

# ELECTRONIC SUPPLEMENTARY MATERIAL

## **Intestinal metagenomes and metabolomes in healthy young males: Inactivity and hypoxia generated negative physiological symptoms precede microbial dysbiosis**

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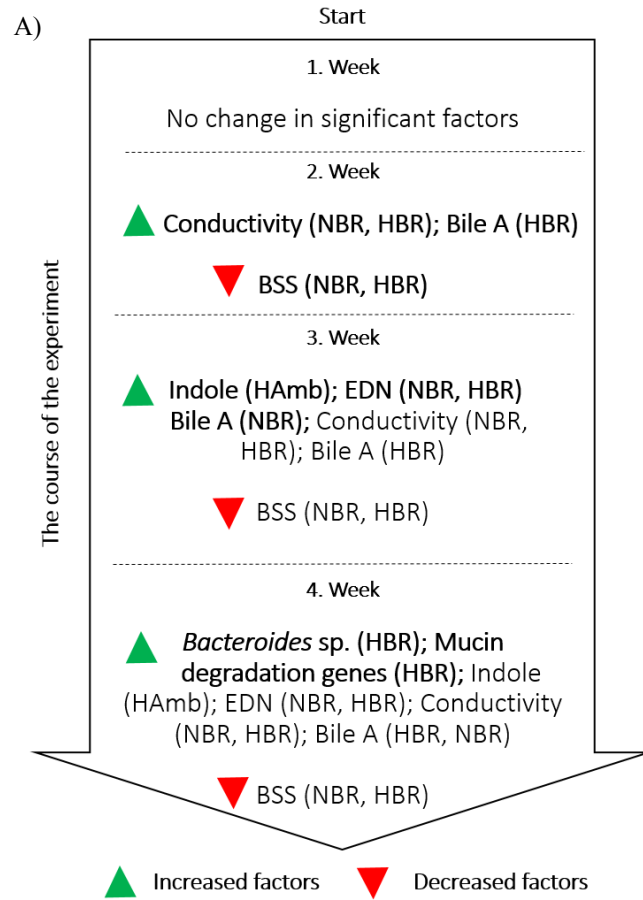
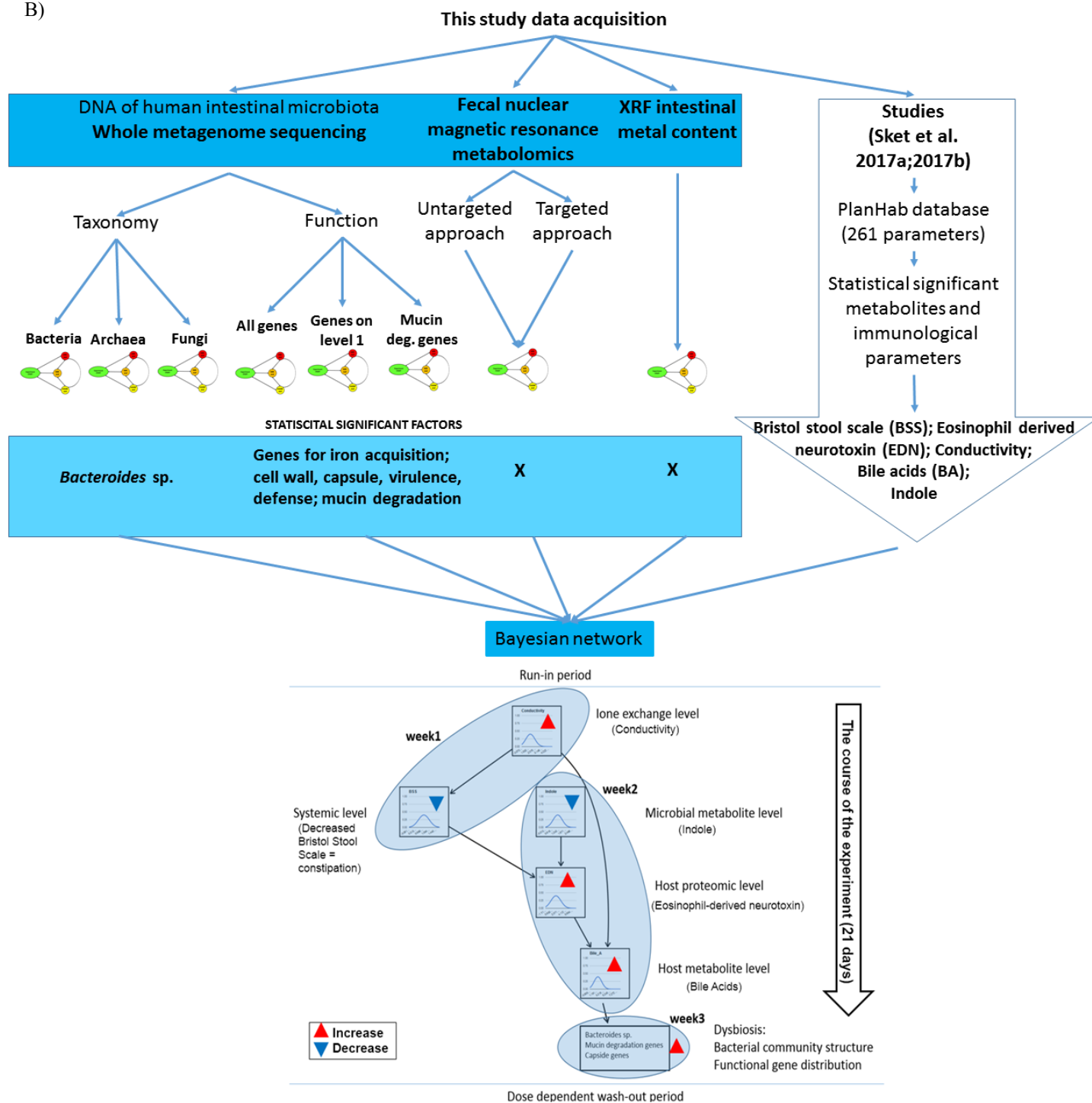
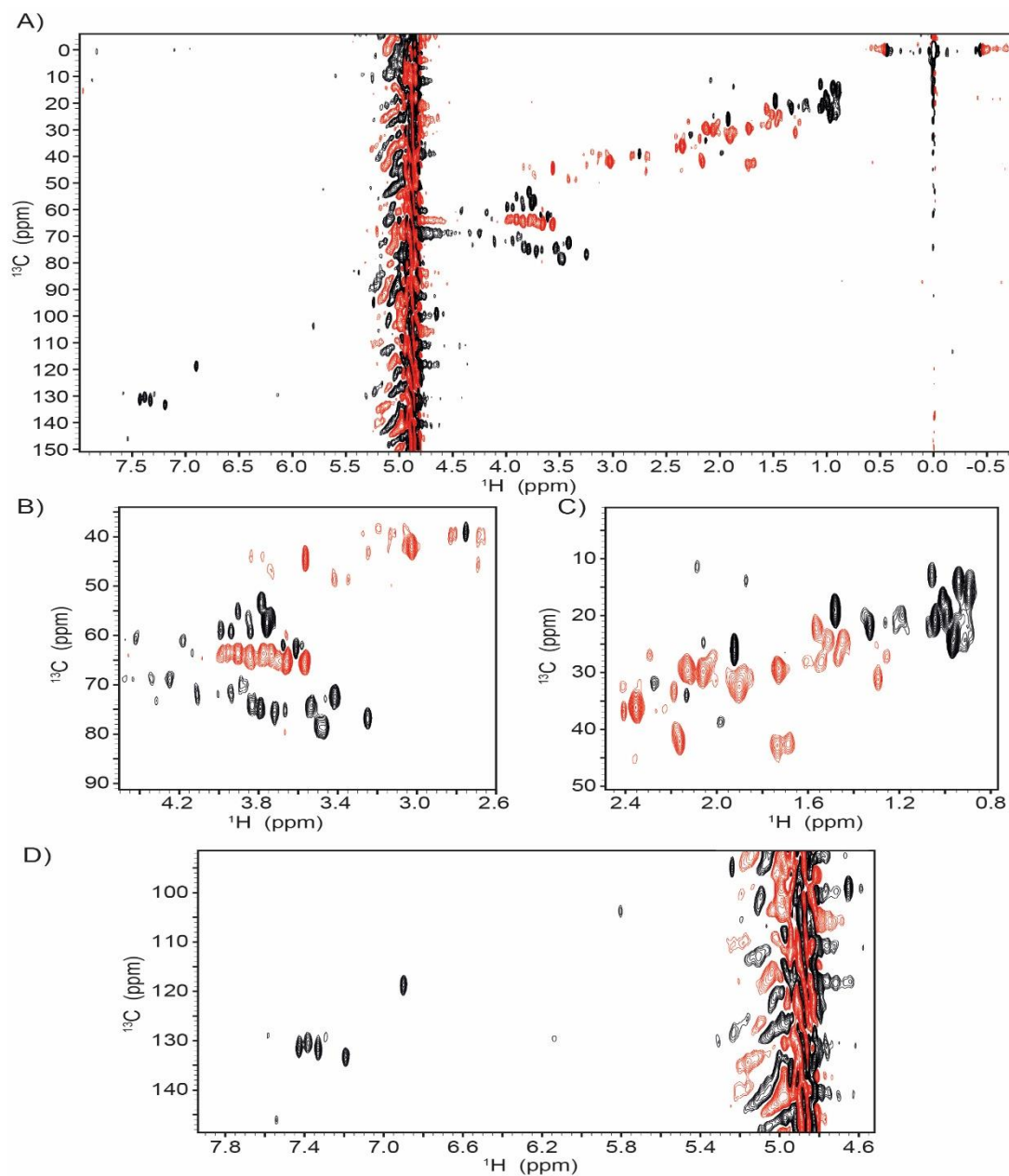


