¹**Title:** Fetal programming by the parental microbiome of offspring behavior, and DNA methylation and gene expression within the hippocampus

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36

³⁷**Abstract**

38 Background
39 The microorg

The microorganisms colonizing the gastrointestinal tract of animals, collectively referred to as 40 the gut microbiome, affect numerous host behaviors dependent on the central nervous system
41 (CNS). Studies comparing germ-free mice to normally colonized mice have demonstrated 41 (CNS). Studies comparing germ-free mice to normally colonized mice have demonstrated
42 influences of the microbiome on anxiety-related behaviors, voluntary activity, and gene 42 influences of the microbiome on anxiety-related behaviors, voluntary activity, and gene
43 expression in the CNS. Additionally, there is epidemiologic evidence supporting an 43 expression in the CNS. Additionally, there is epidemiologic evidence supporting an
44 intergenerational influence of the maternal microbiome on neurodevelopment of offspring and 44 intergenerational influence of the maternal microbiome on neurodevelopment of offspring and
45 behavior later in life. There is limited experimental evidence however directly linking the 45 behavior later in life. There is limited experimental evidence however directly linking the
46 maternal microbiome to long-term neurodevelopmental outcomes, or knowledge regarding 46 maternal microbiome to long-term neurodevelopmental outcomes, or knowledge regarding
47 mechanisms responsible for such effects. 47 mechanisms responsible for such effects.
48

49 Results

50 Here we show that that the maternal microbiome has a dominant influence on several offspring
51 Dhenotypes including anxiety-related behavior, voluntary activity, and body weight, Adverse 51 phenotypes including anxiety-related behavior, voluntary activity, and body weight. Adverse
52 outcomes in offspring were associated with features of the maternal microbiome including bile 52 outcomes in offspring were associated with features of the maternal microbiome including bile
53 salt hydrolase activity gene expression (bsh), abundance of certain bile acids, and hepatic 53 salt hydrolase activity gene expression (*bsh*), abundance of certain bile acids, and hepatic
54 expression of *Slc10a1*. In cross-foster experiments, offspring resembled their birth dam 54 expression of *Slc10a1*. In cross-foster experiments, offspring resembled their birth dam
55 phenotypically, despite faithful colonization in the postnatal period with the surrogate dam 55 phenotypically, despite faithful colonization in the postnatal period with the surrogate dam
56 microbiome. Genome-wide methylation analysis of hippocampal DNA identified microbiome-56 microbiome. Genome-wide methylation analysis of hippocampal DNA identified microbiome-
57 associated differences in methvlation of 196 loci in total. 176 of which show conserved profiles 57 associated differences in methylation of 196 loci in total, 176 of which show conserved profiles
58 between mother and offspring. Further, single-cell transcriptional analysis revealed 58 between mother and offspring. Further, single-cell transcriptional analysis revealed
59 accompanying-differences-in-expression-of-several-differentially-methylated-genes-within-certain 59 accompanying differences in expression of several differentially methylated genes within certain
60 biopocampal cell clusters, and vascular expression of genes associated with bile acid transport. hippocampal cell clusters, and vascular expression of genes associated with bile acid transport.

61 Inferred cell-to-cell communication in the hippocampus based on coordinated ligand-receptor
62 expression revealed differences in expression of neuropeptides associated with satiety.

62 expression revealed differences in expression of neuropeptides associated with satiety.
63

64 Conclusions
65 Collectively,

65 Collectively, these data provide proof-of-principle that the maternal gut microbiome has a
66 dominant influence on the neurodevelopment underlying certain offspring behaviors and 66 dominant influence on the neurodevelopment underlying certain offspring behaviors and
67 activities, and selectively affects genome methylation and gene expression in the offspring CNS 67 activities, and selectively affects genome methylation and gene expression in the offspring CNS
68 in coniunction with that neurodevelopment. 68 in conjunction with that neurodevelopment.
69

⁷⁰**Keywords:** Fetal programming, Maternal microbiome, Gut-brain axis, DNA methylation, 71 Hippocampus, Gene expression, Bile acids, Neurodevelopment
72

72

⁷³**Background**

⁷⁴Neurodevelopmental and behavioral disorders are a growing concern worldwide. According to the World Health Organization, nearly one billion people worldwide live with a mental disorder¹.
76. Behavioral disorders are commonly associated with social impairments, decreased productivity, 76 Behavioral disorders are commonly associated with social impairments, decreased productivity,
77 financial losses, and general maladjustment². Previous studies have found that such disorders financial losses, and general maladjustment² ⁷⁷. Previous studies have found that such disorders contribute substantially to global nonfatal health loss¹. Anxiety disorders (AD) are increasing in
19. prevalence, affecting close to 1 in 10 children and adolescents between the ages of 3 and 17³. prevalence, affecting close to 1 in 10 children and adolescents between the ages of 3 and 17³.
80 Like most mood disorders, AD are multifactorial and often result from a combination of genetic. 80 Like most mood disorders, AD are multifactorial and often result from a combination of genetic,
81 environmental, and experiential factors. Similarly, one in five children in the U.S. are obese or 81 environmental, and experiential factors. Similarly, one in five children in the U.S. are obese or
82 overweight⁴, reflecting the combined influence of western diet, increasingly sedentary lifestyles, overweight⁴, reflecting the combined influence of western diet, increasingly sedentary lifestyles,
83 and other factors. Moreover, AD and obesity/overweight (OO) are reciprocal risk factors, 83 and other factors. Moreover, AD and obesity/overweight (OO) are reciprocal risk factors,
84 frequently occurring as co-morbidities⁵⁻⁹.

frequently occurring as co-morbidities⁵⁻⁹.
85. A growing body of research has linked 85 A growing body of research has linked the gut microbiome (GM) to neurodevelopment and
86 behavior¹⁰⁻¹⁵, and growth rate or weight gain¹⁶⁻¹⁹. Work with germ-free mice shows the behavior¹⁰⁻¹⁵, and growth rate or weight gain¹⁶⁻¹⁹. Work with germ-free mice shows the
87 importance of the GM in normative behavior and metabolism²⁰⁻²², and transfer of an anxiety- 187 importance of the GM in normative behavior and metabolism²⁰⁻²², and transfer of an anxiety-
88 related phenotype or increased energy harvest via fecal microbiome transfer indicates that 88 related phenotype or increased energy harvest via fecal microbiome transfer indicates that
89 certain features within naturally occurring microbiomes influence these phenotypes²³⁻²⁵. There is certain features within naturally occurring microbiomes influence these phenotypes²³⁻²⁵. There is
90. also evidence that indicates that the effects of the GM can go bevond simply influencing the 90 also evidence that indicates that the effects of the GM can go beyond simply influencing the
91 bost. Research in rodents has revealed that the GM of a pregnant dam can influence the fetus 91 bost. Research in rodents has revealed that the GM of a pregnant dam can influence the fetus
92 and phenotvpe of the offspring following birth. Eloguent studies in mice have shown that effects 92 and phenotype of the offspring following birth. Eloquent studies in mice have shown that effects
93 of diet and exercise on the maternal GM can be transferred to the offspring, relieving negative 93 of diet and exercise on the maternal GM can be transferred to the offspring, relieving negative
94 metabolic phenotypes $26, 27$. There are also developmental components to both AD and OO. metabolic phenotypes 26 , 27 . There are also developmental components to both AD and OO,

95 raising the question of how the maternal microbiome during pregnancy affects fetal
96 development and subsequent behavior and energy metabolism in the adult offspring. The 96 development and subsequent behavior and energy metabolism in the adult offspring. The
97 maternal gut microbiome during pregnancy produces metabolites which reach peripheral 97 maternal gut microbiome during pregnancy produces metabolites which reach peripheral
98 circulation and the fetal CNS²⁸, and maternal proteins and peptides produced by 98 circulation and the fetal CNS^{28} , and maternal proteins and peptides produced by
99 enteroendocrine cells in response to the microbiome likely also cross the placenta and reach enteroendocrine cells in response to the microbiome likely also cross the placenta and reach
100 fetal circulation²⁹⁻³¹. Disruption of the maternal GM can affect these processes as demonstrated fetal circulation²⁹⁻³¹. Disruption of the maternal GM can affect these processes as demonstrated
101. by increased anxiety in the offspring of mice with antibiotic- or diet-induced dysbiosis³²⁻³⁵. by increased anxiety in the offspring of mice with antibiotic- or diet-induced dysbiosis³²⁻³⁵.
102. There are still maior gaps in our knowledge however regarding the mechanisms through

102 There are still major gaps in our knowledge however regarding the mechanisms through which
103 the maternal microbiome during pregnancy programs long-lasting changes in offspring behavior 103 the maternal microbiome during pregnancy programs long-lasting changes in offspring behavior
104 and metabolism. These intergenerational effects suggest fetal imprinting by an unknown 104 and metabolism. These intergenerational effects suggest fetal imprinting by an unknown
105 mechanism, while differences in anxiety-related behavior (and other complex behaviors) 105 mechanism, while differences in anxiety-related behavior (and other complex behaviors)
106 indicate a neurodevelopmental basis. Owing to the genetic. dietary, and environmental 106 indicate a neurodevelopmental basis. Owing to the genetic, dietary, and environmental
107 heterogeneity, analysis of these processes in a human population requires very large sample 107 heterogeneity, analysis of these processes in a human population requires very large sample
108 sizes and long-term tracking of mother-child pairs. To circumvent these factors, here we use two 108 sizes and long-term tracking of mother-child pairs. To circumvent these factors, here we use two
109 groups of genotype-, age-, and sex-matched outbred CD-1 mice consuming the same diet. To 109 groups of genotype-, age-, and sex-matched outbred CD-1 mice consuming the same diet. To
110 be clear, all mice in these two colonies are of the same genetic background, and only differ in be clear, all mice in these two colonies are of the same genetic background, and only differ in 111 the two microbiomes they harbor. These microbiomes, originally derived from Jackson
112 Laboratory and Envigo (now known as Inotiv), are characterized by low and high alpha diversity 112 Laboratory and Envigo (now known as Inotiv), are characterized by low and high alpha diversity
113 Frelative to each other and distinct beta diversity. These two colonies were developed at MU 113 Frelative to each other and distinct beta diversity. These two colonies were developed at MU
114 FMutant Mouse Resource and Research Center (MMRRC) by initially transferring CD-1 embryos 114 Mutant Mouse Resource and Research Center (MMRRC) by initially transferring CD-1 embryos
115 into respective C57BL/6 dams and allowing the dams to transfer their GMs to offspring via 115 into respective C57BL/6 dams and allowing the dams to transfer their GMs to offspring via
116 natural postnatal transmission. These CD-1 pups became the founders of these two colonies 116 natural postnatal transmission. These CD-1 pups became the founders of these two colonies
117 Which have been maintained and continually monitored for GM stability within our facility for 117 which have been maintained and continually monitored for GM stability within our facility for
118 over 35 generations. Additionally, a rotational breeding scheme and routine introduction of CD-1 118 over 35 generations. Additionally, a rotational breeding scheme and routine introduction of CD-1
119 ogenetics via embryo transfer from CD-1 mice purchased from Charles River allows for the 119 genetics via embryo transfer from CD-1 mice purchased from Charles River allows for the
120 maintenance of allelic heterozygosity within each colony and ensures these colonies do not 120 maintenance of allelic heterozygosity within each colony and ensures these colonies do not
121 become genetically distinct from each other. Since CD-1 mice that harbor a Jackson Laboratory become genetically distinct from each other. Since CD-1 mice that harbor a Jackson Laboratory 122 origin GM have a GM with low phylogenetic richness and diversity, the GM of these mice was
123 designated GM^{Low}. The CD-1 colony with an Envigo origin GM has relatively high phyologenetic designated GM^{Low}. The CD-1 colony with an Envigo origin GM has relatively high phyologenetic
124. diversity and is thus designated GM^{High}. Phenotypic assessments of these two colonies diversity and is thus designated GM^{High}. Phenotypic assessments of these two colonies
125 revealed differences in anxiety-related behavior, voluntary activity, fetal growth, food intake, and 125 revealed differences in anxiety-related behavior, voluntary activity, fetal growth, food intake, and 126 adult growth^{36, 37}. 126 adult growth $36, 37$.
127 We hypothesized

127 We hypothesized that the maternal GM would influence the neurodevelopment of the offspring
128 via fetal programming while *in utero* by GM-derived metabolites. Taking advantage of the via fetal programming while *in utero* by GM-derived metabolites. Taking advantage of the

129 phenotypic differences in these two colonies, we utilized cross-foster studies to determine the
130 relative influence of the parental (i.e., prenatal) and offspring (i.e., postnatal) microbiome on 130 relative influence of the parental (i.e., prenatal) and offspring (i.e., postnatal) microbiome on
131 offspring phenotypes. Cross-fostering between dams of the reciprocal GM (e.g., pups born to offspring phenotypes. Cross-fostering between dams of the reciprocal GM (e.g., pups born to GM^{Low} cross-fostered to GM^{High}, and vice versa) allows offspring to develop under the influence
133 of the birth dam GM *in utero*, and then acquire the surrogate dam GM during postnatal life. 133 of the birth dam GM *in utero*, and then acquire the surrogate dam GM during postnatal life.
134 Microbiome-associated differential phenotypes in which cross-fostered offspring match the 134 Microbiome-associated differential phenotypes in which cross-fostered offspring match the
135 phenotypes observed in surrogate dams suggest a postnatal influence, while similarities phenotypes observed in surrogate dams suggest a postnatal influence, while similarities 136 between cross-fostered offspring and their birth dams suggest a dominant prenatal influence of
137 the parental microbiome. Here, we expand on previous behavioral phenotyping to include 137 the parental microbiome. Here, we expand on previous behavioral phenotyping to include
138 control and cross-fostered (CF) offspring, demonstrating a dominant influence of the birth dam 138 control and cross-fostered (CF) offspring, demonstrating a dominant influence of the birth dam
139 GM on offspring development and behavior at seven weeks of age. This work was 139 GM on offspring development and behavior at seven weeks of age. This work was
140 complemented by microbial and metabolic profiling of mice in each colony, genome-wide 140 complemented by microbial and metabolic profiling of mice in each colony, genome-wide
141 methylome analysis of hippocampal DNA from dams and control and CF offspring, and single 141 methylome analysis of hippocampal DNA from dams and control and CF offspring, and single
142 nuclei transcriptome analysis of RNA from control and CF offspring. Previously identified nuclei transcriptome analysis of RNA from control and CF offspring. Previously identified 143 phylogenetic differences are now complemented by differences in certain metabolites, including
144 bile acids (BA), and differential ileal and hepatic expression of BA receptors and transporters. bile acids (BA), and differential ileal and hepatic expression of BA receptors and transporters. 145 Analysis of hippocampal DNA revealed dominant effects of the maternal microbiome on CpG
146 methylation, maintained in offspring independent of the postnatal microbiome. Single-nuclei 146 methylation, maintained in offspring independent of the postnatal microbiome. Single-nuclei
147 RNA sequencing (snRNA-seq) of hippocampal RNA confirmed fetal programming of several 147 RNA sequencing (snRNA-seq) of hippocampal RNA confirmed fetal programming of several
148 cell-specific differentially methylated genes, including genes involved in G protein-coupled 148 cell-specific differentially methylated genes, including genes involved in G protein-coupled
149 receptor and orexigenic signaling pathways. receptor and orexigenic signaling pathways.

150
151

¹⁵¹**Methods**

¹⁵³**Mice**

154 All mice tested in the current study were outbred CD-1 mice (Crl:CD1(ICR)) generated from
155 breeders obtained from the Mutant Mouse Resource and Research Center at the University of breeders obtained from the Mutant Mouse Resource and Research Center at the University of 156 Missouri (MU MMRRC). Multiple different cohorts of mice were utilized for various outcomes.
157 CD-1 mice were from two colonies in which the founders were originally purchased from 157 CD-1 mice were from two colonies in which the founders were originally purchased from
158 Charles River (Frederick, MD), and were generated via rederivation to harbor either a high 158 Charles River (Frederick, MD), and were generated via rederivation to harbor either a high
159 richness Envigo (now Inotiv, Indianapolis, IN) origin GM (GM^{High}), or a low richness Jackson ith the section of the individary of the indianapolis, IN) origin GM (GM^{High}), or a low richness Jackson
160 Laboratory origin GM (GM^{Low}) as previously described²⁴. All donor mice were reared at the MU Laboratory origin GM (GM^{Low}) as previously described²⁴. All donor mice were reared at the MU
161 MMRRC and the two colonies have been maintained and continually monitored for GM stability 161 MMRRC and the two colonies have been maintained and continually monitored for GM stability
162 Within our facility for over 35 generations. Additionally, a rotational breeding scheme and routine within our facility for over 35 generations. Additionally, a rotational breeding scheme and routine 163 introduction of CD-1 genetics via embryo transfer from CD-1 mice purchased from Charles
164 River allows for the maintenance of allelic heterozygosity within each colony and ensures these 164 Biver allows for the maintenance of allelic heterozygosity within each colony and ensures these
165 Colonies do not become genetically distinct from each other. Since CD-1 mice that harbor a colonies do not become genetically distinct from each other. Since CD-1 mice that harbor a 166 Jackson Laboratory-origin GM were found to have a GM with low phylogenetic richness and
167 diversity, the GM of these mice was designated GM^{Low}. Similarly, since CD-1 mice that harbored diversity, the GM of these mice was designated GM^{Low}. Similarly, since CD-1 mice that harbored
168 an Envigo-origin GM were found to have a GM with high phylogenetic richness and diversity 168 an Envigo-origin GM were found to have a GM with high phylogenetic richness and diversity
169 relative to GM^{Low}, the GM of these mice was designated GM^{High}. Colonies of mice were housed relative to GM^{Low}, the GM of these mice was designated GM^{High}. Colonies of mice were housed
170. under barrier conditions in microisolator cages with compressed pelleted paper bedding and 170 under barrier conditions in microisolator cages with compressed pelleted paper bedding and
171 nestlets, on ventilated racks with ad libitum access to irradiated chow and acidified, autoclaved 171 nestlets, on ventilated racks with *ad libitum* access to irradiated chow and acidified, autoclaved
172 vater, under a 14:10 light/dark cycle. Mice were determined to be free of all bacterial pathogens 172 water, under a 14:10 light/dark cycle. Mice were determined to be free of all bacterial pathogens
173 including Bordetella bronchiseptica, Filobacterium rodentium, Citrobacter rodentium, Clostridium 173 including *Bordetella bronchiseptica, Filobacterium rodentium, Citrobacter rodentium, Clostridium*
174 *iniiforme, Corynebacterium bovis, Corynebacterium kutscheri, Helicobacter spp., Mycoplasma* ¹⁷⁴*piliforme, Corynebacterium bovis, Corynebacterium kutscheri, Helicobacter* spp., *Mycoplasma* 175 spp., *Rodentibacter* spp., *Pneumocystis carinii, Salmonella* spp., *Streptobacillus moniliformis, 176 Streptococcus pneumoniae*; adventitious viruses including H1, Hantaan, KRV, LCMV, MAD1, ¹⁷⁶*Streptococcus pneumoniae*; adventitious viruses including H1, Hantaan, KRV, LCMV, MAD1, 177 MNV, PVM, RCV/SDAV, REO3, RMV, RPV, RTV, and Sendai viruses; intestinal protozoa
178 including Spironucleus muris, Giardia muris, Entamoeba muris, trichomonads, and other including Spironucleus muris, Giardia muris, Entamoeba muris, trichomonads, and other 179 intestinal flagellates and amoebae; intestinal parasites including helminths; and external
180 parasites including all species of lice and mites, via quarterly sentinel testing performed by 180 parasites including all species of lice and mites, via quarterly sentinel testing performed by
181 IDEXX BioAnalytics (Columbia, MO). Fecal samples were collected from pregnant dams at 19 181 IDEXX BioAnalytics (Columbia, MO). Fecal samples were collected from pregnant dams at 19
182 days of gestation, and from mouse pups at time of weaning (21 days of age) using previously days of gestation, and from mouse pups at time of weaning (21 days of age) using previously
183 described methods³⁸. Briefly, mice were placed in an empty autoclaved cage within a biological described methods³⁸. Briefly, mice were placed in an empty autoclaved cage within a biological
184 safety cabinet and allowed to defecate. Freshly evacuated samples feces were immediately 184 safety cabinet and allowed to defecate. Freshly evacuated samples feces were immediately
185 collected into a sterile collection tube using autoclaved wooden toothpicks discarded after each 185 collected into a sterile collection tube using autoclaved wooden toothpicks discarded after each
186 single usage. All samples were promptly placed on ice. Following fecal sample collection, 186 single usage. All samples were promptly placed on ice. Following fecal sample collection,
187 samples were stored in a -80°C freezer until DNA extraction was performed. Samples were 187 samples were stored in a -80°C freezer until DNA extraction was performed. Samples were
188 collected from all experimental mice at 50 days of age, at time of necropsy. All dams were 188 collected from all experimental mice at 50 days of age, at time of necropsy. All dams were
189 mated with sires of the same GM and were housed together until approximately day 14 of mated with sires of the same GM and were housed together until approximately day 14 of 190 gestation, at which time sires were removed. All dams were singly housed for the last week of
191 gestation to ensure pups were correctly assigned to their birth dams. All dams were handled 191 gestation to ensure pups were correctly assigned to their birth dams. All dams were handled
192 minimally during gestation, and only handled for routine cage changes by vivarium care staff 192 minimally during gestation, and only handled for routine cage changes by vivarium care staff
193 and once for pre-parturition fecal sample collection. During the one week of anxiety-related 193 and once for pre-parturition fecal sample collection. During the one week of anxiety-related
194 behavior testing, only the investigators handled and entered the home cages to avoid unknown 194 behavior testing, only the investigators handled and entered the home cages to avoid unknown
195 and excessive disturbances to the mice. 195 and excessive disturbances to the mice.
196

197 **Gut microbiome analysis**
198 *DNA extraction*. Fecal DN.

¹⁹⁸*DNA extraction*. Fecal DNA was extracted using QIAamp PowerFecal Pro DNA kits (Qiagen), 199 according to the manufacturer's instructions, with the exception that the initial sample
100 disaggregation was performed using a TissueLyser II (Qiagen), rather than a vortex and adaptor 200 disaggregation was performed using a TissueLyser II (Qiagen), rather than a vortex and adaptor
201 as described in the protocol.

201 as described in the protocol.
202 16S rRNA amplicon library p ²⁰²*16S rRNA amplicon library preparation and sequencing*.Extracted fecal DNA was processed at 203 the University of Missouri DNA Core Facility. Bacterial 16S rRNA amplicons were constructed
204 via amplification of the V4 region of the 16S rRNA gene using previously developed universal 204 via amplification of the V4 region of the 16S rRNA gene using previously developed universal
205 primers (U515F/806R), flanked by Illumina standard adapter sequences^{39, 40}. Oligonucleotide 205 primers (U515F/806R), flanked by Illumina standard adapter sequences^{39, 40}. Oligonucleotide
206 sequences are available at proBase⁴¹. Dual-indexed forward and reverse primers were used in sequences are available at proBase⁴¹. Dual-indexed forward and reverse primers were used in 207 all reactions. PCR was performed in 50 µL reactions containing 100 ng metagenomic DNA, 207 all reactions. PCR was performed in 50 µL reactions containing 100 ng metagenomic DNA,
208 primers (0.2 µM each), dNTPs (200 µM each), and Phusion high-fidelity DNA polymerase (1U, 208 primers (0.2 µM each), dNTPs (200 µM each), and Phusion high-fidelity DNA polymerase (1U, 209 Thermo Fisher). Amplification parameters were $98^{\circ}C^{(3 min)} + 198^{\circ}C^{(15 sec)} + 50^{\circ}C^{(30 sec)} + 72^{\circ}C^{(30 sec)}$ Thermo Fisher). Amplification parameters were $98^{\circ}C^{(3 min)} + [98^{\circ}C^{(15 sec)} + 50^{\circ}C^{(30 sec)} + 72^{\circ}C^{(30 sec)}$
210 ^{sec)}] x 25 cycles + 72°C^(7 min). Amplicon pools (5 µL/reaction) were combined, thoroughly mixed, S^{sec} \times 25 cycles + 72°C^(7 min). Amplicon pools (5 µL/reaction) were combined, thoroughly mixed,
211. and then purified by addition of Axygen Axyprep MagPCR clean-up beads to an equal volume of 211 and then purified by addition of Axygen Axyprep MagPCR clean-up beads to an equal volume of 212
212 50 µL of amplicons and incubated for 15 minutes at room temperature. Products were washed 212 50 µL of amplicons and incubated for 15 minutes at room temperature. Products were washed
213 multiple times with 80% ethanol and the dried pellet was resuspended in 32.5 µL EB buffer 213 multiple times with 80% ethanol and the dried pellet was resuspended in 32.5 µL EB buffer
214 (Qiagen), incubated for two minutes at room temperature, and then placed on a magnetic stand 214 (Qiagen), incubated for two minutes at room temperature, and then placed on a magnetic stand
215 for five minutes. The final amplicon pool was evaluated using the Advanced Analytical 215 for five minutes. The final amplicon pool was evaluated using the Advanced Analytical
216 Fragment Analyzer automated electrophoresis system, quantified using quant-iT HS dsDNA 216 Fragment Analyzer automated electrophoresis system, quantified using quant-iT HS dsDNA
217 Freagent kits, and diluted according to Illumina's standard protocol for sequencing on the MiSeg 217 reagent kits, and diluted according to Illumina's standard protocol for sequencing on the MiSeq
218 instrument. 218 instrument.
219 *Bioinformatics*.

²¹⁹*Bioinformatics***.** DNA sequences were assembled and annotated at the MU Informatics 220 Research Core Facility. Primers were designed to match the 5' ends of the forward and reverse
221 reads. Cutadapt⁴² (version 2.6) was used to remove the primer from the 5' end of the forward reads. Cutadapt⁴² ²²¹(version 2.6) was used to remove the primer from the 5' end of the forward 222 read. If found, the reverse complement of the primer to the reverse read was then removed from
223 the forward read as were all bases downstream. Thus, a forward read could be trimmed at both 223 the forward read as were all bases downstream. Thus, a forward read could be trimmed at both
224 ends if the insert was shorter than the amplicon length. The same approach was used on the 224 ends if the insert was shorter than the amplicon length. The same approach was used on the
225 reverse read, but with the primers in the opposite roles. Read pairs were rejected if one read or 225 reverse read, but with the primers in the opposite roles. Read pairs were rejected if one read or
226 the other did not match a 5' primer, and an error-rate of 0.1 was allowed. Two passes were 226 the other did not match a 5' primer, and an error-rate of 0.1 was allowed. Two passes were
227 made over each read to ensure removal of the second primer. A minimal overlap of three bp 227 made over each read to ensure removal of the second primer. A minimal overlap of three bp
228 with the 3' end of the primer sequence was required for removal. The QIIME2⁴³ DADA2⁴⁴ plugin 228 with the 3' end of the primer sequence was required for removal. The QIIME2⁴³ DADA2⁴⁴ plugin
229 (version 1.10.0) was used to denoise, de-replicate, and count ASVs (amplicon sequence 229 (version 1.10.0) was used to denoise, de-replicate, and count ASVs (amplicon sequence
230 variants), incorporating the following parameters: 1) forward and reverse reads were truncated variants), incorporating the following parameters: 1) forward and reverse reads were truncated to 150 bases, 2) forward and reverse reads with number of expected errors higher than 2.0
232 were discarded, and 3) Chimeras were detected using the "consensus" method and removed. R
233 version 3.5.1 and Biom version 2.1 were discarded, and 3) Chimeras were detected using the "consensus" method and removed. R
233 version 3.5.1 and Biom version 2.1.7 were used in QIIME2. Taxonomies were assigned to final
234 sequences using the Silva.v132⁴ version 3.5.1 and Biom version 2.1.7 were used in QIIME2. Taxonomies were assigned to final
234 sequences using the Silva.v132⁴⁵ database, using the classify-sklearn procedure. The
235 cladogram was constructed with GraP sequences using the Silva.v132 45 234 sequences using the Silva.v132⁴⁵ database, using the classify-sklearn procedure. The cladogram was constructed with GraPhIAn using genus-level taxonomic classifications⁴⁶.
236 Branch color depicts phylum-level cla cladogram was constructed with GraPhIAn using genus-level taxonomic classifications⁴⁶ cladogram was constructed with GraPhIAn using genus-level taxonomic classifications⁴⁶.
236 Branch color depicts phylum-level classification. The outer ring denotes Benjamini-Hochberg-
237 corrected *p* values from Wilcox Branch color depicts phylum-level classification. The outer ring denotes Benjamini-Hochberg-
237 corrected p values from Wilcox Rank-Sum tests comparing the relative abundance of each
238 genus between GMs. The color of th corrected *p* values from Wilcox Rank-Sum tests comparing the relative abundance of each
238 genus between GMs. The color of the outer ring indicates the GM with the greater average
239 relative abundance of that genus.
24 genus between GMs. The color of the outer ring indicates the GM with the greater average

239 relative abundance of that genus.

240
 Real-time reverse transcription-polymerase chain reaction (qRT-PCR)

242 Total PNA was

relative abundance of that genus.

240
 Real-time reverse transcription-polymerase chain reaction (qRT-PCR)

242 Total RNA was isolated from tissues using the Qiagen RNeasy Mini Kit per manufacturer's

243 instructions. 241
242
243
244 **Real-time reverse transcription-polymerase chain reaction (qRT-PCR)**
242 Total RNA was isolated from tissues using the Qiagen RNeasy Mini Ki
243 Instructions. RT-PCR was performed using the BioRad iTaq Universal SY
244 Ki Total RNA was isolated from tissues using the Qiagen RNeasy Mini Kit per manufacturer's

243 instructions. RT-PCR was performed using the BioRad iTaq Universal SYBR Green One-Step

244 Kit following the manufacturer instru instructions. RT-PCR was performed using the BioRad iTaq Universal SYBR Green One-Step
244 Kit following the manufacturer instructions. Briefly, each reaction consisted of 5 µL of SYBR
245 Green Supermix, 0.125 µL iScript 244 Kit following the manufacturer instructions. Briefly, each reaction consisted of 5 μL of SYBR
245 Green Supermix, 0.125 μL iScript reverse transcriptase, 0.45 μL of forward and reverse primers,
246 1.475 μL of water, 245 Green Supermix, 0.125 μL iScript reverse transcriptase, 0.45 μL of forward and reverse primers,
246 1.475 μL of water, and 2.5 μL of template RNA. The reaction was run on a BioRad C1000 Touch
247 thermal cycler with 246 1.475 μL of water, and 2.5 μL of template RNA. The reaction was run on a BioRad C1000 Touch
247 thermal cycler with a BioRad CFX384 Real-Time System with the following parameters: 50°C for
248 10 min for reverse trans thermal cycler with a BioRad CFX384 Real-Time System with the following parameters: 50°C for

248 10 min for reverse transcription, 95°C for 1 min for DNA Polymerase activation and DNA

249 denaturation, and 40 cycles of 9 10 min for reverse transcription, 95°C for 1 min for DNA Polymerase activation and DNA

249 denaturation, and 40 cycles of 95°C for 10 sec and 60°C for 30 sec. Melt-curve analysis was

250 performed using the following par performed using the following parameters: $65-95^{\circ}$ C with 0.5° C increments for 5 sec/step. performed using the following parameters: 65-95°C with 0.5°C increments for 5 sec/step.
251 Primers used can be found in **Table S1.**
252 **Bile salt hydrolase metagenomic and metatranscriptomic analysis of mouse feces**
254

251 Primers used can be found in **Table S1**.
252 **Bile salt hydrolase metagenomic and
254 Metagenomic and metatranscriptomic b
255 multi omic analysis of GM^{Low} (GM4)** 253
254
255
256 **Bile salt hydrolase metagenomic and metatranscriptomic analysis of mouse feces**
254 Metagenomic and metatranscriptomic bsh (K01442) read counts were acquired from p
255 multi-omic analysis of GM^{Low} (GM1) and GM^{High} (G Metagenomic and metatranscriptomic *bsh* (K01442) read counts were acquired from previous

255 multi-omic analysis of GM^{Low} (GM1) and GM^{High} (GM4)⁴⁷. Expression counts from

256 metatranscriptomic analysis were norma multi-omic analysis of GM^{Low} (GM1) and GM^{High} (GM4)⁴⁷. multi-omic analysis of GM^{LOW} (GM1) and GM^{High} (GM4)⁴⁷. Expression counts from
256 metatranscriptomic analysis were normalized to bsh metagenomic reads.
257 **Measurement of BSH activity in mouse feces**
258 BSH activit

metatranscriptomic analysis were normalized to *bsh* metagenomic reads.

