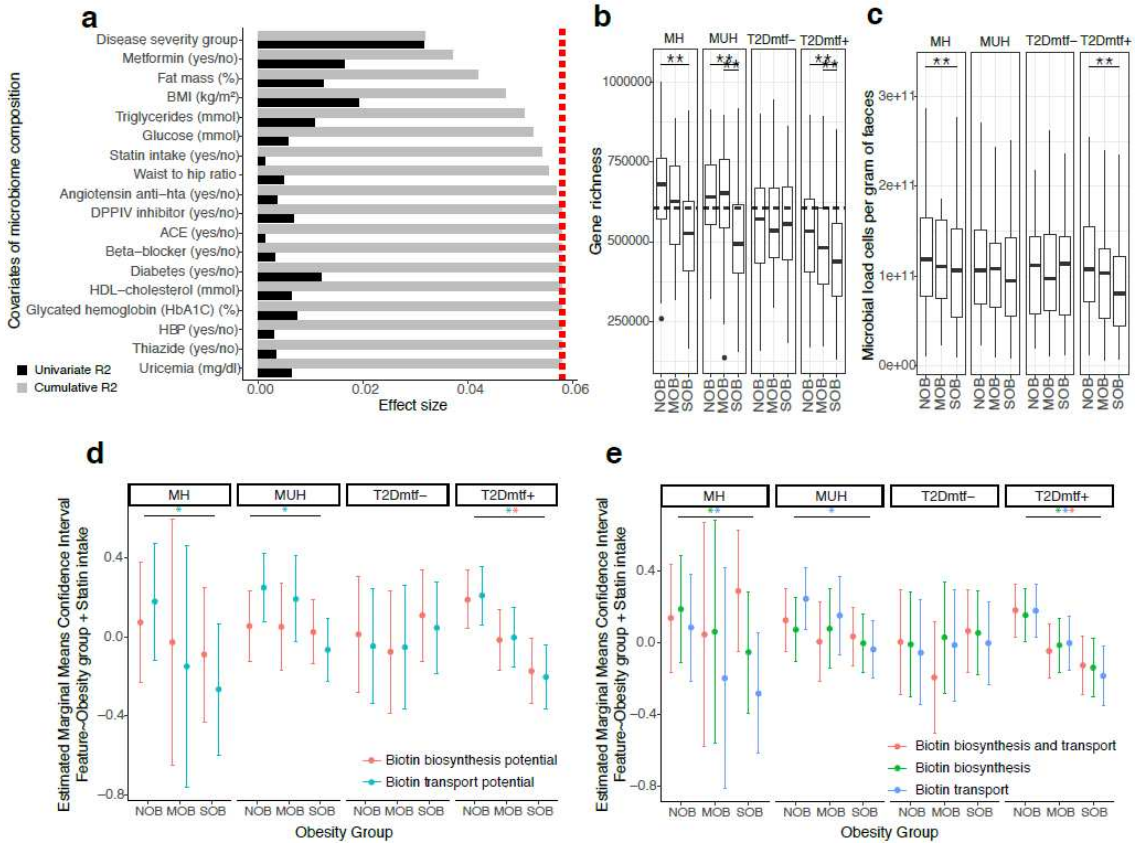


Figure 1: Functional features associated with the severity of obesity in metabolic health groups: effect of bacterial cell load. (a) Major variables explaining the microbiome compositional variation in the MetaCardis cohort subset (distance-based redundancy analyses, dbRDA; genus-level Bray-Curtis dissimilarity), either independently (univariate effect sizes in black) or in a multivariate model (cumulative effect sizes in grey). The cut-off for significant non-redundant contribution to the multivariate model is represented by the red line. BMI: Body Mass Index, ACE: angiotensin-converting enzyme inhibitors, HBP: high-blood pressure. (b) Gene richness distribution across obesity groups (NOB=Non-obese; MOB=Overweight/Moderately obese; SOB=Severely obese) stratified by metabolic health status. (**: P-value<0.05 in Kruskal-Wallis test controlled for country of recruitment and age, FDR<0.05 pairwise Wilcoxon rank-sum tests controlled for country of recruitment and age) The dash line represents the threshold that

