

1 **Genetic and environmental circadian disruption induce metabolic** 2 **impairment through changes in the gut microbiome**

3

4 **Baraa Altaha^{1,2}, Marjolein Heddes^{1,2}, Violetta Pilorz³, Yunhui Niu^{1,2}, Elizaveta**
5 **Gorbunova^{1,2}, Michael Gigl⁴, Karin Kleigrew⁴, Henrik Oster³, Dirk Haller^{1,2}, Silke**
6 **Kiessling^{1,2,5,*}**

7

8 ¹ ZIEL - Institute for Food & Health, Technical University of Munich, Gregor-Mendel Str. 2,
9 85354 Freising, Germany

10 ² Chair of Nutrition and Immunology, Technical University of Munich, Gregor-Mendel-Str.
11 2, 85354 Freising, Germany

12 ³ Institute of Neurobiology, Center of Brain, Behavior & Metabolism, University of Lübeck,
13 Marie Curie Street, 23562, Lübeck, Germany.

14 ⁴ Bavarian Center for Biomolecular Mass Spectrometry, Technical University of Munich,
15 Gregor-Mendel-Str. 4, 85354 Freising, Germany

16 ⁵ Faculty of Health and Biomedical Science, University of Surrey, 388 Stagg Hill Campus,
17 GU27XH, Guildford, UK

18

19 *** Correspondence:**

20 Dr. Silke Kiessling

21 Chair of Nutrition and Immunology

22 Technical University of Munich

23 Gregor-Mendel-Str. 2

24 85354 Freising, Germany

25 Email: silke.kiessling@tum.de

26 Phone: +44 148368-4643

27

28

29

30

31

32

33

34

35

36

37 Abstract

38

39 Objective

40 Internal clocks time behavior and physiology, including the gut microbiome in a
41 circadian (~24 h) manner. Mismatch between internal and external time, e.g. during shift
42 work, disrupts circadian system coordination promoting the development of obesity and
43 type 2 diabetes (T2D). Conversely, body weight changes induce microbiota dysbiosis. The
44 relationship between circadian disruption and microbiota dysbiosis in metabolic diseases,
45 however, remains largely unknown.

46

47 Methods

48 Core and accessory clock gene expression in different gastrointestinal (GI) tissues were
49 determined by qPCR in two different models of circadian disruption - mice with *Bmal1*
50 deficiency in the circadian pacemaker, the suprachiasmatic nucleus (*Bmal1^{SCNfl/-}*), and
51 wild-type mice exposed to simulated shift work (SSW). Body composition and energy
52 balance were evaluated by nuclear magnetic resonance (NMR), bomb calorimetry, food
53 intake and running-wheel activity. Intestinal permeability was measured in an Ussing
54 chamber. Microbiota composition and functionality were evaluated by 16S rRNA gene
55 amplicon sequencing, PICRUST2.0 analysis and targeted metabolomics. Finally,
56 microbiota transfer was conducted to evaluate the functional impact of SSW-associated
57 microbiota on the host's physiology.

58

59 Results

60 Both chronodisruption models show desynchronization within and between peripheral
61 clocks in GI tissues and reduced microbial rhythmicity, in particular in taxa involved in
62 short-chain fatty acid (SCFA) fermentation and lipid metabolism. In *Bmal1^{SCNfl/-}* mice,
63 loss of rhythmicity in microbial functioning associates with previously shown increased
64 body weight, dysfunctional glucose homeostasis and adiposity. Similarly, we observe an
65 increase in body weight in SSW mice. Germ-free colonization experiments with SSW-
66 associated microbiota mechanistically link body weight gain to microbial changes.
67 Moreover, alterations in expression of peripheral clock genes as well as clock-controlled
68 genes (CCGs) relevant for metabolic functioning of the host were observed in recipients,
69 indicating a bidirectional relationship between microbiota rhythmicity and peripheral
70 clock regulation.

71

72 Conclusions

73 Collectively, our data suggest that loss of rhythmicity in bacteria taxa and their products,
74 which likely originates in desynchronization of intestinal clocks, promotes metabolic
75 abnormalities during shift work.

76 **Keywords:** circadian rhythm, SCN, shift work, microbiota, short chain fatty acids, bile
77 acids

78

79 **Abbreviations**

80 BA: bile acid

81 *Bmal1* = Brain and Muscle ARNT-Like 1

82 CCGs: clock-controlled genes

83 *Cry1* = cryptochrome circadian regulator 1

84 CT = circadian time

85 *Dbp* = D Site of Albumin Promoter (Albumin D-Box) Binding Protein

86 DD = constant darkness

87 EC = Enzyme Commission

88 *Efla* = Elongation factor 1-alpha

89 *Fabp2* = Fatty Acid Binding Protein 2

90 GF = Germ-free

91 GI = gastrointestinal

92 GUniFrac = Generalized UniFrac

93 *Glut2* = Glucose transporter 2

94 *Hdac3* = Histone Deacetylase 3

95 *Ifabp* = Intestinal-type fatty acid-binding protein

96 LD = 12 hours light and 12 hours darkness schedule

97 LEFSE = LDA effective score

98 NMR: Nuclear magnetic resonance

99 *Per2* = Period 2

100 PICRUST = Phylogenetic Investigation of Communities by Reconstruction of Unobserved States

101 *Pparγ* = Peroxisome Proliferator Activated Receptor Gamma

102 qRT-PCR = Quantitative real-time PCR

103 *Rev-erba* = Nuclear receptor subfamily 1 group D member 1

104 SCFA: short-chain fatty acid

105 SCN = suprachiasmatic nucleus

106 SPF = specific-pathogen free

107 SSW: simulated shift work

108 T2D = type 2 diabetes

109 UPL = Universal Probe Library system

110 zOTUs = Zero-radius operational taxonomic units

111 ZT = *Zeitgeber* time

112

113 **1 Introduction**

114 Most species have evolved endogenous circadian clocks to facilitate adaption to daily recurring
115 changes. A complex hierarchical circadian system consists of a central clock in the
116 suprachiasmatic nuclei (SCN) of the hypothalamus which regulates rhythmic behavior, such as
117 rest-activity, and synchronizes peripheral clocks via neuronal and humoral signals to adapt to
118 environmental changes [1]. Peripheral circadian clocks have been identified in various organs,
119 including the gastrointestinal (GI) tract, and regulate tissue-specific functions, such as
120 glucocorticoid synthesis and glucose metabolism [2; 3]. On the molecular level, the circadian
121 clock consists of a subset of interconnected clock genes which regulate circadian rhythms of
122 tissue-specific clock-controlled genes (CCGs) and thereby control various aspects of
123 physiology [4].

124 Mismatch between the internal clock and the environmental time, observed in shift workers,
125 induces circadian desynchronization among peripheral clocks [5]. Genetically and
126 environmentally induced circadian disruption has been associated with various metabolic and
127 GI diseases including obesity and diabetes [6-8]. Similarly, lack of the coordinative input from
128 the central clock results in desynchronization between peripheral clocks and causes an increase
129 in body weight and impaired glucose tolerance [6; 9]. These results suggest that peripheral
130 circadian desynchronization might be causal for metabolic alterations.

131 In the context of metabolic disease, human cohort studies have identified altered microbial
132 profiles associated with obesity, insulin resistance, and T2D [10-14]. In agreement with these
133 findings, frequent time zone shifts (jetlag) induce major alterations in overall gut microbiota
134 communities and loss of daytime-dependent oscillation in specific taxa [15]. Importantly, in
135 large human cohorts we showed that microbiota composition and function undergo 24-h
136 rhythmicity and are disrupted in subjects with obesity and/or type 2 diabetes (T2D) [16].
137 Interestingly, our results in prediabetic patients indicate that arrhythmicity of specific taxa

138 precedes the onset of diabetes and a signature of arrhythmic bacteria predicts T2D risk in
139 populations. Of importance, our recent work on mice identified clocks in cells of the GI tract
140 to be the major regulators of microbial rhythmicity and, therefore, GI homeostasis [17].
141 Consequently, we hypothesize that intestinal clock-controlled oscillation of the microbiome
142 provides a functional link to metabolic requirements of the host to maintain metabolic health.
143 Here we investigate the impact of circadian disruption on the synchronization of GI clocks and
144 the rhythmicity of microbiota composition and function. Our results show desynchronization
145 of GI clocks in two independent models of circadian disruption, a genetic approach using mice
146 with central circadian dysfunction and an environmental approach using simulated shift work
147 (SSW) on wild type mice. Arrhythmicity of microbial taxa was observed in both models,
148 although microbiota composition differed between experiments. Importantly, arrhythmic
149 bacterial taxa and metabolites identified in both models shared functionalities relevant for
150 metabolic homeostasis of the host. Microbiota transfer further revealed a cross-talk between
151 oscillating taxa and intestinal clocks, highlighting the physiological relevance of microbial
152 rhythms for metabolic health and as therapeutic target.

153

154 **2 Material and methods**

155 **2.1 Ethics Statement**

156 Experiments were conducted at Technical University of Munich in accordance with Bavarian
157 Animal Care and Use Committee (TVA ROB-55.2Vet-2532.Vet_02-18-14) or were conducted
158 at the University of Lübeck licensed by the Ministry of Agriculture, Environment and Rural
159 Areas (MELUR) of the state of Schleswig-Holstein (project license:42-5/18_Oster).

160 2.2 Mouse models and light conditions

161 2.2.1 *Syt10^{cre}-Bmal1^{IEC +/-}* and *Syt10^{cre}-Bmal1^{IECfl/-}* mice

162 Male SCN-specific *Bmal1* knock-out (Synaptotagmin-10 CRE/wt x *Bmal1^{fl/-}*; referred to as
163 *Bmal1^{SCNfl/-}*) mice and their control littermates (Synaptotagmin-10 CRE/wt x *Bmal1^{+/-}*;
164 referred to as *Bmal1^{SCN+/-}*) on a genetic C57BL/6J background, mice were generated at the
165 University of Lübeck as described before [18]. Male mice were maintained under a 12 hours
166 light and 12 hours darkness schedule (LD) cycle for 2 weeks (age 8-10 weeks), and sacrificed
167 at the indicated time points during the 2nd day in constant darkness (DD).

168 2.2.2 Simulated shift work (SSW)

169 Wild type mice on a genetic C57BL/6J background were bred in house at the Technical
170 University of Munich. Male mice were kept in LD 12:12 cycles (300 lux), with lights turned
171 on at 5am (*Zeitgeber* time (ZT0) to 5pm (ZT12)). Mice were single housed at the age of 8
172 weeks in running wheel-equipped cages with ad libitum access to chow and water and under
173 specific-pathogen free (SPF) conditions according the FELASA recommendation. To
174 minimize cage-related bias in microbiota composition [19], littermates and litters of
175 comparable age from as few as possible breeding pairs and cages were selected. One set of
176 control males was maintained under a LD cycle for 8 weeks (age 8-16 weeks), whereas another
177 set of male mice was first exposed to for 2 weeks (age 8-10 weeks) of LD and then subjected
178 to SSW conditions for at least 6 weeks. During the experiment mice were exposed to 100lux
179 light intensity and shifted every 5th day by 8 hours. On day 1 of the jet lag, the lights-off time
180 (ZT12) was shifted from 5 pm to 9 am (phase advance paradigm) and from 9 am to 5 pm (phase
181 delay paradigm). Using a short day protocol, we defined day 1 as the first advanced dark period
182 as defined previously [5].

183 2.2.3 Germ free colonization experiment Transfer experiments

184 germ-free wild type C57BL6 were gavaged at the age of 10 weeks with cecal microbiota from
185 mixture of cecal content diluted 1:10 in 40% glycerol. Cecal microbiota of 4-5 mice from LD
186 and SSW group were adjusted to 7×10^6 bacteria/ μl and 100 μl of were used for gavaging each
187 mouse at ZT13. Germ free recipient mice kept in LD12:12 and were checked weekly for
188 bodyweight changes. After 6 weeks of the gavage, at age 16 weeks, mice were released in
189 constant darkness and sacrificed at the 2nd day at the indicated time point.

190 2.3 Tissue collection

191 All animals were sacrificed by cervical dislocation followed by decapitation at the age of 16-
192 20 weeks. *Bmal1^{SCN1L/-}* mice were sacrificed during the 2nd day of darkness at the indicated
193 circadian time (CT) points. Control mice in the SSW experiment were sacrificed in LD
194 conditions at the indicated *Zeitgeber* time (ZT) and animals undergoing SSW were sacrificed
195 during the 1st day following the final phase advance of SSW at the indicated time point
196 according to the LD control cohort. Tissues were collected and snap frozen using dry ice and
197 stored in -80 degrees until further processing.

198 2.4 Gut permeability

199 Gut permeability were measured using Ussing chambers as described previously ([20-22]).
200 Briefly, we took 1.5 cm of the proximal colon directly after dissecting the mice. The tissue was
201 cut open and fixed as a flat sheet separating the two halved of the Ussing chamber (six chamber
202 system - Scientific instruments). The tissue was supported from the two sides with carbogen-
203 gassed freshly prepared Krebs buffer (5.4 mM KCl, 114 mM NaCl, 1.2 mM CaCl₂, 21 mM
204 NaHCO₃, 1.2 mM MgCl₂, 2.4 mM Na₂HPO₄, 10 mM glucose, 0.6 mM NaH₂PO₄, pH 7.4) at
205 37°C. We added 250 μl of 1.7673mM fluorescein to the luminal side, then we determined the
206 fluorescence intensity at 45 and 60 minutes from the buffer on the serosal part, to calculate
207 tissue permeability in cm/s.