257
 Measurement of BSH activity in mouse feces

259 BSH activity in the mouse feces was measured using previously reported bioluminescent bile

26 259
260
261 **Measurement of BSH activity in mouse feces**
259 BSH activity in the mouse feces was measured
260 acid activatable luciferin probes (BAL) protocol⁴⁸
261 bioluminescence readout with the recombinan 259 BSH activity in the mouse feces was measured using previously reported bioluminescent bile

260 acid activatable luciferin probes (BAL) protocol⁴⁸ with major modification by replacing whole-cell

261 bioluminescence acid activatable luciferin probes (BAL) protocol⁴⁸ with major modification by replacing whole-cell acid activatable luciferin probes (BAL) protocol⁴⁸ with major modification by replacing whole-cell

261 bioluminescence readout with the recombinant luciferase enzymatic assay⁴⁹. Mouse fecal

262 samples were soaked i bioluminescence readout with the recombinant luciferase enzymatic assay 49 samples were soaked in PBS (pH 7.4, Gibco, ref# 10010-023) supplemented with 2-
263 mercaptoethanol (Acros Organics, 20 mM) at a concentration of 10 mg/mL on ice for 30 min.
264 The mixtures were homogenized by sonication mercaptoethanol (Acros Organics, 20 mM) at a concentration of 10 mg/mL on ice for 30 min. 263 mercaptoethanol (Acros Organics, 20 mM) at a concentration of 10 mg/mL on ice for 30 min.
264 The mixtures were homogenized by sonication in ultrasound cleaner (Elmasonic Easy 40 H, 340 264 The mixtures were homogenized by sonication in ultrasound cleaner (Elmasonic Easy 40 H, 340

The mixtures were homogenized by sonication in ultrasound cleaner (Elmasonic Easy 40 H, 340

The mixtures were homogenized by

265 W) at 0°C for 30 min, stirring every 10 min. Resulting mixtures along with blank buffer (3
266 replicates by 50 μ L) were mixed with working solutions of BAL probes (50 μ L, 20 μ M in PBS)
267 along with a solut 266 replicates by 50 μL) were mixed with working solutions of BAL probes (50 μL, 20 μM in PBS)
267 along with a solution of luciferin (50 μL, 2 μM in PBS) in a 96-well assay plate (Corning, ref#
268 3595) and incubated at along with a solution of luciferin (50 μL, 2 μM in PBS) in a 96-well assay plate (Corning, ref# 268 3595) and incubated at 37°C for 1 h. After incubation, the mixtures were diluted with 2% Triton X-100 in PBS (100 μL) to 268 3595) and incubated at 37°C for 1 h. After incubation, the mixtures were diluted with 2% Triton
269 X-100 in PBS (100 µL) to stop the reaction. In a separate 96-well flat bottom black plate
270 (Corning, ref# 3650), r X-100 in PBS (100 μL) to stop the reaction. In a separate 96-well flat bottom black plate
270 (Corning, ref# 3650), resulting mixtures (5 μL) were diluted with PBS (50 μ). A luciferase
271 solution containing recombinant 270 (Corning, ref# 3650), resulting mixtures (5 μL) were diluted with PBS (50 μ). A luciferase
271 solution containing recombinant luciferase from Photinus pyralis (Sigma-Aldrich, 20 μg/mL), ATP
272 disodium trihydrate (80 solution containing recombinant luciferase from Photinus pyralis (Sigma-Aldrich, 20 μg/mL), ATP
272 disodium trihydrate (Fisher Scientific, 2 mM), and magnesium sulfate heptahydrate (Fisher
273 Scientific, 2 mM) in PBS disodium trihydrate (Fisher Scientific, 2 mM), and magnesium sulfate heptahydrate (Fisher
273 Scientific, 2 mM) in PBS (50 μ L) was added to each well simultaneously. Bioluminescence was
274 measured immediately in an I Scientific, 2 mM) in PBS (50 μL) was added to each well simultaneously. Bioluminescence was

274 measured immediately in an IVIS Spectrum (Xenogen) imaging system for 20 min with 1 min

275 intervals using automatic setti intervals using automatic settings. Raw data were processed using Living Image 4.2 software

276 (Caliper LifeSciences), further data processing was carried out in Excel (Microsoft 365), and

277 finally visualization and 278 Deconjugation potentials or percentage of probe hydrolysis were calculated as the ratio of the
279 signal from the BAL probe to the signal from luciferin in the corresponding fecal extract and 279 signal from the BAL probe to the signal from luciferin in the corresponding fecal extract and
280 reported as the mean value of 3 replicates. The signals from incubation of BAL probes in blank finally visualization and statistical calculations were performed in Prism 9 (GraphPad software).
278 Deconjugation potentials or percentage of probe hydrolysis were calculated as the ratio of the
279 signal from the BAL p Deconjugation potentials or percentage of probe hydrolysis were calculated as the ratio of the
279 signal from the BAL probe to the signal from luciferin in the corresponding fecal extract and
280 reported as the mean valu 279 signal from the BAL probe to the signal from luciferin in the corresponding fecal extract and
280 reported as the mean value of 3 replicates. The signals from incubation of BAL probes in blank
281 buffer provided a bac 280 reported as the mean value of 3 replicates. The signals from incubation of BAL probes in blank
281 buffer provided a background result of nonspecific hydrolysis of the probes.
282 **Metabolite analyses** 281 buffer provided a background result of nonspecific hydrolysis of the probes.
282 **Metabolite analyses**
284 GC-MS. Fecal and serum samples were diluted in 18 volumes of ice-cold 2:2:1

284
285 283 **Metabolite analyses**
284 *GC-MS.* Fecal and
285 methanol/acetonitrile/
286 succinic acid, D8-valir methanol/acetonitrile/water containing a mixture of internal standards (D4-citric acid, D4-
286 succinic acid, D8-valine, and U13C-labeled glutamine, glutamic acid, lysine, methionine, serine,
287 and tryptophan; Cambridge methanol/acetonitrile/water containing a mixture of internal standards (D4-citric acid, D4-
286 succinic acid, D8-valine, and U13C-labeled glutamine, glutamic acid, lysine, methionine, serine,
287 and tryptophan; Cambridge succinic acid, D8-valine, and U13C-labeled glutamine, glutamic acid, lysine, methionine, serine,

287 and tryptophan; Cambridge Isotope Laboratories), where the 1-part water was composed of

288 ample volume + water. Sampl and tryptophan; Cambridge Isotope Laboratories), where the 1-part water was composed of

288 sample volume + water. Sample extraction mixtures were vortexed for 10 minutes at RT and

289 rotated for 1 hour at -20°C. Mixtur 289 rotated for 1 hour at -20°C. Mixtures were centrifuged for 10 minutes at 21,000 \times g, and 150 μ L
290 of the cleared metabolite extracts were transferred to autosampler vials and dried using a
291 SpeedVac vacuum rotated for 1 hour at -20°C. Mixtures were centrifuged for 10 minutes at 21,000 \times g, and 150 μ L
290 of the cleared metabolite extracts were transferred to autosampler vials and dried using a
291 SpeedVac vacuum conc 290 of the cleared metabolite extracts were transferred to autosampler vials and dried using a
291 SpeedVac vacuum concentrator (Thermo). Dried metabolite extracts were reconstituted in 30 μ L
292 of 11.4 mg/mL methoxya SpeedVac vacuum concentrator (Thermo). Dried metabolite extracts were reconstituted in 30 μL
292 of 11.4 mg/mL methoxyamine (MOX) in anhydrous pyridine, vortexed for 5 minutes, and heated
293 for 1 hour at 60°C. Next, to 293 for 1 hour at 60°C. Next, to each sample 20 μ L of N,O-Bis(trimethylsilyl)trifluoroacetamide
294 (TMS) was added, samples were vortexed for 1 minute, and heated for 30 minutes at 60°C.
295 Derivatized samples were a 293 for 1 hour at 60°C. Next, to each sample 20 μ L of N,O-Bis(trimethylsilyl)trifluoroacetamide
294 (TMS) was added, samples were vortexed for 1 minute, and heated for 30 minutes at 60°C.
295 Derivatized samples were a 294 (TMS) was added, samples were vortexed for 1 minute, and heated for 30 minutes at 60°C.
295 Derivatized samples were analyzed by GC-MS. One µL of derivatized sample was injected into
296 a Trace 1300 GC (Thermo) fitted 296 a Trace 1300 GC (Thermo) fitted with a TraceGold TG-5SilMS column (Thermo) operating
297 under the following conditions: split ratio = 20:1. split flow = 24 uL/minute. purge flow = 5 296 a Trace 1300 GC (Thermo) fitted with a TraceGold TG-5SilMS column (Thermo) operating
297 under the following conditions: split ratio = 20:1, split flow = 24 μ L/minute, purge flow = 5 297 under the following conditions: split ratio = 20:1, split flow = 24 μL/minute, purge flow = 5

20:1

20:1, split flow = 24 μL/minute, purge flow = 5

mL/minute, carrier mode = Constant Flow, and carrier flow rate = 1.2 mL/minute. The GC oven

299 temperature gradient was as follows: 80°C for 3 minutes, increasing at a rate of 20°C/minute to

280°C, and holding at a temp 299 temperature gradient was as follows: 80°C for 3 minutes, increasing at a rate of 20°C/minute to
300 280°C, and holding at a temperature at 280°C for 8 minutes. Ion detection was performed by an
301 ISQ 7000 mass spectr 280°C, and holding at a temperature at 280°C for 8 minutes. Ion detection was performed by an
301 ISQ 7000 mass spectrometer (Thermo) operated from 3.90 to 21.00 minutes in EI mode (-70eV)
302 IC-MS SCFA analysis. 18-fold ISQ 7000 mass spectrometer (Thermo) operated from 3.90 to 21.00 minutes in EI mode (-70eV)
302 using select ion monitoring (SIM).
303 LC-MS SCFA analysis. 18-fold (w/v) extraction solvent (Acetonitrile:Methanol:Water (2:2:

302 using select ion monitoring (SIM).
303 LC-MS SCFA analysis. 18-fold (
304 containing deuterated SCFA sta
305 added to each sample and rotate *LC-MS SCFA analysis.* 18-fold (w/v) extraction solvent (Acetonitrile:Methanol:Water (2:2:1))
304 containing deuterated SCFA standards (D3-acetate, D7-butyrate, and D5-propionate) was
305 added to each sample and rotated a 304 containing deuterated SCFA standards (D3-acetate, D7-butyrate, and D5-propionate) was
305 added to each sample and rotated at -20°C for 1 hr and then centrifuged at 21,000 x g for 10
306 min. Supernatant was used for L 305 added to each sample and rotated at -20°C for 1 hr and then centrifuged at 21,000 \times g for 10 min. Supernatant was used for LC-MS SCFA analysis. LC-MS data was acquired on a Thermo Q Exactive hybrid quadrupole Orbit Q Exactive hybrid quadrupole Orbitrap mass spectrometer with a Vanquish Flex UHPLC system
308 or Vanquish Horizon UHPLC system. The LC column used was a ZIC-pHILIC guard column (20
309 x 2.1 mm). The injection volume was 2 308 or Vanquish Horizon UHPLC system. The LC column used was a ZIC-pHILIC guard column (20 \times 2.1 mm). The injection volume was 2 µL. For the Mobile phase, Solvent A consisted of 20 mM ammonium carbonate [(NH4)2CO3] and 311 and Solvent B consisted of Acetonitrile. This method was run at a flow rate of 0.1 mL/min, and
312 the injection volume was 2 µL. Linear gradient was used at 70% solvent B with a 5 min elution \times 2.1 mm). The injection volume was 2 μ L. For the Mobile phase, Solvent A consisted of 20 mM
310 ammonium carbonate [(NH4)2CO3] and 0.1% ammonium hydroxide (v/v) [NH4OH] at pH ~9.1]
311 and Solvent B consisted of Ac 310 ammonium carbonate [(NH4)2CO3] and 0.1% ammonium hydroxide (v/v) [NH4OH] at pH ~9.1]
311 and Solvent B consisted of Acetonitrile. This method was run at a flow rate of 0.1 mL/min, and
312 the injection volume was 2 µL and Solvent B consisted of Acetonitrile. This method was run at a flow rate of 0.1 mL/min, and
312 the injection volume was 2 µL. Linear gradient was used at 70% solvent B with a 5 min elution
313 time. The mass spectromet 312 the injection volume was 2 µL. Linear gradient was used at 70% solvent B with a 5 min elution
313 time. The mass spectrometer was operated in targeted selected ion-monitoring (tSIM) mode
314 from 1 to 5 minutes. An in time. The mass spectrometer was operated in targeted selected ion-monitoring (tSIM) mode
314 from 1 to 5 minutes. An inclusion list for the three short chain fatty acids and their deuterated
315 crsions were used in tSIM m from 1 to 5 minutes. An inclusion list for the three short chain fatty acids and their deuterated
315 versions were used in tSIM method.
316 LC-MS bile acid analysis. Extraction solvent (methanol:acetonitrile:water, 2:2:1)

versions were used in tSIM method.
316 LC-MS bile acid analysis. Extraction
317 (5 µLl/mL) deuterated bile acids Max
318 volume extraction buffer was adde LC-MS bile acid analysis. Extraction solvent (methanol:acetonitrile:water, 2:2:1) was spiked with
317 (5 µLl/mL) deuterated bile acids MaxSpec Mixture (Cayman Chemicals Item no. 33506). 18-fold
318 volume extraction buffer 317 (5 µLI/mL) deuterated bile acids MaxSpec Mixture (Cayman Chemicals Item no. 33506). 18-fold
318 volume extraction buffer was added to each sample. The samples were placed in a -20°C
319 freezer for 1 hour while rotatin volume extraction buffer was added to each sample. The samples were placed in a -20°C
319 freezer for 1 hour while rotating. The samples were then centrifuged at 21,000 x g for 10
320 minutes. Supernatant was transferred t 319 freezer for 1 hour while rotating. The samples were then centrifuged at 21,000 \times g for 10
320 minutes. Supernatant was transferred to LC-MS autosampler vials for analysis. LC-MS data was
321 acquired on a Thermo Q minutes. Supernatant was transferred to LC-MS autosampler vials for analysis. LC-MS data was
acquired on a Thermo Q Exactive hybrid quadrupole Orbitrap mass spectrometer with a
Vanquish Flex UHPLC system or Vanquish Horizo 321 acquired on a Thermo Q Exactive hybrid quadrupole Orbitrap mass spectrometer with a
322 Vanquish Flex UHPLC system or Vanquish Horizon UHPLC system. A Thermo Hypersil GOLD
323 (2.1 x 150 mm, 1.9 µm) UHPLC column was u Vanquish Flex UHPLC system or Vanquish Horizon UHPLC system. A Thermo Hypersil GOLD
323 (2.1 x 150 mm, 1.9 µm) UHPLC column was used with a column Temperature of 30°C. For the
324 Mobile Phase, solvent A consisted of 1% ac $(2.1 \times 150 \text{ mm}, 1.9 \text{ µm})$ UHPLC column was used with a column Temperature of 30°C. For the
324 Mobile Phase, solvent A consisted of 1% acetonitrile in water with 0.1% formic acid, and solvent
325 B is 99% acetonitrile w B is 99% acetonitrile with 0.1% formic acid. The gradient started at 50% Solvent B and was held
for 2.5 minutes; then increased to 100% B at 10 minutes and held for 0.5 minutes before re-
equilibration to 50% solvent B for for 2.5 minutes; then increased to 100% B at 10 minutes and held for 0.5 minutes before re-
equilibration to 50% solvent B for 5.5 min. Flow Rate was 0.4 mL/min, and injection volume was
328 3 µL. The mass spectrometer was 326 for 2.5 minutes; then increased to 100% B at 10 minutes and held for 0.5 minutes before re-
327 equilibration to 50% solvent B for 5.5 min. Flow Rate was 0.4 mL/min, and injection volume was
328 3 µL. The mass spectro equilibration to 50% solvent B for 5.5 min. Flow Rate was 0.4 mL/min, and injection volume was
328 3 µL. The mass spectrometer was operated in full-scan negative mode, with the spray voltage
329 set to 3.0 kV, the heated c 329 set to 3.0 kV, the heated capillary held at 275°C, and the HESI probe held at 350°C. The sheath
330 gas flow was set to 40 units, the auxiliary gas flow was set to 15 units, and the sweep gas flow 329 set to 3.0 kV, the heated capillary held at 275°C, and the HESI probe held at 350°C. The sheath
330 gas flow was set to 40 units, the auxiliary gas flow was set to 15 units, and the sweep gas flow 330 gas flow was set to 40 units, the auxiliary gas flow was set to 15 units, and the sweep gas flow

gas flow

gas flow

gas flow was set to 15 units, and the sweep gas flow

gas flow

resolution set at 70,000, the AGC target at 1 \times 10⁶, and the maximum injection time at 200 ms⁵⁰

was set to 1 unit. MS data acquisition was performed in a range of m/z 70–1,000, with the

resolution set at 70,000, the AGC target at 1 x 10⁶, and the maximum injection time at 200 ms⁵⁰.
 Metabolomic Data Analysis. resolution set at 70,000, the AGC target at 1×10^6 , and the maximum injection time at 200 ms⁵⁰.
333 *Metabolomic Data Analysis.* GC-MS Raw data were analyzed using TraceFinder 5.1 (Thermo).
334 Metabolite identifica Metabolite identification and annotation required at least two ions (target + confirming) and a
335 unique retention time that corresponded to the ions and retention time of a reference standard
336 previously determined i 336 previously determined in-house. A pooled-sample generated prior to derivatization was
337 analyzed at the beginning, at a set interval during, and the end the analytical run to correct peak
338 intensities using the NO unique retention time that corresponded to the ions and retention time of a reference standard
336 previously determined in-house. A pooled-sample generated prior to derivatization was
337 analyzed at the beginning, at a s previously determined in-house. A pooled-sample generated prior to derivatization was
analyzed at the beginning, at a set interval during, and the end the analytical run to correct peak
intensities using the NOREVA tool⁵¹ analyzed at the beginning, at a set interval during, and the end the analytical run to correct peak
intensities using the NOREVA tool⁵¹. NOREVA corrected data were then normalized to the total
signal per sample to contro intensities using the NOREVA tool 51 signal per sample to control for extraction, derivatization, and/or loading effects. Acquired LC-
340 MS data were processed by Thermo Scientific TraceFinder 4.1 software, and metabolites were
341 identified based on the U signal per sample to control for extraction, derivatization, and/or loading effects. Acquired LC-
340 MS data were processed by Thermo Scientific TraceFinder 4.1 software, and metabolites were
341 inhouse library. NOREVA w MS data were processed by Thermo Scientific TraceFinder 4.1 software, and metabolites were
341 identified based on the University of Iowa Metabolomics Core facility standard-confirmed,
342 inhouse library. NOREVA was used identified based on the University of Iowa Metabolomics Core facility standard-confirmed,
342 inhouse library. NOREVA was used for signal drift correction⁵¹. For bile acids, data were
343 normalized to one of the d4-bile inhouse library. NOREVA was used for signal drift correction⁵¹ 343 normalized to one of the d4-bile acid standards. For SCFA, analyte signal was corrected by
344 normalizing to the deuterated analyte signal and the signal obtained from processing blank was
345 subtracted. 343 normalized to one of the d4-bile acid standards. For SCFA, analyte signal was corrected by
344 normalizing to the deuterated analyte signal and the signal obtained from processing blank was
345 subtracted.
346 344 normalizing to the deuterated analyte signal and the signal obtained from processing blank was
345 subtracted.
346 **Cross-fostering** 345 subtracted.
346
347 **Cross-foste**
348 Mice in cros

346
347
348
349
350 347 **Cross-fostering**
348 Mice in cross-fost
349 dam of the recip
350 cages were move Mice in cross-foster (CF) groups were cross-fostered at less than 24 hours of age to a surrogate
349 dam of the reciprocal GM. Following identification of recently birthed litters from both GMs,
350 cages were moved to a b dam of the reciprocal GM. Following identification of recently birthed litters from both GMs,
350 cages were moved to a biosafety cabinet. Litters were removed from the cage of the biological
351 dam and placed onto clean cages were moved to a biosafety cabinet. Litters were removed from the cage of the biological
351 dam and placed onto clean paper towels. Bedding from the cage of the surrogate dam was
352 gently mixed with the pups to tra dam and placed onto clean paper towels. Bedding from the cage of the surrogate dam was
352 gently mixed with the pups to transfer the surrogate dam scent to the pups and reduce the
353 possibility of cannibalism. The pups gently mixed with the pups to transfer the surrogate dam scent to the pups and reduce the

possibility of cannibalism. The pups were then placed into the surrogate dam cage, and cages

were returned to the appropriate hous possibility of cannibalism. The pups were then placed into the surrogate dam cage, and cages
354 were returned to the appropriate housing rack.
355 **Behavior testing**
357 Open Field Exploration. The open field exploration

were returned to the appropriate housing rack.

355
 Behavior testing

357 Open Field Exploration. The open field exploration test was used to evaluate anxiety-related

358 behavior and locomotor function. Environmental 356
357
358
359 356 **Behavior testing**
357 Open Field Explo.
358 behavior and loco
359 Columbus, OH) Open Field Exploration. The open field exploration test was used to evaluate anxiety-related
358 behavior and locomotor function. Environmental control chambers (Omnitech Electronics, Inc.,
359 Columbus, OH) consisting of behavior and locomotor function. Environmental control chambers (Omnitech Electronics, Inc.,
359 Columbus, OH) consisting of 4 separate environmental isolation chambers containing a
360 plexiglass box (41cm x 41cm x 30cm) 360 plexiglass box (41cm \times 41cm \times 30cm) placed onto an infrared grid (41cm \times 41cm) to track
361 locomotion. Lighting for each isolation chamber was set to 159 lux. Mice were allowed to
362 acclimate to the behavi plexiglass box (41cm x 41cm x 30cm) placed onto an infrared grid (41cm x 41cm) to track
361 locomotion. Lighting for each isolation chamber was set to 159 lux. Mice were allowed to
362 acclimate to the behavior room for 1 361 locomotion. Lighting for each isolation chamber was set to 159 lux. Mice were allowed to
362 acclimate to the behavior room for 1 hour prior to testing. Before starting each test, the
363 plexiglass was cleaned with 0. 362 acclimate to the behavior room for 1 hour prior to testing. Before starting each test, the
363 plexiglass was cleaned with 0.25% bleach, followed by 70% ethanol to remove any residual 363 plexiglass was cleaned with 0.25% bleach, followed by 70% ethanol to remove any residual olfactory cues. Each mouse was placed into the middle of the open field exploration test and
recorded for 30 minutes by the Fusion behavior monitoring software (Omnitech Electronics, Inc.,
Columbus, OH). The first 20 minut recorded for 30 minutes by the Fusion behavior monitoring software (Omnitech Electronics, Inc.,
366 Columbus, OH). The first 20 minutes of the test were considered acclimation time, and the final
367 10 minutes were analyz Columbus, OH). The first 20 minutes of the test were considered acclimation time, and the final
367 10 minutes were analyzed following completion of the test. Total distance traveled (cm), time
368 spent in the center zone 10 minutes were analyzed following completion of the test. Total distance traveled (cm), time
368 spent in the center zone (seconds), distance traveled in the center zone, and vertical activity
369 (rearing) were measured.

spent in the center zone (seconds), distance traveled in the center zone, and vertical activity

369 (rearing) were measured.

370 Light/Dark Transition. The light/dark transition test was performed within the environmenta 369 (rearing) were measured.
370 *Light/Dark Transition.* Th
371 control chambers (Omnite
372 of a plexiglass box withi Light/Dark Transition. The light/dark transition test was performed within the environmental
371 control chambers (Omnitech Electronics, Inc., Columbus, OH, USA). The apparatus consisted
372 of a plexiglass box within the control chambers (Omnitech Electronics, Inc., Columbus, OH, USA). The apparatus consisted
372 of a plexiglass box within the environmental chambers (41cm x 41cm x 30cm) that were
373 partitioned into two equal sections by 372 of a plexiglass box within the environmental chambers $(41 \text{cm} \times 41 \text{cm} \times 30 \text{cm})$ that were
373 partitioned into two equal sections by a black plexiglass insert with a door that allowed one half
374 to be a dark zo to be a dark zone, and a second half to be a light zone. The light zone was illumination was set
to 200 lux. Mice were allowed to acclimate to the behavior room for 1 hour prior to testing.
Before the start of each test, t to be a dark zone, and a second half to be a light zone. The light zone was illumination was set
375 to 200 lux. Mice were allowed to acclimate to the behavior room for 1 hour prior to testing.
376 Before the start of each to 200 lux. Mice were allowed to acclimate to the behavior room for 1 hour prior to testing.
376 Before the start of each test, the plexiglass box and insert were cleaned with 0.25% bleach,
377 followed by 70% ethanol to r Before the start of each test, the plexiglass box and insert were cleaned with 0.25% bleach,
377 followed by 70% ethanol to remove any residual olfactory cues. Mice were placed into the light
378 zone facing away from the followed by 70% ethanol to remove any residual olfactory cues. Mice were placed into the light
378 zone facing away from the dark zone and monitored by Fusion behavior monitoring software
379 (Omnitech Electronics, Inc., C zone facing away from the dark zone and monitored by Fusion behavior monitoring software
379 (Omnitech Electronics, Inc., Columbus, OH, USA) for 15 minutes. Time spent in the light zone
380 (sec), distance travelled in the (Omnitech Electronics, Inc., Columbus, OH, USA) for 15 minutes. Time spent in the light zone
380 (sec), distance travelled in the light zone (cm), and number of transitions between light and dark
381 *Elevated Plus Maze.*

380 (sec), distance travelled in the light zone (cm), and number of transitions between light and dark
381 zones were measured.
382 *Elevated Plus Maze*. The elevated plus maze test consisted of an apparatus with two open 381 zones were measured.
382 Elevated Plus Maze. Th
383 (32.5 x 5 cm, with 2-m
384 The open arms were a Elevated Plus Maze. The elevated plus maze test consisted of an apparatus with two open arms
383 (32.5 x 5 cm, with 2-mm ledges) and two closed arms (32.5 x 5 cm, with 14.5 cm high walls).
384 The open arms were arranged p 383 (32.5 \times 5 cm, with 2-mm ledges) and two closed arms (32.5 \times 5 cm, with 14.5 cm high walls).
384 The open arms were arranged perpendicular to the closed arms so the apparatus formed the
385 shape of a plus sign w 385 shape of a plus sign with a center square $(5 \times 5 \text{ cm})$. The entire apparatus was raised 50 cm
386 above the floor. The center zone of the apparatus was illuminated to 50 lux. Mice were allowed
387 to acclimate to the shape of a plus sign with a center square $(5 \times 5 \text{ cm})$. The entire apparatus was raised 50 cm
386 above the floor. The center zone of the apparatus was illuminated to 50 lux. Mice were allowed
387 to acclimate to the beh above the floor. The center zone of the apparatus was illuminated to 50 lux. Mice were allowed
387 to acclimate to the behavior room for 1 hour prior to testing. Prior to testing, the apparatus was
388 cleaned with 0.25% b to acclimate to the behavior room for 1 hour prior to testing. Prior to testing, the apparatus was
388 cleaned with 0.25% bleach followed by 70% ethanol to remove olfactory cues. Each mouse was
399 placed in the center squ cleaned with 0.25% bleach followed by 70% ethanol to remove olfactory cues. Each mouse was
389 placed in the center square facing an open arm and was recorded and monitored for 5 minutes.
390 Distance Traveled in the open placed in the center square facing an open arm and was recorded and monitored for 5 minutes.
390 Distance Traveled in the open arms (cm), time spent in the open arms (sec), and number of
391 entries into the open arms was Distance Traveled in the open arms (cm), time spent in the open arms (sec), and number of
391 entries into the open arms was calculated from distance measurements and entry counts
392 obtained by Any-Maze monitoring softwa

entries into the open arms was calculated from distance measurements and entry counts
392 obtained by Any-Maze monitoring software (Stoelting Co., Wood Dale, IL, USA).
393 *Voluntary running*. New litters of CD-1 GM^{Low} a obtained by Any-Maze monitoring software (Stoelting Co., Wood Dale, IL, USA).
393 *Voluntary running*. New litters of CD-1 GM^{Low} and GM^{High} mice were genera
394 voluntary wheel running assays (the mice used in the beha Voluntary running. New litters of CD-1 GM^{Low} and GM^{High} Voluntary running. New litters of CD-1 GM^{Low} and GM^{High} mice were generated to evaluate
voluntary wheel running assays (the mice used in the behavior assays did not undergo wheel
running evaluation). Litters for running running evaluation). Litters for running wheel experiments were culled to six pups per litter (3) 395 running evaluation). Litters for running wheel experiments were culled to six pups per litter (3 male, 3 female) at birth, and then weaned into cages of same-sex trios at weaning. During wheel set-up at seven weeks of 396 male, 3 female) at birth, and then weaned into cages of same-sex trios at weaning. During
397 wheel set-up at seven weeks of age, mice were transferred from their home cage to a new static
397 397 wheel set-up at seven weeks of age, mice were transferred from their home cage to a new static

1937 Wheel set-up at seven weeks of age, mice were transferred from their home cage to a new static

1937 Wheel set-up at

microisolator cage containing a wireless running wheel (Med Associates, ENV-047) connected
to a wireless hub and laptop computer in the animal room. Only investigators entered the
400 behavior room to check mice and equipm to a wireless hub and laptop computer in the animal room. Only investigators entered the

400 behavior room to check mice and equipment daily during the 12 days of testing to avoid

401 excessive disturbances to the mice. behavior room to check mice and equipment daily during the 12 days of testing to avoid
401 excessive disturbances to the mice. Mice were singly housed during the experiment, assigned
402 to running wheel cages using a rand excessive disturbances to the mice. Mice were singly housed during the experiment, assigned
to running wheel cages using a random number generator, and were placed in alternating order
on the shelf such that microbiome gro to running wheel cages using a random number generator, and were placed in alternating order

403 on the shelf such that microbiome group and sex were consistently alternated. Following five

404 days of acclimation, data on the shelf such that microbiome group and sex were consistently alternated. Following five

404 days of acclimation, data were collected continuously for seven consecutive days using Wheel

405 Manager software, v2.04.00 Manager software, v2.04.00 (Med Associates, SOF-860). Data were analyzed using Wheel
Analysis software, v2.02.01 (Med Associates, SOF-861). No other mice were housed in the
room containing running wheel cages, traffic was Analysis software, v2.02.01 (Med Associates, SOF-861). No other mice were housed in the
room containing running wheel cages, traffic was limited to once daily checks at the same time
of day by one laboratory staff, and no 407 arom containing running wheel cages, traffic was limited to once daily checks at the same time
408 of day by one laboratory staff, and no cage changes were performed during the acclimation and
409 testing period. room containing running wheel cages, traffic was limited to once daily checks at the same time
408 of day by one laboratory staff, and no cage changes were performed during the acclimation and
409 testing period.
A11 Necro of day by one laboratory staff, and no cage changes were performed during the acclimation and

409 testing period.