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

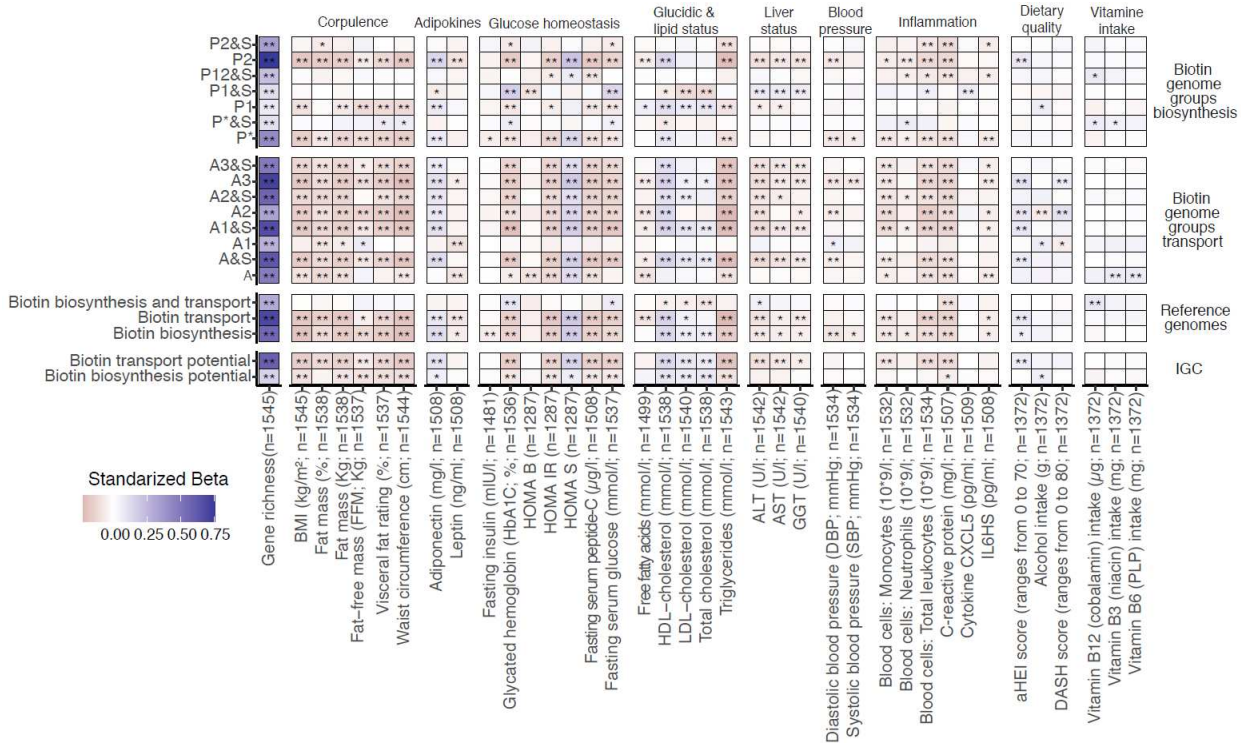


Figure 2: Association between microbiome biotin status and host metabolic and inflammation markers in the MetaCardis subcohort. Heatmap indicating adjusted associations between log-10 transformed QMP abundance profiles of metagenomic signatures regarding biotin production and transport with clinical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

