

1

## 2 **Figure 1: Functional features associated with the severity of obesity in metabolic health**

3 **groups: effect of bacterial cell load.** (a) Major variables explaining the microbiome

4 compositional variation in the MetaCardis cohort subset (distance-based redundancy analyses,

5 dbRDA; genus-level Bray-Curtis dissimilarity), either independently (univariate effect sizes in

6 black) or in a multivariate model (cumulative effect sizes in grey). The cut-off for significant non-

7 redundant contribution to the multivariate model is represented by the red line. BMI: Body Mass

8 Index, ACE: angiotensin-converting enzyme inhibitors, HBP: high-blood pressure. (b) Gene

9 richness distribution across obesity groups (NOB=Non-obese; MOB=Overweight/Moderately

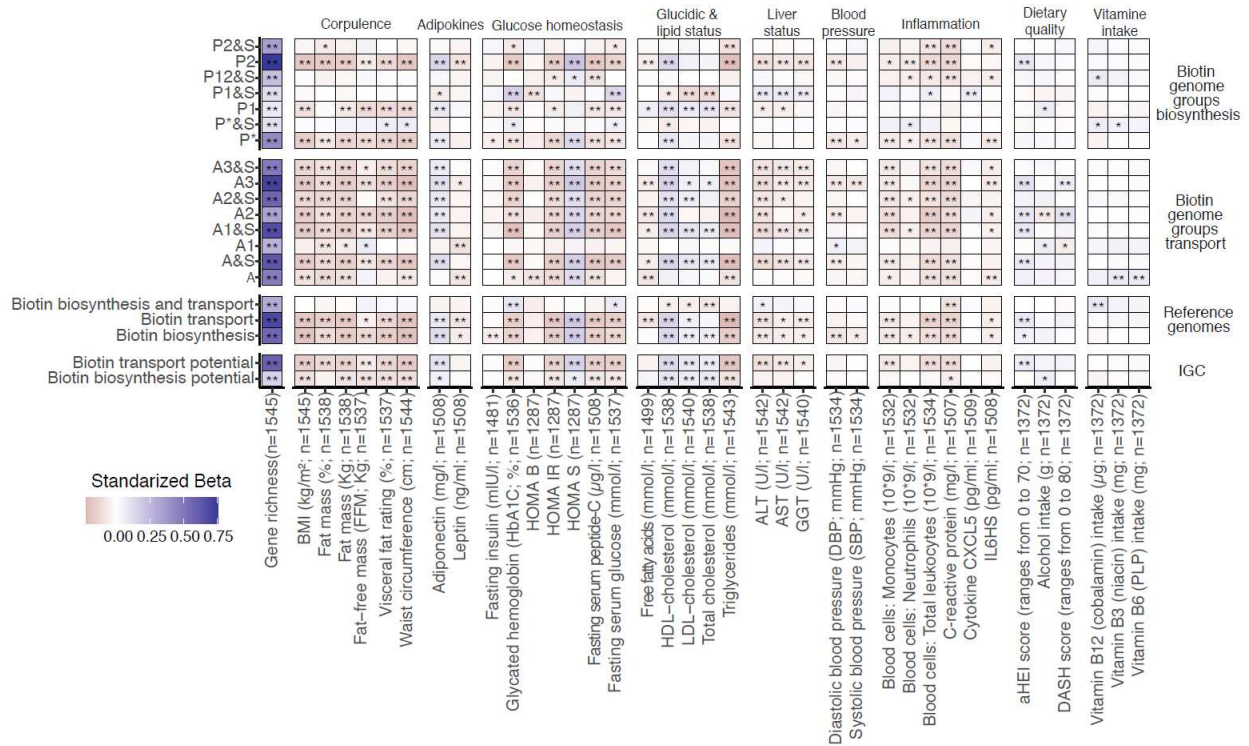
10 obese; SOB=Severely obese) stratified by metabolic health status. (\*\*: P-value<0.05 in Kruskal-

11 Wallis test controlled for country of recruitment and age, FDR<0.05 pairwise Wilcoxon rank-sum

12 tests controlled for country of recruitment and age) The dash line represents the threshold that

1

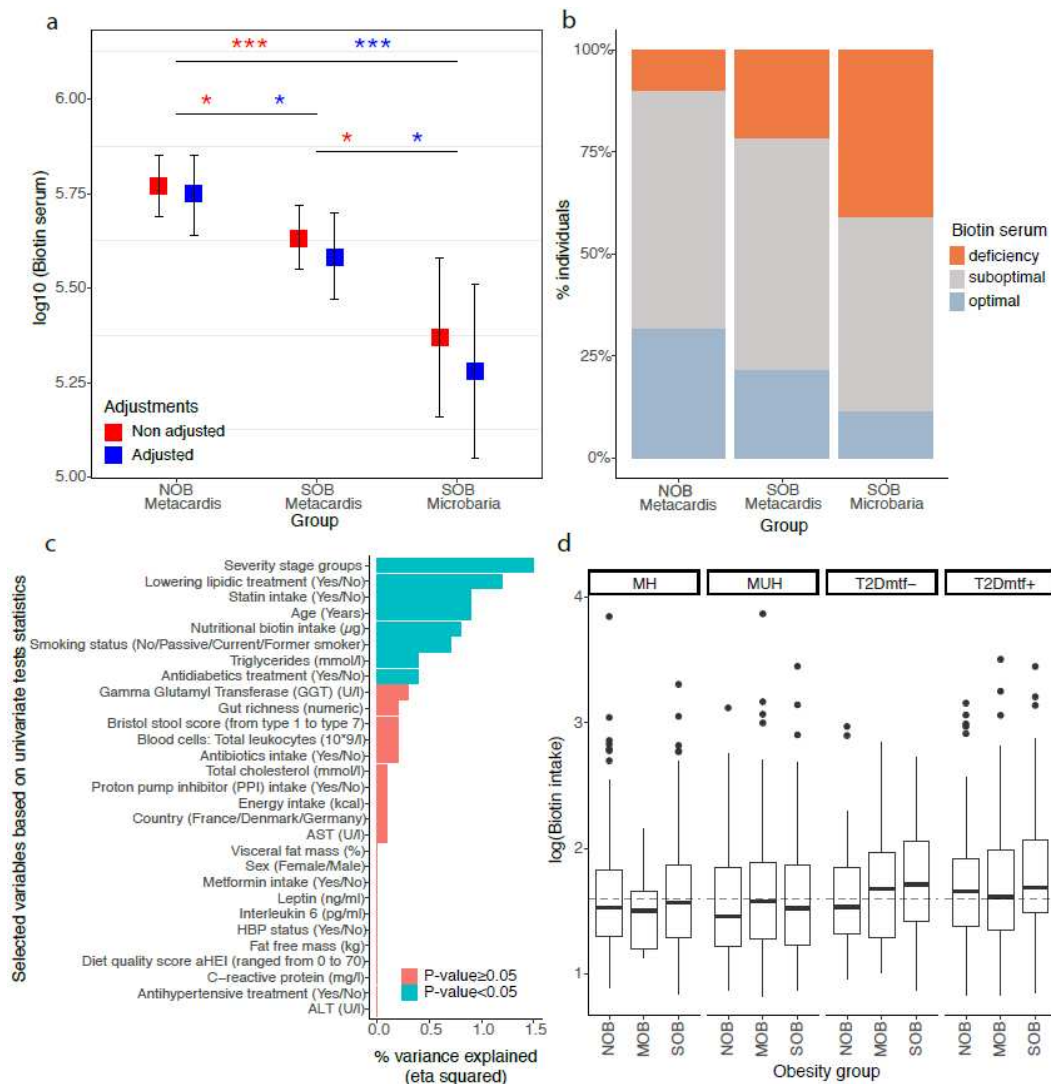
13 stratifies individuals as High vs. Low gene count (HGC/LGC) based on the median of gene  
14 richness in healthy German population (n=91) which exhibit gene richness bimodality (c)  
15 Microbial cell counts distribution across obesity groups stratified by metabolic health status. (\*\*:  
16 P-value<0.05 in Kruskal-Wallis test controlled for country of recruitment, FDR<0.05 pairwise  
17 Wilcoxon rank-sum tests controlled for country of recruitment.) (d) Estimated marginal means  
18 and confidence intervals of log-transformed absolute abundances of microbiome biotin  
19 biosynthesis and consumption potential across obesity groups adjusted by statin intake and  
20 stratified by the metabolic health status. (e) Estimated marginal means and confidence intervals  
21 of log-transformed absolute abundances of biotin producers (e.g. prokaryotic organisms  
22 harboring all biotin biosynthesis genes from pimelate precursor and no biotin biosynthesis  
23 transport genes), biotin transporters (prokaryotic organisms with no biotin biosynthesis genes)  
24 and biotin producers and transporters (prokaryotic organisms with all biotin biosynthesis genes  
25 from pimelate and biotin transport genes) across obesity groups adjusted by statin intake and  
26 stratified by the metabolic health status. (\*: FDR<0.05 on linear regression models of feature  
27 abundance by obesity status adjusted by statin intake, P-adj<0.05 on pairwise Tukey tests  
28 between obesity states).  
29  
30



