

## Supplementary Material

### Development of a gut microbiota model for the analysis of bacterial modifications of xenobiotics

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#### ***Supplementary methods for xenobiotic transformation assays***

All three media used in this work were based on a modified SHIME® salt solution. This solution served as a defined basal medium that replicated the ionic composition and buffering conditions of the intestinal lumen. The fiber-rich medium was used to represent a polysaccharide-rich colonic environment, whereas the Western-style media were designed to mimic a luminal environment richer in starch, protein, and fatty acids, reflecting dietary patterns associated with a Western-style diet. Supplementation with bile salts was included to additionally account for bile exposure under high-fat dietary conditions. Together, these media were selected to cover distinct physiologically relevant nutritional environments for functional regeneration of the isolated colon microbiota.

**Table S1: Composition of the modified SHIME®-based regeneration media.**

|                                        | Fiber-rich medium   | Western-style medium without BS | Western-style medium with BS |
|----------------------------------------|---------------------|---------------------------------|------------------------------|
| NaHCO <sub>3</sub>                     | 29.8 mM             | 29.8 mM                         | 29.8 mM                      |
| NaCl                                   | 34.2 mM             | 34.2 mM                         | 34.2 mM                      |
| NH <sub>4</sub> Cl                     | 18.7 mM             | 18.7 mM                         | 18.7 mM                      |
| K <sub>2</sub> HPO <sub>4</sub>        | 49.5 mM             | 49.5 mM                         | 49.5 mM                      |
| KH <sub>2</sub> PO <sub>4</sub>        | 49.8 mM             | 49.8 mM                         | 49.8 mM                      |
| CaCl <sub>2</sub> x 2 H <sub>2</sub> O | 0.07 mM             | 0.07 mM                         | 0.07 mM                      |
| MgSO <sub>4</sub> x 7 H <sub>2</sub> O | 0.03 mM             | 0.03 mM                         | 0.03 mM                      |
| Mucin                                  | 1 g L <sup>-1</sup> | 1 g L <sup>-1</sup>             | 1 g L <sup>-1</sup>          |
| Pectin                                 | 1 g L <sup>-1</sup> | 1 g L <sup>-1</sup>             | 1 g L <sup>-1</sup>          |
| Xylan                                  | 1 g L <sup>-1</sup> | 1 g L <sup>-1</sup>             | 1 g L <sup>-1</sup>          |
| Starch                                 | 1 g L <sup>-1</sup> | 1 g L <sup>-1</sup>             | 1 g L <sup>-1</sup>          |
| Casein                                 | -                   | 0.5 g L <sup>-1</sup>           | 0.5 g L <sup>-1</sup>        |
| Peptone                                | -                   | 0.5 g L <sup>-1</sup>           | 0.5 g L <sup>-1</sup>        |
| Palmitic acid                          | -                   | 0.25 g L <sup>-1</sup>          | 0.25 g L <sup>-1</sup>       |
| Stearic acid                           | -                   | 0.25 g L <sup>-1</sup>          | 0.25 g L <sup>-1</sup>       |
| Oleic acid                             | -                   | 0.25 g L <sup>-1</sup>          | 0.25 g L <sup>-1</sup>       |
| Linoleic acid                          | -                   | 0.25 g L <sup>-1</sup>          | 0.25 g L <sup>-1</sup>       |
| Acetate                                | -                   | 10 mM                           | 10 mM                        |
| Propionate                             | -                   | 10 mM                           | 10 mM                        |
| Bile salts                             | -                   | -                               | 0.5 g L <sup>-1</sup>        |

To evaluate microbial xenobiotic metabolism under defined conditions, incubations were conducted using model substrates representing major biotransformation classes: 4-acetoxyacetanilide (ester hydrolysis), sulindac (sulfoxide reduction), sulfasalazine (azo bond reduction), and nitrendipine (nitro group reduction). Reaction mixtures were prepared under anaerobic conditions (37°C, 24 h) as follows: (i) 210 µL SHIME® saline containing the fiber-rich substrate formulation with 1 g L<sup>-1</sup> mucin, pectin, and xylan (substrate control), (ii) 150 µL pooled fecal suspension (three individual pools with n = 3 donors each, 1:10 diluted in SHIME® saline) + 60 µL fiber-rich substrate, (iii) 300 µL pooled exoenzyme fraction (three individual pools with n = 3 donors each, 1:10 in SHIME® saline), and (iv) 150 µL bacterial colon model + 60 µL fiber-rich substrate. All incubations contained 500 µM of the respective test compound. At defined time points, samples were mixed 1:1 (v/v) with ethanol, vortexed, and centrifuged

(18,000 × g, 3 min). The resulting supernatant was combined 1:1 (v/v) with acetonitrile (100 %) and centrifuged again.

The clarified supernatant was diluted with the mobile phase prior to injection. Non-polar and UV-absorbing compounds were analyzed on a Knauer Eurospher II C8 column (250 × 4 mm, 5 µm) with a C8 guard cartridge using an AZURA CT 2 system (Knauer, Berlin, Germany) equipped with a SpectraSystem SCM1000 degasser, P4000 pump, AS3000 autosampler, and UVD 2.1L detector. Paracetamol was analyzed on a Shodex Asahipak NH2P-50 3E column (Showa Denko, Japan) with a corresponding guard column and an RI-101 detector (Ercatech AG, Germany). Chromatographic parameters, including mobile-phase composition, column temperature, flow rate, and detection wavelength are summarized in Suppl. Tab. S1. Quantification of parent compounds and metabolites was performed by external calibration using analytical reference standards.

**Table S2: Chromatographic conditions for HPLC-based detection of selected xenobiotics and their microbial transformation products.** Analytes were separated on either C8 or amino columns under compound-specific conditions. Mobile phases consisted of acetonitrile (ACN) with acidic modifiers and were operated under isocratic flow. Detection wavelengths correspond to the absorbance maxima of each compound.

|                             | Column | Mobile phase                                                                     | Temperature [°C] | Flow rate [ml min <sup>-1</sup> ] | UV detection [nm] |
|-----------------------------|--------|----------------------------------------------------------------------------------|------------------|-----------------------------------|-------------------|
| <b>Sulindac</b>             | C8     | 40 % ACN + 0.1 % H <sub>3</sub> PO <sub>4</sub>                                  | 40               | 0.5                               | 340               |
| <b>Sulindac-sulfide</b>     | C8     | 40 % ACN + 0.1 % H <sub>3</sub> PO <sub>4</sub>                                  | 40               | 0.5                               | 340               |
| <b>Sulfasalazin</b>         | C8     | 40 % ACN + 0.1 % H <sub>3</sub> PO <sub>4</sub>                                  | 40               | 0.5                               | 340               |
| <b>Sulfapyridine</b>        | C8     | 15 % ACN + 0.1 % HCOOH                                                           | 40               | 0.5                               | 265               |
| <b>Roxatidine-acetate</b>   | C8     | 50 % KH <sub>2</sub> PO <sub>4</sub> (50 mM) +<br>30 % MeOH + 20 % ACN<br>pH 7.0 | 40               | 0.5                               | 276               |
| <b>Roxatidine</b>           | C8     | 50 % KH <sub>2</sub> PO <sub>4</sub> (50 mM) +<br>30 % MeOH + 20 % ACN<br>pH 7.0 | 40               | 0.5                               | 276               |
| <b>4-Acetoxyacetanilide</b> | C8     | 40 % ACN                                                                         | 40               | 0.5                               | 254               |
| <b>Chloramphenicol</b>      | C8     | 40 % ACN                                                                         | 40               | 0.5                               | 254               |
| <b>Paracetamol</b>          | Amino  | 65 % ACN                                                                         | 60               | 0.5                               | 240               |
| <b>Nitrendipine</b>         | C8     | 60 % ACN                                                                         | 40               | 0.5                               | 254               |

To confirm the identity of selected biotransformation products, representative samples were analyzed by liquid chromatography-mass spectrometry (LC-MS). Samples were mixed 1:1 (v/v) with ethanol and centrifuged (18,000 × g, 5 min). From the resulting supernatant, 50 µL were diluted in 450 µL H<sub>2</sub>O: ACN (70:30, v/v). Prior to analysis, samples were filtered through 0.2 µm Chromafil RC-20/15 MS syringe filters. For chromatographic separation the Acquity

UPLC I-Class system (Waters, Milford, MA, USA), consisting of a binary pump, a sample manager cooled to 10°C and a column oven set at 40°C, was used. The system was equipped with an HSS T3 C18 column (2.1 × 100 mm; 1.8 µm) and a VanGuard HSS T3 C18 precolumn (5 × 2.1 mm, 1.7 µm) (Waters, Milford, MA, USA). Elution was performed with 0.1 % Formic acid in H<sub>2</sub>O (A) and 0.1 % Formic acid in ACN (B) at a flow rate of 0.4 mL min<sup>-1</sup>. The gradient elution was performed as follows: 0-1 min, 80 % B, 1-2.5 min, 50 % B, 2.5-3.7 min, 0 % B, 3.7-4.3 min, 0 % B, 4.3-5.0 min, 80 % B.