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

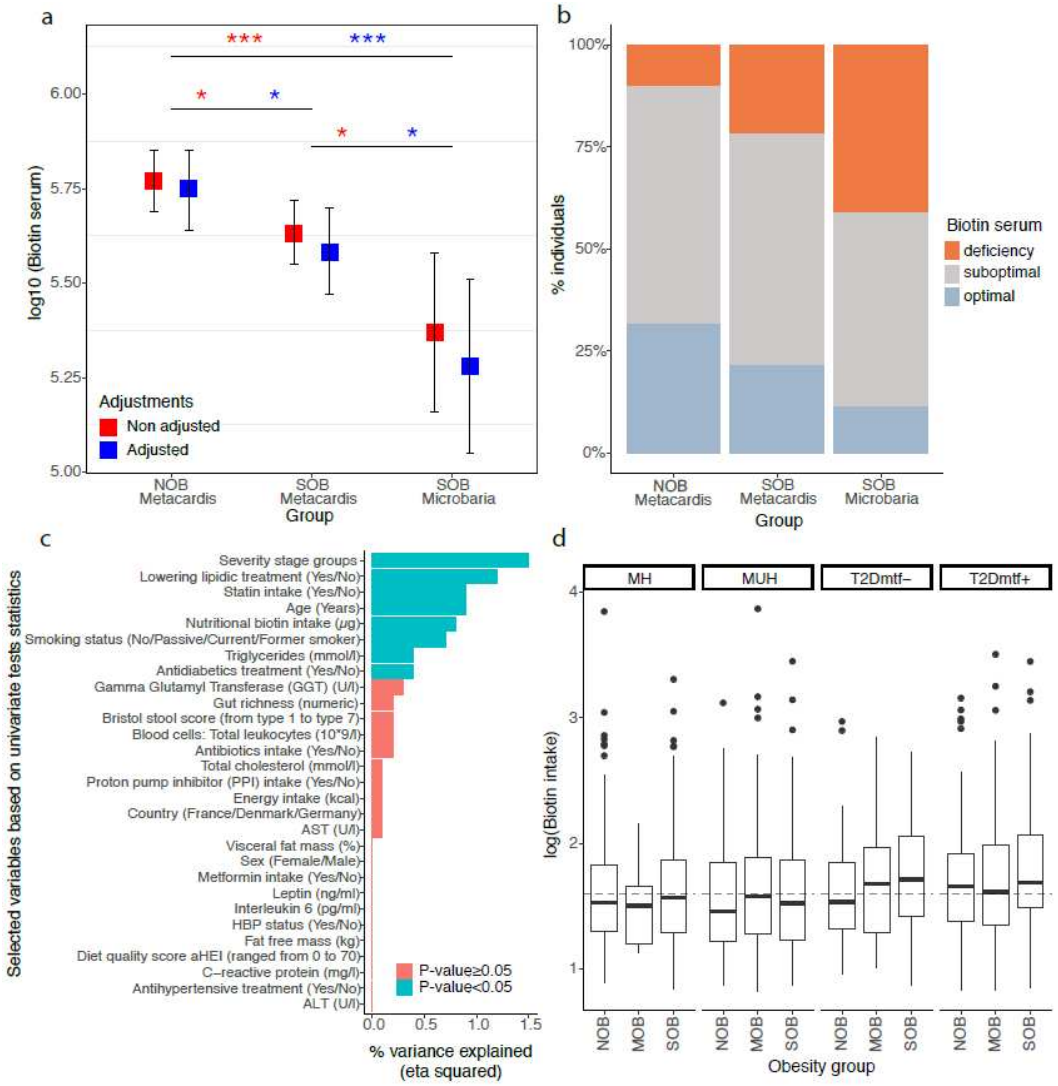


Figure 3: **Systemic and nutritional biotin profiles across obesity groups in MetaCardis subcohort:** (a) Differences of biotin serum levels between obesity groups in 212 individuals from the MetaCardis subcohort (n=107 (NOB), n=105 (SOB)) and 17 more severely obese individuals of the Microbaria study (*: P-value<0.05; ***: P-value<0.001). Significant differences were observed with non-adjusted and adjusted (for diabetes status, metformin, statin and biotin intakes) Generalized Linear Models and lsmeans function, with P-value adjustment for multiple comparisons with Benjamini-Hochberg method. Biotin serum was log10 transformed to enable a normal distribution of the biotin variable. (NOB vs. SOB (MetaCardis and Microbaria) Cohen's D effect size=0.91. NOB vs. SOB MetaCardis Cohen's effect size D =0.18). (b) Distribution of biotin deficiency status between obesity groups according to the following thresholds[28]: deficiency (<200 ng/l), suboptimal levels (200-400 ng/l), optimal levels (>400 ng/l). Significant differences were observed with Chi-2 tests (P-value=1.0x10-2). (c) Association between clinical covariates and biotin status defined by the urinary metabolite 3-hydroxyisovaleric acid. Horizontal bars correspond to the variance in 3-hydroxyisovaleric acid explained by each clinical covariate (measured by the eta squared statistic derived from a multivariate ANCOVA model, n=1545). Statistical significance is indicated for a global model containing all the variables. ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase, GGT: Gamma-Glutyl Transferase, HBP: high-blood pressure. (d) Differences in log10 transformed nutritional biotin intake (µg/day) across obesity groups stratified by metabolic health status (n=284 (NOB-MH), n=130 (NOB-MUH), n=51 (NOB-T2Dmtf-), n=173 (NOB-T2Dmtf+), n=13 (MOB-MH), n=81 (MOB-MUH), n=41 (MOB-T2Dmtf-), n=164 (MOB-T2Dmtf+), n=161 (SOB-MH), n=219 (SOB-MUH), n=85 (SOB-T2Dmtf-), n=143 (SOB-T2Dmtf+)). No significant differences in biotin intake were observed across study groups (FDR>0.05; non-parametric pairwise univariate tests controlled by country or statin intake). Dashed line represents the recommended daily biotin intake according to the European Food Safety Authority (40µg/day)[50].

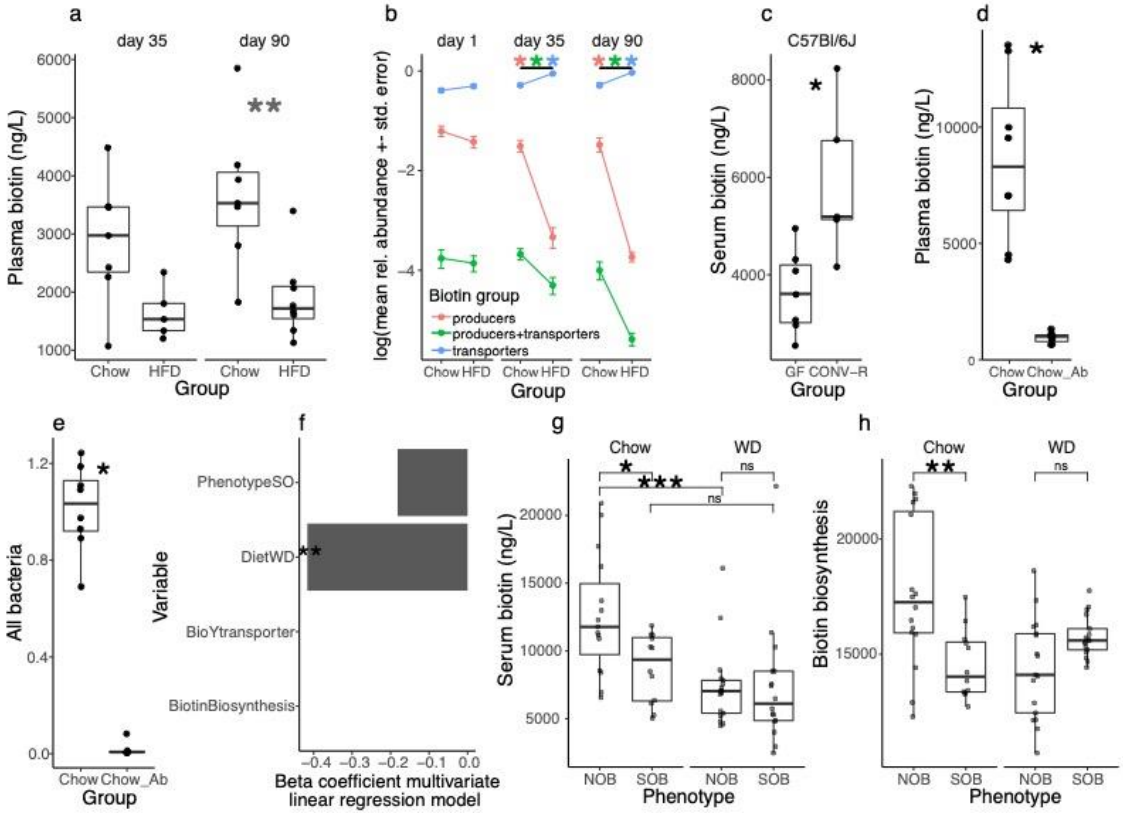


Figure 4: HFD-induced obesity in mice leads to depletion of biotin serum levels together with depletion of bacterial biotin production lineages. (a): Plasma biotin concentration of age-matched Chow-fed and HFD-fed C57BL6/J mice after 4 (left panel) and 13 weeks (right panel) (**: P-value<0.01; Chow n=7 for day 35 and day 90, HFD n=5 for d35 and n=8 for d90, Wilcoxon rank-sum test) (b): Relative abundance profiles of biotin producers (bacteria with all biotin biosynthesis genes from pimelate and no biotin transport gene), biotin transporters (bacteria with no gene involved in biotin biosynthesis) and biotin producers+transporters (bacteria harboring biotin biosynthesis and transport genes) in these same mice at baseline (day 1), day 35 and day 90 (*: P-value and FDR<0.05 pairwise Wilcoxon rank-sum test). (c) Serum biotin concentration of germ-free (GF) and conventionally raised (CONV-R) C57BL6/J mice (*: P-value<0.05, C57BL6/J GF n=7 and CONV-R n=5; Wilcoxon rank-sum tests). (d) Plasma biotin concentration and (e) total bacterial 16S rRNA gene load measured by qPCR in chow-fed mice