Figure S1 (continues below)

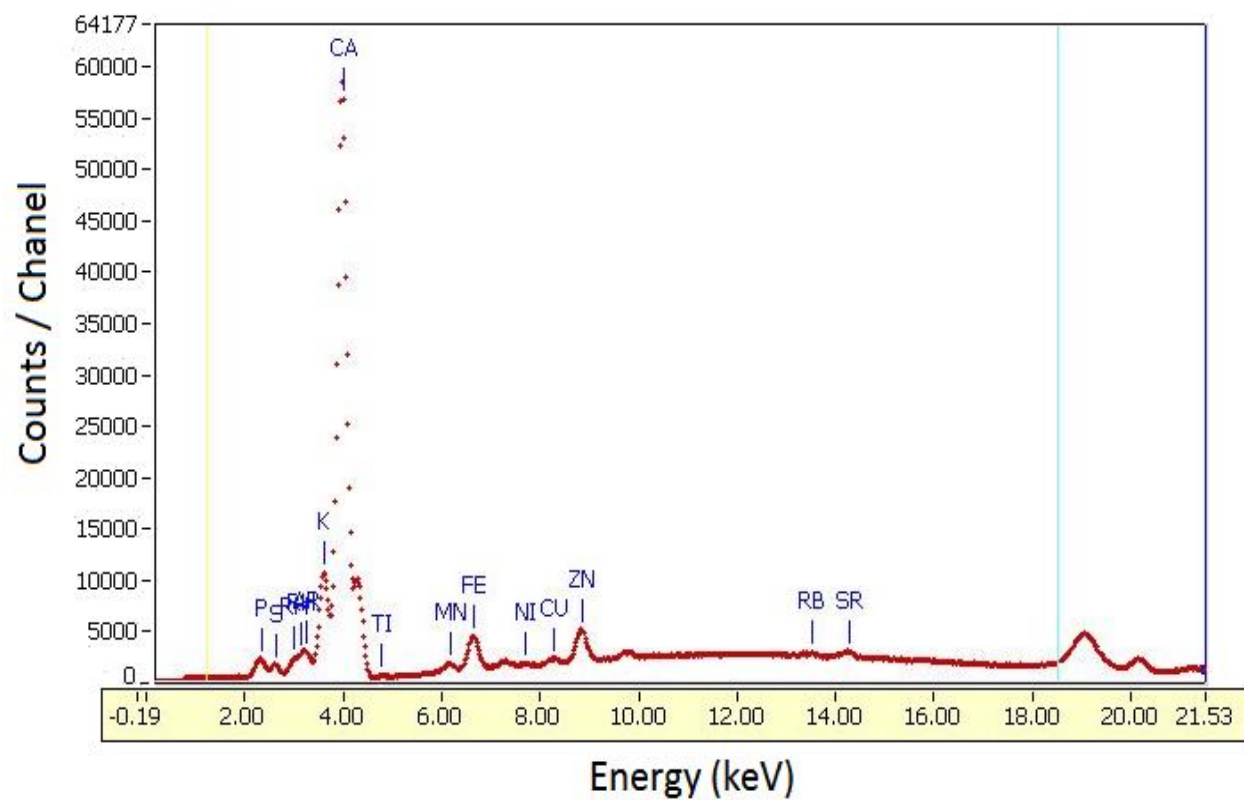
B)



**Figure S1: (A)** A schematic representation of the significant changes in measured parameters of intestinal tract during the 21-day PlanHab experiments in NBR, HBR and HAMB variants reported before (Sket et al., 2017a; 2017b). **(B)** An overview of the analytical approaches adopted in this study, integrating over the different 'omics, spectroscopy, nutrition and physiology layers.



**Figure S2:** 2D  $^1\text{H}$ - $^{13}\text{C}$  NMR HSQC spectrum of sample S12\_1A measured at 25 °C on 800 MHz spectrometer (A), and three zoomed sections showing aliphatic (B and C) as well as aromatic regions (D). HSQC was recorded in multiplicity-edited mode, where correlation signals in black correspond to CH and CH<sub>3</sub> groups, whereas signals in red represent CH<sub>2</sub> groups. Identified metabolites with assigned NMR chemical shifts are shown in Table S1.



