(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
28 January 2021 (28.01.2021)

WIPO | PCT

(10) International Publication Number
WO 2021/016129 A1

(51) International Patent Classification:
A61B 10/00 (2006.01) *A61B 5/07* (2006.01)
A61B 5/00 (2006.01)

(21) International Application Number:
PCT/US2020/042669

(22) International Filing Date:
17 July 2020 (17.07.2020)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/876,388 19 July 2019 (19.07.2019) US

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(54) Title: COMPOSITIONS AND METHODS TO AFFECT HUMAN GUT MICROBES

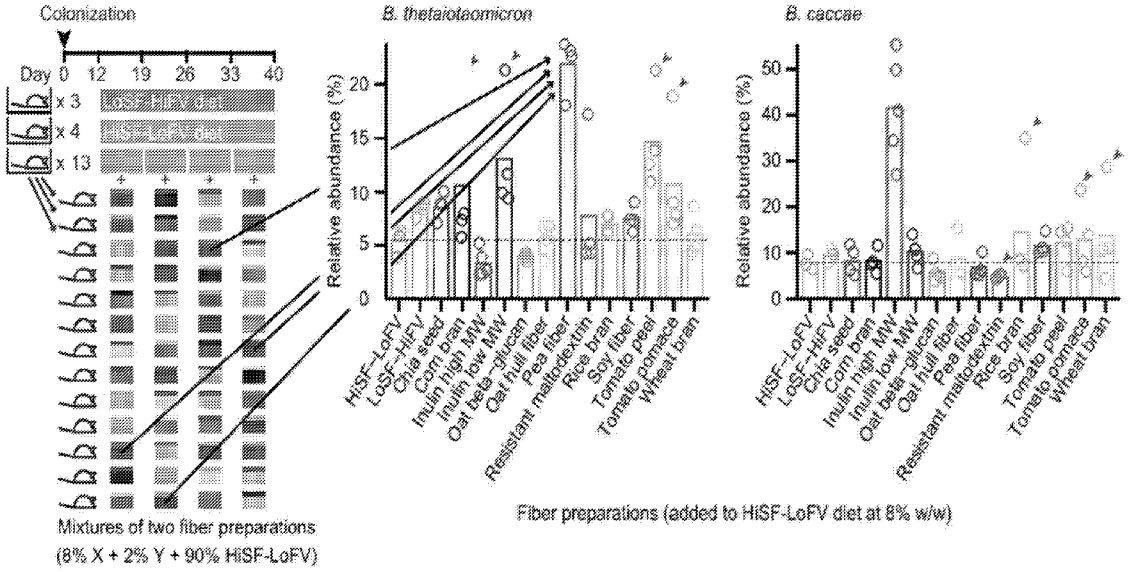


FIG. 1A

(57) Abstract: The present disclosure provides compositions and foods that selectively promote the representation and expressed beneficial function of members of a human gut community in ways that promote a healthy gut microbiota and in turn positively impact health. Various examples of compositions and foods, which comprise one or more fiber preparation, are discussed in detail, as are methods of their use.

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(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

— *with international search report (Art. 21(3))*

COMPOSITIONS AND METHODS TO AFFECT HUMAN GUT MICROBES

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to U.S. Provisional Application No. 62/876,388, filed July 19, 2019, the disclosures of which are incorporated herein by reference.

GOVERNMENTAL RIGHTS

[0002] This invention was made with government support under DK070977, DK078669 and DK107158 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

[0003] Increasing evidence that the gut microbiota impacts multiple features of human biology has catalyzed efforts to develop microbiota-directed interventions that improve health status. Microbiota-directed foods (MDFs) are one approach, as diet has pronounced and rapid effects on microbial community configuration. Dietary carbohydrates provide an important source of energy for gut bacteria, with the products of their metabolism benefiting primary microbial consumers, their syntrophic partners, and the host. Consumption of plant polysaccharides in the form of dietary fiber has been linked to a number of health benefits. In addition, the diminished diversity of complex polysaccharides in the diets of those living in industrialized countries has been associated with loss of bacterial diversity in their microbiota.

[0004] From the perspective of gut microbiota, plant material and fiber preparations prepared from plant material contain active and inactive fractions with different structural features and biophysical availability. Historically, identifying the bioactive components of fiber preparations has been a formidable challenge. Accordingly, there remains a need in the art for compositions that selectively promote the representation and expressed beneficial function of members of a human gut

community in ways that promote a healthy gut microbiota and in turn positively impact health.

SUMMARY OF THE INVENTION

[0005] In an aspect, the present disclosure encompasses a composition comprising a plurality of fiber preparations, each fiber preparation independently selected from the group consisting of a barley fiber preparation or a glycan equivalent thereof, a citrus fiber preparation or a glycan equivalent thereof, a citrus pectin formulation or a glycan equivalent thereof, a high molecular weight inulin preparation or a glycan equivalent thereof, a pea fiber preparation or a glycan equivalent thereof, and a sugar beet fiber preparation or a glycan equivalent thereof, wherein the plurality of fiber preparations is at least 95 wt% of the composition.

[0006] In another aspect, the present disclosure encompasses a composition at least 15 wt% of one or more sugar beet fiber preparation and at least 28 wt% of one or more high molecular weight inulin preparation, and optionally one or more citrus pectin preparation in an amount that does not exceed 10 wt%, one or more citrus fiber preparation in an amount that does not exceed 25 wt%, and one or more barley fiber preparations in an amount does not exceed 45 wt%, wherein the plurality of fiber preparations is at least 95 wt% of the composition.

[0007] In another aspect, the present disclosure encompasses a composition comprising at least 15 wt% of one or more pea fiber preparation or a glycan equivalent thereof; and at least one additional fiber preparation chosen from (i) at least 28 wt% of one or more high molecular weight inulin preparation or a glycan equivalent thereof, (ii) 10 wt% or less of one or more citrus pectin preparation or a glycan equivalent thereof, (iii) 25 wt% or less of one or more citrus fiber preparation or a glycan equivalent thereof, or (iv) 45 wt% or less of one or more barley fiber preparation or a glycan equivalent thereof.

[0008] In another aspect, the present disclosure encompasses a composition comprising about 35 wt% of one or more pea fiber preparation or a glycan equivalent thereof, about 10 wt% of one or more citrus fiber preparation or a glycan equivalent thereof, about 35 wt% of one or more high molecular weight inulin

preparation or a glycan equivalent thereof, and about 20 wt% of one or more barley fiber preparation or a glycan equivalent thereof; and wherein the pea fiber preparation(s), citrus fiber preparation(s), high molecular weight inulin preparation(s), and barley fiber preparation(s) are at least 95 wt% of the composition.

[0009] In another aspect, the present disclosure encompasses a composition comprising about 30-40 wt% of one or more pea fiber preparation or a glycan equivalent thereof, about 9-11 wt% of one or more citrus fiber preparation or a glycan equivalent thereof, about 30-40 wt% of one or more high molecular weight inulin or a glycan equivalent thereof, and about 18-22 wt% of one or more barley fiber preparation or a glycan equivalent thereof; and wherein the pea fiber preparation(s), citrus fiber preparation(s), high molecular weight inulin preparation(s), and barley fiber preparation(s) are at least 95 wt% of the composition.

[0010] In another aspect, the present disclosure encompasses a composition comprising about 30-35 wt% of one or more pea fiber preparation or a glycan equivalent thereof, about 9-11 wt% of one or more citrus fiber preparation or a glycan equivalent thereof, about 35-40 wt% of one or more high molecular weight inulin preparation or a glycan equivalent thereof, and about 18-22 wt% of one or more barley bran preparation or a glycan equivalent thereof; and wherein the pea fiber preparation(s), citrus fiber preparation(s), high molecular weight inulin preparation(s), and barley fiber preparation(s) are at least 95 wt% of the composition.

[0011] In another aspect, the present disclosure encompasses a composition comprising about 33 wt% of one or more pea fiber preparation or a glycan equivalent thereof, about 11 wt% of one or more citrus fiber preparation or a glycan equivalent thereof, about 36 wt% of one or more high molecular weight inulin preparation or a glycan equivalent thereof, and about 20 wt% of one or more barley fiber preparation or a glycan equivalent thereof; and wherein the pea fiber preparation(s), citrus fiber preparation(s), high molecular weight inulin preparation(s), and barley fiber preparation(s) are at least 95 wt% of the composition.

[0012] In another aspect, the present disclosure encompasses a composition comprising about 65 wt% pea fiber or a glycan equivalent thereof, and

about 35 wt% high molecular weight inulin or a glycan equivalent thereof; and wherein the pea fiber preparation(s) and high molecular weight inulin preparation(s) are at least 95 wt% of the composition.

[0013] In another aspect, the present disclosure encompasses food compositions comprising compositions disclosed herein. In some embodiments, the amount of the composition is about 40 wt% to about 50 wt% of the food composition. In some embodiments, the composition provides about 90% or more of the total dietary fibers in the food composition.

[0014] In another aspect, the present disclosure encompasses a pressed, extruded or baked food composition, the food composition comprising about 40 wt% to about 95 wt% of a composition of fiber preparations, the composition of fiber preparations comprising (a) about 25 wt% to about 40 wt% of one or more pea fiber preparation, or a glycan equivalent thereof; about 5 wt% to about 15 wt% of one or more citrus fiber preparation, or a glycan equivalent thereof; about 30 wt% to about 40 wt% of one or more high molecular weight inulin preparation, or a glycan equivalent thereof; and about 10 wt% to about 30 wt% of one or more barley fiber preparation, or a glycan equivalent thereof; or (b) about 55 wt% to about 65 wt% of one or more pea fiber preparation, or a glycan equivalent thereof; and about 30 wt% to about 40 wt% of one or more high molecular weight inulin preparation, or a glycan equivalent thereof; wherein a 30 g serving of the food composition has at least 6 g of total dietary fiber; and wherein the food composition effects an increase in the fiber degrading capacity of a subject's gut microbiota and/or an improvement in the a subject's health, when the subject has consumed the food composition at least once a day for at least 5 days (e.g., at least 6 days, at least 7 days, etc.).

[0015] Other aspects and iterations of the invention are described more thoroughly below.

BRIEF DESCRIPTION OF THE FIGURES

[0016] The application file contains at least one photograph executed in color. Copies of this patent application publication with color photographs will be provided by the Office upon request and payment of the necessary fee.

[0017] **FIG. 1A** and **FIG. 1B** show the design and results of an *in vivo* screen of the effects of food-grade fiber preparations on members of a defined human gut microbiota. **FIG. 1A** includes a schematic design of the screen (one of three similar screens). Individually-housed adult germ-free mice were colonized with a consortium of 20 bacterial strains obtained from a single human donor. Animals received a series of supplemented HiSF-LoFV diets, each containing one fiber preparation at 8% (w/w) and another at 2% (w/w) (colored boxes). Fecal samples were collected during the last two days of each week-long diet period. Control animals received the unsupplemented HiSF-LoFV or LoSF-HiFV diet monotonously for four weeks. Also shown are the average relative abundance values for *B. thetaiotaomicron* and *B. caccae* on days 6 and 7 of treatment with the indicated fiber-supplemented HiSF-LoFV diets. Bars show mean values. Circles denote individual mice. Black arrows point to data obtained from different mice consuming diets containing 8% (w/w) pea fiber consumed at the indicated periods of their diet oscillation sequence. Green arrowheads in panel B mark mice that received pea fiber as the minor fiber type (2% w/w) while purple arrowheads in panel C highlight animals where high molecular weight (MW) inulin was the minor fiber. See Table A for compositional analysis of the 34 fibers. **FIG. 1B** depicts estimates of coefficients from linear models for bacterial strains across the three screening experiments where models produced at least one estimated coefficient > 0.4. Statistically significant coefficients ($P < 0.01$; ANOVA) are shaded according to the color bar.

[0018] **FIG. 2A, FIG. 2B, FIG. 2C, FIG. 2D, FIG. 2E, FIG. 2F, FIG. 2G, FIG. 2H, FIG. 2I** and **FIG. 2J** show the results of proteomics and forward genetic experiments to identify arabinan in pea fiber as a nutrient source for multiple bacterial species. **FIG. 2A** is a schematic representation of polysaccharide structures detected in pea fiber based on monosaccharide and linkage analyses (with stereochemistry of anomeric carbon inferred). **FIG. 2B-FIG. 2E** are graphs showing relative abundance of the indicated bacterial strains. Adult C57BL/6J germ-free mice were colonized with a 15-member community consisting of INSeq libraries representing four *Bacteroides* species together with 10 additional bacterial strains used in screening experiments depicted in FIG. 1. Relative abundance is shown for each bacterial strain at each of the

indicated time points in mice monotonously fed the control HiSF-LoFV diet (grey), or the HiSF-LoFV diet supplemented with 10% (w/w) pea fiber (green). Circles denote individual mice. Shading denotes the 95%CI. The position of the line within the data points for a given time point represents the mean value (n=15 individually-caged mice per group; Tables S4A-S4C). *, $P < 0.05$; (pea fiber supplemented versus unsupplemented HiSF-LoFV diet; ANOVA). **FIG. 2F-FIG. 2I** are graphs showing Proteomic and INSeq analyses of fecal samples collected on experimental day 6. On the x-axis, the position of each dot denotes the mean value for the abundance of a single bacterial protein in samples obtained from animals monotonously fed the pea fiber-supplemented HiSF-LoFV diet (relative to controls fed the unsupplemented diet). The y-axis indicates the mean value for the differential enrichment of mutant strains with Tn disruptions in the gene encoding each protein in the pea fiber versus HiSF-LoFV diet groups. The total number of genes represented in both the protein dataset and INSeq mutant pool is shown in the upper left of each plot, and these genes are plotted as grey dots. Green circles highlight genes that are significantly affected by pea fiber ($P < 0.05$, |fold change| > log2(1.2); limma or limma-voom) as judged by levels of their protein products or their contribution to fitness; open circles mark the subset of these genes that are encoded by PULs. Genes that are present in three homologous arabinan-processing PULs in *B. thetaiotaomicron*, *B. cellulosilyticus*, and *B. vulgatus* are labeled with their PUL number as it appears in PULDB (Terrapon et al., 2018). Genes in an arabinose-processing operon in *B. vulgatus* are labeled with an 'A'. Genes in the *B. ovatus* RGI-processing PUL97 are also labeled. (J) Alignment of *B. thetaiotaomicron* PUL7, *B. cellulosilyticus* PUL5, *B. vulgatus* PUL27, and the *B. vulgatus* arabinose operon. The direction of transcription is left to right (unless marked by a leftward pointing arrowhead). The first and last genes are labeled above with their locus tag number. Genes are color-coded according to their functional annotation (see key). GH families for enzymes in the CAZy database are shown as numbers inside the gene boxes (characterized members of GH51, GH43:4, GH43:29, and GH146 are predominantly arabinanases or arabinofuranosidases). Shaded regions connecting genes denote significant BLAST

homology (E-value $<10^{-9}$); the percent amino acid identity of their protein products is shown.

[0019] **FIG. 3A, FIG. 3B, FIG. 3C,** and **FIG. 3D** show results from experiments that deliberately manipulate a community composition to demonstrate interspecies competition for pea fiber arabinan. **FIG. 3A and FIG. 3C**are graphs showing relative abundance of the indicated bacterial strains. Adult C57BL/6J germ-free mice were colonized with the same defined community that was used for the experiments in FIG. 2, with or without *B. cellulosilyticus* (*B.c.*). Relative abundance of each bacterial strain is shown at each time point in mice fed the control HiSF-LoFV diet in the presence (light grey, closed circles), or absence (dark grey, open circles) of *B. cellulosilyticus*, or fed the HiSF-LoFV diet supplemented with 10% (w/w) pea fiber in the presence (green, closed circles) or absence (magenta, open circles) of *B. cellulosilyticus*. Key: circles, individual mice; lines, mean values; shading, 95%CI (n=4-10 mice per group). *, $P < 0.05$; (diet-by-community interaction; ANOVA). **FIG. 3B** and **FIG. 3D** are plots showing mean values ± SD (vertical shading) (n=5 animals/treatment group) from proteomics analysis of fecal communities sampled on experimental days 6, 12, 19, and 25. Genes in PULs of interest are shown along the x-axis (as locus tag number only; *BT_XXXX* or *BVU_XXXX*). Genes are color-coded according to their functional annotation (see key). GH families for enzymes in the CAZy database are shown as numbers inside the gene boxes. Key for circles is identical to that used in panels A and C. *, $P < 0.05$, |fold change| > log2(1.2) (pea fiber supplemented versus unsupplemented HiSF-LoFV diet; limma).

[0020] **FIG. 4A, FIG. 4B, FIG. 4C, FIG. 4D, and FIG. 4E** show results from experiments to characterize glycan processing as a function of community membership with artificial food particles. **FIG. 4A** is a schematic depiction of a bead-based *in vivo* glycan degradation assay. **FIG. 4B** depicts flow cytometry plots showing levels of fluorescence in a pool of three bead types before and after transit though the guts of mice representing two colonization conditions. Axes are labeled with the fluorophore detected in each channel. **FIG. 4C** graphically depicts the mass of arabinose associated with two types of polysaccharide-coated beads together with empty uncoated beads before (black) and after (green) passage through the intestine of gnotobiotic mice,

mono-colonized with either *B. cellulosilyticus* or *B. vulgatus*. Beads were purified from cecal and colonic contents four hours after gavage. The mass of arabinose associated with beads is plotted before (black) and after (green) passage through the intestine. Circles denote individual animals. Bars show mean values and 95%CI. **FIG. 4D** and **FIG. 4E** graphically depict polysaccharide degradation in mice colonized with the 15-member community (with *B. cellulosilyticus*), or the 14-member community (lacking *B. cellulosilyticus*) fed the HiSF-LoFV diet. The mass of bead-associated arabinose (panel D) or glucose (panel E) is plotted before (black) and after collection from cecal and colonic contents on experimental day 12 (grey, 15-member community group; magenta, minus *B. cellulosilyticus* group). The presence or absence of *B. cellulosilyticus* in each group of mice is noted along the x-axis. Circles denote individual mice. Mean values + 95%CI are shown (n=3-6 animals/group). *, $P < 0.05$ (Mann-Whitney *U* test).

[0021] **FIG. 5A, FIG. 5B, FIG. 5C, FIG. 5D, FIG. 5E,** and **FIG. 5F** show the results of experiments to detect acclimation to the presence of a potential competitor using proteomics and forward genetics. **FIG. 5A** and **FIG. 5B** graphically depict the relative abundance of the indicated bacterial strains after adult C57BL/6J germ-free mice were colonized with the same defined community used for the experiments in FIG. 2, with or without *B. cellulosilyticus* (*B.c.*) or *B. vulgatus* (*B.v.*). Relative abundance of each bacterial strain in fecal samples is shown at each time point in mice colonized with the 15-member community (grey closed circles) or that community lacking *B. cellulosilyticus or B. vulgatus* (open circles; magenta and brown respectively). All mice received the control base HiSF-LoFV diet. Key: circles, individual mice; lines, mean values; shading, 95%CI. **FIG. 5C** and **FIG. 5D** are plots showing protein abundance and INSeq data for genes in arabinoxylan PULs shown along the x-axis (as locus tag number only; *Bovatus_0XXXX*) according to the order in which they appear in the genome. Mean values ± SD (vertical shading) are indicated (n=5 animals/treatment group). Genes are color-coded according to functional annotation. Key for circles: grey, 15-member community; magenta or brown, mice harboring communities without *B. cellulosilyticus* or *B. vulgatus*, respectively. *, $P < 0.05$, |fold change| > log2(1.2) [15-member community versus 14-member (minus *B. cellulosilyticus*); limma or limma-

voom]. **FIG. 5E** is a plot showing a proteomics analysis of fecal communities sampled on experimental day 6. Proteins whose abundances increase significantly in the absence of *B. cellulosilyticus* appear in the upper right; those encoded by genes in PULs are highlighted with open circles while those encoded by genes in arabinoxylan processing PULs are labeled with their PUL number. **FIG. 5F** is a plot showing an INSeq analysis showing the change in abundance of mutant strains from experimental day 2 to day 6 relative to the 15-strain community. Genes that are significantly more important for fitness in the absence of *B. cellulosilyticus* appear in the upper left. Genes in PULs that have a significant effect on fitness are highlighted with open circles; those located in arabinoxylan processing PULs are labeled with their PUL number.