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

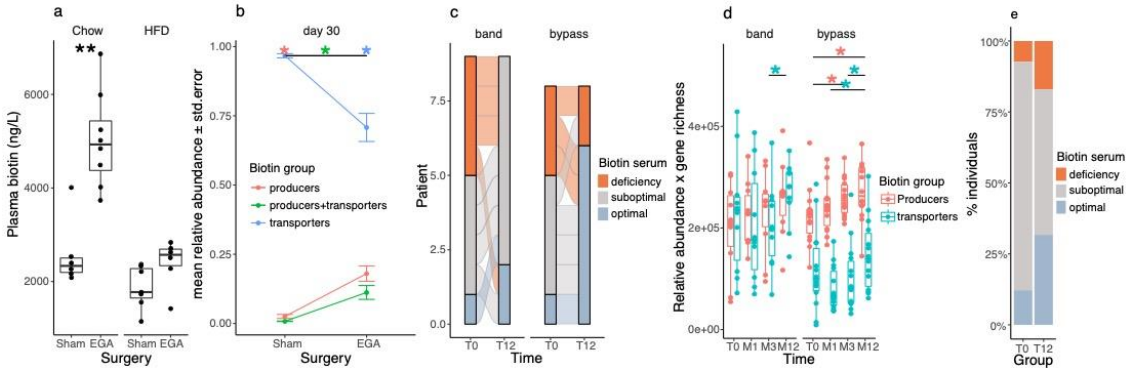


Figure 5: Biotin metabolism after bariatric surgery in mouse and human experiments. (a) Plasma biotin concentration of chow- or high-fat diet (HFD)-fed C57BL/6J mice with sham intervention (Sham) or bariatric surgery (Entero-gastro anastomosis, EGA[35]). Blood was collected 1 month after surgery for the HFD group and 3 months after surgery for the Chow group (**: P-value<0.01 Wilcoxon rank-sum test; Chow-Sham n=6, Chow-EGA n=8, HFD-Sham

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

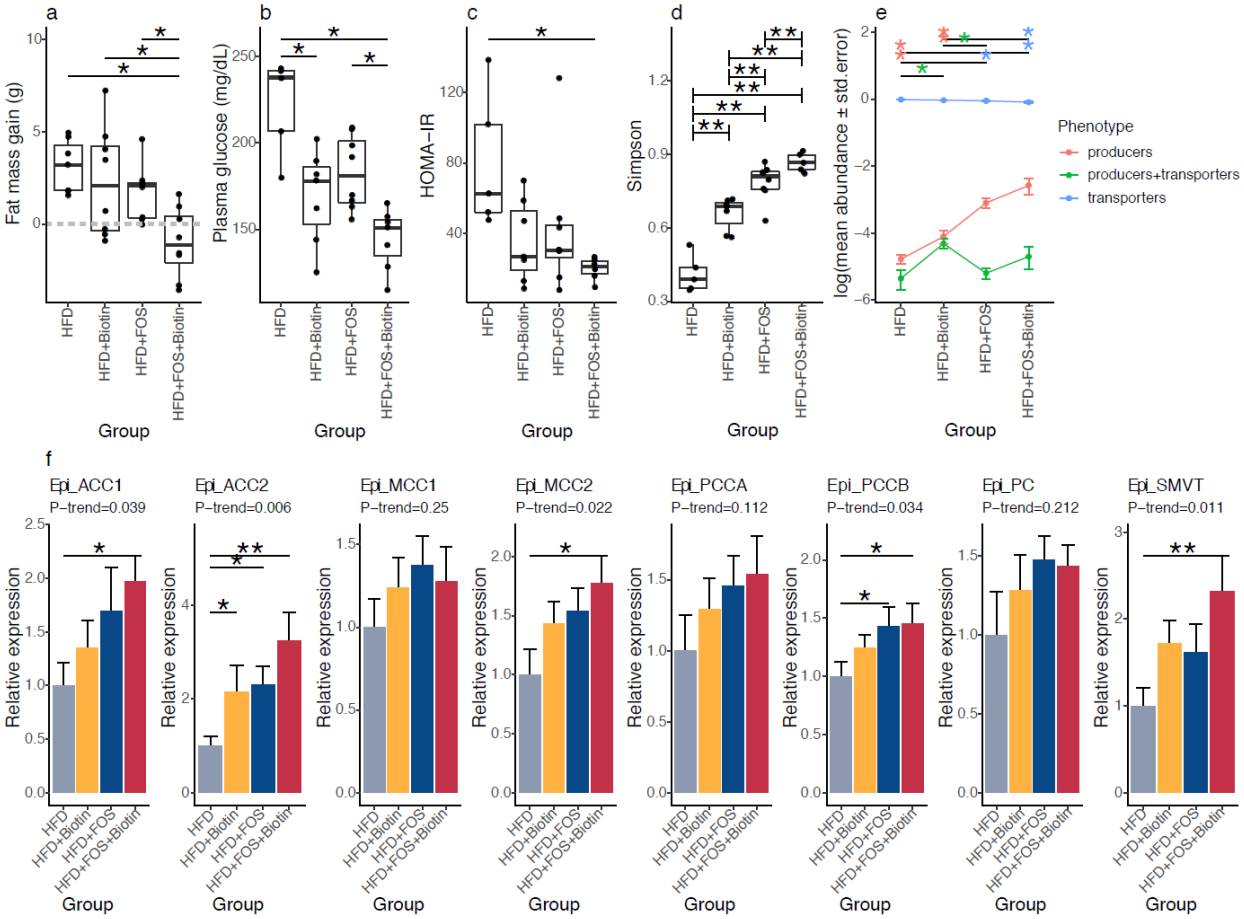
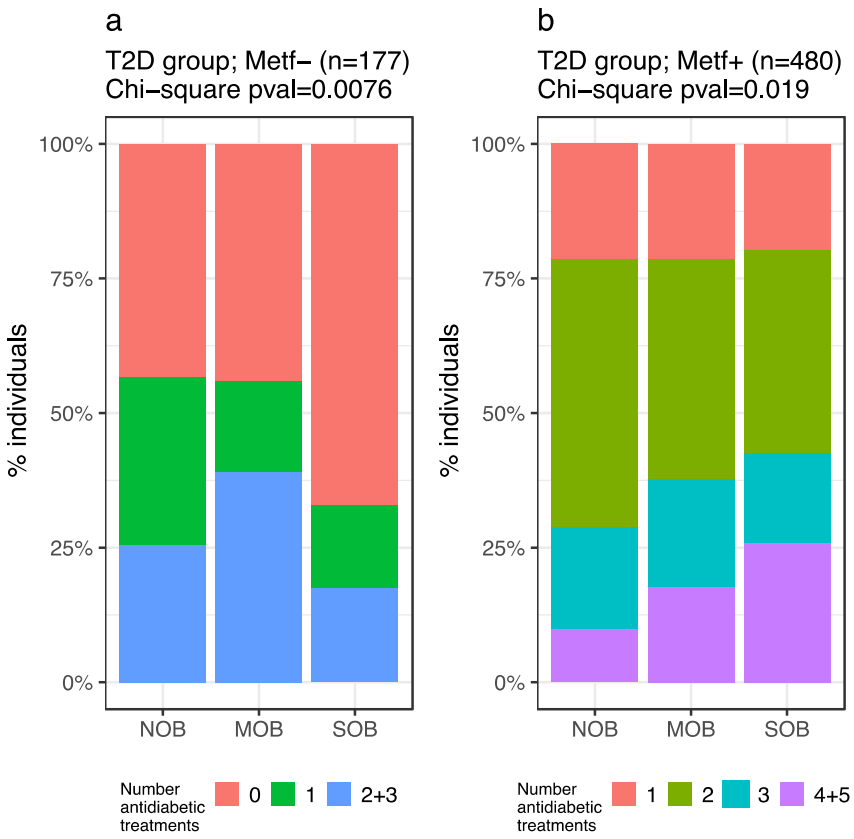