208 2.5 Gene expression analysis (qRT-PCR) Quantitative real-time PCR

209 Snap frozen tissue samples were used to extract RNA samples with Trizol reagent. Next we
210 used 1000ng RNA to synthesize cDNA with cDNA synthesis kit Multiscribe RT
211 (Thermofischer Scientific). We perform qPCR in a Light Cycler 480 system (Roche
212 Diagnostiscs, Mannheim, Germany) using Universal Probe Library system (UPL) according to
213 manufacturer's instructions. We used the following primers and probes to measure gene
214 expression: Brain and Muscle ARNT-Like 1 (*Bmal1*) F 5'-ATTCCAGGGGGAACCAGA-' R
215 5'-GGCGATGACCCTCTTATCC-3' Probe 15, Nuclear receptor subfamily 1 group D
216 member 1 (*Rev-erba*) F 5'- AGGAGCTGGGCCTATTCAC-3' R 5'-
217 CGGTTCTTCAGCACCAGAG-3' probe 1, Period 2 (*Per2*) F 5'-
218 TCCGAGTATATCGTGAAGAACG-3' R 5'- CAGGATCTTCCCAGAAACCA-3' probe 5,
219 D Site Of Albumin Promoter (Albumin D-Box) Binding Protein (*Dbp*) F 5'-
220 ACAGCAAGCCCAAAGAACC-3' R 5'- GAGGGCAGAGTTGCCTTG-3' probe 94, (*Cry1*)
221 F 5'- ATCGTGCGCATTTCACATAC-3' R 5'- TCCGCCATTGAGTTCTATGAT-3' probe
222 85, Glucose transporter 2 (*Glut2*) F 5'-TTACCGACAGCCCATCCT-3' R 5'-
223 TGAAAAATGCTGGTTGAATAGTAAAA-3' probe 3, Fatty Acid Binding Protein 2 (*Fabp2*)
224 F 5'- ACGGAACGGAGCTCACTG-3' R 5'- TGGATTAGTTCATTACCAGAAACCT-3'
225 probe 56, Peroxisome Proliferator Activated Receptor Gamma (*Pparg*) F 5'-
226 AAGACAACGGACAAATCACCA-3' R 5'- GGGGGTGATATGTTTGAAGTTG-3' probe
227 7, Histone Deacetylase 3 (*HDAC3*) F 5'- GAGAGGTCCCGAGGAGAAC-3' R 5'-
228 CGCCATCATAGAACTCATTGG-3' probe 40, Intestinal-type fatty acid-binding protein
229 (*Ifabp*) 5'-GGTTTCTGGTAATGAACTAATCCAG-3' 5'-
230 AAATCTGACATCAGCTTAGCTCTTC-3' probe 1, the housekeeping gene Elongation
231 factor 1-alpha (*Efla*) F 5'- GCCAAT TTCTGGTTGGAATG-3' R 5'-
232 GGTGACTTTCCATCCCTTGA-3' probe 67 was used to normalize gene expression.

233 2.6 Nuclear magnetic resonance (NMR)

234 Body composition (fat, lean mass, free fluid) was measured using a minispec TD-NMR
235 analyser (Bruker Optics, Ettlingen, Germany). Mice were placed in a plastic restrainer and
236 inserted in the minispec for measurements

237 2.7 Energy assimilation

238 Fecal samples were collected from individual mice over 5 days and dried at 55 °C for another
239 5 days. Dried fecal pellets were grinded using the TissueLyserII (Qiagen, Retsch, Haan,
240 Germany) and pressed into pellets of 1 gram (technical duplicates). Gross fecal energy content
241 was measured using a 6400 calorimeter (Parr Instrument Company, Moline, IL, USA).
242 Assimilation efficiency was calculated by recording the food intake and feces production over
243 the fecal collection days as indicated in the formula below.

$$244 \text{ Assimilation efficiency (\%)} = \frac{(\text{Food intake [g]} * \text{Efood [kJ} * \text{g}^{-1}]) - (\text{Feces production [g]} * \text{Efeces [kJ} * \text{g}^{-1}])}{\text{Food intake [g]} * \text{Efood [kJ} * \text{g}^{-1}}} * 100$$

245 2.8 High-Throughput 16S Ribosomal RNA (rRNA) Gene Sequencing and microbial Analysis

246 Snap-frozen fecal samples was possessed in accordance to slightly mpdified protocol from
247 Godon and colleagues to isolate genomic DNA [23]. DNA was purified with DNA NucleoSpin
248 gDNA columns (Machery-Nagel, No. 740230.250). 24ng DNA was used in a two-step PCR
249 using 341F-ovh and 785r-ov primer to amplify V3-V4 region of 16s rRNA. Sampled were
250 pooled and sequenced in pair-end mode (2x250 bp) on Illumina HiSeq using Rapid V2
251 chemistry, as previously described ({Reitmeier, 2020 #48}). For every 45 samples we included
252 two negative controls of DNA stabilizer without fecal samples to insure reproducibility and
253 control for artifacts. High quality sequence of 16s rRNA with >500 read counts were used for
254 microbial data analysis. FASTQ files were further processed with NGSToolkit (Version
255 3.5.2_64) with trim score of 5 at both 5' and 3' end of R1 and R2 read, then chimera was
256 removed with FASTQ mergepair script of USEARCH. Zero-radius operational taxonomic
257 units (zOTUs) were generated after denoising, deduplicating, clustering and merging quality

258 filtered reads. Here we used zOTUs to have the utmost possible resolution of 16s rRNA
259 sequencing by correcting for sequencing error and identifying sequence with 100% similarity
260 as a unique microbial strain. Taxonomy was assigned based on EZBiocloud database, and
261 RHEA pipeline was used to analyze the data. We aligned the sequence by the maximum
262 likelihood approach with MUSCLE from the software MegaX to generate phylogenetic trees
263 and use the online tool Evolview for tree visualization (<http://www.evolgenius.info/evolview>)
264 [24]. For quantitative analysis, we add spike of 12 artificial DNA that mimics 16s rRNA genes
265 in order to determine 16s rRNA genes copy numbers per gram of fecal sample as previously
266 described [17]

267 2.9 PICRUST 2.0

268 Metagenomic functionality were predicted using PICRUST2.0. Briefly, based on zOTUs
269 sequence metagenome was constructed to predict functional genes, Normalized zOTU copy
270 numbers were multiplied by the genes for each zOTU. Finally, enzymatic genes were classified
271 to Enzyme Commission (EC) numbers and were assigned to Metacyc pathways. After
272 removing super-classes, and we used Metacyc pathways for LDA effective score (LEFSE)
273 calculation [25] using the online tool (<http://huttenhower.sph.harvard.edu/galaxy>).

274

275 2.10 Sample preparation for targeted analysis

276 Approximately 20 mg of mouse faeces was weighed in a 2 mL bead beater tube (Lysing Matrix
277 D, MP Biomedicals). 1 mL of methanol-based dehydrocholic acid extraction solvent
278 ($c=1.3 \mu\text{mol/L}$) was added as an internal standard to correct for work-up losses. The samples
279 were extracted 3 times for 20 seconds with 6 m/sec with 30 seconds breaks in using a FastPrep-
280 24 5G bead beating grinder (MP Biomedicals) supplied with a CoolPrep adapter.

281

282 2.11 Targeted bile acid (BA) measurement

283 20 μ L of isotopically labeled bile acids (ca. 7 μ M each) were added to 100 μ L of sample extract.
284 Targeted bile acid measurement was performed using a QTRAP 5500 triple quadrupole mass
285 spectrometer (Sciex, Darmstadt, Germany) coupled to an ExionLC AD (Sciex, Darmstadt,
286 Germany) ultrahigh performance liquid chromatography system according to Reiter et al.[26]
287 . Briefly, a multiple reaction monitoring (MRM) method was used for the detection and
288 quantification of the bile acids. An electrospray ion voltage of -4500 V and the following ion
289 source parameters were used: curtain gas (35 psi), temperature (450 $^{\circ}$ C), gas 1 (55 psi), gas 2
290 (65 psi), and entrance potential (-10 V). For separation of the analytes a 100×2.1 mm, 100 \AA ,
291 1.7 μ m, Kinetex C18 column (Phenomenex, Aschaffenburg, Germany) was used.
292 Chromatographic separation was performed with a constant flow rate of 0.4 mL/min using a
293 mobile phase consisted of water (eluent A) and acetonitrile/water (95/5, v/v, eluent B), both
294 containing 5 mM ammonium acetate and 0.1% formic acid. The gradient elution started with
295 25% B for 2 min, increased at 3.5 min to 27% B, in 2 min to 35% B, which was hold until 10
296 min, increased in 1 min to 43% B, held for 1 min, increased in 2 min to 58% B; held 3 min
297 isocratically at 58% B, then the concentration was increased to 65% at 17.5 min, with another
298 increase to 80% B at 18 min, following an increase at 19 min to 100% B which was hold for 1
299 min, at 20.5 min the column was equilibrated for 4.5 min at starting. The injection volume for
300 all samples was 1 μ L, the column oven temperature was set to 40 $^{\circ}$ C, and the auto-sampler was
301 kept at 15 $^{\circ}$ C. Data acquisition and instrumental control were performed with Analyst 1.7
302 software (Sciex, Darmstadt, Germany).

303

304 2.12 Targeted short chain fatty acid (SCFA) measurement

305 The 3-NPH method was used for the quantitation of SCFAs [27; 28]. Briefly, 40 μ L of the
306 fecal extract and 15 μ L of isotopically labeled standards (ca 50 μ M) were mixed with 20 μ L
307 120 mM EDC HCl-6% pyridine-solution and 20 μ L of 200 mM 3-NPH HCL solution. After

308 30 min at 40°C and shaking at 1000 rpm using an Eppendorf Thermomix (Eppendorf,
309 Hamburg, Germany), 900 µL acetonitrile/water (50/50, v/v) was added. After centrifugation at
310 13000 U/min for 2 min the clear supernatant was used for analysis. The same system as
311 described above was used. The electrospray voltage was set to -4500 V, curtain gas to 35 psi,
312 ion source gas 1 to 55, ion source gas 2 to 65 and the temperature e to 500°C. The MRM-
313 parameters were optimized using commercially available standards for the SCFAs. The
314 chromatographic separation was performed on a 100 × 2.1 mm, 100 Å, 1.7 µm, Kinetex C18
315 column (Phenomenex, Aschaffenburg, Germany) column with 0.1% formic acid (eluent A)
316 and 0.1% formic acid in acetonitrile (eluent B) as elution solvents. An injection volume of 1
317 µL and a flow rate of 0.4 mL/min was used. The gradient elution started at 23% B which was
318 held for 3 min, afterward the concentration was increased to 30% B at 4 min, with another
319 increase to 40%B at 6.5 min, at 7 min 100% B was used which was hold for 1 min, at 8.5 min
320 the column was equilibrated at starting conditions. The column oven was set to 40°C and the
321 autosampler to 15°C. Data acquisition and instrumental control were performed with Analyst
322 1.7 software (Sciex, Darmstadt, Germany).

323

324 2.13 Statistical analysis

325 Statistical analysis was performed using GraphPad Prism, version 9.3.0 (GraphPad Software),
326 R and online platforms (see below). The RHEA pipeline (Lagkourdos) was used to calculate
327 generalized Unifrac distances between sample and consequently to determine microbiota
328 diversity, MDS plots were used to visualize distances between samples [29]. To calculate the
329 circadian pattern of each 24h period graphs, we used cosine-wave equation:
330 $y = \text{baseline} + (\text{amplitude} \cdot \cos(2 \cdot \pi \cdot ((x - [\text{phase shift}]) / 24)))$, with a fixed 24-h period. This equation
331 was used to determine significance of rhythmicity of clock genes, richness, phyla, family and
332 exemplary profiles of zOTUs. Overall rhythmicity of zOTUs was determined with

333 JTK_CYCLE algorithm [30]. For the manhattan plots JTK_CYCLE was used to calculate
334 amplitude and p-value, and the phase was calculated by cosine-wave regression. Evolview was
335 used for tree visualization (<http://www.evolgenius.info/evolview>)[24]. To generate heatmaps
336 with the online tool (heatmapper.ca) [31], we sorted the zOTUs or pathways based on the phase
337 of the control group for visualization. The R package SIAMCAT with the function
338 “check.association” [32] was used to generate abundance plots. In order to compare two
339 groups, the non-parametric Mann-Whitney test was used. Two-way ANOVA was used to
340 compare weight gain, clock genes expression in SSW and transfer experiment with Tukey
341 posthoc test for multiple comparison. P-values ≤ 0.05 were assumed as statistically significant.

