrate-enriched diet or an acetate-containing
 512 diet for ten days prior to inducing colitis by administering DSS. The SCFA
 513 supplementation was continued throughout the entire experiment. The
 514 butyrate-enriched diet consisted of control feed (V1534, ssniff) supplemented
 515 with 10% (w/w) of the butyrate-polymer tributyrin (Sigma Aldrich), as this
 516 ensures the release of butyrate in the colon after metabolization by the intestinal
 517 microbiota instead of its absorption in the proximal small intestine ^{46,47}. The
 518 acetate-enriched diet consisted of control feed but the drinking water was

519 replaced with autoclaved tap water supplemented with 150 mM sodium acetate
 520 (Sigma Aldrich), a concentration used successfully in previous studies ^{26,36}.
 521 Gnotobiotic experiments were performed in the animal facilities of either the
 522 Experimental Biomedicine (EBM) of Gothenburg or Hannover Medical School
 523 (MHH). All animal protocols were approved by the Gothenburg Animal Ethics
 524 Committee or by Lower Saxony State Office for Consumer Protection and Food
 525 Safety. Gnotobiotic mice were housed as described under standard procedures
 526 ^{48,49}. Mice were kept under a 12-h light cycle and fed autoclaved chow diet *ad*
 527 *libitum* (Labdiet, St Louis, MO, USA). Monoassociated mice were generated by
 528 inoculating 12-week-old GF mice with 200 µl of overnight (stationary phase) *in*
 529 *vitro* cultures of *B. thetaiotaomicron*, *E. coli* or *B. longum* by oral gavage and
 530 the mice were sacrificed 14 days post colonization. ASF- and OMM-associated
 531 mice were generated by co-housing of weaned germ-free mice for four weeks
 532 with gnotobiotic donor animals colonized with either ASF or OMM and sacrificed
 533 at an age of 12 weeks.

534 **Bacteria and *in vitro* culture.** *B. thetaiotaomicron* VPI-5482 (ATCC 29148, ⁵⁰),
 535 *E. coli* W3110 ⁵¹ were kindly provided by Dr. Jeffrey Gordon (Edison Family
 536 Center for Genome Sciences and Systems Biology, Washington University
 537 School of Medicine, St. Louis, MO 63110, USA), while *B. longum* NCC 2705
 538 was kindly provided by Dr. Stéphane Duboux, Nestlé Research Center,
 539 Lausanne, Switzerland. Liquid media (TYG, LB and MRS broth supplemented
 540 with 0.05% (w/v) cysteine, for *B. thetaiotaomicron*, *E. coli* and *B. longum*,
 541 respectively) were inoculated with single colonies of cultures on agar plates and
 542 were grown to the stationary phase overnight in an anaerobic jar at 37°C. ASF
 543 is a mix of eight bacteria: two Clostridia species ASF356 & ASF502,

544 *Lactobacillus murinus* ASF361 and spec. ASF360, *Mucispirillum schaedleri*
 545 ASF457, *Eubacterium plexicaudatum* ASF492, *Parabacteroides spec.* ASF519
 546 and an unknown Firmicutes bacterium ASF500⁵². ASF colonized mice were
 547 purchased from Taconic and inoculated transgenerally by co-housing. OMM
 548 (Oligo-Mouse-Microbiota) is a mix of 12 bacteria: *Bacterioides ceacimuris* I48,
 549 *Muribaculum intestinale* YL27, *Akkermansia municipihila* YL44, *Turicimonas*
 550 *muris* YL45, *Limosilactobacillus reuteri* I49, *Enterococcus faecalis* KB1, *Blautia*
 551 *coccoides* YL58, *Clostridium innocuum* I46, *Flavonifractor plautii* YL31,
 552 *Enterocloster clostridioforme* YL32, *Acutalibacter muris* KB18 and
 553 *Bifidobacterium animalis* YL2²¹. Bacteria of the OMM consortium were grown
 554 in single cultures under anaerobic conditions in Anaerobic Akkermansia
 555 Medium (AAM) as previously described²¹.