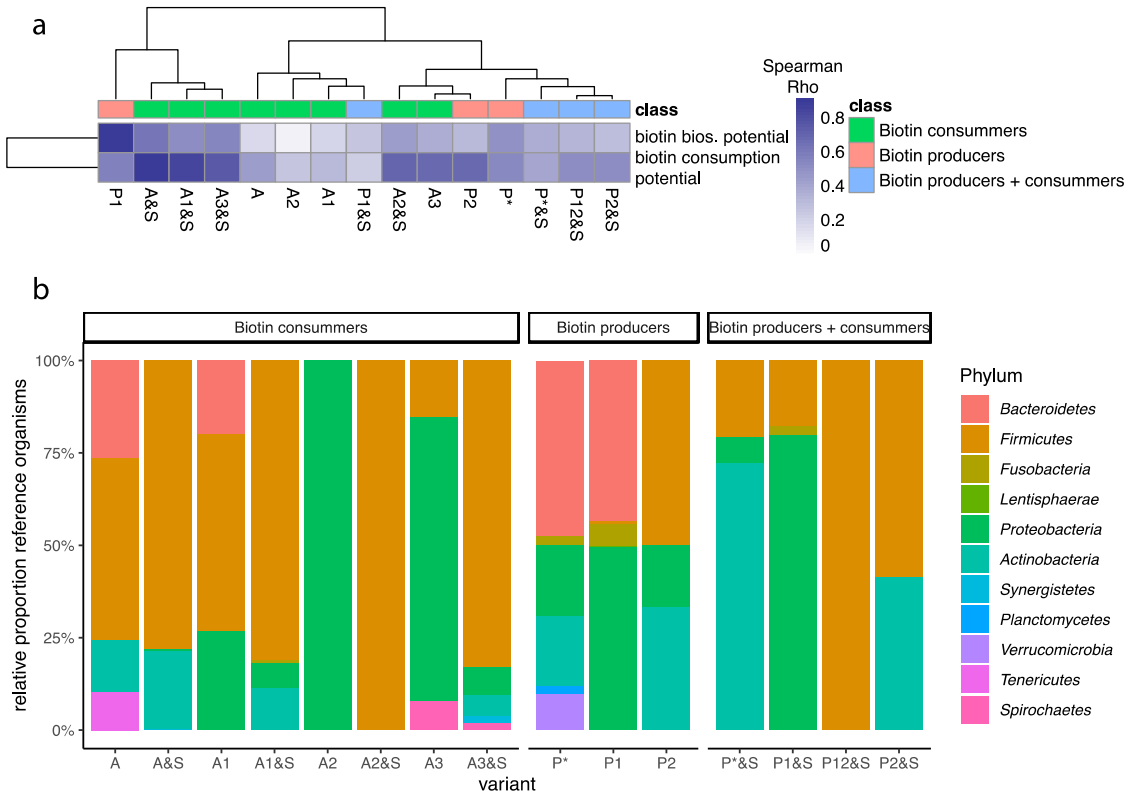
Figure 6: **Effects of biotin and FOS supplementation on host metabolism, biotin status and microbiome composition in established obesity in mouse experiments**. (a) Fat mass gain of mice with established obesity, between day 82 (after twelve weeks of HFD and before treatments) and day 135 (after eight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

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

Supplemental Figures



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



Supplemental Figure 2: Biotin biosynthesis and transport potential of the microbiome is associated to different taxonomic groups. (a) Heatmap of spearman correlations between absolute biotin biosynthesis and consumption potential from the microbiome derived from IGC gene abundances (y-axis) and absolute abundances of 15 different bacterial groups in terms of biotin metabolism (x-axis) derived from Rodionov et al.[15] (n=1545 individuals of MetaCardis cohort). In brief, these included 3 groups of strict biotin producers (P1, P2, P* groups) harboring all 4 genes common to the different pathway variants of biotin biosynthesis from pimeloyl-ACP. This also included 8 groups of strict biotin auxotrophs (A&S/A groups; microorganisms not capable of biotin production and with (A&S groups) or without (A groups) genes involved in biotin transport) with different levels of incompletion in the 4 core biotin biosynthesis genes (harboring from 1 to 3 biosynthetic genes at most), and 4 groups of biotin producers that also harbors

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.