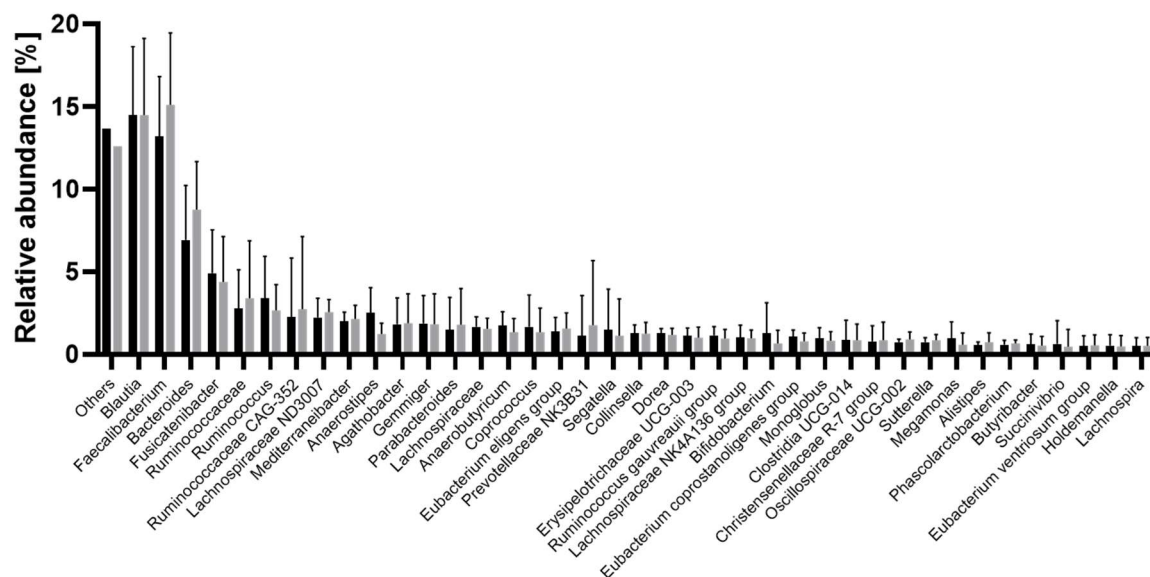
For mass spectrometric analysis the VION IMS QTOF MS was operated in MS full scan acquisition mode with an electrospray ionization (ESI) interface operating in both polarities (positive and negative). The capillary voltage was adjusted to 2.00 kV for both polarities. Nitrogen served as the desolvation, nebulizing, and collision gas. The source temperature was maintained at 120°C, while the desolvation gas temperature was set to 550°C with a flow rate of 700 L h<sup>-1</sup>.

Scan range was 50-500 Da and calibration of the instrument was conducted on a weekly basis using the “Major Mix” (Waters, Milford, MA, USA). For automated mass correction, a leucine-enkephalin solution (100 pg µL<sup>-1</sup>) was used to ensure consistent mass accuracy throughout the analyses. The data was acquired and processed with UNIFI v1.9.2.045 (Waters, Milford, MA, USA).

### ***Supplementary comparison of microbial community composition in native feces and the derived colon model***

To assess whether Nycodenz®-based purification altered the microbial community composition, native fecal samples before purification and the corresponding bacterial colon model obtained after density-gradient centrifugation were compared by 16S rRNA gene amplicon sequencing (Supplementary Fig. S1). Overall, no significant differences were observed between both samples. Alpha-diversity parameters were highly comparable between native feces and the colon model. Specifically, the number of observed features amounted to  $374.8 \pm 83.5$  in native feces and  $353.0 \pm 63.7$  in the colon model ( $H = 0.273$ ,  $p = 0.602$ ,  $q = 0.667$ ). Likewise, Shannon diversity was similar in both groups ( $6.50 \pm 0.24$  and  $6.37 \pm 0.09$ ,  $H = 2.455$ ,  $p = 0.117$ ,  $q = 0.353$ ), as were Pielou’s evenness ( $0.763 \pm 0.019$  and  $0.755 \pm 0.018$ ,  $H = 0.535$ ,  $p = 0.465$ ,  $q = 0.655$ ) and Faith’s phylogenetic diversity ( $19.700 \pm 3.673$  and  $19.188 \pm 3.299$ ,  $H = 0.098$ ,  $p = 0.754$ ,  $q = 0.782$ ) in native feces and the colon model, respectively. Beta-diversity analyses based on Bray-Curtis, Jaccard, weighted UniFrac, and unweighted UniFrac distances likewise did not show a significant separation between native feces and the bacterial colon model. The mean pairwise distances between corresponding native fecal samples and the colon model fractions were  $0.153 \pm 0.032$  for Bray-Curtis,  $0.265 \pm 0.055$  for Jaccard,  $0.053 \pm 0.021$  for weighted UniFrac, and  $0.146 \pm 0.014$  for

unweighted UniFrac. Pairwise comparison of both groups resulted in pseudo-F = 0.158,  $p = 0.892$ ,  $q = 0.892$  for Bray-Curtis, pseudo-F = 0.167,  $p = 0.896$ ,  $q = 0.896$  for Jaccard, pseudo-F = 0.496,  $p = 0.762$ ,  $q = 0.762$  for weighted UniFrac, and pseudo-F = 0.136,  $p = 0.918$ ,  $q = 0.918$  for unweighted UniFrac. Thus, neither abundance-based nor phylogeny-based distance measures indicated a detectable global shift in community structure. This was further supported by genus-level composition profiles, which remained highly similar after Nycodenz® enrichment when considering genera with a relative abundance > 0.5 %. Across the paired native feces and colon model samples, the dominant genera were consistently retained, including *Blautia*, *Faecalibacterium*, and *Bacteroides*, together with other abundant taxa such as *Fusicatenibacter*, *Gemmiger*, *Anaerostipes*, *Dorea*, and *Lachnospira*. Although moderate changes in the relative abundance of individual genera were observed in some sample pairs, the overall taxonomic profiles remained highly similar, with pairwise correlations ranging from  $r = 0.96$  to  $0.99$ . Together, these findings indicate that the purification procedure did not induce a detectable major shift in the overall microbial community composition and that the colon model largely preserved the original fecal microbiota structure while reducing matrix-derived background.



**Fig. S1: Genus-level comparison of native fecal samples and the corresponding bacterial colon model fractions.** Shown are dominant genera with a mean relative abundance > 0.5 %. The overall taxonomic composition remained highly similar between native feces and the derived colon model, indicating preservation of the original microbial community structure during Nycodenz®-based purification. In total, five paired sample pools were analyzed, with each native fecal pool ( $n=3$ ) being compared to the corresponding bacterial colon model pool derived from the same starting material.

**Supplementary dataset from 16S rRNA gene amplicon sequencing (Fig. 2)**

**Table S3: Genus-level hit counts from 16S rRNA gene amplicon sequencing of the bacterial colon model under different substrate conditions.** Taxonomic assignments are based on high-quality reads after denoising and chimera removal (DADA2, Q ≥ 30). Only taxa with valid genus-level classification (“OK hits”) are included. BS = Bile salts.

| Hits                                         | No Substrate T0 n=2 T24 n=3 |       |       |       |      | Fiber-Rich T0 n=2 T24 n=3 |       |       |       |      | Western + BS T0 n=2 T24 n=3 |       |      |      |       | Western - BS T0 n=2 T24 n=3 |       |       |       |       |
|----------------------------------------------|-----------------------------|-------|-------|-------|------|---------------------------|-------|-------|-------|------|-----------------------------|-------|------|------|-------|-----------------------------|-------|-------|-------|-------|
|                                              | n=1                         | n=2   | n=1   | n=2   | n=3  | n=1                       | n=2   | n=1   | n=2   | n=3  | n=1                         | n=2   | n=1  | n=2  | n=3   | n=1                         | n=2   | n=1   | n=2   | n=3   |
| <i>Bacteroides</i>                           | 21970                       | 9986  | 9948  | 9986  | 6810 | 10626                     | 14124 | 9948  | 9986  | 6810 | 17233                       | 14699 | 8766 | 7780 | 15898 | 13725                       | 13016 | 26191 | 13484 | 18174 |
| Bacteria                                     | 0                           | 0     | 0     | 0     | 0    | 0                         | 0     | 0     | 0     | 0    | 0                           | 2     | 0    | 5    | 9     | 11                          | 0     | 0     | 0     | 0     |
| Enterobacteriaceae                           | 0                           | 0     | 19    | 0     | 8    | 0                         | 0     | 19    | 0     | 8    | 0                           | 0     | 620  | 372  | 915   | 0                           | 0     | 68    | 15    | 37    |
| Ruminococcaceae                              | 207                         | 418   | 449   | 418   | 311  | 97                        | 108   | 449   | 418   | 311  | 138                         | 99    | 123  | 104  | 238   | 67                          | 100   | 389   | 395   | 480   |
| Peptococcaceae                               | 7                           | 0     | 0     | 0     | 0    | 0                         | 11    | 0     | 0     | 0    | 6                           | 8     | 2    | 0    | 0     | 0                           | 3     | 0     | 2     | 0     |
| Lachnospiraceae_FCS020                       | 322                         | 352   | 381   | 352   | 241  | 135                       | 179   | 381   | 352   | 241  | 234                         | 197   | 141  | 87   | 229   | 156                         | 158   | 132   | 153   | 166   |
| DTU014                                       | 5                           | 10    | 9     | 10    | 2    | 0                         | 0     | 9     | 10    | 2    | 0                           | 0     | 0    | 0    | 0     | 0                           | 4     | 2     | 0     | 0     |
| RF39                                         | 57                          | 114   | 100   | 114   | 83   | 26                        | 25    | 100   | 114   | 83   | 34                          | 23    | 50   | 23   | 102   | 20                          | 20    | 42    | 27    | 44    |
| <i>Faecalibacterium</i>                      | 22775                       | 6462  | 6291  | 6462  | 4338 | 9849                      | 13102 | 6291  | 6462  | 4338 | 14914                       | 12728 | 3413 | 2215 | 5306  | 10992                       | 11517 | 7522  | 6509  | 6690  |
| <i>Blautia</i>                               | 16152                       | 13518 | 14182 | 13518 | 8952 | 6513                      | 9132  | 14182 | 13518 | 8952 | 11558                       | 8776  | 7369 | 5043 | 14912 | 6586                        | 7785  | 7488  | 5419  | 6804  |
| <i>Dorea</i>                                 | 1955                        | 1941  | 2028  | 1941  | 1307 | 777                       | 1031  | 2028  | 1941  | 1307 | 1258                        | 1073  | 785  | 507  | 1506  | 653                         | 990   | 965   | 700   | 948   |
| [ <i>Bacteroides</i> ] <i>_pectinophilus</i> | 30                          | 39    | 44    | 39    | 11   | 20                        | 15    | 44    | 39    | 11   | 17                          | 13    | 9    | 0    | 10    | 13                          | 11    | 16    | 6     | 16    |
| <i>Fournierella</i>                          | 37                          | 123   | 135   | 123   | 94   | 0                         | 0     | 135   | 123   | 94   | 36                          | 33    | 0    | 9    | 45    | 37                          | 28    | 92    | 150   | 161   |
| Ruminococcaceae                              | 110                         | 128   | 119   | 128   | 104  | 51                        | 66    | 119   | 128   | 104  | 66                          | 62    | 35   | 0    | 66    | 52                          | 67    | 71    | 57    | 57    |
| <i>Acetanaerobacterium</i>                   | 3                           | 3     | 0     | 3     | 0    | 0                         | 0     | 0     | 3     | 0    | 0                           | 0     | 0    | 0    | 0     | 0                           | 0     | 0     | 0     | 0     |
| <i>Lachnospira</i>                           | 1062                        | 712   | 775   | 712   | 469  | 425                       | 610   | 775   | 712   | 469  | 706                         | 597   | 190  | 119  | 381   | 518                         | 571   | 353   | 337   | 333   |
| [ <i>Eubacterium</i> ] <i>_eligens</i>       | 1205                        | 1184  | 1224  | 1184  | 818  | 495                       | 676   | 1224  | 1184  | 818  | 803                         | 643   | 713  | 524  | 1496  | 654                         | 604   | 1032  | 717   | 901   |
| Lachnospiraceae                              | 2124                        | 1791  | 1980  | 1791  | 1171 | 986                       | 1155  | 1980  | 1791  | 1171 | 1377                        | 1197  | 693  | 585  | 1591  | 918                         | 1012  | 920   | 696   | 1015  |