[0022] **FIG. 6A, FIG. 6B, FIG. 6C, FIG. 6D, FIG. 6E, FIG. 6F,** and **FIG. 6G** show the results of experiments to alleviate competition between arabinoxylan consuming *Bacteroides*. **FIG. 6A, FIG. 6B,** and **FIG, 6C** graphically depict the relative abundance of bacterial strains after adult C57BL/6J germ-free mice were colonized with the same defined community used for the experiments in FIG. 2, with or without *B. cellulosilyticus* (*B.c.*) and/or *B. ovatus* (*B.v.*). The relative abundance of each bacterial strain is shown at each time point in mice fed the control HiSF-LoFV diet and colonized with the 15-member community (closed circles) or the derivative communities lacking *B. cellulosilyticus* or *B. ovatus* or both species (open circles; magenta, orange, and cyan respectively). Key: circles, individual mice; lines, mean values; shading, 95%CI. **FIG. 6D** and **FIG. 6E** graphically show the analysis of *B. ovatus* or *B. cellulosilyticus* protein abundances in fecal samples obtained on experimental day 6. Genes in arabinoxylan-processing PULs are shown along the x-axis (as locus tag number only; *Bovatus_0XXXX* or *BcellWH2_0XXXX*) according to the order in which they appear in the genome. Mean values ± SD (vertical shading) are indicated (n=5-7 animals/treatment group). Genes are color-coded according to functional annotation (see key). Key for circles: grey, 15-member community; magenta, orange, or cyan, mice harboring communities without *B. cellulosilyticus, B. ovatus*, or both species, respectively. *, $P < 0.05$ [15-member community versus 14-member (minus *B. cellulosilyticus*); limma]. **FIG. 6F** and **FIG. 6G** graphically show the results of a bead-

based assay of polysaccharide degradation in mice fed the HiSF-LoFV diet and colonized with the complete 15-member community, or a community lacking *B. cellulosilyticus*, *B. ovatus*, or both species. The mass of bead-associated arabinose (**FIG. 6F**) or mannose (**FIG. 6G**) is plotted before (black) and after exposure to the indicated communities (grey, complete 15-member community; magenta, community with *B. cellulosilyticus* omitted; orange, community lacking *B. ovatus*; cyan, community lacking both *Bacteroides* species). The presence or absence of *B. cellulosilyticus* and *B. ovatus* in each group of mice is noted along the x-axis. Circles denote individual mice. Mean values + 95%CI are shown (n=5-7 animals/group). *, $P < 0.05$ (Mann-Whitney *U* test).

[0023] **FIG. 7A, FIG. 7B, FIG. 7C, FIG. 7D, FIG. 7E, FIG. 7F, FIG. 7G, FIG. 7H,** and **FIG. 7I** show the results of proteomics and forward genetic experiments to identify homogalacturonan in citrus pectin as a nutrient source for multiple bacterial species. **FIG. 7A** is a schematic representation of polysaccharide structures detected in citrus pectin based on monosaccharide and linkage analyses (with stereochemistry of anomeric carbons inferred). **FIG. 7B-E** are graphs showing relative abundance of the indicated bacterial strains. Adult C57BL/6J germ-free mice were colonized with a 15-member community consisting of INSeq libraries representing four *Bacteroides* species together with 10 additional bacterial strains used in screening experiments depicted in Figure 1. Relative abundance is shown for each bacterial strain at each of the indicated time points in mice fed the control HiSF-LoFV diet (grey), or the HiSF-LoFV diet supplemented with 10% (w/w) citrus pectin (blue). Circles denote individual mice, lines the mean value and shading the 95% CI (n=15 individually caged mice per group; results pooled from three independent experiments). *, $P < 0.05$ (Mann-Whitney *U* test). **FIG. F-I** are plots showing proteomic and INSeq analyses of fecal samples collected on experimental day 6. On the x-axis, each dot denotes the mean value for the abundance of a single bacterial protein in samples from animals monotonously fed the citrus pectin-supplemented HiSF-LoFV diet (relative to controls fed the unsupplemented diet). The y-axis indicates the mean value for the differential enrichment of mutants with Tn disruptions in the gene encoding each protein in the citrus pectin versus HiSF-LoFV diet

groups. Blue dots represent genes that are significantly affected by citrus pectin (P < 0.05, |fold change| > log2(1.2); limma or limma-voom) as judged by levels of their protein products or their contribution to fitness while open circles mark the subset of these genes that are encoded by PULs. Genes present in predicted homogalacturonan-processing PULs in *B. thetaiotaomicron*, *B. cellulosilyticus*, and *B. vulgatus* are labeled with their PUL number as it appears in PULDB (Terrapon et al., 2018).

[0024] **FIG. 8A, FIG. 8B, FIG. 8C,** and **FIG. 8D** show results from experiments that deliberately manipulate a community composition to demonstrate interspecies competition for homogalacturonan in citrus pectin. **FIG. 8A and FIG. 8B**are graphs showing relative abundance of the indicated bacterial strains. Adult C57BL/6J germ-free mice were colonized with the same defined community that was used for the experiments in FIG. 2, with or without *B. cellulosilyticus* (*B.c.*). Relative abundance of each bacterial strain is shown at each time point in mice fed the control HiSF-LoFV diet in the presence (light grey, closed circles), or absence (dark grey, open circles) of *B. cellulosilyticus*, or fed the HiSF-LoFV diet supplemented with 10% (w/w) citrus pectin in the presence (green, closed circles) or absence (magenta, open circles) of *B. cellulosilyticus*. Key: circles, individual mice; lines, mean values; shading, 95%CI (n=4-10 mice per group). *, P < 0.05; (diet-by-community interaction; ANOVA). **FIG. 8C** and **FIG. 8D** are plots showing mean values ± SD (vertical shading) (n=5 animals/treatment group) from proteomics analysis of fecal communities sampled on experimental days 6, 12, 19, and 25. Genes in predicted homogalacturonan-processing PULs are shown along the x-axis (as locus tag number only; *BT_XXXX, BVU_XXXX*) according to the order in which they appear in the genome. Mean values ± SD (vertical lines) are indicated (n=5 animals/treatment group). Genes are color-coded according to functional annotation (see key). GH families for enzymes in the CAZy database are shown as numbers inside the gene boxes. Key for circles is identical to that used in panels A and B. *, P < 0.05, |fold change| > log2(1.2) (citrus pectin supplemented versus unsupplemented-HiSF-LoFV diet; limma).

[0025] **FIG. 9A, FIG. 9B, FIG. 9C, FIG. 9D, FIG. 9E, FIG. 9F** show results from experiments to characterize glycan processing as a function of community

membership with artificial food particles. **FIG. 9A** and **FIG. 9B** graphically depict the mass of arabinose or glucose associated with three types of polysaccharide-coated beads or with empty uncoated beads. Gnotobiotic mice, mono-colonized with either *B. cellulosilyticus* or *B. vulgatus*, were gavaged with three types of polysaccharide-coated beads together with empty uncoated beads. Beads were purified from cecal and colonic contents 4 hours after gavage. The mass of arabinose (**FIG. 9A**) or glucose (**FIG. 9B**) associated with beads is plotted before (black) and after (green) their transit through the gut. Circles denote individual animals. Bars show mean values with 95%CI. In **FIG. 9C** and **FIG. 9D**, adult C57BL/6J germ-free mice were gavaged with four beads types (labeled on the x-axis). Beads were isolated from fecal samples collected from 4 to 12 hours after gavage. The mass of arabinose (**FIG. 9C**) and glucose (**FIG. 9D**) associated with beads is plotted before (black) and after (blue) their transit through the gut. Circles denote individual mice. Bars show the mean values + 95%CI (n=13 animals). **FIG. 9E** and **FIG. 9F** graphically depict polysaccharide degradation in mice colonized with the 15-member community (with *B. cellulosilyticus*), or the 14-member community lacking *B. cellulosilyticus* fed the HiSF-LoFV diet +10% pea fiber. Beads were recovered from cecal and colonic contents. The mass of bead-associated arabinose (**FIG. 9E**) or glucose (**FIG. 9F**) is plotted before (black) and after transit through the gut (green, 15-member community group; magenta, minus *B. cellulosilyticus* group). In **FIG. 9A, B, E, and F**, and in **FIG. 4D and 4E**, input beads are shared for all plots, since all six groups of mice were analyzed in the same experiment. The presence or absence of *B. cellulosilyticus* in each group of mice is noted along the x-axis. Circles denote individual mice. Mean values + 95%CI are shown (n=3-6 animals/group). *, $P < 0.05$ (Mann-Whitney *U* test).

[0026] **FIG. 10** graphically depicts the results of an adhesion assay using glycan-coated beads and gut microorganisms. The extent of fluorescence (Syto-60+) on the y-axis is measured relative to control beads that are incubated with fluorescent dye but not bacteria.

[0027] **FIG. 11A, FIG. 11B, FIG. 11C, FIG. 11D,** and **FIG. 11E** illustrate various experimental designs described in the examples. **FIG. 11A** - Monotonous

feeding of the unsupplemented HiSF-LoFV diet or the diet supplemented with one of four different fiber preparations. Fecal samples were collected on days 2, 3, 6, 8, 12, 14, 19 and 21. **FIG. 11B** - Monotonous feeding of the unsupplemented HiSF-LoFV diet or the HiSF-LoFV diet supplemented with pea fiber or citrus pectin to mice colonized with the community with or without *B. cellulosilyticus.* Fecal samples were collected on days 2, 3, 6, 8, 12, 14, 19 and 25. **FIG. 11C** - Monotonous feeding of the HiSF-LoFV with or without pea fiber to mice colonized with the community with or without *B. cellulosilyticus.* Fecal samples were collected on days 2, 3, 4, 6, 7, 8, 10, 11, and 12. **FIG. 11D** - Monotonous feeding of HiSF-LoFV with or without citrus pectin to mice colonized with a community with or without *B. cellulosilyticus* or *B. vulgatus.* Fecal samples were collected on days 2, 3, 4, 6, 7, 8, 10, and 12. **FIG. 11E** - Monotonous feeding of the unsupplemented HiSF-LoFV diet to mice harboring communities with or without *B. cellulosilyticus* and/or *B. ovatus.* Fecal samples were collected on days 2, 3, 4, 6, 7, 8, and 10.

[0028] **FIG. 12** is a chemical reaction schematic. Although only a single polysaccharide is used in this depiction, any glycan may be used.

[0029] **FIG. 13A** is a graph depicting the zeta potential of surface modified paramagnetic silica beads. Parent beads and beads modified with only APTS or THPMP were used as standards.

[0030] **FIG. 13B** is a graph depicting bead fluorescence after reaction of each bead type shown with NHS ester fluorescein. Only beads modified with surface amines, and not acetylated, were highly fluorescent.

[0031] **FIG. 14** is a chemical reaction schematic of CDAP activation of polysaccharides and immobilization on the surface of amine phosphonate beads. Although only a single polysaccharide is used in this depiction, any glycan may be used.

[0032] **FIG. 15** is a graph depicting arabinoxylan immobilization on surface modified beads. Beads were reacted with CDAP-activated arabinoxylan in the presence of catalytic TEA. The amount of arabinoxylan bound to each bead type was determined by quantifying xylose and arabinose liberated following acid hydrolysis of a set number of beads.

[0033] **FIG. 16** is a schematic of the use of polysaccharide-coated beads to measure the biochemical function of a gut microbiota within a mouse.

[0034] **FIG. 17** is a graph depicting arabinose release from polysaccharide-coated beads harvested from cecum 4 hours post bead gavage. Each data point represents a single mouse. Mean ± SD. Pairwise Welch's t-test. Benjamini and Hochberg corrected. *$p<0.05$.

[0035] **FIG. 18** diagrams a procedure for fractionation of a pea fiber preparation.

[0036] **FIG. 19** is graph depicting monosaccharide compositions of fractions 1 to 8 of a pea fiber preparation.

[0037] **FIG. 20A** depicts the structure of a pea fiber arabinan. R groups (not shown) are attached to each end, where R may be hydrogen or a pectic fragment. The proposed chemical structure for pea fiber arabinan is derived from partially methylated alditol acetate GC-MS analysis which was supported by the Chemical Sciences, Geosciences and Biosciences Division, Office of Basic Energy Sciences, U.S. Department of Energy grant (DE-SC0015662) to DOE - Center for Plant and Microbial Complex Carbohydrates at the Complex Carbohydrate Research Center. In Fraction 8, R_1 is H and R_2 is a pectic fragment containing galacturonic acid, galactose, and rhamnose.

[0038] **FIG. 20B** depicts the structure of a sugar beet arabinan. An R group (not shown) is attached to the free end, where R may be hydrogen or a pectic fragment.

[0039] **FIG. 21** is graph depicting monosaccharide compositions of sugar beet arabinan and Fraction 8.

[0040] **FIG. 22** is an illustration of the experimental design described in Example 10.

[0041] **FIG. 23** is a graph of a principal component analysis of fecal bacterial community composition in response to diet supplementation. Each data point represents an individual mouse. Shaded regions represent 95% probability region of the s.d. of mean.

[0042] **FIG. 24** graphically depicts the fractional abundance of several bacterial strains following diet supplementation. Each circle represents an individual mouse. Shaded regions are ± SD.

[0043] **FIG. 25** is an illustration of the experimental design described in Example 10.

[0044] **FIG. 26** are graphs depicting arabinose mass following diet supplementation.

[0045] **FIG. 27A**, **FIG. 27B**, and **FIG. 27C** are alignments of arabinan-utilization loci arabinan-utilization loci in *Bacteroides* species (related to FIG. 2). Alignment of *B. thetaiotaomicron* PUL7 (**FIG. 27A**), *B. cellulosilyticus* PUL5 (**FIG. 27B**), and *B. vulgatus* PUL27 (**FIG. 27C**) across multiple strains of each species. The direction of transcription is indicated by the arrowhead. The genes are labeled with their locus tag number and color-coded according to their functional annotation (see key). Shaded regions connecting genes denote (i) significant BLAST homology (E-value $<10^{-9}$) and the percent amino acid identity of their protein products (see key).

[0046] **FIG. 28A**, **FIG. 28B**, and **FIG. 28C** are graphs showing *B. cellulosilyticus*-dependent glycan use by *B. ovatus* in the HiSF-LoFV diet context (related to FIG. 6). Proteomics analysis of fecal communities sampled on experimental days 6, 12, 19, and 25. Genes, color-coded according to their functional annotation including GH family assignments, in the indicated PULs are shown along the x-axis together with their locus tag numbers (*Bovatus_0XXXX*). The abundance of their expressed protein products (mean values ± SD) is plotted along the y-axis (n=5 animals/treatment group). Key for circles: grey, 15-member community; magenta, mice harboring communities without *B. cellulosilyticus*. *, $P < 0.05$, |fold change| > log2(1.2), [15-member community versus 14-member (minus *B. cellulosilyticus*); limma].

[0047] **FIG. 29** diagram a process for making a food composition (e.g., extruded pillow).

[0048] **FIG. 30A** shows an illustration of the study design of Example 12.

[0049] **FIG. 30B** show descriptions of singular value decomposition and higher-order singular value decomposition. The top part of the illustration shows a

matrix M, defined by *n* rows and *m* columns, is analyzed by SVD to create three new matrices: U (dimensions *n* by *n*), E (dimensions *n* by *m*), and V (dimensions *m* by *m*). Columns of the U matrix are termed 'Left Singular Vectors' (LSVs), diagonal entries of E are termed 'Singular Values'`, rows of V are termed 'Right Singular Vectors' (RSVs). Multiplication of the first LSV (LSV1), the first singular value, and the first RSV (RSV1) creates a matrix, M^1, that reflects variation contained within the first singular value exclusively. The bottom part of the illlustration shows a tensor, O, defined by *n* rows, *m* columns, and *p* conditions, is analyzed by HO-SVD to create a core tensor populated by diagonal elements only, G, and three matrices (dimensions *n* by *a*, *m* by *b*, and *p* by *c*). The dimensions of the core tensor G (*a*, *b*, and *c*) are determined from a numeric approximation method used in HO-SVD known as Canonical-Polyadic Alternating Least Squares (CP-ALS). The fractional variance captured by tensor component 1 (TC1) is reflected by the value of the first element of G (a_1, b_1, c_1) (red-shaded cube in core tensor G). The result of HO-SVD on O results in computing contributions, or 'projections' of each degree of freedom (*n*, *m*, or *p*) on each tensor component. The projections of each degree of freedom on TC1 are highlighted in red.

[0050] **FIG. 30C**, **FIG. 30D**, **FIG. 30E**, and **FIG. 30F** show the results of testing for the effects of dietary fibers in gnotobiotic mice colonized with nine different obese human donor microbiota and fed a HiSF-LoFV USA diet. **FIG. 30C** shows a plot of microbiome configurations on TC1 and TC2 as a function of diet treatment resulting from HO-SVD applied to CAZymes in fecal microbiomes of mice colonized with nine different obese human donor microbial communities during the pea fiber phase of the diet oscillation experiment. **FIG. 30D** shows a histogram of CAZyme projections on TC1 where the CAZyme genes that project within the most positive and negative 10^{th} percentiles are highlighted in red and yellow respectively. **FIG. 30E** and **FIG. 30F** show heatmaps of $\log_2$ fold-change discriminatory CAZymes shown in FIG. 30D. Data are averaged for animals containing a given human donor microbiota sampled at the indicated time points and normalized to day 14 values. The depicted order of CAZymes ranked from top to bottom of the heatmap (beginning with FIG. 30E and ending with FIG. 30F) is based on their projections along TC1 in FIG. 30D, beginning with the most

negatively projecting CAZyme (GH102 positioned at the top of the left-most column) and ending with the most positively projecting CAZymes (PL6 and GH99 positioned at the bottom of the right-most column).

[0051] **FIG. 31A**, **FIG. 31B**, **FIG. 31C**, **FIG. 31D**, **FIG. 31E**, **FIG. 31F**, **FIG. 31G**, **FIG. 31H**, **FIG. 31I**, **FIG. 31J**, and **FIG. 31K** show results of a controlled diet study of the effects of fiber-snack food prototypes on the fecal microbiomes of overweight and obese humans. **FIG. 31A** shows an illustration of the study design of Example 14. **FIG. 31B** shows an illustration of the study design of Example 15. **FIG. 31C-E** show HO-SVD analyses of changes in microbiome configurations as a function of fiber snack prototype, defined by the representation of discriminatory CAZymes where **FIG. 31C** was defined by the CAZyme pea fiber; **FIG. 31D** was defined by the CAZyme pea fiber and inulin; and **FIG. 31E** was defined by the CAZyme pea fiber, inulin, orange fiber and barley bran. **FIG. 31F-K** show heatmaps plotting the $\log_2$ fold-change in the abundances of these discriminatory CAZymes relative to the time of initiation of pea fiber snack consumption (day 14) (**FIG. 31F**, **FIG. 31G**); initiation of pea fiber and inulin snack consumption (day 11) (**FIG. 31H**, **FIG. 31I**); and initiation of pea fiber, inulin, orange fiber and barley bran snack consumption (day 11) (**FIG. 31J**, **FIG. 31K**). In **FIG. 31F-K**, hierarchical clustering (Canberra distance) provided a way to operationally group participant microbiomes as responsive or hypo-responsive to the intervention (branches colored red and black, respectively, in the dendrograms shown).