**Figure S3:** Fluorescence spectrum of intestinal metal content measured by X-ray fluorescence spectrometry (XRF).

## Comparison: Function (all genes)



▲ **1\*:** 2'-3'-cyclic-nucleotide 2'-phosphodiesterase (EC 3.1.4.16) (p = 0.047); 5'-nucleotidase (EC 3.1.3.5) (p = 0.015); 6-phosphogluconate dehydrogenase: decarboxylating (EC 1.1.1.44) (p = 0.049); Acetylornithine deacetylase (EC 3.5.1.16) (p = 0.028); Biotin carboxyl carrier protein (p = 0.036); Biotin carboxylase of acetyl-CoA carboxylase (EC 6.3.4.14) (p = 0.013); Butyrate kinase (EC 2.7.2.7) (p = 0.045); Chromosome (plasmid) partitioning protein ParA (p = 0.018); COG3866 Pectate lyase (p = 0.036); Cytochrome d ubiquinol oxidase subunit I (EC 1.10.3.-) (p = 0.037); Di-/tripeptide transporter (p = 0.037); DNA primase (EC 2.7.7.-) (p = 0.049); Glutamine synthetase type III. GlnN (EC 6.3.1.2) (p = 0.029); Glycosyl transferase: group 1 (p = 0.035); GTP pyrophosphokinase (EC 2.7.6.5): (p)ppGpp synthetase II (p = 0.020); Guanosine-3'-5'-bis(diphosphate) 3'-pyrophosphohydrolase (EC 3.1.7.2) (p = 0.037); Inner membrane protein translocase component YidC: long form (p = 0.024); Maltose O-acetyltransferase (EC 2.3.1.79) (p = 0.014); NADP-dependent malic enzyme (EC 1.1.1.40) (p = 0.032); PaaD-like protein (DUF59) involved in Fe-S cluster assembly (p = 0.045); Phosphate butyryltransferase (EC 2.3.1.19) (p = 0.028); Rubrerythrin (p = 0.040); Sporulation initiation inhibitor protein Soj (p = 0.028); Topoisomerase IV subunit A (EC 5.99.1.-) (p = 0.027)