556 **Isolation of primary cells and intestinal organoids.** IECs were isolated from
 557 intestinal tissue using the Lamina Propria Dissociation Kit (Miltenyi BioTech,
 558 Bergisch Gladbach, Germany) according to the manufacturer's protocol with
 559 minor deviations as described before⁵³. In brief, intestinal epithelial cells were
 560 isolated by disruption of the structural integrity of the epithelium using
 561 ethylenediaminetetraacetic acid (EDTA) and dithiothreitol (DTT). Purity of
 562 individual IEC fractions was analyzed by flow cytometry on a FACS Calibur flow
 563 cytometer (B&D, Heidelberg, Germany) with Cellquest analysis software from
 564 Becton Dickinson. We used the Anti-EpCam-PE (Clone: G8.8, Biolegend, San
 565 Diego, USA) antibody for analysis of IEC purity. FACS-data was analysed using
 566 Flowing Software (Perttu Terho, Turku Centre for Biotechnology, Finland).
 567 Intestinal organoids were generated following procedures described earlier by
 568 Sato et al., 2009⁵⁴. Organoids were cultivated in ENR-conditioned medium

569 supplemented with human recombinant EGF as described by Sato et al., 2011
570 ⁵⁵.

571 **Generation and culture of HK2-deficient Caco-2 cells.** A CRISPR plasmid
572 targeting human *Hk2* was generated using the GeneArt® CRISPR Nuclease
573 Vector Kit from Thermo Fisher. Caco-2 cells were purchased from DSMZ (ACC-
574 169) and transfected with the *Hk2* CRISPR plasmid using Lipofectamin reagent
575 kit (Thermo Fisher Scientific). Positive clones were screened via Western blot
576 to generate a monoclonal population termed Caco-2^{ΔHk2}. Caco-2 cells that were
577 also subjected to the CRISPR transfection and selection procedure, but which
578 still showed a HK2 band as per western blot analysis were used as controls
579 (Caco-2^{WT}). Caco-2^{WT} and Caco-2^{ΔHk2} cells were cultured in MEM with 20%
580 (v/v) FCS purchased from Gibco/Life Technologies. Cells were seeded with
581 70% confluency and 24 hours in advance of stimulation.

582 **RNA isolation and qPCR.** Total RNA was extracted using the RNeasy Mini Kit
583 (Qiagen) according to the manufacturer's protocol. RNA concentration was
584 measured using a NanoDrop ND-1000 spectrophotometer (PeqLab
585 Biotechnologie). 1μg of total RNA was reverse-transcribed to cDNA using the
586 Maxima H Minus First Strand cDNA Synthesis Kit (ThermoFisher Scientific).
587 qPCR was carried out using SYBR Select Master Mix (Applied Biosystems)
588 according to the manufacturer's instructions on a Viia 7 Real-Time PCR System
589 (ThermoFisher Scientific). Expression levels were normalized to *Actb* (β-actin
590 or *Rpl32* (ribosomal protein L32). Primer sequences are listed in Extended data
591 table 3.

592 **RNA sequencing.** Total RNA was extracted as described above from isolated
593 IECs from *Hk2*^{ΔIEC} and littermate control mice under untreated conditions and

594 after administering DSS for 3 or 7 days. RNA libraries were prepared using
 595 TruSeq stranded mRNA Kit (Illumina) according to manufacturer's instructions.
 596 All samples were sequenced using an Illumina NovaSeq 6000 sequencer
 597 (Illumina, San Diego, CA) with an average of 23 million paired-end reads (2x 50
 598 bp) at IKMB NGS core facilities. The RNA-seq data was processed using an in-
 599 house pipeline (<https://github.com/nf-core/rnaseq>). Briefly, adapters and low-
 600 quality bases from the RNA-seq reads were removed using Trim Galore
 601 (version 0.4.4). The filtered reads were mapped to the mouse genome
 602 (GRCm38) using STAR aligner (version 2.5.2b). Expression counts of the
 603 transcripts were estimated using featureCounts (version 1.5.2) and then
 604 normalized across samples using the DESeq normalization method. DESeq2⁵⁶
 605 was used to determine differentially expressed genes. Genes were considered
 606 as significant differentially expressed if the adjusted *p-value* (Benjamini-
 607 Hochberg (BH) multiple test correction method) was less than 0.05. Gene
 608 Ontology (GO) enrichment analysis was conducted using the Bioconductor
 609 package topGO (version 2.32.0) and a Fisher.elim *p-value* (weight algorithm)
 610 of 0.05 was used as significance threshold.