| Hits                              | No Substrate T0 n=2 T24 n=3 |      |      |      |      | Fiber-Rich T0 n=2 T24 n=3 |      |      |      |      | Western + BS T0 n=2 T24 n=3 |      |      |      |      | Western - BS T0 n=2 T24 n=3 |      |      |      |      |
|-----------------------------------|-----------------------------|------|------|------|------|---------------------------|------|------|------|------|-----------------------------|------|------|------|------|-----------------------------|------|------|------|------|
|                                   | n=1                         | n=2  | n=1  | n=2  | n=3  | n=1                       | n=2  | n=1  | n=2  | n=3  | n=1                         | n=2  | n=1  | n=2  | n=3  | n=1                         | n=2  | n=1  | n=2  | n=3  |
| Lachnospiraceae_ND3007            | 4574                        | 3618 | 3769 | 3618 | 2594 | 2046                      | 2588 | 3769 | 3618 | 2594 | 3017                        | 2740 | 1691 | 1177 | 3360 | 2532                        | 2361 | 1987 | 1464 | 1476 |
| <i>Sutterella</i>                 | 1876                        | 622  | 558  | 622  | 430  | 925                       | 1185 | 558  | 622  | 430  | 1416                        | 1315 | 1067 | 798  | 2229 | 1146                        | 1152 | 1185 | 2085 | 1189 |
| <i>Ruminococcus</i>               | 2115                        | 1635 | 1420 | 1635 | 1197 | 988                       | 1188 | 1420 | 1635 | 1197 | 1192                        | 1015 | 324  | 271  | 609  | 896                         | 1080 | 502  | 526  | 467  |
| Lachnospiraceae                   | 0                           | 0    | 0    | 0    | 0    | 0                         | 2    | 0    | 0    | 0    | 0                           | 0    | 0    | 0    | 0    | 0                           | 0    | 0    | 0    | 0    |
| <i>[Eubacterium]_nodatum</i>      | 0                           | 0    | 11   | 0    | 0    | 0                         | 3    | 11   | 0    | 0    | 0                           | 9    | 0    | 0    | 0    | 0                           | 0    | 0    | 9    | 12   |
| Anaerovoracaceae                  | 175                         | 168  | 150  | 168  | 151  | 43                        | 67   | 150  | 168  | 151  | 98                          | 76   | 44   | 27   | 88   | 72                          | 65   | 212  | 114  | 160  |
| Lachnospiraceae                   | 526                         | 649  | 712  | 649  | 396  | 212                       | 324  | 712  | 649  | 396  | 319                         | 302  | 197  | 179  | 538  | 190                         | 217  | 296  | 375  | 404  |
| <i>Romboutsia</i>                 | 77                          | 56   | 64   | 56   | 38   | 40                        | 38   | 64   | 56   | 38   | 33                          | 39   | 55   | 26   | 98   | 43                          | 33   | 38   | 34   | 29   |
| <i>Intestinibacter</i>            | 44                          | 38   | 29   | 38   | 12   | 20                        | 26   | 29   | 38   | 12   | 25                          | 21   | 17   | 4    | 25   | 15                          | 24   | 16   | 16   | 20   |
| <i>Terrisporobacter</i>           | 17                          | 13   | 16   | 13   | 8    | 0                         | 1    | 16   | 13   | 8    | 9                           | 0    | 15   | 11   | 21   | 0                           | 0    | 0    | 8    | 11   |
| <i>Eubacterium</i>                | 877                         | 1325 | 1274 | 1325 | 838  | 387                       | 555  | 1274 | 1325 | 838  | 638                         | 594  | 556  | 407  | 1193 | 383                         | 496  | 745  | 658  | 666  |
| <i>Lachnoclostridium</i>          | 1630                        | 2062 | 2085 | 2062 | 1428 | 654                       | 932  | 2085 | 2062 | 1428 | 1110                        | 936  | 2265 | 1602 | 4353 | 820                         | 863  | 1928 | 1241 | 1926 |
| Lachnospiraceae_UCG003            | 17                          | 15   | 14   | 15   | 0    | 5                         | 10   | 14   | 15   | 0    | 12                          | 12   | 0    | 0    | 8    | 9                           | 18   | 7    | 7    | 8    |
| <i>Agathobacter</i>               | 8962                        | 7007 | 7545 | 7007 | 4624 | 3756                      | 4905 | 7545 | 7007 | 4624 | 6025                        | 5107 | 3133 | 2188 | 6196 | 4300                        | 4401 | 3784 | 2993 | 3666 |
| <i>Actinomyces</i>                | 0                           | 8    | 8    | 8    | 0    | 0                         | 0    | 8    | 8    | 0    | 5                           | 0    | 0    | 5    | 8    | 0                           | 0    | 2    | 0    | 4    |
| <i>Citrobacter</i>                | 0                           | 8    | 35   | 8    | 11   | 0                         | 0    | 35   | 8    | 11   | 0                           | 0    | 62   | 41   | 137  | 0                           | 0    | 20   | 0    | 0    |
| <i>Enterobacter</i>               | 8                           | 0    | 9    | 0    | 0    | 0                         | 0    | 9    | 0    | 0    | 6                           | 9    | 345  | 213  | 446  | 0                           | 10   | 59   | 27   | 36   |
| <i>Escherichia-Shigella</i>       | 48                          | 141  | 129  | 141  | 104  | 34                        | 41   | 129  | 141  | 104  | 51                          | 36   | 3540 | 3374 | 5846 | 33                          | 39   | 6139 | 1003 | 3099 |
| <i>[Eubacterium]_hallii_group</i> | 3690                        | 4537 | 4786 | 4537 | 3074 | 1593                      | 2076 | 4786 | 4537 | 3074 | 2325                        | 2081 | 4344 | 2723 | 8367 | 1939                        | 1912 | 4808 | 3579 | 4462 |
| <i>Klebsiella</i>                 | 0                           | 18   | 12   | 18   | 8    | 0                         | 0    | 12   | 18   | 8    | 0                           | 0    | 1795 | 1008 | 2709 | 0                           | 0    | 92   | 7    | 56   |
| <i>Bifidobacterium</i>            | 3047                        | 3242 | 3400 | 3242 | 2474 | 1563                      | 1828 | 3400 | 3242 | 2474 | 1861                        | 1575 | 3356 | 2464 | 6601 | 1710                        | 1653 | 3909 | 2765 | 2933 |
| <i>Phascolarctobacterium</i>      | 1029                        | 817  | 900  | 817  | 631  | 549                       | 697  | 900  | 817  | 631  | 1381                        | 1174 | 2297 | 1715 | 3922 | 772                         | 722  | 1745 | 982  | 1316 |