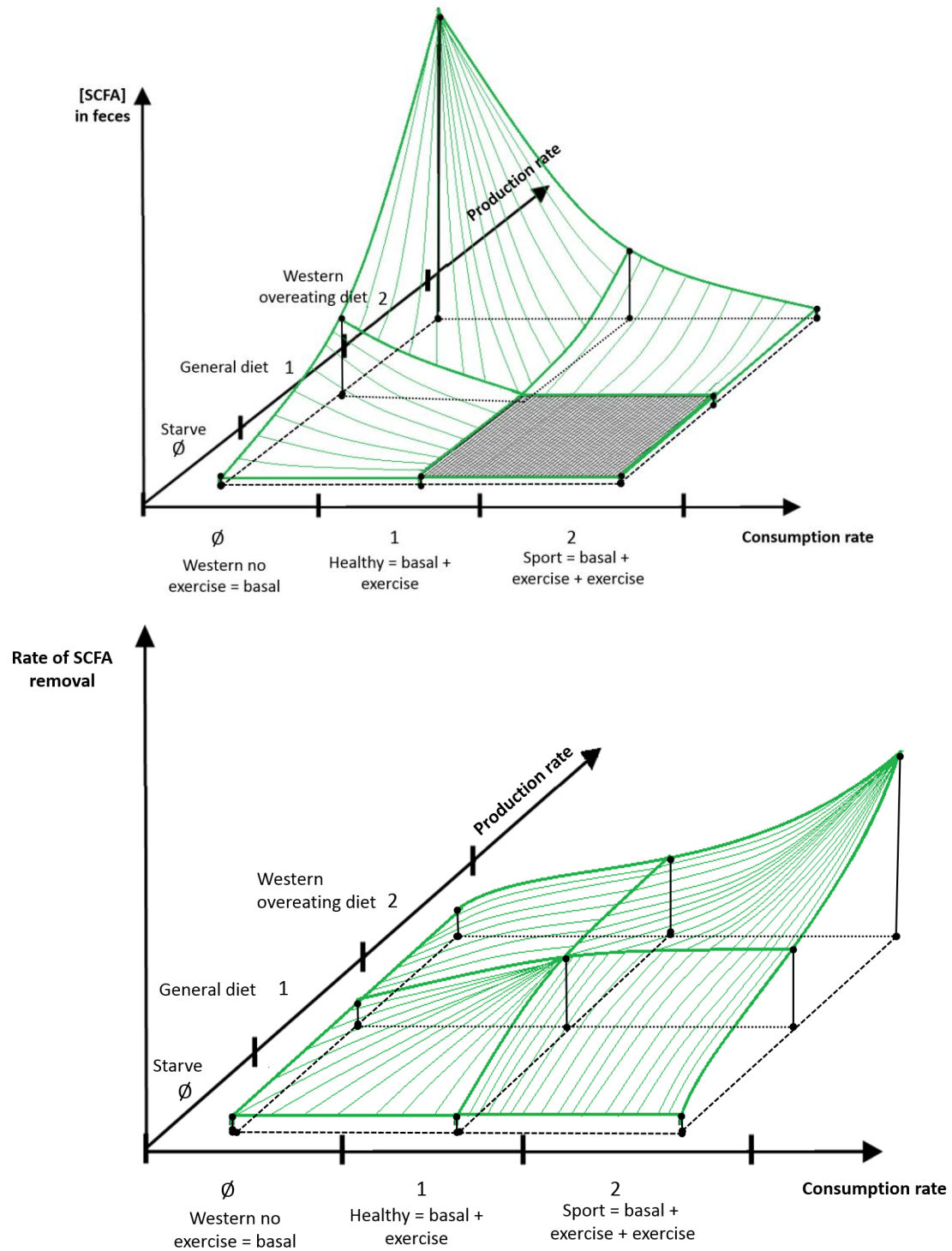


▲ **2\*:** 2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase (EC 4.6.1.12) (p = 0.046); 2-keto-3-deoxy-D-arabino-heptulosonate-7-phosphate synthase I alpha (EC 2.5.1.54) (p = 0.026); Aspartyl-tRNA synthetase (EC 6.1.1.12) (p = 0.029); ATPase component of general energizing module of ECF transporters (p = 0.045); ATP-dependent nuclease: subunit A (p = 0.038); D-alanyl-D-alanine carboxypeptidase (EC 3.4.16.4) (p = 0.049); FIG001583. hypothetical protein: contains S4-like RNA binding domain (p = 0.024); FtsK/SpoIIIE family protein: putative secretion system component EssC/YukA (p = 0.031); Glycerate kinase (EC 2.7.1.31) (p = 0.018); RNA binding methyltransferase FtsJ like (p = 0.040); rRNA small subunit methyltransferase I (p = 0.033); Septum formation protein Maf (p = 0.030); Substrate-specific component BioY of biotin ECF transporter (p = 0.035); Type II/IV secretion system ATP hydrolase TadA/VirB11/CpaF: TadA subfamily (p = 0.045)



▲ **3\*:** Peptide chain release factor 2 (p = 0.042); Pyruvate phosphate dikinase (EC 2.7.9.1) (p = 0.042); Recombination inhibitory protein MutS2 (p = 0.022); Ribosomal-protein-S18p-alanine acetyltransferase (EC 2.3.1.-) (p = 0.033)

**Figure S4:** Statistically significantly increased genes at the functional level at the end point of NBR, HBR and HAMB variants, as described in Figure 3A.



**Figure S5:** A conceptual framework for schematic comparison between the observed concentration of SCFA in feces (A) and the potential rates of SCFA removal into host tissue (B). These results are in line with the generally elevated SCFA concentrations in the obese population.

**Table S1:**  $^1\text{H}$  and  $^{13}\text{C}$  NMR chemical shifts of metabolites identified in sample S12\_1A (in ppm).

| Metabolite         | $^1\text{H}$ (ppm) | $^{13}\text{C}$ (ppm) |
|--------------------|--------------------|-----------------------|
| A-Glucose          | 3.841              | 63.958                |
|                    | 3.777              | 63.684                |
|                    | 3.830              | 74.210                |
|                    | 3.408              | 72.501                |
|                    | 3.723              | 75.666                |
|                    | 3.533              | 74.244                |
|                    | 5.240              | 94.907                |
| B-Glucose          | 3.898              | 63.797                |
|                    | 3.737              | 63.726                |
|                    | 3.471              | 78.744                |
|                    | 3.410              | 72.508                |
|                    | 3.249              | 76.929                |
|                    | 4.650              | 98.891                |
| Butyric acid       | 0.898              | 15.947                |
|                    | 1.558              | 22.224                |
|                    | 2.161              | 42.150                |
| Dimethylamine      | 2.754              | 39.197                |
| Ethanol            | 1.187              | 19.925                |
|                    | 3.661              | 59.898                |
| Glycerol           | 3.790              | 75.042                |
|                    | 3.569              | 65.45                 |
|                    | 3.569              | 65.45                 |
| Guanidoacetic acid | 3.740              | 46.541                |
|                    | 3.789              | 53.416                |
| L-Alanine          | 1.479              | 19.144                |
|                    | 3.789              | 53.416                |
| L-Aspartic acid    | 3.904              | 55.112                |
|                    | 2.808              | 39.528                |
|                    | 2.685              | 39.627                |
| L-Isoleucine       | 3.673              | 62.175                |



|                 |       |         |
|-----------------|-------|---------|
|                 | 1.985 | 38.711  |
|                 | 0.998 | 17.558  |
|                 | 1.257 | 27.278  |
|                 | 1.466 | 27.278  |
|                 | 0.942 | 13.934  |
| L-Lactic acid   |       |         |
|                 | 1.327 | 22.842  |
|                 | 4.111 | 71.939  |
| L-Leucine       |       |         |
|                 | 3.733 | 56.208  |
|                 | 1.687 | 42.608  |
|                 | 1.703 | 26.581  |
|                 | 0.961 | 24.805  |
|                 | 0.947 | 23.754  |
| L-Lysine        |       |         |
|                 | 3.025 | 42.129  |
|                 | 1.707 | 29.328  |
|                 | 1.449 | 24.312  |
|                 | 1.499 | 24.309  |
|                 | 1.900 | 32.622  |
|                 | 3.756 | 57.541  |
| L-Phenylalanine |       |         |
|                 | 3.989 | 59.016  |
|                 | 3.125 | 39.274  |
|                 | 3.272 | 39.274  |
|                 | 7.332 | 131.925 |
|                 | 7.427 | 131.571 |
|                 | 7.383 | 130.531 |
| L-Serine        |       |         |
|                 | 3.837 | 59.388  |
|                 | 3.971 | 63.654  |
| L-Threonine     |       |         |
|                 | 3.579 | 62.150  |
|                 | 4.250 | 68.852  |
|                 | 1.327 | 22.085  |
| L-Tyrosine      |       |         |
|                 | 3.934 | 59.169  |
|                 | 3.192 | 38.496  |
|                 | 3.061 | 38.545  |
|                 | 7.193 | 133.387 |
|                 | 6.901 | 118.702 |

