**SUPPLEMENTARY INFORMATION**



**S1.** A PLS-DA scores plot (A, Q2*Y*=0.83) derived from UPLC-MS(-)data of faecal samples shows a separation between 6 sham (blue) and 6 RYGB (red)-operated rats at week 8. Plot (B) indicates cholic acid levels in both sham- and RYGB-operated rats at 1 week pre-operation, 6 and 8 weeks post operation, which is the main contributor to the group separation post surgery. Relative concentrations of observed unconjugated (C) and taurine-conjugated (D) bile acids show a significant difference between sham and RYGB-operated rats at weeks 6 and 8. Error bar indicates standard error of the mean and the significance value, p, was calculated by Student’s t-Test.

**S2.** Phylogenetic tree (neighbor-joining from a jukes-cantor matrix) of the main taxonomic groups which varied between the two test groups, only the higher taxonomic groups from which they were clustered is shown. The barchart to the right of the tree shows the reads associated with a representative from its taxonomic group. \* *Enterobacter hormaechei*.



**S3.** Typical 600 MHz 1H NMR spectra of urine obtained from a sham control rat (A) and a Roux-en-Y Gastric Bypass (RYGB)-operated rat (B) 8-week post operation. The spectra in the aromatic region (δ1H 6.4-9.3) and the region δ1H 0.6-2.28 were magnified twice compared to the region δ1H 2.28-4.7. Keys: Ace: acetate; Ala: alanine; AP: 2-oxoadipate; AV: 5-aminovalerate; BT: butyrate; Cit: citrate; Cre: creatine; Crn: creatinine; DMA: dimethylamine; FA: formate; FM: fumarate; GT: 2-oxoglutarate; HA: *p*-hydroxyphenylacetate; HP: hippurate; IS: indoxyl sulfate; Lac: lactate; MA: methylamine; MNA: 1-methylnicotinamide; PAG: phenylacetylglycine; PG: *p*-cresyl glucuronide; PS: *p*-cresyl sulfate; Suc: succinate; Tau: taurine; TMAO: trimethylamine *N*-oxide.



**S4.** Typical 600 MHz 1H NMR spectra of faecal extracts obtained from a sham control rat (A) and a Roux-en-Y Gastric Bypass (RYGB)-operated rat (B) 8-week post operation. The spectra in the aromatic region (δ1H 5.75-8.5) were magnified 4 times compared to the region δ1H 0.5-4.7. Keys: Ace: acetate; Ala: alanine; AP: 2-oxoadipate; Asp: aspartate; AV: 5-aminovalerate; BT: butyrate; DE: diaminoethane; ET: ethanol; FA: formate; FM: fumarate; GABA: -animo-*N*-butyrate; Glu: glutamate; Gly: glycine; Ileu: isoleucine; Lac: lactate; Leu: leucine; MA: methylamine; MT: methanol; OS: oligosaccharides; Phe: phenylalanine; PP: propionate; PT: putrescine; Suc: succinate; TMA: trimethylamine; Tyr: tyrosine; Ura: uracil; Val: valine.



**S5.** O-PLS regression loadings plot shows the correlation between the combination of urinary and faecal NMR spectral data and *Enterobacter hormaechei* (Q2*Y*=0.7; R2*X*=28%) level.

**A**



**B**



**S6.** (A) O-PLS regression coefficient plot derived from 1H NMR urinary (Q2*Y*=0.58; R2*X*=31.4%) and faecal (Q2*Y*=0.61; R2*X*=28%) spectral data against body weight. (B) Scatter plots of bacterial family levels and body weight of rats (red: RYGB-operated; blue: sham control) at week 2 (circles) and 8 (stars).



**S7.** Cross correlation plots between selected urinary and faecal metabolites and the abundance levels of 37 bacterial families across matched samples (correlations significant at *p* < 0.01) Key: PS, *p*-cresyl sulfate; PG, *p*-cresyl glucuronide; PAG, phenylacetylglycine.

**Table S1** 454 primers use to amplify the V1-V3 regions of the 16S rRNA gene, the portion in bold is the unique barcode (5mer) that distinguished each sample and allows for multiplexing of the sample on the 454. To the right of the barcode is the target primer which anneals to the 16S rRNA gene.

|  |  |
| --- | --- |
| Sample | Forward primer and barcode in bold |
| S1 | CCATCTCATCCCTGCGTGTCTCCGACTCAG GATCT GCCTAACACATGCAAGTC |
| S2 | CCATCTCATCCCTGCGTGTCTCCGACTCAG ATCAG GCCTAACACATGCAAGTC |
| S3 | CCATCTCATCCCTGCGTGTCTCCGACTCAG ACACT GCCTAACACATGCAAGTC |
| S4 | CCATCTCATCCCTGCGTGTCTCCGACTCAG AGCTA GCCTAACACATGCAAGTC |
| S5 | CCATCTCATCCCTGCGTGTCTCCGACTCAG CACAC GCCTAACACATGCAAGTC |
| S6 | CCATCTCATCCCTGCGTGTCTCCGACTCAG ACAGA GCCTAACACATGCAAGTC |
| S7 | CCATCTCATCCCTGCGTGTCTCCGACTCAG AGATG GCCTAACACATGCAAGTC |
| S8 | CCATCTCATCCCTGCGTGTCTCCGACTCAG CACTG GCCTAACACATGCAAGTC |
| S9 | CCATCTCATCCCTGCGTGTCTCCGACTCAG CAGAG GCCTAACACATGCAAGTC |
| S10 | CCATCTCATCCCTGCGTGTCTCCGACTCAG CGCAG GCCTAACACATGCAAGTC |
| S11 | CCATCTCATCCCTGCGTGTCTCCGACTCAG CTGTG GCCTAACACATGCAAGTC |
| S12 | CCATCTCATCCCTGCGTGTCTCCGACTCAG GTGAG GCCTAACACATGCAAGTC |
| B1 | CCATCTCATCCCTGCGTGTCTCCGACTCAG TCATG GCCTAACACATGCAAGTC |
| B2 | CCATCTCATCCCTGCGTGTCTCCGACTCAG AGCAT GCCTAACACATGCAAGTC |
| B3 | CCATCTCATCCCTGCGTGTCTCCGACTCAG CAGCT GCCTAACACATGCAAGTC |
| B4 | CCATCTCATCCCTGCGTGTCTCCGACTCAG CATGT GCCTAACACATGCAAGTC |
| B5 | CCATCTCATCCCTGCGTGTCTCCGACTCAG CTGAT GCCTAACACATGCAAGTC |
| B6 | CCATCTCATCCCTGCGTGTCTCCGACTCAG CTGCA GCCTAACACATGCAAGTC |
| B7 | CCATCTCATCCCTGCGTGTCTCCGACTCAG GATGA GCCTAACACATGCAAGTC |
| B8 | CCATCTCATCCCTGCGTGTCTCCGACTCAG TACGC GCCTAACACATGCAAGTC |
| B9 | CCATCTCATCCCTGCGTGTCTCCGACTCAG ACTGC GCCTAACACATGCAAGTC |
| B10 | CCATCTCATCCCTGCGTGTCTCCGACTCAG GTCAC GCCTAACACATGCAAGTC |
| B11 | CCATCTCATCCCTGCGTGTCTCCGACTCAG CGTAC GCCTAACACATGCAAGTC |
| B12 | CCATCTCATCCCTGCGTGTCTCCGACTCAG TGCGT GCCTAACACATGCAAGTC |
|  |  |
| Reverse primer | CCTATCCCCTGTGTGCCTTGGCAGTCTCAG ATTACCGCGGCTGCTGG |