| Hits                                     | No Substrate T0 n=2 T24 n=3 |      |      |      |      | Fiber-Rich T0 n=2 T24 n=3 |      |      |      |      | Western + BS T0 n=2 T24 n=3 |     |      |      |      | Western - BS T0 n=2 T24 n=3 |     |      |      |      |
|------------------------------------------|-----------------------------|------|------|------|------|---------------------------|------|------|------|------|-----------------------------|-----|------|------|------|-----------------------------|-----|------|------|------|
|                                          | n=1                         | n=2  | n=1  | n=2  | n=3  | n=1                       | n=2  | n=1  | n=2  | n=3  | n=1                         | n=2 | n=1  | n=2  | n=3  | n=1                         | n=2 | n=1  | n=2  | n=3  |
| <i>Kocuria</i>                           | 0                           | 0    | 4    | 0    | 0    | 0                         | 0    | 4    | 0    | 0    | 0                           | 0   | 0    | 0    | 0    | 0                           | 0   | 0    | 0    | 0    |
| Anaerovoracaceae                         | 56                          | 64   | 58   | 64   | 43   | 39                        | 40   | 58   | 64   | 43   | 38                          | 27  | 38   | 21   | 66   | 34                          | 34  | 65   | 51   | 47   |
| <i>Clostridia</i>                        | 0                           | 0    | 0    | 0    | 0    | 0                         | 0    | 0    | 0    | 0    | 0                           | 0   | 0    | 0    | 0    | 0                           | 0   | 2    | 0    | 0    |
| Lachnospiraceae_UCG010                   | 97                          | 123  | 157  | 123  | 80   | 41                        | 55   | 157  | 123  | 80   | 52                          | 57  | 0    | 0    | 0    | 43                          | 49  | 350  | 285  | 332  |
| GCA-900066755                            | 0                           | 10   | 0    | 10   | 0    | 0                         | 0    | 0    | 10   | 0    | 0                           | 0   | 0    | 0    | 11   | 0                           | 0   | 6    | 0    | 5    |
| <i>Tyzzerella</i>                        | 52                          | 38   | 39   | 38   | 26   | 29                        | 34   | 39   | 38   | 26   | 30                          | 25  | 18   | 11   | 26   | 23                          | 31  | 88   | 77   | 81   |
| UCG-009                                  | 4                           | 0    | 6    | 0    | 0    | 0                         | 0    | 6    | 0    | 0    | 0                           | 0   | 0    | 0    | 0    | 0                           | 0   | 0    | 0    | 0    |
| Lachnospiraceae_NK4A136                  | 1267                        | 1127 | 1308 | 1127 | 804  | 516                       | 684  | 1308 | 1127 | 804  | 809                         | 690 | 100  | 79   | 259  | 426                         | 508 | 405  | 474  | 475  |
| Lachnospiraceae_UCG001                   | 920                         | 525  | 531  | 525  | 308  | 383                       | 532  | 531  | 525  | 308  | 628                         | 548 | 149  | 118  | 303  | 496                         | 397 | 211  | 114  | 180  |
| <i>Megasphaera</i>                       | 515                         | 486  | 473  | 486  | 350  | 288                       | 391  | 473  | 486  | 350  | 423                         | 381 | 1824 | 1280 | 3719 | 372                         | 353 | 434  | 325  | 341  |
| Lachnospiraceae_NK4B4                    | 54                          | 24   | 24   | 24   | 22   | 18                        | 26   | 24   | 24   | 22   | 35                          | 12  | 0    | 0    | 8    | 28                          | 20  | 11   | 11   | 10   |
| <i>Butyricoccus</i>                      | 401                         | 346  | 336  | 346  | 209  | 176                       | 248  | 336  | 346  | 209  | 279                         | 235 | 155  | 103  | 274  | 207                         | 194 | 203  | 198  | 199  |
| Erysipelotrichaceae                      | 1652                        | 1482 | 1483 | 1482 | 1131 | 829                       | 1110 | 1483 | 1482 | 1131 | 1135                        | 975 | 767  | 713  | 1869 | 938                         | 909 | 1041 | 726  | 852  |
| <i>Clostridium_sensu_1</i>               | 27                          | 12   | 5    | 12   | 8    | 13                        | 8    | 5    | 12   | 8    | 7                           | 14  | 16   | 11   | 26   | 4                           | 11  | 11   | 4    | 21   |
| <i>Paludicola</i>                        | 0                           | 0    | 0    | 0    | 0    | 0                         | 0    | 0    | 0    | 0    | 0                           | 0   | 0    | 0    | 0    | 0                           | 0   | 0    | 3    | 0    |
| <i>Finegoldia</i>                        | 3                           | 0    | 0    | 0    | 0    | 0                         | 0    | 0    | 0    | 0    | 0                           | 0   | 0    | 0    | 0    | 0                           | 0   | 0    | 0    | 0    |
| <i>Coprococcus</i>                       | 1629                        | 2190 | 2411 | 2190 | 1496 | 690                       | 960  | 2411 | 2190 | 1496 | 1065                        | 958 | 583  | 503  | 1549 | 752                         | 811 | 3075 | 1998 | 2834 |
| <i>Flavonifractor</i>                    | 117                         | 191  | 160  | 191  | 96   | 68                        | 87   | 160  | 191  | 96   | 72                          | 67  | 42   | 16   | 126  | 67                          | 73  | 98   | 101  | 82   |
| [ <i>Eubacterium</i> ] <i>ventriosum</i> | 284                         | 211  | 206  | 211  | 113  | 100                       | 159  | 206  | 211  | 113  | 172                         | 175 | 96   | 63   | 165  | 134                         | 120 | 128  | 82   | 104  |
| GCA-900066575                            | 58                          | 36   | 42   | 36   | 34   | 20                        | 34   | 42   | 36   | 34   | 32                          | 38  | 11   | 0    | 18   | 37                          | 42  | 21   | 15   | 24   |
| <i>Lactonifractor</i>                    | 6                           | 12   | 12   | 12   | 11   | 0                         | 7    | 12   | 12   | 11   | 0                           | 0   | 0    | 0    | 11   | 0                           | 0   | 0    | 0    | 0    |
| <i>Frisingicoccus</i>                    | 7                           | 39   | 47   | 39   | 32   | 4                         | 0    | 47   | 39   | 32   | 0                           | 4   | 0    | 0    | 9    | 0                           | 0   | 18   | 14   | 28   |

| Hits                             | No Substrate T0 n=2 T24 n=3 |      |      |      |      | Fiber-Rich T0 n=2 T24 n=3 |      |      |      |      | Western + BS T0 n=2 T24 n=3 |      |      |      |      | Western - BS T0 n=2 T24 n=3 |      |      |      |      |
|----------------------------------|-----------------------------|------|------|------|------|---------------------------|------|------|------|------|-----------------------------|------|------|------|------|-----------------------------|------|------|------|------|
|                                  | n=1                         | n=2  | n=1  | n=2  | n=3  | n=1                       | n=2  | n=1  | n=2  | n=3  | n=1                         | n=2  | n=1  | n=2  | n=3  | n=1                         | n=2  | n=1  | n=2  | n=3  |
| <i>Catenibacillus</i>            | 0                           | 4    | 0    | 4    | 0    | 0                         | 0    | 0    | 4    | 0    | 0                           | 0    | 0    | 0    | 5    | 0                           | 0    | 0    | 0    | 0    |
| <i>Subdoligranulum</i>           | 4288                        | 4387 | 4442 | 4387 | 3024 | 1885                      | 2544 | 4442 | 4387 | 3024 | 2673                        | 2436 | 3320 | 2214 | 6639 | 2338                        | 2308 | 4029 | 3204 | 3443 |
| <i>[Eubacterium]_fissicatena</i> | 13                          | 5    | 7    | 5    | 0    | 11                        | 8    | 7    | 5    | 0    | 0                           | 2    | 0    | 0    | 0    | 5                           | 4    | 0    | 0    | 0    |
| CHKCI001                         | 0                           | 0    | 0    | 0    | 0    | 0                         | 0    | 0    | 0    | 0    | 3                           | 0    | 0    | 0    | 0    | 0                           | 0    | 0    | 0    | 0    |
| <i>Roseburia</i>                 | 3314                        | 1560 | 1590 | 1560 | 967  | 1276                      | 1668 | 1590 | 1560 | 967  | 2204                        | 1698 | 267  | 214  | 610  | 1559                        | 1529 | 974  | 879  | 1013 |
| <i>[Ruminococcus]_torques</i>    | 1791                        | 1731 | 1825 | 1731 | 1112 | 827                       | 1026 | 1825 | 1731 | 1112 | 1190                        | 976  | 785  | 547  | 1576 | 775                         | 882  | 657  | 502  | 792  |
| <i>[Ruminococcus]_gauvreauii</i> | 895                         | 695  | 708  | 695  | 427  | 338                       | 454  | 708  | 695  | 427  | 558                         | 508  | 258  | 127  | 297  | 341                         | 425  | 55   | 149  | 132  |
| <i>Marvinbryantia</i>            | 115                         | 75   | 81   | 75   | 44   | 23                        | 52   | 81   | 75   | 44   | 72                          | 45   | 69   | 43   | 147  | 57                          | 62   | 67   | 54   | 99   |
| CAG-56                           | 329                         | 284  | 310  | 284  | 155  | 118                       | 167  | 310  | 284  | 155  | 194                         | 166  | 30   | 14   | 70   | 134                         | 154  | 49   | 76   | 59   |
| <i>Shuttleworthia</i>            | 7                           | 10   | 0    | 10   | 7    | 0                         | 8    | 0    | 10   | 7    | 5                           | 0    | 13   | 0    | 10   | 16                          | 0    | 8    | 0    | 0    |
| <i>Oscillospirales</i>           | 0                           | 6    | 4    | 6    | 2    | 0                         | 0    | 4    | 6    | 2    | 0                           | 0    | 0    | 0    | 0    | 7                           | 0    | 3    | 3    | 0    |
| <i>Fusicatenibacter</i>          | 6840                        | 4688 | 4979 | 4688 | 3134 | 2816                      | 3559 | 4979 | 4688 | 3134 | 4772                        | 3958 | 1218 | 932  | 2190 | 3023                        | 3364 | 1664 | 1238 | 1759 |
| <i>Fenollaria</i>                | 0                           | 0    | 0    | 0    | 2    | 0                         | 0    | 0    | 0    | 2    | 0                           | 0    | 0    | 0    | 0    | 0                           | 0    | 0    | 0    | 0    |
| <i>[Ruminococcus]_gnavus</i>     | 178                         | 96   | 96   | 96   | 78   | 69                        | 81   | 96   | 96   | 78   | 98                          | 89   | 11   | 14   | 18   | 50                          | 71   | 27   | 40   | 41   |
| Christensenellaceae_R-7          | 948                         | 1270 | 1289 | 1270 | 882  | 478                       | 563  | 1289 | 1270 | 882  | 757                         | 612  | 650  | 613  | 1523 | 461                         | 478  | 1058 | 659  | 835  |
| Candidatus_Soleaferrea           | 12                          | 25   | 18   | 25   | 11   | 6                         | 4    | 18   | 25   | 11   | 3                           | 9    | 0    | 0    | 14   | 5                           | 0    | 5    | 7    | 19   |
| <i>Anaerostipes</i>              | 1711                        | 1795 | 2028 | 1795 | 1227 | 775                       | 958  | 2028 | 1795 | 1227 | 1177                        | 1048 | 1642 | 1041 | 3319 | 903                         | 866  | 1164 | 874  | 969  |
| <i>Eisenbergiella</i>            | 5                           | 8    | 11   | 8    | 0    | 0                         | 6    | 11   | 8    | 0    | 0                           | 0    | 10   | 20   | 60   | 0                           | 0    | 28   | 13   | 23   |
| Lachnospiraceae_UCG008           | 0                           | 0    | 6    | 0    | 0    | 0                         | 0    | 6    | 0    | 0    | 0                           | 0    | 0    | 0    | 0    | 0                           | 0    | 0    | 0    | 0    |
| <i>Eubacterium_xylanophilum</i>  | 416                         | 45   | 42   | 45   | 34   | 165                       | 249  | 42   | 45   | 34   | 277                         | 268  | 9    | 7    | 14   | 215                         | 179  | 25   | 18   | 17   |
| <i>[Eubacterium]_ruminantium</i> | 374                         | 447  | 488  | 447  | 300  | 149                       | 231  | 488  | 447  | 300  | 190                         | 169  | 45   | 45   | 94   | 128                         | 172  | 241  | 235  | 226  |
| <i>Moryella</i>                  | 23                          | 26   | 41   | 26   | 22   | 13                        | 27   | 41   | 26   | 22   | 17                          | 12   | 10   | 9    | 33   | 15                          | 13   | 16   | 20   | 35   |