611 **Microbiota analysis.** MiSeq 16S amplicon sequence data was analyzed using
 612 MacQIIME v1.9.1 (<http://www.wernerlab.org/software/macqiime>,⁵⁷) as
 613 described previously^{58,59}. Briefly, all sequencing reads were trimmed keeping
 614 only nucleotides with a Phred quality score of at least 20, then paired-end
 615 assembled and mapped onto the different samples using the barcode
 616 information. Sequences were assigned to operational taxonomic units (OTUs)
 617 using uclust and the greengenes reference database (gg_13_8 release) with
 618 97% identity^{60,61}. Representative OTUs were picked and taxonomy assigned

619 using uclust and the greengenes database. Quality filtering was performed by
 620 removing chimeric sequences using ChimeraSlayer ⁶² and by removing
 621 singletons and sequences that failed to align with PyNAST ⁶³. The reference
 622 phylogenetic tree was constructed using FastTree 2 ⁶⁴. All samples within a
 623 single analysis were normalized by rarefaction to the minimum shared read
 624 count to account for differential sequencing depth among samples (10,174
 625 sequences per sample). Relative abundance was calculated by dividing the
 626 number of reads for an OTU by the total number of sequences in the sample.
 627 Alpha diversity measures were computed and beta diversity was calculated
 628 using Unweighted Unifrac and visualized by principal coordinate analysis.
 629 Significance of differences in abundances of various taxonomic units between
 630 *Hk2^{ΔIEC}* and littermate control mice was calculated using t-test and p values
 631 were adjusted for multiple testing using FDR correction (q-value).

632 **Western blot analyses.** Caco-2 cells were lysed using RIPA buffer. Organoids
 633 were lysed using SDS-based DLB buffer + 1% Halt Protease inhibitor cocktail
 634 (Thermo Fisher Scientific). Lysates were heated to 95°C for 5 min centrifuged
 635 at 16,000 g for 15 min at 4°C to remove cell remnants. Protein concentrations
 636 were measured by DC Protein Assay (BioRad) according to the manufacturers
 637 protocol. Equal amounts of lysates containing Laemmli buffer were heated at
 638 95°C and electrophoresed on 12% polyacrylamide gels under standard SDS-
 639 PAGE conditions before being transferred onto a polyvinylidene fluoride
 640 membranes (GE Healthcare). Protein loaded membranes were blocked with
 641 5% (w/v) non-fat dry milk or bovine serum albumin (BSA) in Tris-buffered saline
 642 (TBS) supplemented with 0,1% (v/v) Tween 20 for 1 hour, incubated with
 643 primary antibody (mouse anti-HK2, Novus Biologicals, #NBP2-02272; rabbit

644 anti-PARP1/cPARP1, Cell Signaling Technology, #9542; rabbit anti-
645 Caspase3/cCaspase3, Cell Signaling Technology, #9662, mouse anti-
646 betaActin, Abcam, #ab20272) overnight, washed three times with TBS-Tween-
647 20 and then incubated with the secondary horseradish peroxidase (HRP)-
648 conjugated antibody for 1 hour at room temperature. Proteins were detected
649 using the Pierce ECL and ECL Plus Substrate Kits (ThermoFisher).

650 **Histology and immunostaining.** Tissue specimen were fixed in 10% formalin
651 solution over night at 4°C and then embedded in paraffin. 5 µm thick sections
652 were cut and stained with hematoxylin and eosin (H&E) or subjected to
653 immunostaining using the Vectastain ABC kit (Vector Laboratories) including
654 antigen retrieval in boiling citrate buffer. Primary antibodies were incubated
655 overnight. For immunostaining of HK2 we used a 1:1000 diluted antibody
656 (Novus Biologicals, #NBP2-02272). For immunostaining of Ki67 we used a
657 1:500 diluted mouse anti-Ki67 antibody (BD Biosciences, cat.no. 556003). The
658 TUNEL assay was performed using the ApopTag Plus Peroxidase In Situ
659 Apoptosis Detection Kit (Merck Millipore) according to the manufacturer's
660 instructions. Slides were visualized using a Zeiss Imager Z1 microscope (Zeiss)
661 and pictures were taken using ZEN pro (Zeiss) software.

662 **Seahorse analysis.** To perform real-time ECAR and OCR-analyses, Caco-2
663 cells were analyzed using the Seahorse XF24 Analyzer from Agilent
664 Technologies by Mito Stress Test Kit or Glycolysis Stress Test Kit according to
665 the manufacturer's instructions. 4×10^4 cells were used in each assay with n=9
666 replicates in three independent experiments.