342

343 **3 Results**

344 **3.1 Central clock dysfunction induces circadian desynchronization in the GI tract**

345 Recently we showed that when mice lacking a functional central clock are released into
346 constant darkness (DD), peripheral clocks such as the adrenal, liver, kidney, heart, pancreas,
347 and white adipose tissue gradually desynchronize [6; 9]. As a consequence of system-wide
348 circadian desynchronization, these mice develop obesity and altered glucose metabolism [6].
349 Of importance, metabolic homeostasis is partially controlled by GI functions regulated by the
350 circadian system [33]. To investigate the degree of circadian desynchronization in peripheral
351 clocks within the GI tract, we compared clock gene expression rhythms in the jejunum, cecum
352 and proximal colon between mice lacking the major clock gene *Bmal1* specifically in the SCN
353 (*Bmal1^{SCN^{fl}/-}*) and their littermate controls (*Bmal1^{SCN^{+/+}}*) on the 2nd day of DD (**Fig. 1, Table 1**).
354 Circadian rhythmicity analysis revealed that the expression of the core clock genes *Bmal1*,
355 *Per2* and *Rev-erba* followed circadian oscillation in the jejunum in both genotypes (cosine-
356 wave regression, control: p=0.004, p=0.02, p=0.03, *Bmal1^{SCN^{fl}/-}*: p=0.01, p=0.01, p=0.04) (**Fig.**
357 **1A, Table 1**). However, the circadian phases in all clock genes examined in *Bmal1^{SCN^{fl}/-}* were

358 significantly advanced (*Bmal1*: 2.7h, *Per2*: 3.6h, *Rev-erba*: 5.7h). In addition, the baseline of
359 *Rev-erba* was reduced, *Dbp* did not show significant rhythmicity using cosine regression, but
360 a significant time effect was found in both genotypes by two-way ANOVA analysis (p=0.01).
361 *Cry1* lost rhythmicity in *Bmal1^{SCN^{fl/-}}* mice (*Cry1*: p=0.009, p=0.42). In the cecum, all clock
362 genes examined lost rhythmicity in *Bmal1^{SCN^{fl/-}}* mice, although a time effect was found for both
363 genotypes by two-way ANOVA (time: *Bmal1* p=0.006, *Per2* p=0.002, *Rev-erba* p=0.0009,
364 *Dbp* p=0.03, *Cry1* p=0.003) (**Fig. 1B, Table 1**). In contrast, rhythmicity of *Bmal1*, *Per2* and
365 *Cry1* gene expression in the proximal colon was undistinguishable between genotypes, and the
366 amplitude of *Rev-erba* expression was significantly reduced (cosine regression, p=0.02).
367 Similar to results obtained from jejunum, *Dbp* lost rhythmicity in *Bmal1^{SCN^{fl/-}}* mice (**Fig. 1C,**
368 **Table 1**). Altogether, these results suggest that in *Bmal1^{SCN^{fl/-}}* mice the jejunal clock free-runs
369 with a reduced amplitude, the cecal clock slowly loses its functionality, whereas the colon clock
370 is functional, albeit with a dampened amplitude. Consequently, these data demonstrate
371 profound disruption of GI clocks in the absence of a functional central clock, which appears at
372 a very early stage following release into constant darkness.

373

374 3.2 Disruption of microbiota rhythmicity in SCN-specific *Bmal1*-deficient mice

375 GI clocks are dominant regulators of circadian microbiome fluctuations and thereby balance
376 GI homeostasis, as previously shown by us [17]. This prompted us to determine whether
377 circadian desynchronization in GI tissues in *Bmal1^{SCN^{fl/-}}* mice affects circadian microbiota
378 composition and function. Indeed, 16s rRNA analysis of fecal samples revealed significant
379 clustering according to genotype (**Fig. 2A**), suggesting a different microbiota composition in
380 *Bmal1^{SCN^{fl/-}}* mice. Moreover, circadian rhythmicity in community diversity (species richness)
381 observed in control mice was abolished in *Bmal1^{SCN^{fl/-}}* mice, although Generalized UniFrac
382 distance (GUniFrac) quantification to CT1 identified a time difference in both genotypes (two-

383 way ANOVA, $p=0.0037$) (**Fig. 2B**). Relative abundance of the two major phyla, *Firmicutes*
384 and *Bacteroidetes*, showed circadian rhythmicity with similar patterns in both genotypes (**Fig.**
385 **2C**). However, previous research, including from our own group, showed that rhythmicity in
386 relative abundance can be masked due to oscillations of highly abundant taxa [17; 34]. Thus,
387 we used synthetic DNA spikes to determine quantitative microbiota composition as previously
388 described [35]. Indeed, both phyla lost rhythmicity in quantitative abundance in *Bmal1*^{SCNfl/-}
389 mice compared to controls (**Fig. 2C**). Central clock disruption led to loss of rhythmicity of the
390 families *Lactobacillaceae* and *Clostridiales* independent of the analysis (**Suppl. Fig.1A**). Then
391 we set out to determine rhythmicity of zero-radius OTUs (zOTUs) after removal of low-
392 abundance taxa (mean relative abundance < 0.1%; prevalence < 10%). The heatmaps illustrate
393 disrupted circadian oscillations of zOTUs in *Bmal1*^{SCNfl/-} mice for both analyses (**Fig. 2D,**
394 **Suppl. Fig. 1B**). The amount of rhythmic zOTUs was reduced by three quarters in mice with
395 SCN-specific *Bmal1* deficiency (JTK_CYCLE, adj. p-value < 0.05) (**Fig. 2E, Suppl. Fig.1C,**
396 **Suppl. Table. 1**). For example, we identified zOTUs which lost rhythmicity in *Bmal1*^{SCNfl/-}
397 mice predominantly belonging to mucus foragers (*Muribaculaceae*) and to the secondary bile
398 acid and SCFA producing family *Ruminococcaceae* [36; 37] (**Fig. 2F, Suppl. Fig.1D**). In
399 particular, SCFA producing taxa, including *Faecalibaculum* and *Agathobaculum* [38], were
400 arrhythmic in *Bmal1*^{SCNfl/-} mice (**Fig. 2F, G, Suppl. Fig.1D, E**). Of note, bacteria belonging to
401 *Alloprevotella*, *Muribaculaceae* and *Faecalibaculum* lost rhythmicity and additionally differed
402 in their abundance between genotypes (**Suppl. Fig. 1F**).

403

404 3.3 SCN clock-controlled microbial functions balance metabolic homeostasis

405 To address the potential physiological relevance of microbial rhythmicity we performed
406 PICRUST 2.0 analysis on zOTUs which lost rhythmicity in *Bmal1*^{SCNfl/-} mice [39]. SCN clock-
407 deficient mice develop adiposity and impaired glucose handling [6]. In this context, genotype

408 differences and loss of rhythmicity was observed in predicted pathways related to sugar
409 metabolism, SCFA fermentation and fatty acid metabolism (**Fig. 3A, Suppl. Fig. 2A**).
410 Targeted metabolite analysis further revealed that alterations in taxa identified in *Bmal1^{SCNfl/-}*
411 mice led to changes in key bacterial products involved in sugar and lipid signaling, such as
412 SCFAs and (BAs (**Fig. 3B-F, Suppl. Fig. 2B-D**). In particular, propionic acid, important for
413 lipid metabolism [40], showed reduced levels in *Bmal1^{SCNfl/-}* mice (**Fig. 3B**). Moreover,
414 branched-chain fatty acids including isovaleric acid, isobutyric acid and 2-methylbutyric acid
415 were reduced in *Bmal1^{SCNfl/-}* mice, whereas total SCFA concentrations were undistinguishable
416 between genotypes (**Fig. 3B, Suppl. Fig. 2B**). Rhythmicity of total SCFAs as well as of major
417 microbial derived products such as acetic acid, propionic acid and lactic acid was absent in
418 *Bmal1^{SCNfl/-}* mice (cosine regression, control: $p=0.003$, $p=0.001$, $p=0.02$, $p=0.0009$, *Bmal1^{SCNfl/-}*
419 : $p=0.32$, $p=0.5$, $p=0.49$, $p=0.93$) (**Fig. 3C**). Of note, other SCFAs, including butyric acid and
420 valeric acid, showed rhythmicity in both genotypes (cosine regression, control $p=0.0001$,
421 $p=0.007$, *Bmal1^{SCNfl/-}*, $p=0.02$, $p=0.01$, respectively) (**Suppl. Fig. 2C**). In addition, BA
422 concentrations were altered in mice lacking a functional central clock (**Fig. 3D, Suppl. Fig.**
423 **2D**). For example, 6-ketolithocholic acid concentrations were reduced, whereas concentrations
424 of b-muricholic acid and tauro-a-muricholic acid were significantly elevated in *Bmal1^{SCNfl/-}*
425 mice (**Fig. 3D**). Although other BAs measured had comparable concentrations in both
426 genotypes, rhythmicity of various BAs was disrupted in *Bmal1^{SCNfl/-}* including, 7-sulfocholic
427 acid, ursodeoxycholic acid, taurocholic acid and allolithocholic acid (**Fig. 3E, F, Suppl. Fig.**
428 **2D**), suggesting altered fat and cholesterol metabolism [41].
429 Taken together, our results highlight the importance of the central clock in synchronizing
430 peripheral clocks located in GI tissues. In addition, these results show for the first time loss of
431 microbial taxa and their functional outputs, in particular SCFAs and BAs, in mice lacking

432 central clock function, which associates with adiposity and impaired glucose metabolism in
433 these animals [6].

434

435 3.4 Simulated shift work induces circadian desynchrony between GI clocks

436 Epidemiological and experimental studies indicate that frequent circadian desynchronization
437 increases the risk of developing metabolic diseases and weight gain [42; 43], similar to the
438 phenotype observed in central clock-deficient mice [6]. Circadian desynchronization among
439 tissue clocks, as observed in mice lacking the central clock [9], can be induced by misalignment
440 between internal and environmental time, such as during jetlag or shift work [5]. To investigate
441 whether shift work induces circadian desynchrony among GI clocks similar to the effects of a
442 loss of central clock function in *Bmal1^{SCNfl/-}* mice, wild type mice were exposed to phase shifts
443 of 8 hours every 5th day for 6-8 weeks to SSW (**Fig. 4A**). The activity profiles gradually
444 advanced during the first days in SSW (**Fig. 4A, B**). In particular, in comparison to the LD
445 profiles before SSW and the control cohort kept in LD, the activity onset advanced by less than
446 3 hours at the 1st day (**Fig. 4A, B**). This resulted in an equal distribution of activity between
447 prior day and night, although total activity was unaffected (**Fig. 4B, Suppl. Fig. 3A**). In line
448 with previous studies, mice in SSW significantly increased their body weight ($P < 0.0001$) [15]
449 (**Fig. 4C**). In addition, colon permeability was enhanced at CT13 during SSW, although no
450 difference in energy assimilation or total food intake was detected (**Fig. 4D-F**).

451 Differences in the resetting speed of circadian clocks and between clock genes within the same
452 tissue have been reported [5]. To test whether GI clocks are affected differentially by SSW,
453 clock gene expression in GI tissues was measured at ZT1 and ZT13 (1 and 13 hours after the
454 lights on in controls). Indeed, diurnal expression of clock genes in GI tissues and the liver as
455 control was differentially affected at the 1st day during the last phase advance in SSW (**Fig.**
456 **4A, I, J**). Although *Bmal1* and *Per2* in the liver, jejunum and proximal colon showed daytime

457 dependent expression in both genotypes, *Dbp*, *Cry1* and *Rev-erba* were affected only in
458 specific tissues. For example, *Dbp* was dramatically reduced at ZT13 in the liver and jejunum
459 of mice exposed to SSW, whereas no daytime effect, but enhanced expression during SSW,
460 was found for *Dbp* (**Fig. 4I, J**). In contrast, in the cecum daytime differences of *Bmall* were
461 absent and *Cry1* significantly enhanced its expression at ZT13 during SSW, while *Per2*, *Rev-*
462 *erba* and *Dbp* were unaffected (**Fig. 4I**). Moreover, in the colon of mice undergoing SSW, a
463 time difference in the expression of almost all clock genes examined (except of *Cry1*) was
464 found, although *Bmall*, *Rev-Erba* and *Dbp* expression was significantly suppressed at ZT13
465 (**Fig. 4I**). These results indicate that all peripheral clocks examined were in different resetting
466 stages of the phase advance and consequently circadian desynchronization was evident
467 between GI clocks.

468 3.5 Simulated shift work disrupts rhythmicity of microbiota composition and function

469 Previous research, including from our own group, indicates that changes in environmental
470 conditions can modify microbial community composition and cause arrhythmicity of specific
471 taxa [15; 17; 44]. In accordance, we found significantly different fecal microbial communities
472 between mice exposed to LD and SSW conditions ($p=0.014$) (**Fig. 5A**). Rhythmicity of
473 GUniFrac distance quantification as well as the relative and quantitative abundance of major
474 phyla and families was phase shifted in line with the advanced behavioral rhythm (**Fig. 4A, B,**
475 **Fig. 5B-D, Suppl. Fig. 3B, C**). Importantly, the quantitative abundance of *Bacteroidetes* lost
476 rhythmicity in SSW (**Fig. 5C**). Heatmaps of bacterial abundances over the course of the 24-
477 hour day illustrate phase advanced rhythms of zOTUs during SSW independent of the analysis
478 (**Suppl. Fig. 3D, E**). Moreover, arrhythmicity was identified during SSW in ~50% of all
479 rhythmic zOTUs in LD conditions, including *Lactobacillus*, *Ruminococcus* and *Odoribacter*
480 (**Fig. 5E, G, Suppl. Fig. 3D-F, Suppl. Table 1**). zOTUs which lost rhythmicity in quantitative
481 and relative analyses included taxa belonging to *Eubacterium*, *Bacteroides* and *Ruminococcus*

482 (Fig. 5G, H, Suppl. Fig. 3G, Suppl. Table 1). The phase of the remaining rhythmic zOTUs in
483 SSW advanced by 3.7 - 6.4h, including the genera *Alistipes*, *Duncaniella*, *Roseburia*,
484 *Oscillibacter* and the family *Lachnospiraceae*, (Fig. 5F, Suppl. Fig. 3D, F, Suppl. Table 1).
485 Of note, the average abundance of arrhythmic zOTUs belonging to the *Ruminococcaceae* and
486 *Muribaculaceae* families as well as the genus *Lactobacillus* significantly differed between
487 SSW and LD conditions (Fig. 5G, Suppl. Fig 3F) in accordance with results obtained from
488 mice exposed to chronic jetlag or sleep deprivation [15; 44-46].

489 To evaluate whether GI clock desynchronization during SSW might have induced similar
490 disturbance of microbial oscillations as observed in mice with central clock disruption, we
491 analyzed rhythmicity of the microbiome in mice undergoing SSW. Of note, overall microbiota
492 composition was not comparable between these two experiments performed in different animal
493 facilities (Suppl. Fig. 3H). However, this is in accordance with frequent reports illustrating
494 that the housing situation dramatically influences microbiota composition [47]. To consider
495 microbiota function rather than composition, we performed PICRUST analysis of zOTUs
496 which lost rhythmicity in SSW (Fig. 5H). Their predicted functionality was then compared to
497 results obtained from arrhythmic taxa identified in *Bmal1^{SCN^{fl}/-}* mice (Fig. 3A, Fig. 5H, Suppl.
498 Fig. 3I). Independent of the approach of inducing circadian desynchronization, disrupted
499 rhythmicity and changes in abundance were found in pathways related to amino acids, fatty
500 acids as well as sugar metabolism and SCFA fermentation (Fig. 5I), suggesting a functional
501 link between circadian microbiota regulation and GI physiology.