|                 |       |         |
|-----------------|-------|---------|
| L-Valine        | 3.609 | 63.169  |
|                 | 2.274 | 32.088  |
|                 | 0.989 | 19.49   |
|                 | 1.041 | 20.78   |
| L-glutamic acid | 2.352 | 36.258  |
|                 | 2.057 | 30.00   |
|                 | 2.129 | 29.64   |
|                 | 3.758 | 57.524  |
| Malonic acid    | 3.130 | 49.906  |
| Pimelic acid    | 2.170 | 40.067  |
|                 | 1.540 | 28.132  |
|                 | 1.291 | 31.216  |
| Propionic acid  | 1.055 | 12.956  |
|                 | 2.184 | 33.390  |
| Uracil          | 5.807 | 103.704 |
|                 | 7.544 | 146.132 |
| acetate         | 1.921 | 26.195  |
| glycine         | 3.563 | 44.457  |

**Table S2:** Groups of annotated genes used for statistical analyses in Figure 3: (A) Subsystem database level 1; (B) Degradation of host mucins; (C) Butyrate synthesis pathways; (D) Aerobic and anaerobic respiration capacities.

**(A) Subsystem database level 1:** Amino Acids and Derivatives ; Carbohydrates ; Cell Division and Cell Cycle ; Cell Wall and Capsule ; Clustering-based subsystems ; Cofactors Vitamins Prosthetic Groups Pigments ; DNA Metabolism ; Dormancy and Sporulation ; Fatty Acids Lipids and Isoprenoids ; Iron acquisition and metabolism ; Membrane Transport ; Metabolism of Aromatic Compounds ; Miscellaneous ; Motility and Chemotaxis ; Nitrogen Metabolism ; Nucleosides and Nucleotides ; Phages Prophages Transposable elements Plasmids ; Phosphorus Metabolism ; Photosynthesis ; Potassium metabolism ; Protein Metabolism ; Regulation and Cell signaling ; Respiration ; RNA Metabolism ; Secondary Metabolism ; Stress Response ; Sulfur Metabolism ; Virulence Disease and Defense.

**(B) Degradation of host mucins:** Sialidase (EC 3.2.1.18); Beta-galactosidase (EC 3.2.1.23); Beta-galactosidase (EC 3.2.1.23) LacA family; Beta-galactosidase (EC 3.2.1.23) LacZ family; Beta-galactosidase 3; Beta-galactosidase large subunit (EC 3.2.1.23); Beta-galactosidase small subunit (EC 3.2.1.23); Alpha-N-acetylglucosaminidase (EC 3.2.1.50); Alpha-L-fucosidase (EC 3.2.1.51); Beta-hexosaminidase (EC 3.2.1.52); Alpha-galactosidase (EC 3.2.1.22); Alpha-galactosidase precursor (EC 3.2.1.22).

**(C) Butyrate synthesis pathways:** Butyrate kinase (EC 2.7.2.7); Butyrate-acetoacetate CoA-transferase subunit A (EC 2.8.3.9); Butyrate-acetoacetate CoA-transferase subunit B (EC 2.8.3.9); Butyryl-CoA dehydrogenase (EC 1.3.99.2); D-beta-hydroxybutyrate dehydrogenase (EC 1.1.1.30); D-beta-hydroxybutyrate permease; 3-hydroxybutyryl-CoA dehydratase (EC 4.2.1.55); 3-hydroxybutyryl-CoA dehydrogenase (EC 1.1.1.157); 2-amino-3-ketobutyrate coenzyme A ligase (EC 2.3.1.29); Acetyl-CoA:acetoacetyl-CoA transferase. alpha subunit (EC 2.8.3.8); Lysine 2,3-aminomutase (EC 5.4.3.2); L-beta-lysine 5,6-aminomutase alpha subunit (EC 5.4.3.3); L-beta-lysine 5,6-aminomutase beta subunit (EC 5.4.3.3); 3-keto-5-aminohexanoate cleavage enzyme; 3-aminobutyryl-CoA ammonia lyase (EC 4.3.1.14); Phosphate butyryltransferase (EC 2.3.1.19) ; 3-ketoacyl-CoA thiolase (EC 2.3.1.16).