667 **Metabolic modelling of SCFA production by OMM bacteria.** Metabolic
668 networks were reconstructed using gapseq⁶⁵ based on the genomes of OMM

bacteria, which were downloaded from NCBI bioproject PRJNA289613, and the Anaerobic Akkermansia Medium ²¹. The formation of fermentation products was determined via an extended flux balance analysis that minimizes the total flux through all reactions as a proxy for the parsimonious enzyme usage ⁶⁶. The variability of predictions was taken into account by randomly sampling the space of alternative optimal solutions via the function ACHR from the R package sybilcycleFreeFlux (W=5000, nPoints=10000) ⁶⁷ to derive the distribution of fermentation products for each bacteria. All predicted fermentation products were then used as explanatory variables to estimate the *Hk2* expression via linear regression.

Quantification of SCFAs. OMM bacteria were grown as described and 1 mL of each bacterial culture supernatant was homogenized using NucleoSpin Bead Tubes (Macherey-Nagel, Düren, Germany) and a Precellys Evolution Homogenizer (Bertin Corp., Rockville, Maryland, USA). Homogenates were cleared by centrifugation for 10 min at 21,000 × g and 4°C. SCFA standards including acetic, propionic and butyric acid (purchased from Sigma Aldrich, St Louis, MO, USA) were prepared in methanol to a concentration of 100 ppm. Derivatization of SCFAs was performed with 3-nitrophenylhydrazine as described ⁶⁸. SCFA concentrations in the samples were then measured via Ultra-High Performance Liquid Chromatography (Acquity UPLC, Waters, Milford, MA, USA) coupled to Mass Spectrometry (amaZon ETD IonTrap, Bruker Daltonics GmbH, Bremen, Germany). Sample separation was performed using a C8 column and solvent system consisting of ammonium acetate (5 mM, Sigma Aldrich, St Louis, MO, USA) combined with acetic acid (0.1%, pH 4.2, Biosolve, Valkenswaard, Netherlands) in water or acetonitrile

(LC-MS CHROMASOLV, FLUKA, Sigma Aldrich, St Louis, MO, USA). Mass spectrometry analysis was performed in negative electrospray ionization mode. Peak areas and concentrations of SCFAs in the bacterial supernatants were calculated using QuantAnalysis (Bruker, Daltonics, Bremen, Germany) software.

Statistical analysis. Biostatistical analyses were performed using GraphPad Prism (version 8) software (GraphPad, Inc, La Jolla, CA), MacQIIME v1.9.2 or R (v 3.2.5). Specific comparisons and analyses are described in the individual method sections. Differences between the groups were considered significant at $P < 0.05$ and the data are presented as means $\pm$ SEM.

Data Availability Statement. All data is either included in this manuscript or deposited on public databases. The RNA sequencing data has been deposited at NCBI's Sequence Read Archive under the accession number GSE158026. The 16S amplicon sequencing data are accessible through the European Nucleotide Archive (ENA, <https://www.ebi.ac.uk/ena>) under the study accession number PRJEB40281. Additional data that support the findings of this study are available from the corresponding author upon reasonable request.

Code Availability Statement. All codes used to generate the bioinformatic analyses are available from the corresponding author upon reasonable request.

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801

802 **Author contributions**

803 JH, FH, LS, PR and FS designed research. JH, FH, LS, NS and FS managed
 804 the conventional mouse colony, monitored the mice, performed animal experi-
 805 ments and collected samples. MB, VT, FB maintained the gnotobiotic mouse
 806 facilities, performed the colonizations of GF mice with single bacteria and min-
 807 imal consortia and provided samples. JH, FH, LS, AW, KS, NS, KK, DP, SK
 808 and PR conducted the wet lab experiments. JH, FH, LS, NM, KS, NS, KK, RH,
 809 SK, FB, PR and FS analyzed and interpreted the data. JH, NM, AW, JZ, RH
 810 and FS performed the bioinformatics analyses. JH, FH, LS and FS prepared
 811 the figures. PR and FS obtained funding. JH, FH, LS, PR and FS co-wrote the
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814 **Competing interest declaration**

815 The authors have no competing interests to declare. All authors have read and
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819