410 **Necropsy**

Mice were transported to the pecropsy room at 50 days of age and allowed to acclimate to t

409 testing period.
410
411 **Necropsy**
412 Mice were tran 411
412
413
414 411 **Necropsy**
412 Mice were
413 room for 1
414 other mice Fig. 2412 Mice were transported to the necropsy room at 50 days of age and allowed to acclimate to the room for 1 hour. Mice were then euthanized one at a time by CO_2 asphyxiation out of sight of other mice. The euthana 113 room for 1 hour. Mice were then euthanized one at a time by CO_2 asphyxiation out of sight of
114 other mice. The euthanasia chamber was cleaned with 70% ethanol between mice to eliminate
115 olfactory cues. Followin other mice. The euthanasia chamber was cleaned with 70% ethanol between mice to eliminate
145 olfactory cues. Following loss of paw pinch and righting reflexes, blood was collected by cardiac
146 puncture and placed into s olfactory cues. Following loss of paw pinch and righting reflexes, blood was collected by cardiac
416 puncture and placed into serum separator tubes. The brain was then removed, and the
417 hippocampus was gently dissected puncture and placed into serum separator tubes. The brain was then removed, and the
417 hippocampus was gently dissected out, placed in a 2 mL tube, and promptly plunged into liquid
418 nitrogen to flash freeze. Liver and 417 hippocampus was gently dissected out, placed in a 2 mL tube, and promptly plunged into liquid
418 nitrogen to flash freeze. Liver and ileum were isolated, placed in a 2 mL tube, and promptly
419 plunged into liquid nit plunged into liquid nitrogen. Two fecal pellets were collected from the colon for 16S rRNA

420 amplicon sequencing. Blood was allowed to clot for 30 minutes at room temperature and was

421 then centrifuged at 4,000 RPM f plunged into liquid nitrogen. Two fecal pellets were collected from the colon for 16S rRNA
420 amplicon sequencing. Blood was allowed to clot for 30 minutes at room temperature and was
421 then centrifuged at 4,000 RPM for amplicon sequencing. Blood was allowed to clot for 30 minutes at room temperature and was

421 then centrifuged at 4,000 RPM for 15 minutes, and serum placed into a 1.5 mL microcentrifuge

422 tube. Hippocampus, feces, and then centrifuged at 4,000 RPM for 15 minutes, and serum placed into a 1.5 mL microcentrifuge

422 tube. Hippocampus, feces, and serum were promptly placed into -80°C freezer for storage.

424 **Methylome analysis**

425 Due

123
124 **Methylome analysis**
125 Due to constraints on resources, methylome analysis were performed in only one sex. To
126 assess intergenerational effects on DNA methylation, we analyzed dams and female offspring in 424
425
426
427 **Methylome analysis

425 Due to constraints of

426 assess intergeneration

427 both control and CF m

428 WOTO CULLO to Six form** assess intergenerational effects on DNA methylation, we analyzed dams and female offspring in
both control and CF mice. Dams from both colonies were time mated, and following birth, litters
were culled to six female pups. assess intergenerational effects on DNA methylation, we analyzed dams and female offspring in

427 both control and CF mice. Dams from both colonies were time mated, and following birth, litters

428 were culled to six fem both control and CF mice. Dams from both colonies were time mated, and following birth, litters

428 were culled to six female pups. Three of the pups from each litter remained with the birth dam,

429 and three were cross were culled to six female pups. Three of the pups from each litter remained with the birth dam,
and three were cross-fostered onto a dam of the opposite GM so that every dam from both GMs
had three of their birth pups and and three were cross-fostered onto a dam of the opposite GM so that every dam from both GMs
had three of their birth pups and three cross-foster surrogate pups. Hippocampi were collected
from all dams following weaning, fl 430 had three of their birth pups and three cross-foster surrogate pups. Hippocampi were collected
431 from all dams following weaning, flash frozen in liquid nitrogen, and stored at -80°C. At seven 431 from all dams following weaning, flash frozen in liquid nitrogen, and stored at -80°C. At seven

1491 from all dams following weaning, flash frozen in liquid nitrogen, and stored at -80°C. At seven weeks of age, hippocampi from the offspring were collected, flash frozen in liquid nitrogen, and
stored at -80°C. Hippocampal genomic DNA was isolated from adult female CF and control
offspring hippocampi using the DNeasy stored at -80°C. Hippocampal genomic DNA was isolated from adult female CF and control
434 offspring hippocampi using the DNeasy kit (Qiagen, Valencia, CA) following manufacturer
435 instructions. For studying genome-wide offspring hippocampi using the DNeasy kit (Qiagen, Valencia, CA) following manufacturer

1435 instructions. For studying genome-wide DNA methylation profiles, 1 µg of genomic DNA was

1436 treated with sodium bisulfite (Zy instructions. For studying genome-wide DNA methylation profiles, 1 μg of genomic DNA was
treated with sodium bisulfite (Zymo Research, Irvine, CA). Converted DNA was analyzed using
Infinium Mouse Methylation BeadChip assay treated with sodium bisulfite (Zymo Research, Irvine, CA). Converted DNA was analyzed using

1437 Infinium Mouse Methylation BeadChip assay (Illumina, San Diego, CA). This array includes over

1438 285,000 CpG sites coveri Infinium Mouse Methylation BeadChip assay (Illumina, San Diego, CA). This array includes over

438 285,000 CpG sites covering all RefSeq genes, including CpG islands, translation start sites,

439 enhancers, imprinted loc 285,000 CpG sites covering all RefSeq genes, including CpG islands, translation start sites,

439 enhancers, imprinted loci, and other regions⁵². All data analyses were conducted using the R

440 environment version 4.2. enhancers, imprinted loci, and other regions⁵² enhancers, imprinted loci, and other regions⁵². All data analyses were conducted using the R
environment version 4.2.0. Microarray data was processed using the *ENmix* version.1.34.02⁵³
and *minfi* v.1.44.0⁵⁴ packag environment version 4.2.0. Microarray data was processed using the *ENmix* version.1.34.02⁵³ 440
441
442
443
444 and *minfi* v.1.44.0⁵⁴ and *minfi* v.1.44.0⁵⁴ packages. Quantile normalization of U or M intensities for Infinium I or II
probes were performed, respectively. A model-based correction was performed, and a dye-bias
correction was conducted usi probes were performed, respectively. A model-based correction was performed, and a dye-bias
correction was conducted using *RELIC*⁵⁵. β -values representing the averaged total intensity
value per CG position was calcu correction was conducted using *RELIC* ⁵⁵. β-values representing the averaged total intensity
value per CG position was calculated as unmethylated intensity (U) + methylated intensity (M)
[M / (U + M + 100)]. Probes wi value per CG position was calculated as unmethylated intensity (U) + methylated intensity (M)

445 [M / (U + M + 100)]. Probes with a detection $p < 1 \times 10^{-6}$ and less than 3 beads were defined as

446 low quality. Sample [M / (U + M + 100)]. Probes with a detection $p < 1 \times 10^{-6}$ and less than 3 beads were defined as $[M / (U + M + 100)]$. Probes with a detection $p < 1 \times 10^{-6}$ and less than 3 beads were defined as
 1445 low quality. Samples with low quality methylation measurements > 5% or low intensity bisulfite
 1447 conversion probes Fold Changes in the logarithmic scale (log EQ) and the significance of the difference as a EDP-
449 **COMRs**) between the experimental groups were determined using the *ENmix* version.1.34.02⁵³ package. For each position conversion probes were removed from further analysis. Differentially methylated regions

448 (DMRs) between the experimental groups were determined using the *ENmix* version.1.34.02⁵³

449 package. For each position, the (DMRs) between the experimental groups were determined using the *ENmix* version.1.34.02⁵³ 448
449
450
451 package. For each position, the magnitude of the DNA methylation difference was expressed as
450 Fold Changes in the logarithmic scale (logFC) and the significance of the difference as a FDR-
451 corrected p value (q value

453 Single nuclei were isolated from mouse hippocampal tissue samples as follows. Briefly, Nuclei **Isolation of hippocampal nuclei**
453 Single nuclei were isolated from mouse hippocampal tissue samples as follows. Briefly, Nuclei
454 Lysis Buffer was prepared by adding 12 mL of Nuclei EZ Prep Lysis Buffer (Sigma-Aldric **Isolation of hippocampal nuclei**

453 Single nuclei were isolated from n

454 Lysis Buffer was prepared by add

455 Louis, MO, USA) to a 15mL tube a

456 MO USA) and allowing tablet to d Single nuclei were isolated from mouse hippocampal tissue samples as follows. Briefly, Nuclei
454 Lysis Buffer was prepared by adding 12 mL of Nuclei EZ Prep Lysis Buffer (Sigma-Aldrich, St.
455 Louis, MO, USA) to a 15mL t Lysis Buffer was prepared by adding 12 mL of Nuclei EZ Prep Lysis Buffer (Sigma-Aldrich, St.

455 Louis, MO, USA) to a 15mL tube and adding 1 cOmplete Ultra tablet (Sigma-Aldrich, St. Louis,

456 MO, USA) and allowing tabl Louis, MO, USA) to a 15mL tube and adding 1 cOmplete Ultra tablet (Sigma-Aldrich, St. Louis,
456 MO, USA) and allowing tablet to dissolve. Two 4-mL aliquots of the Nuclei EZ Prep Lysis Buffer
457 + cOmplete Ultra tablets w MO, USA) and allowing tablet to dissolve. Two 4-mL aliquots of the Nuclei EZ Prep Lysis Buffer
457 + cOmplete Ultra tablets were then placed in 15 mL tubes. Twenty (20) µL of Protector RNase
458 inhibitor (Sigma-Aldrich, S + cOmplete Ultra tablets were then placed in 15 mL tubes. Twenty (20) μL of Protector RNase

inhibitor (Sigma-Aldrich, St. Louis, MO, USA) and 20 uL of Superase-In (MilliporeSigma,

Burlington, MA, USA) were added to one inhibitor (Sigma-Aldrich, St. Louis, MO, USA) and 20 uL of Superase-In (MilliporeSigma,
459 Burlington, MA, USA) were added to one 4 mL aliquot to make Nuclei Lysis Buffer 1 (NLB1).
460 Four (4) µL of Protector RNase inhib Burlington, MA, USA) were added to one 4 mL aliquot to make Nuclei Lysis Buffer 1 (NLB1).

460 Four (4) µL of Protector RNase inhibitor (Sigma-Aldrich, St. Louis, MO, USA) and 4 µL of

461 Superase-In (MilliporeSigma, Burl Four (4) µL of Protector RNase inhibitor (Sigma-Aldrich, St. Louis, MO, USA) and 4 µL of
461 Superase-In (MilliporeSigma, Burlington, MA, USA) were added to the second -mL aliquot to
462 make Nuclei Lysis Buffer 2 (NLB2). make Nuclei Lysis Buffer 2 (NLB2). Suspension Buffer (SB) was prepared by adding 1 mL of
fetal bovine serum (Sigma-Aldrich, St. Lous, MO, USA) to 9 mL of 1x phosphate-buffered saline
with 4 µL of Protector RNase inhibitor fetal bovine serum (Sigma-Aldrich, St. Lous, MO, USA) to 9 mL of $1 \times$ phosphate-buffered saline with 4 μ L of Protector RNase inhibitor (Sigma-Aldrich, St. Louis, MO, USA). Eight (8) with 4 µL of Protector RNase inhibitor (Sigma-Aldrich, St. Louis, MO, USA). Eight (8)
465 hippocampi halves from eight individual mice were pooled and homogenized to a single cell
465 hippocampi halves from eight individua 465 hippocampi halves from eight individual mice were pooled and homogenized to a single cell

suspension in a 2 mL dounce homogenizer with 2 mL of NBL1. The sample was then strained
through a 200 µm strainer (pluriSelect Life Science, El Cajon, CA, USA) and the strained cell
suspension returned to the 2 mL dounce a through a 200 µm strainer (pluriSelect Life Science, El Cajon, CA, USA) and the strained cell
468 suspension returned to the 2 mL dounce and homogenized to a nuclei suspension. The nuclei
469 were strained through a 40 µm suspension returned to the 2 mL dounce and homogenized to a nuclei suspension. The nuclei

469 were strained through a 40 µm strainer (pluriSelect Life Science, El Cajon, CA, USA) and

470 centrifuged at 500 RCF at 4°C for were strained through a 40 µm strainer (pluriSelect Life Science, El Cajon, CA, USA) and

470 centrifuged at 500 RCF at 4°C for 5 minutes. Supernatant was removed, and pellet was

471 resuspended with NLB2 and incubated at centrifuged at 500 RCF at 4°C for 5 minutes. Supernatant was removed, and pellet was
471 resuspended with NLB2 and incubated at 4°C for 5 minutes. Nuclei were then centrifuged at
472 500 RCF at 4°C for 5 minutes, supernata 471 resuspended with NLB2 and incubated at 4°C for 5 minutes. Nuclei were then centrifuged at
472 500 RCF at 4°C for 5 minutes, supernatant was removed, and nuclei were resuspended in
474 10x Genomics single cell 3' RNA-Se 500 RCF at 4°C for 5 minutes, supernatant was removed, and nuclei were resuspended in
473 suspension buffer.
474 **10x Genomics single cell 3' RNA-Seq library preparation**
475 Libraries were constructed by following the man

473 suspension buffer.
474
475 **10x Genomics sin**
476 Libraries were con 474
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476
477
478 10x Genomics single cell 3' RNA-Seq library preparation

475 Libraries were constructed by following the manufacturer's protocol with reagents supplied in

477 10x Genomics Chromium Next GEM Single Cell 3' Kit v3.1. Briefl Libraries were constructed by following the manufacturer's protocol with reagents supplied in

477 10x Genomics Chromium Next GEM Single Cell 3' Kit v3.1. Briefly, nuclei suspension

478 concentration was measured with an 10x Genomics Chromium Next GEM Single Cell 3' Kit v3.1. Briefly, nuclei suspension
178 concentration was measured with an Invitrogen Countess II automated cell counter. Nuclei
179 suspension (1,200 nuclei per microliter), concentration was measured with an Invitrogen Countess II automated cell counter. Nuclei
479 suspension (1,200 nuclei per microliter), reverse transcription master mix, and partitioning oil
480 were loaded on a Chromium Ne suspension (1,200 nuclei per microliter), reverse transcription master mix, and partitioning oil
480 were loaded on a Chromium Next GEM G chip with a capture target of 8,000 nuclei per library.
481 Post-Chromium controller were loaded on a Chromium Next GEM G chip with a capture target of 8,000 nuclei per library.

481 Post-Chromium controller GEMs were transferred to a PCR strip tube and reverse transcription

482 performed on an Applied Bi Post-Chromium controller GEMs were transferred to a PCR strip tube and reverse transcription
performed on an Applied Biosystems Veriti thermal cycler at 53°C for 45 minutes. cDNA was
amplified for 13 cycles and purified us performed on an Applied Biosystems Veriti thermal cycler at 53°C for 45 minutes. cDNA was
483 amplified for 13 cycles and purified using Axygen AxyPrep MagPCR Clean-up beads.
484 Fragmentation of cDNA, end-repair, A-tailin amplified for 13 cycles and purified using Axygen AxyPrep MagPCR Clean-up beads.
484 Fragmentation of cDNA, end-repair, A-tailing and ligation of sequencing adaptors was
485 performed according to manufacturer specificatio Fragmentation of cDNA, end-repair, A-tailing and ligation of sequencing adaptors was

485 performed according to manufacturer specifications. The final library was quantified with the

486 Qubit HS DNA kit and the fragment Qubit HS DNA kit and the fragment size determined using an Agilent Fragment Analyzer
187 system. Libraries were pooled and sequenced on an Illumina NovaSeq 6000 to generate
188 50,000 reads per nuclei. 486 Qubit HS DNA kit and the fragment size determined using an Agilent Fragment Analyzer
487 system. Libraries were pooled and sequenced on an Illumina NovaSeq 6000 to generate
488 50,000 reads per nuclei.
489 Single cell 487 system. Libraries were pooled and sequenced on an Illumina NovaSeq 6000 to generate
488 50,000 reads per nuclei.
490 **Single cell bioinformatics**
491 FASTO files were generated from the raw base call outputs using Cell

490 Single cell bioinformatics
491 FASTQ files were generated from the raw base call outputs using Cell Ranger (10x Genomics) 492
493 **Single cell bioinformatics**
491 FASTQ files were generated
492 pipeline, *mkfastq* v3.0. Us
493 sample was obtained using
494 kent genes that were detect FASTQ files were generated from the raw base call outputs using Cell Ranger (10x Genomics)

pipeline, *mkfastq* v3.0. Using default parameters, a UMI (gene-barcode) count matrix per

sample was obtained using the built-in pipeline, *mkfastq* v3.0. Using default parameters, a UMI (gene-barcode) count matrix per
493 sample was obtained using the built-in Cell Ranger count pipeline. To reduce noise, we only
494 kept genes that were detected in sample was obtained using the built-in Cell Ranger count pipeline. To reduce noise, we only

494 kept genes that were detected in at least three barcodes, and subsequently removed ribosomal-

495 encoded genes from the cou kept genes that were detected in at least three barcodes, and subsequently removed ribosomal-

495 encoded genes from the count matrix. Scrublet⁵⁶ was then used to identify potential multiplet-

497 analyses. The files w encoded genes from the count matrix. Scrublet⁵⁶ encoded genes from the count matrix. Scrublet⁵⁶ was then used to identify potential multiplet-
barcodes and only those with a doublet score of less than 0.15 were used for downstream
analyses. The files were then combin barcodes and only those with a doublet score of less than 0.15 were used for downstream
analyses. The files were then combined in a single embedding for the control and CF groups
separately, following the Seurat v3 integra analyses. The files were then combined in a single embedding for the control and CF groups
498 separately, following the Seurat v3 integration workflow⁵⁷. SCTransform was used to normalize
499 each sample, followed by th separately, following the Seurat v3 integration workflow⁵⁷. SCT ransform was used to normalize separately, following the Seurat v3 integration workflow^o'. SCTransform was used to normalize

499 each sample, followed by the identification of integration anchors and variable features using

499 each sample, followed 499 each sample, followed by the identification of integration anchors and variable features using

199 each sample, followed by the identification of integration anchors and variable features using

199 each sample, follo

the Seurat workflow. Dimension reduction was performed scaled data after 4000 highly variable
501 genes across the samples were identified (SelectIntegrationFeatures function). The
502 IntegrateData function was then used 501 genes across the samples were identified (*SelectIntegrationFeatures* function). The
502 *IntegrateData* function was then used to obtain a combined and centered matrix, where the top
503 30 components were used to car IntegrateData function was then used to obtain a combined and centered matrix, where the top
503 30 components were used to carry out ordination analyses. These components were used to
504 build a SNN (shared nearest neigh 30 components were used to carry out ordination analyses. These components were used to
504 build a SNN (shared nearest neighbour) graph which was subsequently clustered using the
505 Louvain algorithm for speed and comput 505 Louvain algorithm for speed and computational efficiency. The principal components were then
506 mapped into two dimensions using the default uniform manifold approximation and projection
507 (UMAP) algorithm, where th Louvain algorithm for speed and computational efficiency. The principal components were then
506 mapped into two dimensions using the default uniform manifold approximation and projection
507 (UMAP) algorithm, where the n mapped into two dimensions using the default uniform manifold approximation and projection

507 (UMAP) algorithm, where the $n = 30$ neighbours was set, with a minimum distance of 0.4.

508 Finally, the *FindAllMarkers* fu 507 (UMAP) algorithm, where the n = 30 neighbours was set, with a minimum distance of 0.4.
508 Finally, the *FindAllMarkers* function was used to identify marker genes for each cluster. The top
510 marker genes were used Finally, the *FindAllMarkers* function was used to identify marker genes for each cluster. The top
509 marker genes were used manually based on literature searches to assign cell type annotations
510 for each cluster. This marker genes were used manually based on literature searches to assign cell type annotations
510 for each cluster. This was further corroborated by cluster annotations using the Azimuth mouse
511 reference datasets⁵⁷. Th fit for each cluster. This was further corroborated by cluster annotations using the Azimuth mouse
511 reference datasets⁵⁷. The cell types across samples and groups were combined with their
512 pseudo bulk profiles, and reference datasets⁵⁷ 511 reference datasets⁵⁷. The cell types across samples and groups were combined with their
512 pseudo bulk profiles, and the resulting gene-cell type matrix was normalized by estimating
513 transcripts per million and 512 pseudo bulk profiles, and the resulting gene-cell type matrix was normalized by estimating
513 transcripts per million and transformed (log2) for downstream analyses. To obtain statistically
514 enriched differential transcripts per million and transformed (log2) for downstream analyses. To obtain statistically
514 enriched differential gene expression, we used generalized additive regression models, where
515 in the control or CF vari enriched differential gene expression, we used generalized additive regression models, where
515 in the control or CF variables, alongside the GM^{Low} or GM^{High} status were encoded as
516 independent variables. The mode in the control or CF variables, alongside the GM^{Low} or GM^{High} in the control or CF variables, alongside the GM^{Low} or GM^{high} status were encoded as
516 independent variables. The models were analyzed for each cluster independent of the other,
517 where per gene log2 fold-change was 516 independent variables. The models were analyzed for each cluster independent of the other,
517 where per gene log2 fold-change was determined. Significance was identified as those genes
518 with an adjusted p value of where per gene log2 fold-change was determined. Significance was identified as those genes
518 with an adjusted p value of less than 0.05, following Benjamini-Hochberg correction. All figures
519 and statistical analyses and statistical analyses were performed using R $v4.1^{58}$.

with an adjusted p value of less than 0.05, following Benjamini-Hochberg correction. All figures
519 and statistical analyses were performed using R v4.1⁵⁸.
520 Cell-to-cell communication was inferred using log10-transfo and statistical analyses were performed using R v4.1⁵⁸.
520 Cell-to-cell communication was inferred using log1
521 collected from snRNA-seq of the mouse hippocampu
522 identified above were grouped into the following cel Cell-to-cell communication was inferred using log10-transformed gene expression data
521 collected from snRNA-seq of the mouse hippocampus using CellChat^{59, 60}. Cell clusters as
522 identified above were grouped into the collected from snRNA-seq of the mouse hippocampus using CellChat^{59, 60} collected from snRNA-seq of the mouse hippocampus using CellChat^{59, 60}. Cell clusters as
identified above were grouped into the following cell types based on Azimuth classification:
glutamatergic and GABAergic neurons, p 524 oligodendrocytes. Ligand-receptor interactions were inferred using the mouse reference
525 database provided by CellChat (Accessed May 25, 2023). Overexpressed genes and
526 interactions were determined using default s glutamatergic and GABAergic neurons, periendothelial cells, microglia, astrocytes, and
524 oligodendrocytes. Ligand-receptor interactions were inferred using the mouse reference
525 database provided by CellChat (Accessed 524 oligodendrocytes. Ligand-receptor interactions were inferred using the mouse reference
525 database provided by CellChat (Accessed May 25, 2023). Overexpressed genes and
526 interactions were determined using default s database provided by CellChat (Accessed May 25, 2023). Overexpressed genes and
526 interactions were determined using default settings. Cell-cell communication probability was
527 inferred using default settings. Informati 526 interactions were determined using default settings. Cell-cell communication probability was
527 inferred using default settings. Information flow was determined by the summation of
528 communication probability for ea 527 inferred using default settings. Information flow was determined by the summation of
528 communication probability for each pathway.
530 **Statistics**
531 Two-way Analysis of Variance (ANOVA) followed by Tukey's post-be

communication probability for each pathway.
529 **Statistics**
530 **Statistics**
531 Two-way Analysis of Variance (ANOVA) followed by Tukey's *post hoc* analysis was used to test
532 for main effects of GM and sex in OFE, LDT 530
531
532
533 530 **Statistics**
531 Two-way A
532 for main e
533 parameters for main effects of GM and sex in OFE, LDT, and EPM behavior tests for all behavior testing 532 for main effects of GM and sex in OFE, LDT, and EPM behavior tests for all behavior testing
533 parameters. Due to lack of normality, CF LDT parameter distance travelled in the light zone and 533 parameters. Due to lack of normality, CF LDT parameter distance travelled in the light zone and

in the light zone and

CF EPM parameters time spent in open arms and distance travelled in open arms were
535 normalized by square root transformation. Two-way ANOVA followed by Tukey's *post hoc* was
536 used to test for main effects of GM and normalized by square root transformation. Two-way ANOVA followed by Tukey's *post hoc* was
536 used to test for main effects of GM and Time (day) for voluntary running distance data. Two-way
537 ANOVA followed by Tukey's used to test for main effects of GM and Time (day) for voluntary running distance data. Two-way

ANOVA followed by Tukey's *post hoc* was used to test for main effects of GM and sex in the

body weight data. Two-way Permut 537 ANOVA followed by Tukey's *post hoc* was used to test for main effects of GM and sex in the
538 body weight data. Two-way Permutational analysis of variance (PERMANOVA) was used to test
539 group beta-diversity for mai body weight data. Two-way Permutational analysis of variance (PERMANOVA) was used to test
539 group beta-diversity for main effects of GM and sex. Two-way ANOVA followed by *Tukey's post*
540 *hoc was used to test Chao-1 r* group beta-diversity for main effects of GM and sex. Two-way ANOVA followed by *Tukey's post*
540 *hoc was used to test Chao-1 richness for main effects of GM and treatment. Since it was not*
541 possible to include a male *hoc* was used to test Chao-1 richness for main effects of GM and treatment. Since it was not

possible to include a male dam group, the main effect of sex was excluded from Chao-1

analysis. Chao-1 richness was calculated possible to include a male dam group, the main effect of sex was excluded from Chao-1

analysis. Chao-1 richness was calculated using PAST 4.03 software⁶¹. Differences in family-

and genus-level relative abundance betwe analysis. Chao-1 richness was calculated using PAST 4.03 software 61 analysis. Chao-1 richness was calculated using PAST 4.03 software⁶¹. Differences in family-

and genus-level relative abundance between GMs was assessed using Wilcoxon Rank-Sum

tests with a Benjamini-Hochberg correctio and genus-level relative abundance between GMs was assessed using Wilcoxon Rank-Sum
544 tests with a Benjamini-Hochberg correction for multiple comparisons. Due to uniform lack of
545 normality across metabolites, a Mann-W tests with a Benjamini-Hochberg correction for multiple comparisons. Due to uniform lack of
545 normality across metabolites, a Mann-Whitney U test was used to test for differences in
546 metabolite concentrations between normality across metabolites, a Mann-Whitney U test was used to test for differences in
546 metabolite concentrations between GM^{Low} and GM^{High} treatment groups. Spearman's rank
547 correlation was used to test correlat metabolite concentrations between GM^{Low} and GM^{High} correlation was used to test correlations between genus-level abundance and statistically

significant metabolites. Two-way ANOVA followed by Tukey's *post hoc* was used to test for main

effects of GM and sex in the gene correlation was used to test correlations between genus-level abundance and statistically
548 significant metabolites. Two-way ANOVA followed by Tukey's *post hoc* was used to test for main
550 differences in the GM^{Low} a significant metabolites. Two-way ANOVA followed by Tukey's *post hoc* was used to test for main
549 effects of GM and sex in the gene expression data. A student's t-test was used to test for
550 differences in the GM^{Low} Exercise of GM and sex in the gene expression data. A student's t-test was used to test for
550 differences in the GM^{Low} and GM^{High} groups in the *bsh* metagenomic and metatranscriptomic
551 read counts. A two-way ANO differences in the GM^{Low} and GM^{High} read counts. A two-way ANOVA followed by Tukey's *post hoc* was used to test for main effects
552 of GM and sex in the BSH activity data. All univariant data analysis was performed using
553 SigmaPlot 14.0 (Systat Software read counts. A two-way ANOVA followed by Tukey's *post hoc* was used to test for main effects
552 of GM and sex in the BSH activity data. All univariant data analysis was performed using
553 SigmaPlot 14.0 (Systat Software of GM and sex in the BSH activity data. All univariant data analysis was performed using
553 SigmaPlot 14.0 (Systat Software, Inc, San Jose, CA). Shapiro-Wilk test was used to test for
554 normality using SigmaPlot 14.0. T SigmaPlot 14.0 (Systat Software, Inc, San Jose, CA). Shapiro-Wilk test was used to test for
554 normality using SigmaPlot 14.0. Two-way PERMANOVA testing was based on Bray-Curtis
555 dissimilarities using the *adonis2* lib normality using SigmaPlot 14.0. Two-way PERMANOVA testing was based on Bray-Curtis
555 dissimilarities using the *adonis2* library from the *vegan* library⁶².
556
Results dissimilarities using the *adonis2* library from the *vegan* library⁶².
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553 . Results
558 . Taxonomic differences are associated with different le

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557 **Results
558 Taxonor
560 metabol**
561 Composi

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562 Taxonomic differences are associated with different levels of biologically relevant
560 metabolites
561 Compositional differences between GM^{Low} and GM^{High} have been described previously^{36, 37, 63},
562 included Ensine 560 **metabolites**
561 Compositiona
562 included *Ery*
564 Acholoniasm Compositional differences between GM^{Low} and GM^{High} have been described previously^{36, 37, 63} Compositional differences between GM^{Low} and GM^{High} have been described previously^{36, 37, 63},
562 including greater richness in GM^{High} compared to GM^{Low} (**Fig. 1A**). Families enriched in GM^{Low}
563 included *Erys* including greater richness in GM^{High} compared to GM^{Low} (**Fig. 1A**). Families enriched in GM^{Low} 563
564
565
566 563 included *Erysipelatoclostridiaceae*, *Erysipelotrichaceae*, *Sutterellaceae*, *Saccharimonadaceae*,
564 *Acholeplasmataceae*, and unresolved members of orders *Tissierellales* and RF39; while
565 families enriched in 564 *Acholeplasmataceae*, and unresolved members of orders *Tissierellales* and RF39; while
565 families enriched in GM^{High} included *Prevotellaceae*, *Marinifilaceae*, *Clostridiaceae*,
566 *Desulfovibrionaceae, Deferri* families enriched in GM^{High} 565 families enriched in GM^{rign} included *Prevotellaceae*, *Marinifilaceae*, *Clostridiaceae,*
566 *Desulfovibrionaceae, Deferribacteraceae*, and an unresolved family within the order
567 *Rhodospirillales* (**Table S2**). 566 *Desulfovibrionaceae*, *Deferribacteraceae*, and an unresolved family within the order *Rhodospirillales* (**Table S2**). Genera that were enriched in GM^{Low} included *Anaeroplasma,* $\frac{1}{2}$ and $\frac{1}{2}$ and

Lachnoclostridium, and *Oscillospira*; while genera that were enriched in GM^{High} included Lachnoclostridium, and Oscillospira; while genera that were enriched in GM^{rign} included
569 Odoribacter, Alloprevotella, Rikenella, Bilophila, and Desulfovibrio (**Fig. S1**; **Table S3**). To
570 determine whether these phy *Odoribacter, Alloprevotella, Rikenella, Bilophila,* and *Desulfovibrio* (Fig. S1; Table S3). To
570 determine whether these phylogenetic differences were associated with different metabolite
571 profiles, fecal samples we determine whether these phylogenetic differences were associated with different metabolite
571 profiles, fecal samples were collected from both male and female GM^{Low} and GM^{High} mice, and a
572 combination of mass spect profiles, fecal samples were collected from both male and female GM^{Low} and GM^{High} profiles, fecal samples were collected from both male and female GM^{LOW} and GM^{nign} mice, and a
combination of mass spectrometry (MS)-based platforms were used to measure short-chain
fatty acids; branched chain fatty aci combination of mass spectrometry (MS)-based platforms were used to measure short-chain
573 fatty acids; branched chain fatty acids, branched chain keto-acids, and other lipids;
574 unconjugated primary and secondary bile a 573 fatty acids; branched chain fatty acids, branched chain keto-acids, and other lipids;
574 unconjugated primary and secondary bile acids; all proteinogenic amino acids and several
575 nonproteinogenic and acetylated ami unconjugated primary and secondary bile acids; all proteinogenic amino acids and several
575 nonproteinogenic and acetylated amino acids; tryptophan derivatives including kynurenine,
576 serotonin, and several indoles; B v nonproteinogenic and acetylated amino acids; tryptophan derivatives including kynurenine,
576 serotonin, and several indoles; B vitamins; dicarboxylic acids; glucose, fructose, and other
577 compounds within glycolysis; al serotonin, and several indoles; B vitamins; dicarboxylic acids; glucose, fructose, and other

compounds within glycolysis; all ribonucleosides and nitrogenous bases; compounds within the

pentose phosphate pathway; compoun pentose phosphate pathway; compounds within the classic TCA cycle; and other biologically

relevant microbial metabolites. Analysis of fecal metabolites revealed differences in sugar

molecules involved in glycolysis, mul 581 **S4**). Specifically, glucose-6-phosphate, fructose-6-phophate, ribulose-5-phosphate, and β -
582 alanine were enriched in GM^{Low} feces, while cysteine, succinate, lactate, chenodeoxycholic acid For the vant microbial metabolites. Analysis of fecal metabolites revealed differences in sugar

580 molecules involved in glycolysis, multiple amino acids, and primary bile acids (**Fig. 1B**; **Table**

581 **S4**). Specifica molecules involved in glycolysis, multiple amino acids, and primary bile acids (**Fig. 1B**; **Table**

581 **S4**). Specifically, glucose-6-phosphate, fructose-6-phophate, ribulose-5-phosphate, and β-

alanine were enriched in **S4**). Specifically, glucose-6-phosphate, fructose-6-phophate, ribulose-5-phosphate, and β-
alanine were enriched in GM^{Low} feces, while cysteine, succinate, lactate, chenodeoxycholic acid
(CDCA) and deoxycholic acid (DC alanine were enriched in GM $^{\mathsf{Low}}$ sas alanine were enriched in GM^{Low} feces, while cysteine, succinate, lactate, chenodeoxycholic acid
583 (CDCA) and deoxycholic acid (DCA) were enriched in GM^{High} feces. These differences were
584 particularly apparent (CDCA) and deoxycholic acid (DCA) were enriched in GM^{High} feces. These differences were (CDCA) and deoxycholic acid (DCA) were enriched in GM^{Hign} feces. These differences were

584 particularly apparent in the feces of male mice (**Fig. S2A**), while female mice also showed a

585 single secondary bile acid (particularly apparent in the feces of male mice (**Fig. S2A**), while female mice also showed a
585 single secondary bile acid (lithocholic acid, LCA) enriched in the feces of GM^{Low} (**Fig. S2B**).
586 When genus-level taxon single secondary bile acid (lithocholic acid, LCA) enriched in the feces of GM^{Low} single secondary bile acid (lithocholic acid, LCA) enriched in the feces of GM^{LOW} (Fig. S2B).
586 When genus-level taxonomic abundances were compared to each of the significant metabolites,
587 numerous significant corre When genus-level taxonomic abundances were compared to each of the significant metabolites,
587 numerous significant correlations were identified indicating that taxonomic features in the GM
588 were strongly associated wi numerous significant correlations were identified indicating that taxonomic features in the GM
588 were strongly associated with the differentially abundant metabolites (Fig. S3; Table S5).
590 Metabolites were also measur were strongly associated with the differentially abundant metabolites (**Fig. S3; Table S5**).
589 Metabolites were also measured in serum, collected at the same time as fecal samples.
590 Remarkably, the only significant di Metabolites were also measured in serum, collected at the same time as fecal samples.