**(D) Aerobic and anaerobic respiration capacities:** 2,4-dienoyl-CoA reductase (NADPH) (EC 1.3.1.34); 2-dehydropanoate 2-reductase (EC 1.1.1.169); 2-hydroxy-3-oxopropionate reductase (EC 1.1.1.60); 2-polyprenylphenol hydroxylase and related flavodoxin oxidoreductases; 3-oxoacyl-(acyl-carrier protein) reductase (EC 1.1.1.100); 3-oxoacyl-(acyl-carrier protein) reductase paralog (EC 1.1.1.100) in cluster with unspecified monosaccharide transporter; 4-hydroxy-3-methylbut-2-enyl diphosphate reductase (EC 1.17.1.2); 5,10-methylenetetrahydrofolate reductase (EC 1.5.1.20); 5-amino-6-(5-phosphoribosylamino)uracil reductase (EC 1.1.1.193); 5-keto-D-gluconate 5-reductase (EC 1.1.1.69); Acetoacetyl-CoA reductase (EC 1.1.1.36); Adenylylsulfate reductase alpha-subunit (EC 1.8.99.2); Adenylylsulfate reductase beta-subunit (EC 1.8.99.2); Aldo-keto reductase family 1 member B10 (EC 1.1.1.-); Alkyl hydroperoxide reductase protein C (EC 1.6.4.-); Alkyl hydroperoxide reductase protein F (EC 1.6.4.-); Alkyl hydroperoxide reductase subunit C-like protein; Altronate oxidoreductase (EC 1.1.1.58); Anaerobic dimethyl sulfoxide reductase chain A (EC 1.8.99.-); Anaerobic dimethyl sulfoxide reductase chain B (EC 1.8.99.-); Arsenate reductase (EC 1.20.4.1); Benzoyl-CoA reductase subunit BadE (EC 1.3.99.15); Benzoyl-CoA reductase subunit BadF (EC 1.3.99.15); Benzoyl-CoA reductase subunit BadG (EC 1.3.99.15); CoA-disulfide reductase (EC 1.8.1.14); Cob(II)alamin reductase; Cob(III)alamin reductase; Cobalt-precorrin-6x reductase (EC 1.3.1.54); CoB--CoM heterodisulfide reductase subunit A (EC 1.8.98.1); CoB--CoM heterodisulfide reductase subunit B (EC 1.8.98.1); CoB--CoM heterodisulfide reductase subunit C (EC 1.8.98.1); CoB--CoM heterodisulfide reductase subunit D (EC 1.8.98.1); Coenzyme A disulfide reductase; Cytochrome c nitrite reductase, small subunit NrfH; Cytochrome c-type biogenesis protein DsbD, protein-disulfide reductase (EC 1.8.1.8); Dihydrodipicolinate reductase (EC 1.3.1.26); Dihydroflavonol-4-reductase (EC 1.1.1.219); Dihydrofolate reductase (EC 1.5.1.3); Dissimilatory sulfite reductase (desulfoviridin), alpha and beta subunits; D-mannonate oxidoreductase (EC 1.1.1.57); D-proline reductase, 23 kDa subunit (EC 1.21.4.1); D-proline reductase, 45 kDa subunit (EC 1.21.4.1); dTDP-4-dehydrothamnose reductase (EC 1.1.1.133); Enoyl-(acyl-carrier-protein) reductase (FMN) (EC 1.3.1.9); Enoyl-(acyl-carrier-protein) reductase (NADH) (EC 1.3.1.9); Ferredoxin reductase; Ferredoxin-type protein NapG (periplasmic nitrate reductase); Fumarate reductase flavoprotein subunit (EC 1.3.99.1); Gamma-glutamyl phosphate reductase (EC 1.2.1.41); Glutamyl-tRNA reductase (EC 1.2.1.70); Glycine reductase component B alpha subunit (EC 1.21.4.2); Glycine reductase component B beta subunit (EC 1.21.4.2); Glycine reductase component B gamma subunit (EC 1.21.4.2);