| Hits                       | No Substrate T0 n=2 T24 n=3 |     |     |     |     | Fiber-Rich T0 n=2 T24 n=3 |     |     |     |     | Western + BS T0 n=2 T24 n=3 |     |      |      |      | Western - BS T0 n=2 T24 n=3 |     |     |     |     |
|----------------------------|-----------------------------|-----|-----|-----|-----|---------------------------|-----|-----|-----|-----|-----------------------------|-----|------|------|------|-----------------------------|-----|-----|-----|-----|
|                            | n=1                         | n=2 | n=1 | n=2 | n=3 | n=1                       | n=2 | n=1 | n=2 | n=3 | n=1                         | n=2 | n=1  | n=2  | n=3  | n=1                         | n=2 | n=1 | n=2 | n=3 |
| UBA1819                    | 28                          | 29  | 18  | 29  | 20  | 11                        | 10  | 18  | 29  | 20  | 22                          | 14  | 0    | 6    | 39   | 12                          | 17  | 26  | 21  | 14  |
| Lachnospiraceae_NC2004     | 0                           | 0   | 0   | 0   | 0   | 0                         | 0   | 0   | 0   | 0   | 0                           | 0   | 2    | 0    | 0    | 0                           | 0   | 2   | 0   | 0   |
| <i>Enterorhabdus</i>       | 0                           | 3   | 11  | 3   | 5   | 0                         | 0   | 11  | 3   | 5   | 7                           | 0   | 6    | 5    | 12   | 6                           | 4   | 0   | 8   | 5   |
| Defluviitaleaceae UCG011   | 25                          | 37  | 61  | 37  | 25  | 9                         | 18  | 61  | 37  | 25  | 9                           | 5   | 9    | 0    | 11   | 13                          | 13  | 16  | 22  | 15  |
| <i>Oscillibacter</i>       | 211                         | 212 | 209 | 212 | 171 | 87                        | 118 | 209 | 212 | 171 | 176                         | 107 | 138  | 94   | 257  | 113                         | 102 | 157 | 118 | 126 |
| <i>Clostridia</i> _UCG-014 | 423                         | 582 | 569 | 582 | 375 | 207                       | 239 | 569 | 582 | 375 | 313                         | 248 | 218  | 157  | 504  | 244                         | 178 | 335 | 233 | 259 |
| Desulfovibrionaceae        | 2                           | 3   | 4   | 3   | 0   | 3                         | 3   | 4   | 3   | 0   | 4                           | 0   | 0    | 0    | 3    | 4                           | 0   | 6   | 0   | 2   |
| <i>Desulfovibrio</i>       | 8                           | 12  | 7   | 12  | 6   | 4                         | 8   | 7   | 12  | 6   | 5                           | 10  | 20   | 13   | 18   | 7                           | 4   | 7   | 16  | 9   |
| <i>Bilophila</i>           | 207                         | 227 | 231 | 227 | 169 | 128                       | 134 | 231 | 227 | 169 | 163                         | 121 | 204  | 165  | 397  | 127                         | 104 | 239 | 158 | 167 |
| <i>Anaerofustis</i>        | 5                           | 3   | 0   | 3   | 0   | 2                         | 4   | 0   | 3   | 0   | 0                           | 3   | 0    | 0    | 0    | 8                           | 2   | 0   | 3   | 7   |
| UCG-008                    | 26                          | 15  | 21  | 15  | 7   | 18                        | 12  | 21  | 15  | 7   | 8                           | 6   | 20   | 6    | 20   | 12                          | 6   | 18  | 10  | 9   |
| <i>Monoglobus</i>          | 516                         | 405 | 388 | 405 | 282 | 256                       | 293 | 388 | 405 | 282 | 414                         | 362 | 115  | 77   | 216  | 301                         | 327 | 203 | 244 | 193 |
| <i>Haemophilus</i>         | 16                          | 6   | 11  | 6   | 7   | 8                         | 10  | 11  | 6   | 7   | 15                          | 13  | 0    | 0    | 0    | 4                           | 9   | 4   | 0   | 4   |
| Desulfovibrionaceae        | 3                           | 10  | 4   | 10  | 3   | 0                         | 3   | 4   | 10  | 3   | 3                           | 0   | 5    | 4    | 5    | 6                           | 0   | 0   | 0   | 3   |
| <i>Fusobacterium</i>       | 332                         | 79  | 75  | 79  | 45  | 173                       | 282 | 75  | 79  | 45  | 257                         | 228 | 1875 | 1431 | 3667 | 244                         | 186 | 700 | 159 | 472 |
| <i>Marmoricola</i>         | 0                           | 0   | 0   | 0   | 0   | 0                         | 0   | 0   | 0   | 0   | 0                           | 0   | 0    | 0    | 0    | 0                           | 0   | 0   | 0   | 0   |
| <i>Novosphingobium</i>     | 0                           | 4   | 0   | 4   | 0   | 0                         | 0   | 0   | 4   | 0   | 0                           | 0   | 0    | 0    | 0    | 0                           | 0   | 0   | 0   | 0   |
| Sphingomonadaceae          | 0                           | 0   | 0   | 0   | 0   | 0                         | 0   | 0   | 0   | 0   | 0                           | 0   | 0    | 0    | 9    | 0                           | 0   | 0   | 0   | 0   |
| <i>Pseudomonas</i>         | 4                           | 0   | 0   | 0   | 0   | 0                         | 0   | 0   | 0   | 0   | 0                           | 0   | 0    | 0    | 0    | 0                           | 0   | 0   | 0   | 0   |
| <i>Hungatella</i>          | 0                           | 26  | 47  | 26  | 27  | 0                         | 0   | 47  | 26  | 27  | 0                           | 0   | 101  | 78   | 252  | 0                           | 0   | 54  | 27  | 55  |
| <i>Roseomonas</i>          | 0                           | 0   | 0   | 0   | 0   | 0                         | 0   | 0   | 0   | 0   | 0                           | 0   | 0    | 0    | 3    | 0                           | 0   | 0   | 0   | 0   |
| <i>Methylobacterium</i>    | 0                           | 0   | 0   | 0   | 0   | 0                         | 0   | 0   | 0   | 0   | 0                           | 0   | 0    | 0    | 5    | 0                           | 0   | 0   | 0   | 0   |

| Hits                            | No Substrate T0 n=2 T24 n=3 |      |      |      |      | Fiber-Rich T0 n=2 T24 n=3 |      |      |      |      | Western + BS T0 n=2 T24 n=3 |     |      |      |      | Western - BS T0 n=2 T24 n=3 |      |      |      |      |
|---------------------------------|-----------------------------|------|------|------|------|---------------------------|------|------|------|------|-----------------------------|-----|------|------|------|-----------------------------|------|------|------|------|
|                                 | n=1                         | n=2  | n=1  | n=2  | n=3  | n=1                       | n=2  | n=1  | n=2  | n=3  | n=1                         | n=2 | n=1  | n=2  | n=3  | n=1                         | n=2  | n=1  | n=2  | n=3  |
| <i>Enhydrobacter</i>            | 2                           | 0    | 4    | 0    | 0    | 0                         | 0    | 4    | 0    | 0    | 0                           | 0   | 0    | 0    | 0    | 0                           | 0    | 0    | 0    | 0    |
| <i>Johnsonella</i>              | 0                           | 0    | 2    | 0    | 0    | 0                         | 0    | 2    | 0    | 0    | 0                           | 0   | 0    | 0    | 0    | 0                           | 0    | 0    | 0    | 0    |
| <i>Clostridia_vadin</i> BB60    | 34                          | 72   | 60   | 72   | 48   | 13                        | 17   | 60   | 72   | 48   | 16                          | 6   | 0    | 0    | 0    | 10                          | 13   | 32   | 54   | 38   |
| <i>Acinetobacter</i>            | 0                           | 0    | 0    | 0    | 0    | 0                         | 0    | 0    | 0    | 0    | 0                           | 0   | 0    | 0    | 0    | 0                           | 0    | 0    | 2    | 0    |
| UCG-010                         | 37                          | 78   | 61   | 78   | 36   | 13                        | 21   | 61   | 78   | 36   | 34                          | 27  | 6    | 4    | 25   | 22                          | 20   | 26   | 32   | 19   |
| <i>Oscillospira</i>             | 18                          | 16   | 18   | 16   | 8    | 5                         | 24   | 18   | 16   | 8    | 19                          | 18  | 8    | 0    | 12   | 11                          | 8    | 10   | 10   | 15   |
| Oscillospiraceae                | 39                          | 37   | 27   | 37   | 27   | 15                        | 15   | 27   | 37   | 27   | 23                          | 36  | 28   | 29   | 75   | 24                          | 16   | 102  | 64   | 69   |
| <i>Rhodospirillales</i>         | 31                          | 9    | 14   | 9    | 6    | 14                        | 21   | 14   | 9    | 6    | 20                          | 23  | 3    | 0    | 0    | 20                          | 15   | 7    | 6    | 0    |
| Oscillospiraceae                | 252                         | 230  | 236  | 230  | 146  | 110                       | 137  | 236  | 230  | 146  | 154                         | 149 | 85   | 42   | 160  | 130                         | 109  | 164  | 150  | 112  |
| <i>Intestinimonas</i>           | 27                          | 46   | 64   | 46   | 42   | 6                         | 22   | 64   | 46   | 42   | 23                          | 16  | 6    | 15   | 11   | 10                          | 6    | 44   | 20   | 28   |
| NK4A214_group                   | 433                         | 638  | 598  | 638  | 388  | 222                       | 294  | 598  | 638  | 388  | 401                         | 329 | 170  | 144  | 368  | 298                         | 311  | 603  | 365  | 461  |
| Christensenellaceae             | 14                          | 22   | 22   | 22   | 9    | 6                         | 13   | 22   | 22   | 9    | 10                          | 0   | 0    | 0    | 11   | 5                           | 15   | 10   | 4    | 14   |
| UCG-002                         | 1958                        | 3107 | 3019 | 3107 | 2175 | 916                       | 1116 | 3019 | 3107 | 2175 | 1127                        | 972 | 993  | 809  | 2119 | 959                         | 1066 | 2566 | 1867 | 2302 |
| UCG-003                         | 708                         | 794  | 828  | 794  | 557  | 381                       | 391  | 828  | 794  | 557  | 525                         | 410 | 432  | 345  | 671  | 322                         | 421  | 614  | 357  | 439  |
| <i>Eubacterium oxidoreducen</i> | 0                           | 0    | 0    | 0    | 0    | 0                         | 0    | 0    | 0    | 0    | 0                           | 0   | 0    | 0    | 0    | 2                           | 0    | 0    | 0    | 0    |
| <i>Colidextribacter</i>         | 340                         | 285  | 246  | 285  | 174  | 143                       | 212  | 246  | 285  | 174  | 193                         | 153 | 73   | 39   | 146  | 161                         | 189  | 159  | 160  | 137  |
| <i>Megamonas</i>                | 1553                        | 1672 | 1620 | 1672 | 1217 | 811                       | 1045 | 1620 | 1672 | 1217 | 1069                        | 899 | 1588 | 1094 | 2862 | 1033                        | 845  | 1445 | 1016 | 1140 |
| <i>Pseudoflavonifractor</i>     | 8                           | 0    | 15   | 0    | 9    | 0                         | 6    | 15   | 0    | 9    | 0                           | 0   | 7    | 6    | 0    | 0                           | 0    | 0    | 5    | 7    |
| UCG-005                         | 433                         | 627  | 644  | 627  | 422  | 214                       | 250  | 644  | 627  | 422  | 288                         | 217 | 168  | 115  | 364  | 229                         | 229  | 277  | 310  | 278  |
| <i>Christensenella</i>          | 4                           | 0    | 0    | 0    | 0    | 0                         | 0    | 0    | 0    | 0    | 0                           | 0   | 0    | 0    | 0    | 0                           | 0    | 3    | 0    | 0    |
| <i>Papillibacter</i>            | 6                           | 0    | 0    | 0    | 0    | 0                         | 0    | 0    | 0    | 0    | 0                           | 0   | 0    | 0    | 5    | 0                           | 0    | 0    | 0    | 0    |
| <i>Akkermansia</i>              | 197                         | 244  | 170  | 244  | 172  | 91                        | 137  | 170  | 244  | 172  | 156                         | 93  | 525  | 488  | 861  | 123                         | 106  | 780  | 264  | 473  |