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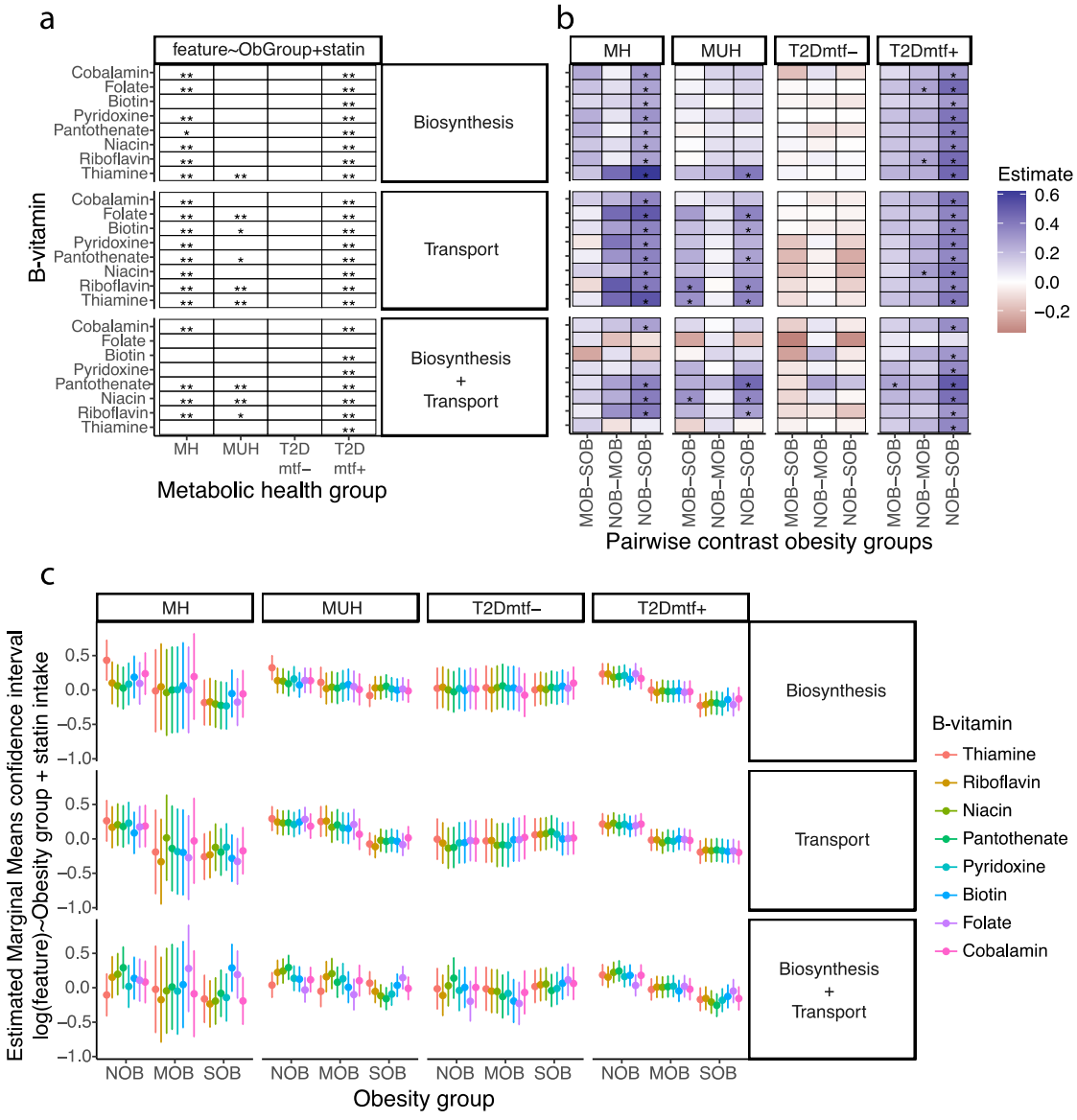