[0052] **FIG. 32A**, **FIG. 32B**, **FIG. 32C**, **FIG. 32D**, **FIG. 32E**, and **FIG. 32F** show host responses defined by plasma proteomic features in a controlled diet study. **FIG. 32A, FIG. 32C, and FIG. 32E** show HO-SVD analyses of changes in the plasma proteomes of subjects in each of the indicated treatment groups sampled at the indicated time points where **FIG. 32A** is the group that consumed pea fiber snacks, **FIG. 32C** is the group that consumed pea fiber and inulin snacks, and **FIG. 32E** is the group that consumed pea fiber, inulin, orange fiber and barley bran snacks. **FIG. 32B, FIG. 32D, and FIG. 32F** show $\log_2$ fold-changes in the abundances of 25 discriminatory plasma proteins assigned to the KEGG insulin and glucagon signaling pathways, in each subject as a function of the different snack fiber prototype treatments changes

normalized to day 14 in the pea fiber study (**FIG. 32B**) and day 11 for the effects of the two- (**FIG. 32D**) and four-fiber (**FIG. 32F**) formulations. The direction of change in the abundance of each of these proteins that is indicative of movement towards a healthier state is denoted by the vertical bar on right side of the heatmaps (increase in red or a decrease in blue). Subjects were classified as responsive or hypo-responsive to the snack food prototype interventions based on an aggregate change of ≥ 50% of these 25 protein markers towards a healthier state plus the results of hierarchical clustering (Canberra distance).

[0053] **FIG. 33A**, **FIG. 33B**, **FIG. 33C** and **FIG. 33D** show cross correlation singular value decompositions (CC-SVD) relating host proteomic responses to changes in CAZyme gene representation in the microbiomes of subjects consuming the four-fiber snack prototype. **FIG. 33A and FIG. 33B** show a summary of the CC-SVD method where each element of the cross-correlation matrix contains the Spearman rank-correlation between CAZyme i and protein j (**FIG. 33A**). The singular value decomposition (SVD) of the cross-correlation matrix is shown in **FIG. 33B**. The left singular matrix contains projections of CAZymes along SV1 while the right singular matrix contains projections of proteins along SV1. Singular values, which relate the left and right SVs (and by extension, the CAZymes proteins with the plasma proteins) are housed in the central matrix. Histograms of projections of CAZymes (orange) and proteins (blue) along SV1. CAZymes and proteins within the same tail of the distribution are strongly positively correlated while those in opposite tails of the distribution are strongly negatively correlated. **FIG. 33C-D** show heatmaps plotting the Spearman correlation coefficient between CAZymes and proteins for the four-fiber, two-fiber, and pea-fiber alone snack prototypes. The blank column in **FIG. 33C** indicates that measurement of TFF2 in the plasma proteomes of study 1 participants consuming pea fiber did not pass quality control criteria. The coloring indicated in FIG. 33C for the enzymes / CAZyme designation applies to FIG. 33D. The key in FIG. 33D applies to FIG. 33C.

[0054] **FIG. 34A**, **FIG. 34B**, **FIG. 34C**, **FIG. 34D** and **FIG. 34E** show monosaccharide content (**FIG. 34A**, **FIG. 34B**, **FIG. 34C**, **FIG. 34D**) and glycosyl

linkages (**FIG. 34E**) present in unsupplemented and fiber-supplemented HiSF-LoFV diets fed to gnotobiotic mice. Data shown are *p<0.01, ***, p<0.001 as determined by a one-way ANOVA with Holm-Sidak multiple comparison correction. Linkages shown are represented by their methylated monosaccharide derivatives Abbreviations. Glc, glucose; Gal, galactose; GalA, galacturonic acid; GlcA, glucuronic acid; Ara, arabinose; Xyl, xylose; Fru, fructose; Fuc, fucose; Rha, rhamnose; Rib, ribose; Hex, hexose; dHex, deoxyhexose; T, terminal; f, furanose; p, pyranose; X, undefined linkage.

[0055] **FIG. 35A**, **FIG. 35B**, **FIG. 35C**, **FIG. 35D**, **FIG. 35E**, **FIG. 35F**, **FIG. 35G**, **FIG. 35H**, and **FIG. 35I** show results of HO-SVD applied to ASV and mcSEED metabolic pathway datasets generated from the fecal microbiota of mice harboring nine different obese human donor microbial communities during the pea-fiber phase of the diet oscillation. **FIG. 35A** shows projections of microbiota configuration as defined by representation on TC1 and TC2. **FIG. 35B** shows a histogram of ASV projections on TC1; taxa that project within the most positive and negative 10^{th} percentiles are highlighted in red and yellow, respectively. **FIG. 35C, FIG. 35D, FIG. 35CE** show heatmaps of fractional abundances of a subset of the taxa highlighted in Fig. 35B where each column indicates the human donor microbiota used to colonize the mice. Data are averaged for all mice in a given treatment group on the indicated experimental days. **FIG. 35F** shows microbiome configurations as defined by the representation of mcSEED metabolic pathways. **FIG. 35G** shows a histogram that highlights pathways that project within the most positive and negative 10^{th} percentiles. **FIG. 35H** and **FIG. 35I** show heatmaps depicting the $\log_2$ fold-change for representation of discriminatory mcSEED metabolic pathways identified in **FIG. 35G**. Data are averaged for all mice in the indicated treatment groups at the indicated time points and normalized to day 14 values.

[0056] **FIG. 36A**, **FIG. 36B**, **FIG. 36C**, **FIG. 36D**, **FIG. 36E** and **FIG. 36F** show results of HO-SVD applied to genes encoding CAZymes present in the fecal microbiomes of mice during the orange fiber treatment phase of their diet oscillation. **FIG. 36A** shows changes in microbiome configuration as defined by CAZyme gene abundances. **FIG. 36B** shows a histogram of CAZyme projections on TC1 and TC2 with

those projecting within the most positive and negative 10th percentiles highlighted in red and yellow. **FIG. 36C**, **FIG. 36D**, **FIG. 36E** and **FIG. 36F** show a heatmaps of log_2 fold-change for the discriminatory CAZymes shown in FIG. 36B. Data are averaged for all mice in the indicated treatment groups at the indicated time points and normalized to day 14 values. FIG. 36C and FIG. 36D are day 44; FIG. 36E and FIG. 36F are day 54.

[0057] **FIG. 37A**, **FIG. 37B**, **FIG. 37C**, **FIG. 37D**, **FIG. 37E**, **FIG. 37F**, **FIG. 37G**, and **FIG. 37H** show results of HO-SVD applied to the ASV and mcSEED pathway datasets generated from mice during the orange fiber phase of the diet oscillation. **FIG. 37A** shows projections of microbiota configuration as defined by representation on TC1 and TC2. **FIG. 37B** shows a histogram of ASV projections on TC1; taxa that project within the most positive and negative 10th percentiles are highlighted in red and yellow, respectively. **FIG. 37C**, **FIG. 37D**, **FIG. 37E** show heatmaps of fractional abundances of a subset of the taxa highlighted in FIG. 37B. Each column indicates the human donor microbiota used to colonize the mice. Data are averaged for all mice in a given treatment group on the indicated experimental days. **FIG. 37F** shows microbiome configurations as defined by the representation of mcSEED metabolic pathways. **FIG. 37G** shows a histogram that highlights pathways that project within the most positive and negative 10th percentiles. **FIG. 37H** shows a heatmap depicting the log_2 fold-change in the representation of discriminatory mcSEED metabolic pathways identified *in* Fig. 37G. Data are averaged for all mice in the indicated treatment groups at the indicated time points and normalized to day 14 values.

[0058] **FIG. 38A**, **FIG. 38B**, **FIG. 38C**, **FIG. 38D**, **FIG. 38E**, and **FIG. 38F** show results of HO-SVD applied to CAZymes genes represented in the fecal microbiomes of mice colonized with the obese human donor microbial communities during the barley bran fiber phase of the diet oscillation. **FIG. 38A** shows changes in microbiome configuration as defined by CAZyme gene abundances. **FIG. 38B** shows a histogram of CAZyme projections on TC1 and TC2 with those projecting within the most positive and negative 10th percentiles highlighted in red and yellow, respectively. **FIG. 38C**, **FIG. 38D**, **FIG. 38E** and **FIG. 38F** show heatmaps of log_2 fold-change for the discriminatory CAZymes shown in FIG. 38B at day 54 (FIG. 38C and FIG. 38D) and day

65 (FIG. 38E and FIG. 38F). Data are averaged for all mice in the indicated treatment groups at the indicated time points and normalized to day 14 values.

[0059] **FIG. 39A**, **FIG. 39B**, **FIG. 39C**, **FIG. 39D**, **FIG. 39E**, **FIG. 39F**, and **FIG. 39G** show results of HO-SVD of ASV and mcSEED pathway representation in the fecal communities of mice during the barley bran fiber phase of the diet oscillation. **FIG. 39A** shows projections of microbiota configuration as defined by representation on TC1 and TC2. **FIG. 39B** shows a histogram ASV projections on TC1; taxa that project within the most positive and negative 10^{th} percentiles are highlighted in red and yellow, respectively. **FIG. 39C** and **FIG. 39D**show heatmaps of fractional abundances of a subset of the taxa highlighted in **FIG. 39B**. Each column indicates the human donor microbiota used to colonize the mice. Data are averaged for all mice in a given treatment group on the indicated experimental days. **FIG. 39E** shows microbiome configurations as defined by the representation of mcSEED metabolic pathways. **FIG. 39F** shows a histogram that highlights pathways positioned within the most positive 10^{th} percentile and most negative 20^{th} percentile of projections along TC1. **FIG. 39G** shows a heatmap depicting the $\log_2$ fold-change in representation of discriminatory mcSEED metabolic pathways identified in **FIG. 39F**. Data are averaged for all mice in the indicated treatment groups at the indicated time points and normalized to day 14 values.

[0060] **FIG. 40A**, **FIG. 40B**, and **FIG. 40C**show CAZymes identified by HO-SVD analysis as discriminatory for microbiome responses to the different fiber snack food prototypes. **FIG. 40A**, **FIG. 40B**, and **FIG. 40C** show histograms of CAZyme projections on the indicated tensor components for pea fiber snack food (**FIG. 40A**) and the two- (**FIG. 40B**) and four-fiber (**FIG. 40C**) snack food formulations. CAZyme genes that project within the most positive and negative 20^{th} percentiles are highlighted in red and yellow, respectively. The dashed box relates the rank order of CAZymes from top to bottom of the heatmaps shown in **FIG. 31F-K** to their projections along the tensor components described in these histograms, beginning with the most positively projecting CAZyme at the bottom of the right-most column and proceeding to the upper most CAZyme in the first column encompassed within the box (i.e., for pea fiber, the most positive projecting CAZyme, CMB77, is located at the top of the heatmap

displayed in **FIG. 31F,G** while CBM3 is positioned at the bottom of the heatmap; for the two-fiber formulation, PL29 is located at the top and GH89 at the bottom of the heatmap in **FIG. 31H,I**; for the four-fiber formulation, PL38 is at the top and CMB77 is at the bottom of the heatmap in **FIG. 31J,K**).

[0061] **FIG. 41A**, **FIG. 41B**, **FIG. 41C**, **FIG. 41D**, **FIG. 41E**, **FIG. 41F**, **FIG. 41G**, **FIG. 41H**, and **FIG. 41I** show results of HO-SVD analysis of the effects of the pea fiber snack prototype on representation of pea fiber treatment-discriminatory mcSEED metabolic pathways and ASV taxa present in the fecal microbiomes of subjects enrolled in human study 1. **FIG. 41A** shows projections of microbiome configuration of mcSEED metabolic pathways on TC1 and TC3. **FIG. 41B** shows a histogram of mcSEED metabolic pathways projections on TC3; metabolic pathways that project within the most positive and negative 20^{th} percentiles are highlighted in red and yellow, respectively. **FIG. 41C** shows a heatmap of the $\log_2$ fold-change at day 29 (consumption of the maximum dose of the pea fiber snack prototype) in the representation of discriminatory mcSEED metabolic pathways identified in **FIG. 41B**, normalized to day 14 (last pre-treatment timepoint). Each column indicates a subject. **FIG. 41D** shows microbiota configurations. **FIG. 41E** shows a histogram that highlights ASVs that project within the most positive and negative 20^{th} percentiles on TC2. **FIG. 41F**, **FIG. 41G**, **FIG. 41H**, and **FIG. 41I** show heatmaps depicting the fractional abundances of ASVs identified in **FIG. 41E** for days 14 (last day of pre-treatment, FIG. 41F and FIG. 41G), and day 29 (consumption of the maximum dose of the pea fiber snack prototype, FIG. 41H and FIG. 41I). Each row indicates a participant, the rows are identified in FIG. 41F and FIG. 41H, and the identifiers also apply to FIG. 41G and FIG. 41I..

[0062] **FIG. 42A** and **FIG. 42B** show spearman-rank cross-correlation analyses of representation of CAZymes by monosaccharides and glycosyl linkages in the fecal communities of subjects consuming the pea fiber snack prototype. Correlations between the $\log_2$ fold-change of HO-SVD defined discriminatory CAZyme gene abundances (matched by time and subject) to the $\log_2$ fold-change in levels of monosaccharides (**FIG. 42A**) and glycosidic linkages (**FIG. 42B**) normalized to day 14 (pre-intervention phase). Monosaccharides abundant in pea fiber that are significantly

positively correlated with discriminatory CAZymes which increased during pea fiber supplementation are highlighted by the green boxes in **FIG. 42A**. Each row of the heatmap is a monosaccharide. From top to bottom, the rows are Rib, Xyl, GalA, Ara, Fru, Fuc, Glc, Man, GlcA, Gal, All, Rha, GlcNAc, GalNAc. Each column is a CAZyme. From left to right, the columns are: GH43_4, PL27, GH43_37, PL11, GH115, GH43_19, GT101, GH43_29, CBM6, CBM27, CBM23, GH43_5, GH10, GH82, CBM61, CBM22, CBM4, GH5_2, CBM72, GT17, GT76, GH30_5, GH97, PL8, GH43_2, PL6, GH5_5, GH50, PL17, PL15, PL13, PL12, GH5_21, GH43_1, GH67, GH108, GH5_1, GH30_8, GH43_7, CBM37, CBM2, GH30, PL30, GH26, PL1, PL9, CBM77, GH13_8, GT3, GH19, GH13_38, GT30, GH5_7, GH30_3, GH57, GH9, GH5_8, GH5_4, GH44, CBM3, CBM79, CBM78. **FIG. 42B** provides evidence that subject microbiomes contain CAZymes that cleave multiple branches of pea fiber arabinan, resulting in accumulation of its 1,5-arabinofuranose backbone in feces. Each row indicates a glycosidic linkage. From top to bottom, the rows are: 5-Ara(f); 2-Xyl; X,X-Hex (I); 2-Gal; 4-Man/ 3-Man; 4-Glc; 4,6-Glc/ 3,6-Gal; X,X,X-Hex (I); 3-Xyl; 2,X1-Xyl; 2,X-Hex (I); 2,X,X-Hex (I); 4-Xyl(p); 2,X,X-Hex (II); 2,X2-Ara; 2-Ara(f); 3-Ara(f); 3,4,6-Man, 3,4,6-Gal; 3,6-Man; 6-Glc/ 6-Gal; 4-Gal/ 6-Man; 2,X-dHex (III); T-Man; 3-Glc/ 3-Gal; T-Gal; T-Rha; 2,X-dHex (II); T-Ara(f); T-Glc; T-Fuc; X-Hex; 2-Man; 2-Glc; 4,6-Man; 3,4-Xyl(p); Xyl(p); 2,X1-Ara; 2,X2-Rib; 2,X-Hex (II); 2-dHex (I); X-dHex (I); 3,4-Fuc; X-dHex (II); T-fru; 2,X-dHex (I). Each column is a CAZyme. From left to right, the columns are: GH5_2, GH5_7, CBM4, GH5_21, GH67, GH10, GH57, GH97, GT19, GH13_38, GH13_8, GT3, GT30, GH43_2, GT101, GH43_29, PL1, CBM6, GH82, PL11, GH43_1, GH43_19, GH115, GH43_4, GH43_37, GT76, CBM27, CBM77, GH43_7, GH5_8, CMB2, CMB79, GH44, GH5_1, CMB78, CMB3, GH5_37, GH30_8, GH26, GH5_4, GH108, GH30_3, GT17, PL17, CBM61, PL27, GH30, GH9, PL30, PL6, GH5_5, PL8, CBM23, CBM22, PL9, GH50, PL12, PL15, PL13, GH43_5, CBM72, CBM37, GH30_5. Abbreviations: glucose (Glc), galacturonic acid (GalA), arabinose (Ara), xylose (Xyl), galactose (Gal), mannose (Man), rhamnose (Rha), fucose (Fuc), fructose (Fru), glucuronic acid (GlcA), N-acetylglucosamine (GlcNAc), N-acetylgalactosamine (GalNAc), allose (All), ribose (Rib), hexose (Hex), deoxyhexose (dHex), terminal (T), pyranose (p), furanose (f), undefined linkage (X).

[0063] **FIG. 43A**, **FIG. 43B**, **FIG. 43C**, **FIG. 43D**, **FIG. 43E**, **FIG. 43F**, and **FIG. 43G** show HO-SVD analysis of the effects of the two-fiber snack prototype on the representation of treatment discriminatory mcSEED metabolic pathways and ASVs in the fecal communities of subjects enrolled in human study 2. **FIG. 43A** shows projections of microbiome configuration based on mcSEED metabolic pathway composition. **FIG. 43B** shows a histogram of mcSEED metabolic pathways projections on TC2; pathways that project within the most positive and negative 20^{th} percentiles are highlighted in red and yellow, respectively. **FIG. 43C** shows a heatmap of the $\log_2$ fold-change at day 25 (maximum dose of the two-fiber snack prototype) in the representation of discriminatory mcSEED metabolic pathways identified in **FIG. 43B**, normalized to day 11 (last day of pre-treatment). Each column indicates a subject. **FIG. 43D** shows microbiota configurations based on ASV composition. **FIG. 43E** shows a histogram that highlights taxa that project within the most positive and negative 20^{th} percentiles on TC2. **FIG. 43F**, **FIG. 43G**, **FIG. 43H**, and **FIG. 43I** show heatmaps depicting the fractional abundances of discriminatory ASVs identified in **FIG. 43E** for days 11 (last day of pre-treatment, FIG. 43F and FIG. 43G), and day 25 (consumption of the maximum dose of the two-fiber snack prototype, FIG. 43H and FIG. 43I). Each row indicates a participant. The rows are identified in FIG. 43F and FIG. 43H, and the identifiers also apply to FIG. 43G and FIG. 43I.