| Hits                     | No Substrate T0 n=2 T24 n=3 |      |      |      |      | Fiber-Rich T0 n=2 T24 n=3 |     |      |      |      | Western + BS T0 n=2 T24 n=3 |     |      |      |       | Western - BS T0 n=2 T24 n=3 |     |      |      |      |
|--------------------------|-----------------------------|------|------|------|------|---------------------------|-----|------|------|------|-----------------------------|-----|------|------|-------|-----------------------------|-----|------|------|------|
|                          | n=1                         | n=2  | n=1  | n=2  | n=3  | n=1                       | n=2 | n=1  | n=2  | n=3  | n=1                         | n=2 | n=1  | n=2  | n=3   | n=1                         | n=2 | n=1  | n=2  | n=3  |
| <i>Cloacibacillus</i>    | 0                           | 0    | 0    | 0    | 0    | 0                         | 0   | 0    | 0    | 0    | 0                           | 0   | 0    | 2    | 0     | 0                           | 0   | 0    | 0    | 0    |
| Lachnospiraceae UCG004   | 183                         | 163  | 200  | 163  | 120  | 57                        | 93  | 200  | 163  | 120  | 104                         | 104 | 130  | 103  | 219   | 106                         | 90  | 112  | 76   | 136  |
| <i>Prevotella</i>        | 719                         | 118  | 122  | 118  | 34   | 364                       | 509 | 122  | 118  | 34   | 522                         | 444 | 23   | 39   | 123   | 539                         | 497 | 134  | 26   | 63   |
| Muribaculaceae           | 106                         | 31   | 38   | 31   | 32   | 58                        | 61  | 38   | 31   | 32   | 85                          | 74  | 0    | 5    | 3     | 59                          | 47  | 9    | 16   | 9    |
| <i>Odoribacter</i>       | 199                         | 137  | 148  | 137  | 135  | 88                        | 118 | 148  | 137  | 135  | 144                         | 138 | 72   | 58   | 161   | 149                         | 121 | 192  | 113  | 105  |
| <i>Alistipes</i>         | 1400                        | 5148 | 4893 | 5148 | 3881 | 727                       | 884 | 4893 | 5148 | 3881 | 1058                        | 917 | 5613 | 4296 | 11486 | 968                         | 863 | 3252 | 1604 | 2229 |
| <i>Barnesiella</i>       | 307                         | 394  | 381  | 394  | 258  | 157                       | 192 | 381  | 394  | 258  | 197                         | 196 | 168  | 140  | 336   | 201                         | 180 | 480  | 321  | 366  |
| <i>Parabacteroides</i>   | 1454                        | 1095 | 1170 | 1095 | 743  | 698                       | 889 | 1170 | 1095 | 743  | 1176                        | 980 | 3237 | 2696 | 6627  | 955                         | 913 | 3107 | 1205 | 1877 |
| <i>Coprobacter</i>       | 31                          | 9    | 0    | 9    | 5    | 25                        | 35  | 0    | 9    | 5    | 25                          | 29  | 4    | 0    | 0     | 36                          | 26  | 8    | 0    | 0    |
| <i>Holdemanella</i>      | 43                          | 36   | 29   | 36   | 24   | 17                        | 14  | 29   | 36   | 24   | 19                          | 18  | 12   | 7    | 27    | 14                          | 15  | 36   | 22   | 11   |
| <i>Butyrivibrio</i>      | 66                          | 106  | 114  | 106  | 68   | 32                        | 24  | 114  | 106  | 68   | 59                          | 38  | 128  | 80   | 205   | 41                          | 32  | 156  | 68   | 88   |
| Barnesiellaceae          | 5                           | 0    | 0    | 0    | 0    | 0                         | 0   | 0    | 0    | 0    | 0                           | 0   | 0    | 2    | 0     | 0                           | 0   | 3    | 0    | 0    |
| <i>Sanguibacteroides</i> | 4                           | 12   | 13   | 12   | 6    | 2                         | 4   | 13   | 12   | 6    | 0                           | 0   | 17   | 17   | 19    | 3                           | 0   | 13   | 2    | 8    |
| <i>Rikenella</i>         | 0                           | 3    | 0    | 3    | 0    | 0                         | 0   | 0    | 3    | 0    | 0                           | 0   | 0    | 0    | 0     | 0                           | 0   | 0    | 0    | 0    |
| <i>Paraprevotella</i>    | 263                         | 47   | 35   | 47   | 42   | 122                       | 174 | 35   | 47   | 42   | 184                         | 139 | 12   | 0    | 27    | 156                         | 164 | 29   | 61   | 20   |
| Puniceicoccaceae         | 14                          | 17   | 36   | 17   | 20   | 9                         | 9   | 36   | 17   | 20   | 8                           | 9   | 37   | 23   | 69    | 9                           | 6   | 56   | 24   | 30   |
| Rhodobacteraceae         | 0                           | 3    | 0    | 3    | 0    | 0                         | 0   | 0    | 3    | 0    | 0                           | 0   | 0    | 0    | 0     | 0                           | 0   | 0    | 0    | 0    |
| <i>Paracoccus</i>        | 0                           | 0    | 0    | 0    | 0    | 0                         | 0   | 0    | 0    | 0    | 0                           | 0   | 0    | 0    | 0     | 0                           | 0   | 2    | 0    | 0    |
| <i>Lactobacillus</i>     | 24                          | 30   | 34   | 30   | 39   | 24                        | 14  | 34   | 30   | 39   | 29                          | 27  | 36   | 19   | 51    | 21                          | 21  | 29   | 19   | 23   |
| <i>Allisonella</i>       | 5                           | 0    | 0    | 0    | 0    | 5                         | 2   | 0    | 0    | 0    | 0                           | 5   | 25   | 10   | 43    | 4                           | 5   | 7    | 0    | 0    |
| <i>Acidaminococcus</i>   | 17                          | 32   | 30   | 32   | 15   | 12                        | 16  | 30   | 32   | 15   | 28                          | 30  | 0    | 0    | 10    | 24                          | 17  | 12   | 10   | 0    |
| <i>Veillonella</i>       | 23                          | 13   | 38   | 13   | 24   | 22                        | 17  | 38   | 13   | 24   | 28                          | 16  | 55   | 42   | 146   | 19                          | 12  | 86   | 27   | 54   |