31

32 **Figure 2: Association between microbiome biotin status and host metabolic and**  
 33 **inflammation markers in the MetaCardis subcohort.** Heatmap indicating adjusted  
 34 associations between log-10 transformed QMP abundance profiles of metagenomic signatures  
 35 regarding biotin production and transport with clinical and lifestyle factors. The y-axis represents  
 36 independent variables and the variables in the x-axis are the dependent variable (n=1545  
 37 individuals). These models were adjusted for the country of recruitment and age. (\*: P-  
 38 value<0.05; \*\*: FDR<0.05. Clinical and lifestyle variables for which no association with  
 39 FDR<0.05 was found are not included in the heatmap). The color tones correspond to effect  
 40 sizes represented by standardized beta coefficients from the adjusted linear regression models.  
 41 Biosynthesis and transport genome groups were defined according to the nomenclature defined  
 42 in Rodionov et al.<sup>15</sup>. Briefly, these included 3 groups of strict biotin producers (P1, P2, P\* groups)  
 43 harboring all 4 genes common to the different pathway variants of biotin biosynthesis from  
 44 pimelate (P2) or pimeloyl-ACP (P1, P\*). This also included 8 groups of strict biotin auxotrophs

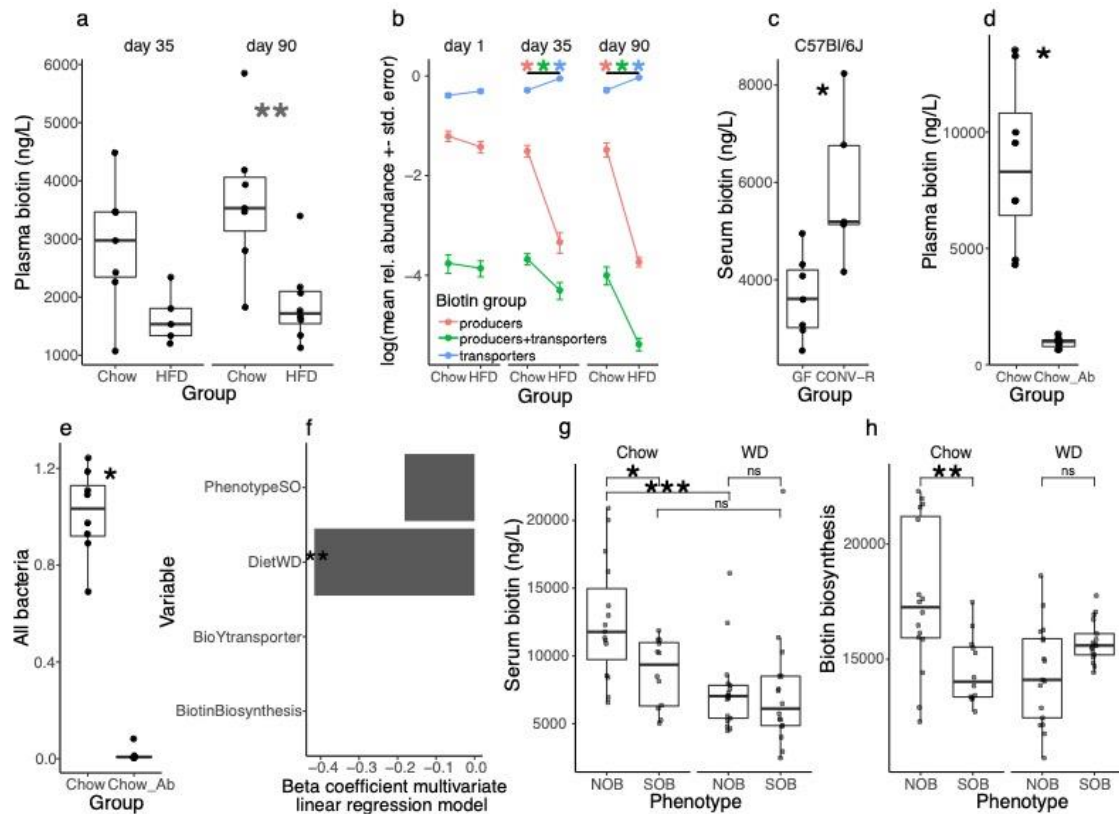
45 (A&S/A groups; microorganisms not capable of biotin production and with (A&S groups) or  
 46 without (A groups) genes involved in biotin transport) with different levels of incompleteness in the 4  
 47 core biotin biosynthesis genes (harboring from 1 to 3 biosynthetic genes at most), and 4 groups  
 48 of biotin producers that also harbors genes coding for biotin transport (P&S groups). BMI: Body  
 49 Mass Index, ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase, GGT: Gamma-  
 50 Glutyl Transferase, PLP: pyridoxal 5'-phosphate.