[0064] **FIG. 44A**, **FIG. 44B**, **FIG. 44C**, **FIG. 44D**, **FIG. 44E**, **FIG. 44F**, **FIG. 44G**, **FIG. 44H**, and **FIG. 44I** show HO-SVD analysis of the effects of the four-fiber snack prototype on the representation of treatment discriminatory mcSEED metabolic pathways and ASVs in the fecal communities of subjects enrolled in human study 2. **FIG. 44A** shows projections of microbiome configuration based on mcSEED metabolic pathways composition. **FIG. 44B** shows a histogram of mcSEED metabolic pathways projections on TC1; metabolic pathways that project within the most positive and negative 20^{th} percentiles are highlighted in red and yellow, respectively. **FIG. 44C** shows a heatmap of the $\log_2$ fold-change at day 49 (consumption of the maximum dose of the four-fiber snack prototype) in the representation of discriminatory mcSEED metabolic pathways identified in **FIG. 44B**, normalized to day 11 (last day of pre-

treatment). Each column indicates a subject. **FIG. 44D** shows microbiota configurations as defined by the representation of ASVs. **FIG. 44E** shows a histogram that highlights taxa that project within the most positive and negative 20th percentiles on TC1. **FIG. 44F, FIG. 44G**, **FIG. 44H**, and **FIG. 44I** show heatmaps depicting the fractional abundances of discriminatory ASVs, identified in **FIG. 44E**, for days 35 (last day of washout-phase; FIG. 44E and FIG. 44F), and day 49 (consumption of the maximum dose of the four-fiber snack prototype; FIG. 44G, and FIG. 44H). Each row indicates a participant. The rows are identified in FIG. 44F and FIG. 44H, and the identifiers also apply to FIG. 44G and FIG. 44I.

[0065] **FIG. 45A**, **FIG. 45B**, and **FIG. 45C** show LC-QTOF-MS analysis of a biomarker of orange fiber consumption present in gnotobiotic mouse and human fecal samples**. FIG. 45A** shows a comparison of levels of the m/z 274.1442 analyte in colonized and germ-free mice fed the unsupplemented, orange fiber-supplemented or pea fiber-supplemented HiSF-LoFV diet for 10 days. The analyte is only detectable when orange fiber is consumed and is not dependent upon on the donor microbiome for its generation. **FIG. 45B and FIG. 45C** show comparisons of levels of the analyte in fecal samples obtained from participants in human study 2 on days 25 and 49 when they were consuming the maximum dose of the two-fiber (pea and inulin) and four-fiber (pea fiber, inulin, orange fiber plus barley bran) snack food prototypes where **FIG. 45B** shows the average analyte amount and **FIG. 45C** shows the analyte amount in the fecal samples of each individual. The horizontal dashed line in **FIG. 45C** denotes a baseline value operationally defined as the highest level of detection of the analyte in subjects consuming the two-fiber snack food prototype lacking orange fiber.

DETAILED DESCRIPTION

[0066] The present disclosure provides compositions and foods that selectively promote the representation and expressed beneficial function of members of a human gut community in ways that promote a healthy gut microbiota (e.g., improve fiber degrading capacity) and in turn positively impact health. The effects of the fiber supplements on gut microbial community configuration (representation of microbial taxa, genes encoding carbohydrate-active enzymes and genes encoding proteins and

enzymes in various metabolic pathways), gut microbial function (activity of genes encoding carbohydrate-active enzymes and/or genes encoding proteins and enzymes in various metabolic pathways) and host biology (which may be defined by changes in the levels of plasma proteins representing biomarkers and mediators of numerous physiologic, metabolic, and immune functions) are shown to be specific. Therefore, these specific effects can be considered discriminatory features and can be used to provide a rigorous scientific foundation for claims about the benefits of these products for different consumers with different weights, body mass indices, diets, and health. For instance, responders may be defined as those subjects with an aggregate change of ≥ 50% towards a healthier state for a collection of plasma protein markers (e.g., protein markers of chronic inflammation, protein markers of insulin and/or glucagon signaling, protein markers of satiety, protein markers of weight management, protein markers of cardiovascular health, etc.). The collection of plasma protein biomarkers in the Examples are defined by the proteomic assay (e.g., SOMAscan Assay 1.3k) but other assays can be used. Alternatively, or in addition, responders may be defined as those subjects with an aggregate change of ≥ 50% towards a healthier state in the representation of health discriminatory CAZymes, mcSEED subsystem proteins, or microbial taxa. Various examples of compositions and foods of the present disclosure, which comprise one or more fiber preparation, are discussed in detail below, as are methods of their use. In addition, Applicants have identified bioactive components in compositionally complex food ingredients that increase the fiber degrading capacity of the gut microbiota.

[0067] While specific implementations are discussed, it should be understood that this is done for illustration purposes only. A person skilled in the relevant art will recognize that other components and configurations may be used without parting from the spirit and scope of the disclosure. Additional features and advantages of the disclosure will be set forth in the description which follows, and in part will be obvious from the description, or can be learned by practice of the herein disclosed principles. These and other features of the disclosure will become more fully

apparent from the following description and appended claims, or can be learned by the practice of the principles set forth herein.

[0068] Several definitions that apply throughout this disclosure will now be presented.

[0069] As used herein, "about" refers to numeric values, including whole numbers, fractions, percentages, etc., whether or not explicitly indicated. The term "about" generally refers to a range of numerical values, for instance, ± 0.5-1%, ± 1-5% or ± 5-10% of the recited value, that one would consider equivalent to the recited value, for example, having the same function or result. In some instances, the term "about" may include numerical values that are rounded to the nearest significant figure.

[0070] The term "comprising" means "including, but not necessarily limited to"; it specifically indicates open-ended inclusion or membership in a so-described combination, group, series and the like. The terms "comprising" and "including" as used herein are inclusive and/or open-ended and do not exclude additional, unrecited elements or method processes.

[0071] As used herein, the term "fiber preparation" refers to a composition comprising dietary fiber that (i) is intended as an ingredient in a food, and (ii) has been prepared from a plant source including, but not limited to, fruits, vegetables, legumes, oilseeds, and cereals; or has been otherwise manufactured to have a composition similar to a fiber preparation prepared from a plant source. "Prepared from a plant source," as used herein, indicates plant material has undergone one or more treatment step prior to its utilization to make a composition disclosed herein (e.g., grinding, milling, shelling, hulling, extraction, extrusion, fractionation, etc.).

[0072] The term "dietary fiber" refers to edible parts of plants, or analogous glycans and carbohydrates, that are resistant to digestion and adsorption in the human small intestine with complete or partial fermentation in the large intestine. The term "dietary fiber" includes glycans, lignin, and associated plant substances. Total dietary fiber, soluble dietary fiber, and insoluble dietary fiber are terms of art defined by the methodology used to measure their relative amount. As used herein, total dietary fiber is

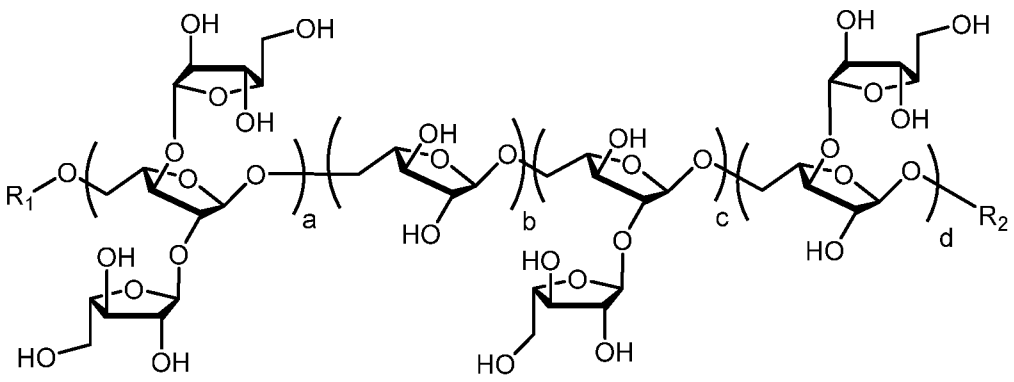
defined by AOAC method 2009.01; soluble dietary fiber and insoluble dietary fiber are defined by AOAC method 2011.25.

[0073] The term "carbohydrate" refers to an organic compound with the formula $C_m(H_2O)_n$, where *m* and *n* may be the same or different number, provided the number is greater than 3.

[0074] As used herein, the term "glycan" refers to a homo- or heteropolymer of two or more monosaccharides linked glycosidically. As such, the term "glycan" includes disaccharides, oligosaccharides and polysaccharides. The term also encompasses a polymer that has been modified, whether naturally or otherwise; non-limiting examples of such modifications include acetylation, alkylation, esterification, etherification, oxidation, phosphorylation, selenization, sulfonation, or any other manipulation. Glycans may be linear or branched, may be produced synthetically or obtained from a natural source, and may or may not be purified or processed prior to use.

[0075] A glycan may be defined, in part, in terms of its monosaccharide content and its glycosyl linkages. For example, plant arabinans are composed of 1,5-α-linked L-arabinofuranosyl residues, and these can be branched at O-2 or O-3 by single arabinosyl residues or short side chains (Beldman et al., 1997; Ridley et al., 2001; Mohnen, 2008). 1,5-Linked arabinan structures exist as free polymers unattached to pectic domains or attached to pectic domains (Beldman et al., 1997; Ridley et al., 2001).

[0076] As is understood in the art, due to the mechanism of side chain synthesis, a plant glycan is not a single chemical entity but is rather a mixture of glycans that have a defined backbone and variable amounts of substituents / branching. It is routine in the art to indicate the presence of variable amounts of a substituent by indicating its fractional abundance. For instance, when R_1 and R_2 are each H, the glycan depicted below is an arabinan – specifically, a polymer consisting of 1,5-α-linked L-arabinofuranosyl residues:



(I).

The formula indicates that (1) the polymer backbone consists of 1,5-α-linked L-arabinofuranosyl residues, and (2) there are 4 types of arabinose components – namely, component a – 2,3,5-arabinofuranose, component b – 5-arabinofuranose, component c – 2,5-arabinofuranose, and component d – 3,5-arabinofuranose. The fractional abundance of each component is indicated by the values assigned to a, b, c, and d, respectively. The sum of all the values is about 1 (allowing for a small amount of error in the measurements). A value of zero (0) indicates the component is never present in the polymer. A value of one (1) indicates the component accounts for 100% of the polymer. A value of 0.5 indicates that the component accounts for 50% of the polymer. The arrangement of the components within the polymer can vary, as is understood in the art, and is not defined by the order depicted.

[0077] The term "compositional glycan equivalent" refers to a fiber preparation with a substantially similar glycan content as the composition to which it is being compared. A compositional glycan equivalent may be substituted about 1:1 for its comparison composition because the compositional glycan equivalent has a glycan content similar to the composition it is replacing. For instance, if about 30 wt% of pea fiber preparation is to be replaced with a compositional glycan equivalent thereof, one of skill in the art would use about 30 wt% of the pea fiber glycan equivalent. A compositional glycan equivalent may be defined in terms of its monosaccharide content and optionally by an analysis of the glycosidic linkages. Methods for measuring monosaccharide content and analyzing glycosidic linkages are known in the art, and described herein.

[0078] The term "functional glycan equivalent" refers to a fiber preparation with substantially similar function as the composition to which it is being compared. The amount of a functional glycan equivalent needed to achieve a substantially similar function may be about the same as the comparison composition, or may be less. For instance, a compositional glycan equivalent will typically have substantially similar function as its comparison composition on a 1:1 (weight) basis. However, an enriched bioactive fraction of a composition may have substantially similar function as the initial composition, but comprise less material, and therefore, less weight than the initial composition. The present disclosure contemplates these and other functional glycan equivalents, as illustrated in Example 10. Substantially similar function may be measured by any method detailed in the Examples herein, in particular the ability to affect total abundance(s) of microbial community members, relative abundance(s) of microbial community members, expression of microbial genes, abundance of microbial gene products (e.g. proteins), activity of microbial proteins, and/or observed biological function of a microbial community.

[0079] A "food" or a "food composition" is an article to be taken by mouth. The form of the food or food composition can vary, and includes but is not limited to a powder form which may be reconstituted or sprinkled on a different food; a bar; a drink; a gel, a gummy, a candy, or the like; a cookie, a cracker, a cake, or the like; and a dairy product (e.g., yogurt, ice cream or the like). The term also encompasses a pill, capsule, tablet, or liquid. A "microbiota-directed food," as used herein, refers to a food that selectively promotes the representation and/or expressed beneficial functions of targeted human gut microbes.

[0080] The term "microbiota" refers to microorganisms that are found within a specific environment, and the term "microbiome" refers to a collection of genes in the genomes of all the microorganisms found in a particular environment. Accordingly, the term "gut microbiota" refers to microorganisms that are found within a gastrointestinal tract of a subject, and a "gut microbiome" refers to a collection of genomes from all the microorganisms found in the gastrointestinal tract of a subject.

[0081] The "health" of a subject's gut microbiota may be defined by its features, namely its compositional state and/or its functional state. The "compositional state" of a gut microbiota refers to the presence, absence or abundance (relative or absolute) of microbial community members. The community members can be described by different methods of classification typically based on 16S rRNA sequences, including but not limited to operational taxonomic units (OTUs) and amplicon sequence variants (ASVs). The "functional state" of a gut microbiota refers to expression of microbial genes, observed biological functions, and/or phenotypic states of the community. A subject with an unhealthy gut microbiota has a measure of at least one feature of the gut microbiota or microbiome that deviates by 1.5 standard deviation or more (e.g., 2 std. deviation, 2.5 std. deviation, 3 std. deviation, etc.) from that of healthy subjects with similar environmental exposures, such as geography, diet, and age. To "promote a healthy gut microbiota in a subject" means to change the feature of the microbiota or microbiome of the subject with the unhealthy gut microbiota in a manner towards the healthy subjects, and encompasses complete repair (i.e., the measure of gut microbiota health does not deviate by 1.5 standard deviation or more) and levels of repair that are less than complete. Promoting a healthy gut microbiota in a subject also includes preventing the development of an unhealthy gut microbiota in a subject.

[0082] The "fiber degrading capacity" of a subject's gut microbiota may be defined by its compositional state and/or its functional state. For instance, the compositional stage of a subject's gut microbiota may be defined by the absence, presence and abundance of primary and secondary consumers of dietary fiber, while the functional state may be defined by the representation of relevant genomic loci (polysaccharide utilization loci (PULs), carbohydrate-active enzymes (CAZymes), etc.), expression from these loci, and/or activity of proteins encoded by these loci. An increase in the fiber degrading capacity of a subject may be effected by increasing the abundance of microorganisms with genomic loci for import and metabolism of glycans, as exemplified by PULs and/or loci encoding CAZymes; and/or increasing the abundance or expression of one or more proteins encoded by a PUL and/or one or more CAZyme (with or without concomitant changes in microorganism abundance).

[0083] As used herein, "statistically significant" is a p-value <0.05, or a comparable value calculated by other suitable methods.

[0084] The term "substantially similar" generally refers to a range of numerical values, for instance, ± 0.5-1%, ± 1-5% or ± 5-10% of the recited value, that one would consider equivalent to the recited value, for example, having the same function or result.

[0085] The terms "relative abundance" and "fractional abundance" as used herein describe an amount of one or more microorganism. Relative abundance means the percent composition of a microorganism of a particular kind relative to the total number of microorganisms in the area. Fractional abundance is the relative abundance divided by 100. For example, the "relative abundance of *Bacteroides* in a subject's gut microbiota" is the percent of all *Bacteroides* species relative to the total number of bacteria constituting the subject's gut microbiota, as measured in a suitable sample. "Total abundance" refers to the total number of microorganisms. Suitable samples for quantifying gut microbiota include a fecal sample, a cecal sample or other sample of the lumen. A variety of methods are known in the art for quantifying gut microbiota. For example, a fecal sample, a cecal sample or other sample of the lumenal contents of the large intestine may be collected, processed, plated on appropriate growth media, cultured under suitable conditions (i.e., temperature, presence or absence of oxygen and carbon dioxide, agitation, etc.), and colony forming units may be determined. Alternatively, sequencing methods or arrays may be used to determine abundance. The examples detail one method, COPRO-Seq, where relative abundance is defined by the number of sequencing reads that can be unambiguously assigned to the species' genome after adjusting for genome uniqueness. 16S rRNA gene sequencing methods can also be used and are well known in the art.

[0086] These and other aspects of the present disclosure are detailed further below.

I. COMPOSITIONS OF FIBER PREPARATIONS (FIBER BLENDS)

[0087] In one aspect, the present disclosure provides compositions comprising a plurality of fiber preparations. Compositions of this section may also be

referred to herein as "a fiber blend." Each fiber preparation can be independently selected from the group consisting of a barley fiber preparation, a citrus fiber preparation, a citrus pectin preparation, a high molecular weight inulin preparation, a pea fiber preparation, a sugar beet fiber preparation, and glycan equivalents thereof, wherein the plurality of fiber preparations is at least 95 wt%, at least 97 wt%, or at least 99 wt% of the composition. The present disclosure also provides compositions consisting essentially of a plurality of fiber preparations, each fiber preparation independently selected from the group consisting of a barley fiber preparation, a citrus fiber preparation, a citrus pectin preparation, a high molecular weight inulin preparation, a pea fiber preparation, a sugar beet fiber preparation and glycan equivalents thereof, wherein the plurality of fiber preparations is at least 95 wt%, at least 97 wt%, or at least 99 wt%, of the composition, and the remaining weight percent (if any) of the composition is comprised of one or more additional food ingredient that lacks dietary fibers. The amount of the plurality of fiber preparations in a composition may also be expressed as a range, for instance about 95 wt% to about 97 wt%, about 97 wt% to about 100 wt%, or about 98 wt% to about 100 wt%, etc.; or as individual values, for instance, 95 wt%, 96 wt%, 97 wt%, 98 wt%, 99 wt%, or 100 wt%. The glycan equivalent may be a functional glycan equivalent or a compositional glycan equivalent. The plurality of fiber preparations may be 2, 3, 4, 5, 6, 7, 8, 9, 10 or more different fiber preparations selected from the group consisting of a barley fiber preparation or a glycan equivalent thereof, a citrus fiber preparation or a glycan equivalent thereof, citrus pectin or a glycan equivalent thereof, a high molecular weight inulin preparation or a glycan equivalent thereof, a pea fiber preparation or a glycan equivalent thereof, and a sugar beet fiber preparation or a glycan equivalent thereof. In some embodiments, a composition may contain 2 or more different barley fiber preparations, 2 or more different citrus fiber preparations, etc. Various embodiments are described in further detail below.

[0088] In another aspect, the present disclosure provides compositions comprising at least 15 wt% of one or more pea fiber preparation or a glycan equivalent thereof; and at least one additional fiber preparation chosen from (i) at least 28 wt% of

one or more high molecular weight inulin preparation or a glycan equivalent thereof, (ii) between 0 wt% and 10 wt% (inclusive) of one or more citrus pectin preparation or a glycan equivalent thereof, (iii) between 0 wt% and 25 wt% (inclusive) of one or more citrus fiber preparation or a glycan equivalent thereof, or (iv) between 0 wt% and 45 wt% (inclusive) of one or more barley fiber preparation or a glycan equivalent thereof. The composition may contain 2, 3, 4, 5, 6, 7, 8, 9, 10 or more different fiber preparations. Various embodiments are described in further detail below.