| Hits                                    | No Substrate T0 n=2 T24 n=3 |      |      |      |      | Fiber-Rich T0 n=2 T24 n=3 |      |      |      |      | Western + BS T0 n=2 T24 n=3 |      |     |     |      | Western - BS T0 n=2 T24 n=3 |      |      |     |     |
|-----------------------------------------|-----------------------------|------|------|------|------|---------------------------|------|------|------|------|-----------------------------|------|-----|-----|------|-----------------------------|------|------|-----|-----|
|                                         | n=1                         | n=2  | n=1  | n=2  | n=3  | n=1                       | n=2  | n=1  | n=2  | n=3  | n=1                         | n=2  | n=1 | n=2 | n=3  | n=1                         | n=2  | n=1  | n=2 | n=3 |
| <i>Granulicatella</i>                   | 0                           | 0    | 0    | 0    | 2    | 0                         | 0    | 0    | 0    | 2    | 0                           | 0    | 0   | 0   | 2    | 0                           | 0    | 0    | 0   | 0   |
| <i>Dialister</i>                        | 241                         | 204  | 227  | 204  | 123  | 133                       | 148  | 227  | 204  | 123  | 199                         | 188  | 195 | 141 | 342  | 144                         | 137  | 165  | 158 | 160 |
| <i>Peptococcus</i>                      | 5                           | 7    | 11   | 7    | 4    | 3                         | 0    | 11   | 7    | 4    | 4                           | 7    | 0   | 0   | 5    | 0                           | 5    | 5    | 0   | 9   |
| <i>Lactococcus</i>                      | 5                           | 3    | 3    | 3    | 0    | 0                         | 0    | 3    | 3    | 0    | 0                           | 7    | 3   | 0   | 0    | 0                           | 5    | 4    | 3   | 5   |
| <i>Streptococcus</i>                    | 76                          | 50   | 56   | 50   | 45   | 40                        | 38   | 56   | 50   | 45   | 46                          | 55   | 76  | 51  | 113  | 41                          | 47   | 57   | 33  | 40  |
| <i>Turicibacter</i>                     | 35                          | 38   | 27   | 38   | 22   | 16                        | 29   | 27   | 38   | 22   | 35                          | 29   | 53  | 25  | 81   | 27                          | 30   | 39   | 24  | 25  |
| Paenibacillaceae                        | 3                           | 0    | 0    | 0    | 0    | 0                         | 0    | 0    | 0    | 0    | 0                           | 0    | 0   | 0   | 0    | 0                           | 0    | 0    | 0   | 0   |
| [ <i>Eubacterium</i> ] <i>_brachy</i>   | 5                           | 4    | 9    | 4    | 5    | 3                         | 0    | 9    | 4    | 5    | 3                           | 0    | 2   | 0   | 3    | 4                           | 4    | 4    | 0   | 0   |
| <i>Olsenella</i>                        | 0                           | 8    | 0    | 8    | 0    | 0                         | 0    | 0    | 8    | 0    | 0                           | 0    | 0   | 0   | 0    | 0                           | 0    | 3    | 0   | 0   |
| <i>Collinsella</i>                      | 2169                        | 1690 | 1684 | 1690 | 1229 | 1089                      | 1339 | 1684 | 1690 | 1229 | 1451                        | 1282 | 805 | 596 | 1706 | 1251                        | 1191 | 1128 | 845 | 875 |
| <i>Enorma</i>                           | 4                           | 5    | 0    | 5    | 0    | 0                         | 0    | 0    | 5    | 0    | 0                           | 0    | 5   | 6   | 8    | 0                           | 0    | 0    | 0   | 0   |
| <i>Victivallis</i>                      | 0                           | 0    | 0    | 0    | 0    | 0                         | 0    | 0    | 0    | 0    | 0                           | 0    | 0   | 0   | 0    | 2                           | 4    | 0    | 0   | 0   |
| <i>Mastigocladopsis</i> PC10914         | 0                           | 0    | 0    | 0    | 0    | 0                         | 0    | 0    | 0    | 0    | 0                           | 0    | 0   | 0   | 0    | 0                           | 0    | 0    | 0   | 0   |
| <i>Chloroplast</i>                      | 3                           | 16   | 0    | 16   | 0    | 0                         | 0    | 0    | 16   | 0    | 0                           | 0    | 0   | 0   | 0    | 0                           | 0    | 0    | 0   | 2   |
| <i>Anaeroplasma</i>                     | 13                          | 10   | 11   | 10   | 3    | 3                         | 4    | 11   | 10   | 3    | 11                          | 10   | 0   | 0   | 0    | 12                          | 3    | 0    | 0   | 0   |
| <i>Erysipelatoclostridium</i>           | 63                          | 42   | 48   | 42   | 20   | 10                        | 26   | 48   | 42   | 20   | 22                          | 16   | 26  | 11  | 47   | 28                          | 19   | 30   | 16  | 16  |
| <i>Izemoplasma</i> tales                | 0                           | 22   | 13   | 22   | 14   | 0                         | 3    | 13   | 22   | 14   | 4                           | 4    | 0   | 0   | 0    | 3                           | 7    | 0    | 0   | 4   |
| Erysipelatoclostridiaceae               | 0                           | 6    | 0    | 6    | 6    | 0                         | 4    | 0    | 6    | 6    | 5                           | 0    | 0   | 0   | 0    | 0                           | 8    | 0    | 0   | 0   |
| <i>Dielma</i>                           | 0                           | 0    | 0    | 0    | 0    | 0                         | 0    | 0    | 0    | 0    | 0                           | 0    | 0   | 0   | 0    | 0                           | 0    | 0    | 0   | 0   |
| [ <i>Clostridium</i> ] <i>_innocuum</i> | 0                           | 5    | 5    | 5    | 0    | 1                         | 0    | 5    | 5    | 0    | 5                           | 0    | 3   | 0   | 7    | 4                           | 6    | 6    | 10  | 3   |
| <i>Merdibacter</i>                      | 4                           | 0    | 4    | 0    | 8    | 0                         | 5    | 4    | 0    | 8    | 8                           | 0    | 0   | 3   | 6    | 5                           | 3    | 6    | 0   | 0   |
| <i>Holdemanella</i>                     | 732                         | 544  | 561  | 544  | 380  | 364                       | 442  | 561  | 544  | 380  | 611                         | 534  | 208 | 139 | 368  | 465                         | 413  | 324  | 223 | 219 |

| Hits                             | No Substrate T0 n=2 T24 n=3 |     |     |     |     | Fiber-Rich T0 n=2 T24 n=3 |     |     |     |     | Western + BS T0 n=2 T24 n=3 |     |     |     |     | Western - BS T0 n=2 T24 n=3 |     |     |     |     |
|----------------------------------|-----------------------------|-----|-----|-----|-----|---------------------------|-----|-----|-----|-----|-----------------------------|-----|-----|-----|-----|-----------------------------|-----|-----|-----|-----|
|                                  | n=1                         | n=2 | n=1 | n=2 | n=3 | n=1                       | n=2 | n=1 | n=2 | n=3 | n=1                         | n=2 | n=1 | n=2 | n=3 | n=1                         | n=2 | n=1 | n=2 | n=3 |
| Erysipelotrichaceae              | 0                           | 0   | 0   | 0   | 0   | 0                         | 0   | 0   | 0   | 0   | 0                           | 0   | 0   | 0   | 0   | 5                           | 0   | 0   | 0   | 0   |
| <i>Faecalitalea</i>              | 33                          | 38  | 34  | 38  | 18  | 7                         | 19  | 34  | 38  | 18  | 24                          | 16  | 21  | 15  | 34  | 14                          | 15  | 24  | 14  | 20  |
| <i>Anaerococcus</i>              | 3                           | 0   | 0   | 0   | 0   | 0                         | 0   | 0   | 0   | 0   | 0                           | 0   | 0   | 0   | 0   | 0                           | 0   | 0   | 0   | 0   |
| Coriobacteriales                 | 12                          | 9   | 0   | 9   | 0   | 5                         | 3   | 0   | 9   | 0   | 6                           | 4   | 3   | 0   | 7   | 4                           | 8   | 4   | 6   | 0   |
| Eggerthellaceae                  | 0                           | 13  | 0   | 13  | 0   | 0                         | 0   | 0   | 13  | 0   | 7                           | 0   | 7   | 3   | 10  | 5                           | 3   | 13  | 69  | 31  |
| <i>Adlercreutzia</i>             | 29                          | 53  | 71  | 53  | 41  | 31                        | 42  | 71  | 53  | 41  | 29                          | 26  | 41  | 34  | 90  | 25                          | 20  | 53  | 52  | 50  |
| Eggerthellaceae                  | 26                          | 46  | 29  | 46  | 33  | 9                         | 13  | 29  | 46  | 33  | 19                          | 25  | 31  | 16  | 58  | 24                          | 24  | 19  | 19  | 23  |
| Coriobacteriales                 | 0                           | 2   | 0   | 2   | 0   | 0                         | 0   | 0   | 2   | 0   | 0                           | 0   | 0   | 0   | 0   | 0                           | 0   | 0   | 0   | 0   |
| <i>Senegalimassilia</i>          | 52                          | 48  | 50  | 48  | 47  | 23                        | 20  | 50  | 48  | 47  | 46                          | 21  | 41  | 32  | 77  | 28                          | 31  | 53  | 29  | 29  |
| <i>Eggerthella</i>               | 42                          | 47  | 48  | 47  | 40  | 15                        | 23  | 48  | 47  | 40  | 25                          | 22  | 54  | 29  | 96  | 30                          | 20  | 50  | 61  | 34  |
| <i>Slackia</i>                   | 0                           | 2   | 4   | 2   | 0   | 6                         | 5   | 4   | 2   | 0   | 4                           | 3   | 0   | 0   | 4   | 8                           | 9   | 0   | 0   | 3   |
| <i>Gordonibacter</i>             | 29                          | 27  | 18  | 27  | 18  | 12                        | 13  | 18  | 27  | 18  | 11                          | 11  | 27  | 15  | 63  | 14                          | 16  | 42  | 153 | 65  |
| <i>Delftia</i>                   | 0                           | 0   | 3   | 0   | 2   | 0                         | 0   | 3   | 0   | 2   | 0                           | 0   | 0   | 0   | 4   | 0                           | 2   | 0   | 0   | 0   |
| <i>Parasutterella</i>            | 37                          | 49  | 51  | 49  | 36  | 21                        | 33  | 51  | 49  | 36  | 29                          | 30  | 68  | 58  | 141 | 19                          | 18  | 79  | 61  | 70  |
| <i>Oxalobacter</i>               | 6                           | 11  | 10  | 11  | 9   | 0                         | 9   | 10  | 11  | 9   | 11                          | 11  | 12  | 16  | 21  | 10                          | 0   | 12  | 12  | 14  |
| <i>Herbaspirillum</i>            | 0                           | 0   | 0   | 0   | 0   | 0                         | 0   | 0   | 0   | 0   | 698                         | 627 | 84  | 81  | 268 | 646                         | 670 | 85  | 98  | 58  |
| <i>Burkholderia-Caballeronia</i> | 0                           | 0   | 0   | 0   | 0   | 0                         | 0   | 0   | 0   | 0   | 160                         | 102 | 30  | 21  | 90  | 101                         | 101 | 131 | 87  | 129 |
| Oxalobacteraceae                 | 0                           | 0   | 0   | 0   | 2   | 0                         | 0   | 0   | 0   | 2   | 0                           | 0   | 0   | 0   | 0   | 0                           | 0   | 0   | 0   | 0   |
| vadinBE97                        | 0                           | 7   | 0   | 7   | 0   | 0                         | 0   | 0   | 7   | 0   | 0                           | 3   | 8   | 3   | 20  | 3                           | 0   | 12  | 3   | 5   |
| UCG-004                          | 5                           | 0   | 0   | 0   | 0   | 0                         | 0   | 0   | 0   | 0   | 0                           | 0   | 0   | 0   | 0   | 2                           | 0   | 0   | 0   | 0   |
| Erysipelatoclostridiaceae        | 0                           | 0   | 5   | 0   | 0   | 0                         | 2   | 5   | 0   | 0   | 2                           | 0   | 0   | 0   | 0   | 0                           | 0   | 0   | 0   | 0   |
| Incertae_Sedis                   | 318                         | 290 | 309 | 290 | 185 | 128                       | 177 | 309 | 290 | 185 | 224                         | 179 | 170 | 88  | 363 | 162                         | 135 | 227 | 150 | 163 |