502

503 3.6 Simulated shift work-associated microbiota promote weight gain and suppress GI clocks

504 In order to directly investigate the effect of SSW-induced arrhythmicity of the microbiome on
505 the host, we performed cecal microbiota transfer from donor mice undergoing 6 weeks of SSW
506 and controls kept in LD into germ-free (GF) wild type recipients (Fig. 6A). Mice receiving

507 SSW-associated microbiota significantly increased their body weight (**Fig. 6B**), in line with
508 observations following fecal microbiota transplantation from mice exposed to chronic jetlag
509 [15]. Interestingly, 6 weeks after transfer, body weight as well as most organ weights were
510 undistinguishable between recipients (**Fig. 6A-C**), indicating that microbial alterations are
511 temporary in rhythmic hosts. Of note, an increased cecum weight was observed even 6 weeks
512 after transfer (**Fig. 6C**). Microbial derived products, especially SCFAs and BAs have been
513 described to alter clock gene expression in GI tissues [48; 49]. This prompted us to measure
514 clock gene expression in recipients as well as in GF controls. Indeed, mice receiving SSW-
515 associated microbiota showed altered GI clock gene expression 6 weeks after the transfer (**Fig.**
516 **6D**). Although most clock genes examined in the proximal colon fluctuated between daytimes
517 independent of the genotype of the donor, *Per2* expression was highly suppressed at ZT13 and
518 the daytime difference of *Rev-erba* expression in controls was absent in mice receiving SSW-
519 associated microbiota (**Fig. 6D**). Similarly, *Per2*, *Cry1* and *Dbp* expression in jejunum as well
520 as *Per2*, *Rev-erba* and *Dbp* expression in cecum was suppressed at ZT13 in mice receiving
521 SSW-associated microbiota. Dampened daytime differences in GI clock gene expression
522 followed similar trends than observations made in donor mice exposed to SSW and in GF mice
523 (**Fig. 4I, Fig. 6D**). These results suggest that the microbiome can at least partly transfer the GI
524 clock phenotype from the donor to the host and thus directly impact GI physiology. In mice
525 receiving SSW-associated microbiota, we then investigated the effect of clock gene
526 suppression on clock-controlled genes related to glucose and fat metabolism, such as *Fabp2*,
527 *Hdac3*, *Ifab*, *Glut2* and *Ppary* [50-52]. Indeed, in the jejunum, suppressed expression was
528 found for *Fabp2* involved in lipid uptake [52] and *Glut2* a regulator for glucose uptake [53]. In
529 the colon, enhanced expression was found for *Ppary*, a transcriptional regulator of glucose and
530 lipid metabolism [50] (**Fig. 6E, F**) and SCFAs were shown to modulate the metabolic state of
531 the host through PPARs [54]. Altogether, these results demonstrate the physiological relevance

532 of the GI clock-microbiome crosstalk, specifically for maintenance of the host's metabolic
533 health.

534

535 **4 Discussion**

536 Mice with central clock dysfunction were shown to develop a metabolic phenotype and
537 desynchrony in peripheral clocks, such as the adrenal, the liver, the heart, the pancreas and
538 eWAT [6; 9]. In addition, we provide evidence that GI clocks desynchronize in the absence of
539 a functional central clock. Moreover, we demonstrate that desynchronization among GI clocks
540 also appears in wild type mice exposed to SSW conditions. Our results comply with alterations
541 in colonic clock gene expression following chronic jet lag [15] and suggest that GI clock
542 desynchrony is a common feature during circadian disruption. Of note, distinct sections of the
543 GI circadian system responded differentially to circadian disturbances, which was evident in
544 the genetic model and during environmentally induced circadian disruption. Considering
545 previous research indicating a temporal phase gradient of clock gene rhythms along the gut
546 cranio-caudal axis [55], the circadian response to circadian disturbance might differ between
547 gut sections. However, 24-hour profiling of clock gene expression over multiple days would
548 be necessary to compare the kinetics of resetting between intestinal tissue clocks.

549 Recently, we identified that GI clocks are prominent drivers of gut microbiota rhythmicity [17].
550 Consequently, arrhythmicity of the microbiota observed in mice with central clock disruption
551 and in mice kept in SSW was likely induced by desynchronization among GI clocks. Indeed,
552 in line with recent results obtained from mice with dysfunctional intestinal clocks [17], taxa
553 belonging to the families *Rikenellaceae*, *Ruminococcaceae* and *Muribaculaceae* as well as to
554 the genera *Lactobacillus* and *Alistipes* lost rhythmicity in *Bmal1^{SCNfl/-}* mice and in mice
555 undergoing SSW. Of note, disruption of rhythmicity was more severe in mice lacking a
556 functional central clock. Here, arrhythmicity was found in microbial diversity and on the level

557 of phyla and families. During SSW the abundance of the phylum *Bacteroidetes* and, thus, a
558 substantial amount of taxa remained rhythmic, although with an advanced phase. This
559 discrepancy between both models might be explained by the arrhythmic food intake behaviour
560 documented in *Bmal1^{SCNfl/-}* mice upon release in DD [18], whereas in SSW the daily patterns
561 of food intake were rhythmic but phase shifted. Manipulating the timing of food intake has
562 been shown to phase shift specific taxa belonging to *Alistipes*, *Lactobacillus* and *Bacteroides*
563 [15; 17]. Therefore, a phase-advanced food intake rhythm in SSW could have changed the
564 phase of bacterial oscillations. Nevertheless, a substantial amount of taxa lost rhythmicity upon
565 exposure to SSW and were also found to lose rhythmicity in mice with SCN-specific and GI
566 clock disruption [17], indicating that loss of synchrony between GI clocks may be responsible
567 for microbial arrhythmicity during circadian disruption.

568 Recently we discovered a link between microbiota rhythmicity, obesity and T2D development
569 in humans [16], suggesting that microbial rhythms may play a causative role for disease
570 development. Accordingly, transfer of microbiota from an obese human donor as well as from
571 lean donors undergoing jetlag induces an obesity-associated phenotype in GF recipient mice
572 [15; 45; 56]. However, these studies did not address whether obesity associated loss of
573 microbial rhythmicity or general changes in abundance of bacteria are the underlying cause.
574 Transfer experiments using mouse models with circadian dysfunction provide direct evidence
575 for the physiological relevance of microbiota rhythms for metabolic health. For example,
576 transfer of arrhythmic microbiota from gut-clock deficient mice disrupts GI homeostasis in
577 recipient animals [17], and microbiota from mice exposed to environmentally induces circadian
578 disruption promoting body weight gain in wild type mice. Similar results were obtained
579 following microbiota transfer from jet lagged mice [15]. Together, these results suggest that on
580 top of peripheral clock disruption in the fat and liver[6; 9], the rhythmicity of the microbiome
581 is a critical factor for the development of metabolic disease.

582 GI metabolism is strongly influenced by bacterially derived products, such as SCFAs and BAs
583 [57; 58]. After both genetic and environmental circadian disruption, loss of microbial
584 rhythmicity was reflected by arrhythmicity of predicted microbial functionality, such as SCFA
585 fermentation, as well as sugar, fatty acid and amino acid metabolism. Targeted metabolite
586 analysis further confirmed lack of rhythmicity of key microbial derived products in *Bmal1^{SCN^{fl}/-}*
587 mice, namely SCFAs and BAs. For example, arrhythmicity was found for the SCFAs Propionic
588 acid and Acetic acid. Both play a major role in fat and glucose metabolism and are capable in
589 preventing diet induced obesity and insulin resistance [40]. Additionally, alterations in either
590 rhythmicity or abundance of taurine-conjugated bile acids as well as the secondary BA
591 Ursodeoxycholic acid were observed. These metabolites are known to impact signaling through
592 the nuclear bile acid receptor FXR, resulting in the transcription of target genes important for
593 lipid and glucose homeostasis (reviewed by [41]). Importantly, bacterial metabolites, such as
594 SCFAs and BAs, are controlled by the circadian clock, and alterations in SCFA and BA
595 oscillations were previously reported in mice exposed to chronic jet lag and in GI clock
596 deficient animals [17; 59]. Loss of rhythmicity of SCFAs as well as BAs which are both
597 involved in sugar and fatty acid metabolism (reviewed by [60]) might alter metabolic
598 functionalities of the host following circadian disruption, since both bacterial products are
599 known to balance host metabolism (reviewed by [61]). In this regard, we previously reported
600 an increased body weight gain, when *Bmal1^{SCN^{fl}/-}* mice were kept in DD for multiple weeks [6].
601 Of importance, loss of microbiota rhythms and subsequent microbial functions predominantly
602 involved in glucose and lipid metabolism, such as Ursodeoxycholic acid, Propionic acid and
603 Acetic acid [57; 62; 63], were already found at the 2nd day of DD and thus precede the obesity
604 phenotype reported in these mice. Consequently, the observed microbial changes might
605 represent an early event in the development of the metabolic phenotype of *Bmal1^{SCN^{fl}/-}* mice
606 [6].

607 Interestingly, shift work associated bacteria directly affect the host's GI clock function. In
608 particular, GI clock dysregulation in donor mice following circadian disruption was partly
609 reflected in recipients. For example, suppression of daytime differences in colonic *Rev-Erba*
610 and *Dbp* expression in jejunum was evident in both donor and recipient, indicating that
611 microbiota transfer the circadian phenotype from the donor to recipients. Peripheral circadian
612 clocks are known to control organ functions through regulation of tissue-specific CCGs ([4]).
613 Accordingly, GI clock disruption in recipients altered the expression levels of CCGs in jejunum
614 and colon, such as *Fabp2*, *Glut2* and *Ppary*, both involved in glucose and fat metabolism [50;
615 53]. The mechanisms linking microbiota rhythms with functions of the GI tissue likely involve
616 local epithelial-microbial interactions. Indeed, SCFAs and BAs have been reported to directly
617 impact rhythmicity in intestinal epithelial cells and affect metabolic responses of the host [48;
618 59; 64; 65]. Consequently, arrhythmicity of the transferred microbiota likely resulted in
619 arrhythmicity of bacterial products, capable to alter GI clock function and, subsequently,
620 metabolic CCGs. Therefore, our results provide first mechanistic insights into microbiota-
621 dependent metabolic abnormalities during circadian disruption.

622

623 **5 Conclusions**

624 Taken together, the comparison of two models of genetic and environmentally induced
625 circadian disruption revealed shared disruption at the level of GI clocks and identified
626 microbial taxa and their functionalities involved in metabolic abnormalities of the host. Further,
627 microbial alterations during SSW appear to be causal for the metabolic phenotype of the host.
628 Our data provide first evidence that molecular alterations of GI clock function during circadian
629 disruption are transferrable between organisms through the microbiome. Thereby our data
630 highlight the intestinal clock-bacteria dialogue as a potent underlying factor in the development
631 of metabolic diseases in humans exposed to circadian disruption due to their lifestyle.

632 **6 Author contribution**

633 SK conceived and coordinated the project. BA, VP, MH, YN and EG performed mouse
634 experiments and fecal samples collection. YN and MH measured epithelial membrane
635 properties. MH conducted bomb calorimetry and NMR. SK and MH analyzed activity and food
636 intake behavior. BA and MH performed 16S rRNA gene sequencing and bioinformatics
637 analysis. BA analyzed gene expression, predicted microbial functionality and conducted germ
638 free mouse colonization. KK, MG and BA performed targeted metabolomics and data analyses.
639 SK supervised the work and data analysis. SK, HO and DH secured funding. BA, SK and MH
640 wrote the manuscript. All authors reviewed and revised the manuscript.

641

642 **7 Funding**

643 SK was supported by the German Research Foundation (DFG, project KI 19581) and the
644 European Crohn's and Colitis Organisation (ECCO, grant 5280024). SK and DH received
645 funding by the Funded by the Deutsche Forschungsgemeinschaft (DFG, German Research
646 Foundation) – Projektnummer 395357507 – SFB 1371). HO was funded by the DFG (project
647 OS353-11/1).

648

649 **8 Data availability**

650 Microbiota sequencing data and metabolite data will be available from the Sequence Read Archive
651 (SRA) and the MetaboLights database for Metabolomics experiments (<https://www.ebi.ac.uk/metabolights>) upon request.

652

653 **9 Declaration of interest**

655 The authors declare no competing interests.

656

657 **10 Acknowledgements**

658 The Technical University of Munich provided funding for the ZIEL Institute for Food &
659 Health, animal facility support, technical assistance and support for 16S rRNA gene amplicon
660 sequencing. Qu Guojing provided assistance with bomb calorimetry experiments and
661 preliminary data collection.

662

663 **11 References**

- 664 [1] Mohawk, J.A., Green, C.B., Takahashi, J.S., 2012. Central and peripheral circadian
665 clocks in mammals. *Annu Rev Neurosci* 35:445-462.
- 666 [2] Oster, H., Damerow, S., Kiessling, S., Jakubcakova, V., Abraham, D., Tian, J., et al.,
667 2006. The circadian rhythm of glucocorticoids is regulated by a gating mechanism residing in
668 the adrenal cortical clock. *Cell Metab* 4(2):163-173.
- 669 [3] Lamia, K.A., Storch, K.F., Weitz, C.J., 2008. Physiological significance of a peripheral
670 tissue circadian clock. *Proc Natl Acad Sci U S A* 105(39):15172-15177.
- 671 [4] Zhang, R., Lahens, N.F., Ballance, H.I., Hughes, M.E., Hogenesch, J.B., 2014. A
672 circadian gene expression atlas in mammals: implications for biology and medicine. *Proc Natl*
673 *Acad Sci U S A* 111(45):16219-16224.
- 674 [5] Kiessling, S., Eichele, G., Oster, H., 2010. Adrenal glucocorticoids have a key role in
675 circadian resynchronization in a mouse model of jet lag. *J Clin Invest* 120(7):2600-2609.