51

52

53 **Figure 3: Systemic and nutritional biotin profiles across obesity groups in MetaCardis**  
54 **subcohort:** (a) Differences of biotin serum levels between obesity groups in 212 individuals  
55 from the MetaCardis subcohort (n=107 (NOB), n=105 (SOB)) and 17 more severely obese  
56 individuals of the Microbaria study (\*: P-value<0.05; \*\*\*: P-value<0.001). Significant differences  
57 were observed with non-adjusted and adjusted (for diabetes status, metformin, statin and biotin  
58 intakes) Generalized Linear Models and lsmeans function, with P-value adjustment for multiple  
59 comparisons with Benjamini-Hochberg method. Biotin serum was log<sub>10</sub> transformed to enable a  
60 normal distribution of the biotin variable. (NOB vs. SOB (MetaCardis and Microbaria) Cohen's D  
61 effect size=0.91. NOB vs. SOB MetaCardis Cohen's effect size D =0.18). (b) Distribution of  
62 biotin deficiency status between obesity groups according to the following thresholds<sup>28</sup>:  
63 deficiency (<200 ng/l), suboptimal levels (200-400 ng/l), optimal levels (>400 ng/l). Significant  
64 differences were observed with Chi-2 tests (P-value=1.0x10<sup>-2</sup>). (c) Association between clinical  
65 covariates and biotin status defined by the urinary metabolite 3-hydroxyisovaleric acid.  
66 Horizontal bars correspond to the variance in 3-hydroxyisovaleric acid explained by each clinical  
67 covariate (measured by the eta squared statistic derived from a multivariate ANCOVA model,  
68 n=1545). Statistical significance is indicated for a global model containing all the variables. ALT:  
69 Alanine Aminotransferase, AST: Aspartate Aminotransferase, GGT: Gamma-Glutyl Transferase,  
70 HBP: high-blood pressure. (d) Differences in log<sub>10</sub> transformed nutritional biotin intake (µg/day)  
71 across obesity groups stratified by metabolic health status (n=284 (NOB-MH), n=130 (NOB-  
72 MUH), n=51 (NOB-T2Dmtf-), n=173 (NOB-T2Dmtf+), n=13 (MOB-MH), n=81 (MOB-MUH), n=41  
73 (MOB-T2Dmtf-), n=164 (MOB-T2Dmtf+), n=161 (SOB-MH), n=219 (SOB-MUH), n=85 (SOB-  
74 T2Dmtf-), n=143 (SOB-T2Dmtf+)). No significant differences in biotin intake were observed  
75 across study groups (FDR>0.05; non-parametric pairwise univariate tests controlled by country  
76 or statin intake). Dashed line represents the recommended daily biotin intake according to the  
77 European Food Safety Authority (40µg/day)<sup>50</sup>.  
78



79

80 **Figure 4: HFD-induced obesity in mice leads to depletion of biotin serum levels together**81 **with depletion of bacterial biotin production lineages.** (a): Plasma biotin concentration of

82 age-matched Chow-fed and HFD-fed C57BL6/J mice after 4 (left panel) and 13 weeks (right

83 panel) (\*\*: P-value&lt;0.01; Chow n=7 for day 35 and day 90, HFD n=5 for d35 and n=8 for d90,

84 Wilcoxon rank-sum test) (b): Relative abundance profiles of biotin producers (bacteria with all

85 biotin biosynthesis genes from pimelate and no biotin transport gene), biotin transporters

86 (bacteria with no gene involved in biotin biosynthesis) and biotin producers+transporters

87 (bacteria harboring biotin biosynthesis and transport genes) in these same mice at baseline (day

88 1), day 35 and day 90 (\*: P-value and FDR&lt;0.05 pairwise Wilcoxon rank-sum test). (c) Serum

89 biotin concentration of germ-free (GF) and conventionally raised (CONV-R) C57BL6/J mice (\*:

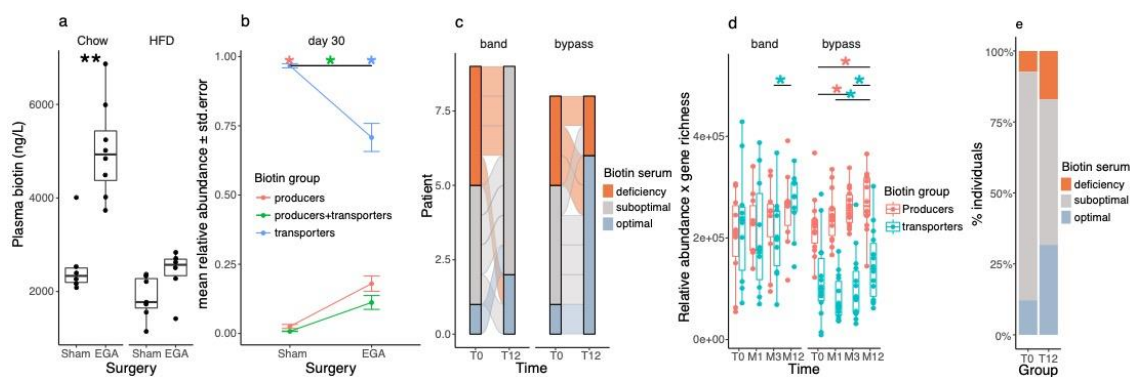
90 P-value&lt;0.05, C57BL6/J GF n=7 and CONV-R n=5; Wilcoxon rank-sum tests). (d) Plasma biotin

91 concentration and (e) total bacterial 16S rRNA gene load measured by qPCR in chow-fed mice

6

92 with (n=7) and without (n=8) large spectrum antibiotics (100mg/kg of vancomycin and 200 mg/kg  
 93 of ampicillin, neomycin and metronidazole)<sup>33</sup> diluted in water for 14 days (\*: P-value<0.05;  
 94 Wilcoxon rank-sum test). (f) Beta-coefficients obtained with multivariate linear regression models  
 95 between diet, phenotype and the abundances of biotin production and transport inferred from  
 96 16S data and serum biotin in a same global model with all covariates (\*: P-value<0.05) from  
 97 fecal transfer experiments in mice from panels g and h. (g) Serum biotin levels of Swiss Webster  
 98 mice colonized with faecal slurries of 4 subjects from the MetaCardis subcohort (2 NOB; 2 SOB).  
 99 Mice were colonized for 28 days and were fed either chow (NOB, n=16; SOB, n=12) or western  
 100 diet (NOB, n=17; SOB, n=17) (\*: P-value and FDR<0.05; \*\*\*: P-value<0.001 and FDR<0.05;  
 101 Wilcoxon rank-sum test). (h) Abundance of biotin production module inferred from PICRUST  
 102 functional profiles of 16S rRNA gene amplicon data of mice from panel f (\*: P-value<0.05;  
 103 Wilcoxon rank-sum test).