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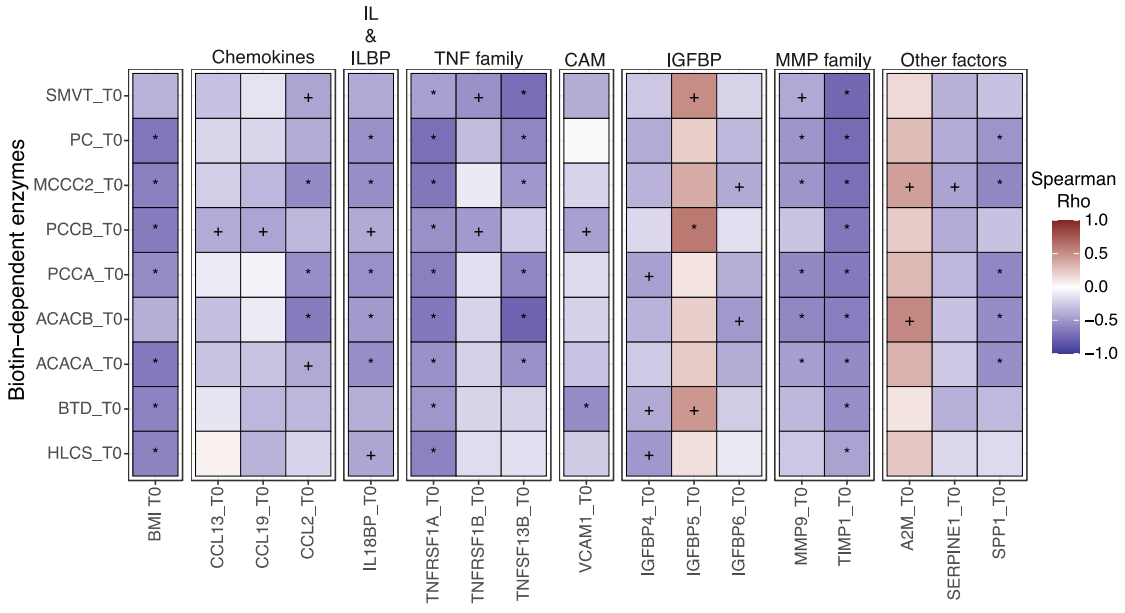
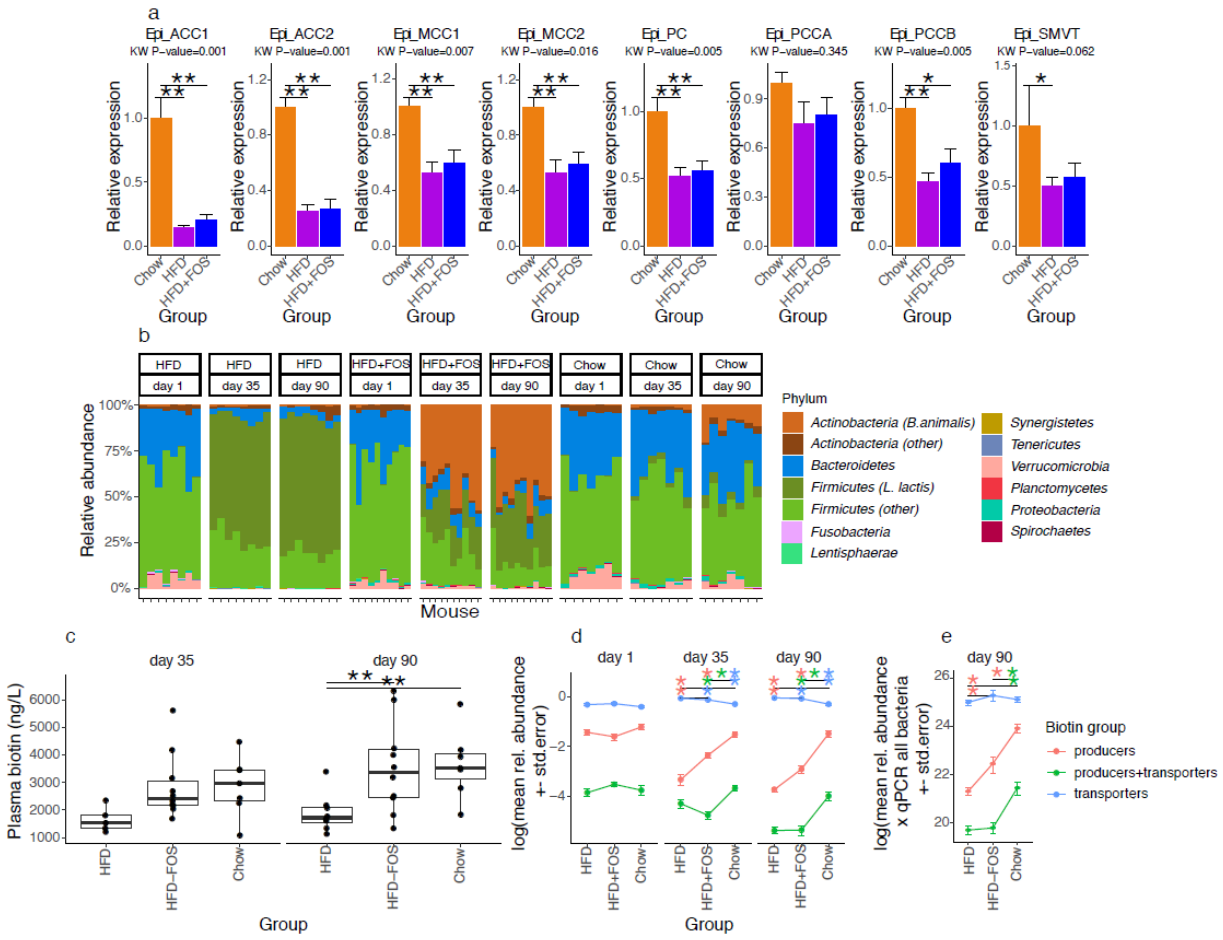
179 **Supplemental Figure 3**: **Absolute abundances of producers and transporters of different**

180 **B-vitamins across obesity stage of severity**. (a) Representation of significant associations

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

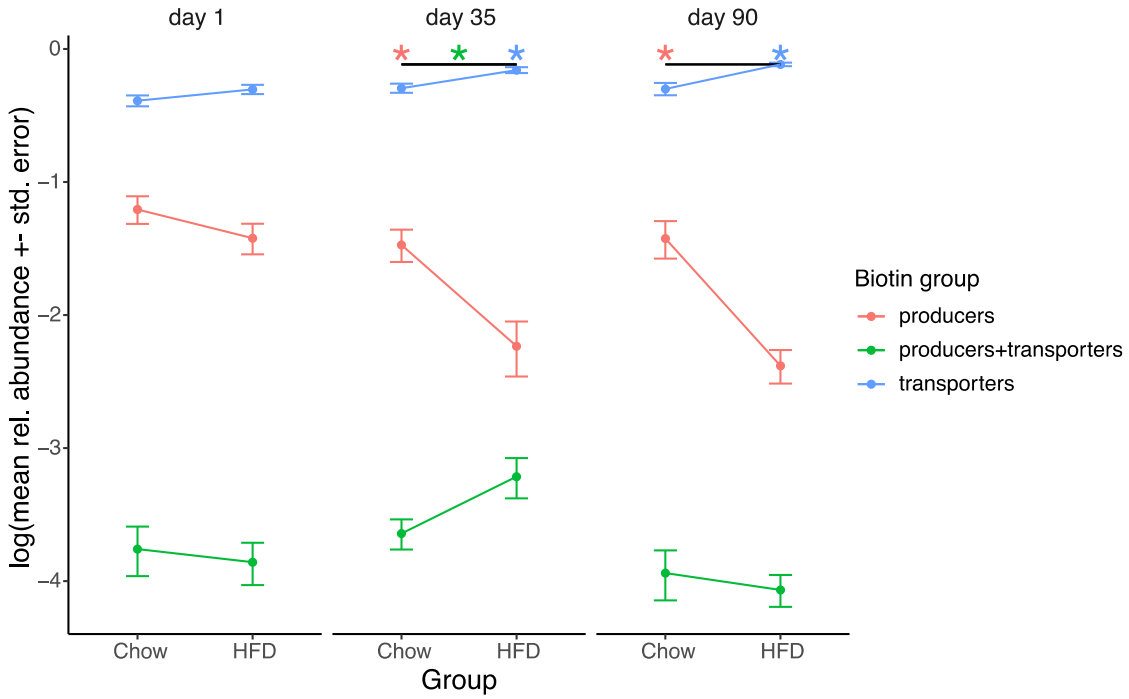


Supplemental Figure 4: Subcutaneous adipose tissue gene expression of biotin-dependent carboxylases and SMVT in relation to obesity and inflammatory factors in bariatric surgery cohort. Spearman correlations of BMI and gene expression of inflammatory factors in subcutaneous adipose tissue samples (measured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