[0089] In some embodiments, a composition comprises (a) at least 15 wt% of one or more pea fiber preparation and/or at least 15 wt% of one or more sugar beet fiber preparation, and (b) at least 28 wt% of one or more high molecular weight inulin preparation, an amount of one or more citrus pectin preparation that does not exceed 10 wt%, an amount of one or more citrus fiber preparation that does not exceed 25 wt%, an amount of one or more barley fiber preparation that does not exceed 45 wt%, and no sugar beet fiber preparations. In another embodiment, a composition consists essentially of (a) at least 15 wt% of one or more pea fiber preparation and/or at least 15 wt% of one or more sugar beet fiber preparation, and (b) at least 28 wt% of one or more high molecular weight inulin preparation, an amount of one or more citrus pectin preparation that does not exceed 10 wt%, an amount of one or more citrus fiber preparation that does not exceed 25 wt%, an amount of one or more barley fiber preparation that does not exceed 45 wt%, and no sugar beet fiber preparations. In some embodiments, the citrus pectin preparation(s) is less than 1 wt%, or citrus pectin is absent from the composition. In further embodiments, the one or more citrus fiber is in an amount that does not exceed 15 wt%, or in an amount that does not exceed 12 wt%. In still further embodiments, the one or more barley fiber preparation is in an amount that does not exceed 30 wt%, or in an amount that does not exceed 20 wt%. In still further embodiments, there is either one or more pea fiber preparation or one or more sugar beet fiber preparation.

[0090] In some embodiments, a composition comprises (a) at least 28 wt% of one or more pea fiber preparation and/or at least 15 wt% of one or more sugar beet fiber preparation, and (b) at least 28 wt% of one or more high molecular weight inulin

preparation, an amount of one or more citrus pectin preparation that does not exceed 10 wt%, an amount of one or more citrus fiber that does not exceed 25 wt%, an amount of one or more barley fiber preparation that does not exceed 45 wt%, and no sugar beet fiber preparation. In another embodiment, a composition consists essentially of (a) at least 28 wt% of one or more pea fiber preparation and/or at least 15 wt% of one or more sugar beet fiber preparation, and (b) at least 28 wt% of one or more high molecular weight inulin preparation, an amount of one or more citrus pectin preparation that does not exceed 10 wt%, an amount of one or more citrus fiber preparation that does not exceed 25 wt%, an amount of one or more barley fiber preparation that does not exceed 45 wt%, and no sugar beet fiber preparation. In some embodiments, the citrus pectin preparation(s) is less than 1 wt%, or citrus pectin is absent from the composition. In further embodiments, the one or more citrus fiber preparation is in an amount that does not exceed 15 wt%, or in an amount that does not exceed 12 wt%. In still further embodiments, the one or more barley fiber preparation is in an amount that does not exceed 30 wt%, or in an amount that does not exceed 20 wt%. In still further embodiments, there is either one or more pea fiber preparation or one or more sugar beet fiber preparation.

[0091] In some embodiments, a composition comprises (a) at least 30 wt% of one or more pea fiber preparation and/or at least 15 wt% of one or more sugar beet fiber preparation, and (b) at least 30 wt% of one or more high molecular weight inulin preparation, an amount of one or more citrus pectin preparation that does not exceed 10 wt%, an amount of one or more citrus fiber preparation that does not exceed 25 wt%, an amount of one or more barley fiber preparation that does not exceed 45 wt%, and no sugar beet fiber preparation. In another embodiment, a composition consists essentially of (a) at least 15 wt% of one or more pea fiber preparation and/or at least 15 wt% of one or more sugar beet fiber preparation, and (b) at least 28 wt% of one or more high molecular weight inulin preparation, an amount of one or more citrus pectin preparation that does not exceed 10 wt%, an amount of one or more citrus fiber preparation that does not exceed 25 wt%, an amount of one or more barley fiber preparation that does not exceed 45 wt%, and is no sugar beet fiber preparation(s). In some embodiments,

the citrus pectin preparation(s) is less than 1 wt%, or citrus pectin is absent from the composition. In further embodiments, the one or more citrus fiber preparation is in an amount that does not exceed 15 wt%, or in an amount that does not exceed 12 wt%. In still further embodiments, the one or more barley fiber preparation is in an amount that does not exceed 30 wt%, or in an amount that does not exceed 20 wt%. In still further embodiments, there is either one or more pea fiber preparation or one or more sugar beet fiber preparation.

[0092] In some embodiments, a composition comprises (a) at least 35 wt% of one or more pea fiber preparation and/or at least 15 wt% of one or more sugar beet fiber preparation, and (b) at least 35 wt% of one or more high molecular weight inulin preparation, an amount of one or more citrus pectin preparation that does not exceed 10 wt%, an amount of one or more citrus fiber preparation that does not exceed 25 wt%, an amount of one or more barley fiber preparation that does not exceed 45 wt%, and no sugar beet fiber preparations. In another embodiment, a composition consists essentially of (a) at least 15 wt% of one or more pea fiber and/or at least 15 wt% of one or more sugar beet fiber preparation, and (b) at least 28 wt% of one or more high molecular weight inulin preparation, an amount of one or more citrus pectin preparation that does not exceed 10 wt%, an amount of one or more citrus fiber preparation that does not exceed 25 wt%, an amount of one or more barley fiber preparation that does not exceed 45 wt%, and no sugar beet fiber preparations. In some embodiments, the citrus pectin preparation(s) is less than 1 wt%, or citrus pectin is absent from the composition. In further embodiments, the one or more citrus fiber preparation is in an amount that does not exceed 15 wt%, or in an amount that does not exceed 12 wt%. In still further embodiments, the one or more barley fiber preparation is in an amount that does not exceed 30 wt%, or in an amount that does not exceed 20 wt%. In still further embodiments, there is either one or more pea fiber preparation or one or more sugar beet fiber preparation.

[0093] In some embodiments, a composition comprises (a) at least 15 wt% of one or more pea fiber preparation, at least 15 wt% of one or more sugar beet fiber preparation, or a glycan equivalent thereof, and (b) at least one additional fiber

preparation chosen from: at least 28 wt% of one or more high molecular weight inulin preparation or a glycan equivalent thereof, 10 wt% of less of one or more citrus pectin preparation or a glycan equivalent thereof, 25 wt% or less of one or more citrus fiber preparation or a glycan equivalent thereof, and 45 wt% or less of one or more barley fiber preparation or a glycan equivalent thereof. In another embodiment, a composition consists essentially of (a) at least 15 wt% of one or more pea fiber preparation, at least 15 wt% of one or more sugar beet fiber preparation, or a glycan equivalent thereof, and (b) at least one additional fiber preparation chosen from: at least 28 wt% of one or more high molecular weight inulin preparation or a glycan equivalent thereof, 10 wt% of less of one or more citrus pectin preparation or a glycan equivalent thereof, 25 wt% or less of one or more citrus fiber preparation or a glycan equivalent thereof, and 45 wt% or less of one or more barley fiber preparation or a glycan equivalent thereof. In some embodiments, the amount of one or more citrus pectin or a glycan equivalent thereof is less than 1 wt%, or citrus pectin or a glycan equivalent thereof is absent from the composition. In further embodiments, the one or more citrus fiber preparation or a glycan equivalent thereof is in an amount that does not exceed 15 wt%, or in an amount that does not exceed 12 wt%. In still further embodiments, the one or more barley fiber preparation or a glycan equivalent thereof is in an amount that does not exceed 30 wt%, or in an amount that does not exceed 20 wt%. In still further embodiments, there is either (i) one or more pea fiber preparation or a glycan equivalent thereof, or (ii) one or more sugar beet fiber preparation or a glycan equivalent thereof. The glycan equivalent can be a functional glycan equivalent or a compositional glycan equivalent.

[0094] In some embodiments, a composition comprises (a) at least 28 wt% of one or more pea fiber preparation, at least 28 wt% of one or more sugar beet fiber preparation, or a glycan equivalent thereof, and (b) at least one additional fiber preparation chosen from: at least 28 wt% of one or more high molecular weight inulin preparation or a glycan equivalent thereof, 10 wt% of less of one or more citrus pectin preparation or a glycan equivalent thereof, 25 wt% or less of one or more citrus fiber preparation or a glycan equivalent thereof, and 45 wt% or less of one or more barley fiber preparation or a glycan equivalent thereof. In another embodiment, a composition

consists essentially of (a) at least 28 wt% of one or more pea fiber preparation, at least 28 wt% of one or more sugar beet fiber preparation, or a glycan equivalent thereof, and (b) at least one additional fiber preparation chosen from: at least 28 wt% of one or more high molecular weight inulin preparation or a glycan equivalent thereof, 10 wt% of less of one or more citrus pectin preparation or a glycan equivalent thereof, 25 wt% or less of one or more citrus fiber preparation or a glycan equivalent thereof, and 45 wt% or less of one or more barley fiber preparation or a glycan equivalent thereof. In some embodiments, the amount of one or more citrus pectin preparation or a glycan equivalent thereof is less than 1 wt%, or citrus pectin or a glycan equivalent thereof is absent from the composition. In further embodiments, the one or more citrus fiber preparation or a glycan equivalent thereof is in an amount that does not exceed 15 wt%, or in an amount that does not exceed 12 wt%. In still further embodiments, the one or more barley fiber preparation or a glycan equivalent thereof is in an amount that does not exceed 30 wt%, or in an amount that does not exceed 20 wt%. In still further embodiments, there is either (i) one or more pea fiber preparation or a glycan equivalent thereof, or (ii) one or more sugar beet fiber preparation or a glycan equivalent thereof. The glycan equivalent can be a functional glycan equivalent or a compositional glycan equivalent.

[0095] In some embodiments, a composition comprises (a) at least 30 wt% of one or more pea fiber preparation, at least 30 wt% of one or more sugar beet fiber preparation, or a glycan equivalent thereof, and (b) at least one additional fiber preparation chosen from: at least 30 wt% of one or more high molecular weight inulin preparation or a glycan equivalent thereof, 10 wt% of less of one or more citrus pectin preparation or a glycan equivalent thereof, 25 wt% or less of one or more citrus fiber preparation or a glycan equivalent thereof, and 45 wt% or less of one or more barley fiber preparation or a glycan equivalent thereof. In another embodiment, a composition consists essentially of (a) at least 30 wt% of one or more pea fiber preparation, at least 30 wt% of one or more sugar beet fiber preparation, or a glycan equivalent thereof, and (b) at least one additional fiber preparation chosen from: at least 30 wt% of one or more high molecular weight inulin preparation or a glycan equivalent thereof, 10 wt% of less

of one or more citrus pectin preparation or a glycan equivalent thereof, 25 wt% or less of one or more citrus fiber preparation or a glycan equivalent thereof, and 45 wt% or less of one or more barley fiber preparation or a glycan equivalent thereof. In some embodiments, the citrus pectin preparation or a glycan equivalent thereof is less than 1 wt%, or citrus pectin preparation or a glycan equivalent thereof is absent from the composition. In further embodiments, the one or more citrus fiber preparation or a glycan equivalent thereof is in an amount that does not exceed 15 wt%, or in an amount that does not exceed 12 wt%. In still further embodiments, the one or more barley fiber preparation or a glycan equivalent thereof is in an amount that does not exceed 30 wt%, or in an amount that does not exceed 20 wt%. In still further embodiments, there is either (i) one or more pea fiber preparation or a glycan equivalent thereof, or (ii) one or more sugar beet fiber preparation or a glycan equivalent thereof. The glycan equivalent can be a functional glycan equivalent or a compositional glycan equivalent.

[0096] In some embodiments, a composition comprises about 30 wt% to about 40 wt% of one or more pea fiber preparation or a glycan equivalent thereof, about 30 wt% to about 40 wt% of one or more high molecular weight inulin preparation or a glycan equivalent thereof, about 9 wt% to about 11 wt% of one or more citrus fiber preparation or a glycan equivalent thereof, and about 18 wt% to about 22 wt% of one or more barley fiber preparation or a glycan equivalent thereof. In another embodiment, a composition consists essentially of about 30 wt% to about 40 wt% of one or more pea fiber preparation or a glycan equivalent thereof, about 30 wt% to about 40 wt% of one or more high molecular weight inulin preparation or a glycan equivalent thereof, about 9 wt% to about 11 wt% of one or more citrus fiber preparation or a glycan equivalent thereof, and about 18 wt% to about 22 wt% of one or more barley fiber preparation or a glycan equivalent thereof. The glycan equivalent can be a functional glycan equivalent or a compositional glycan equivalent.

[0097] In some embodiments, a composition comprises about 30 wt% to about 35 wt% of one or more pea fiber preparation or a glycan equivalent thereof, about 35 wt% to about 40 wt% of one or more high molecular weight inulin preparation or a glycan equivalent thereof, about 9 wt% to about 11 wt% of one or more citrus fiber

preparation or a glycan equivalent thereof, and about 18 wt% to about 22 wt% of one or more barley fiber preparation or a glycan equivalent thereof. In another embodiment, a composition consists essentially of about 30 wt% to about 35 wt% of one or more pea fiber preparation or a glycan equivalent thereof, about 35 wt% to about 40 wt% of one or more high molecular weight inulin preparation or a glycan equivalent thereof, about 9 wt% to about 11 wt% of one or more citrus fiber preparation or a glycan equivalent thereof, and about 18 wt% to about 22 wt% of one or more barley fiber preparation or a glycan equivalent thereof. The glycan equivalent can be a functional glycan equivalent or a compositional glycan equivalent.

[0098] In some embodiments, a composition comprises about 35 wt% of one or more pea fiber preparation or a glycan equivalent thereof, about 35 wt% of one or more high molecular weight inulin preparation or a glycan equivalent thereof, about 10 wt% of one or more citrus fiber preparation or a glycan equivalent thereof, and about 20 wt% of one or more barley fiber preparation or a glycan equivalent thereof. In another embodiment, a composition consists essentially of about 35 wt% of one or more pea fiber preparation or a glycan equivalent thereof, about 35 wt% of one or more high molecular weight inulin preparation or a glycan equivalent thereof, about 10 wt% of one or more citrus fiber preparation or a glycan equivalent thereof, and about 20 wt% of one or more barley fiber preparation or a glycan equivalent thereof. The glycan equivalent can be a functional glycan equivalent or a compositional glycan equivalent.

[0099] In some embodiments, a composition comprises about 33 wt% of one or more pea fiber preparation or a glycan equivalent thereof, about 36 wt% of one or more high molecular weight inulin preparation or a glycan equivalent thereof, about 11 wt% of one or more citrus fiber preparation or a glycan equivalent thereof, and about 20 wt% of one or more barley fiber preparation or a glycan equivalent thereof. In another embodiment, a composition consists essentially of about 33 wt% of one or more pea fiber preparation or a glycan equivalent thereof, about 36 wt% of one or more high molecular weight inulin preparation or a glycan equivalent thereof, about 11 wt% of one or more citrus fiber preparation or a glycan equivalent thereof, and about 20 wt% of one

or more barley fiber preparation or a glycan equivalent thereof. The glycan equivalent can be a functional glycan equivalent or a compositional glycan equivalent.

[0100] In some embodiments, a composition comprises or consists essentially of about 60 wt% to about 70 wt% of one or more pea fiber preparation or a glycan equivalent thereof and about 30 wt% to about 40 wt% of one or more high molecular weight inulin preparation or a glycan equivalent thereof. The glycan equivalent can be a functional glycan equivalent or a compositional glycan equivalent.

[0101] In some embodiments, a composition comprises or consists essentially of about 65 wt% of one or more pea fiber preparation or a glycan equivalent thereof, about 35 wt% of one or more high molecular weight inulin preparation or a glycan equivalent thereof. The glycan equivalent can be a functional glycan equivalent or a compositional glycan equivalent.

[0102] Fiber preparations may be prepared from plant material by methods known in the art. Plant-derived fiber preparations that are economical for use in human foods typically are mixtures of diverse molecular composition comprising not only dietary fiber but also protein, fat, carbohydrate, etc. A skilled artisan will appreciate that fiber preparations prepared by different manufacturing processes may have different compositions, and a proximate analysis may be used to evaluate the suitability of a fiber preparation. A proximate analysis of a composition (e.g., a fiber preparation, a food item) refers to an analysis of the composition's moisture, protein, fat, ash, and carbohydrate content, which are expressed as the content (wt%) in the composition, respectively. Protein, fat, ash, and moisture content can be measured by methods established by Association of Official Analytical Chemists (AOAC) 2009.01, AOAC 920.123, AOAC 933.05, AOAC 935.42, and AOAC 926.08, respectively, and carbohydrate can be defined as (100 - (Protein + Fat + Ash + Moisture). Analysis of the dietary fiber, which is measured separately, may provide further information by which to evaluate the suitability of a preparation. For instance, soluble and insoluble dietary fiber, and high molecular weight and low molecular weight dietary fiber, can be measured by AOAC method 2011.25. Further details are provided in the Examples. Suitable fiber preparations will be substantially similar to those disclosed herein. As demonstrated

herein, a fiber preparation contains active and inactive fractions with different structural features and biophysical availability, from the perspective of the gut microbiota. Accordingly, preferred fiber preparations may also have substantially similar monosaccharide content and/or glycosidic linkages. Methods for measuring monosaccharide content and performing a glycosidic linkage analysis are known in the art, and described herein.

(a) barley fiber preparations

[0103] Barley fiber preparations may be prepared according to methods known in the art, and evaluated as described herein. Commercial sources may also be used.

[0104] In some embodiments, a composition comprises one or more barley fiber preparation in an amount that does not exceed 45 wt% of the composition. The amount may also be expressed as individual values or a range. For instance, the barley fiber preparation(s) in these embodiments may be about 1 wt%, 2 wt%, 3 wt%, 4 wt%, 5 wt%, 6 wt%, 7 wt%, 8 wt%, 9 wt%, 10 wt%, 11 wt%, 12 wt%, 13 wt%, 14 wt%, 15 wt%, 16 wt%, 17 wt%, 18 wt%, 19 wt%, 20 wt%, 21 wt%, 22 wt%, 23 wt%, 24 wt%, 25 wt%, 26 wt%, 27 wt%, 28 wt%, 29 wt%, 30 wt%, 31 wt%, 32 wt%, 33 wt%, 34 wt%, 35 wt%, 36 wt%, 37 wt%, 38 wt%, 39 wt%, 40 wt%, 41 wt%, 42 wt%, 43 wt%, 44 wt%, or 45 wt%. In some examples, the barley fiber preparation(s) may be about 1 wt% to about 45 wt%, about 10 wt% to about 45 wt%, or about 20 wt% to about 45 wt% of the composition. In some examples, the barley fiber preparation(s) may be about 1 wt% to about 25 wt% or about 10 wt% to about 25 wt% of the composition, or about 1 wt% to about 20 wt% or about 10 wt% to about 20 wt% of the composition.

[0105] In an exemplary embodiment of a suitable barley fiber preparation, the total dietary fiber is comprised of about 5 wt% to about 15 wt%, or about 10 wt% to about 15% of insoluble dietary fiber and/or about 40 wt% to about 50 wt%, or about 42 wt% to about 47 wt% of high molecular weight dietary fiber. In some embodiments, the total dietary fiber is about 35 wt% to about 55 wt%, about 40 wt% to about 55 wt%, or about 45 wt% to about 55 wt% of the preparation. In other embodiments, the total dietary fiber is about 35 wt% to about 50 wt% or about 30 wt% to about 45 wt% of the

preparation. In still further embodiments, the barley fiber preparation comprises about 15 wt% to about 20 wt% protein, about 2 wt% to about 5 wt% fat, about 65 wt% to about 75 wt% carbohydrate, about 2 wt% to about 7 wt% moisture, and about 1 wt% to about 3 wt% ash.