590 Remarkably, the only significant difference in the serum was a primary bile acid (CDCA) (**Fig.**

591 **1C**; **Table S6**). There were Remarkably, the only significant difference in the serum was a primary bile acid (CDCA) (**Fig.**
591 **1C**; **Table S6**). There were also several bile acids, both primary and secondary, that while not
592 **GM^{High} mice (Fig. 1C**; **Table S6**). There were also several bile acids, both primary and secondary, that while not

reaching statistical significance, had greater levels within the serum of female GM^{Low} and male

GM^{High} mice (Fig. S2Creaching statistical significance, had greater levels within the serum of female GM Low GM^{High} mice (Fig. S2C-D).

reaching statistical significance, had greater levels within the serum of female GM^{LOW} and male
593 GM^{High} mice (**Fig. S2C-D**).
594 To determine whether these differences in bile acids are associated with differences i 593 GM^{High} mice (**Fig. S2C-D**).
594 To determine whether thes
595 cellular transport and rece
596 both ileal and hepatic tissu To determine whether these differences in bile acids are associated with differences in bile acid
cellular transport and receptor signaling, quantitative RT-PCR was performed with mRNA from
both ileal and hepatic tissue. 598 a gene involved in transport of bile acids from the ileal epithelium into vascular circulation,
599 showed greater expression in ileum of GM^{High} mice (Fig. 1E). Gpbar1, a G protein-coupled both ileal and hepatic tissue. Asbt, a gene involved in transporting bile acid from the ileal lumen
597 into the ileal epithelium did not show a difference in gene expression (**Fig. 1D**). However, Ost- β ,
598 a gene inv into the ileal epithelium did not show a difference in gene expression (**Fig. 1D**). However, *Ost-β*,
598 a gene involved in transport of bile acids from the ileal epithelium into vascular circulation,
599 showed greater 598 a gene involved in transport of bile acids from the ileal epithelium into vascular circulation,
599 showed greater expression in ileum of GM^{High} mice (**Fig. 1E**). *Gpbar1*, a G protein-coupled
600 receptor (GPCR) als showed greater expression in ileum of GM^{High} 599 showed greater expression in ileum of GM^{Hign} mice (**Fig. 1E**). *Gpbar1*, a G protein-coupled
600 receptor (GPCR) also known as TGR5, was found to have greater expression in the ileum of
601 GM^{Low} mice (**Fig. 1F**). 600 receptor (GPCR) also known as TGR5, was found to have greater expression in the ileum of GM^{Low} mice (Fig. 1F). *Fxr*, a gene critically involved in regulation of hepatic bile acid synthesis, 601 GM^{Low} mice (**Fig. 1F**). *Fxr*, a gene critically involved in regulation of hepatic bile acid synthesis,
strategies, the regulation of hepatic bile acid synthesis, 602 showed no difference in ileal expression (**Fig. 1G**). Accordingly, *Cyp7a1*, a gene downstream of
603 ileal Fxr signaling that encodes the rate-limiting protein in bile acid synthesis, also showed no
604 difference in fieal Fxr signaling that encodes the rate-limiting protein in bile acid synthesis, also showed no
604 difference in hepatic expression (**Fig. 1H**). Expression of *Slc10a1*, a bile acid transport protein,
605 was higher in difference in hepatic expression (**Fig. 1H**). Expression of *Slc10a1*, a bile acid transport protein,
605 was higher in the liver of GM^{Low} mice (**Fig. 1I**). *S1pr2*, a GPCR activated by bile acids, was also
606 expressed was higher in the liver of GM $^{\mathsf{Low}}$ was higher in the liver of GM^{Low} mice (**Fig. 1I**). *S1pr2*, a GPCR activated by bile acids, was also
606 expressed at a greater level in the liver of GM^{Low} mice (**Fig 1J**). These data indicated that the
607 differentia expressed at a greater level in the liver of GM^{Low} mice (Fig 1J). These data indicated that the expressed at a greater level in the liver of GM^{Low} mice (Fig 1J). These data indicated that the
607 differential abundance of BAs detected in feces and serum of GM^{Low}- and GM^{High}-colonized mice
608 are also associate differential abundance of BAs detected in feces and serum of GM^{Low}- and GM^{High} differential abundance of BAs detected in feces and serum of GM^{Low} - and GM^{High} -colonized mice

608 are also associated with differential ileal and hepatic expression of bile acid receptors and

609 transporters.

610 We

are also associated with differential ileal and hepatic expression of bile acid receptors and

transporters.

We next examined whether bile salt hydrolase (BSH), the bacterial enzyme used to deconjugate

bile acids, levels 609 transporters.
610 We next exar
611 bile acids, le
612 colonized fen 610 We next examined whether bile salt hydrolase (BSH), the bacterial enzyme used to deconjugate
611 bile acids, levels showed differential expression or activity between GM^{Low} and GM^{High}
612 colonized female mice. While bile acids, levels showed differential expression or activity between GM^{Low} - and GM^{High} bile acids, levels showed differential expression or activity between GM^{Low} - and GM^{ring} -
612 colonized female mice. While no difference was detected in total gene read count (**Fig. S2E**),
613 microbial expression o colonized female mice. While no difference was detected in total gene read count (**Fig. S2E**),
613 microbial expression of *bsh* was significantly higher in GM^{Low} (**Fig. S2F)**. The BSH family of
614 enzymes is widely exp microbial expression of *bsh* was significantly higher in GM^{Low} microbial expression of *bsh* was significantly higher in GM^{Low} (Fig. S2F). The BSH family of
614 enzymes is widely expressed among gut bacteria, and prone to significant variations in structure
615 with some isoforms ex enzymes is widely expressed among gut bacteria, and prone to significant variations in structure
615 with some isoforms exhibiting different deconjugation activity toward various bile acids and
616 possessing various level with some isoforms exhibiting different deconjugation activity toward various bile acids and
616 possessing various levels of enzymatic activities^{64, 65}. Using a panel of highly sensitive
617 bioluminescent assays⁴⁸, possessing various levels of enzymatic activities^{64, 65} possessing various levels of enzymatic activities^{64, 65}. Using a panel of highly sensitive
617 bioluminescent assays⁴⁸, we compared BSH enzymatic activities of both microbiomes toward
618 various bile acids. Our resul bioluminescent assays⁴⁸, we compared BSH enzymatic activities of both microbiomes toward
618 various bile acids. Our results demonstrated significantly greater BSH activity specific for cholic
619 acid in GM^{Low} (**Fig.** various bile acids. Our results demonstrated significantly greater BSH activity specific for cholic
619 acid in GM^{Low} (**Fig. S2G**), but no other differences were detected in BSH activities towards other
620 bile acids e acid in GM^{Low} (Fig. S2G), but no other differences were detected in BSH activities towards other acid in GM^{Low} (**Fig. S2G**), but no other differences were detected in BSH activities towards other
620 bile acids examined (**Fig. S2H-K**). Collectively, the differences in bacterial *bsh* expression and
621 fiecal and se 620 bile acids examined (**Fig. S2H-K**). Collectively, the differences in bacterial *bsh* expression and
621 fiecal and serum bile acid levels suggest greater uptake by GM^{Low} mice and greater fecal loss by
622 GM^{High} mi fecal and serum bile acid levels suggest greater uptake by GM $^{\mathsf{Low}}$ fecal and serum bile acid levels suggest greater uptake by GM^{Low} mice and greater fecal loss by
622 GM^{High} mice. This is supported by greater hepatic expression of bile acid receptors and
624 **Complex microbiome-depend** GM^{High} mice. This is supported by greater hepatic expression of bile acid receptors and
623 transporters.
624 **Complex microbiome-dependent behaviors determined by the parental microbiome**
626 **Dright Work has revealed r**

623 transporters.
624 **Complex mides**
626 Prior work ha 624
625
626
627
628 **Complex microbiome-dependent behaviors determined by the parental microbiome**
626 Prior work has revealed reproducible differences in behavior and growth between GM^{Lov}
627 GM^{High}-colonized mice^{36, 37}. To determine t Prior work has revealed reproducible differences in behavior and growth between GM^{Low} - and Frior work has revealed reproducible differences in behavior and growth between GM^{Low} - and
627 GM^{High} -colonized mice^{36, 37}. To determine the developmental period in which these phenotypic
628 differences are establishe GM^{High}-colonized mice^{36, 37} 627 GM^{High}-colonized mice^{36, 37}. To determine the developmental period in which these phenotypic
628 differences are established, we used an experimental design with four groups, comprising mice
629 born to dams har differences are established, we used an experimental design with four groups, comprising mice

born to dams harboring GM^{Low} or GM^{High} and remaining with their birth dams until weaning

(control), or cross-fostered at born to dams harboring GM^{Low} or GM^{High} 629 born to dams harboring GM^{Low} or GM^{High} and remaining with their birth dams until weaning
630 (control), or cross-fostered at birth to nursing dams harboring the reciprocal microbiome (**Fig.**
2A). These groups are d (control), or cross-fostered at birth to nursing dams harboring the reciprocal microbiome (**Fig.** 2A). These groups are denoted as CF^{Low} and CF^{High}, with the microbiome designation indicating the postnatal offspring co **2A**). These groups are denoted as $\mathsf{CF}^{\mathsf{Low}}$ and $\mathsf{CF}^{\mathsf{High}}$ **2A**). These groups are denoted as CF^{Low} and CF^{High} , with the microbiome designation indicating
the postnatal offspring composition acquired via cross-foster (CF). Comparisons were then
made between the two control group made between the two control groups, and between the two CF groups in behavior tests made between the two control groups, and between the two CF groups in behavior tests
634 associated with anxiety-related behavior and voluntary activity between five and seven weeks of
635 age, and body weight (BW) at thre 634 associated with anxiety-related behavior and voluntary activity between five and seven weeks of
635 age, and body weight (BW) at three and seven weeks of age.
1999. 635 age, and body weight (BW) at three and seven weeks of age.

Aller and seven weeks of age.

In agreement with previous studies³⁷, age-, sex-, and genotype-matched CD-1 mice colonized 636 In agreement with previous studies³⁷, age-, sex-, and genotype-matched CD-1 mice colonized
637 with GM^{Low} spent less time and traveled less distance in the light portion of a light-dark transition
638 (LDT) test (with GM^{Low} spent less time and traveled less distance in the light portion of a light-dark transition with GM^{Low} spent less time and traveled less distance in the light portion of a light-dark transition
638 (LDT) test (**Fig. 2B, Fig. S4A**), and the open arms of an elevated plus maze (EPM) test (**Fig.**
2C, Fig. S4B), (LDT) test (**Fig. 2B, Fig. S4A**), and the open arms of an elevated plus maze (EPM) test (**Fig.**
 2C, Fig. S4B), relative to mice colonized with GM^{High}, indicating differential effects on anxiety-

related behavior of t 2C, Fig. S4B), relative to mice colonized with GM^{High} **2C, Fig. S4B**), relative to mice colonized with GM^{Hign} , indicating differential effects on anxiety-
related behavior of the two microbiomes. No behavioral differences were observed in the open-
field exploration test be For the two microbiomes. No behavioral differences were observed in the open-
641 field exploration test between GM^{Low} and GM^{High} mice (Fig. S4C-D). To assess voluntary
642 physical activity, mice were singly housed with field exploration test between GM^{Low} and GM^{High} field exploration test between GM^{Log} and GM^{Log} mice (**Fig. S4C-D**). To assess voluntary

physical activity, mice were singly housed with bluetooth wireless running wheels for a five-day

acclimation period followe 642 physical activity, mice were singly housed with bluetooth wireless running wheels for a five-day
643 acclimation period followed by a seven-day test period. Both male (**Fig. 2D**) and female (**Fig.**
25. This colonize 643 acclimation period followed by a seven-day test period. Both male (**Fig. 2D**) and female (**Fig. 2E**) mice colonized with GM^{High} ran significantly more than mice with GM^{Low} ($p < 0.0001$, $F = 29.2$, and $p = 0.0002$, **2E**) mice colonized with GM^{High} ran significantly more than mice with GM^{Low} **2E**) mice colonized with GM^{High} ran significantly more than mice with GM^{Low} ($p < 0.0001$, F = 29.2, and $p = 0.0002$, F = 14.4, respectively). Previously reported differences in body weight (BW) at weaning and adultho 645 29.2, and $p = 0.0002$, $F = 14.4$, respectively). Previously reported differences in body weight
646 (BW) at weaning and adulthood were also reproducible in the GM^{Low} and GM^{High} groups³⁷ (**Fig.**
2F, Fig. S4E). (BW) at weaning and adulthood were also reproducible in the GM^{Low} and GM^{High} groups³⁷ 2F, Fig. S4E). Collectively, these data confirmed microbiome-associated differences in anxiety-
related behavior, voluntary physical activity, and body weight, in sex-, age-, and genotype-
matched mice.
Microbial 16S rRNA related behavior, voluntary physical activity, and body weight, in sex-, age-, and genotype-
matched mice.
Kicrobial 16S rRNA amplicon sequencing was used to confirm that CF mice harbored a
microbiome of comparable richnes

650 Microbial 16S rRNA amplicon sequencing was used to confirm that CF mice harbored a
651 microbiome of comparable richness and beta-diversity to that present in surrogate dams, in both
652 directions of CF (Fig. S5A-C). 649 matched mice.
650 Microbial 16S
651 microbiome of c
652 directions of CF Microbial 16S rRNA amplicon sequencing was used to confirm that CF mice harbored a
651 microbiome of comparable richness and beta-diversity to that present in surrogate dams, in both
652 directions of CF (**Fig. S5A-C**). B microbiome of comparable richness and beta-diversity to that present in surrogate dams, in both
652 directions of CF (**Fig. S5A-C**). Behavioral analysis showed the GM-associated differences in the
653 LDT, independent of directions of CF (**Fig. S5A-C**). Behavioral analysis showed the GM-associated differences in the

LDT, independent of sex, were reversed in the comparison between CF^{Low} and CF^{High} mice, with

the CF^{High} mice demonst LDT, independent of sex, were reversed in the comparison between $\mathsf{CF}^{\mathsf{Low}}$ and $\mathsf{CF}^{\mathsf{High}}$ LDT, independent of sex, were reversed in the comparison between CF^{Low} and CF^{High} mice, with
the CF^{High} mice demonstrating behavior suggestive of greater anxiety (Fig. 2G, Fig. S4F).
Similarly, the robust GM-dependent di the CF^{High} the CF^{High} mice demonstrating behavior suggestive of greater anxiety (Fig. 2G, Fig. S4F).

Similarly, the robust GM-dependent differences in the EPM were reversed in the CF^{Low} and

CF^{High} mice (Fig. 2H, Fig. S4G) ind Similarly, the robust GM-dependent differences in the EPM were reversed in the CF^{Low} and Similarly, the robust GM-dependent differences in the EPM were reversed in the CF^{Low} and
656 CF^{High} mice (**Fig. 2H, Fig. S4G**) indicating that the birth dam microbiome has a substantial, if not
657 dominant, influence on $\mathsf{CF}^{\mathsf{High}}$ 656 CF^{High} mice (**Fig. 2H, Fig. S4G**) indicating that the birth dam microbiome has a substantial, if not
657 dominant, influence on neurodevelopmental events contributing to anxiety-related behavior in
658 the offspring. dominant, influence on neurodevelopmental events contributing to anxiety-related behavior in
658 the offspring. While a significant difference of total distance traveled in the OFE test was
659 observed, distance traveled 658 the offspring. While a significant difference of total distance traveled in the OFE test was
659 observed, distance traveled in the center was not found to be significant in the cross-foster
660 groups (Fig. S4H-I). T 659 observed, distance traveled in the center was not found to be significant in the cross-foster
660 groups (Fig. S4H-I). The significant GM-associated differences in voluntary physical activity
661 were absent in male a groups (Fig. S4H-I). The significant GM-associated differences in voluntary physical activity
661 were absent in male and female CF mice (Fig. 2I-J). Comparison of BW revealed no difference
662 at weaning (Fig. S4J) and re were absent in male and female CF mice (Fig. 2I-J). Comparison of BW revealed no difference
662 at weaning (Fig. S4J) and reversal in adulthood (Fig. 4K), such that CF^{High} mice weighed more
663 than age-matched CF^{Low} m at weaning (**Fig. S4J**) and reversal in adulthood (**Fig. 4K**), such that CF^{High} at weaning (**Fig. S4J**) and reversal in adulthood (**Fig. 4K**), such that CF^{trign} mice weighed more
than age-matched CF^{Low} mice. Collectively, these data supported an equivalent or dominant
effect of the birth dam than age-matched CF^{Low} than age-matched CF^{Low} mice. Collectively, these data supported an equivalent or dom
effect of the birth dam GM on offspring behavioral phenotypes and body weight.
665
Fetal programming of gene methylation in hippocampu

667 Reasoning that an influence on offspring behavior by the parental microbiome must have a
668 biological foundation in the CNS, and a mechanism by which cellular function and gene 666
667
668
669 Fetal programming of gene methylation in hippocampus by the parental microbiome
Reasoning that an influence on offspring behavior by the parental microbiome must ha
biological foundation in the CNS, and a mechanism by whic 667 Reasoning that an influence on offspring behavior by the parental microbiome must have a
668 biological foundation in the CNS, and a mechanism by which cellular function and gene
669 expression are established during f 668 biological foundation in the CNS, and a mechanism by which cellular function and gene
669 expression are established during fetal development, we next examined gene expression and
669 669 expression are established during fetal development, we next examined gene expression and

670 its epigenetic regulation in offspring hippocampus, given its central role in anxiety-related
671 behavior⁶⁶⁻⁷¹. To identify effects of the parental microbiome on fetal programming of DNA
672 methylation in the hipp behavior⁶⁶⁻⁷¹ 671 behavior⁶⁶⁻⁷¹. To identify effects of the parental microbiome on fetal programming of DNA
672 methylation in the hippocampus, we performed genome-wide DNA methylation profiling in
673 female mice. Analysis of methyl 672 methylation in the hippocampus, we performed genome-wide DNA methylation profiling in
673 female mice. Analysis of methylation across the entire array, and parallel comparisons between
674 samples from GM^{Low} or GM^H 673 female mice. Analysis of methylation across the entire array, and parallel comparisons between
674 samples from GM^{Low} or GM^{High} mice, and from CF^{Low} or CF^{High} mice, identified only 196
675 differentially methy samples from GM^{Low} or GM^{High} mice, and from CF^{Low} or CF^{High} 574 samples from GM^{Low} or GM^{High} mice, and from CF^{Low} or CF^{High} mice, identified only 196

675 differentially methylated regions (DMRs) with beta values differing by $log_2FC > 1$ (**Table S7**).

676 Remarkably, 176 of 675 differentially methylated regions (DMRs) with beta values differing by $log_2FC > 1$ (**Table S7**).
676 Remarkably, 176 of those 196 sites (89.8%) showed a difference in methylation at the same site
677 in the reciprocal c 676 Remarkably, 176 of those 196 sites (89.8%) showed a difference in methylation at the same site
677 in the reciprocal contrast, such that offspring methylation matched that of their birth dam (**Fig.**
678 **3A, R² = -0** 677 in the reciprocal contrast, such that offspring methylation matched that of their birth dam (**Fig.** 3A, $R^2 = -0.644$, $p = 2 \times 10^{-7}$). DMRs identified in offspring hippocampi were distributed across the genome, occurr $3A, R^2 = -0.644, p = 2 \times 10^{-7}$ the genome, occurring most often early in gene bodies or enhancer/promoter regions and
680 roughly correlating in frequency to chromosomal gene content, with the exception of an
681 apparent enrichment for DMRs on chromos 681 apparent enrichment for DMRs on chromosome 14 (**Fig. 3B,** $p = 0.003$). Differential methylation
682 was detected at five contiguous markers mapped to promoters, enhancers, and exon 1 of the
683 *Pde1c* gene, encoding 680 roughly correlating in frequency to chromosomal gene content, with the exception of an apparent enrichment for DMRs on chromosome 14 (**Fig. 3B,** $p = 0.003$). Differential methylation was detected at five contiguous ma 681 apparent enrichment for DMRs on chromosome 14 (**Fig. 3B,** $p = 0.003$). Differential methylation
682 was detected at five contiguous markers mapped to promoters, enhancers, and exon 1 of the
683 *Pde1c* gene, encoding was detected at five contiguous markers mapped to promoters, enhancers, and exon 1 of the
683 *Pde1c* gene, encoding phosphodiesterase 1c, a regulator of Ca²⁺ and cGMP-dependent
684 intracellular signaling. This methyla Pde1c gene, encoding phosphodiesterase 1c, a regulator of Ca²⁺ For *Pde1c* gene, encoding phosphodiesterase 1c, a regulator of Ca²⁺ and cGMP-dependent
684 intracellular signaling. This methylation pattern differed between hippocampus from GM^{Low} and
685 GM^{High} mice, and was cons intracellular signaling. This methylation pattern differed between hippocampus from GM^{Low} intracellular signaling. This methylation pattern differed between hippocampus from GM^{Low} and
685 GM^{High} mice, and was conserved between birth dam and offspring, regardless of postnatal
686 colonization (**Fig. 3C**). Simi GM^{High} 685 GM^{High} mice, and was conserved between birth dam and offspring, regardless of postnatal colonization (**Fig. 3C**). Similarly, microbiome-associated differences in methylation were identified at multiple closely space identified at multiple closely spaced markers on both strands of chromosome 14, including
688 markers associated with the genes *Ang5* and *Ang6* (**Fig. 3D**), encoding members 5 and 6,
689 respectively, of the angiogenin, markers associated with the genes *Ang5* and *Ang6* (Fig. 3D), encoding members 5 and 6,
689 respectively, of the angiogenin, ribonuclease A family.
690 To identify shared pathways or commonalities among the functional pro

690 To identify shared pathways or commonalities among the functional products (i.e., proteins)
691 encoded by protein-coding genes among the DMRs, a STRING analysis was performed using a
692 final input of 144 gene names 689 respectively, of the angiogenin, ribonuclease A family.
690 To identify shared pathways or commonalities amore
691 encoded by protein-coding genes among the DMRs, a
692 final input of 144 gene names⁷². Interaction a Fo identify shared pathways or commonalities among the functional products (i.e., proteins)

encoded by protein-coding genes among the DMRs, a STRING analysis was performed using a

final input of 144 gene names⁷². Inte 691 encoded by protein-coding genes among the DMRs, a STRING analysis was performed using a
692 final input of 144 gene names⁷². Interaction analysis resulted in assembly of one large network
693 with 32 nodes, a smalle final input of 144 gene names⁷² with 32 nodes, a smaller network with six nodes, and four dyads (Fig. 3E). While enrichment
analysis failed to detect greater network connectivity than would occur at random, stratified
analysis of the 32 nodes in the lar with 32 nodes, a smaller network with six nodes, and four dyads (**Fig. 3E**). While enrichment

analysis failed to detect greater network connectivity than would occur at random, stratified

analysis of the 32 nodes in the analysis failed to detect greater network connectivity than would occur at random, stratified

695 analysis of the 32 nodes in the large network revealed 10 significantly enriched Gene Ontology

696 (GO) Biological Proces analysis of the 32 nodes in the large network revealed 10 significantly enriched Gene Ontology
696 (GO) Biological Processes; five GO Molecular Functions including TGF- β receptor binding
697 (GO:0005160, strength 1.94, 696 (GO) Biological Processes; five GO Molecular Functions including TGF-β receptor binding
697 (GO:0005160, strength 1.94, FDR-adjusted $p = 0.015$) and GTPase activity (GO:0003924,
698 trength = 1.09, FDR-adjusted $p = 0$ 697 (GO:0005160, strength 1.94, FDR-adjusted $p = 0.015$) and GTPase activity (GO:0003924, strength = 1.09, FDR-adjusted $p = 0.042$); five KEGG pathways including Gap junction (mmu04540, strength = 1.38, FDR-adjusted $p = 0$ 698 strength = 1.09, FDR-adjusted $p = 0.042$); five KEGG pathways including Gap junction
699 (mmu04540, strength = 1.38, FDR-adjusted $p = 0.033$) and TGF- β signaling pathway
700 (mmu04350, strength = 1.32, FDR-adjusted 699 (mmu04540, strength = 1.38, FDR-adjusted $p = 0.033$) and TGF-β signaling pathway
700 (mmu04350, strength = 1.32, FDR-adjusted $p = 0.033$); and four Reactome pathways including
701 axon guidance (MMU-422475, strength 700 (mmu04350, strength = 1.32, FDR-adjusted $p = 0.033$); and four Reactome pathways including
701 axon guidance (MMU-422475, strength = 1.08, FDR-adjusted $p = 0.048$). Network STRING
702 analysis results provided in **Tab** 701 axon guidance (MMU-422475, strength = 1.08, FDR-adjusted *p* = 0.048). Network STRING
702 analysis results provided in **Table S8**.
703 702 analysis results provided in **Table S8**.

Fetal programming of gene expression in hippocampus by parental microbiome

705 We next performed single nuclei RNAseq on hippocampus collected from control mice and

706 cross-fostered mice. Based on expression of cell-sp We next performed single nuclei RNAseq on hippocampus collected from control mice and

706 cross-fostered mice. Based on expression of cell-specific markers, 11 cell clusters were

707 identified in the control mice (**Fig.** cross-fostered mice. Based on expression of cell-specific markers, 11 cell clusters were

107 identified in the control mice (**Fig. S6A-B**) and 24 cell clusters were identified in the cross-

108 fostered mice (**Fig. S6C**identified in the control mice (**Fig. S6A-B**) and 24 cell clusters were identified in the cross-

fostered mice (**Fig. S6C-D**). Gene expression in GM^{Low} and GM^{High} control mice was compared

using generalized additive fostered mice (**Fig. S6C-D**). Gene expression in GM^{Low} and GM^{High} fostered mice (**Fig. S6C-D**). Gene expression in GM^{Low} and GM^{high} control mice was compared

709 using generalized additive regression models, and differentially expressed genes (DEGs) within

710 each treatment group we using generalized additive regression models, and differentially expressed genes (DEGs) within

rach treatment group were identified (**Table S9**). Similarly, mice from CF^{Low} and CF^{High} were

raxamined for DEGs within each treatment group were identified (Table S9). Similarly, mice from CF^{Low} and CF^{High} each treatment group were identified (**Table S9**). Similarly, mice from CF^{Low} and CF^{High} were

711 examined for DEGs within each cell cluster (**Table S10**). After DEGs from each cell cluster had

712 been determined usin For Deen determined using a cutoff magnitude difference of $Log_2FC > 1.5$, STRING analysis was

113 used to determine the protein-protein interactions of the DEGs from each treatment⁷². The

114 number of DEGs from each ce been determined using a cutoff magnitude difference of $Log_2FC > 1.5$, STRING analysis was

713 used to determine the protein-protein interactions of the DEGs from each treatment⁷². The

714 number of DEGs from each cell c used to determine the protein-protein interactions of the DEGs from each treatment⁷² number of DEGs from each cell cluster were compared to the mean node degree received from

the STRING analysis results to determine which cell clusters contained a high number of DEGs

that were most likely to interact wit 714 number of DEGs from each cell cluster were compared to the mean node degree received from
715 the STRING analysis results to determine which cell clusters contained a high number of DEGs
716 that were most likely to in the STRING analysis results to determine which cell clusters contained a high number of DEGs

T16 that were most likely to interact within protein pathways. Interestingly the cell cluster with the

T17 highest number of DE that were most likely to interact within protein pathways. Interestingly the cell cluster with the

717 highest number of DEGs, as well as the highest number of mean node degree of protein-protein

718 interactions, in bot interactions, in both control and cross-foster mice was identified as hippocampal endothelial

719 cells (Fig. S7A-B). STRING analysis was used to generate interaction networks using DEGs

720 identified in the endothelial cells (Fig. S7A-B). STRING analysis was used to generate interaction networks using DEGs
identified in the endothelial cells of control mice as well as the cross-fostered mice (Fig. 4A-B).
Of these interactions, six DEGs (identified in the endothelial cells of control mice as well as the cross-fostered mice (Fig. 4A-B).