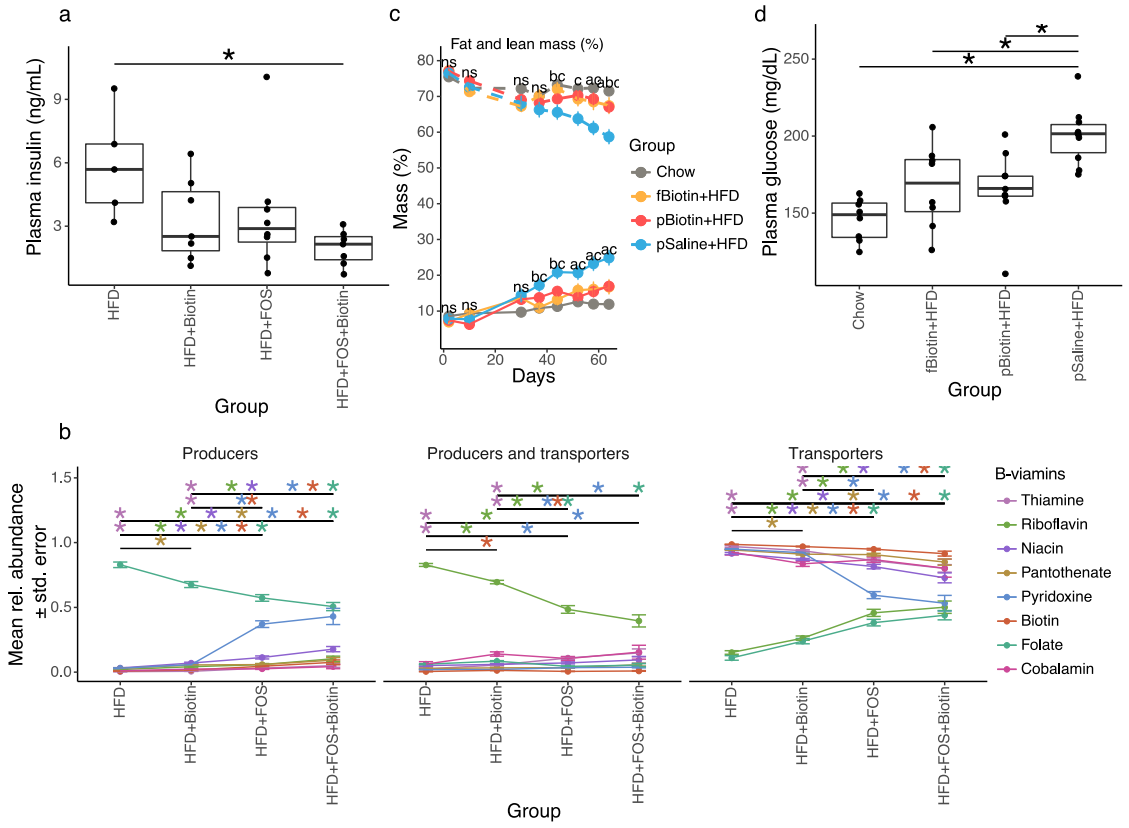


Supplemental Figure 5: Adipose tissue gene expression, serum biotin and abundance of biotin producers and transporters in mice experiments of HFD-induced obesity with FOS supplementation. (a) mRNA expression of biotin carboxylases (ACCA, ACCB, MCC1, MCC2, PCCA, PCCB, PC, SMVT) in epididymal adipose tissue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

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



Supplemental Figure 6: Impact of *L. lactis* removal on the abundances of biotin producers and transporters in mouse experiments of HFD-induced obesity. Abundance profiles of biotin producers (bacteria with all biotin biosynthesis genes from pimelate and no biotin transport gene), biotin transporters (bacteria with no gene involved in biotin biosynthesis) and biotin producers+transporters (bacteria harbouring biotin biosynthesis and transport genes) in age-matched Chow-fed and HFD-fed C57BL6/J mice in baseline (day 1) 4 weeks (day 35) and 13 weeks (day 90) of treatment represented in Figure 4b of the manuscript after excluding *Lactococcus lactis* from the computation of group abundances (*: FDR<0.05 pairwise Wilcoxon rank-sum test; Supplemental Discussion).



Supplemental Figure 7: **Effects of biotin supplementation in mice on body corpulence, insulin and glucose levels and abundance of producers and transporters of different B-vitamins.** (a) Fasting insulinemia of mice with long-term established obesity supplemented with FOS and/or Biotin measured after 6 weeks of treatment by ELISA (*: P-value and FDR<0.05, Kruskal Wallis rank test with Dunn's multiple comparison test). (b) Mean abundances of producers, producers and transporters and transporters of different B-vitamins across mice groups of panel a (*:P-value and FDR<0.05 on Kruskal Wallis tests and in pairwise Wilcoxon rank-sum test within each bacterial group) (c) Body composition: percentage of lean (dashed lines) and fat (plain lines) mass of animals fed a HFD and supplemented by biotin either via subcutaneous osmotic pumps (pBiotin+HFD, n=9), or food (fBiotin+HFD, n=8), as well as two control groups one fed a HFD with subcutaneous osmotic pumps delivering the vehicle solution

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