104



105

106

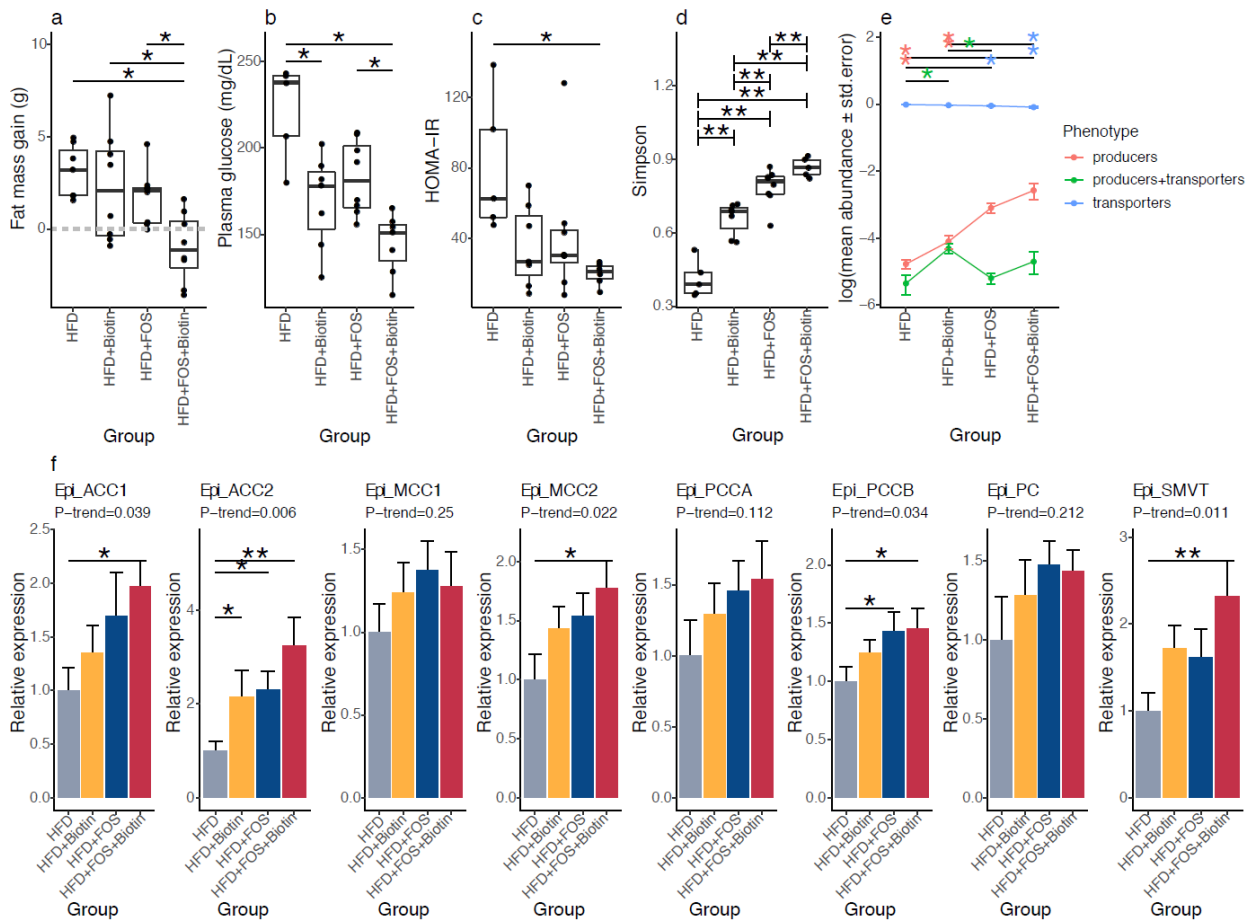
107 **Figure 5: Biotin metabolism after bariatric surgery in mouse and human experiments.** (a)

108 Plasma biotin concentration of chow- or high-fat diet (HFD)-fed C57BL/6J mice with sham  
 109 intervention (Sham) or bariatric surgery (Entero-gastro anastomosis, EGA<sup>35</sup>). Blood was  
 110 collected 1 month after surgery for the HFD group and 3 months after surgery for the Chow  
 111 group (\*\*: P-value<0.01 Wilcoxon rank-sum test; Chow-Sham n=6, Chow-EGA n=8, HFD-Sham

112 n=7, HFD-EGA n=6). (b) Mean abundances of biotin producers (bacteria with all biotin  
113 biosynthesis genes from pimelate and no biotin transport gene), biotin transporters (bacteria with  
114 no gene involved in biotin biosynthesis) and biotin producers+transporters (bacteria harbouring  
115 biotin biosynthesis and transport genes) in sham and EGA mice of the HFD group 30 days after  
116 surgery (\*: FDR<0.05 pairwise Wilcoxon rank-sum test). (c) Distribution of biotin deficiency  
117 groups between baseline and month 12 in 17 individuals of the Microbaria study stratified by  
118 surgery group (n=9, gastric banding; n=8, Roux-en-Y gastric bypass) according to the following  
119 thresholds<sup>28</sup>: deficiency (<200 ng/l), suboptimal levels (200-400 ng/l), optimal levels (>400 ng/l).  
120 P-value=2.4x10<sup>-2</sup> (bypass), P-value=1.1x10<sup>-1</sup> (band); Fisher's test. (d) Change of biotin  
121 producers and biotin transporters abundances (relative abundances multiplied by gene richness  
122 as a surrogate of microbial cell count to simulate QMP data) in 24 individuals of the Microbaria  
123 study stratified by surgery type (adjustable gastric banding, n=10; Roux-en-Y gastric, n=14) with  
124 metagenomics data at baseline, 1, 3, and 12 months after bariatric surgery (\*: P-value<0.05;  
125 Wilcoxon signed-rank test). (e) Distribution of biotin deficiency groups at baseline (T0) and 12  
126 months (T12) after bypass surgery in the BARICAN cohort (n=41; P-value=2.0x10<sup>-2</sup>, Chi2 test)



127



128

129 **Figure 6: Effects of biotin and FOS supplementation on host metabolism, biotin status**130 **and microbiome composition in established obesity in mouse experiments. (a) Fat mass**131 **gain of mice with established obesity, between day 82 (after twelve weeks of HFD and before**132 **treatments) and day 135 (after eight weeks of treatment by FOS and/or biotin) (a: HFD+FOS**133 **(n=10) vs. HFD (n=5); b: HFD+FOS vs. HFD+Biotin (n=9); c: HFD+Biotin vs. HFD; d:**134 **HFD+FOS+Biotin (n=5) vs. HFD; \*P-value<0.05, Kruskal-Wallis rank test with Dunn's multiple**135 **comparison test) (b)Fasting glycaemia of these same animals measured after 6 weeks of**136 **treatment by FOS and/or biotin (\*: P-value<0.05, Kruskal Wallis rank test with Dunn's multiple**137 **comparison test). (c) HOMA-IR index calculated after 6 weeks of treatment by FOS and/or biotin**