721 Of these interactions, six DEGs (*Fas, Fzd6, Gja1, Ntng1, Pik3r3, Sox17*) showed a pattern of

722 fetal programming. 721 Of these interactions, six DEGs (*Fas, Fzd6, Gja1, Ntng1, Pik3r3, Sox17*) showed a pattern of
1722 fetal programming. Of note, numerous DEGs (including *Pde1c, Dock1, and Pdzrn3*) identified
1723 were also found to be 721 Of these interactions, six DEGs (*Fas, Fzd6, Gja1, Ntng1, Pik3r3, Sox17*) showed a pattern of
722 fetal programming. Of note, numerous DEGs (including *Pde1c, Dock1, and Pdzrn3*) identified
723 were also found to be di fetal programming. Of note, numerous DEGs (including *Pde1c, Dock1, and Pdzrn3*) identified

123 were also found to be differentially methylated or closely related to differentially methylated

124 genes. When L5 IT glutam were also found to be differentially methylated or closely related to differentially methylated

724 genes. When L5 IT glutamatergic neurons and astrocytes were examined using interaction

725 networks, they also containe genes. When L5 IT glutamatergic neurons and astrocytes were examined using interaction

T25 networks, they also contained multiple DEGs that showed a pattern of fetal programming as well

T26 as DEGs that were identified The metworks, they also contained multiple DEGs that showed a pattern of fetal programming as well

T26 as DEGs that were identified as differentially methylated (**Fig. S8A-B; Fig. S9A-B**). When we

T27 examined the Log₂ 276 as DEGs that were identified as differentially methylated (**Fig. S8A-B; Fig. S9A-B**). When we
277 examined the Log₂FC of *Dock1* expression of CF^{Low} and CF^{High} , *Dock1* was shown to be
278 upregulated in the cluste examined the Log₂FC of *Dock1* expression of CF^{Low} and CF^{High} examined the Log₂FC of *Dock1* expression of CF^{Low} and CF^{High} , *Dock1* was shown to be upregulated in the clusters of endothelial cells, oligodendrocyte, microglial cells, and subsets of astrocytes and glutamatergic ne quare upregulated in the clusters of endothelial cells, oligodendrocyte, microglial cells, and subsets of

729 astrocytes and glutamatergic neurons of CF^{High} mice compared with CF^{Low} (**Fig. 4C**). Similarly,

730 when we astrocytes and glutamatergic neurons of CF^{High} mice compared with CF^{Low} astrocytes and glutamatergic neurons of CF^{rign} mice compared with CF^{Low} (Fig. 4C). Similarly,
730 when we examined expression of *Pde1c*, it too was found to be increased in the endothelial cell
731 cluster of CF^{High} when we examined expression of *Pde1c*, it too was found to be increased in the endothelial cell
cluster of CF^{High} mice, though not consistent with the expression patterns of other cells seen with
Dock1 (**Fig. 4D**). We cluster of CF^{High} mice, though not consistent with the expression patterns of other cells seen with cluster of CF^{High} mice, though not consistent with the expression patterns of other cells seen with
732 *Dock1* (**Fig. 4D**). We next used CellChat software⁵⁹ to impute cell-cell communication via
733 patterns of coord Dock1 (Fig. 4D). We next used CellChat software⁵⁹ Dock1 (Fig. 4D). We next used CellChat software⁵⁹ to impute cell-cell communication via

patterns of coordinated ligand-receptor expression in each control and cross-foster group, to

identify cell signaling pathways th identify cell signaling pathways that show a pattern of fetal programming. There were 42 cell-cell
communication pathways that were shared among the control mice and the cross-foster mice
(Fig. 4E). Of the 42 pathways iden identify cell signaling pathways that show a pattern of fetal programming. There were 42 cell-cell

communication pathways that were shared among the control mice and the cross-foster mice

(Fig. 4E). Of the 42 pathways id communication pathways that were shared among the control mice and the cross-foster mice
736 (Fig. 4E). Of the 42 pathways identified, 15 showed a pattern of fetal programming including
737 VEGF, IGF, IL2, TGFβ, WNT, and 736 (Fig. 4E). Of the 42 pathways identified, 15 showed a pattern of fetal programming including
737 VEGF, IGF, IL2, TGFβ, WNT, and NPY (Fig. 4F-G). Interestingly, three cell-cell communication
737 VEGF, IGF, IL2, TGFβ, W 737 VEGF, IGF, IL2, TGFβ, WNT, and NPY (**Fig. 4F-G**). Interestingly, three cell-cell communication

pathways that were identified in only GM^{Low} and CF^{High} were appetite-stimulating orexigenic
739 neuropeptide pathways.
740 **Discussion** neuropeptide pathways.
140
741 **Discussion**
142 Studies comparing germ-free and SPF mice demonstrate that the parental microbiome can

742
743 741 **Discussion**
742 Studies com
743 affect offspri
744 obesity⁷⁴, an The Studies comparing germ-free and SPF mice demonstrate that the parental microbiome can

The affect offspring phenotypes associated with neurodevelopment⁷³, metabolic diseases including

The obesity⁷⁴, and organogen affect offspring phenotypes associated with neurodevelopment⁷³ The current data demonstrate that differences among native

743 affect offspring phenotypes associated with neurodevelopment⁷³, metabolic diseases including

744 obesity⁷⁴, and organogenesis in the CNS and intestines obesity⁷⁴, and organogenesis in the CNS and intestines⁷⁵ besity⁷⁴, and organogenesis in the CNS and intestines⁷⁵. While challenging to study in human

cohorts, recent retrospective analyses suggest a dominant influence of the maternal microbiome

on offspring phenotypes rel cohorts, recent retrospective analyses suggest a dominant influence of the maternal microbiome
on offspring phenotypes related to asthma⁷⁶, neurodevelopment⁷⁷, and metabolic diseases
including obesity and diabetes⁷⁸ on offspring phenotypes related to asthma⁷⁶, neurodevelopment⁷⁷, and metabolic diseases on offspring phenotypes related to asthma⁷⁶, neurodevelopment⁷⁷, and metabolic diseases

including obesity and diabetes⁷⁸. The current data demonstrate that differences among native

parental microbiomes can influen including obesity and diabetes⁷⁸ including obesity and diabetes⁷⁸. The current data demonstrate that differences among native

parental microbiomes can influence neurodevelopment and behavioral outcomes in the

offspring. The current findings and prior parental microbiomes can influence neurodevelopment and behavioral outcomes in the

749 offspring. The current findings and prior studies^{36, 37} show reproducible effects of these native

750 SPF microbiomes on certain p offspring. The current findings and prior studies^{36, 37} show reproducible effects of these native offspring. The current findings and prior studies^{36, 37} show reproducible effects of these native
750 SPF microbiomes on certain phenotypes. GM^{Low} -colonized CD-1 mice are consistently heavier
751 than age- and sex-matc SPF microbiomes on certain phenotypes. GM^{Low} The SPF microbiomes on certain phenotypes. GM^{Low} -colonized CD-1 mice are consistently heavier

751 than age- and sex-matched mice colonized with GM^{High} , and the same effect is observed in

752 inbred C57BL/6J and BTBR T than age- and sex-matched mice colonized with GM^{High} than age- and sex-matched mice colonized with GM^{Hign} , and the same effect is observed in

752 inbred C57BL/6J and BTBR T⁺ ltpr3[#]/J mice⁷⁹. Behavior and BW data from the cross-foster (CF)

753 mice provide strong inbred C57BL/6J and BTBR T $^{\text{\tiny{+}}}$ Itpr3 $^{\text{\tiny{tf}}}\!$ J mice 79 The provide S7BL/6J and BTBR T⁺ Itpr3[#]/J mice⁷⁹. Behavior and BW data from the cross-foster (CF)
The provide strong evidence of a dominant effect of the parental GM on these behavioral
The phenotypes in offspring. W mice provide strong evidence of a dominant effect of the parental GM on these behavioral

754 phenotypes in offspring. While we did not measure food intake in the current study, previous

755 work showed that the heavier G phenotypes in offspring. While we did not measure food intake in the current study, previous

755 work showed that the heavier GM^{Low}-colonized mice consume more food (normalized to BW)

756 than age- and sex-matched GM^H work showed that the heavier GM^{Low} -colonized mice consume more food (normalized to BW) work showed that the heavier GM^{Low} -colonized mice consume more food (normalized to BW)

756 than age- and sex-matched GM^{High} -colonized at all timepoints examined³⁶. The differences in

757 BW between CF and control off than age- and sex-matched GM^{High}-colonized at all timepoints examined³⁶ than age- and sex-matched GM^{High}-colonized at all timepoints examined³⁶. The differences in
757 BW between CF and control offspring would suggest that these feeding behaviors are similarly
758 programmed during fetal, BW between CF and control offspring would suggest that these feeding behaviors are similarly

1758 programmed during fetal, embryonic, or even pre-fertilization events. As such, these findings

1759 raise the possibility o programmed during fetal, embryonic, or even pre-fertilization events. As such, these findings

raise the possibility of a connection between the anxiety-related behaviors and the behaviors

underlying the difference in BW

762 The GM can influence host physiology through microbially derived metabolites in peripheral underlying the difference in BW and voluntary physical activity, representing a constellation of

761 behavioral phenotypes influenced by common features within the parental microbiomes.

762 The GM can influence host phys behavioral phenotypes influenced by common features within the parental microbiomes.

The GM can influence host physiology through microbially derived metabolites in pe

circulation⁷³, interactions with the immune system The GM can influence host physiology through microbially derived metabolites in peripheral

circulation⁷³, interactions with the immune system⁸⁰, and stimulation of the vagus nerve or

enteric nervous system by microb circulation⁷³, interactions with the immune system⁸⁰, and stimulation of the vagus nerve or

764 enteric nervous system by microbially derived neurotransmitters⁸¹ and other molecules. Gut

765 metabolites have been enteric nervous system by microbially derived neurotransmitters⁸¹ enteric nervous system by microbially derived neurotransmitters⁸¹ and other molecules. Gut

T65 metabolites have been implicated as a means by which the parental microbiome can influence

T66 fetal development^{73, 82}. T metabolites have been implicated as a means by which the parental microbiome can influence

fetal development^{73, 82}. The present data provide evidence of a functional difference between

these native SPF microbiomes, inc fetal development^{73, 82} fetal development^{73, 82}. The present data provide evidence of a functional difference between

167 these native SPF microbiomes, including differential abundance of several bile acids. Bile acids

168 stored in the gallb stored in the gallbladder are released into the duodenum following food intake, and the observed differences in fecal bile acids may reflect differences in bsh expression, food intake, or other factors. Regardless, the observed differences in *ileal and hepatic bile acid transporters* 770 other factors. Regardless, the observed differences in ileal and hepatic bile acid transporters

The acid transporters

The observed differences in ileal and hepatic bile acid transporters

The observed differences in 271 and receptors indicate that the differences in bile acid levels are physiologically relevant to the

272 host. The observed difference in expression of $S/c10a1$ (Ntcp) may reflect a mechanism to

273 regulate reabsorp host. The observed difference in expression of *Slc10a1 (Ntcp)* may reflect a mechanism to
regulate reabsorption of bile acids. GM^{Low} mice also demonstrated greater hepatic expression of
S1pr2, a GPCR that when bound t regulate reabsorption of bile acids. GM $^{\mathsf{Low}}$ regulate reabsorption of bile acids. GM^{Low} mice also demonstrated greater hepatic expression of
774 S1pr2, a GPCR that when bound to primary conjugated bile acids is involved in the regulation of
775 hepatic lipid metabo $51pr2$, a GPCR that when bound to primary conjugated bile acids is involved in the regulation of
 775 hepatic lipid metabolism⁸³. There is considerable interest in the role of bile acids in anxiety and
 776 depressi hepatic lipid metabolism⁸³. There is considerable interest in the role of bile acids in anxiety and metabolism⁸³. There is considerable interest in the role of bile acids in anxiety and
depressive disorders^{84, 85}, and causative links have been shown between bile acids, bile acid
receptor signaling, and these outcome depressive disorders^{84, 85} depressive disorders^{84, 85}, and causative links have been shown between bile acids, bile acid

777 receptor signaling, and these outcomes⁸⁶⁻⁸⁹. CDCA, present at greater levels in the serum of

778 GM^{Low}-colonized fem receptor signaling, and these outcomes⁸⁶⁻⁸⁹ receptor signaling, and these outcomes⁸⁶⁻⁸⁹. CDCA, present at greater levels in the serum of
778 \cdot GM^{Low}-colonized females, has been shown to readily cross the blood brain barrier⁹⁰ and
779 influence the expressi GM^{Low} -colonized females, has been shown to readily cross the blood brain barrier 90 GM^{Low} -colonized females, has been shown to readily cross the blood brain barrier⁹⁰ and

179 influence the expression of transcription factors CREB and BDNF through FXR activation, which

179 when down-regulated can lea influence the expression of transcription factors CREB and BDNF through FXR activation, which
780 when down-regulated can lead to decreased neuroplasticity and mood disorders including
781 anxiety⁹¹. The current findings when down-regulated can lead to decreased neuroplasticity and mood disorders including

181 anxiety⁹¹. The current findings suggest that differences in the native microbiome, independent of

182 dietary challenge or host anxiety⁹¹

anxiety⁹¹. The current findings suggest that differences in the native microbiome, independent of
782 dietary challenge or host insult, can have intergenerational effects on these outcomes.
783 Analyses of hippocampal DN dietary challenge or host insult, can have intergenerational effects on these outcomes.

283 Analyses of hippocampal DNA methylation and gene expression were performed to c

284 and compare the effects of the parental or o Analyses of hippocampal DNA methylation and gene expression were performed to document

284 and compare the effects of the parental or offspring GM on those processes, and identify

285 specific genes, pathways, and cell t and compare the effects of the parental or offspring GM on those processes, and identify

785 specific genes, pathways, and cell types associated with the observed phenotypic differences. It

786 is well-established that t specific genes, pathways, and cell types associated with the observed phenotypic differences. It

786 is well-established that the GM can influence the epigenome of the host⁹²⁻⁹⁴. The distribution

787 and relationship is well-established that the GM can influence the epigenome of the host⁹²⁻⁹⁴ is well-established that the GM can influence the epigenome of the host⁹²⁻⁹⁴. The distribution

and relationship of genes affected by differential methylation reflects a semi-stochastic effect

across the genome, with e and relationship of genes affected by differential methylation reflects a semi-stochastic effect across the genome, with enrichment of genes and pathways associated with TGF-β signaling and GTPase activity, both of which were identified in the single-cell transcriptome data as well. Four of the 144 protein-coding genes (<i>Dlx5</i>, <i>Drd1</i>, <i>Zfp64</i>, and <i>BC034090</i>) identified as DMRs here are included among a comprehensive list of 384 genes known to undergo total imprinting⁹⁵ 288 across the genome, with enrichment of genes and pathways associated with TGF-β signaling

289 and GTPase activity, both of which were identified in the single-cell transcriptome data as well.

290 Four of the 144 pro and GTPase activity, both of which were identified in the single-cell transcriptome data as well.

The Four of the 144 protein-coding genes (*Dlx5, Drd1, Zfp64*, and *BC034090*) identified as DMRs

The are included among a This suggests that the GM-associated effects on methylation of those and perhaps other DMRs
This suggests that the GM-associated effects on methylation of those and perhaps other DMRs
This suggests that the GM-associated e here are included among a comprehensive list of 384 genes known to undergo fetal imprinting⁹⁵. here are included among a comprehensive list of 384 genes known to undergo fetal imprinting⁹⁵.

This suggests that the GM-associated effects on methylation of those and perhaps other DMRs

occurred in germline cells pre This suggests that the GM-associated effects on methylation of those and perhaps other DMRs

occurred in germline cells pre-fertilization. Indeed, several recent studies have revealed the

influence of the paternal microbi occurred in germline cells pre-fertilization. Indeed, several recent studies have revealed the

794 influence of the paternal microbiome on germline methylation and offspring outcomes⁹⁶⁻⁹⁸. As all

795 matings in the cur influence of the paternal microbiome on germline methylation and offspring outcomes⁹⁶⁻⁹⁸ matings in the current study were between mice sharing the same microbiome, it is unclear

795 whether the effects of the GM on offspring DNA methylation occurred pre- or post-fertilization

797 and whether the maternal lo matings in the current study were between mice sharing the same microbiome, it is unclear
796 whether the effects of the GM on offspring DNA methylation occurred pre- or post-fertilization
797 and whether the maternal lor whether the effects of the GM on offspring DNA methylation occurred pre- or post-fertilization

297 and whether the maternal lor paternal microbiome had a dominant or selective influence. As

298 even transient co-housing and whether the maternal lor paternal microbiome had a dominant or selective influence. As

reven transient co-housing to breed mice results in sharing of the GM, *in vitro* fertilization or

similar methods would be neede even transient co-housing to breed mice results in sharing of the GM, *in vitro* fertilization or

799 similar methods would be needed to investigate those questions. There was an incredibly high

800 degree of conservatio 5799 similar methods would be needed to investigate those questions. There was an incredibly high

800 degree of conservation across all mice in the degree of methylation at the vast majority of CpG

801 sites included in sites included in the BeadChip array. However, we also observed a high degree of conservation
802 between dams and offspring in the specific DMRs affected by the GM, suggesting the affected
803 loci are not the result of r sites included in the BeadChip array. However, we also observed a high degree of conservation

802 between dams and offspring in the specific DMRs affected by the GM, suggesting the affected

803 loci are not the result of 802 between dams and offspring in the specific DMRs affected by the GM, suggesting the affected
803 loci are not the result of random DNA methyltransferase (DNMT) activity, but rather an outcome
804 with a teleological exp 803 loci are not the result of random DNA methyltransferase (DNMT) activity, but rather an outcome
804 with a teleological explanation. While speculative, the methylation and gene expression profiles 804 with a teleological explanation. While speculative, the methylation and gene expression profiles

following a pattern of fetal programming may represent an intergenerational feedback

1806 mechanism wherein nutrient availability in the parent may program the trafficking of, or receptor

1807 response to, microbial meta mechanism wherein nutrient availability in the parent may program the trafficking of, or receptor
807 response to, microbial metabolites as a way of fine-tuning offspring metabolism.
809 It is worth noting that the number

response to, microbial metabolites as a way of fine-tuning offspring metabolism.
808
It is worth noting that the number and connectivity of DEGs were greatest in enc
810 both control and CF mice. These cells supply blood t 810
811
812 It is worth noting that the number and connectivity of DEGs were greatest in endothelial cells, in

810 both control and CF mice. These cells supply blood to tissues within the CNS and comprise the

811 blood-brain barrier both control and CF mice. These cells supply blood to tissues within the CNS and comprise the
811 blood-brain barrier. The greater size of GM^{Low}-colonized mice would necessitate greater amount
812 of peripheral vasculatu blood-brain barrier. The greater size of GM $^{\mathsf{Low}}$ blood-brain barrier. The greater size of GM^{Low} -colonized mice would necessitate greater amount
812 of peripheral vasculature to adequately perfuse tissues. Indeed, prior work found the total
813 cardiac weight of $GM<$ of peripheral vasculature to adequately perfuse tissues. Indeed, prior work found the total

813 cardiac weight of GM^{Low} -colonized mice to be significantly greater than age- and sex-matched

814 GM^{High} -colonized mice, an cardiac weight of GM^{Low} cardiac weight of GM^{Low} -colonized mice to be significantly greater than age- and sex-matched
814 GM^{High} -colonized mice, and no difference in cardiac weight when normalized to total BW³⁷,
815 indicating differential gro GM^{High}-colonized mice, and no difference in cardiac weight when normalized to total BW³⁷ GM^{righ}-colonized mice, and no difference in cardiac weight when normalized to total BW³⁷,
815 indicating differential growth of the circulatory system commensurate with the difference in BW.
816 Moreover, the same stud indicating differential growth of the circulatory system commensurate with the difference in BW.
816 Moreover, the same study found no difference in body composition based on DEXA scanning,
817 and a significant correlatio Moreover, the same study found no difference in body composition based on DEXA scanning,
817 and a significant correlation between BW and crown-to-rump length, further indicating that the
818 observed phenotypic difference and a significant correlation between BW and crown-to-rump length, further indicating that the
818 observed phenotypic difference is associated with somatic growth rather than adiposity. Several
819 of the pathways identi observed phenotypic difference is associated with somatic growth rather than adiposity. Several
819 of the pathways identified in the CellChat analysis showing patterns of fetal programming
820 represented growth factors i of the pathways identified in the CellChat analysis showing patterns of fetal programming
820 represented growth factors including TGF-β, vascular endothelial growth factor (VEGF) and
821 insulin-like growth factor (IGF).

represented growth factors including TGF-β, vascular endothelial growth factor (VEGF) and
821 insulin-like growth factor (IGF).
822 Two of the DEGs identified in the endothelial cells included *Pde1c* and *Dock1*, two gen 821 insulin-like growth factor (IGF).
822 Two of the DEGs identified in 1
823 were also found to be different
824 family of enzymes involved in 1 Two of the DEGs identified in the endothelial cells included *Pde1c* and *Dock1*, two genes that

were also found to be differentially methylated. PDE1C is a member of the phosphodiesterase

family of enzymes involved in t were also found to be differentially methylated. PDE1C is a member of the phosphodiesterase
824 family of enzymes involved in the production of cyclic guanosine monophosphate (cGMP) and
825 cyclic adenosine monophosphate (family of enzymes involved in the production of cyclic guanosine monophosphate (cGMP) and
825 cyclic adenosine monophosphate (cAMP). The production of cAMP is necessary to maintain the
826 integrity of the blood-brain barr cyclic adenosine monophosphate (cAMP). The production of cAMP is necessary to maintain the
826 integrity of the blood-brain barrier (BBB)⁹⁹, and excessive levels of cGMP are associated with
827 anxiety and depression¹⁰⁰ integrity of the blood-brain barrier (BBB)⁹⁹ integrity of the blood-brain barrier (BBB)⁹⁹, and excessive levels of cGMP are associated with
anxiety and depression¹⁰⁰. DOCK1 is a protein belonging to the dedicator of cytokinesis (DOCK)
family of guanine exchange anxiety and depression¹⁰⁰. DOCK1 is a protein belonging to the dedicator of cytokinesis (DOCK) anxiety and depression¹⁰⁰. DOCK1 is a protein belonging to the dedicator of cytokinesis (DOCK)
828 family of guanine exchange factors (GEFs) involved in activation of G proteins. DOCK1 is
829 involved in neuronal develo Family of guanine exchange factors (GEFs) involved in activation of G proteins. DOCK1 is

829 involved in neuronal development and angiogenesis¹⁰¹. The difference in expression of *Pde1c*

830 and *Dock1* in the endothe involved in neuronal development and angiogenesis¹⁰¹ involved in neuronal development and angiogenesis¹⁰¹. The difference in expression of *Pde1c* and *Dock1* in the endothelial cells may indicate a difference in permeability of the BBB within the hippocampus. It is also w the hippocampus. It is also worth noting that a number of other phosphodiesterase and DOCK
1832 genes were identified as DEGs in endothelial cells, with a largely uniform direction of difference.
1833 This suggests a consi genes were identified as DEGs in endothelial cells, with a largely uniform direction of difference.

This suggests a consistent differential effect of these microbiomes on two major mechanisms of

regulating intracellular 835 across a sizeable range of surface receptors, including GPCRs. GPCRs are among the largest
836 classes of receptors and common drug targets¹⁰², responding to neurotransmitters, hormones, This suggests a consistent differential effect of these microbiomes on two major mechanisms of

834 regulating intracellular signal transduction (i.e., cyclic nucleotide generation and GEF activity)

835 across a sizeable regulating intracellular signal transduction (i.e., cyclic nucleotide generation and GEF activity)
across a sizeable range of surface receptors, including GPCRs. GPCRs are among the largest
classes of receptors and common across a sizeable range of surface receptors, including GPCRs. GPCRs are among the largest
classes of receptors and common drug targets¹⁰², responding to neurotransmitters, hormones,
and a wide range of sensory cues. Wi classes of receptors and common drug targets 102 classes of receptors and common drug targets¹⁰², responding to neurotransmitters, hormones,

837 and a wide range of sensory cues. With widespread and strong expression in the CNS¹⁰³, and a wide range of sensory cues. With widespread and strong expression in the CNS^{103} , and a wide range of sensory cues. With widespread and strong expression in the CNS^{103} ,

GPCRs are also broadly expressed by enteroendocrine cells¹⁰⁴, vagal efferents¹⁰⁵, and other GPCRs are also broadly expressed by enteroendocrine cells¹⁰⁴, vagal efferents¹⁰⁵, and other
839 cells throughout the gut¹⁰⁶.
840 Another gene of interest found to be differentially expressed in astrocytes is the gene cells throughout the qut^{106} .

839 cells throughout the gut¹⁰⁶.
840 **Another gene of interest for brain-derived neurotrop**
842 **anxiety-related behavior in** 840
841
842
843
844 Another gene of interest found to be differentially expressed in astrocytes is the gene encoding

842 for brain-derived neurotrophic factor (BDNF). BDNF over-expression leads to a decrease in

843 anxiety-related behavior for brain-derived neurotrophic factor (BDNF). BDNF over-expression leads to a decrease in
anxiety-related behavior in mice¹⁰⁷. However, we observed relatively greater *Bdnf* expression in
GM^{Low} and CF^{High} mice which anxiety-related behavior in mice¹⁰⁷ anxiety-related behavior in mice^{tor}. However, we observed relatively greater *Bdnf* expression in
844 GM^{Low} and CF^{High} mice which were found to have relatively increased anxiety-related behavior,
845 and these findin $\mathsf{GM}^{\mathsf{Low}}$ and $\mathsf{CF}^{\mathsf{High}}$

and these findings therefore need to be explored further.
846 micrestingly, CellChat analysis detected coordinated expression of genes involved in orexigenic
848 mathways including *Ghrelin, Npr,* and *Qrfp* only in GM^{Low} and these findings therefore need to be explored further.
846 **Interestingly, CellChat analysis detected coordinated exp**
848 **pathways including Ghrelin, Npr, and Qrfp only in GM^{Lov}
849 Propriet BW** These differences i 847
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850 Interestingly, CellChat analysis detected coordinated expression of genes involved in orexigenic

848 pathways including *Ghrelin, Npr,* and *Qrfp* only in GM^{Low} and CF^{High} mice, the two groups with

850 per BW. These pathways including *Ghrelin, Npr*, and *Qrfp* only in GM^{Low} and CF^{High} greater BW. These differences in intercellular signaling may provide an explanation for the
850 observed differences in feed intake. These findings highlight cell type specific differences in
851 hippocampal gene expressio 850 observed differences in feed intake. These findings highlight cell type specific differences in
851 hippocampal gene expression in genes identified within DMRs and genes in pathways
852 associated with growth and feedi hippocampal gene expression in genes identified within DMRs and genes in pathways
852 associated with growth and feeding behavior, giving a possible reason for the increased weight
853 noted in these two groups.
854 We rec associated with growth and feeding behavior, giving a possible reason for the increased weight
noted in these two groups.
We recognize a number of limitations within this study. For example, methylome analysis was
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associated with growth and feeding behavior, giving a possible reason for the increased weight

853 noted in these two groups.

854 We recognize a number of limitations within this study. For example, methylome analysis wa 853 noted in these two groups.
854 We recognize a number of
855 restricted to samples from
856 Similarly, methylome and We recognize a number of limitations within this study. For example, methylome analysis was

strestricted to samples from female offspring and their dams due to resource constraints.

Similarly, methylome and transcriptome restricted to samples from female offspring and their dams due to resource constraints.
856 Similarly, methylome and transcriptome analyses were limited to hippocampal tissue. While
857 these data provide strong proof-of-p Similarly, methylome and transcriptome analyses were limited to hippocampal tissue. While

857 these data provide strong proof-of-principle and demonstrate the utility of the experimental

858 model, additional work is nee 857 these data provide strong proof-of-principle and demonstrate the utility of the experimental
858 model, additional work is needed to determine whether the observed differences in hippocampal
859 methylation and gene ex methylation and gene expression are conserved in other regions of the CNS or even other
tissues. Lastly, the parental generation was represented by the dams in all analyses presented
here. There has been growing evidence f methylation and gene expression are conserved in other regions of the CNS or even other
860 tissues. Lastly, the parental generation was represented by the dams in all analyses presented
861 here. There has been growing ev tissues. Lastly, the parental generation was represented by the dams in all analyses presented

here. There has been growing evidence for paternal programming of offspring through the

epigenome^{96, 98}, and additional stu here. There has been growing evidence for paternal programming of offspring through the

862 epigenome^{96, 98}, and additional studies are needed to determine whether the observed effects

863 are due to pre- or post-ferti epigenome^{96, 98}, and additional studies are needed to determine whether the observed effects

are due to pre- or post-fertilization events..

864
 Conclusion

865 **Conclusion**

are due to pre- or post-fertilization events..

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 Conclusion

In total, the findings presented here demonstrate that features within healthy native GMs exert

867 an intergenerational effect on offspring behavior, gro 865
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868 865 **Conclusion**
866 In total, the
867 an intergene
868 within the co an intergenerational effect on offspring behavior, growth, DNA methylation, and gene expression
868 within the central nervous system, and strongly suggest a relationship between these factors
869 during fetal development. an intergenerational effect on offspring behavior, growth, DNA methylation, and gene expression
868 within the central nervous system, and strongly suggest a relationship between these factors
869 during fetal development. within the central nervous system, and strongly suggest a relationship between these factors
869 during fetal development. Moreover, these findings implicate bile acids as potential mediators of
870 these effects, includin during fetal development. Moreover, these findings implicate bile acids as potential mediators of
870 these effects, including changes in GPCR signal transduction and pathways involved in feeding
871 behavior. 870 these effects, including changes in GPCR signal transduction and pathways involved in feeding
871 behavior.
171 behavior. 871 behavior.