820 **Additional information**

821

822 **Abbreviations:**

823 ASF, altered Schaedler Flora; BSA, bovine serum albumin; Caco-2, colon car-
 824 cinoma cell line 2; CD, Crohn's disease; CR, conventionally raised mice; DAI,
 825 disease activity index; DSS, dextran sodium sulfate; ECAR, extracellular acidi-
 826 fication rate; EWAT, epididymal white adipose tissue; FCCP, cyanide-4-(trifluo-
 827 romethoxy)-phenylhydrazine; FFAR, free fatty-acid receptor; GF, germ-free;
 828 GO, gene ontology; GPR, G protein-coupled receptor; H&E, hematoxylin and
 829 eosin; HDAC, histone deacetylases; HK, hexokinase; HRP, horseradish perox-
 830 idase; IBD, inflammatory bowel disease; IEC, intestinal epithelial cell; MPTP,
 831 mitochondrial permeability transition pore; OCR, oxygen consumption rate;
 832 OMM, oligo Mouse Microbiota; PARP1, Poly(ADP-Ribose)-Polymerase 1;
 833 PPIF, peptidyl-prolyl cis-trans isomerase; qPCR, quantitative real-time poly-
 834 merase chain reaction; SCFA, short-chain fatty acid; SPF, specific pathogen
 835 free.; TBS, Tris-buffered saline; TNF, tumor necrosis factor; TUNEL, terminal
 836 deoxynucleotidyl transferase dUTP nick end labelling; UC, ulcerative colitis;
 837 WT, wild-type

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840 **Extended data figure/table legends**

841 **Extended data figure 1: *Hk2*^{ΔIEC} mice do not display an evident** 842 **immunological or metabolic phenotype under baseline conditions. a-g)**

843 10- or 90-week-old WT and *Hk2*^{ΔIEC} mice were sacrificed and organ measures
844 taken. **a)** Body weight. **b)** Cecum weight. **c)** Liver weight. **d)** Spleen weight. **e)**
845 Epididymal white adipose tissue (EWAT) weight. **f)** Small intestine length. **g)**
846 Colon length. **h)** Relative expression of all hexokinase family members in
847 unfractionated colon tissue of 10-week-old WT and *Hk2*^{ΔIEC} mice as per qPCR.
848 **i)** Intestinal permeability as measured by fluorescein isothiocyanate (FITC)
849 levels in blood of 10-week-old WT and *Hk2*^{ΔIEC} mice 1h after oral gavage. **j)**
850 Blood glucose levels during oral glucose tolerance test in 10-week-old WT and
851 *Hk2*^{ΔIEC} mice. **k)** Colonic histological score of 10-week-old WT and *Hk2*^{ΔIEC}
852 mice. **l)** TUNEL-positive cells per colon crypt of 10-week-old WT and *Hk2*^{ΔIEC}
853 mice. **m)** Ki-67-positive cells per colon crypt of 10-week-old WT and *Hk2*^{ΔIEC}
854 mice including representative images. The scale bar represents 50 μm.

855 **Extended data figure 2: Phenotyping of WT and *Hk2*^{ΔIEC} mice during DSS-**

856 **induced colitis. a-h)** Organ and histological data from WT and *Hk2*^{ΔIEC} mice
857 sacrificed at the end (day 10) of DSS colitis. **a)** Cecum weight. **b)** Liver weight.
858 **c)** Spleen weight. **d)** Epididymal white adipose tissue (EWAT) weight. **e)** Small
859 intestine length. **f)** Colon length. **g)** TUNEL-positive cells per colon crypt. **h)** Ki-
860 67-positive cells per colon crypt. **i)** HK2 protein levels in colon epithelium of WT
861 mice at baseline (day 0) and at day 10 during DSS colitis as determined per
862 immunohistochemistry. **j)** Ki-67-positive cells per colon crypt and **k)** histological
863 score of WT and *Hk2*^{ΔIEC} mice at day 3 and 7 during DSS colitis.

864 **Extended data figure 3: Heatmap of genes differentially expressed in**
 865 **colon of WT and *Hk2*^{ΔIEC} mice at days 0, 3 and 7 during DSS colitis.** WT
 866 and *Hk2*^{ΔIEC} mice were given 2% DSS in drinking water and analyzed on day 0,
 867 3 and 7 of treatment. RNA was isolated from unfractionated colon and
 868 sequenced to identify transcripts with differential expression dependent on the
 869 loss of epithelial HK2 during the onset of inflammation.