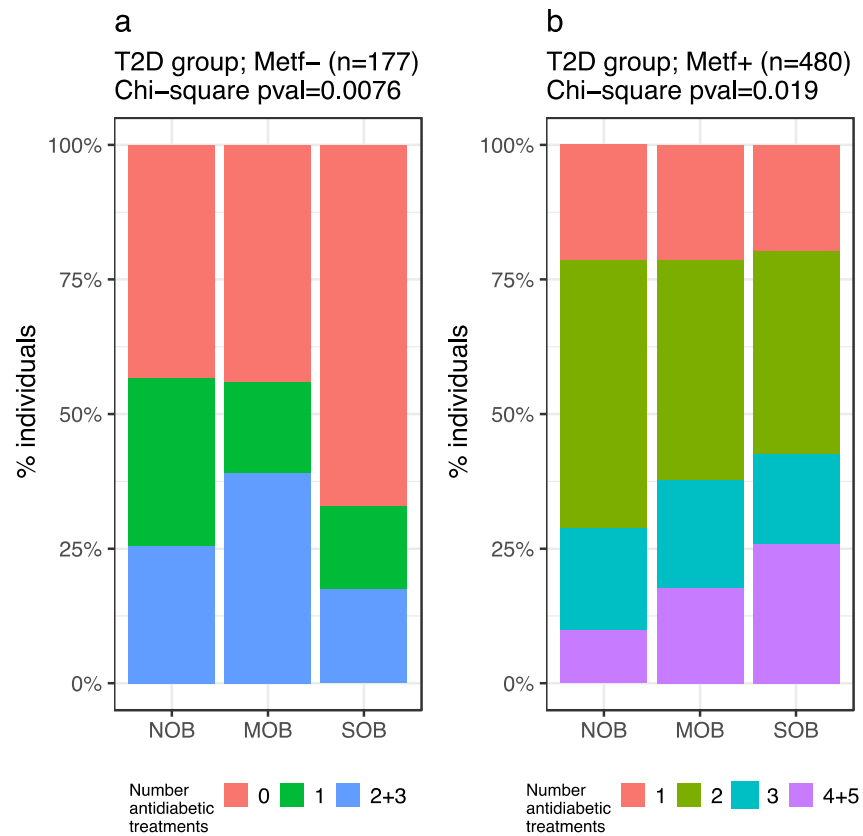
9

138 (\*: P-value<0.05, Kruskal Wallis rank test with Dunn's multiple comparison test). (d) Simpson  
139 diversity distribution in different groups of mice with long-term established obesity (\*\*: P-  
140 value<0.01 and FDR<0.05; pairwise Wilcoxon rank-sum test). (e) Mean abundances of biotin  
141 producers (bacteria with all biotin biosynthesis genes from pimelate and no biotin transport  
142 gene), biotin transporters (bacteria with no gene involved in biotin biosynthesis) and biotin  
143 producers+transporters (bacteria harbouring biotin biosynthesis and transport genes) in different  
144 groups of mice with long-term established obesity (\*:P-value and FDR<0.05 pairwise Wilcoxon  
145 rank-sum test). (f) mRNA expression of biotin carboxylases (ACCA, ACCB, MCC1, MCC2,  
146 PCCA, PCCB, PC) and biotin transporter SMVT in epididymal adipose tissue of mice with long-  
147 term established obesity supplemented with FOS and/or biotin after 20 weeks of total follow-up  
148 (Kruskal-Wallis rank test, with Dunn's multiple comparison; \*: P-value and FDR<0.05, \*\*: P-value  
149 and FDR<0.01, pairwise comparisons and P-trend were calculated using linear contrast tests).

150

## 151 **Supplemental Figures**

152

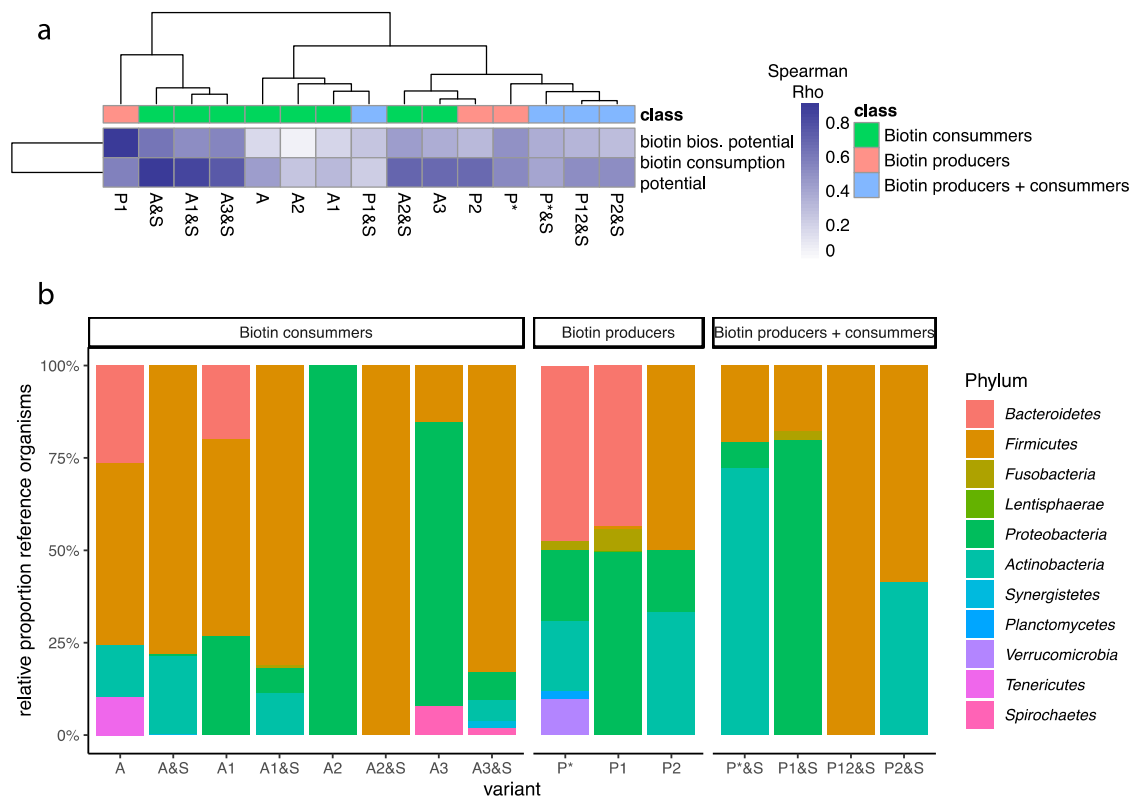


153

154 **Supplemental Figure 1: Antidiabetic medication profiles across 657 T2D individuals of the**  
 155 **cohort.** (a) Distribution of number of antidiabetic treatments in T2D individuals not treated with  
 156 metformin across obesity severity stages groups. (b) Distribution of the number of antidiabetic  
 157 treatments in T2D individuals treated with Metformin across obesity severity stages groups. Chi-  
 158 square tests on contingency tables were used to test for differences in the number of antidiabetic  
 159 treatments between obesity groups (P-values shown).

160

161



162

163 **Supplemental Figure 2: Biotin biosynthesis and transport potential of the microbiome is**164 **associated to different taxonomic groups.** (a) Heatmap of spearman correlations between

165 absolute biotin biosynthesis and consumption potential from the microbiome derived from IGC

166 gene abundances (y-axis) and absolute abundances of 15 different bacterial groups in terms of

167 biotin metabolism (x-axis) derived from Rodionov et al.<sup>15</sup> (n=1545 individuals of MetaCardis

168 cohort). In brief, these included 3 groups of strict biotin producers (P1, P2, P\* groups) harboring

169 all 4 genes common to the different pathway variants of biotin biosynthesis from pimeloyl-ACP.