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- 873 **List of abbreviations**
874 AD Anxiety disorders
875 BSH Bile salt hydrola
876 *bsh* Bile salt hydrolas
- 874 AD Anxiety disorders
875 BSH Bile salt hydrolase enzyme
876 bsh Bile salt hydrolase gene
877 CF Cross-fostered offspring
878 CNS Central Nervous System 875 BSH – Bile salt hydrolase enzyme
876 bsh – Bile salt hydrolase gene
877 CF – Cross-fostered offspring
878 CNS – Central Nervous System
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- 876 *bsh* Bile salt hydrolase gene
877 CF Cross-fostered offspring
878 CNS Central Nervous Systen
879 DEGs Differentially expresse 877 CF – Cross-fostered offspring
878 CNS – Central Nervous Syster
879 DEGs – Differentially expresse
880 DMRs – Differentially methylat
881 – EPM – Elovated plus maze tos 878 CNS – Central Nervous System
879 DEGs – Differentially expressed
880 DMRs – Differentially methylated
881 EPM – Elevated plus maze test 879 DEGs – Differentially expressed genes
880 DMRs – Differentially methylated region
881 EPM – Elevated plus maze test
882 GM – Gut microbiome
- 880 DMRs Differentially methylated regions
881 EPM Elevated plus maze test
882 GM Gut microbiome
883 LDT Light/dark transition test
884 OFE Open field expleration test
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- 882 GM Gut microbiome
883 LDT Light/dark transi
884 OFE Open-field exple
885 OO Obesity/Overwei
- 881 EPM Elevated plus maze test
882 GM Gut microbiome
883 LDT Light/dark transition test
884 OFE Open-field exploration test
885 OO Obesity/Overweight 883 LDT – Light/dark transition test
884 OFE – Open-field exploration te
885 OO – Obesity/Overweight
886 snRNA-seq – Single nuclei RN/
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- 884 OFE Open-field exploration test

885 OO Obesity/Overweight

886 snRNA-seq Single nuclei RNA sequencing

887 Declarations 885 OO – Obesity/Overweight
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888 **Declarations**
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889 **Ethics Appro**

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- 889 **Ethics Approval and consent to participate**
890 All activities described here were performed in accordance with the quidelines put forth in the
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- 891 Guide for the Care and Use of Laboratory Animals and were approved by the Institutional
892 Animal Care and Use Committee (IACUC) of the University of Missouri, an AAALAC accre
893 institution. All activities described here were performed in accordance with the guidelines put forth in the
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897 **Availability of data and material**
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898 **Availability of**
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901 All data supporting our analyses and reported conclusions have been deposited in the

900 appropriate data repositories and are publicly available. Metagenomic, metatranscriptomic, 16S

901 RNA amplicon sequencing, and sin 890 appropriate data repositories and are publicly available. Metagenomic, metatranscriptomic, 16S
891 RNA amplicon sequencing, and single nuclei RNAseq data are available at the National Center
802 for Biotechnology and I r FRNA amplicon sequencing, and single nuclei RNAseq data are available at the National Center
902 for Biotechnology and Informatics (NCBI) Sequence Read Archive (SRA) under the BioProject
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903 accession number PRJNA885816. Mouse Methylation BeadChip array data are available at the
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904 Gene Expression Omnibus (GEO) under accession GSE239371.
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962 E.A.G. performed expe
963 analysis, and figure g K.L.G and A.C.E conceived the project and wrote the manuscript. K.L.G., Z.L.M., R.E.M., P.K.,
962 E.A.G. performed experiments and analyzed data. Z.L.M. assisted in microbiome bioinformatic
963 analysis, and figure generat E.A.G. performed experiments and analyzed data. Z.L.M. assisted in microbiome bioinformatic

963 analysis, and figure generation and editing. S.B.B., Z.L.M, M.R, and L.M.C performed the

964 snRNAseq bioinformatic analysis analysis, and figure generation and editing. S.B.B., Z.L.M, M.R, and L.M.C performed the
964 snRNAseq bioinformatic analysis. R.C. assisted in designing and collecting data for the
965 methylome analysis experiment, and pe 964 snRNAseq bioinformatic analysis. R.C. assisted in designing and collecting data for the
965 methylome analysis experiment, and performed the methylome bioinformatic analysis. N.J.B.
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966 assisted in designing and collecting data for the snRNAseq experiment. P.W. contributed
967 resources and assisted with snRNAseq assisted in designing and collecting data for the snRNAseq experiment. P.W. contributed
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989 986 Organization, 2022.

987 2. Freidl EK, Stroeh OM, Elkins RM, et al. Assessment and Treatment of Anxiety Among Children and Adolescents. Focus (Am Psychiatr Publ) 2017;15:144-156.

989 3. Prevention CfDCa. Anxiety and d 987 2. Freidl EK, Stroeh Organization
988 and Adolescents. Fo
989 3. Prevention CfDCa. A
990 Volume 2023: cdc.go
- 989 3. Prevention CfDCa. Anxiety and depression in children: Get th
990 Volume 2023: cdc.gov, 2023.
991 4. Prevention CfDCa. Prevalence of Childhood Obesity in the
992 5. Gariepy G, Nitka D, Schmitz N. The association betw
- 988 and Adolescents. Focus (Am Psychiatr Publ) 2017;15:144-156.

989 3. Prevention CfDCa. Anxiety and depression in children: Get the facts. Children's Mental Health.

990 Volume 2023: cdc.gov, 2023.

991 4. Prevention CfD 989 3. Prevention CfDCa. Anxiety and depression in children: Get the facts. Children's Mental Health.

990 Volume 2023: cdc.gov, 2023.

991 4. Prevention CfDCa. Prevalence of Childhood Obesity in the United States. Overwei 991 4. Prevention CfDCa. Prevalend
992 0besity. Volume 2023. cdc.gov
993 5. Gariepy G, Nitka D, Schmitz
994 population: a systematic revie
995 6. Grundy A, Cotterchio M,
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- 992 6. Previously, Volume 2023. cdc.gov, 2023.

992 5. Gariepy G, Nitka D, Schmitz N. The association between obesity and anxiety disorders in the population: a systematic review and meta-analysis. Int J Obes (Lond) 2010;3 population: a systematic review and meta-analysis. Int J Obes (Lond) 2010;34:407-19.

995 6. Grundy A, Cotterchio M, Kirsh VA, et al. Associations between anxiety, depression,

996 antidepressant medication, obesity and we 994 population: a systematic review and meta-analysis. Int J Obes (Lond) 2010;34:407-19.
995 6. Grundy A, Cotterchio M, Kirsh VA, et al. Associations between anxiety, depression,
996 antidepressant medication, obesity and 995 6. Grundy A, Cotterchio M, Kirsh VA, et al. Associations between anxiety, contract antidepressant medication, obesity and weight gain among Canadian women.
997 2014;9:e99780.
998 7. Lindberg L, Hagman E, Danielsson P, 996 antidepressant medication, obesity and weight gain among Canadian women. PLoS One
997 2014;9:e99780.
998 7. Lindberg L, Hagman E, Danielsson P, et al. Anxiety and depression in children and adolescents
999 with obesity
- 997 2014;9:e99780.
998 7. Lindberg L, Hagman E, Danielsson P, et al. Anxiety and depression in children and adolescents
999 with obesity: a nationwide study in Sweden. BMC Med 2020;18:30.
8. Sharafi SE, Garmaroudi G, Ghafo 998 7. Lindberg L, Hag

999 with obesity: a n

000 8. Sharafi SE, Garn

001 with overweight

002 9. Wang S, Sun Q
-
- with obesity: a nationwide study in Sweden. BMC Med 2020;18:30.

8. Sharafi SE, Garmaroudi G, Ghafouri M, et al. Prevalence of anxiety and depression in patients

with overweight and obesity. Obesity Medicine 2020;17:1-5.
 8. Sharafi SE, Garmaroudi G, Ghafouri M, et al. Prevalence of anxiet

9. With overweight and obesity. Obesity Medicine 2020;17:1-5.

9. Wang S, Sun Q, Zhai L, et al. The Prevalence of Depression ana

9. Overweight/Obese an 1001 with overweight and obesity. Obesity Medicine 2020;17:1-5.

1002 9. Wang S, Sun Q, Zhai L, et al. The Prevalence of Depression and Anxiety Symptoms among

1003 Overweight/Obese and Non-Overweight/Non-Obese Children/Ad with overweight and obesity. Obesity Medicine 2020;17:1-5.

1002 9. Wang S, Sun Q, Zhai L, et al. The Prevalence of Depression and Anxiety Symptoms among

1003 Overweight/Obese and Non-Overweight/Non-Obese Children/Adolesc Overweight/Obese and Non-Overweight/Non-Obese Children/Adolescents in China: A

1004 Systematic Review and Meta-Analysis. Int J Environ Res Public Health 2019;16.

1005 10. Bercik P, Denou E, Collins J, et al. The intestin
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- Systematic Review and Meta-Analysis. Int J Environ Res Public Health 2019;16.

1005 10. Bercik P, Denou E, Collins J, et al. The intestinal microbiota affect central levels of brain-derived

1006 neurotropic factor and beh 1005 10. Bercik P, Denou E, Collins J, et al. The intestinal microbiota affect central leve
1006 neurotropic factor and behavior in mice. Gastroenterology 2011;141:599-609,
1007 11. Bravo JA, Forsythe P, Chew MV, et al. In 1006 11. Bravo JA, Forsythe P, Chew MV, et al. Ingestion of Lactobacillus strain regulates emotional

1007 11. Bravo JA, Forsythe P, Chew MV, et al. Ingestion of Lactobacillus strain regulates emotional

1008 behavior and 1007 11. Bravo JA, Forsythe P, Chew MV, et al. Ingestion of Lactobacillus strain regulates emotional
1008 behavior and central GABA receptor expression in a mouse via the vagus nerve. Proc Natl Acad
1009 Sci U S A 2011;108 1008 behavior and central GABA receptor expression in a mouse via the vagus nerve. Proc Natl Acad
1009 Sci U S A 2011;108:16050-5. 1009 behavior and central GABA receptor expression in a mouse via the value of $\frac{1}{2}$ or $\frac{1}{2}$ and \frac 1009 Sci U S A 2011;108:16050-5.

- Buffington SA, Di Prisco GV, Auchtung TA, et al. Microbial Reconstitution Reverses Maternal Diet-1010 12.
-
- 1012 13. Hsiao EY, McBride SW, Hsien S, et al. Microbiota modulate behavior abnormalities associated with neurodevelopmental disorders. Cell 2013;155
1014 14. Catron TR, Swank A, Wehmas LC, et al. Microbiota alter metab
10
- 1015 1015 12. Buffington Sa, Buffangetta Governmental toxicity of 17 beta-estradiol. Sci Rep 2019;9:7064.
1016 15. Kim E, Paik D, Ramirez RN, et al. Maternal gut bacteria drive intestinal abnormalities associated with neurodevelopmental disorders. Cell 2013;155:1451-63.

1014 14. Catron TR, Swank A, Wehmas LC, et al. Microbiota alter metabolism and mediate

1015 15. Kim E, Paik D, Ramirez RN, et al. Materna 1014 14. Catron TR, Swank A, Wehmas LC, et al. Microbiota alter metabolism and
1015 abnormalities are in the a-estradiol. Scill epi 2019;9:7064.
1016 15. Kim E, Paik D, Ramirez RN, et al. Maternal gut bacteria drive intest ntial 1015

1015 meurodevelopmental toxicity of 17beta-estradiol. Sci Rep 2019;9:7064.

1016 15. Kim E, Paik D, Ramirez RN, et al. Maternal gut bacteria drive intestinal inflammation in offspring

1017 with neurodevelopmen 1016 15. Kim E, Paik D, Ramirez RN, et al. Maternal gut bacteria drive intestinal
1017 with neurodevelopmental disorders by altering the chromatin land
1018 Immunity 2022;55:145-158 e7.
1019 16. Cho HM, Gonzalez-Ortiz G, M
- with neurodevelopmental disorders by altering the chromatin landscape of CD4(+) T cells.

1018 Immunity 2022;55:145-158 e7.

1019 16. Cho HM, Gonzalez-Ortiz G, Melo-Duran D, et al. Stimbiotic supplementation improved

1020 1018 Immunity 2022;55:145-158 e7.

1019 16. Cho HM, Gonzalez-Ortiz G, Melo-Duran D, et al. Stimbiotic supplementation improved

1020 performance and reduced inflammatory response via stimulating fiber fermenting microbiome 1019 16. Cho HM, Gonzalez-Ortiz G,
1020 performance and reduced infla
1021 in weaner pigs housed in a poc
1022 diet. PLoS One 2020;15:e02402
1023 17. Everard A, Belzer C, Geurts L, e 1020 performance and reduced inflammatory response via stimulating fiber fermenting microbiome

1021 in weaner pigs housed in a poor sanitary environment and fed an antibiotic-free low zinc oxide

1022 diet. PLoS One 2020; 1021 in weaner pigs housed in a poor sanitary environment and fed an antibiotic-free low zinc oxide

1022 diet. PLoS One 2020;15:e0240264.

1023 17. Everard A, Belzer C, Geurts L, et al. Cross-talk between Akkermansia muci
- diet. PLoS One 2020;15:e0240264.

1022 diet. PLoS One 2020;15:e0240264.

1023 17. Everard A, Belzer C, Geurts L, et al. Cross-talk between Akkermansia muciniphila and intestinal

1024 epithelium controls diet-induced obesi 1023 17. Everard A, Belzer C, Geurts L, et a
1024 epithelium controls diet-induced of
1025 18. Lee H, Lee Y, Kim J, et al. Modulat
1026 profiles in aged obese mice. Gut Mi
1027 19. Rodrigues RR, Greer RL, Dong X,
- epithelium controls diet-induced obesity. Proc Natl Acad Sci U S A 2013;110:9066-71.
1025 18. Lee H, Lee Y, Kim J, et al. Modulation of the gut microbiota by metformin improves metabolic
1026 profiles in aged obese mice. G 1025 18. Lee H, Lee Y, Kim J, et al. Modulation of the gut microbiota by metformin improve-
1026 profiles in aged obese mice. Gut Microbes 2018;9:155-165.
1027 19. Rodrigues RR, Greer RL, Dong X, et al. Antibiotic-Induced
- 1025 18. Lee H, Lee Y, Kim J, et al. Modulation of the gut microbiota by metformin improves metabolic

1026 profiles in aged obese mice. Gut Microbes 2018;9:155-165.

1027 19. Rodrigues RR, Greer RL, Dong X, et al. Antibio 1027 19. Rodrigues RR, Greer RL, Dong X, et al. Antibiotic-Induced
1028 Associated with Changes in Glucose Metabolism in Healthy
1029 20. Desbonnet L, Clarke G, Shanahan F, et al. Microbiota is es.
1030 mouse. Mol Psychiat
-
- Associated with Changes in Glucose Metabolism in Healthy Mice. Front Microbiol 2017;8:2306.

1029 20. Desbonnet L, Clarke G, Shanahan F, et al. Microbiota is essential for social development in the

1030 1031 21. Diaz Heij 20. Desbonnet L, Clarke G, Shanahan F, et al. Microbiota is essential for social development in th
1030 mouse. Mol Psychiatry 2014;19:146-8.
1031 21. Diaz Heijtz R, Wang S, Anuar F, et al. Normal gut microbiota modulates b
- mouse. Mol Psychiatry 2014;19:146-8.

1030 mouse. Mol Psychiatry 2014;19:146-8.

1031 21. Diaz Heijtz R, Wang S, Anuar F, et al. Normal gut microbiota modulates brain development and

1032 22. Rabot S, Membrez M, Bruneau A 1031 21. Diaz Heijtz R, Wang S, Anuar F, et al. N
1032 behavior. Proc Natl Acad Sci U S A 2011
1033 22. Rabot S, Membrez M, Bruneau A, et a
1034 induced insulin resistance and have alte
1035 23. Collins SM, Kassam Z, Berci 1032

1032 behavior. Proc Natl Acad Sci U S A 2011;108:3047-52.

1033 22. Rabot S, Membrez M, Bruneau A, et al. Germ-free C57BL/6J mice are resistant to high-fat-diet-

1034 23. Collins SM, Kassam Z, Bercik P. The adoptive behavior. Proc Natl Acad Sci U S A 2011;108:3047-52.

1033 22. Rabot S, Membrez M, Bruneau A, et al. Germ-free C57BL/6J mice are resistant to high-fat-diet-

1034 induced insulin resistance and have altered cholesterol met
- 23. Collins SM, Kassam Z, Bercik P. The adoptive transfer of behavioral phenotype via the intestir
1036 microbiota: experimental evidence and clinical implications. Curr Opin Microbiol 2013;16:240-5
1037 24. De Palma G, Ly
- 1038 bowel syndrome alters gut function and behavior in recipient mice. Sci Transl Med 2017;9.
1039 25. Turnbaugh PJ, Ley RE, Mahowald MA, et al. An obesity-associated gut microbiome with
- microbiota: experimental evidence and clinical implications. Curr Opin Microbiol 2013;16:240-5.

1037 24. De Palma G, Lynch MD, Lu J, et al. Transplantation of fecal microbiota from patients with irritable

1038 bowel synd 24. De Palma G, Lynch MD, Lu J, et al. Transplantation of fecal microbiota from patients with irritable

1038 bowel syndrome alters gut function and behavior in recipient mice. Sci Transl Med 2017;9.

1039 25. Turnbaugh PJ 1038 bowel syndrome alters gut function and behavior in recipient mice. Sci Transl Med 2017;9.

1039 25. Turnbaugh PJ, Ley RE, Mahowald MA, et al. An obesity-associated gut microbiome with

1040 increased capacity for ener 25. Turnbaugh PJ, Ley RE, Mahowald MA, et al. An obesity-associated gut microbiome

1040 increased capacity for energy harvest. Nature 2006;444:1027-31.

26. Bhagavata Srinivasan SP, Raipuria M, Bahari H, et al. Impacts of
-
- increased capacity for energy harvest. Nature 2006;444:1027-31.
1041 26. Bhagavata Srinivasan SP, Raipuria M, Bahari H, et al. Impacts of Diet and Exercise on Maternal
1042 Gut Microbiota Are Transferred to Offspring. Fron 26. Bhagavata Srinivasan SP, Raipuria M, Bahari H, et al. Impacts of Gut Microbiota Are Transferred to Offspring. Front Endocrinol (Lau 1043 27. Bruce-Keller AJ, Fernandez-Kim S-O, Townsend RL, et al. Mater differentially Gut Microbiota Are Transferred to Offspring. Front Endocrinol (Lausanne) 2018;9:716.
1043 27. Bruce-Keller AJ, Fernandez-Kim S-O, Townsend RL, et al. Maternal obese-type gut microbiota
1044 differentially impact cognition, 1043 27. Bruce-Keller AJ, Fernandez-Kim S-O, Townsend RL, et al. Maternal obese-type gut

1044 differentially impact cognition, anxiety and compulsive behavior in male and female c

1045 mice. PLOS ONE 2017;12:e0175577.

1 differentially impact cognition, anxiety and compulsive behavior in male and female offspring in

1045 28. Pessa-Morikawa T, Husso A, Karkkainen O, et al. Maternal microbiota-derived metabolic profile

1047 1048 29. Deng X
-
- mice. PLOS ONE 2017;12:e0175577.

1046 28. Pessa-Morikawa T, Husso A, Karkkainen O, et al. Maternal microbiota-derived metabolic profile

1047 in fetal murine intestine, brain and placenta. BMC Microbiol 2022;22:46.

1048 1046 28. Pessa-Morikawa T, Husso A, Karkkai
1047 in fetal murine intestine, brain and p
1048 29. Deng X, Guarita DR, Pedroso MR, et
1049 the area postrema in unanesthe
1050 2001;281:R645-53. 28. Pessa-Morikawa T, Husso A, Karkkainen O, et al. Maternal microbiota-derived metabolic profile

1047 in fetal murine intestine, brain and placenta. BMC Microbiol 2022;22:46.

29. Deng X, Guarita DR, Pedroso MR, et al. P 1048 29. Deng X, Guarita DR, Pedroso MR, et al. PYY inhibits CCK-stimulated pances.

1049 the area postrema in unanesthetized rats. Am J Physiol Regul

1050 2001;281:R645-53.

1051 30. Cabral A, Cornejo MP, Fernandez G, et 1049 1121 2001;281:R645-53.

1050 1050 2001;281:R645-53.

2001;281:R645-53.

2001;281:R645-53.

2001;281:R645-53.

2001;281:R645-53.

2001;281:R645-53.

2001;281:R645-53.

2001;281:R645-53.

2001;281:R645-53.

2001;281:R64
-
- 2001;281:R645-53.

1051 30. Cabral A, Cornejo MP, Fernandez G, et al. Circulating Ghrelin Acts on GABA Neurons of the Area

1052 Postrema and Mediates Gastric Emptying in Male Mice. Endocrinology 2017;158:1436-1449.

1053 1051 30. Cabral A, Cornejo N
1052 Postrema and Medi
1053 31. Yamamoto H, Kishi
1054 the area postrema I
1055 Neurosci 2003;23:2 Postrema and Mediates Gastric Emptying in Male Mice. Endocrinology 2017;158:1436-1449.

1053 31. Vamamoto H, Kishi T, Lee CE, et al. Glucagon-like peptide-1-responsive catecholamine neurons in

1054 the area postrema link 1053 31. Yamamoto H, Kishi T, Lee CE, et al. Glucagon-like peptide-1-responsive catecholamine neurons in
1054 the area postrema link peripheral glucagon-like peptide-1 with central autonomic control sites. J
1055 Neurosci the area postrema link peripheral glucagon-like peptide-1 with central autonomic control sites. J 1055 Neurosci 2003;23:2939-46. 1055 Neurosci 2003;23:2939-46.

21056 32. Arslanova A, Tarasova A, Alexandrova A, et al. Protective Effects of Probiotics on Cognitive and

1057 Motor Functions, Anxiety Level, Visceral Sensitivity, Oxidative Stress and Microbiota in Mice with

1058 Anti

- 2058 Antibiotic-Induced Dysbiosis. Life (Basel) 2021;11.

1059 33. O'Connor R, Moloney GM, Fulling C, et al. Maternal antibiotic administration during a critical

1060 developmental window has enduring neurobehavioural eff
- 1059 33. O'Connor R, Moloney GM, Fulling C, et al. Maternal antibiotic administration during a critical
1060 developmental window has enduring neurobehavioural effects in offspring mice. Behav Brain
1061 Res 2021;404:11315 Res 2021;404:113156.

1061 Res 2021;404:113156.

1062 34. Sun Y, Zhu H, Cheng R, et al. Outer membrane protein Amuc_1100 of Akkermansia muciniphila

1063 alleviates antibiotic-induced anxiety and depression-like behavior i 1062 34. Sun Y, Zhu H, Cheng R,
1063 alleviates antibiotic-in
1064 2023;258:114023.
1065 35. Bruce-Keller AJ, Ferna
1066 differentially impact co
- 1063

1063 alleviates antibiotic-induced anxiety and depression-like behavior in mice. Physiol Behav

2023;258:114023.

1065 35. Bruce-Keller AJ, Fernandez-Kim SO, Townsend RL, et al. Maternal obese-type gut microbiota

10 2023;258:114023.

1065 35. Bruce-Keller AJ, Fernandez-Kim SO, Townsend RL, et al. Maternal obese-type gut microbiota

1066 differentially impact cognition, anxiety and compulsive behavior in male and female offspring in

1 1065 35. Bruce-Keller AJ, Fe

1066 differentially impace

1067 mice. PLoS One 20

1068 36. Cheatham CN, Gu

1069 Influence Fetal Gro
- differentially impact cognition, anxiety and compulsive behavior in male and female offspring in

1067 1068 36. Cheatham CN, Gustafson KL, McAdams ZL, et al. Standardized Complex Gut Microbiomes

1069 1069 1070 2023;11. mice. PLoS One 2017;12:e0175577.

1067 mice. PLoS One 2017;12:e0175577.

1068 36. Cheatham CN, Gustafson KL, McAdams ZL, et al. Standardized Complex Gut Microbiomes

1069 Influence Fetal Growth, Food Intake, and Adult Body 1068 36. Cheatham CN, Gustafson KL, McA
1069 Influence Fetal Growth, Food Intake
1070 2023;11.
1071 37. Ericsson AC, Hart ML, Kwan J, et al
1072 Iocomotor and anxiety-related bel
- 1069 Influence Fetal Growth, Food Intake, and Adult Body Weight in Outbred Mice. Microorganisms
1070 2023;11.
1071 37. Ericsson AC, Hart ML, Kwan J, et al. Supplier-origin mouse microbiomes significantly influence
1072 loc 2023;11.
1071 37. Ericsson AC, Hart ML, Kwan J, et al. Supplier-origin mouse microbiomes significantly influence
1072 locomotor and anxiety-related behavior, body morphology, and metabolism. Commun Biol
1073 2021;4:716. Wo 1071 37. Ericsson
1072 locomoto
1073 2021;4:7:
1074 38. Wolfe AE
1075 (Candidat
- 1072 Iocomotor and anxiety-related behavior, body morphology, and metabolism. Commun Biol
1073 2021;4:716.
1074 38. Wolfe AE, Moskowitz JE, Franklin CL, et al. Interactions of Segmented Filamentous Bacteria
1075 (Candidatu 2021;4:716.
1073 2021;4:716.
1074 38. Wolfe AE, Moskowitz JE, Franklin CL, et al. Interactions of Segmented Filamentous Bacteria
1075 (Candidatus Savagella) and bacterial drivers in colitis-associated colorectal cancer dev 1074 38. Wolfe AE, N
1075 (Candidatus
1076 PLoS One 20
1077 39. Walters WA,
1078 analysis of b 1074 38. Wolfe AE, Moskowitz JE, Franklin CL, et al. Interactions of Segmented Filamentous Bacteria

1075 (Candidatus Savagella) and bacterial drivers in colitis-associated colorectal cancer development.

1076 PLoS One 202
- 1077 39. Walters WA, Caporaso JG, La
1078 analysis of barcoded polymera
1079 40. Caporaso JG, Lauber CL, Walt
1080 millions of sequences per sam
1081 41. Loy A, Maixner F, Wagner
- 1077 39. Walters WA, Caporaso JG, Lauber CL, et al. PrimerProspector: de novo design and taxonomic

1078 analysis of barcoded polymerase chain reaction primers. Bioinformatics 2011;27:1159-61.

1079 40. Caporaso JG, Lauber
- 1077 39. Walters WA, Caporaso JG, Lauber CL, et al. PrimerProspector: de novo design and taxonomic 1079 40. Caporaso JG, Lauber CL, Walters WA, et al. Global patterns of 16S rRNA diversity at a de

1080 millions of sequences per sample. Proc Natl Acad Sci U S A 2011;108 Suppl 1:4516-22.

1081 41. Loy A, Maixner F, Wagne millions of sequences per sample. Proc Natl Acad Sci U S A 2011;108 Suppl 1:4516-22.

1081 41. Loy A, Maixner F, Wagner M, et al. probeBase--an online resource for rRNA-targeted

1082 oligonucleotide probes: new features 2
- 1081 41. Loy A, Maixner F, Wagner M, et al. probeBase--an online resource for rRN
1082 bilgonucleotide probes: new features 2007. Nucleic Acids Res 2007;35:D800-4.
1083 42. Martin M. Cutadapt removes adapter sequences from
- oligonucleotide probes: new features 2007. Nucleic Acids Res 2007;35:D800-4.

1083 42. Martin M. Cutadapt removes adapter sequences from high-throughput sequencing reads.

1084 EMBnet journal 2011;17:10-12.

1085 43. Bolye 42. Martin M. Cutadapt removes adapter sequences from high-throughput s
1084 EMBnet.journal 2011;17:10-12.
1085 43. Bolyen E, Rideout JR, Dillon MR, et al. Reproducible, interactive, scalabl
1086 microbiome data science us EMBnet.journal 2011;17:10-12.

1084 EMBnet.journal 2011;17:10-12.

1085 43. Bolyen E, Rideout JR, Dillon MR, et al. Reproducible, interactive, scalable and extensible

1086 microbiome data science using QIIME 2. Nat Biotec 1085 43. Bolyen E, Rideout JR, Dillon
1086 microbiome data science using (
1087 44. Callahan BJ, McMurdie PJ, Ros
1088 Illumina amplicon data. Nat Met
1089 45. Pruesse E, Quast C, Knittel K, et
-
- microbiome data science using QIIME 2. Nat Biotechnol 2019;37:852-857.
1087 44. Callahan BJ, McMurdie PJ, Rosen MJ, et al. DADA2: High-resolution sample inference from
1088 Hlumina amplicon data. Nat Methods 2016;13:581-3. 1087 44. Callahan BJ, McMurdie PJ, Rosen MJ, et al. DADA2: High-resolution s:
1088 lllumina amplicon data. Nat Methods 2016;13:581-3.
1089 45. Pruesse E, Quast C, Knittel K, et al. SILVA: a comprehensive online resour
1090 1087 44. Callahan BJ, McMurdie PJ, Rosen MJ, et al. DADA2: High-resolution sample inference from 1089 45. Pruesse E, Quast C, Knittel K, et al. SILVA: a comprehensive and aligned ribosomal RNA sequence data con
1091 2007;35:7188-96.
1092 46. Asnicar F, Weingart G, Tickle TL, et al. Compact gra
1093 and metadata with G and aligned ribosomal RNA sequence data compatible with ARB. Nucleic Acids Res

1091 2007;35:7188-96.

1092 46. Asnicar F, Weingart G, Tickle TL, et al. Compact graphical representation of phylogenetic data

1093 and metad
-
- 2007;35:7188-96.
1092 46. Asnicar F, Weingart G, Tickle TL, et al. Compact graphical representation of phylogenetic data
1093 and metadata with GraPhlAn. Peerl 2015;3:e1029.
1094 47. Zachary L McAdams SBB, Kevin L Gustafso 1092 46. Asnicar F, Weinga
1093 and metadata with
1094 47. Zachary L McAdan
1095 C Ericsson. Mult
1096 functional differen 46. Asnicar F, Weingart G, Tickle TL, et al. Compact graphical representation of phylogenetic data

1093 and metadata with GraPhlAn. Peerl 2015;3:e1029.

1094 47. Zachary L McAdams SBB, Kevin L Gustafson, Nathan Bivens, Cr
- 1096 functional differences and novel metagenome-assembled genomes. bioRxiv 2022.
1097 48. Khodakivskyi PV, Lauber CL, Yevtodiyenko A, et al. Noninvasive imaging and quant
1098 salt hydrolase activity: From bacteria to hum C Ericsson. Multi-omics analysis of mouse fecal microbiome reveals supplier-dependent

1096 functional differences and novel metagenome-assembled genomes. bioRxiv 2022.

1097 48. Khodakivskyi PV, Lauber CL, Yevtodiyenko A, 1096 functional differences and novel metagenome-assembled genomes. bioRxiv 2022.
1097 48. Khodakivskyi PV, Lauber CL, Yevtodiyenko A, et al. Noninvasive imaging and quantification of bile
1098 5alt hydrolase activity: Fro 48. Khodakivskyi PV, Lauber CL, Yevtodiyenko A, et al. Noninvasive imaging and quanti

1098 salt hydrolase activity: From bacteria to humans. Sci Adv 2021;7.

1099 49. Vorobyeva AG, Stanton M, Godinat A, et al. Development
- salt hydrolase activity: From bacteria to humans. Sci Adv 2021;7.