870 **Extended data Figure 4: Expression pattern of the HK2 protein in the**
 871 **colonic epithelium of untreated 10-week-old WT and *Hk2*^{ΔIEC} mice.** The
 872 scale bar represents 50 μm. The dotted line indicates the tip epithelium area
 873 used for quantification of HK2 protein expression. Note the dominant HK2
 874 expression in the colonic tip epithelium.

875 **Extended data Figure 5: Ablation of HK2 in IECs does not alter the**
 876 **composition of the intestinal microbiota. a)** Principal coordinate analysis of
 877 fecal microbiota from WT and *Hk2*^{ΔIEC} mice. **b)** Taxonomic overview on genus
 878 level. **c)** Alpha diversity (the variation of microorganisms in a single sample).

879 **Extended data Figure 6: Role of SCFAs and HDACs in butyrate-mediated**
 880 **repression of *Hk2* expression. a)** Metabolic modeling of the production of
 881 acetate, butyrate and propionate by the individual OMM bacteria based on their
 882 published genome information and the used *in-vitro* growth conditions, and
 883 correlation of metabolite production with *Hk2* expression. R denotes the
 884 Pearson correlation coefficient. **b)** Prediction of linear models incorporating the
 885 acetate, butyrate and propionate levels to explain the observed changes in *Hk2*
 886 expression upon stimulation of Caco-2 cells with the bacterial culture
 887 supernatants (shown in Fig. 3d). **c)** SCFA levels quantified in culture
 888 supernatants of single OMM bacteria, which were used to stimulate Caco-2

889 cells (shown in Fig. 3d). **d)** Relative *Hk2* expression in Caco-2 cells after
 890 transfection with a mix of siRNA targeting the three G protein-coupled receptors
 891 (GPR) GPR41 (*Ffar3*), GPR43 (*Ffar2*) and GPR109a (*Hca2*) and subsequent
 892 stimulation with butyrate. **e)** Expression of *Ffar3*, *Ffar2* and *Hcar2* in Caco-2
 893 cells after transfection with the siRNA targeting these three GPRs to test for
 894 successful knockdown. **f)** Relative *Hk2* expression in Caco-2 cells first treated
 895 with general or specific HDAC inhibitors and then incubated with butyrate.
 896 HDACi refers to a mixture of SAHA and NAM and was used to inhibit all HDAC
 897 classes. A mixture of PCI-34051 and TC-H106 were used to inhibit class I
 898 HDACs. TMP269, NAM (nicotinamide) and SIS17 were used to inhibit class II,
 899 III and IV, respectively. Valproic acid (VPA) and PCI-34051 were used to inhibit
 900 only HDAC1 and HDAC8, respectively. All expression values were normalized
 901 to the mean of DMSO-PBS.

902 **Extended data figure 7: Organ measures and histological data of WT and**
 903 ***Hk2*^{ΔIEC} mice at the end of the dietary SCFA supplementation and DSS-**
 904 **induced colitis experiment. a)** Cecum weight. **b)** Liver weight. **c)** Spleen
 905 weight. **d)** Epididymal white adipose tissue (EWAT) weight. **e)** Small intestine
 906 length. **f)** Colon length. **g)** Ki-67-positive cells per colon crypt.

907 **Extended data table 1: Complete normalized read counts and statistics**
 908 **from RNA sequencing of IECs isolated from colon of WT and *Hk2*^{ΔIEC}**
 909 **mice at day 0 (baseline) and days 3 and 7 of DSS colitis.**

910 **Extended data table 2: Statistics of linear modelling of *Hk2* expression**
 911 **induced by metabolites produced by single OMM bacteria grown *in vitro*.**

912 AIC = Akaike Information Criteria. Sigma = standard deviation of the residuals.

913 R² = coefficient of determination, referring to the proportion of variance in the

914 dependent variable that is predictable from independent variables. R = Pearson
 915 correlation coefficient of predicted metabolite production with *Hk2* expression
 916 data.

917 **Extended data table 3: List of primers used in this study.**

918

919

Fig.1

Fig.2

Fig.3

Fig.S1

Fig.S2

Fig.S3

a Prediction of SCFA production & correlation with *Hk2*

b Linear model estimation

c Quantified SCFA levels

Fig.S7