170 This also included 8 groups of strict biotin auxotrophs (A&amp;S/A groups; microorganisms not

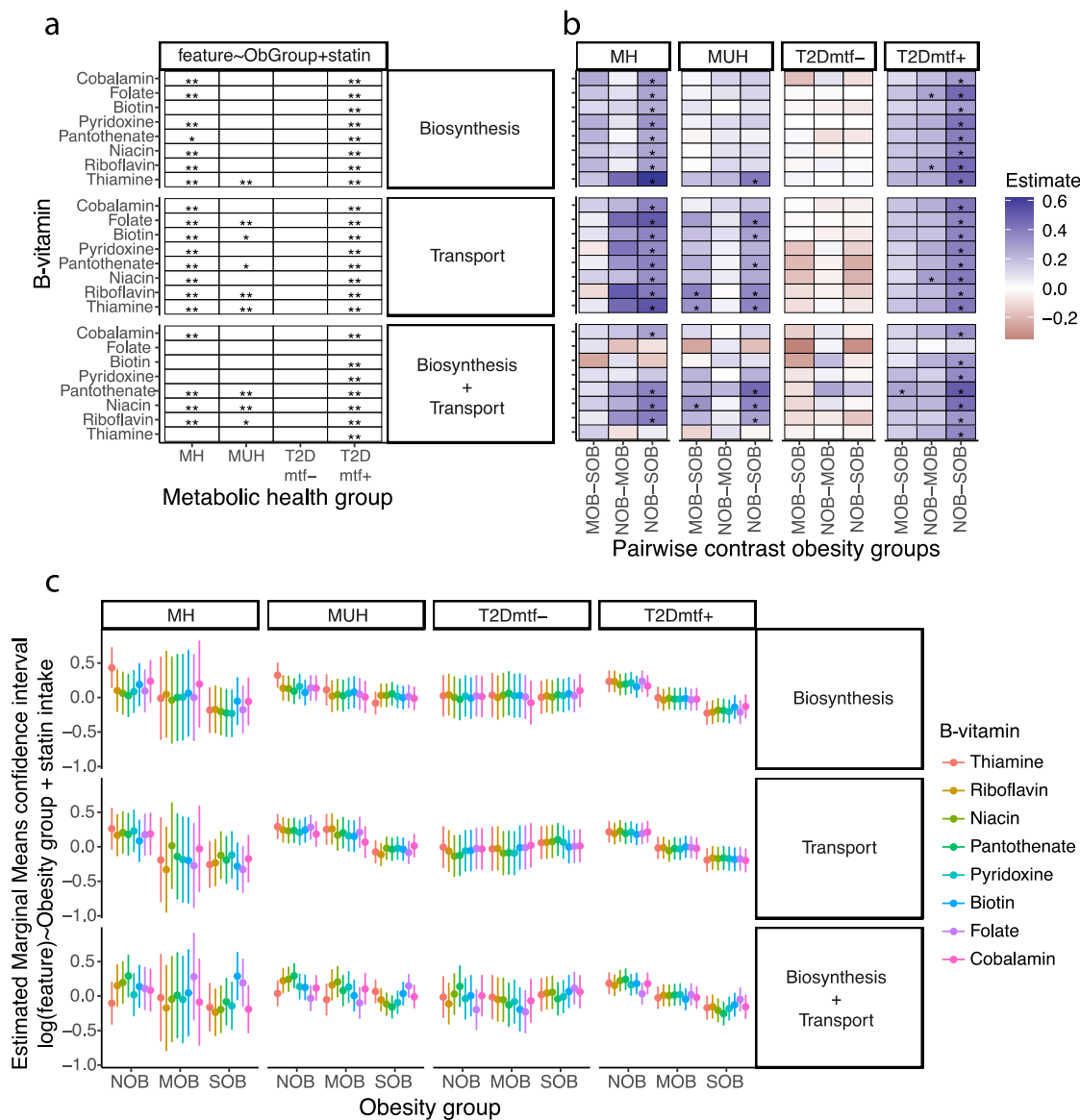
171 capable of biotin production and with (A&amp;S groups) or without (A groups) genes involved in biotin

172 transport) with different levels of incompleteness in the 4 core biotin biosynthesis genes (harboring

173 from 1 to 3 biosynthetic genes at most), and 4 groups of biotin producers that also harbors

12

174 genes coding for biotin transport (P&S groups).(b) Phylum-level taxonomic profile of the 15  
 175 bacterial groups in x-axis of panel a.  
 176  
 177



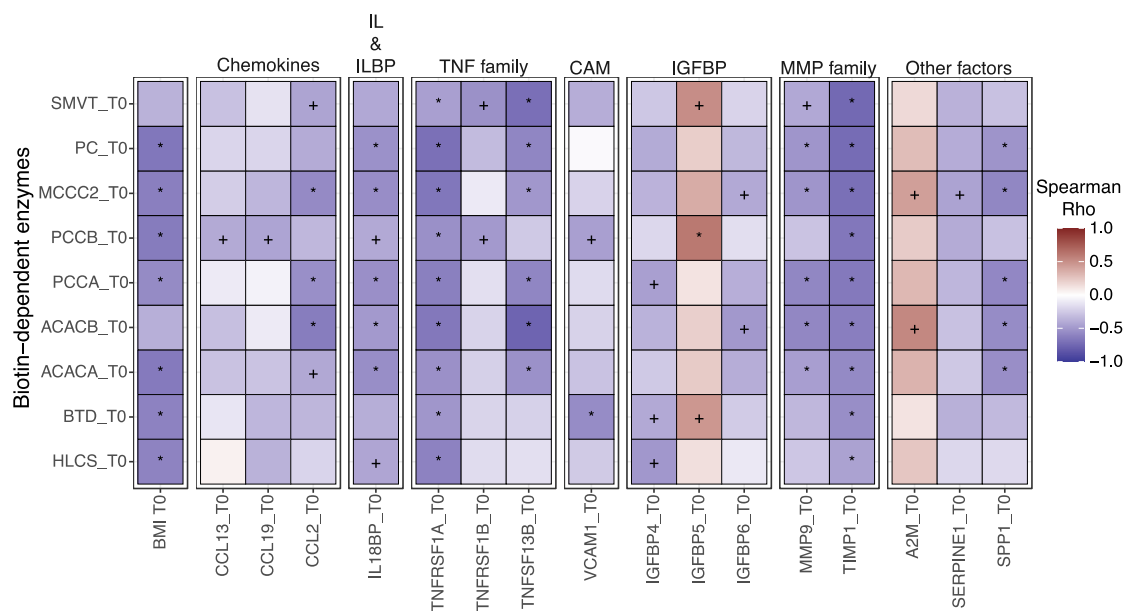
178  
 179 **Supplemental Figure 3: Absolute abundances of producers and transporters of different**  
 180 **B-vitamins across obesity stage of severity. (a) Representation of significant associations**

181 between the absolute abundances of different bacterial groups of producers and transporters of  
 182 8 B-vitamins and obesity status based on linear regression models adjusted by statin intake on  
 183 each metabolic health group (\*\*=FDR<0.05; \*=P-value<0.05). (b) Heatmap representing the beta  
 184 coefficients product of pairwise comparisons of statin-adjusted expected marginal means  
 185 (EMMs) of absolute abundances of B-vitamin producers and transporters between levels of the  
 186 obesity status variable (\* P-adjusted<0.05, Tukey method). (c) EMM confidence intervals of  
 187 pairwise comparisons represented in b to illustrate the sense of the associations. Sample sizes  
 188 of clinical groups: n=284 (NOB-MH), n=130 (NOB-MUH), n=51 (NOB-T2Dmtf-), n=173 (NOB-  
 189 T2Dmtf+), n=13 (MOB-MH), n=81 (MOB-MUH), n=41 (MOB-T2Dmtf-), n=164 (MOB-T2Dmtf+),  
 190 n=161 (SOB-MH), n=219 (SOB-MUH), n=85 (SOB-T2Dmtf-), n=143 (SOB-T2Dmtf+).