| Hits                          | No Substrate T0 n=2 T24 n=3 |      |      |      |      | Fiber-Rich T0 n=2 T24 n=3 |     |      |      |      | Western + BS T0 n=2 T24 n=3 |     |     |     |     | Western - BS T0 n=2 T24 n=3 |     |     |     |     |
|-------------------------------|-----------------------------|------|------|------|------|---------------------------|-----|------|------|------|-----------------------------|-----|-----|-----|-----|-----------------------------|-----|-----|-----|-----|
|                               | n=1                         | n=2  | n=1  | n=2  | n=3  | n=1                       | n=2 | n=1  | n=2  | n=3  | n=1                         | n=2 | n=1 | n=2 | n=3 | n=1                         | n=2 | n=1 | n=2 | n=3 |
| <i>Caproiciproducens</i>      | 0                           | 5    | 7    | 5    | 0    | 0                         | 0   | 7    | 5    | 0    | 0                           | 0   | 0   | 0   | 0   | 0                           | 0   | 0   | 0   | 0   |
| <i>Anaerotruncus</i>          | 0                           | 0    | 9    | 0    | 0    | 4                         | 0   | 9    | 0    | 0    | 4                           | 3   | 0   | 0   | 0   | 0                           | 0   | 0   | 0   | 0   |
| <i>Negativibacillus</i>       | 54                          | 44   | 36   | 44   | 35   | 9                         | 18  | 36   | 44   | 35   | 40                          | 29  | 14  | 19  | 47  | 23                          | 29  | 19  | 14  | 31  |
| <i>Phoceia</i>                | 11                          | 7    | 4    | 7    | 4    | 4                         | 4   | 4    | 7    | 4    | 0                           | 7   | 0   | 0   | 4   | 8                           | 5   | 0   | 6   | 0   |
| DTU089                        | 10                          | 14   | 16   | 14   | 4    | 0                         | 4   | 16   | 14   | 4    | 6                           | 3   | 3   | 2   | 10  | 14                          | 5   | 11  | 5   | 6   |
| <i>Oscillospirales</i>        | 0                           | 0    | 0    | 0    | 0    | 0                         | 0   | 0    | 0    | 0    | 2                           | 0   | 0   | 0   | 0   | 0                           | 0   | 0   | 0   | 0   |
| <i>Anaerofilum</i>            | 0                           | 0    | 6    | 0    | 0    | 0                         | 0   | 6    | 0    | 0    | 0                           | 0   | 0   | 0   | 0   | 0                           | 0   | 0   | 0   | 0   |
| CAG-352                       | 1067                        | 1445 | 1388 | 1445 | 1091 | 487                       | 620 | 1388 | 1445 | 1091 | 690                         | 593 | 521 | 386 | 997 | 490                         | 491 | 723 | 629 | 656 |
| <i>[Eubacterium]_siraenum</i> | 238                         | 104  | 88   | 104  | 56   | 90                        | 125 | 88   | 104  | 56   | 151                         | 113 | 16  | 11  | 34  | 116                         | 104 | 58  | 40  | 31  |

### ***Supplementary statistical analysis (Fig. 2)***

To quantitatively evaluate the compositional trends shown in Fig. 2, a statistical analysis ( $\Delta T24-T0$ ) was performed for all bacterial genera under each substrate condition to determine which composition most effectively preserved the original microbial community structure. For each genus, the condition with the smallest absolute deviation from baseline was identified, and group-level differences were statistically tested using the Kruskal-Wallis test. Among all tested conditions, the fiber-rich medium minimized compositional drift most reliably, being identified as the best-performing condition in 11 out of 34 genera, with statistical significance ( $p < 0.05$ ) in 91 % of those cases. By contrast, the substrate-free condition showed the highest number of best-performer counts (14 genera), but with a notably lower significance rate (64 %), suggesting potential contributions from background effects or low selective pressure. The Western-style substrate conditions, with or without bile salts, yielded lower frequencies (5 and 7 genera, respectively), yet achieved statistical significance in all relevant comparisons, indicating condition-specific but strong compositional effects. These results collectively demonstrate that the fiber-rich formulation provided the most robust support for microbial stability and recovery in vitro, outperforming all other tested substrate compositions in both consistency and statistical reliability. In contrast, exposure to Western-style substrates induced shifts toward a dysbiotic community profile enriched in bile-tolerant and facultative anaerobic taxa.

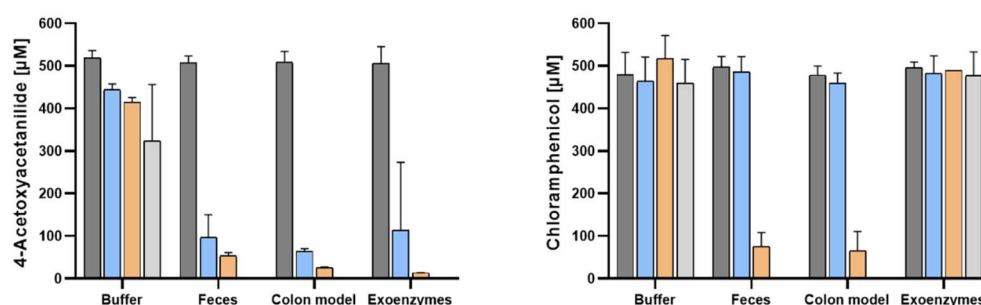
### ***Supplementary xenobiotic biotransformation datasets (Fig. 7)***

To provide a parameter-based comparison of xenobiotic turnover across matrices, apparent first-order rate constants ( $k$ ) and half-lives ( $t_{1/2}$ ) were calculated from parent compound depletion data and are summarized in Supplementary Table S4. The kinetic evaluation confirmed the qualitative trends observed in the depletion plots: all compounds were essentially stable in buffer, whereas clear turnover occurred in fecal slurry and in the colon model. For roxatidine-acetate and chloramphenicol, kinetic parameters were nearly identical between fecal slurry and the colon model, while sulindac, sulfasalazine, and nitrendipine showed the same overall transformation pattern with minor quantitative differences. The exoenzyme fraction showed relevant activity only for ester hydrolysis substrates, particularly roxatidine-acetate and 4-acetoxyacetanilide, whereas the other compounds remained largely stable. These data support both the functional similarity between fecal slurry and the colon model and the mechanistic specificity of the system.

**Table S4: Apparent first-order rate constants ( $k$ ) and corresponding half-lives ( $t_{1/2}$ ) for xenobiotic depletion in buffer, fecal slurry, colon model, and the corresponding exoenzyme fraction.** Kinetic parameters were calculated from linear regression of  $\ln$  (remaining precursor compound) versus time using quantifiable parent-compound concentrations only. Rate constants are given in  $\text{h}^{-1}$  and half-lives in h.

|                    | Roxatidine-acetate |           | Sulindac |           | Sulfasalazine |           | Nitrendipine |           | 4-Acetoxy-acetanilide |           | Chloramphenicol |           |
|--------------------|--------------------|-----------|----------|-----------|---------------|-----------|--------------|-----------|-----------------------|-----------|-----------------|-----------|
|                    | $k$                | $t_{1/2}$ | $k$      | $t_{1/2}$ | $k$           | $t_{1/2}$ | $k$          | $t_{1/2}$ | $k$                   | $t_{1/2}$ | $k$             | $t_{1/2}$ |
| <b>Buffer</b>      | 0.005              | > 24      | 0.0003   | > 24      | 0.002         | > 24      | 0.0009       | > 24      | 0.02                  | > 24      | 0.002           | > 24      |
| <b>Feces</b>       | 1.16               | 0.60      | 0.12     | 5.78      | 0.93          | 0.75      | 0.44         | 1.58      | 15.38                 | 0.05      | 0.45            | 1.54      |
| <b>Colon model</b> | 1.13               | 0.61      | 0.20     | 3.47      | 1.20          | 0.58      | 0.92         | 0.75      | 21.18                 | 0.03      | 0.47            | 1.47      |
| <b>Exoenzymes</b>  | 0.16               | 4.33      | 0.003    | > 24      | 0.003         | > 24      | 0.002        | > 24      | 13.46                 | 0.05      | 0.003           | > 24      |

Two additional xenobiotics representing distinct microbial reaction pathways are shown in Supplementary Figure S2. 4-Acetoxyacetanilide, used as a model substrate for ester hydrolysis, was rapidly degraded in fecal slurry and in the bacterial colon model, while the corresponding exoenzyme fraction also showed clear hydrolytic activity, consistent with an extracellular enzyme-driven reaction. In contrast, chloramphenicol, representing nitroreduction, was strongly transformed in fecal slurry and in the bacterial colon model, but remained essentially stable in the exoenzyme fraction, indicating that this reaction depended on intact bacterial cells rather than released extracellular enzymes. Overall, the degradation kinetics observed in fecal slurry and the colon model were highly comparable for both compounds, further supporting that the bacterial colon model preserves the relevant functional microbial activities while also allowing mechanistic discrimination between extracellular and cell-associated reactions.



**Fig. S2: Microbial biotransformation of xenobiotic model compounds in buffer, fecal slurry, colon model, and the corresponding exoenzyme fraction.** Representative substrates were selected to cover different microbial transformation reactions commonly observed in the human colon: 4-acetoxyacetanilide for ester hydrolysis (A) and chloramphenicol for nitroreduction (B). Precursor-compound concentrations were quantified by HPLC at 0 h (dark grey), 0.1 h (blue), 4 h (orange), and 24 h (light grey) under anaerobic conditions ( $37^\circ\text{C}$ ). Data represent mean  $\pm$  SD of three independent biological pools, each composed of three human donors.