- 676 [6] Kolbe, I., Leinweber, B., Brandenburger, M., Oster, H., 2019. Circadian clock network
677 desynchrony promotes weight gain and alters glucose homeostasis in mice. *Mol Metab* 30:140-
678 151.
- 679 [7] Wendeu-Foyet, M.G., Menegaux, F., 2017. Circadian Disruption and Prostate Cancer
680 Risk: An Updated Review of Epidemiological Evidences. *Cancer Epidemiol Biomarkers Prev*
681 26(7):985-991.
- 682 [8] Marcheva, B., Ramsey, K.M., Peek, C.B., Affinati, A., Maury, E., Bass, J., 2013.
683 Circadian clocks and metabolism. *Handb Exp Pharmacol*(217):127-155.
- 684 [9] Husse, J., Leliavski, A., Tsang, A.H., Oster, H., Eichele, G., 2014. The light-dark cycle
685 controls peripheral rhythmicity in mice with a genetically ablated suprachiasmatic nucleus
686 clock. *FASEB J* 28(11):4950-4960.
- 687 [10] Turnbaugh, P.J., Ley, R.E., Mahowald, M.A., Magrini, V., Mardis, E.R., Gordon, J.I.,
688 2006. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature*
689 444(7122):1027-1031.
- 690 [11] Karlsson, F.H., Tremaroli, V., Nookaew, I., Bergstrom, G., Behre, C.J., Fagerberg, B.,
691 et al., 2013. Gut metagenome in European women with normal, impaired and diabetic glucose
692 control. *Nature* 498(7452):99-103.
- 693 [12] Qin, J., Li, Y., Cai, Z., Li, S., Zhu, J., Zhang, F., et al., 2012. A metagenome-wide
694 association study of gut microbiota in type 2 diabetes. *Nature* 490(7418):55-60.
- 695 [13] Pedersen, H.K., Gudmundsdottir, V., Nielsen, H.B., Hyotylainen, T., Nielsen, T.,
696 Jensen, B.A., et al., 2016. Human gut microbes impact host serum metabolome and insulin
697 sensitivity. *Nature* 535(7612):376-381.
- 698 [14] Thingholm, L.B., Ruhlemann, M.C., Koch, M., Fuqua, B., Laucke, G., Boehm, R., et
699 al., 2019. Obese Individuals with and without Type 2 Diabetes Show Different Gut Microbial
700 Functional Capacity and Composition. *Cell Host Microbe* 26(2):252-264 e210.

- 701 [15] Thaïss, C.A., Zeevi, D., Levy, M., Zilberman-Schapira, G., Suez, J., Tengeler, A.C., et
702 al., 2014. Transkingdom control of microbiota diurnal oscillations promotes metabolic
703 homeostasis. *Cell* 159(3):514-529.
- 704 [16] Reitmeier, S., Kiessling, S., Clavel, T., List, M., Almeida, E.L., Ghosh, T.S., et al.,
705 2020. Arrhythmic Gut Microbiome Signatures Predict Risk of Type 2 Diabetes. *Cell Host*
706 *Microbe* 28(2):258-272 e256.
- 707 [17] Heddes, M., Altaha, B., Niu, Y., Reitmeier, S., Kleigrewe, K., Haller, D., et al., 2021.
708 The intestinal circadian clock drives microbial rhythmicity to maintain gastrointestinal
709 homeostasis. *bioRxiv*:2021.2010.2018.464061.
- 710 [18] Husse, J., Zhou, X., Shostak, A., Oster, H., Eichele, G., 2011. Synaptotagmin10-Cre, a
711 driver to disrupt clock genes in the SCN. *J Biol Rhythms* 26(5):379-389.
- 712 [19] Ubeda, C., Lipuma, L., Gobourne, A., Viale, A., Leiner, I., Equinda, M., et al., 2012.
713 Familial transmission rather than defective innate immunity shapes the distinct intestinal
714 microbiota of TLR-deficient mice. *J Exp Med* 209(8):1445-1456.
- 715 [20] Muller, V.M., Zietek, T., Rohm, F., Fiamoncini, J., Lagkouvardos, I., Haller, D., et al.,
716 2016. Gut barrier impairment by high-fat diet in mice depends on housing conditions. *Mol Nutr*
717 *Food Res* 60(4):897-908.
- 718 [21] Clarke, L.L., 2009. A guide to Ussing chamber studies of mouse intestine. *Am J Physiol*
719 *Gastrointest Liver Physiol* 296(6):G1151-1166.
- 720 [22] Ussing, H.H., Zerahn, K., 1951. Active transport of sodium as the source of electric
721 current in the short-circuited isolated frog skin. *Acta Physiol Scand* 23(2-3):110-127.
- 722 [23] Godon, J.J., Zumstein, E., Dabert, P., Habouzit, F., Moletta, R., 1997. Molecular
723 microbial diversity of an anaerobic digester as determined by small-subunit rDNA sequence
724 analysis. *Appl Environ Microbiol* 63(7):2802-2813.

- 725 [24] Subramanian, B., Gao, S., Lercher, M.J., Hu, S., Chen, W.-H., 2019. Evolvview v3: a
726 webserver for visualization, annotation, and management of phylogenetic trees. *Nucleic Acids*
727 *Research* 47(W1):W270-W275.
- 728 [25] Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W.S., et al.,
729 2011. Metagenomic biomarker discovery and explanation. *Genome Biol* 12(6):R60.
- 730 [26] Reiter, S., Dunkel, A., Metwaly, A., Panes, J., Salas, A., Haller, D., et al., 2021.
731 Development of a Highly Sensitive Ultra-High-Performance Liquid Chromatography Coupled
732 to Electrospray Ionization Tandem Mass Spectrometry Quantitation Method for Fecal Bile
733 Acids and Application on Crohn's Disease Studies. *J Agric Food Chem* 69(17):5238-5251.
- 734 [27] Han, J., Lin, K., Sequeira, C., Borchers, C.H., 2015. An isotope-labeled chemical
735 derivatization method for the quantitation of short-chain fatty acids in human feces by liquid
736 chromatography–tandem mass spectrometry. *Analytica Chimica Acta* 854:86-94.
- 737 [28] Han, J., Lin, K., Sequeira, C., Borchers, C.H., 2015. An isotope-labeled chemical
738 derivatization method for the quantitation of short-chain fatty acids in human feces by liquid
739 chromatography-tandem mass spectrometry. *Anal Chim Acta* 854:86-94.
- 740 [29] Lagkouvardos, I., Fischer, S., Kumar, N., Clavel, T., 2017. Rhea: a transparent and
741 modular R pipeline for microbial profiling based on 16S rRNA gene amplicons. *PeerJ* 5:e2836.
- 742 [30] Hughes, M.E., Hogenesch, J.B., Kornacker, K., 2010. JTK_CYCLE: an efficient
743 nonparametric algorithm for detecting rhythmic components in genome-scale data sets. *J Biol*
744 *Rhythms* 25(5):372-380.
- 745 [31] Babicki, S., Arndt, D., Marcu, A., Liang, Y., Grant, J.R., Maciejewski, A., et al., 2016.
746 Heatmapper: web-enabled heat mapping for all. *Nucleic Acids Res* 44(W1):W147-153.
- 747 [32] Wirbel, J., Zych, K., Essex, M., Karcher, N., Kartal, E., Salazar, G., et al., 2020.
748 Microbiome meta-analysis and cross-disease comparison enabled by the SIAMCAT machine-
749 learning toolbox. *bioRxiv:2020.2002.2006.931808*.

- 750 [33] Segers, A., Depoortere, I., 2021. Circadian clocks in the digestive system. *Nat Rev*
751 *Gastroenterol Hepatol* 18(4):239-251.
- 752 [34] Liang, X., Bushman, F.D., FitzGerald, G.A., 2015. Rhythmicity of the intestinal
753 microbiota is regulated by gender and the host circadian clock. *Proc Natl Acad Sci U S A*
754 112(33):10479-10484.
- 755 [35] Tourlousse, D.M., Yoshiike, S., Ohashi, A., Matsukura, S., Noda, N., Sekiguchi, Y.,
756 2017. Synthetic spike-in standards for high-throughput 16S rRNA gene amplicon sequencing.
757 *Nucleic Acids Res* 45(4):e23.
- 758 [36] Biddle, A., Stewart, L., Blanchard, J., Leschine, S., 2013. Untangling the Genetic Basis
759 of Fibrolytic Specialization by Lachnospiraceae and Ruminococcaceae in Diverse Gut
760 Communities. *Diversity* 5(3):627-640.
- 761 [37] Gerard, P., 2013. Metabolism of cholesterol and bile acids by the gut microbiota.
762 *Pathogens* 3(1):14-24.
- 763 [38] Tian, B., Geng, Y., Wang, P., Cai, M., Neng, J., Hu, J., et al., 2022. Ferulic acid
764 improves intestinal barrier function through altering gut microbiota composition in high-fat
765 diet-induced mice. *Eur J Nutr*.
- 766 [39] Douglas, G.M., Maffei, V.J., Zaneveld, J.R., Yurgel, S.N., Brown, J.R., Taylor, C.M.,
767 et al., 2020. PICRUSt2 for prediction of metagenome functions. *Nat Biotechnol* 38(6):685-
768 688.
- 769 [40] Lin, H.V., Frassetto, A., Kowalik, E.J., Jr., Nawrocki, A.R., Lu, M.M., Kosinski, J.R.,
770 et al., 2012. Butyrate and propionate protect against diet-induced obesity and regulate gut
771 hormones via free fatty acid receptor 3-independent mechanisms. *PLoS One* 7(4):e35240.
- 772 [41] Dawson, P.A., Karpen, S.J., 2015. Intestinal transport and metabolism of bile acids. *J*
773 *Lipid Res* 56(6):1085-1099.

- 774 [42] Qiao, H., Beibei, Z., Chong, T., Tieying, Z., Yuzhi, G., Jing, M., et al., 2020. Both
775 frequency and duration of rotating night shifts are associated with metabolic parameters: a
776 cross-sectional study. *Sleep Med* 71:89-96.
- 777 [43] Parsons, M.J., Moffitt, T.E., Gregory, A.M., Goldman-Mellor, S., Nolan, P.M.,
778 Poulton, R., et al., 2015. Social jetlag, obesity and metabolic disorder: investigation in a cohort
779 study. *Int J Obes (Lond)* 39(5):842-848.
- 780 [44] Bowers, S.J., Vargas, F., Gonzalez, A., He, S., Jiang, P., Dorrestein, P.C., et al., 2020.
781 Repeated sleep disruption in mice leads to persistent shifts in the fecal microbiome and
782 metabolome. *PLoS One* 15(2):e0229001.
- 783 [45] Voigt, R.M., Forsyth, C.B., Green, S.J., Mutlu, E., Engen, P., Vitaterna, M.H., et al.,
784 2014. Circadian disorganization alters intestinal microbiota. *PLoS One* 9(5):e97500.
- 785 [46] Deaver, J.A., Eum, S.Y., Toborek, M., 2018. Circadian Disruption Changes Gut
786 Microbiome Taxa and Functional Gene Composition. *Front Microbiol* 9:737.
- 787 [47] Parker, K.D., Albeke, S.E., Gigley, J.P., Goldstein, A.M., Ward, N.L., 2018.
788 Microbiome Composition in Both Wild-Type and Disease Model Mice Is Heavily Influenced
789 by Mouse Facility. *Front Microbiol* 9:1598.
- 790 [48] Tahara, Y., Yamazaki, M., Sukigara, H., Motohashi, H., Sasaki, H., Miyakawa, H., et
791 al., 2018. Gut Microbiota-Derived Short Chain Fatty Acids Induce Circadian Clock
792 Entrainment in Mouse Peripheral Tissue. *Sci Rep* 8(1):1395.
- 793 [49] Leone, V., Gibbons, S.M., Martinez, K., Hutchison, A.L., Huang, E.Y., Cham, C.M.,
794 et al., 2015. Effects of diurnal variation of gut microbes and high-fat feeding on host circadian
795 clock function and metabolism. *Cell Host Microbe* 17(5):681-689.
- 796 [50] Duszka, K., Picard, A., Ellero-Simatos, S., Chen, J., Defernez, M., Paramalingam, E.,
797 et al., 2016. Intestinal PPARgamma signalling is required for sympathetic nervous system
798 activation in response to caloric restriction. *Sci Rep* 6:36937.