191

192

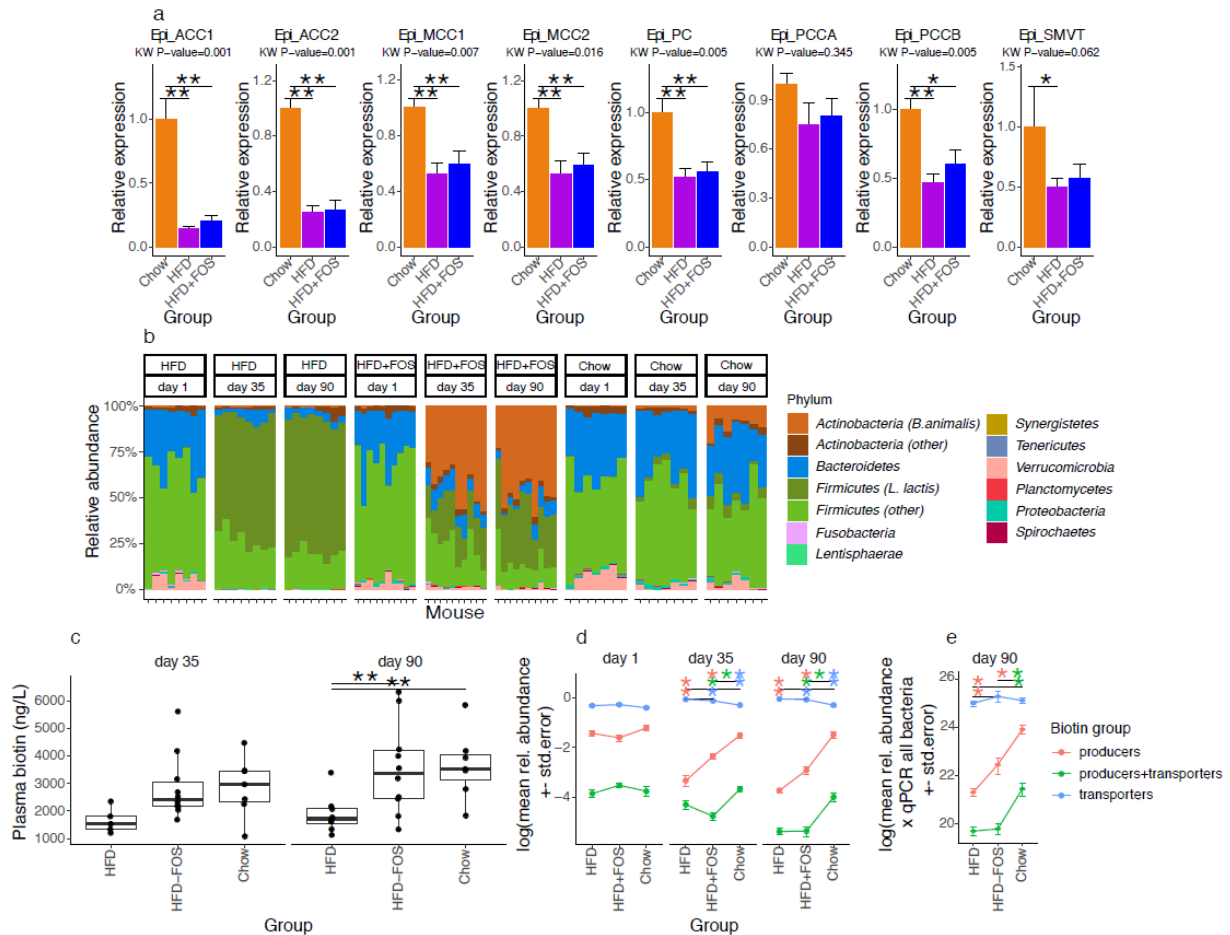
193



194

195 **Supplemental Figure 4: Subcutaneous adipose tissue gene expression of biotin-**  
196 **dependent carboxylases and SMVT in relation to obesity and inflammatory factors in**  
197 **bariatric surgery cohort.** Spearman correlations of BMI and gene expression of inflammatory  
198 factors in subcutaneous adipose tissue samples (measured by a microarray assay) with gene  
199 expression of biotin-dependent carboxylases and SMVT (measured by qPCR, relative to HRPT1  
200 expression) at baseline (T0, e.g., before bariatric surgery). Numbers of observations per  
201 displayed correlation: n=24 for correlations with HLCS, BTM, ACACA, ACACB, PCCA, PCCB,  
202 MCCC2 and PC (except for results concerning TNFRSF11B: n=23) and n=23 for correlations  
203 with SMVT (except for results concerning TNFRSF11B: n=22). Tested variables that showed no  
204 association with biotin-related genes (17 inflammatory factors and %body fat) are not displayed.  
205 Abbreviations: HLCS (gene encoding enzyme holocarboxylase synthetase), BTM (gene  
206 encoding biotinidase), ACACA and ACACB (genes encoding Acetyl-CoA carboxylases 1 and 2),  
207 PCCA and PCCB (genes encoding Propionyl-CoA carboxylase alpha chain and beta chain),  
208 MCCC2 (gene encoding Methylcrotonoyl-CoA carboxylase beta chain,), PC (gene encoding  
209 pyruvate carboxylase), SLC5A6 (gene encoding the biotin transporter SMVT).

210



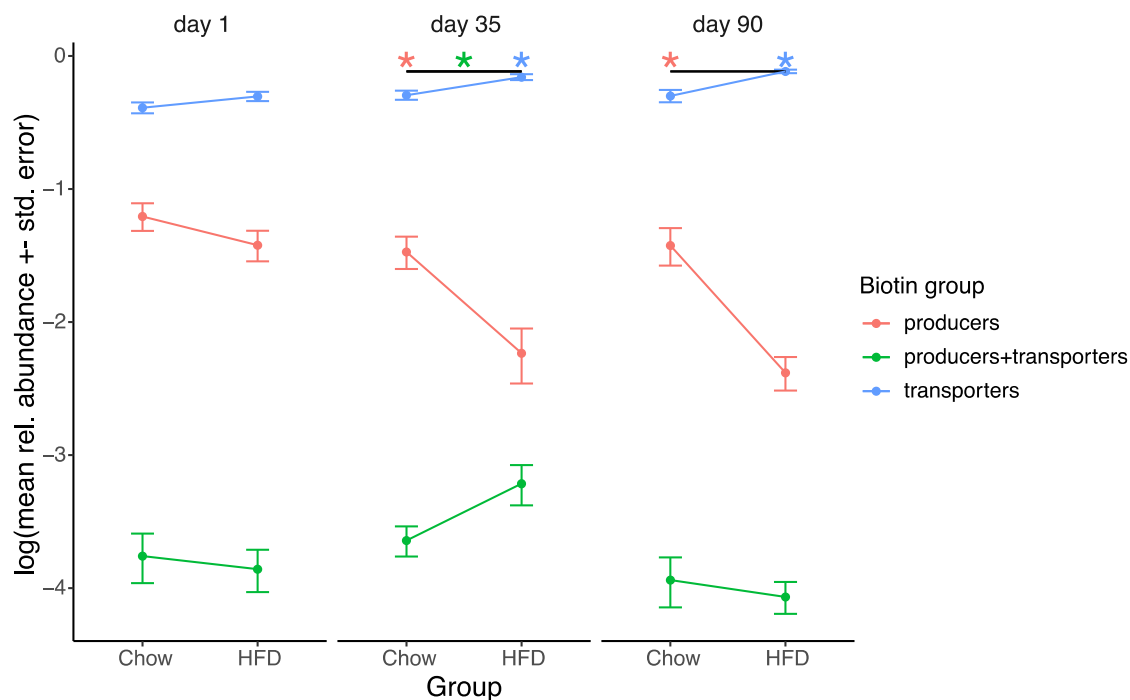
211

212 **Supplemental Figure 5: Adipose tissue gene expression, serum biotin and abundance of**  
 213 **biotin producers and transporters in mice experiments of HFD-induced obesity with FOS**  
 214 **supplementation.** (a) mRNA expression of biotin carboxylases (ACCA, ACCB, MCC1, MCC2,  
 215 PCCA, PCCB, PC, SMVT) in epididymal adipose tissue of mice fed either a Chow diet or a HFD  
 216 with or without FOS supplementation (HFD+FOS) after 13 weeks of follow-up (Kruskal-Wallis  
 217 rank test, with Dunn's multiple comparison; \*: P-value<0.05; \*\*: FDR<0.05, pairwise  
 218 comparisons). (b) Gut microbiome composition at phylum level of Chow, HFD and HFD+FOS  
 219 groups at days 1, 35 and 90 of diet and treatment. The fractions contributed by *Lactococcus*  
 220 *lactis* and *Bifidobacterium animalis* are differentiated in the Firmicutes and Actinobacteria phyla