1099 49. Vorobyeva AG, Stanton M, Godinat A, et al. Development of a Bioluminescent Nitroreductase

1100 Probe for Preclinical Imaging. PLoS One 2015;10:e0 1099 49. Vorobyeva AG, Stanton M, Godinat A, et al. Development of a
1100 Probe for Preclinical Imaging. PLoS One 2015;10:e0131037.
1101 50. Cantor JR, Abu-Remaileh M, Kanarek N, et al. Physiologic Mediu
1102 and Reveals U
- 1100 Frobe for Preclinical Imaging. PLoS One 2015;10:e0131037.
1101 50. Cantor JR, Abu-Remaileh M, Kanarek N, et al. Physiologic Medium Rewires Cellular Metabolism
1102 and Reveals Uric Acid as an Endogenous Inhibitor of U 1101 50. Cantor JR, Abu-Remaileh M, Kanarek N, et al. Physiologic N
1102 and Reveals Uric Acid as an Endogenous Inhibitor of UMP Sy
7. 1101 50. Cantor JR, Abu-Remaileh M, Kanarek N, et al. Physiologic Medium Rewires Cellular Metabolisn
1102 **1102** and Reveals Uric Acid as an Endogenous Inhibitor of UMP Synthase. Cell 2017;169:258-272.e17. 1102 and Reveals Uric Acid as an Endogenous Inhibitor of UMP Synthase. Cell 2017;169:258-272.e17.
-
- 1103 51. Li B, Tang J, Yang Q, et al. NOREVA: normalization and evaluation of MS-based metabolomics

1104 data. Nucleic Acids Res 2017;45:W162-w170.

1105 52. Zhou W, Hinoue T, Barnes B, et al. DNA methylation dynamics and 1105 52. Zhou W, Hinoue T, Barnes B, et al. DNA meth
1106 high-throughput profiling in the mouse. Cell G
1107 53. Xu Z, Niu L, Li L, et al. ENmix: a nov
1108 HumanMethylation450 BeadChip. Nucleic Acid
1109 54. Aryee MJ, Ja
-
- high-throughput profiling in the mouse. Cell Genom 2022;2.

1107 53. Xu Z, Niu L, Li L, et al. ENmix: a novel background correction method for Illumina

1108 HumanMethylation 450 BeadChip. Nucleic Acids Res 2016;44:e20.

1 1107 53. Xu Z, Niu L, Li L, et al. ENmix: a novel background
1108 HumanMethylation450 BeadChip. Nucleic Acids Res 2016;44
1109 54. Aryee MJ, Jaffe AE, Corrada-Bravo H, et al. Minfi: a flexibl
1110 package for the analysis 1108

1108 HumanMethylation450 BeadChip. Nucleic Acids Res 2016;44:e20.

1109 54. Aryee MJ, Jaffe AE, Corrada-Bravo H, et al. Minfi: a flexible and comprehensive Bioconductor

1110 package for the analysis of Infinium DNA 1109 54. Aryee MJ, Jaffe AE, Corrada-Bravo H, et al. Minfi: a flexible and package for the analysis of Infinium DNA methylation microarrays
1111 9.
1112 55. Xu Z, Langie SA, De Boever P, et al. RELIC: a novel dye-bias cont matrice Matter Matter Matter Matter Matter Matter Correction 1110
1110 spackage for the analysis of Infinium DNA methylation microarrays. Bioinformatics 2014;30:1363-
1112 55. Xu Z, Langie SA, De Boever P, et al. RELIC: a
- 1111 package for the analysis of the analysis of 1111
1112 55. Au Z, Langie SA, De Boever P, et al. RELIC: a novel dye-bias correction method for Illumina
1113 Methylation BeadChip. BMC Genomics 2017;18:4.
1114 56. Wolock 1112 55. Xu
1113 M₁
1114 56. W₁
1115 Ce
1116 57. Stu
-
- 1113 Methylation BeadChip. BMC Genomics 2017;18:4.
1114 56. Wolock SL, Lopez R, Klein AM. Scrublet: Computational Identification of Cell Doublets in Single-
1115 57. Stuart T, Butler A, Hoffman P, et al. Comprehensive Inte 1114 56. Wolock SL, Lopez R, Klein AM. Scrublet: Computat
1115 Cell Transcriptomic Data. Cell Syst 2019;8:281-291
1116 57. Stuart T, Butler A, Hoffman P, et al. Compreh
1117 2019;177:1888-1902 e21.
1118 58. Team RDC. R: A 1116 57. Stuart T, Butler A, Hoffman P, et al. Comprehens
1117 2019;177:1888-1902 e21.
1118 58. Team RDC. R: A Language and Environment for Statistic
1119 59. Jin S, Guerrero-Juarez CF, Zhang L, et al. Inference and
1120 C
-
- 1119 59. Jin S, Guerrero-Juarez CF, Zhang L, et al. Inference and analysis of cell-cell communication using
1120 CellChat. Nat Commun 2021;12:1088. 2019;177:1888-1902 e21.

1118 58. Team RDC. R: A Language and Environment for Statistical Computing. 2010.

1119 59. Jin S, Guerrero-Juarez CF, Zhang L, et al. Inference and analysis of cell-cell communication using

1121 1118 58. Team RDC. R: A Language
1119 59. Jin S, Guerrero-Juarez CF,
1120 CellChat. Nat Commun 202
1121 60. Suoqin J, Maksim VP, Qin
1122 single-cell and spatially res 1119 59. Jin S, Guerrero-Juarez CF, Zhang L, et al. Inference and analysis of cell-cell c
1120 CellChat. Nat Commun 2021;12:1088.
1121 60. Suoqin J, Maksim VP, Qing N. CellChat for systematic analysis of cell-cell c
1122 s
-
- CellChat. Nat Commun 2021;12:1088.

1120 CellChat. Nat Commun 2021;12:1088.

1121 60. Suoqin J, Maksim VP, Qing N. CellChat for systematic analysis of cell-cell communication from

1122 single-cell and spatially resolved t 1121 60. Suoqin J, Maksim VP, Qing N. CellCha
1122 single-cell and spatially resolved transc
1123 61. Hammer Ø, Harper DA. Past: paleonto
1124 anlysis. Palaeontologia electronica 200
1125 62. Dixon P. VEGAN, A Package of R single-cell and spatially resolved transcriptomics. bioRxiv 2023:2023.11.05.565674.

1123 61. Hammer Ø, Harper DA. Past: paleontological statistics software package for educaton and data

1124 anlysis. Palaeontologia elect single-cell and spatially resolved transcriptomics. bioRxiv 2023:2023.11.05.565674.

1123 61. Hammer Ø, Harper DA. Past: paleontological statistics software package for educaton and data

1124 anlysis. Palaeontologia elect
-
- 1124 anlysis. Palaeontologia electronica 2001;4:1.
1125 62. Dixon P. VEGAN, A Package of R Functions for Community Ecology. Journal of Vegetation Science
1126 2003;14:927-930.
1127 63. Ericsson AC, Davis JW, Spollen W, et 1125 62. Dixon P. VEGAN, A Package of R Functions fo
1126 2003;14:927-930.
1127 63. Ericsson AC, Davis JW, Spollen W, et al. E
1128 composition of the fecal microbiota of inbrec
1129 64. Song Z, Cai Y, Lao X, et al. Taxono 2003;14:927-930.

1126 2003;14:927-930.

Ericsson AC, Davis JW, Spollen W, et al. Effects of vendor and genetic background on the

1128 64. Song Z, Cai Y, Lao X, et al. Taxonomic profiling and populational patterns of bact 1127 63. Ericsson AC, Davi

1128 composition of the

1129 64. Song Z, Cai Y, Lao

1130 hydrolase (BSH) ge

1131 65. Abdulrab S, Al-Ma
-
- composition of the fecal microbiota of inbred mice. PLoS One 2015;10:e0116704.

1129 64. Song Z, Cai Y, Lao X, et al. Taxonomic profiling and populational patterns of bacterial bile salt

1130 hydrolase (BSH) genes based o 64. Song Z, Cai Y, Lao X, et al. Taxonomic profiling and populational patterns of ba
1130 hydrolase (BSH) genes based on worldwide human gut microbiome. Microbiome 2
1131 65. Abdulrab S, Al-Maweri S, Halboub E. Ursodeoxych
- hydrolase (BSH) genes based on worldwide human gut microbiome. Microbiome 2019;7:9.

1131 65. Abdulrab S, Al-Maweri S, Halboub E. Ursodeoxycholic acid as a candidate therapeutic to alleviate

1132 and/or prevent COVID-19-a 1131 65. Abdulrab S, Al-Maweri S, Halboub E. Ursodeoxycholic acid as a candidate therapeutic to all and/or prevent COVID-19-associated cytokine storm. Med Hypotheses 2020;143:109897.
1133 66. Adhikari A, Topiwala MA, Gordo
- and/or prevent COVID-19-associated cytokine storm. Med Hypotheses 2020;143:109897.

1133 66. Adhikari A, Topiwala MA, Gordon JA. Synchronized activity between the ventral hippocampus

1134 and the medial prefrontal cortex 66. Adhikari A, Topiwala MA, Gordon JA. Synchronized activity between the ventral hippod
1134 and the medial prefrontal cortex during anxiety. Neuron 2010;65:257-69.
1135 67. Baksh RA, Ritchie CW, Terrera GM, et al. The as 66. Adhikari A, Topiwala MA, Gordon JA. Synchronized activity between the ventral hippocampus

1134 and the medial prefrontal cortex during anxiety. Neuron 2010;65:257-69.

1135 67. Baksh RA, Ritchie CW, Terrera GM, et al.
- hippocampal volume in older adults. Psychol Aging 2021;36:288-297.

1137 68. Jimenez JC, Su K, Goldberg AR, et al. Anxiety Cells in a Hippocal

1138 Neuron 2018;97:670-683 e6.

1139 69. Mineur YS, Obayemi A, Wigestrand MB,
- 1136 hippocampal volume in older adults. Psychol Aging 2021;36:288-297.

1137 68. Jimenez JC, Su K, Goldberg AR, et al. Anxiety Cells in a Hippocampal-Hypothalamic Circuit.

1138 69. Mineur YS, Obayemi A, Wigestrand MB, et 1137 68. Jimenez JC, Su K, Goldberg AR, et al. Anxiety Cells in a Hippocar
1138 Neuron 2018;97:670-683 e6.
1139 69. Mineur YS, Obayemi A, Wigestrand MB, et al. Cholinergic signaling in
1140 social stress resilience and anx Neuron 2018;97:670-683 e6.

1139 69. Mineur YS, Obayemi A, Wigestrand MB, et al. Cholinergic signaling in the hippocampus regulates

1140 social stress resilience and anxiety- and depression-like behavior. Proc Natl Acad S 1139 69. Mineur YS, Obayemi A, Wiges
1140 social stress resilience and
1141 2013;110:3573-8.
1142 70. Revest JM, Dupret D, Koehl
1143 related behaviors. Mol Psychi social stress resilience and anxiety- and depression-like behavior. Proc Natl Acad Sci U S A
1141 2013;110:3573-8.
1142 70. Revest JM, Dupret D, Koehl M, et al. Adult hippocampal neurogenesis is involved in anxiety-
1143 7
- 1141 2013;110:3573-8.

1142 70. Revest JM, Dupret D, Koehl M, et al. Adult hippocampal neurogenesis is involved in anxiety-

1143 71. Satpute AB, Mumford JA, Naliboff BD, et al. Human anterior and posterior hippocampus res 1142 70. Revest JM, Dupre

1143 related behaviors.

1144 71. Satpute AB, Mumf

1145 distinctly to state a

1146 72. Szklarczyk D, Gab
-
- 1147 1147 increased coverage, supporting functional discovery in genome-wide experimental datasets.
1148 1148 Nucleic Acids Res 2019;47:D607-D613. 1144 71. Satpute AB, Mumford JA, Naliboff BD, et al. Humar
1145 distinctly to state and trait anxiety. Emotion 2012;1
1146 72. Szklarczyk D, Gable AL, Lyon D, et al. STRING v1
1147 increased coverage, supporting functional distinctly to state and trait anxiety. Emotion 2012;12:58-68.

1146 72. Szklarczyk D, Gable AL, Lyon D, et al. STRING v11: protein-protein association networks with

1147 increased coverage, supporting functional discovery 1146 72. Szklarczyk D, Gable AL, Lyon D, et al. STRING v11: protei
1147 increased coverage, supporting functional discovery in g
1148 Nucleic Acids Res 2019;47:D607-D613.
1149 73. Vuong HE, Pronovost GN, Williams DW, et al 1147 Increased coverage, supporting functional discovery in genome-wide experimental datasets.

1148 Nucleic Acids Res 2019;47:D607-D613.

1149 73. Vuong HE, Pronovost GN, Williams DW, et al. The maternal microbiome modula
- 1148 Nucleic Acids Res 2019;47:D607-D613.
1149 73. Vuong HE, Pronovost GN, Williams DW, et al. The maternal microbiome modulates fetal
1150 **1150** neurodevelopment in mice. Nature 2020;586:281-286. 1149 73. Vuong HE, Pronovost GN, Williams DW, et al. The
1150 meurodevelopment in mice Nature 2020;586:281-286. 1150 neurodevelopment in mice. Nature 2020;586:281-286.

The materials of materials and material model in microbiome model with all the materials feeds of the materials fetales fetales fetales fetales fetales fetales fetal 1150 neurodevelopment in mice. Nature 2020;586:281-286.

-
-
- 1152 offspring metabolic phenotype in mice. Science 2020;367.

1153 75. Husso A, Pessa-Morikawa T, Koistinen VM, et al. Impacts of maternal microbiota and microbial

1154 metabolites on fetal intestine, brain, and placenta 1153 75. Husso A, Pessa-Morikawa T, Koistinen VM, et al. Impacts
1154 metabolites on fetal intestine, brain, and placenta. BMC Bior
1155 76. Lee-Sarwar KA, Chen YC, Chen YY, et al. The maternal
1156 microbiome of childhood
- metabolites on fetal intestine, brain, and placenta. BMC Biol 2023;21:207.
1155 76. Lee-Sarwar KA, Chen YC, Chen YY, et al. The maternal prenatal and offspring early-life gut
1156 microbiome of childhood asthma phenotypes. 2155 76. Lee-Sarwar KA, Chen YC, Chen YY, et al. The maternal prenatal and c
1156 microbiome of childhood asthma phenotypes. Allergy 2023;78:418-428.
1157 77. Sun Z, Lee-Sarwar K, Kelly RS, et al. Revealing the importance 1156 microbiome of childhood asthma phenotypes. Allergy 2023;78:418-428.

1157 77. Sun Z, Lee-Sarwar K, Kelly RS, et al. Revealing the importance of prenatal gut microbiome in

1158 offspring neurodevelopment in humans. EB 1157 77. Sun Z, Lee-Sarwar K, Kelly RS, et al. Revealing the importance of prer
1158 offspring neurodevelopment in humans. EBioMedicine 2023;90:104491.
1159 78. Seneviratne SN, Rajindrajith S. Fetal programming of obesity
-
- 1158 offspring neurodevelopment in humans. EBioMedicine 2023;90:104491.

1159 78. Seneviratne SN, Rajindrajith S. Fetal programming of obesity and type 2 diabetes. World J

1160 Diabetes 2022;13:482-497.

1161 79. McAdams 1158 offspring neurodevelopment in humans. EBioMedicine 2023;90:104491.
- 78. Seneviratne SN, Rajindrajith S. Fetal programming of obesity and type 2 diabetes. World J

1160 Diabetes 2022;13:482-497.

1161 79. McAdams ZL, Gustafson KL, Russell AL, et al. Supplier-origin gut microbiomes affect ho 1161 79. McAdams ZL, Gustafson KL
1162 weight and select autism-re
1163 80. Martini E, Krug SM, Siegr
1164 Relationship With Mucosal
1165 Hepatol 2017;4:33-46. weight and select autism-related behaviors. Gut Microbes 2024;16.
1163 80. Martini E, Krug SM, Siegmund B, et al. Mend Your Fences: The Epithelial Barrier and its
1164 Relationship With Mucosal Immunity in Inflammatory Bow 1163 80. Martini E, Krug SM, Siegmund B, et al. Mend Your Fences: The Relationship With Mucosal Immunity in Inflammatory Bowel Dise
1165 Hepatol 2017;4:33-46.
1166 81. Kaelberer MM, Rupprecht LE, Liu WW, et al. Neuropod Ce 1163 80. Martini E, Krug SM, Siegmund B, et al. Mend Your Fences: The Epithelial Barrier and its
-
- Hepatol 2017;4:33-46.

1166 81. Kaelberer MM, Rupprecht LE, Liu WW, et al. Neuropod Cells: The Emerging Biology of Gut-Brain

1167 Sensory Transduction. Annu Rev Neurosci 2020;43:337-353.

1168 82. Hellmuth C, Lindsay KL, 1166 81. Kaelberer MM, Ruppre
1167 Sensory Transduction.
1168 82. Hellmuth C, Lindsay KI
1169 Offspring Adiposity: Ide
1170 2019;63:e1700889. 1167 Sensory Transduction. Annu Rev Neurosci 2020;43:337-353.
1168 82. Hellmuth C, Lindsay KL, Uhl O, et al. Maternal Metabolomic Profile and Fetal Programming of
1169 Offspring Adiposity: Identification of Potentially Pro 1168 82. Hellmuth C, Lindsay KL, Uhl O, et al. Maternal Metabolom
1169 Offspring Adiposity: Identification of Potentially Protective L
1170 2019;63:e1700889.
1171 83. Nagahashi M, Takabe K, Liu R, et al. Conjugated bile ac 82. Hellmuth C, Lindsay KL, Uhl O, et al. Maternal Metabolomic Profile and Fetal Programming of
1169 Offspring Adiposity: Identification of Potentially Protective Lipid Metabolites. Mol Nutr Food Res
1170 2019;63:e1700889.
-
- 2019;63:e1700889.
1171 83. Nagahashi M, Takabe K, Liu R, et al. Conjugated bile acid-activated S1P receptor 2 is a key
1172 regulator of sphingosine kinase 2 and hepatic gene expression. Hepatology 2015;61:1216-26.
1173 84 1171 83. Nagahashi M, Takal
1172 regulator of sphingo
1173 84. MahmoudianDehko
1174 in the Pathobiology
1175 Neurosci 2022;16:93 1172 regulator of sphingosine kinase 2 and hepatic gene expression. Hepatology 2015;61:1216-26.

1173 84. MahmoudianDehkordi S, Bhattacharyya S, Brydges CR, et al. Gut Microbiome-Linked Metabolites

1174 in the Pathobiolog 1173 84. MahmoudianDehkordi S, Bhattacharyya S, Brydges CR, et al. Gut Microbiome-Linked Metabol
1174 in the Pathobiology of Major Depression With or Without Anxiety-A Role for Bile Acids. Fr
1175 Neurosci 2022;16:937906.
 1174 in the Pathobiology of Major Depression With or Without Anxiety-A Role for Bile Acids. Front
1175 Neurosci 2022;16:937906.
1176 85. Chen S, Shao Q, Chen J, et al. Bile acid signalling and its role in anxiety disorders
-
- Neurosci 2022;16:937906.

1175 Neurosci 2022;16:937906.

1176 85. Chen S, Shao Q, Chen J, et al. Bile acid signalling and its role in anxiety disorders. Front

1177 Endocrinol (Lausanne) 2023;14:1268865.

1178 86. Weng H, 1176 85. Chen S, Shao Q, Chen J,
1177 Endocrinol (Lausanne) 202:
1178 86. Weng H, Deng L, Wang
1179 impairing gut microbiota ar
1180 87. Wu L, Han Y, Zheng Z, et
- Endocrinol (Lausanne) 2023;14:1268865.
1178 86. Weng H, Deng L, Wang T, et al. Humid heat environment causes anxiety-like disorder via
1179 1180 87. Wu L, Han Y, Zheng Z, et al. Obeticholic Acid Inhibits Anxiety via Allevi 1178 86. Weng H, Deng L, Wang T, et al. Humi
1179 impairing gut microbiota and bile acid me
1180 87. Wu L, Han Y, Zheng Z, et al. Obeticholic
1181 Mediated Microglia Accumulation in th
1182 2021;13. 1179 1180. Wu L, Han Y, Zheng Z, et al. Obeticholic Acid Inhibits Anxiety via Alleviating Gut Microbiota-
1180 187. Wu L, Han Y, Zheng Z, et al. Obeticholic Acid Inhibits Anxiety via Alleviating Gut Microbiota-
1181 182 20 1180 87. Wu L, Han Y, Zheng Z, et al. Obeticholic Acid Inhibits Anxiety via Alleviating Gut Microbiotal Microglia Accumulation in the Brain of High-Fat High-Sugar Diet Mice. Nat Communication in the Brain of High-Fat High-
-
- 1185 89. Wang H, Tan YZ, Mu RH, et al. Takeda G Protein-Coupled Receptor 5 Modulates Depression-like
1186 Behaviors via Hippocampal CA3 Pyramidal Neurons Afferent to Dorsolateral Septum. Biol 2021;13.
1183 88. Tao Y, Zhou H, Li Z, et al. TGR5 deficiency-induced anxiety and depression-like behaviors: The role
1184 of gut microbiota dysbiosis. J Affect Disord 2024;344:219-232.
1185 89. Wang H, Tan YZ, Mu RH, et a 1183 88. Tao Y, Zho
1184 of gut mid
1185 89. Wang H,
1186 Behaviors
1187 Psychiatry 1184 61 of gut microbiota dysbiosis. J Affect Disord 2024;344:219-232.

1185 89. Wang H, Tan YZ, Mu RH, et al. Takeda G Protein-Coupled Receptor 5 Modulates Depression-like

1186 Behaviors via Hippocampal CA3 Pyramidal Neu 1185 89. Wang H, Tan YZ, Mu RH, et al. Takeda G Protein-Coupled Recentilation Behaviors via Hippocampal CA3 Pyramidal Neurons Affere
1187 Psychiatry 2021;89:1084-1095.
1188 90. Kamp F, Hamilton JA, Kamp F, et al. Movement Behaviors via Hippocampal CA3 Pyramidal Neurons Afferent to Dorsolateral Septum. Biol
1187 89. Wang F, Hamilton JA, Kamp F, et al. Movement of fatty acids, fatty acid analogues, and bile acids
1189 81. Abdo Qaid EY, Abdull
-
- Phychiatry 2021;89:1084-1095.

1188 90. Kamp F, Hamilton JA, Kamp F, et al. Movement of fatty acids, fatty acid analogues, and bile acids

1189 1. Abdo Qaid EY, Abdullah Z, Zakaria R, et al. Minocycline Attenuates Lipopoly 1188 90. Kamp F, Hamilton JA, Kamp F, e
1189 across phospholipid bilayers. Bio
1190 91. Abdo Qaid EY, Abdullah Z, Zaka
1191 Locomotor Deficit and Anxiety-
1192 the Rat Medial Prefrontal Corte 1189 across phospholipid bilayers. Biochemistry 1993;32:11074-86.

1190 91. Abdo Qaid EY, Abdullah Z, Zakaria R, et al. Minocycline Attenuates Lipopolysaccharide-Induced

1191 Locomotor Deficit and Anxiety-like Behavior an 1190 91. Abdo Qaid EY, Abdullah Z, Zakaria R, et al. Minocycline Atten
1191 Locomotor Deficit and Anxiety-like Behavior and Related Expre
1192 the Rat Medial Prefrontal Cortex (mPFC). Int J Mol Sci 2022;23.
1193 92. Obata Locomotor Deficit and Anxiety-like Behavior and Related Expression of the BDNF/CREB Protein in
1192 the Rat Medial Prefrontal Cortex (mPFC). Int J Mol Sci 2022;23.
1193 92. Obata Y, Furusawa Y, Endo TA, et al. The epigenet
- the Rat Medial Prefrontal Cortex (mPFC). Int J Mol Sci 2022;23.

1193 92. Obata Y, Furusawa Y, Endo TA, et al. The epigenetic regulator Uhrf1 facilitates the proliferation

1194 and maturation of colonic regulatory T cells 92. Chata Y, Furusawa Y, Endo TA, et al. The epigenetic regulator
1194 and maturation of colonic regulatory T cells. Nat Immunol 2014
1195 93. Ansari I, Raddatz G, Gutekunst J, et al. The microbiota programitestinal homeos
- 1194 and maturation of colonic regulatory T cells. Nat Immunol 2014;15:571-9.

1195 93. Ansari I, Raddatz G, Gutekunst J, et al. The microbiota programs DNA methylation to control

1196 intestinal homeostasis and inflammat 1195 93. Ansari I, Raddatz G, Gutekunst J, et al. The microbiota programs DNA intestinal homeostasis and inflammation. Nat Microbiol 2020;5:610-619.
1197 94. Ramos-Molina B, Sanchez-Alcoholado L, Cabrera-Mulero A, et al. G
- 1196 1196 intestinal homeostasis and inflammation. Nat Microbiol 2020;5:610-619.
1197 94. Ramos-Molina B, Sanchez-Alcoholado L, Cabrera-Mulero A, et al. Gut Microbiota Composition Is
1198 Associated With the Global DNA Met 1197 94. Ramos-Molina B, Sanchez-Alcoholado L, Cabrera-Mulero A, et al. Gut Microbiol 2020
1198 Associated With the Global DNA Methylation Pattern in Obesity. Front Ge
1198 1198 Associated With the Global DNA Methylation Pattern in Obesity. Front Genet 2019;10:613.
Associated With the Global DNA Methylation Pattern in Obesity. Front Genet 2019;10:613. 1198 Associated With the Global DNA Methylation Pattern in Obesity. Front Genet 2019;10:613.

-
-
- 1200 predominantly associated with H3K27me3. Nat Commun 2021;12:3804.
1201 96. Argaw-Denboba A, Schmidt TSB, Di Giacomo M, et al. Paternal microbiome perturbations impact
1202 offspring fitness. Nature 2024;629:652-659.
12 1201 96. Argaw-Denboba A, Schmidt TSB, Di Giacomo M, et al. Paternal microbior
1202 offspring fitness. Nature 2024;629:652-659.
1203 97. Kilama J, Dahlen CR, Reynolds LP, et al. Contribution of the seminal
1204 programming
- offspring fitness. Nature 2024;629:652-659.

1203 97. Kilama J, Dahlen CR, Reynolds LP, et al. Contribution of the seminal microbiome to paternal

1204 programming. Biol Reprod 2024;111:242-268.

1205 98. Masson BA, Kiride 1203 97. Kilama J, Dahlen CR, Reynolds LP, et al. Compress. Natural programming. Biol Reprod 2024;111:242-263.
1205 98. Masson BA, Kiridena P, Lu D, et al. Depletion
1206 RNAs and impacts offspring physiology and
1207 305. 1204 programming. Biol Reprod 2024;111:242-268.

1205 98. Masson BA, Kiridena P, Lu D, et al. Depletion of the paternal gut microbiome alters sperm small

1206 RNAs and impacts offspring physiology and behavior in mice. Br 1205 98. Masson BA, Kiridena P, Lu D, et al. Depletion (1206 RNAs and impacts offspring physiology and be
1207 305.
1208 99. Radeva MY, Waschke J. Mind the gap: mec
1209 Physiol (Oxf) 2018;222. 1206 88. MASSON BA, MASCHE, MIND A
1208 99. Radeva MY, Waschke J. Mind the gap: mechanisms regulating the endothelial barrier. Acta
1209 Physiol
-
- 1207 305.
1208 99. Radeva MY, Waschke J. Mind the gap: mechanisms regulating the endothelial barrier. Acta
1209 Physiol (Oxf) 2018;222.
1210 100. Gutierrez-Rodelo C, Martinez-Tolibia SE, Morales-Figueroa GE, et al. Modulat 1208 99. Rade
1209 Physi
1210 100. Gutie
1211 nucle
1212 Moll 1209 9. Physiol (Oxf) 2018;222.
1210 100. Gutierrez-Rodelo C, Martinez-Tolibia SE, Morales-Figueroa GE, et al. Modulating cyclic
1211 nucleotides pathways by bioactive compounds in combatting anxiety and depression disorde 1210 100. Gutierrez-Rodelo C, N
1211 nucleotides pathways b
1212 Mol Biol Rep 2023;50:7
1213 101. Hernandez-Vasquez MI
1214 adhesion G protein-co
- nucleotides pathways by bioactive compounds in combatting anxiety and depression disorders.

1212 Mol Biol Rep 2023;50:7797-7814.

1213 101. Hernandez-Vasquez MN, Adame-Garcia SR, Hamoud N, et al. Cell adhesion controlled Mol Biol Rep 2023;50:7797-7814.

1213 101. Hernandez-Vasquez MN, Adame-Garcia SR, Hamoud N, et al. Cell adhesion controlled by

1214 adhesion G protein-coupled receptor GPR124/ADGRA2 is mediated by a protein complex

1215 1213 101. Hernandez-Vasquez MN, Adame
1214 adhesion G protein-coupled rec
1215 comprising intersectins and Elmo-
1216 102. Insel PA, Snead A, Murray F, et al.
1217 being used as drug targets? Br J Pl 214 adhesion G protein-coupled receptor GPR124/ADGRA2 is mediated by a protein complex
1215 comprising intersectins and Elmo-Dock. J Biol Chem 2017;292:12178-12191.
1216 102. Insel PA, Snead A, Murray F, et al. GPCR expres
-
- 1215 comprising intersectins and Elmo-Dock. J Biol Chem 2017;292:12178-12191.

1216 102. Insel PA, Snead A, Murray F, et al. GPCR expression in tissues and cells: are the optimal receptors

1217 being used as drug targets? 1216 102. Insel PA, Snead A, Murray F, et al. GPCR expression in tissues and cells: are th
1217 being used as drug targets? Br J Pharmacol 2012;165:1613-1616.
1218 103. Hakak Y, Shrestha D, Goegel MC, et al. Global analysi being used as drug targets? Br J Pharmacol 2012;165:1613-1616.

1218 103. Hakak Y, Shrestha D, Goegel MC, et al. Global analysis of G-protein-coupled receptor signaling in

1220 104. Smith CA, O'Flaherty EAA, Guccio N, et 1218 103. Hakak Y, Shrestha D, Goegel MC, et al. Global analysis of G-prote
1219 human tissues. FEBS Lett 2003;550:11-7.
1220 104. Smith CA, O'Flaherty EAA, Guccio N, et al. Single-cell transcript
1221 cells along the muri
-
- 1219 103. Haman Yissues. FEBS Lett 2003;550:11-7.
1220 104. Smith CA, O'Flaherty EAA, Guccio N, et al. Single-cell transcriptomic atlas of enteroendocrine
1221 cells along the murine gastrointestinal tract. PLoS One 2024;1 1220 104. Smith CA, O'Flaherty EAA, Guccio N, et
1221 cells along the murine gastrointestinal tra
1222 105. Egerod KL, Petersen N, Timshel PN, et
1223 afferents reveals novel gut-to-brain sensii
1224 106. Ito J, Ito M, Nam
- 1220 104. Smith CA, O'Flaherty EAA, Guccio N, et al. Single-cell transcriptomic atlas of enteroendocrine

1221 cells along the murine gastrointestinal tract. PLoS One 2024;19:e0308942.

1222 105. Egerod KL, Petersen N, Tim cells along the murine gastrointestinal tract. PLoS One 2024;19:e0308942.

1222 105. Egerod KL, Petersen N, Timshel PN, et al. Profiling of G protein-coupled receptors in vagal

1223 afferents reveals novel gut-to-brain se 1223 afferents reveals novel gut-to-brain sensing mechanisms. Mol Metab 2018;12:62-75.

1224 106. Ito J, Ito M, Nambu H, et al. Anatomical and histological profiling of orphan G-protein-coupled

1225 receptor expression in
- 1224 106. Ito J, Ito M, Nambu H, et al. Anatomical and histological profiling of orphan G-prot
1225 receptor expression in gastrointestinal tract of C57BL/6J mice. Cell Tissue Res 2009;33
1226 107. Quesseveur G, David D, G receptor expression in gastrointestinal tract of C57BL/6J mice. Cell Tissue Res 2009;338:257-69.