[0106] In another exemplary embodiment of a suitable barley fiber preparation, the total dietary fiber is comprised of about 5 wt% to about 15 wt%, or about 10 wt% to about 15% of insoluble dietary fiber and about 40 wt% to about 50 wt%, or about 42 wt% to about 47 wt% of high molecular weight dietary fiber; the total dietary fiber is about 35 wt% to about 55 wt%, about 40 wt% to about 55 wt%, or about 45 wt% to about 55 wt% of the preparation; and the barley fiber preparation comprises about 15 wt% to about 20 wt% protein, about 2 wt% to about 5 wt% fat, about 65 wt% to about 75 wt% carbohydrate, about 2 wt% to about 7 wt% moisture, and about 1 wt% to about 3 wt% ash.

[0107] In another exemplary embodiment, a suitable barley fiber preparation is substantially similar to the preparation described in **Table A**.

[0108] In each of the above embodiments, a suitable barley fiber preparation may also have a monosaccharide content substantially similar to the preparation described in **Table B**, glycosidic linkages substantially similar to the preparation exemplified in **Table E**, or both.

[0109] In another exemplary embodiment, a suitable barley fiber preparation has a monosaccharide content substantially similar to the preparation exemplified in **Table B** and glycosyl linkages that are substantially similar to the preparation exemplified in **Table E**.

[0110] In another exemplary embodiment, a suitable barley fiber preparation is substantially similar to the preparation described in **Table G.**

(b) citrus fiber preparations

[0111] Citrus fiber preparations may be prepared according to methods known in the art from citrus fruits including, but not limited to, clementine, citron, grapefruit, kumquat, lemon, lime, orange, tangelo, tangerine, and yuzu, and evaluated as described herein. Commercial sources may also be used.

[0112] In some embodiments, a composition comprises one or more citrus fiber preparation in an amount that does not exceed 25 wt% of the composition. The amount may also be expressed as individual values or a range. For instance, the citrus fiber preparation(s) in these embodiments may be about 1 wt%, 2 wt%, 3 wt%, 4 wt%, 5 wt%, 6 wt%, 7 wt%, 8 wt%, 9 wt%, 10 wt%, 11 wt%, 12 wt%, 13 wt%, 14 wt%, 15 wt%, 16 wt%, 17 wt%, 18 wt%, 19 wt%, 20 wt%, 21 wt%, 22 wt%, 23 wt%, 24 wt%, or 25 wt%. In some examples, the citrus fiber preparation(s) may be about 1 wt% to about 25 wt%, about 1 wt% to about 20 wt%, or about 1 wt% to about 15 wt% of the composition. In some examples, the citrus fiber preparation(s) may be about 5 wt% to about 25 wt%, about 5 wt% to about 20 wt%, or about 5 wt% to about 15 wt% of the composition. In some examples, the citrus fiber preparation(s) may be about 10 wt% to about 25 wt%, about 10 wt% to about 20 wt%, or about 10 wt% to about 15 wt% of the composition.

[0113] In an exemplary embodiment of a suitable citrus fiber preparation, the total dietary fiber is comprised of about 30 wt% to about 40 wt%, or about 30 wt% to about 35% of insoluble dietary fiber and/or about 65 wt% to about 75 wt%, or about 65 wt% to about 70 wt% of high molecular weight dietary fiber. In some embodiments, the total dietary fiber is about 60 wt% to about 80 wt%, about 60 wt% to about 75 wt%, or about 60 wt% to about 70 wt% of the preparation. In other embodiments, the total dietary fiber is about 65 wt% to about 80 wt%, about 65 wt% to about 75 wt%, or about 65 wt% to about 70 wt% of the preparation. In still further embodiments, the citrus fiber preparation comprises about 5 wt% to about 10 wt% protein, about 1 wt% to about 3 wt% fat, about 75 wt% to about 85 wt% carbohydrate, about 5 wt% to about 10 wt% moisture, and about 1 wt% to about 4 wt% ash.

[0114] In another exemplary embodiment of a suitable citrus fiber preparation, the total dietary fiber is comprised of about 30 wt% to about 40 wt%, or about 30 wt% to about 35% of insoluble dietary fiber and/or about 65 wt% to about 75 wt%, or about 65 wt% to about 70 wt% of high molecular weight dietary fiber; the total dietary fiber is about 65 wt% to about 80 wt%, about 65 wt% to about 75 wt%, or about 65 wt% to about 70 wt% of the preparation; and the citrus fiber preparation comprises about 5 wt% to about 10 wt% protein, about 1 wt% to about 3 wt% fat, about 75 wt% to

about 85 wt% carbohydrate, about 5 wt% to about 10 wt% moisture, and about 1 wt% to about 4 wt% ash.

[0115] In another exemplary embodiment, a suitable citrus fiber preparation is substantially similar to the preparation described in **Table A**.

[0116] In each of the above embodiments, a suitable citrus fiber preparation may also have monosaccharide content substantially similar to a preparation described in **Table B**, glycosidic linkages substantially similar to a preparation exemplified in **Table F1** or **F2**, or both.

[0117] In another exemplary embodiment, a suitable citrus fiber preparation has a monosaccharide content is substantially similar to a preparation exemplified in **Table B** and glycosyl linkages that are substantially similar to a preparation exemplified in **Table F1** or **F2**.

[0118] In another exemplary embodiment, a suitable citrus fiber preparation is substantially similar to the preparation described in **Table G**

(c) citrus pectin preparations

[0119] Citrus pectin preparations may be prepared according to methods known in the art from citrus fruits including, but not limited to, clementine, citron, grapefruit, kumquat, lemon, lime, orange, tangelo, tangerine, and yuzu, and evaluated as described herein. Commercial sources may also be used.

[0120] In some embodiments, a composition comprises one or more citrus pectin preparation in an amount that does not exceed 10 wt% of the composition. The amount may also be expressed as individual values or a range. For instance, the amount of citrus pectin in these embodiments may be about 1 wt%, 2 wt%, 3 wt%, 4 wt%, 5 wt%, 6 wt%, 7 wt%, 8 wt%, 9 wt%, or 10 wt%. In some examples, the citrus pectin preparation(s) may be about 1 wt% to about 10 wt%, about 1 wt% to about 8 wt%, or about 1 wt% to about 6 wt% of the composition. In some examples, the citrus pectin preparation(s) may be about 1 wt% to about 4 wt%, or about 1 wt% to about 2 wt% of the composition.

[0121] In an exemplary embodiment of a suitable citrus pectin preparation, the total dietary fiber is comprised of about 1 wt% to about 10 wt%, or about 1 wt% to

about 5% of insoluble dietary fiber and/or about 85 wt% to about 95 wt%, or about 90 wt% to about 95 wt% of high molecular weight dietary fiber. In some embodiments, the total dietary fiber is about 75 wt% to about 95 wt%, about 80 wt% to about 95 wt%, or about 85 wt% to about 95 wt% of the preparation. In other embodiments, the total dietary fiber is about 85 wt% to about 90 wt% or about 90 wt% to about 95 wt% of the preparation. In still further embodiments, the citrus pectin preparation comprises about 2 wt% or less of protein, about 1 wt% to about 2 wt% fat, about 85 wt% to about 95 wt% carbohydrate, about 1 wt% to about 6 wt% moisture, and about 3 wt% to about 6 wt% ash.

[0122] In another exemplary embodiment of a suitable citrus pectin preparation, the total dietary fiber is comprised of about 1 wt% to about 10 wt%, or about 1 wt% to about 5% of insoluble dietary fiber and about 85 wt% to about 95 wt%, or about 90 wt% to about 95 wt% of high molecular weight dietary fiber; the total dietary fiber is about 85 wt% to about 95 wt%, about 85 wt% to about 90 wt%, or about 90 wt% to about 95 wt% of the preparation; and the citrus pectin preparation comprises about 2 wt% or less of protein, about 1 wt% to about 2 wt% fat, about 85 wt% to about 95 wt% carbohydrate, about 1 wt% to about 6 wt% moisture, and about 3 wt% to about 6 wt% ash.

[0123] In another exemplary embodiment, a suitable citrus pectin preparation is substantially similar to the preparation described in **Table A**.

[0124] In each of the above embodiments, a suitable citrus pectin preparation may also have a monosaccharide content substantially similar to the preparation exemplified in **Table B**, glycosyl linkages substantially similar to the preparation exemplified in **Table D**, or both.

[0125] In another exemplary embodiment, a suitable citrus pectin preparation has a monosaccharide content substantially similar to the preparation exemplified in **Table B** and glycosyl linkages that are substantially similar to the preparation exemplified in **Table D**.

(d) high molecular weight inulin preparations

[0126] High molecular weight inulin preparations may be prepared according to methods known in the art, and evaluated as described herein. Commercial sources may also be used. Inulin is defined by AOAC method 999.03. High molecular weight inulin is comprised of fructose units linked together by ß-(2,1)-linkages, which are typically terminated by a glucose unit.

[0127] In some embodiments, a composition comprises one or more high molecular weight inulin preparation in an amount that is at least 28 wt% of the composition. The amount may also be expressed as individual values or a range. For instance, the high molecular weight inulin preparation(s) in these embodiments may be about 29 wt%, 30 wt%, 31 wt%, 32 wt%, 33 wt%, 34 wt%, 35 wt%, 36 wt%, 37 wt%, 38 wt%, 39 wt%, 40 wt%, 41 wt%, 42 wt%, 43 wt%, 44 wt%, 45 wt%, 46 wt%, 47 wt%, 48 wt%, 49 wt%, 50 wt%, or more. In some examples, the high molecular weight inulin preparation(s) may be about 30 wt% to about 50 wt%, about 30 wt% to about 45 wt%, or about 30 wt% to about 40 wt% of the composition. In some examples, the high molecular weight inulin preparation(s) may be about 35 wt% to about 50 wt%, about 35 wt% to about 45 wt%, or about 35 wt% to about 40 wt% of the composition. Inulin is defined by AOAC method 999.03.

[0128] In an exemplary embodiment of a suitable high molecular weight inulin preparation, the total dietary fiber is comprised of about 0.5 wt% or less of insoluble dietary fiber and/or about 55 wt% to about 65 wt%, or about 57 wt% to about 62 wt% of high molecular weight dietary fiber. In some embodiments, the total dietary fiber is about 75 wt% to about 95 wt%, about 80 wt% to about 95 wt%, or about 85 wt% to about 95 wt% of the preparation. In other embodiments, the total dietary fiber is about 85 wt% to about 99 wt%, 90 wt% to about 99 wt%, or about 95 wt% to about 99 wt% of the preparation. In still further embodiments, the high molecular weight inulin preparation comprises no more than 1 wt% of protein, about 2 wt% to about 5 wt% fat, about 85 wt% to about 95 wt% carbohydrate, about 2 wt% to about 7 wt% moisture, and no more than 2 wt% ash.

[0129] In an exemplary embodiment of a suitable high molecular weight inulin preparation, the total dietary fiber is comprised of about 0.5 wt% insoluble dietary fiber and about 55 wt% to about 65 wt%, or about 57 wt% to about 62 wt% of high molecular weight dietary fiber; the total dietary fiber is about 85 wt% to about 99 wt%, 90 wt% to about 99 wt%, or about 95 wt% to about 99 wt% of the preparation; and the high molecular weight inulin preparation comprises no more than 1 wt% of protein, about 2 wt% to about 5 wt% fat, about 85 wt% to about 95 wt% carbohydrate, about 2 wt% to about 7 wt% moisture, and no more than 2 wt% ash.

[0130] In another exemplary embodiment, a suitable high molecular weight inulin preparation is substantially similar to the preparation described in **Table A**.

[0131] In another exemplary embodiment, a suitable high molecular weight inulin preparation is substantially similar to the preparation described in **Table G.**

[0132] In each of the above embodiments, about 99% of the inulin in a suitable high molecular weight inulin preparation may have a degree of polymerization (DP) that is greater than or equal to 5. In some example, the DP for the inulin in a suitable preparation may range from 5 to 60. Alternatively or in addition, the average DP may be less than or equal to 23.

(e) pea fiber preparations

[0133] Pea fiber preparations may be prepared according to methods known in the art, and evaluated as described herein. Commercial sources may also be used.

[0134] In some embodiments, a composition comprises one or more pea fiber preparation in an amount that is at least 15 wt% of the composition. The amount may also be expressed as individual values or a range. For instance, the pea fiber preparation(s) in these embodiments may be about 15 wt%, 16 wt%, 17 wt%, 18 wt%, 19 wt%, 20 wt%, 21 wt%, 22 wt%, 23 wt%, 24 wt%, 25 wt%, 26 wt%, 27 wt%, 28 wt%, 29 wt%, 30 wt%, 31 wt%, 32 wt%, 33 wt%, 34 wt%, 35 wt%, 36 wt%, 37 wt%, 38 wt%, 39 wt%, 40 wt%, 41 wt%, 42 wt%, 43 wt%, 44 wt%, 45 wt%, 46 wt%, 47 wt%, 48 wt%, 49 wt%, 50 wt%, 51 wt%, 52 wt%, 53 wt%, 54 wt%, 55 wt%, 56 wt%, 57 wt%, 58 wt%, 59 wt%, 60 wt%, 61 wt%, 62 wt%, 63 wt%, 64 wt%, 65 wt%, or more. In some

examples, the pea fiber preparation(s) may be about 15 wt% to about 75 wt%, about 25 wt% to about 75 wt%, or about 35 wt% to about 75 wt% of the composition. In some examples, the pea fiber preparation(s) may be about 15 wt% to about 65 wt%, about 25 wt% to about 65 wt%, or about 35 wt% to about 65 wt% of the composition. In some examples, the pea fiber preparation(s) may be about 30 wt% to about 85 wt%, about 40 wt% to about 85 wt%, or about 50 wt% to about 85 wt% of the composition.

[0135] In an exemplary embodiment of a suitable pea fiber preparation, the total dietary fiber is comprised of about 55 wt% to about 65 wt%, or about 60 wt% to about 65% of insoluble dietary fiber and/or about 60 wt% to about 70 wt%, or about 65 wt% to about 70 wt% of high molecular weight dietary fiber. In some embodiments, the total dietary fiber is about 60 wt% to about 80 wt%, about 60 wt% to about 75 wt%, or about 60 wt% to about 70 wt% of the preparation. In other embodiments, the total dietary fiber is about 65 wt% to about 80 wt%, about 65 wt% to about 75 wt%, or about 65 wt% to about 70 wt% of the preparation. In still further embodiments, the pea fiber preparation comprises about 7 wt% to about 12 wt% protein, no more than 2 wt% fat, about 75 wt% to about 85 wt% carbohydrate, about 5 wt% to about 10 wt% moisture, and about 1 wt% to about 4 wt% ash.

[0136] In an exemplary embodiment of a suitable pea fiber preparation, the total dietary fiber is comprised of about 55 wt% to about 65 wt%, or about 60 wt% to about 65% of insoluble dietary fiber and about 60 wt% to about 70 wt%, or about 65 wt% to about 70 wt% of high molecular weight dietary fiber; the total dietary fiber is about 65 wt% to about 80 wt%, about 65 wt% to about 75 wt%, or about 65 wt% to about 70 wt% of the preparation; and the pea fiber preparation comprises about 7 wt% to about 12 wt% protein, no more than 2 wt% fat, about 75 wt% to about 85 wt% carbohydrate, about 5 wt% to about 10 wt% moisture, and about 1 wt% to about 4 wt% ash.

[0137] In another exemplary embodiment, a suitable pea fiber preparation is substantially similar to the preparation described in **Table A**.

[0138] In each of the above embodiments, a suitable pea fiber preparation may also have a monosaccharide content substantially similar to a preparation

exemplified in **Table B**; glycosyl linkages substantially similar to the preparation exemplified in **Table C1**, **Table C2**, **Table 13**, **Table 14**, **Table 16**, or **Table 17**; or both.

[0139] In another exemplary embodiment, a suitable pea fiber preparation has a monosaccharide content substantially similar to a preparation exemplified in **Table B** and glycosyl linkages substantially similar to the preparation exemplified in **Table C1**, **Table C2**, **Table 13**, **Table 14**, **Table 16**, or **Table 17**.

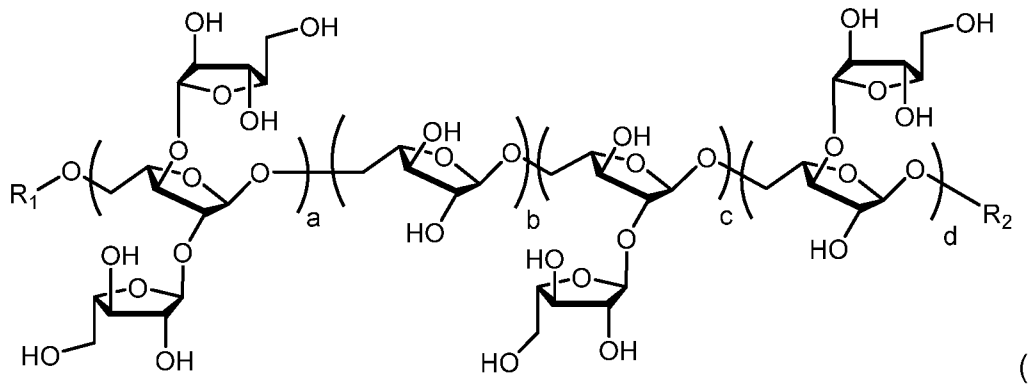
[0140] In another exemplary embodiment a suitable pea fiber preparation has a monosaccharide content that has about 10 wt% to about 90 wt% arabinose, and arabinose linkages that are substantially similar to the preparation exemplified in **Table C1**, **Table C2**, **Table 13**, **Table 14**, **Table 16**, or **Table 17**. In some examples, arabinose may be about 10 wt% to 20 wt%, or about 15 wt% to about 20 wt%. In some examples, arabinose may be about 20 wt% to 30 wt%, about 20 wt% to about 25 wt%, or about 25 wt% to about 30 wt%. In some examples, arabinose may be about 50 wt% to 90 wt%, about 60 wt% to about 90 wt%, or about 70 wt% to about 90 wt%. In some examples, arabinose may be about 50 wt% to 80 wt%, about 60 wt% to about 80 wt%, or about 70 wt% to about 80 wt%.

[0141] In another exemplary embodiment, a suitable pea fiber preparation has a monosaccharide content that has a substantially similar arabinose content as the preparation exemplified in **Table B** and arabinose glycosyl linkages that are substantially similar to the preparation exemplified in **Table C1**, **Table C2**, **Table 13**, **Table 14**, **Table 16**, or **Table 17**.

[0142] In another exemplary embodiment, a suitable pea fiber preparation is substantially similar to the Fiber 8 fraction or the enzymatically destarched Fiber 8 fraction described in Example 10.

[0143] In another exemplary embodiment, a suitable pea fiber preparation is substantially similar to the preparation described in **Table G**.