- 799 [51] Kuang, Z., Wang, Y., Li, Y., Ye, C., Ruhn, K.A., Behrendt, C.L., et al., 2019. The
800 intestinal microbiota programs diurnal rhythms in host metabolism through histone deacetylase
801 3. *Science* 365(6460):1428-1434.
- 802 [52] Lau, E., Marques, C., Pestana, D., Santoalha, M., Carvalho, D., Freitas, P., et al., 2016.
803 The role of I-FABP as a biomarker of intestinal barrier dysfunction driven by gut microbiota
804 changes in obesity. *Nutr Metab (Lond)* 13:31.
- 805 [53] Gouyon, F., Caillaud, L., Carriere, V., Klein, C., Dalet, V., Citadelle, D., et al., 2003.
806 Simple-sugar meals target GLUT2 at enterocyte apical membranes to improve sugar
807 absorption: a study in GLUT2-null mice. *J Physiol* 552(Pt 3):823-832.
- 808 [54] Oh, H.Y.P., Visvalingam, V., Wahli, W., 2019. The PPAR-microbiota-metabolic organ
809 trilogy to fine-tune physiology. *FASEB J* 33(9):9706-9730.
- 810 [55] Polidarova, L., Sotak, M., Sladek, M., Pacha, J., Sumova, A., 2009. Temporal gradient
811 in the clock gene and cell-cycle checkpoint kinase Wee1 expression along the gut. *Chronobiol*
812 *Int* 26(4):607-620.
- 813 [56] Ridaura, V.K., Faith, J.J., Rey, F.E., Cheng, J., Duncan, A.E., Kau, A.L., et al., 2013.
814 Gut microbiota from twins discordant for obesity modulate metabolism in mice. *Science*
815 341(6150):1241214.
- 816 [57] Rios-Covian, D., Ruas-Madiedo, P., Margolles, A., Gueimonde, M., de Los Reyes-
817 Gavilan, C.G., Salazar, N., 2016. Intestinal Short Chain Fatty Acids and their Link with Diet
818 and Human Health. *Front Microbiol* 7:185.
- 819 [58] Just, S., Mondot, S., Ecker, J., Wegner, K., Rath, E., Gau, L., et al., 2018. The gut
820 microbiota drives the impact of bile acids and fat source in diet on mouse metabolism.
821 *Microbiome* 6(1):134.

- 822 [59] Desmet, L., Thijs, T., Segers, A., Verbeke, K., Depoortere, I., 2021. Chronodisruption
823 by chronic jetlag impacts metabolic and gastrointestinal homeostasis in male mice. *Acta*
824 *Physiol (Oxf)* 233(4):e13703.
- 825 [60] Nieuwdorp, M., Gilijamse, P.W., Pai, N., Kaplan, L.M., 2014. Role of the microbiome
826 in energy regulation and metabolism. *Gastroenterology* 146(6):1525-1533.
- 827 [61] Wahlstrom, A., Sayin, S.I., Marschall, H.U., Backhed, F., 2016. Intestinal Crosstalk
828 between Bile Acids and Microbiota and Its Impact on Host Metabolism. *Cell Metab* 24(1):41-
829 50.
- 830 [62] Haase, S., Maurer, J., Duscha, A., Lee, D.H., Balogh, A., Gold, R., et al., 2021.
831 Propionic Acid Rescues High-Fat Diet Enhanced Immunopathology in Autoimmunity via
832 Effects on Th17 Responses. *Front Immunol* 12:701626.
- 833 [63] Fan, N., Meng, K., Zhang, Y., Hu, Y., Li, D., Gao, Q., et al., 2020. The effect of
834 ursodeoxycholic acid on the relative expression of the lipid metabolism genes in mouse
835 cholesterol gallstone models. *Lipids Health Dis* 19(1):158.
- 836 [64] Segers, A., Desmet, L., Thijs, T., Verbeke, K., Tack, J., Depoortere, I., 2019. The
837 circadian clock regulates the diurnal levels of microbial short-chain fatty acids and their
838 rhythmic effects on colon contractility in mice. *Acta Physiol (Oxf)* 225(3):e13193.
- 839 [65] Govindarajan, K., MacSharry, J., Casey, P.G., Shanahan, F., Joyce, S.A., Gahan, C.G.,
840 2016. Unconjugated Bile Acids Influence Expression of Circadian Genes: A Potential
841 Mechanism for Microbe-Host Crosstalk. *PLoS One* 11(12):e0167319.

842

843

844

845

846 **12 Figure Legends**

847

848 **Figure 1 Central clock dysfunction induces circadian desynchronization in the GI tract**

849 Relative expression of core and accessory clock genes in the jejunum (A), cecum (B), proximal
850 colon (C) of *Bmal1^{SCN^{fl/-}}* mice (blue) and their controls *Bmal1^{SCN^{+/-}}* (black). Significant rhythms
851 according to cosine-wave regression analysis (p -value ≤ 0.05) are visualized with a solid line,
852 while data connected by dashed line indicate arrhythmicity. Significant phase shifts ($p \leq 0.05$)
853 are indicated with the number of hours of phase shift. $n = 3$ -4 mice/time point/genotype. Data
854 are represented as mean \pm SEM.

855

856 **Figure 2 Disruption of microbiota rhythmicity in SCN-specific *Bmal1*-deficient mice (A)**

857 Beta-diversity MDS plot based on generalized UniFrac distances (GUniFrac) of fecal
858 microbiota stratified by genotype. (B-C) Circadian profile of alpha diversity (B) and the
859 relative and absolute abundance of major phyla (C). (D) Heatmap illustrating the relative
860 abundance of 412 zOTUs (mean relative abundance $> 0.1\%$; prevalence $> 10\%$). Data are
861 ordered based on the zOTUs phase in the controls and normalized based in the peak of each
862 zOTU. (E) Significance and amplitude (based on JTK_CYCLE) of all zOTUs (left) and phase
863 (based on cosine regression) distribution (right) in both genotype. Dashed line represent adj. p -
864 value = 0.05 (JTK_CYCLE). (F) Taxonomic tree of zOTUs losing rhythmicity in *Bmal1^{SCN^{fl/-}}*
865 mice based on quantitative analyses. Taxonomic ranks were indicated as phylum (outer dashed
866 ring), families (inner circle) and genera (middle names). Each zOTU is represented by
867 individual branches. (G) Circadian profiles of absolute abundance of example zOTUs losing
868 rhythmicity in *Bmal1^{SCN^{fl/-}}* mice. Significant rhythms according to cosine-wave regression
869 analysis (p -value ≤ 0.05) are visualized with a solid line, while data connected by dashed line
870 indicate arrhythmicity. $n = 6$ mice/time point/genotype. Data are represented as mean \pm SEM.

871 **Figure 3 SCN clock-controlled microbial functions balance metabolic homeostasis (A)**

872 Heatmap representing MetaCyc Pathways predicted by PICRUST2.0 from zOTUs losing
873 rhythmicity in *Bmal1*^{SCN^{fl/-}} mice. Pathways are ordered by the phase of the control and
874 normalized to the peak abundance of each pathway. We color-coded the pathways according
875 to their sub-classes. **(B)** Fecal SCFA concentrations in both genotype. **(C)** Circadian profiles
876 of fecal SCFA. **(D)** Fecal bile acid concentrations in both genotype. **(E-F)** Circadian profiles
877 of fecal bile acids. Significant rhythms according to cosine-wave regression analysis (p-value
878 ≤ 0.05) are visualized with a solid line, while data connected by dashed line indicate
879 arrhythmicity. Mann Whitney U test was used to assess concentration difference. n = 6
880 mice/time point/genotype. Data are represented as mean \pm SEM. Significance * $p \leq 0.05$, ** p
881 ≤ 0.01 , *** $p \leq 0.001$, **** $p \leq 0.0001$

882

883 **Figure 4 Simulated shift work induces circadian desynchrony between GI clocks (A)**

884 Representative actogram of a control mouse in 12-hour light/12-hour dark (LD) and under
885 simulated shift work (SSW) condition. Tick marks represent running wheel activity. Yellow
886 and grey shadings represent light and darkness respectively. Red arrows indicate fecal sample
887 collection time points. **(B)** Diurnal total wheel-running activity profiles (top) and 24-h
888 summary (bottom). **(C)** Normalized body weight gain of mice in SSW and LD condition. Total
889 daily food intake **(D)**, gut permeability **(E)** and energy assimilation **(F)**. **(I-J)** Relative
890 expression of core and accessory clock genes in GI tract **(I)** and liver **(J)** of WT mice in SSW
891 (red) and their LD controls (black). N = 4-5 mice/time point/light condition. Data are
892 represented as mean \pm SEM. Mann Whitney U test was used to assess food intake and energy
893 assimilation differences. Two-way ANOVA was used to assess the change in body weight and
894 gene expression. Significance * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$

895

896

897 **Figure 5 Simulated shift work disrupts rhythmicity of microbiota composition and**

898 **function** (A) Beta-diversity MDS plot based on generalized UniFrac distances (GUniFrac) of
899 fecal microbiota stratified by light condition. (B) Circadian profile of generalized unifrac
900 distance normalized towards ZT1 of the controls. (C-D) Circadian profiles of the absolute
901 abundance of major phyla (C) and families (D). (E) Significance and amplitude (based on
902 JTK_CYCLE) of all zOTUs (E) and phase (based on cosine regression) distribution (F) in both
903 genotype, dashed line represents adj. p-value = 0.05 (JTK_CYCLE). (G) Taxonomic tree of
904 zOTUs losing rhythmicity in SSW based on quantitative analyses. Taxonomic ranks were
905 indicated as phylum (outer dashed ring), then family (inner circle) and genera (middle names).
906 Each zOTU is represented by individual branches. (H) Circadian profiles of absolute
907 abundance of example zOTUs losing rhythmicity in SSW. (I) Heatmap representing MetaCyc
908 Pathways predicted by PICRUST2.0 from zOTUs losing rhythmicity in SSW. Pathways are
909 ordered by the phase of the control and normalized to the peak abundance of each pathway.
910 We colored the pathways according to their sub-classes. (J) Bar chart representing the number
911 of shared pathways losing rhythmicity in SSW and *Bmal1^{SCNfl/-}* mice. Significant rhythms
912 according to cosine-wave regression analysis ($p\text{-value} \leq 0.05$) are visualized with a solid line,
913 while data connected by dashed line indicate arrhythmicity. Significant phase shifts ($p \leq 0.05$)
914 are indicated with the number of hours of phase shift. $n = 4\text{-}5$ mice/time point/genotype. Data
915 are represented as mean \pm SEM.

916

917 **Figure 6 Simulated shift work-associated microbiota promote weight gain and suppress**

918 **GI clocks** (A) Schematic illustration of cecal microbiota transfer from SSW and LD donors
919 ($n=4\text{-}5$) into germ free wild type mice. (B) Normalized body weight gain of recipient mice.
920 (C) bar chart illustrated cecum weight in recipient mice. (D-E) Relative expression of clock

921 genes (**D**) and clock controlled gene (**E, F**) in the GI tract of germ free mice (green), germ free
922 receiving SSW (red) and LD controls (black) microbiota. N = 5-6 mice/time point/light
923 condition. Data are represented as mean \pm SEM. Mann Whitney U test was used to assess the
924 different in cecum weight. Two-way ANOVA was used to assess the change in body weight
925 and differences in gene expression. Significance * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, ****
926 $p \leq 0.0001$

927

928 **13 Tables:**

929 **Table 1** Summary of results for phase, amplitude, baseline and rhythmicity of core and
930 accessory clock gene expression based on cosine regression analysis in the GI tract of
931 *Bmal1^{SCN1/-}* mice and control. Bold p-values indicate significant difference between genotype.

932

933

934

935

936

937

938

939

940

941

942

943

944 **14 Supplementary Material**

945 **14.1 Supplementary Figure Legends**

946 **Supplementary Figure 1** (A) Circadian profiles of relative and quantitative abundance of
947 bacterial families in *Bmal1^{SCNfl/-}* mice and their controls. (B) Heatmap illustrating the
948 quantitative abundance of 412 zOTUs (mean relative abundance > 0.1%; prevalence > 10%).
949 Data are ordered based on the zOTUs phase in the controls and normalized based in the peak
950 of each zOTU. (E) Significance and amplitude (based on JTK_CYCLE) of all zOTUs (top) and
951 phase (based on cosine regression) distribution (bottom) in both genotype, dashed line
952 represents adj. p-value = 0.05 (JTK_CYCLE). (D) Taxonomic tree of zOTUs losing
953 rhythmicity in *Bmal1^{SCNfl/-}* mice based on relative analyses. Taxonomic ranks were indicated
954 as phylum (outer dashed ring), then family (inner circle) and genera (middle names), each
955 zOTU represented by individual branches. (E) Circadian profile of relative abundance of
956 example zOTUs losing rhythmicity in *Bmal1^{SCNfl/-}* mice. (F) Bar charts illustrate the alteration
957 in abundance (adj. p-value ≤ 0.05) and fold change of zOTUs losing rhythmicity in *Bmal1^{SCNfl/-}*
958 mice. Significant rhythms according to cosine-wave regression analysis (p-value ≤ 0.05) are
959 visualized with a solid line, while data connected by dashed line indicate arrhythmicity. n = 6
960 mice/time point/genotype. Data are represented as mean \pm SEM.