Glycine/sarcosine/betaine reductase component C chain 1; Glycine/sarcosine/betaine reductase component C chain 2; Glyoxylate reductase (EC 1.1.1.26); Glyoxylate reductase (EC 1.1.1.79); Heterodisulfide reductase. cytochrome reductase subunit; heterodisulfide reductase. iron-sulfur binding subunit. putative; heterodisulfide reductase. subunit A/methylviologen reducing hydrogenase. subunit delta; Hydroxylamine reductase (EC 1.7.-.-); Hydroxymethylglutaryl-CoA reductase (EC 1.1.1.34); Hydroxypyruvate reductase (EC 1.1.1.81); Hypothetical oxidoreductase YdjG (EC 1.-.-.-); Indolepyruvate oxidoreductase subunit IorA (EC 1.2.7.8); Indolepyruvate oxidoreductase subunit IorB (EC 1.2.7.8); Ketoisovalerate oxidoreductase subunit VorA (EC 1.2.7.7); Ketoisovalerate oxidoreductase subunit VorB (EC 1.2.7.7); Lactaldehyde reductase (EC 1.1.1.77); L-sorbose 1-phosphate reductase (EC 1.1.1.-); Mercuric ion reductase (EC 1.16.1.1); Na(+)-translocating NADH-quinone reductase subunit A (EC 1.6.5.-); Na(+)-translocating NADH-quinone reductase subunit B (EC 1.6.5.-); Na(+)-translocating NADH-quinone reductase subunit C (EC 1.6.5.-); Na(+)-translocating NADH-quinone reductase subunit D (EC 1.6.5.-); Na(+)-translocating NADH-quinone reductase subunit E (EC 1.6.5.-); Na(+)-translocating NADH-quinone reductase subunit F (EC 1.6.5.-); N-acetyl-gamma-glutamyl-phosphate reductase (EC 1.2.1.38); NAD(P)H oxidoreductase YRKL (EC 1.6.99.-); NADH ubiquinone oxidoreductase chain A (EC 1.6.5.3); NADH-ubiquinone oxidoreductase chain B (EC 1.6.5.3); NADH-ubiquinone oxidoreductase chain C (EC 1.6.5.3); NADH-ubiquinone oxidoreductase chain D (EC 1.6.5.3); NADH-ubiquinone oxidoreductase chain E (EC 1.6.5.3); NADH-ubiquinone oxidoreductase chain F (EC 1.6.5.3); NADH-ubiquinone oxidoreductase chain H (EC 1.6.5.3); NADH-ubiquinone oxidoreductase chain I (EC 1.6.5.3); NADH-ubiquinone oxidoreductase chain J (EC 1.6.5.3); NADH-ubiquinone oxidoreductase chain K (EC 1.6.5.3); NADH-ubiquinone oxidoreductase chain L (EC 1.6.5.3); NADH-ubiquinone oxidoreductase chain M (EC 1.6.5.3); NADH-ubiquinone oxidoreductase chain N (EC 1.6.5.3); NADPH-dependent 7-cyano-7-deazaguanine reductase (EC 1.7.1.-); Nitric oxide reductase activation protein NorD; Nitric oxide reductase activation protein NorQ; Nitrite reductase (NAD(P)H) large subunit (EC 1.7.1.4); Nitrite reductase probable (NAD(P)H) subunit (EC 1.7.1.4); Nitrite reductase probable electron transfer 4Fe-S subunit (EC 1.7.1.4); Nitrogenase (molybdenum-iron) reductase and maturation protein NifH; Peptide methionine sulfoxide reductase MsrA (EC 1.8.4.11); Peptide methionine sulfoxide reductase MsrB (EC 1.8.4.12); PF00070 family. FAD-dependent NAD(P)-disulphide oxidoreductase; Polyferredoxin NapH (periplasmic nitrate reductase); Predicted L-lactate dehydrogenase. Fe-S

oxidoreductase subunit YkgE; Probable electron transfer flavoprotein-quinone oxidoreductase FixC (EC 1.5.5.-); Probable thiol oxidoreductase with 2 cytochrome c heme-binding sites; PUA-PAPS reductase like fusion; Putative oxidoreductase linked to yggC; Putative oxidoreductase YdjL; Pyrroline-5-carboxylate reductase (EC 1.5.1.2); Pyruvateferredoxin oxidoreductase. alpha subunit (EC 1.2.7.1); Pyruvateferredoxin oxidoreductase. beta subunit (EC 1.2.7.1); Pyruvateferredoxin oxidoreductase. delta subunit (EC 1.2.7.1); Pyruvateferredoxin oxidoreductase. gamma subunit (EC 1.2.7.1); Pyruvate-flavodoxin oxidoreductase (EC 1.2.7.-); Respiratory nitrate reductase alpha chain (EC 1.7.99.4); Ribonucleotide reductase of class Ia (aerobic). alpha subunit (EC 1.17.4.1); Ribonucleotide reductase of class Ia (aerobic). beta subunit (EC 1.17.4.1); Ribonucleotide reductase of class Ib (aerobic). alpha subunit (EC 1.17.4.1); Ribonucleotide reductase of class Ib (aerobic). beta subunit (EC 1.17.4.1); Ribonucleotide reductase of class II (coenzyme B12-dependent) (EC 1.17.4.1); Ribonucleotide reductase of class III (anaerobic). activating protein (EC 1.97.1.4); Ribonucleotide reductase of class III (anaerobic). large subunit (EC 1.17.4.2); Ribonucleotide reductase transcriptional regulator NrdR; Superoxide reductase (EC 1.15.1.2); Thioldisulfide oxidoreductase related to ResA; Thioredoxin reductase (EC 1.8.1.9); Trimethylamine-N-oxide reductase (EC 1.6.6.9); UDP-N-acetylenolpyruvoylglucosamine reductase (EC 1.1.1.158)