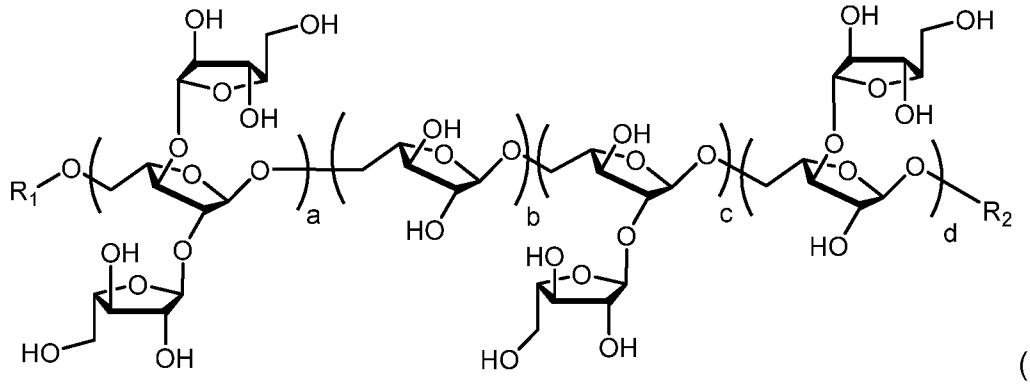
[0144] In all the aforementioned embodiments, a suitable pea fiber preparation may also comprise arabinan of formula (I):



(I)

wherein a is about 0.1 to about 0.3, b is about 0.4 to about 0.6, c is about 0.1 to about 0.4, d is about 0.04 to about 0.06 (calculated from the fractional abundance of arabinose linkages where the arabinose contained a 5-linkage, as determined by partially methylated alditol acetate GC-MS analysis); and wherein R_1 and R_2 are each independently selected from H, a glycosyl, a sugar moiety (modified or not), an oligosaccharide (branched or not), or a polysaccharide (branched or not), and a polysaccharide containing galacturonic acid, galactose, and rhamnose.

[0145] Alternatively, in all the aforementioned embodiments, a suitable pea fiber preparation may also comprise arabinan of formula (I):

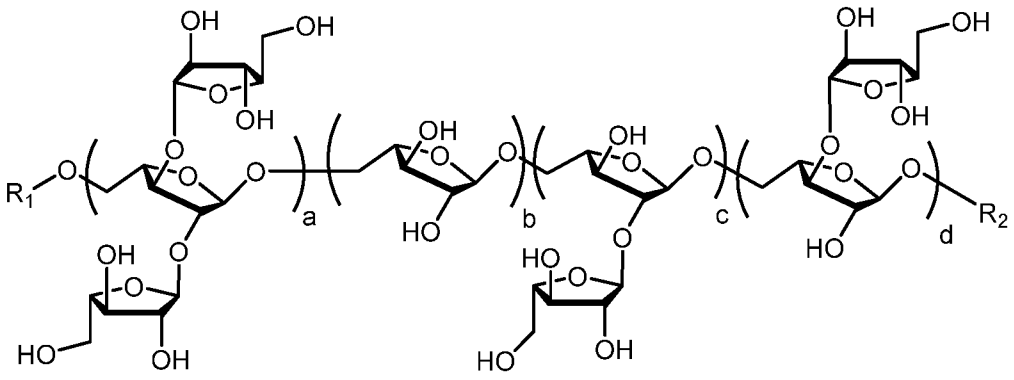


(I)

wherein a is about 0.2 to about 0.3, b is about 0.5 to about 0.6, c is about 0.2 to about 0.4, d is about 0.04 to about 0.06 (calculated from the fractional abundance of arabinose linkages where the arabinose contained a 5-linkage, as determined by partially methylated alditol acetate GC-MS analysis); and wherein R_1 and R_2 are each independently selected from H, a glycosyl, a sugar moiety (modified or not), an

oligosaccharide (branched or not), or a polysaccharide (branched or not), and a polysaccharide containing galacturonic acid, galactose, and rhamnose.

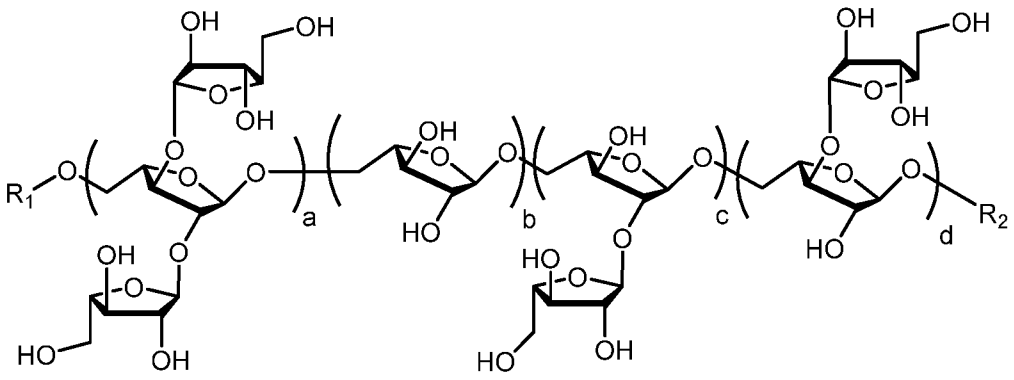
[0146] Alternatively, in all the aforementioned embodiments, a suitable pea fiber preparation may also comprise arabinan of formula (I):



(I)

wherein a is about 0.1 to about 0.2, b is about 0.4 to about 0.5, c is about 0.2 to about 0.4, d is about 0.04 to about 0.06 (calculated from the fractional abundance of arabinose linkages where the arabinose contained a 5-linkage, as determined by partially methylated alditol acetate GC-MS analysis); and wherein R_1 and R_2 are each independently selected from H, a glycosyl, a sugar moiety (modified or not), an oligosaccharide (branched or not), or a polysaccharide (branched or not), and a polysaccharide containing galacturonic acid, galactose, and rhamnose.

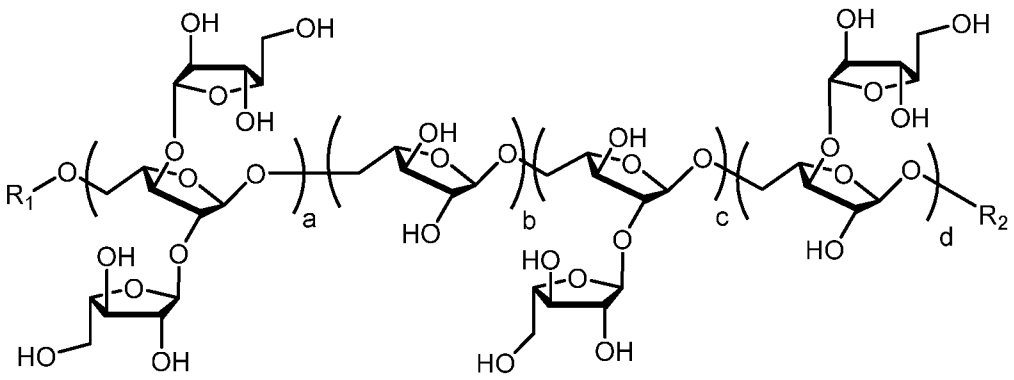
[0147] Alternatively, in all the aforementioned embodiments, a suitable pea fiber preparation may also comprise arabinan of formula (I):



(I)

wherein a is about 0.2 to about 0.3, b is about 0.4 to about 0.5, c is about 0.3 to about 0.4, d is about 0.04 to about 0.06 (calculated from the fractional abundance of arabinose linkages where the arabinose contained a 5-linkage, as determined by partially methylated alditol acetate GC-MS analysis); wherein R_1 and R_2 are each independently selected from H, a glycosyl, a sugar moiety (modified or not), an oligosaccharide (branched or not), or a polysaccharide (branched or not), and a polysaccharide containing galacturonic acid, galactose, and rhamnose.

[0148] Alternatively, in all the aforementioned embodiments, a suitable pea fiber preparation may also comprise arabinan of formula (I):



(I)

wherein a is about 0.20, b is about 0.47, c is about 0.28, d is about 0.05 (calculated from the fractional abundance of arabinose linkages where the arabinose contained a 5-linkage, as determined by partially methylated alditol acetate GC-MS analysis); wherein R_1 and R_2 are each independently selected from H, a glycosyl, a sugar moiety (modified or not), an oligosaccharide (branched or not), or a polysaccharide (branched or not), and a polysaccharide containing galacturonic acid, galactose, and rhamnose.

[0149] The molecular weight of the arabinan may be about 2 kDa to about 500,000 kDa, or more. In one example, the molecular weight of the arabinan may be about 1000 kDa to about 500,000 kDa. In one example, the molecular weight of the arabinan may be about 1000 kDa to about 200,000 kDa. In one example, the molecular weight of the arabinan may be about 1000 kDa to about 100,000 kDa. In one example, the molecular weight of the arabinan may be about 1000 kDa to about 10,000 kDa. In one example, the molecular weight of the arabinan may be about 10,000 kDa to about 500,000 kDa. In one example, the molecular weight of the arabinan may be about

10,000 kDa to about 200,000 kDa. In one example, the molecular weight of the arabinan may be about 100,000 kDa to about 500,000 kDa.

[0150] The total amount of all arabinans of formula (I) in a suitable pea fiber preparation may vary. In some embodiments, the total amount may be at least 10 wt%. For example, the total amount may be about 10 wt%, about 15 wt%, about 20 wt%, about 25 wt%, about 30 wt%, about 35 wt%, about 40 wt%, about 45 wt%, about 50 wt%, about 55 wt%, about 60 wt%, about 65 wt%, about 70 wt%, about 75 wt%, about 80 wt%, about 85 wt%, about 90 wt%, about 95 wt%. In some embodiments, the total amount may be at least 20 wt%, at least 30 wt%, at least 40 wt%, at least 50 wt%, at least, 60 wt%, at least, 70 wt%, at least, 80 wt%, at least 90 wt%. In some embodiments, the total amount may be about 10 wt% to about 50 wt%, about 20 wt% to about 50 wt%, about 30 wt% to about 50 wt%, about 40 wt% to about 50 wt %. In some embodiments, the total amount may be about 30 wt% to about 70 wt%, about 40 wt% to about 70 wt%, about 50 wt% to about 70 wt%, about 60 wt% to about 70 wt %. In some embodiments, the total amount may be about 50 wt% to about 90 wt%, about 60 wt% to about 90 wt%, about 70 wt% to about 90 wt%, about 80 wt% to about 90 wt%.

(f) sugar beet fiber preparations

[0151] Sugar beet fiber preparations may be prepared according to methods known in the art, and evaluated as described herein. Commercial sources may also be used.

[0152] In some embodiments, a composition comprises one or more sugar beet fiber preparation in an amount that is at least 15 wt% of the composition. The amount may also be expressed as individual values or a range. For instance, the pea fiber preparation(s) in these embodiments may be about 15 wt%, 16 wt%, 17 wt%, 18 wt%, 19 wt%, 20 wt%, 21 wt%, 22 wt%, 23 wt%, 24 wt%, 25 wt%, 26 wt%, 27 wt%, 28 wt%, 29 wt%, 30 wt%, 31 wt%, 32 wt%, 33 wt%, 34 wt%, 35 wt%, 36 wt%, 37 wt%, 38 wt%, 39 wt%, 40 wt%, 41 wt%, 42 wt%, 43 wt%, 44 wt%, 45 wt%, 46 wt%, 47 wt%, 48 wt%, 49 wt%, 50 wt%, 51 wt%, 52 wt%, 53 wt%, 54 wt%, 55 wt%, 56 wt%, 57 wt%, 58 wt%, 59 wt%, 60 wt%, 61 wt%, 62 wt%, 63 wt%, 64 wt%, 65 wt%, or more. In some examples, the sugar beet fiber preparation(s) may be about 15 wt% to about 65 wt%,

about 25 wt% to about 65 wt%, or about 35 wt% to about 65 wt% of the composition. In some examples, the sugar beet fiber preparation(s) may be about 15 wt% to about 55 wt%, about 25 wt% to about 55 wt%, or about 35 wt% to about 55 wt% of the composition. In some examples, the sugar beet fiber preparation(s) may be about 15 wt% to about 45 wt%, about 25 wt% to about 45 wt%, or about 35 wt% to about 45 wt% of the composition.

[0153] In an exemplary embodiment of a suitable sugar beet fiber preparation, the total dietary fiber is comprised of about 55 wt% to about 65 wt%, or about 60 wt% to about 65% of insoluble dietary fiber and/or about 75 wt% to about 85 wt%, or about 80 wt% to about 85 wt% of high molecular weight dietary fiber. In some embodiments, the total dietary fiber is about 70 wt% to about 90 wt%, about 70 wt% to about 85 wt%, or about 70 wt% to about 80 wt% of the preparation. In other embodiments, the total dietary fiber is about 75 wt% to about 90 wt%, about 80 wt% to about 90 wt%, or about 80 wt% to about 85 wt% of the preparation. In still further embodiments, the sugar beet fiber preparation comprises about 7 wt% to about 12 wt% protein, about 1 wt% to about 3 wt% fat, about 75 wt% to about 85 wt% carbohydrate, about 5 wt% to about 10 wt% moisture, and about 3 wt% to about 6 wt% ash.

[0154] In an exemplary embodiment of a suitable sugar beet fiber preparation, the total dietary fiber is comprised of about 55 wt% to about 65 wt%, or about 60 wt% to about 65% of insoluble dietary fiber and about 75 wt% to about 85 wt%, or about 80 wt% to about 85 wt% of high molecular weight dietary fiber, the total dietary fiber is about 75 wt% to about 90 wt%, about 80 wt% to about 90 wt%, or about 80 wt% to about 85 wt% of the preparation; and the sugar beet fiber preparation comprises about 7 wt% to about 12 wt% protein, about 1 wt% to about 3 wt% fat, about 75 wt% to about 85 wt% carbohydrate, about 5 wt% to about 10 wt% moisture, and about 3 wt% to about 6 wt% ash.

[0155] In another exemplary embodiment, a suitable sugar beet preparation is substantially similar to the preparation described in **Table A**.

(g) glycan equivalents

[0156] In each of the above embodiments, a compositional glycan equivalent thereof and/or a functional glycan equivalent thereof may be used as an alternative for a barley fiber preparation, a citrus fiber preparation, a citrus pectin preparation, a high molecular weight inulin preparation, a pea fiber preparation, and/or a sugar beet fiber preparation.

[0157] In some embodiments, a suitable functional glycan equivalent of a barley fiber preparation, a citrus fiber preparation, a citrus pectin preparation, a high molecular weight inulin preparation, a pea fiber preparation, or a sugar beet fiber preparation has a substantially similar function as a respective preparation identified in **Table 2A**. Substantially similar function may be measured by any one or more method detailed in the Examples herein, in particular the ability to affect relative or total abundances of microbial community members, in particular primary and secondary fiber degrading microbes, more particularly *Bacteroides* species; and/or expression of one or more microbial genes or gene product, in particular one or more gene or gene product encoded by polysaccharide utilization loci (PULs) and/or one or more CAZyme. In an exemplary embodiment, a suitable functional glycan equivalent is a fiber preparation that is enriched for one or more bioactive glycan, as compared to a barley fiber preparation, a citrus fiber preparation, a citrus pectin preparation, a high molecular weight inulin preparation, a pea fiber preparation, or a sugar beet fiber preparation used in the Examples.

[0158] For instance, a suitable functional glycan equivalent of a fiber preparation may have a similar effect on the relative abundance of *Bacteroides* species in a subject's gut microbiota. In another example, a suitable functional glycan equivalent of a fiber preparation may have a similar effect on the total abundance of *Bacteroides* species in a subject's gut microbiota. In another example, a suitable functional glycan equivalent of a fiber preparation may have a similar effect on the relative abundance of a subset of *Bacteroides* species. In another example, a suitable functional glycan equivalent of a fiber preparation may have a similar effect on the total abundance of a subset of *Bacteroides* species. In one example, the subset of *Bacteroides* species may

include one or more species chosen from *B. caccae*, *B. cellulosilyticus*, *B. finegoldii*, *B. massiliensis*, *B. ovatus*, *B. thetaiotaomicron*, and *B. vulgatus*. In another example, a suitable functional glycan equivalent may have a similar effect on the relative abundance of one or more species chosen from *Bacteroides ovatus*, *Bacteroides cellulosilyticus*, *Bacteroides thetaiotaomicron*, *Bacteroides vulgatus*, *Bacteroides caccae*, *Bacteroides finegoldii*, *Bacteroides massiliensis*, *Collinsella aerofaciens*, *Escherichia coli*, *Odoribacter splanchnicus*, *Parabacteroides distasonis*, a *Ruminococcaceae sp.,* and *Subdoligranulum variabile*.

[0159] Alternatively or in addition, a suitable functional glycan equivalent may have a similar effect on the abundance or activity of one or more protein encoded by one or more polysaccharide utilization locus (PUL) and/or one or more CAZyme. In some examples, the PULs are chosen from PUL5, PUL6, PUL7, PUL27, PUL31, PUL34, PUL35, PUL38, PUL42, PUL43, PUL73, PUL75, PUL83, and PUL97.

[0160] Although the Examples utilize a gnotobiotic mouse model where the mouse is colonized with a defined consortium of cultured, sequenced gut bacteria, the methods detailed in the Examples may also be used to measure effects in a gnotobiotic mouse model where the mouse is colonized with intact uncultured gut microbiota obtained from human(s), as well as to measure effects directly in humans.

(h) additional food ingredients

[0161] In each of the above embodiments, the remaining weight percent (if any) of the composition is comprised of one or more additional food ingredients. Non-limiting examples include anti-caking agents, preservatives, pH control agents, color additives, flavors, flavor enhancers, and the like.

(i) food compositions

[0162] The present disclosure also provides food compositions comprising a composition of this section. The food composition may further comprise one or more additional food ingredients including, but not limited to, flours, meals, sweeteners, preservatives, color additives, flavors, spices, flavor enhancers, fats, oils, fat replacers (including components of formulations used to replace fats), nutrients, vitamins,

minerals, emulsifiers, stabilizers, thickeners, binders, texturizers, pH control agents, leavening agents, anti-caking agents, humectants, firming agents, probiotics, and enzyme preparations, as well as inclusions, fruits, vegetables and grains.

[0163] Flours or meals may be made from a variety of sources, including but not limited to grains, legumes, roots, nuts or seeds.

[0164] Non-limiting examples of sweeteners include sucrose (sugar), glucose, fructose, sugar polyols (e.g., sorbitol, mannitol, etc.), syrups (e.g., corn syrup, high fructose corn syrup, etc.,) saccharin, aspartame, sucralose, acesulfame potassium (acesulfame-K), and neotame.

[0165] Preservatives include but are not limited to ascorbic acid, citric acid, sodium benzoate, calcium propionate, sodium erythorbate, sodium nitrite, calcium sorbate, potassium sorbate, BHA, BHT, EDTA, and tocopherols (Vitamin E).

[0166] Inclusions are substitutional or interstitial ingredients in the composition matrix. Non-limiting examples include candies, chips (chocolate, butterscotch, etc.), nuts, seeds, herbs, and the like.

[0167] Flavors may be natural, synthetic or artificial. Non-limiting examples of flavor enhancers include Monosodium glutamate (MSG), hydrolyzed soy protein, autolyzed yeast extract, disodium guanylate and inosinate.

[0168] Non-limiting examples of fat replacers include olestra, cellulose gel, carrageenan, polydextrose, modified food starch, microparticulated egg white protein, guar gum, xanthan gum, and whey protein concentrate. Emulsifiers may include lecithin, mono- and diglycerides, egg yolks, polysorbates, sorbitan monostearate, and glycerol monostearate.