961

962 **Supplementary Figure 2** LDA score of MetaCyc Pathways characterizing the differences
963 between *Bmal1^{SCNfl/-}* mice and their control. (B) Fecal Lactic and Valeric acid concentrations
964 in both genotype. (C) Circadian profile of fecal SCFA. (D) Fecal bile acid concentration.
965 Significant rhythms according to cosine-wave regression analysis (p-value ≤ 0.05) are
966 visualized with a solid line, while data connected by dashed line indicate arrhythmicity. Mann
967 Whitney U test was used to determine the differences between groups. n = 6 mice/time

968 point/genotype. Data are represented as mean \pm SEM. Significance * $p \leq 0.05$, ** $p \leq 0.01$, ***
969 $p \leq 0.001$, **** $p \leq 0.0001$

970

971 **Supplementary Figure 3** (A) Summary of running wheel activity in day and night of LD and
972 SSW group of mice. (B-C) Diurnal profile of relative abundance of major phyla (B) and family.
973 (D-E) Heatmap illustrating the relative (D) and absolute (E) abundance of 473 zOTUs (mean
974 relative abundance $> 0.1\%$; prevalence $> 10\%$). Data are ordered based on the zOTUs phase in
975 the controls and normalized based in the peak of each zOTU. Significance and amplitude
976 (based on JTK_CYCLE) of all zOTUs (bottom) and phase (based on cosine regression)
977 distribution (top) in both light condition, dashed line represent JTK_CYCLE adj. p. value =
978 0.05. (F) Diurnal profile of example zOTUs. (G) Taxonomic tree of zOTUs losing rhythmicity
979 in SSW mice based on relative analyses. Taxonomic ranks were indicated as phylum (outer
980 dashed ring), then family (inner circle) and genera (middle names), each zOTU represented by
981 individual branches. (H) Microbial composition analysis on the phyla and family level of the
982 fecal microbiota in Lübeck and Munich. LDA score of MetaCyc Pathways characterizing the
983 differences LD and SSW. Significant rhythms according to cosine-wave regression analysis
984 (p -value ≤ 0.05) are visualized with a solid line, while data connected by dashed line indicate
985 arrhythmicity. Significant phase shifts ($p \leq 0.05$) are indicated with the number of hours of
986 phase shift. Two-way ANOVA was used to assess the change in activity. $n = 4-5$ mice/time
987 point/genotype. Data are represented as mean \pm SEM. Significance * $p \leq 0.05$, ** $p \leq 0.01$, ***
988 $p \leq 0.001$, **** $p \leq 0.0001$

989

990 **Supplementary Figure 4 SSW has no impact on GI weight and body composition** (A) Bar
991 charts representing colon and jejunum weight and density (B) Bar charts illustrating NMR data
992 of SSW and control group. $n = 4-5$ mice/light condition. Mann Whitney U test was used to

993 determine the differences between groups. Data are represented as mean \pm SEM. Significance

994 * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$

995

996 **14.2 Supplementary Tables**

997 **Supplementary Table 1: Microbial rhythmicity analysis of genetic and environmental**

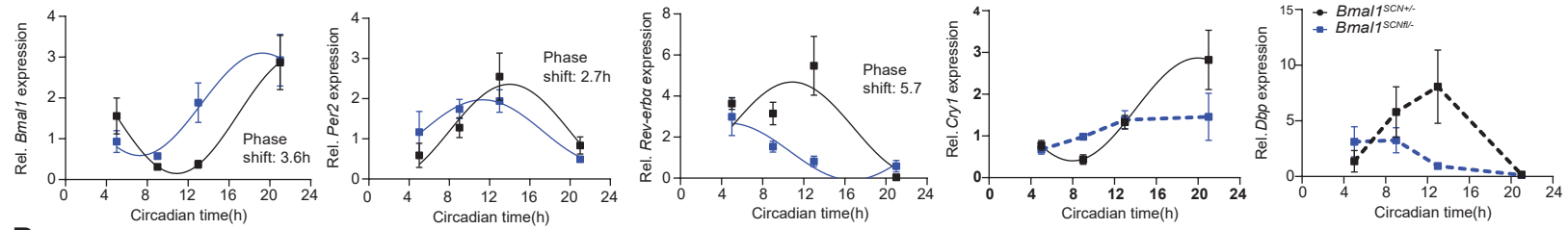
998 **circadian disruption mouse models:** showing microbial rhythmicity and amplitude according