1226 107. Quesseveur G, David D, Gaillard M, et al. BDNF overexpression in mouse hippocampal astrocytes

1227 promotes local 1226 107. Quesseveur G, David D, Gaillard M, et al. BDNF overexpression in mouse hippocampal astrocyte
1227 http://www.fasterointer. Cell neurogenesis and elicits anxiolytic-like activities. Translational psychiatr
1229 ht 1227 promotes local neurogenesis and elicits anxiolytic-like activities. Translational psychiatry
1228 2013;3:e253-e253.
1229 **Figure legends** 1228 promotes local neurogenesis and elicits and elicity, and elicities and psychiatry
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1232 **Figure 1. Micr**e
1233 s<mark>ynthesis or c</mark> 1232
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1234 **Figure 1. Microbiomes linked to fetal programming of complex behaviors differ in synthesis or catabolism of several metabolites including bile acids. (A) Hierarchical clustering of data from adult GM^{Low}- or GM^{High}-col synthesis or catabolism of several metabolites including bile acids. (A)** Hierarchical
1234 clustering of data from adult GM^{Low}- or GM^{High}-colonized mice, demonstrating segregation of
1235 GMs by richness and composit clustering of data from adult GM^{Low} - or GM^{High} -colonized mice, demonstrating segregation of clustering of data from adult GM^{Low} - or GM^{High} -colonized mice, demonstrating segregation of GMs by richness and composition (legend at right). Volcano plots showing metabolites enriched in GM^{Low} (red dots) or GM^{High} (bl 1235 GMs by richness and composition (legend at right). Volcano plots showing metabolites enriched

1236 in GM^{Low} (red dots) or GM^{High} (blue dots) **(B)** feces or **(C)** serum. Horizontal dashed line

1237 indicates sig in GM^{Low} (red dots) or GM^{High} in GM^{Low} (red dots) or GM^{High} (blue dots) **(B)** feces or **(C)** serum. Horizontal dashed line

indicates significance of p<0.05 between GM^{Low} and GM^{High} mice by Wilcox rank sum test.

Normalized ileal expression of indicates significance of p<0.05 between GM^{Low} and GM^{High} indicates significance of p<0.05 between GM^{Low} and $GM^{\text{"}}$ mice by Wilcox rank sum test.
1238 Normalized ileal expression of Asbt (D), Ost β (E), Gpbar1 (F), and Fxr (G) male (M) and female
1239 (F) mice colonized 1238 Normalized ileal expression of *Asbt* (**D**), *Ostβ* (**E**), *Gpbar1* (**F**), and *Fxr* (**G**) male (M) and female (F) mice colonized with GM^{Low} (red dots) or GM^{High} (blue dots). Normalized hepatic expression of *Cyp* (F) mice colonized with GM^{Low} (red dots) or GM^{High} 1239 (F) mice colonized with GM^{Low} (red dots) or GM^{Hign} (blue dots). Normalized hepatic expression
1240 of *Cyp7a1* (H), *Slc10a1* (I), and *S1pr2* (J) in the same groups of mice. Gene expression 1240 of *Cyp7a1* (**H**), *Slc10a1* (**I**), and *S1pr2* (**J**) in the same groups of mice. Gene expression 1241 normalized to *β-Actin* expression. Results indicate main effect of GM in one- or two-way

1242 ANOVA, following correction for multiple tests. * p<0.05, ** p<0.01, **** p<0.0001. Abbreviations:

1243 2-HBA (2-hydrox ANOVA, following correction for multiple tests. * p<0.05, ** p<0.01, **** p<0.0001. Abbreviations:

1243 2-HBA (2-hydroxybutyrate), α-MCA (alpha-Muricholic Acid), β-ALA (beta-Alanine), BUTY

1244 (Butyrate), CA (Cholic Ac 1243 2-HBA (2-hydroxybutyrate), α-MCA (alpha-Muricholic Acid), β-ALA (beta-Alanine), BUTY
1244 (Butyrate), CA (Cholic Acid), CDCA (Chendeoxycholic Acid), CYS (Cysteine), DCA (Deoxycholic
1245 Acid), F6P (Fructose-6-Phosph 1244 (Butyrate), CA (Cholic Acid), CDCA (Chendeoxycholic Acid), CYS (Cysteine), DCA (Deoxycholic
1245 Acid), F6P (Fructose-6-Phosphate), G6P (Glucose-6-Phosphate), GCA (Glycocholic Acid), GLY
1246 (Glycerate), INO (Inosito Acid), F6P (Fructose-6-Phosphate), G6P (Glucose-6-Phosphate), GCA (Glycocholic Acid), GLY
1246 (Glycerate), INO (Inositol), LAC (Lactate), LEU (Leucine), MVA (Mevalonic Acid), R5P
1247 (Ribulose-5-Phosphate), SUC (Succinat 1246 (Glycerate), INO (Inositol), LAC (Lactate), LEU (Leucine), MVA (Mevalonic Acid), R5P
1247 (Ribulose-5-Phosphate), SUC (Succinate), TCA (Taurocholic Acid), TCDCA
1248 (Taurochenodeoxycholic Acid), TDCA (Taurodeoxycholi 1247 (Ribulose-5-Phosphate), SUC (Succinate), TCA (Taurocholic Acid), TCDCA
1248 (Taurochenodeoxycholic Acid), TDCA(Taurodeoxycholic Acid), TLCA(Taurolithocholic Acid),
1249 UDCA(Ursodeoxycholic Acid).
1250 **Figure 2. Anxi**

1248 (Taurochenodeoxycholic Acid), TDCA (Taurodeoxycholic Acid), TLCA (Taurolithocholic Acid),
1249 UDCA (Ursodeoxycholic Acid).
1250 **Figure 2. Anxiety-related behavior and other outcomes in offspring are influenced by th** 1249 UDCA (Ursodeoxycholic Acid).
1250 **Figure 2. Anxiety-related beh**
1251 **maternal gut microbiome. (1**
1252 outcome measures. Behavior Figure 2. Anxiety-related behavior and other outcomes in offspring are influenced by the
1251 **maternal gut microbiome. (A)** Schematic showing the experimental groups and timing of
1252 or GM^{High}, including (B) time spen maternal gut microbiome. (A) Schematic showing the experimental groups and timing of
1252 outcome measures. Behavior outcomes in male (M) and female (F) mice colonized with GM^{Low}
1253 or GM^{High}, including (B) time spen outcome measures. Behavior outcomes in male (M) and female (F) mice colonized with GM^{Low} 1253
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1256 or $\mathsf{GM}^{\mathsf{High}}$ or GM^{High}, including (**B**) time spent in the light portion of a light/dark test, (**C**) time spent in the
1254 open arms of an elevated plus maze, and distance run per day (Km) by (**D**) male and (**E**) female
1255 mice. (open arms of an elevated plus maze, and distance run per day (Km) by (**D**) male and (**E**) female
1255 mice. (**F)** Body weight per mouse (grams) at day 50 in control mice. (**G-J)** Behavior outcomes in
1256 CF^{Low} and CF^{Hi} mice. (**F)** Body weight per mouse (grams) at day 50 in control mice. (**G-J)** Behavior outcomes in
1256 CF^{Low} and CF^{High} mice as those shown in panels **B-E. (K)** Body weight per mouse at day 50 in
1257 cross-fostered mi $\mathsf{CF}^{\mathsf{Low}}$ and $\mathsf{CF}^{\mathsf{High}}$ 1256 CF^{Low} and CF^{High} mice as those shown in panels **B-E. (K)** Body weight per mouse at day 50 in
1257 cross-fostered mice. p and F values represent effect of GM in two-way ANOVA with Tukey post
1258 hoc. Significant s cross-fostered mice. p and F values represent effect of GM in two-way ANOVA with Tukey post
1258 hoc. Significant sex-dependent differences were detected in distance on running wheels and
1259 body weight in both control a hoc. Significant sex-dependent differences were detected in distance on running wheels and
1259 body weight in both control and cross-fostered mice, with no significant interactions between
1260 GM and sex in any tests. *

1262 **hippocampus. (A)** Dot plot showing normalized difference $[Log_2(FC)]$ in mean methylation 1260 GM and sex in any tests. * p<0.05, ** p<0.01, **** p<0.0001
1261 **Figure 3. Maternal microbiome is associated with**
1262 **hippocampus. (A)** Dot plot showing normalized difference
1263 between GM^{Low} and GM^{High}, an **Figure 3. Maternal microbiome is associated with gene methylation in offspring hippocampus. (A)** Dot plot showing normalized difference $[Log_2(FC)]$ in mean methylation between GM^{Low} and GM^{High} , and between CF^{Low} and CF^{High **hippocampus. (A)** Dot plot showing normalized difference [Log₂(FC)] in mean methylation
1263 between GM^{Low} and GM^{High}, and between CF^{Low} and CF^{High}, of all CpG markers achieving a
1264 Log₂(FC) > 1 in either between GM^{Low} and GM^{High}, and between CF^{Low} and CF^{High} between GM^{Low} and GM^{High}, and between CF^{Low} and CF^{High}, of all CpG markers achieving a
1264 Log₂(FC) > 1 in either comparison. **(B)** Ratio of observed to predicted differentially methylated
1265 regions (DMRs), Log₂(FC) > 1 in either comparison. **(B)** Ratio of observed to predicted differentially methylated
1265 regions (DMRs), and locations of DMRs relative to gene bodies, on each chromosome. **(C)**
1266 Mean beta values of 27 regions (DMRs), and locations of DMRs relative to gene bodies, on each chromosome. **(C)**
1266 Mean beta values of 27 CpG sites spanning the enhancers, promoters, introns and exons
1267 across the *Pde1c* gene in all four g 1266 Mean beta values of 27 CpG sites spanning the enhancers, promoters, introns and exons

1267 across the Pde1c gene in all four groups, with specific sites indicated above (upper); and

1268 $log_2(FC)$ between control an across the *Pde1c* gene in all four groups, with specific sites indicated above (upper); and
1268 log₂(FC) between control and cross-fostered groups (lower). Arrows on X-axis indicate direction
1269 of transcription. Num 1268 log₂(FC) between control and cross-fostered groups (lower). Arrows on X-axis indicate direction
1269 of transcription. Numbers on the X-axis indicated number of CpG sites analyzed. **(D)** Mean beta
1270 values (uppe of transcription. Numbers on the X-axis indicated number of CpG sites analyzed. **(D)** Mean beta
1270 values (upper) and Log₂(FC) between groups (lower) across adjacent regions of the positive
1271 (left) and negative (ri 1270 values (upper) and Log₂(FC) between groups (lower) across adjacent regions of the positive
1271 (left) and negative (right) strand of a region of chromosome 14 containing genes for *Ang5*,
1272 *Ang6*, and other ge 1271 (left) and negative (right) strand of a region of chromosome 14 containing genes for *Ang5*,
1272 *Ang6*, and other genes. (E) Protein interaction networks among products of DMRs, with node
color indicating Log₂(FC) 1272 Ang6, and other genes. **(E)** Protein interaction networks among products of DMRs, with node
1273 color indicating Log₂(FC) in methylation between GM^{Low} and GM^{High}. color indicating $Log_2(FC)$ in methylation between GM^{Low} and GM^{High} . 1273 color indicating Log₂(FC) in methylation between GM^{Low} and GM^{High}.

The color indicating Log₂(FC) in methylation between GM^{Low} and GM^{High}.

1274 **Figure 4.** Interaction networks constructed using differentially expressed genes (DEGs) identified in hippocampal endothelial and perivascular cells of control (A) and cross-fostered (B) mice. Highlighted nodes inclu identified in hippocampal endothelial and perivascular cells of control (**A**) and cross-fostered (**B**)
1276 mice. Highlighted nodes include DEGs identified in both comparisons and showing a pattern of
1277 fetal imprinting mice. Highlighted nodes include DEGs identified in both comparisons and showing a pattern of

1277 fetal imprinting, and genes that are also differentially methylated (*Pde1c*, *Dock1*), or closely

1278 related (PDE clus fetal imprinting, and genes that are also differentially methylated (*Pde1c*, *Dock1*), or closely

1278 related (PDE cluster, *Dcc, Elmo1*, *Dock4*, *Dock5*). **c, d.** Log₂FC in expression of (**C**) Pde1c and

1279 (D) Do related (PDE cluster, *Dcc*, *Elmo1*, *Dock4*, *Dock5*). **c, d.** Log₂FC in expression of (**C**) Pde1c and (1279 (D) Dock1 in hippocampal cell clusters of male and female CF^{Low} and CF^{High} mice, as determined via real-t (D) Dock1 in hippocampal cell clusters of male and female CF^{Low} and CF^{High} 1279 **(D)** Dock1 in hippocampal cell clusters of male and female CF^{row} and CF^{right} mice, as
1280 determined via real-time snRNAseq. **(E)** Venn diagram showing number of inferred cell-to-cell
1281 communication pathways id determined via real-time snRNAseq. **(E)** Venn diagram showing number of inferred cell-to-cell
1281 communication pathways identified in hippocampus of GM^{Low}, GM^{High}, CF^{Low}, and CF^{High} mice.
1282 Pathways listed abo communication pathways identified in hippocampus of GM^{Low} , GM^{High} , CF^{Low} , and CF^{High} mice, 1281 communication pathways identified in hippocampus of GM^{Low}, GM^{High}, CF^{Low}, and CF^{High} mice.
1282 Pathways listed above and below the diagram were selectively identified in the indicated
1283 groups. Bar charts Pathways listed above and below the diagram were selectively identified in the indicated
1283 groups. Bar charts showing the relative degree of cell-to-cell communication between (F) GM^{Low}
1284 and GM^{High}, and (G) CF^L groups. Bar charts showing the relative degree of cell-to-cell communication between (F) GM^{Low} 1284
1285
1286 and GM^{High}, and (G) CF^{Low} , and CF^{High} mice in the indicated pathways.

Supplementary Figures
 Figure S1. Cladogram representing genera taxa identified within each phyla. Arrows indicate

genus with statistically significant abundance differences. Wilcox rank sum test with Benjamini-1287
1288 1286 **Supplementary Figures**
1287 **Figure S1.** Cladogram re
1288 genus with statistically sig
1289 Hochberg corrected p valu Figure S1. Cladogram representing genera taxa identified within each phyla. Arrows indicate
1288 genus with statistically significant abundance differences. Wilcox rank sum test with Benjamini-
1289 Hochberg corrected p va

genus with statistically significant abundance differences. Wilcox rank sum test with Benjamini-
1289 Hochberg corrected p values. List of genera identified provided in **Table S3**.
Figure S2. Volcano plots showing the me Hochberg corrected p values. List of genera identified provided in **Table S3**.
1290 **Figure S2.** Volcano plots showing the metabolites enriched in feces of G
1291 **GM^{High} (blue dots) (A)** males, and **(B)** females. Volcan **Figure S2.** Volcano plots showing the metabolites enriched in feces of GM^{Low} (red dots) and GM^{High} (blue dots) (A) males, and (B) females. Volcano plots showing the metabolites enriched in serum of GM^{Low} (red dots) a GM^{High} 1291 **GM^{High}** (blue dots) **(A)** males, and **(B)** females. Volcano plots showing the metabolites enriched
1292 in serum of GM^{Low} (red dots) and GM^{High} (blue dots) **(C)** males, and **(D)** females and Dot plots
1293 rep in serum of GM^{Low}(red dots) and GM^{High} in serum of GM^{Low} (red dots) and GM^{high} (blue dots) **(C)** males, and **(D)** females and Dot plots
representing **(E)** gene and **(F)** the normalized (transcript/gene) expression in the feces of adult
female mice colonized w representing **(E)** gene and **(F)** the normalized (transcript/gene) expression in the feces of adult
1294 female mice colonized with GM^{Low} or GM^{High} (*n* = 3/GM). Box plots and individual data showing
1295 hydrolytic ac female mice colonized with GM^{Low} or GM^{High} 1294 female mice colonized with GM^{Low} or GM^{High} ($n = 3$ /GM). Box plots and individual data showing
1295 hydrolytic activity of bile salt hydrolase (BSH) in adult male and female mice colonized with
1296 GM^{Low} or GM hydrolytic activity of bile salt hydrolase (BSH) in adult male and female mice colonized with

1296 GM^{Low} or GM^{High}, determined using five different bile acid-conjugated bioluminescent probes

1297 specific for (G) ch $\mathsf{GM}^{\mathsf{Low}}$ or $\mathsf{GM}^{\mathsf{High}}$ 1296 GM^{Low} or GM^{High} , determined using five different bile acid-conjugated bioluminescent probes

1297 specific for (G) cholic acid (CA), (H) deoxycholic acid (DCA), (I) chenodeoxycholic acid (CDCA),

1298 (J) ursodeoxyc 1297 specific for **(G)** cholic acid (CA**), (H)** deoxycholic acid (DCA), **(I)** chenodeoxycholic acid (CDCA), 1298 **(J)** ursodeoxycholic acid (UDCA), and **(K)** lithocholic acid (LCA). *p<0.05, **** p<0.0001.

Abbreviations: (**J)** ursodeoxycholic acid (UDCA), and (**K**) lithocholic acid (LCA). *p<0.05, **** p<0.0001.

Abbreviations: 2-HGA (2-hydroxygluterate), 3-HPA (3-Hydroxyproprionate), α-KGA (alpha-

Ketogluterate), α-KIC (alpha-Ketoisocap 1299 Abbreviations: 2-HGA (2-hydroxygluterate), 3-HPA (3-Hydroxyproprionate), α-KGA (alpha-
1300 Ketogluterate), α-KIC (alpha-Ketoisocaproate), ASP (Asparagine), β-ALA (beta-Alanine), CDCA
1301 (Chendeoxycholic Acid), CYS Retogluterate), α-KIC (alpha-Ketoisocaproate), ASP (Asparagine), β-ALA (beta-Alanine), CDCA
1301 (Chendeoxycholic Acid), CYS (Cysteine), DCA (Deoxycholic Acid), F6P (Fructose-6-Phosphate),
1302 G6P (Glucose-6-Phosphate), (Chendeoxycholic Acid), CYS (Cysteine), DCA (Deoxycholic Acid), F6P (Fructose-6-Phosphate),
1302 G6P (Glucose-6-Phosphate), GLY (Glycerate), IAA (Indoleacetic Acid), ISO (Isocitrate), LAC
1303 (Lactate), LCA (Lithocholic A 1302 G6P (Glucose-6-Phosphate), GLY (Glycerate), IAA (Indoleacetic Acid), ISO (Isocitrate), LAC
1303 (Lactate), LCA (Lithocholic Acid), LEU (Leucine), MAL (Malate), MET (Methionine), ORN
1304 (Ornithine), PHEN (Phenylalani 1303 (Lactate), LCA (Lithocholic Acid), LEU (Leucine), MAL (Malate), MET (Methionine), ORN
1304 (Ornithine), PHEN (Phenylalanine), PRO (Proline), PTA (Pantothenic Acid), R5P (Ribulose-5-
1305 Phosphate), SUC (Succinate), T 1304 (Ornithine), PHEN (Phenylalanine), PRO (Proline), PTA (Pantothenic Acid), R5P (Ribulose-5-
1305 Phosphate), SUC (Succinate), TDCA (Taurodeoxycholic Acid), TRY (Tryptamine), VAL (Valine).
1305 Phosphate), SUC (Succinat 1305 Phosphate), SUC (Succinate), TDCA (Taurodeoxycholic Acid), TRY (Tryptamine), VAL (Valine).

1306 **Figure S3.** Spearman correlation coefficient plots comparing relative abundance at the genera

1307 level and metabolite concentrations to the p value of the Spearman correlation. Spearman

1308 correlation coeffici 1307 level and metabolite concentrations to the p value of the Spearman correlation. Spearman
1308 correlation coefficient is plotted along the x-axis for each metabolite, and -Log₁₀(p value) of the
1309 correlation bet 1308 correlation coefficient is plotted along the x-axis for each metabolite, and -Log₁₀(p value) of the correlation between relative taxa abundance and metabolite concentration is plotted on the y-
1310 axis. Red dots 1309 correlation between relative taxa abundance and metabolite concentration is plotted on the y-
1310 axis. Red dots represent genera with an increased relative abundance in GM^{Low}, and blue dots
1311 represent genera axis. Red dots represent genera with an increased relative abundance in GM^Low 2310 axis. Red dots represent genera with an increased relative abundance in GM^{Low} , and blue dots
1311 represent genera with an increased relative abundance in GM^{High} . Dotted lines in each plot
1312 represent statistical represent genera with an increased relative abundance in GM^{High}

represent genera with an increased relative abundance in GM^{Hign} . Dotted lines in each plot
1312 represent statistical significance of p<0.05.
1313 **Figure S4.** Dot and bar plots showing other results of behavior tests i 1312 represent statistical significance of p<0.05.
1313 **Figure S4.** Dot and bar plots showing of
1314 female (F) GM^{Low} and GM^{High} mice include
1315 light/dark test and (B) open arms of the ele **Figure S4.** Dot and bar plots showing other results of behavior tests in adult male (M) and
1314 female (F) GM^{Low} and GM^{High} mice including (A) distance traveled in the light portion of the
1315 light/dark test and (female (F) GM^{Low} and GM^{High} 1314 female (F) GM^{Low} and GM^{righ} mice including (**A**) distance traveled in the light portion of the light/dark test and (**B**) open arms of the elevated plus maze. (**C**) Total distance traveled and (**D**) time spent in 1315 light/dark test and (**B**) open arms of the elevated plus maze. (**C**) Total distance traveled and (**D**) time spent in the center zone during OFE. (**E**) Body weight at 21 days of age of GM^{Low} and GM^{High} mice. (**F-J** time spent in the center zone during OFE. (E) Body weight at 21 days of age of GM^{Low} and 1316 time spent in the center zone during OFE. (**E**) Body weight at 21 days of age of GM^{LOW} and
1317 GM^{High} mice. (**F-J**) Same outcomes in CF^{LOW} and CF^{High} mice as those shown in panels **A-D. (J)**
1318 Body weight GM^{High} mice. (**F-J**) Same outcomes in CF^{Low} and CF^{High}

1317 GM^{High} mice. (F-J) Same outcomes in CF^{Low} and CF^{High} mice as those shown in panels **A-D. (J)**
1318 Body weight at 21 days of age of CFLow and CFHigh mice. * p<0.05, ** p<0.01, **** p<0.0001.
1319 **Figure S5.** Do 1318 Body weight at 21 days of age of CFLow and CFHigh mice. * p<0.05, ** p<0.01, **** p<0.0001.

1319 **Figure S5.** Dot and bar plots showing differences in (A) richness and principal coordinate

1320 analysis (PCoA) plots **Figure S5.** Dot and bar plots showing differences in (A) richness and principal coordinate analysis (PCoA) plots showing differences in beta-diversity based on (B) Jaccard and (C) Bray-
Curtis distances, between adult mal 1320 analysis (PCoA) plots showing differences in beta-diversity based on (**B**) Jaccard and (**C**) Bray-
1321 Curtis distances, between adult male (M) and female (F) mice colonized with GM^{Low} or GM^{High},
1323 cognate bir Curtis distances, between adult male (M) and female (F) mice colonized with GM^{Low} or GM^{High} Curtis distances, between adult male (M) and female (F) mice colonized with GM^{Low} or GM^{High},
1322 and similarities in all of the above metrics between CF mice at seven weeks of age and their
1323 cognate birth dams.

1322 and similarities in all of the above metrics between CF mice at seven weeks of age and their
1323 cognate birth dams.
1324 **Figure S6.** UMAP projections of hippocampal cell clusters in (A) control GM^{Low} and (B) GM 1323 cognate birth dams.
1324 **Figure S6.** UMAP pl
1325 and cross-foster (C)
1326 **Figure S7.** Dot plot **Figure S6.** UMAP projections of hippocampal cell clusters in (A) control GM^{Low} and (B) GM^{High} and cross-foster **(C)** CF^{Low} and **(D)** CF^{High}

Figure S6. UMAP projections of hippocampal cell clusters in (A) control GM^{row} and (B) GM^{right} ,
1325 and cross-foster (C) CF^{Low} and (D) CF^{High} mice.
1326 Figure S7. Dot plot correlation between the number of differentiall 1325 and cross-foster (C) CF^{Low} and (D) CF^{High} mice.
1326 **Figure S7.** Dot plot correlation between the nu
1327 and the mean node degree determined by p
1328 comparisons of (A) GM^{Low} and GM^{High} offspring **Figure S7.** Dot plot correlation between the number of differentially expressed genes (DEGs)

and the mean node degree determined by protein interaction analysis of those DEGs, in

1328 comparisons of (A) GM^{Low} and GM^H 1327 and the mean node degree determined by protein interaction analysis of those DEGs, in

1328 comparisons of (A) GM^{Low} and GM^{High} offspring and in (B) CF^{Low} and CF^{High} offspring. Marker

1329 shapes denote cell ty comparisons of **(A)** GM^{Low} and GM^{High} offspring and in **(B)** CF^{Low} and CF^{High} 1328 comparisons of (A) GM^{Low} and GM^{Log} offspring and in (B) CF^{Low} and CF^{Log} offspring. Marker
1329 shapes denote cell type including endothelial/perivascular cells (Endo, red squares); astrocytes
1330 and oligodendrocytes (Glial, grey diamonds); GABAergic neurons (GABA, dark blue triangles);
1331 and glutamatergic neurons (Gluta, brown triangles).
1332 **Figure S8.** Interaction networks constructed using differentially e

and oligodendrocytes (Glial, grey diamonds); GABAergic neurons (GABA, dark blue triangles);
1331 and glutamatergic neurons (Gluta, brown triangles).
1332 **Figure S8.** Interaction networks constructed using differentially e and glutamatergic neurons (Gluta, brown triangles).
1332 **Figure S8.** Interaction networks constructed usi
1333 identified in hippocampal level five intratelencepha
1334 from (A) control and (B) cross-fostered mice. Lab identified in hippocampal level five intratelencephalon (L5 IT)-projecting glutamatergic neurons
from (A) control and (B) cross-fostered mice. Labeled nodes include DEGs identified in both
comparisons and showing a pattern from (A) control and (B) cross-fostered mice. Labeled nodes include DEGs identified in both 1334 from **(A)** control and **(B)** cross-fostered mice. Labeled nodes include DEGs identified in both
1335 comparisons and showing a pattern of fetal imprinting, and genes that are also differentially
1336 methylated (*Dock* 1335 comparisons and showing a pattern of fetal imprinting, and genes that are also differentially
1336 methylated (*Dock1*), or closely related (PDE cluster, *Adcy8*, *Dcc*, *Ntn1*). 1336 methylated (*Dock1*), or closely related (PDE cluster, *Adcy8*, *Dcc*, *Ntn1*).

Figure S9. Interaction networks constructed using differentially expressed genes (DEGs) identified in hippocampal astrocyte subsets from (A) control and (B) cross-fostered mice. Labeled nodes include DEGs identified in b

- Labeled nodes include DEGs identified in both comparisons and showing a pattern of fetal
- Labeled nodes include DEGs identified in both comparisons and showing a pattern of fetal
1340 imprinting, and genes that are also differentially methylated (*Dock1*), or closely related (PDE
1341 cluster, Adcy8, Dcc, Ntn1) 1340 imprinting, and genes that are also differentially methylated (*Dock1*), or closely related (PDE
1341 cluster, *Adcy8*, *Dcc*, *Ntn1*).
1342 **Supplementary Tables**
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1343 **Supplementary Tables**
1344 **Table S1.** Primer pairs used for qRT-PCR analysis.
- 1342 1343 **Supplementary Tables**
1344 **Table S1.** Primer pairs u
1345 **Table S2.** Relative abune
1346 **Table S3.** Fecal metabol Table S1. Primer pairs used for qRT-PCR analysis.
1345 **Table S2.** Relative abundance of family taxa harbore
1346 **Table S3.** Fecal metabolite concentrations from GM
1347 **Table S4.** Serum metabolite concentrations from GM **Table S2.** Relative abundance of family taxa harbored by either GM^{Low} or GM^{High}
- Table S3. Fecal metabolite concentrations from GM^{Low} and GM^{High} mice.
- **Table S4.** Serum metabolite concentrations from GM^{Low} and GM^{High} mice.
- Table S2. Relative abundance of family taxa harbored by either GM^{Low} or GM^{High}.
1346 **Table S3.** Fecal metabolite concentrations from GM^{Low} and GM^{High} mice.
1347 **Table S4.** Serum metabolite concentrations from GM **Table S5.** Relative abundance of identified genera in GM^{Low} and GM^{High}
- Table S3. Fecal metabolite concentrations from GM^{Low} and GM^{High} mice.
1347 **Table S4.** Serum metabolite concentrations from GM^{Low} and GM^{High} mice
1348 **Table S5.** Relative abundance of identified genera in GM^{Low} and GM **Table S4.** Serum metabolite concentrations from GM^{Low} and GM^{High} mice.

1348 **Table S5.** Relative abundance of identified genera in GM^{Low} and GM^{High} m
 1349 Table S6. Comparison of identified bacterial genera abundan Table S5. Relative abundance of identified genera in GM^{Low} and GM^{High} mice.

1349 Table S6. Comparison of identified bacterial genera abundance to statistically significant fecal

1350 metabolite concentrations between metabolite concentrations between GM^{Low} and GM^{High} mice.
- Table S6. Comparison of identified bacterial genera abundance to statistically significant fecal
1350 metabolite concentrations between GM^{Low} and GM^{High} mice.
1351 Table S7. Differentially methylated regions identified metabolite concentrations between GM^{Low} and GM^{High} mice.
1351 **Table S7.** Differentially methylated regions identified within
1352 **GM^{High} mouse hippocampi.**
1353 **Table S8.** Network STRING analysis results utilizing Table S7. Differentially methylated regions identified within the genomes of female GM^{Low}
- GM^{High} mouse hippocampi.
- **Table S8.** Network STRING analysis results utilizing the 196 DMRs identified in the methylome
1354 analysis
1355 **Table S9.** Differentially expressed genes identified within the hippocampal cells of GM^{Low} and 1352 GM^{High} mouse hippocampi.
1353 **Table S8.** Network STRING
1354 analysis
1355 **Table S9.** Differentially exp **Table S8.** Network STRING analysis results utilizing the 196 DMRs identified in the methylome
1354 analysis
Table S9. Differentially expressed genes identified within the hippocampal cells of GM^{Low} and
1356 **GM^{High}**
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- 1354 analysis
1355 **Table S**
1356 GM^{High} c
1357 **Table S** Table S9. Differentially expressed genes identified within the hippocampal cells of GM^{Low}
- GM^{High} control male and female mice.
- Table S9. Differentially expressed genes identified within the hippocampal cells of GM^{Low} and
1356 GM^{High} control male and female mice.
1357 Table S10. Differentially expressed genes identified within the hippocampal c 1356 GM^{High} control male and female mice.
1357 **Table S10.** Differentially expressed g
1358 CF^{High} male and female mice.
1359 **Table S10.** Differentially expressed genes identified within the hippocampal cells of CF^{Low} and
1358 CF^{High} male and female mice.
1359
- CF^{High} male and female mice. 1358 CF^{High} male and female mice.
1359
Mart and Steps of Alger
-

Ileum

Liver

Phylum

Bacteroidota Firmicutes Deferribacterota Proteobacteria Patescibacteria Cyanobacteria **Desulfobacterota** Actinobacteriota Verrucomicrobiota **Bacteria**

M F

GM^{High}

M F

 GM^{Low}

Endo

Glial

GABA

Gluta