16



221 respectively (c) Plasma biotin concentration of age-matched Chow, HFD, HFD+FOS C57BL6/j  
222 mice after 4 (left panel) and 13 weeks (right panel) of diet alone and FOS treatments (\*  
223 FDR<0.05 Wilcoxon rank-sum test; Chow n=7 for day 35 and d90, HFD n=5 for day 35 and n=8  
224 for day 90, HFD+FOS n=10 for day 35 and day 90 (d) Abundance profiles of biotin producers  
225 (bacteria with all biotin biosynthesis genes from pimelate and no biotin transport gene), biotin  
226 transporters (bacteria with no gene involved in biotin biosynthesis) and biotin  
227 producers+transporters (bacteria harbouring biotin biosynthesis and transport genes) in the  
228 same mice at baseline (day 1), day 35 and day 90 (\*: P-value Kruskal Wallis tests, FDR<0.05  
229 pairwise Wilcoxon rank-sum test within each bacterial group). (e) Absolute abundance profile of  
230 biotin producers (bacteria with all biotin biosynthesis genes from pimelate and no biotin transport  
231 gene), biotin transporters (bacteria with no gene involved in biotin biosynthesis) and biotin  
232 producers+transporters (bacteria harbouring biotin biosynthesis and transport genes) in the  
233 same mice at day 90. Absolute abundances were calculated by multiplying relative metagenomic  
234 abundances by total bacteria abundance obtained by qPCR (\*: P-value Kruskal Wallis tests,  
235 FDR<0.05 pairwise Wilcoxon rank-sum test within each bacterial group).  
236



237

238 **Supplemental Figure 6: Impact of *L. lactis* removal on the abundances of biotin producers**239 **and transporters in mouse experiments of HFD-induced obesity.** Abundance profiles of

240 biotin producers (bacteria with all biotin biosynthesis genes from pimelate and no biotin transport

241 gene), biotin transporters (bacteria with no gene involved in biotin biosynthesis) and biotin

242 producers+transporters (bacteria harbouring biotin biosynthesis and transport genes) in age-

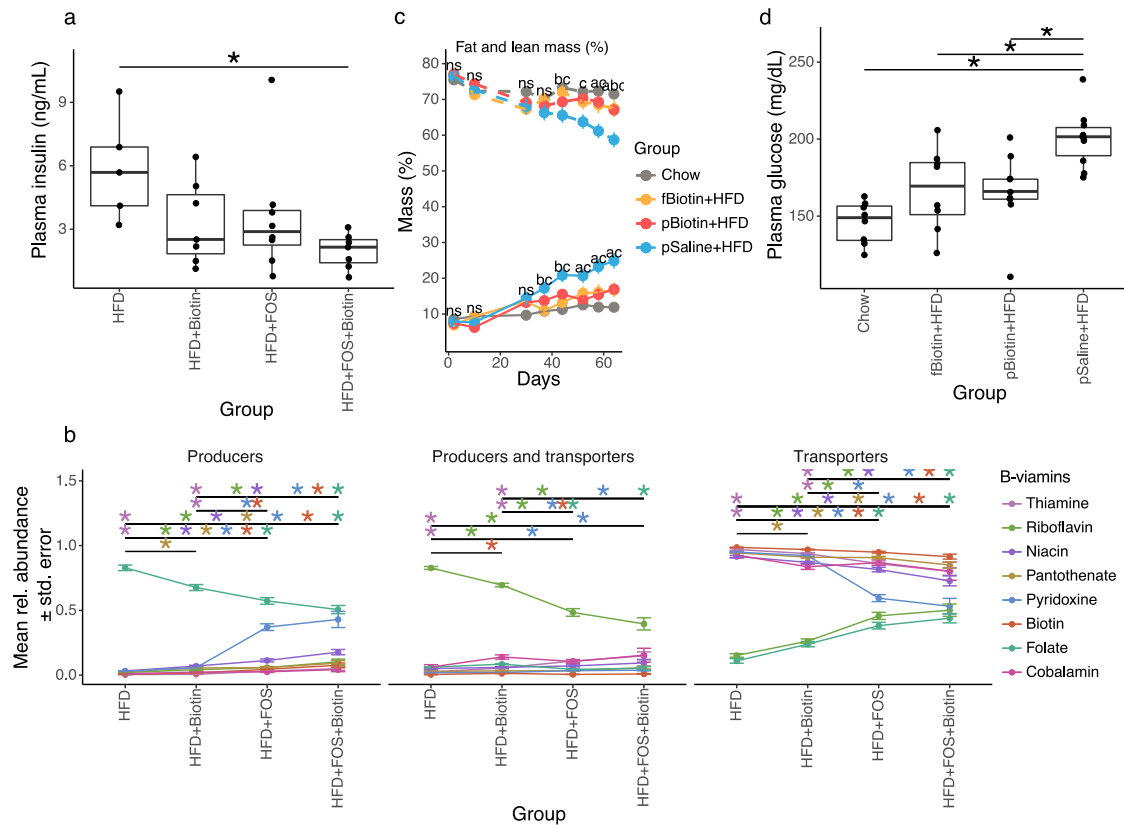
243 matched Chow-fed and HFD-fed C57BL6/J mice in baseline (day 1) 4 weeks (day 35) and 13

244 weeks (day 90) of treatment represented in Figure 4b of the manuscript after excluding

245 *Lactococcus lactis* from the computation of group abundances (\*: FDR<0.05 pairwise Wilcoxon

246 rank-sum test; Supplemental Discussion).

247



248

249 **Supplemental Figure 7: Effects of biotin supplementation in mice on body corpulence,**250 **insulin and glucose levels and abundance of producers and transporters of different B-**251 **vitamins.** (a) Fasting insulinemia of mice with long-term established obesity supplemented with

252 FOS and/or Biotin measured after 6 weeks of treatment by ELISA (\*: P-value and FDR&lt;0.05,

253 Kruskal Wallis rank test with Dunn's multiple comparison test). (b) Mean abundances of

254 producers, producers and transporters and transporters of different B-vitamins across mice

255 groups of panel a (\*:P-value and FDR&lt;0.05 on Kruskal Wallis tests and in pairwise Wilcoxon

256 rank-sum test within each bacterial group) (c) Body composition: percentage of lean (dashed

257 lines) and fat (plain lines) mass of animals fed a HFD and supplemented by biotin either via

258 subcutaneous osmotic pumps (pBiotin+HFD, n=9), or food (fBiotin+HFD, n=8), as well as two

259 control groups one fed a HFD with subcutaneous osmotic pumps delivering the vehicle solution

260 (pSaline+HFD, n=10) and one group fed a standard Chow diet (Chow, n=8). (a:pSaline+HFD vs.  
261 pBiotin+HFD; b:pSaline+HFD vs. fBiotin+HFD; c:pSaline+HFD vs. Chow; d:pBiotin+HFD vs.  
262 fBiotin+HFD; e:pBiotin+HFD vs. Chow; f:fBiotin+HFD vs. Chow, P-value and FDR<0.05 Two  
263 Way ANOVA with Dunn's multiple comparison test). (d) Fasting glycaemia of these same mice,  
264 after 2 months of diet and treatment (\*: P-value and FDR<0.05, Kruskal-Wallis rank test, with  
265 Dunn's multiple comparison test).  
266  
267