999 to JTK analysis of relative and absolute abundance of

Figure 1

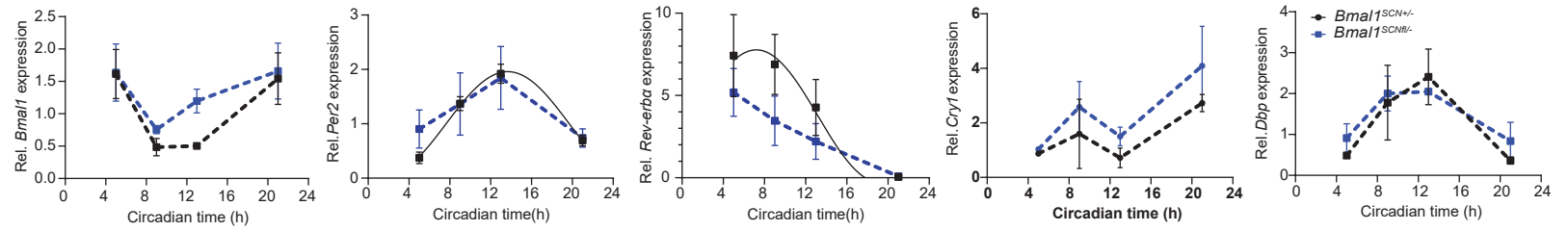
A

Jejunum



B

Cecum



C

Proximal colon

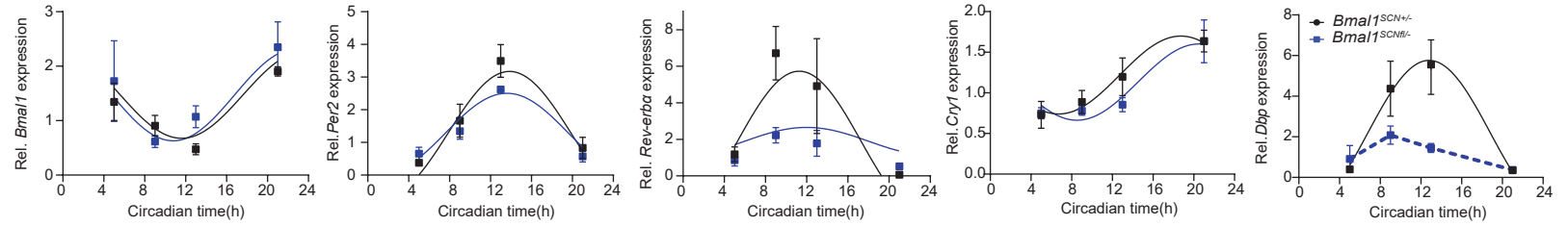


Figure 2

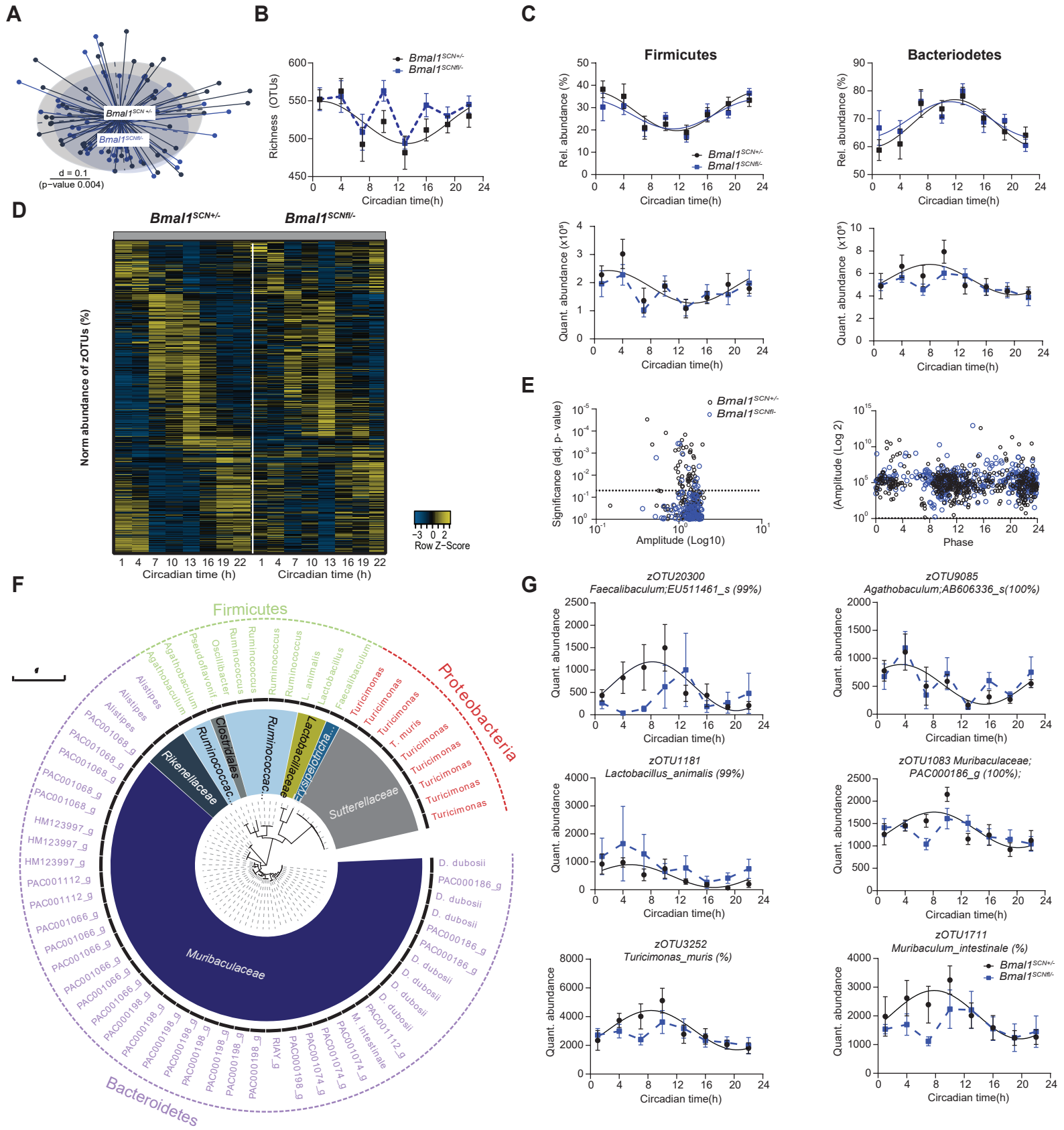


Figure 3

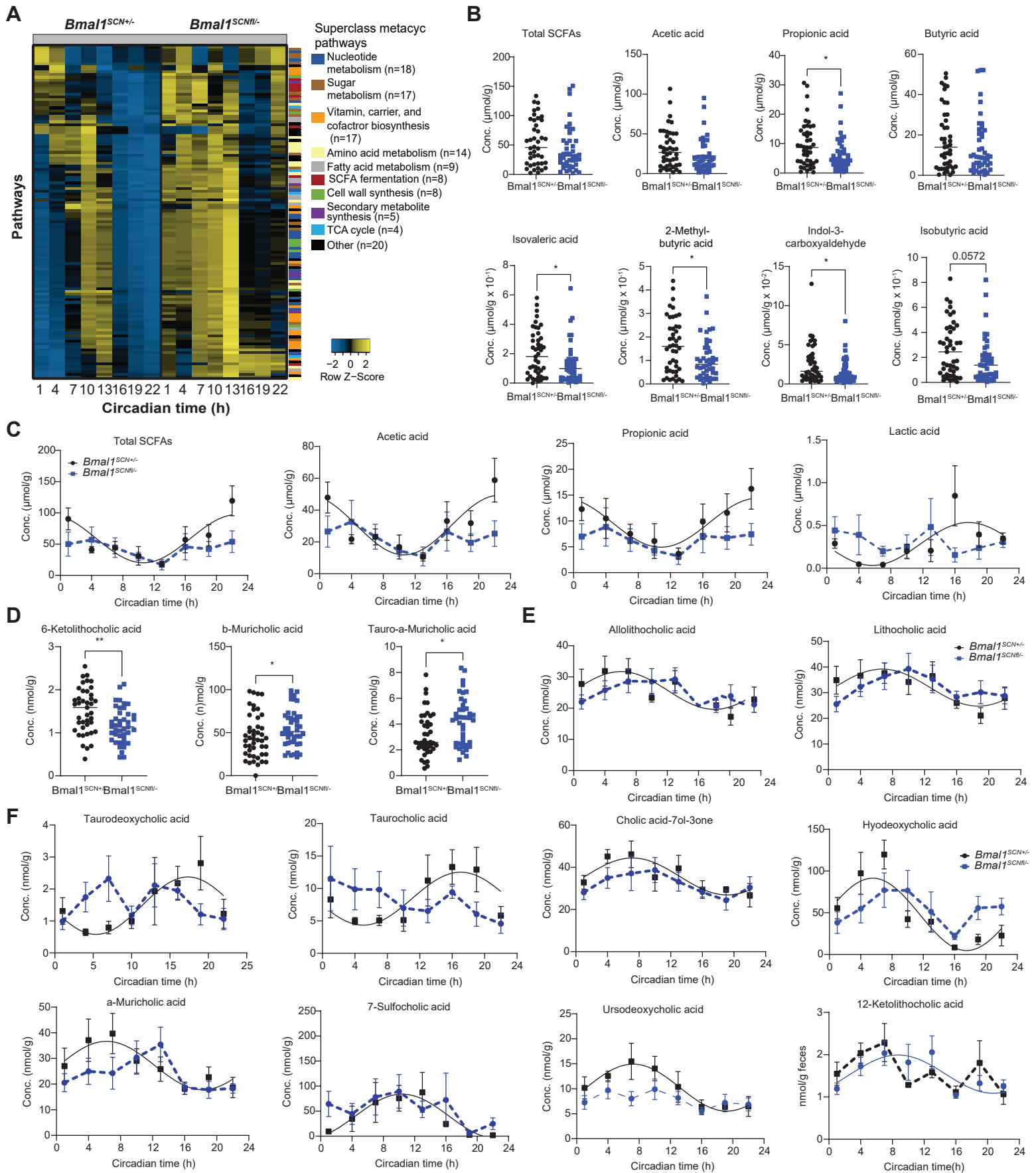


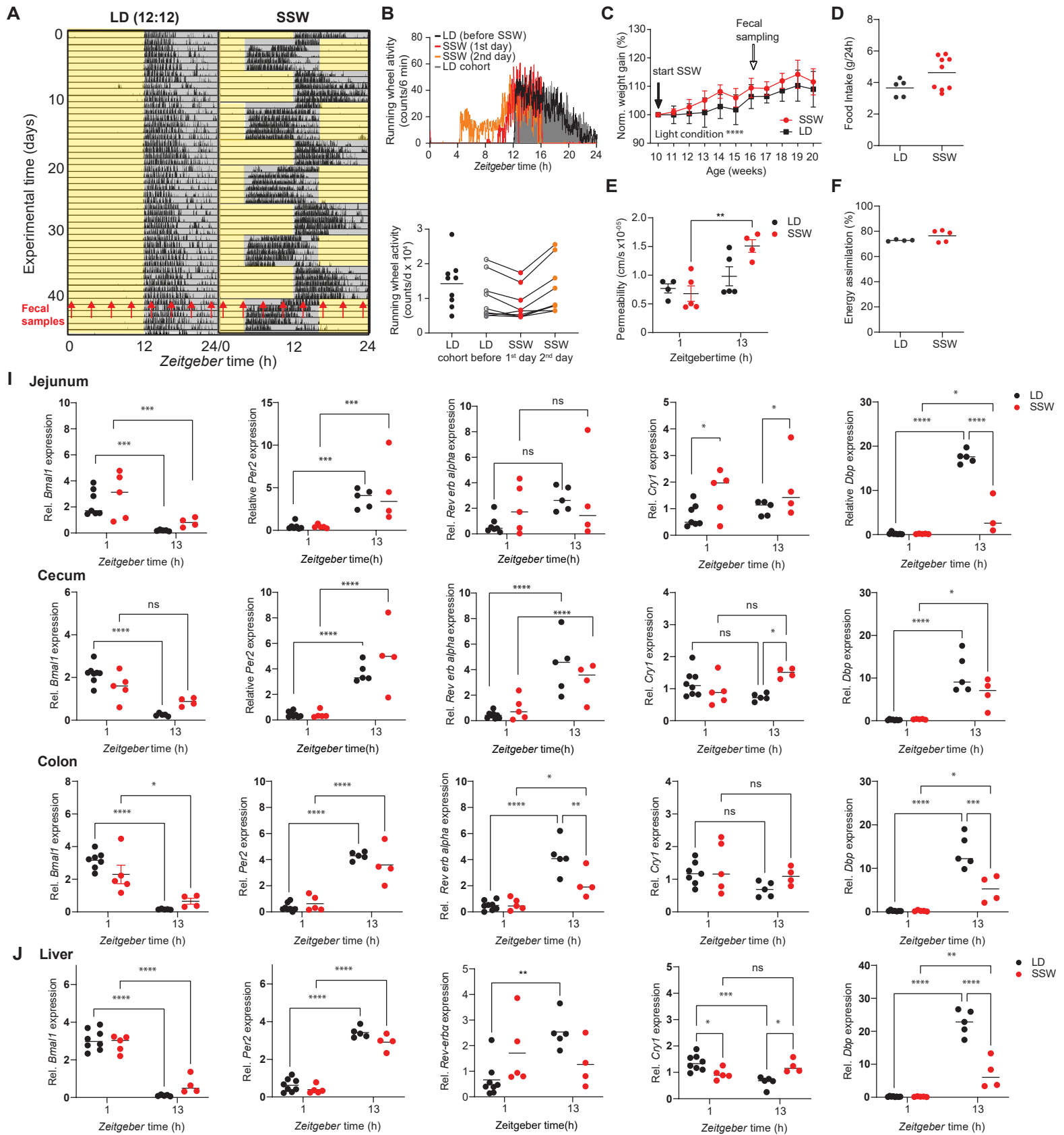
Figure 4

Figure 5

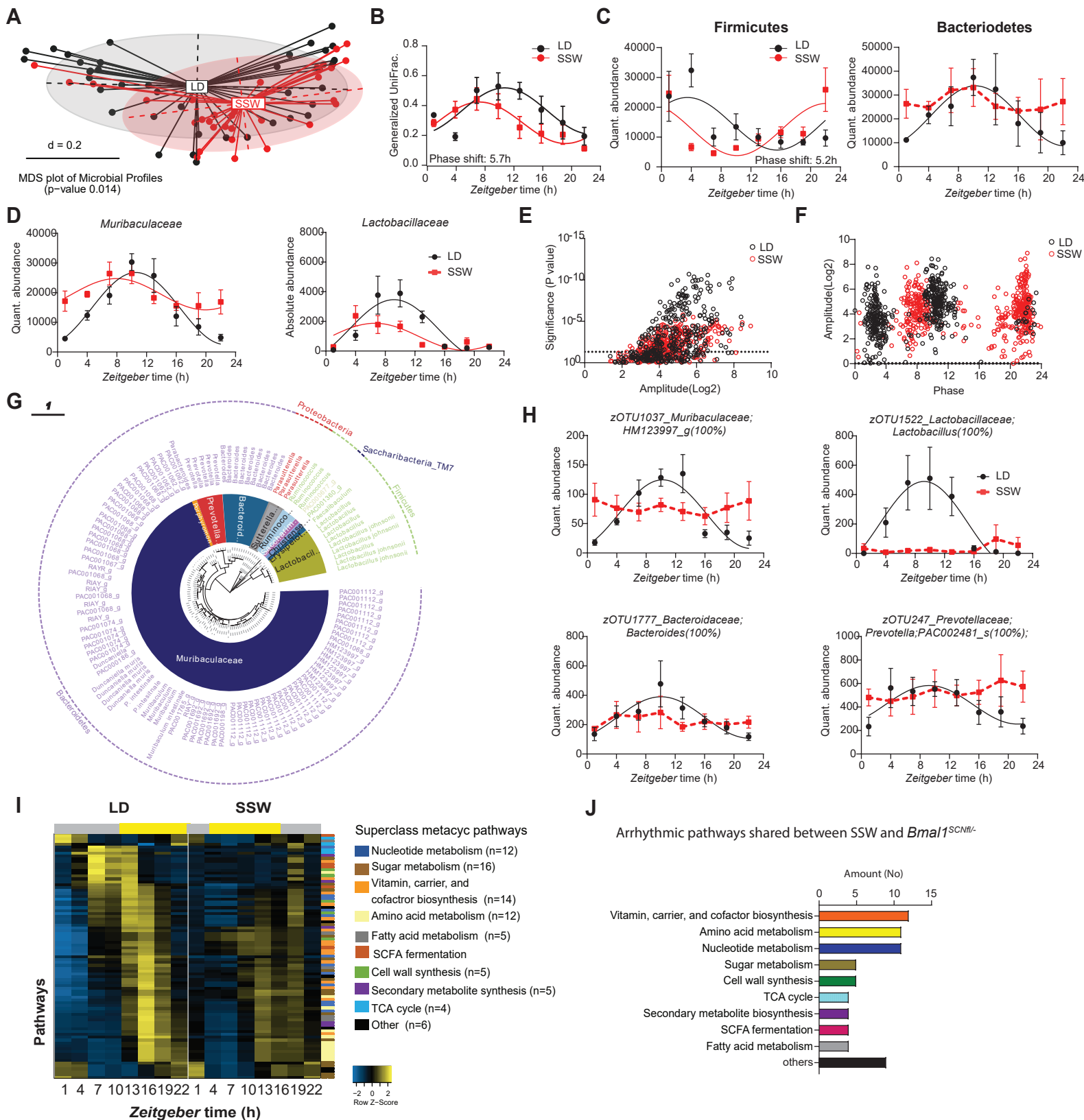
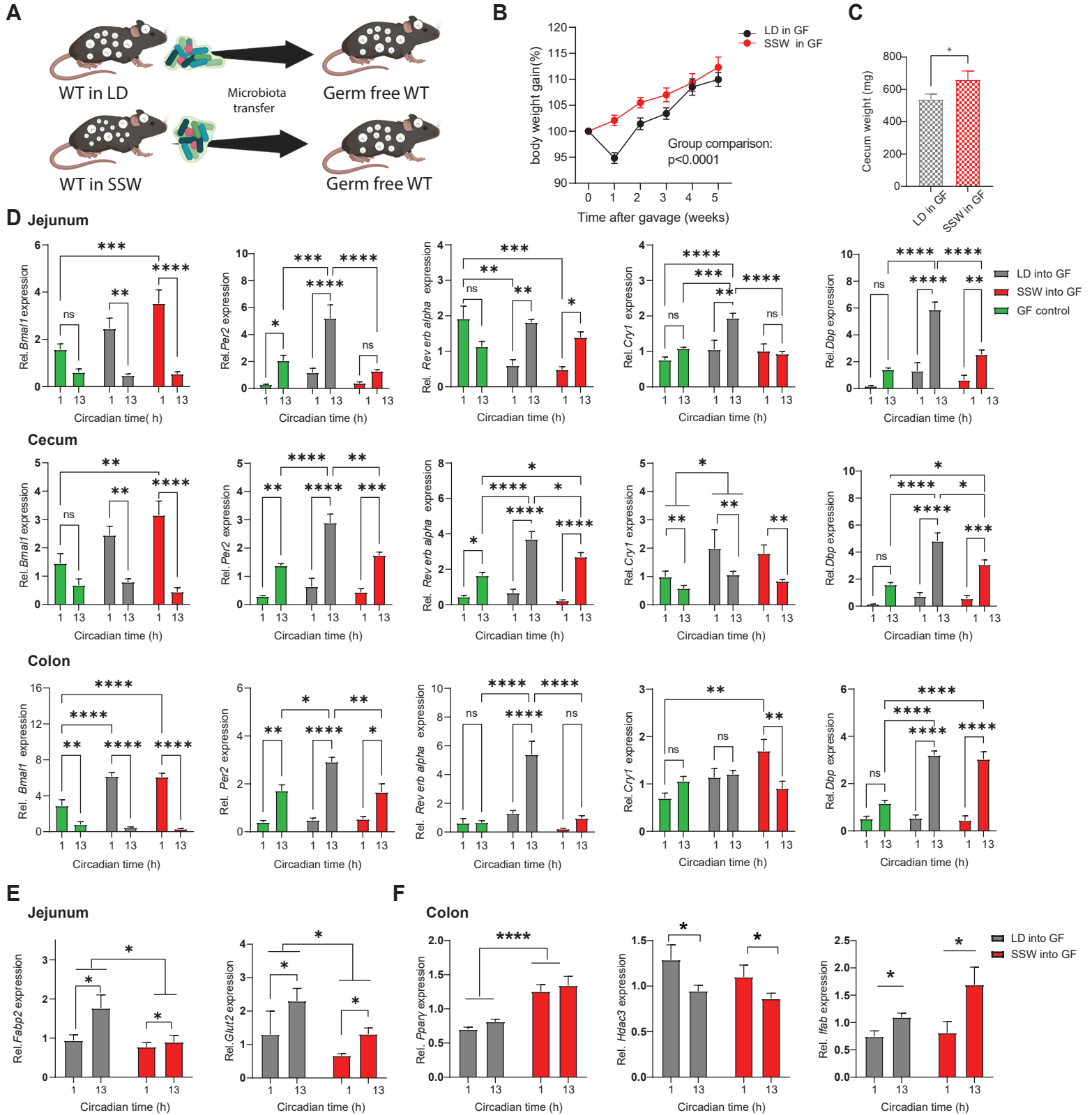


Figure 6



Gastrointestinal tissue	Gene	Group	Rhythmicity (P. value)	Phase shift (P. value)	Amplitude difference (P. value)	Baseline difference (P. value)
Proximal colon	<i>Bmal</i>	<i>Bmall</i> ^{SCN} +/-	0.01	0.53	0.67	0.22
		<i>Bmall</i> ^{SCN} fl/-	0.04			
	<i>Per2</i>	<i>Bmall</i> ^{SCN} +/-	0.004	0.56	0.13	0.25
		<i>Bmall</i> ^{SCN} fl/-	0.001			
	<i>Rev-erba</i>	<i>Bmall</i> ^{SCN} +/-	0.03	0.98	0.02	0.07
<i>Bmall</i> ^{SCN} fl/-		0.03				
<i>Cry1</i>	<i>Bmall</i> ^{SCN} +/-	0.01	0.27	0.94	0.47	
	<i>Bmall</i> ^{SCN} fl/-	0.01				
<i>Dbp</i>	<i>Bmall</i> ^{SCN} +/-	0.008	0.39	0.002	0.04	
	<i>Bmall</i> ^{SCN} fl/-	0.07				
Cecum	<i>Bmal</i>	<i>Bmall</i> ^{SCN} +/-	0.06	0.53	0.34	0.3
		<i>Bmall</i> ^{SCN} fl/-	0.33			
	<i>Per2</i>	<i>Bmall</i> ^{SCN} +/-	0.00006	0.54	0.37	0.59
		<i>Bmall</i> ^{SCN} fl/-	0.31			
	<i>Rev-erba</i>	<i>Bmall</i> ^{SCN} +/-	0.02	0.59	0.29	0.12
<i>Bmall</i> ^{SCN} fl/-		0.08				
<i>Cry1</i>	<i>Bmall</i> ^{SCN} +/-	0.37	0.76	0.74	0.15	
	<i>Bmall</i> ^{SCN} fl/-	0.52				
<i>Dbp</i>	<i>Bmall</i> ^{SCN} +/-	0.056	0.78	0.34	0.45	
	<i>Bmall</i> ^{SCN} fl/-	0.1				

Jejunum	<i>Bmal</i>	<i>Bmall</i> ^{SCN} +/-	0.004	0.02	0.64	0.42
		<i>Bmall</i> ^{SCN} fl/-	0.01	Phase shift=2.7		
	<i>Per2</i>	<i>Bmall</i> ^{SCN} +/-	0.02	0.04	0.28	0.83
		<i>Bmall</i> ^{SCN} fl/-	0.01	Phase shift=3.6		
	<i>Rev-erba</i>	<i>Bmall</i> ^{SCN} +/-	0.03	0.03	0.28	0.04
		<i>Bmall</i> ^{SCN} fl/-	0.04	Phase shift=5.7		
<i>Cry1</i>	<i>Bmall</i> ^{SCN} +/-	0.009	0.33	0.07	0.11	
	<i>Bmall</i> ^{SCN} fl/-	0.42				
<i>Dbp</i>	<i>Bmall</i> ^{SCN} +/-	0.056	0.08	0.08	0.14	
	<i>Bmall</i> ^{SCN} fl/-	0.06				