[0169] Non-limiting examples of stabilizers, thickeners, binders, and texturizers include gelatin, pectin, guar gum, carrageenan, xanthan gum, and whey. Leavening agents include but are not limited to baking soda, monocalcium phosphate, calcium carbonate, ammonium bicarbonate, mono calcium phosphate monohydrate, sodium acid pyrophosphate, sodium aluminum phosphate, organic acids, and yeast. Humectants may be glycerin, sorbitol, and the like. Non-limiting examples of firming agents include calcium chloride and calcium lactate.

[0170] The amount of the composition in the food may vary. In some embodiments, a composition of this section may be about 5 wt% to about 60 wt% of the ingredients used to make the food (excluding any added water). In some embodiments, a composition of this section may be about 40 wt% to about 60 wt% of the ingredients used to make the food (excluding any added water). In some embodiments, a composition of this section may be about 40 wt% to about 50% wt%, about 45 wt% to about 50 wt%, or about 50 wt% to about 60 wt% of the ingredients used to make the food (excluding any added water). In some embodiments, a composition of this section may be about 45 wt% to about 50 wt% of the ingredients used to make the food (excluding any added water).

[0171] In certain embodiments, a composition of this section provides about 90% or more of the total dietary fiber in the food composition. For instance, the composition may provide about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100% of the dietary fiber in the food composition. In one example, the composition provides about 95% or more of the total dietary fiber in the food composition. In another example, the composition provides about 98% or more of the total dietary fiber in the food composition.

[0172] In further embodiments, the food composition provides at least 6 g of dietary fiber per serving. In some examples, the food composition may provide at least 7 grams, at least 8 grams, at least 9 grams, or at least 10 grams of dietary fiber per serving. In other examples, the food composition may provide about 6 g to about 20 g, about 6 g to about 15 g, or about 6 g to about 10 g of dietary fiber per serving. A serving size may be at least 6 grams, for instance about 10 grams, about 15 grams, about 20 grams, about 25 grams, about 30 grams, about 35 grams, about 40 grams, about 45 grams, about 50 grams, etc. In certain embodiments, a serving is about 30 grams.

[0173] In some embodiments, a food composition is in a baked form. In some embodiments, a food composition is in a pressed or extruded form. In some embodiments, a food composition is in a powder form which may be reconstituted or

sprinkled on a different food. In some embodiments, a food composition is a bar; a drink; a gel, a gummy, a candy, or the like; a cookie, a cracker, a cake, or the like; a dairy product (e.g., yogurt, ice cream or the like).

(j) alternative forms of administration

[0174] The present disclosure also provides other oral dosage forms comprising a composition of this section. Suitable dosage forms include a tablet, including a suspension tablet, a chewable tablet, an effervescent tablet or caplet; a pill; a powder such as a sterile packaged powder, a dispensable powder, and an effervescent powder; a capsule including both soft or hard gelatin capsules such as HPMC capsules; a lozenge; pellets; granules; liquids; suspensions; emulsions; or semisolids and gels. Capsule and tablet formulations may include, but are not limited to binders, lubricants, and diluents. Capsules and tablets may be coated according to methods well known in the art. Aqueous suspension formulations may include but are not limited to dispersants, flavor-modifying agents, taste-masking agents, and coloring agents.

Table A – Compositional Analysis of Exemplary Fiber Preparations

Fiber Preparation	% Total DF	% Insoluble DF	% Soluble DF	HMW DF	LMW DF	% Protein	% Fat	% Carb	% Moisture	% Ash
Barley bran	46	11.1	20.8	45.2	0.9	18.72	4.13	69.28	5.69	1.96
Citrus fiber	68.5	33.2	29.5	68.5	0.6	7.47	2.16	80.92	5.69	1.96
Citrus pectin	91	4.7	85.3	91	0.6	0.61	1.23	90.07	3.51	4.58
HMW inulin	98.5	<0.5	98.5	86	12.5	0.28	3.71	91.44	4.28	0.29
Pea fiber	67.2	61.4	4.9	66.3	0.8	9.49	0.93	79.75	7.37	2.46
Sugar beet	83.2	61.6	20.4	82	1.1	8.5	2.45	77.97	6.66	4.42

DF = dietary fiber, HMW = high molecular weight, LMW = low molecular weight

Protein, fat, ash, and moisture content are measured by methods established by Association of Official Analytical Chemists (AOAC) 2009.01, AOAC 920.123, AOAC 933.05, AOAC 935.42, and AOAC 926.08, respectively. Carbohydrate is calculated as (100 - (Protein + Fat + Ash + Moisture). Total dietary fiber is measured by AOAC method 2009.01. Soluble and insoluble dietary fiber, and high molecular weight and low molecular weight dietary fiber, are measured by AOAC method 2011.25.

Table B – Monosaccharide Analysis of Fiber Preparations

	Barley fiber		Citrus fiber (raw)		Citrus fiber (extruded)		Citrus pectin		Pea fiber (raw)		Pea fiber (extruded)	
	2 h	6 h	2 h	6 h	2 h	6 h	2 h	6 h	2 h	6 h	2 h	6 h
Rhamnose	0	0.4	1	1.8	1.3	1.6	0.8	1.5	0	1.7	0.9	1.1
Fucose	0	0	0	0.3	0	0.2	0	0	0	0.1	0	0
Arabinose	4.5	5.3	9.9	10.7	8.3	7.3	4.1	3.8	17.3	20.4	13.8	13.5
Xylose	6.1	7.4	2.3	2.7	2.4	2.2	0	0	4.8	5.6	3.3	3.2
Mannose	0.9	0.9	2.7	3.2	2.7	2.9	1.4	3.9	0.5	0.5	0.5	0.6
Galactose	0.5	0.5	4.5	5.0	3.8	3.6	4.1	4.1	2.6	3.0	2.3	2.2
Glucose	48.9	60.0	17.5	20.4	32.8	30.3	0.4	0.3	38.9	46.9	47.3	44.3
Uronic acids	0.8	0	45.9	33.1	32.2	26.3	78.8	80.6	13.4	12.7	9.5	8.8
Total Carbohydrates	61.7	74.5	83.8	77.1	83.4	74.4	89.6	94.0	77.5	91.1	77.6	73.7
Water	5.7		7.2		4.5		8.0		7.4		6.6	
Degree of methylation (%)	0		29		45		72		16		20	
Starch	22.0		ND		ND		ND		16.6		28.5	
Beta-glucans	17.0		ND		ND		ND		ND		ND	
Cellulose	6.7		16.1		8.6		0		28.1		13.3	

ND = none detected

Monosaccharide Method: Sugar composition analysis combines an acid hydrolysis, and then reduction and acetylation of the free sugars prior to GC analysis. Total acid hydrolysis was performed with 1 M sulfuric acid (2 h or 6h, 100 °C) after a pre- hydrolysis step with concentrated sulfuric acid 72% (30 min, 30°C). The neutral sugar derivation method used follows that published by Blakeney et al. (1983). Alditol acetates were chromatographed on a DB 225 capillary column (J&W Scientific, Folsorn, CA, USA; temperature 205 °C, carrier gas H2). Inositol was used as internal standard. Response factors were determined using standard sugars. Samples were determined in duplicate. Uronic acids in hydrolyzates were quantified using the metahydroxydiphenyl colorimetric acid method (Blumenkrantz & Asboe-Hansen, 1973).

Table C1 – Glycosyl-linkage analysis of a pea fiber preparation (see Example 8 for a description of the methodology)

	Σ linked-sugars/ DW	Σ linked-sugars/ sugars	Deduced linkage	% / Σ sugars							
				Rha	Fuc	Ara	Xyl	Gal	Glc	Man	UA
Rha		1.3%	Terminal Rha(p)	0.1							
			2-Rha(p)	0.4							
			2,4-Rha(p)	0.8							
Fuc		ND	–		ND						
Ara		26.6%	Terminal Ara(f)			9.4					

			5-Ara(f)			12.2					
			2,5-Ara(f)			3.5					
			3,5-Ara(f)			1.4					
Xyl		8.2%	Terminal Xyl(p)				1.3				
			4-Xyl(p)				6.9				
Gal		5.1%	Terminal Gal(p)					0.5			
			3-Gal(p)					1.5			
			4-Gal(p)					3.0			
			2,3,4-Gal(p)					0.1			
Glc		50.2%	Terminal Glc(p)						1.3		
			4-Glc(p)						46.5		
			3,4-Glc(p)						0.4		
			4,6-Glc(p)						1.7		
			2,3,4,6-Glc(p)						0.2		
Man		ND	–							ND	
UA		8.4%	4-GalA(p)								6.8
			4-GalA(p)-methyl ester								0.7
			3,4-GalA(p)								0.9
			Calculated DM								8.0
Total linked-sugars/ DW	19.7%										

Rha = rhamnose, Ara = arabinose, Xyl = xylose, Gal = galactose, Glc = glucose, Man = mannose, UA = uronic acids,
ND = none detected, DM = degree of methylation
Data are expressed in % of the total sugars identified (/ Σ sugars). Yields of mass percentages are indicated in each
table as % of total sugars identified per dried weight (%/DW).
Deduced linkage - 2018, Double reduction (Pettolino et al.)

Table C2 – Glycosyl-linkage analysis of an extruded pea fiber preparation (see Example 8 for a description of the methodology)

	Σ linked-sugars/ DW	**Σ linked-sugars/ sugars**	**Deduced linkage**	**% / Σ sugars**							
				Rha	**Fuc**	**Ara**	**Xyl**	**Gal**	**Glc**	**Man**	**UA**
Rha		1.0%	2-Rha(p)	0.5							
			2,4-Rha(p)	0.5							
Fuc		ND	–		ND						
Ara		27.2%	Terminal Ara(f)			13.3					

			5-Ara(f)			10.4					
			2,5-Ara(f)			2.8					
			3,5-Ara(f)			0.7					
Xyl		5.6%	Terminal Xyl(p)				2.8				
			4-Xyl(p)				2.8				
Gal		3.2%	Terminal Gal(p)					0.4			
			3-Gal(p)					0.5			
			4-Gal(p)					2.3			
Glc		58.1%	Terminal Glc(p)						2.6		
			4-Glc(p)						51.7		
			3,4-Glc(p)						0.6		
			4,6-Glc(p)						3.2		
Man		ND	–							ND	
UA		5.0%	4-GalA(p)								3.7
			4-GalA(p)-methyl ester								0.5
			3,4-GalA(p)								0.5
			3,4-GalA(p)-methyl ester								0.2
			Calculated DM								14.2
Total linked-sugars/ DW	46.7%										

Rha = rhamnose, Ara = arabinose, Xyl = xylose, Gal = galactose, Glc = glucose, Man = mannose, UA = uronic acids,
ND = none detected, DM = degree of methylation
Data are expressed in % of the total sugars identified (/ Σ sugars). Yields of mass percentages are indicated in each
table as % of total sugars identified per dried weight (%/DW).
Deduced linkage - 2018, Double reduction (Pettolino et al.)
Note: the pea fiber preparation of Table C1 was extruded to obtain this preparation.

Table D – Glycosyl-linkage analysis of a citrus pectin preparation (see Example 8 for a description of the methodology)

	Σ linked-sugars/ DW	**Σ linked-sugars/ sugars**	**Deduced linkage**	**% / Σ sugars**							
				Rha	**Fuc**	**Ara**	**Xyl**	**Gal**	**Glc**	**Man**	**UA**
Rha		1.5%	2-Rha(p)	1.2							
			2,4-Rha(p)	0.3							
Fuc		ND	–		ND						

Ara		3.4%	Terminal Ara(f)			0.8					
			5-Ara(f)			1.2					
			2,5-Ara(f)			0.2					
			3,5-Ara(f)			1.2					
Xyl		ND	–				ND				
Gal		6.0%	4-Gal(p)					4.4			
			3,4-Gal					0.1			
			4,6-Gal					0.9			
			2,3,4-Gal					0.7			
Glc		ND	–						ND		
Man		ND	–							ND	
UA		88.6%	4-GalA(p)								26.3
			4-GalA(p)-methyl ester								60.8
			2,4-GalA(p)-methyl ester								0.5
			3,4-GalA(p)								0.3
			3,4-GalA(p)-methyl ester								0.8
			Calculated DM								70.1
Total linked-sugars/ DW	42.6%										

Rha = rhamnose, Ara = arabinose, Xyl = xylose, Gal = galactose, Glc = glucose, Man = mannose, UA = uronic acids,
ND = none detected, DM = degree of methylation
Data are expressed in % of the total sugars identified (/ Σ sugars). Yields of mass percentages are indicated in each
table as % of total sugars identified per dried weight (%/DW).
Deduced linkage - 2018, Double reduction (Pettolino et al.)

Table E – Glycosyl-linkage analysis of a barley fiber preparation (see Example 8 for a description of the methodology)

	Σ linked-sugars/ DW	**Σ linked-sugars/ sugars**	**Deduced linkage**	**% / Σ sugars**								
				Rha	**Fuc**	**Ara**	**Xyl**	**Gal**	**Glc**	**Man**	**UA**	**Hex**
Rha		ND	–	ND								
Fuc		ND	–		ND							
Ara		1.6%	Terminal Ara(f)			1.6						
Xyl		6.3%	4-Xyl(p)				3.0					

			3,4-Xyl(p)				0.9						
			2,3,4-Xyl(p)				2.4						
Gal		1.0%	4-Gal(p)					1.0					
Glc		84.5%	Terminal Glc(p)						3.0				
			3-Glc(p)						5.2				
			4-Glc(p)						71.3				
			4,6-Glc(p)						3.4				
			2,3,4,6-Glc(p)						1.5				
Man		ND	–							ND			
UA		ND	–								ND		
Hex		6.6%	2,4-Hex									1.1	
			3,4-Hex									2.9	
			2,3,4-Hex									0.9	
			3,4,6-Hex									1.7	
Total linked-sugars/ DW	18.2%												

Rha = rhamnose, Ara = arabinose, Xyl = xylose, Gal = galactose, Glc = glucose, Man = mannose, UA = uronic acids,

Hex = hexose, ND = none detected, DM = degree of methylation

Data are expressed in % of the total sugars identified (/ Σ sugars). Yields of mass percentages are indicated in each table as % of total sugars identified per dried weight (%/DW).

Deduced linkage - 2018, Double reduction (Pettolino et al.)

Table F1 – Glycosyl-linkage analysis of a citrus fiber preparation (see Example 8 for a description of the methodology)

	Σ linked-sugars/ DW	**Σ linked-sugars/ sugars**	**Deduced linkage**	**% / Σ sugars**							
				Rha	**Fuc**	**Ara**	**Xyl**	**Gal**	**Glc**	**Man**	**UA**
Rha		0.9%	2-Rha(p)	0.9							
Fuc		ND	–		ND						
Ara		15.4%	Terminal Ara(f)			2.4					
			5-Ara(f)			8.3					
			3,5-Ara(f)			4.7					
Xyl		2.4%	4-Xyl(p)				2.4				
Gal		10.3%	Terminal Gal(p)					0.9			
			3-Gal(p)					1.2			
			4-Gal(p)					8.1			
			4,6-Gal(p)					0.2			
Glc		13.0%	4-Glc(p)						13.0		

Man		ND	–							ND	
UA		57.1%	Terminal GalA(p)								0.1
			Terminal GalA(p)-methyl ester								0.4
			4-GalA(p)								24.0
			4-GalA(p)-methyl ester								31.5
			3,4-GalA(p)								0.4
			3,4-GalA(p)-methyl ester								0.2
			4,6-GalA(p)								0.1
			4,6-GalA(p)-methyl ester								0.4
			Calculated DM								57.1
Total linked-sugars/ DW	9.5%										

Rha = rhamnose, Ara = arabinose, Xyl = xylose, Gal = galactose, Glc = glucose, Man = mannose, UA = uronic acids,
ND = none detected, DM = degree of methylation
Data are expressed in % of the total sugars identified (/ Σ sugars). Yields of mass percentages are indicated in each
table as % of total sugars identified per dried weight (%/DW).
Deduced linkage - 2018, Double reduction (Pettolino et al.)

Table F2 – Glycosyl-linkage analysis of an extruded citrus fiber preparation (see Example 8 for a description of the methodology)

	Σ linked-sugars/ DW	**Σ linked-sugars/ sugars**	**Deduced linkage**	**% / Σ sugars**							
				Rha	**Fuc**	**Ara**	**Xyl**	**Gal**	**Glc**	**Man**	**UA**
Rha		0.7%	2-Rha(p)	0.7							
Fuc		ND	–		ND						
Ara		20.1%	Terminal Ara(f)			8.8					
			5-Ara(f)			8.5					
			3,5-Ara(f)			2.8					
Xyl		3.2%	4-Xyl(p)				3.2				
Gal		5.1%	Terminal Gal(p)					0.5			
			3-Gal(p)					0.7			
			4-Gal(p)					3.7			

Glc		53.1%	Terminal Glc(p)						1.5		
			4-Glc(p)						49.4		
			3,4-Glc(p)						0.3		
			4,6-Glc(p)						1.9		
			2,3,4,6-Glc(p)						0.1		
Man		0.2%	4,6-Man(p)							0.2	
UA		17.7%	4-GalA(p)								6.3
			4-GalA(p)-methyl ester								11.1
			Calculated DM								64.2
Total linked-sugars/ DW	18.7%										

Rha = rhamnose, Ara = arabinose, Xyl = xylose, Gal = galactose, Glc = glucose, Man = mannose, UA = uronic acids,

ND = none detected, DM = degree of methylation

Data are expressed in % of the total sugars identified (/ Σ sugars). Yields of mass percentages are indicated in each

table as % of total sugars identified per dried weight (%/DW).

Deduced linkage - 2018, Double reduction (Pettolino et al.)

Note: the citrus fiber preparation of Table F1 was extruded to obtain this preparation.

Table G

Test Method	%	Pea Fiber	Citrus Fiber	Inulin	Barley Bran
CODEX 2011	Total Dietary Fiber	63.7 - 65.4	74.4 - 77.1	95.3 - 100	28.4 - 44.5
	High Molecular Weight (Insoluble)	61.0 - 63.1	37.2 - 41.7	N.D.	11.2 - 12.3
	High Molecular weight (Soluble)	1.2 - 1.6	30.6 - 36.2	75.3 - 76.8	15.9 - 31.4
	Low Molecular Weight	1.1 - 1.7	1.4 - 2.4	18.2 - 23.5	1.1 - 3.4
Gravimetric	Cellulose	16.2 - 18.2	15.6 - 21.1	N.D.	0.5
Gravimetric	Lignin	0.70 - 0.86	4.3 - 6.0	0.6 - 0.7	1.1
	Free Sugars				
AOAC 2018.16	Fructose	N.D.	1.8 - 1.9	N.D.	0.1
	Galactose	N.D.	N.D.	N.D.	N.D.
	Glucose	N.D.	1.6 - 2.6	N.D.	0.1
	Sucrose	N.D.	2.9 - 3.0	N.D.	1.0 - 1.9
	Lactose	N.D.	0.5	N.D.	N.D.
	Isomaltulose	N.D.	N.D.	N.D.	N.D.
	Maltose	N.D.	N.D.	N.D.	0.6 - 0.9
	Total Sugars		6.8 - 7.4		2.0 - 2.8

	Bound Sugars				
Acid Hydrolysis GC-FID*	Arabinose	20.2 -21.5	7.4 - 8.2	N.D.	2.8
	Glucose	16.8 - 21.6	4.5 - 5.0	3.4	65.1 - 66.9
	Galactose	2.9 -3.3	3.6 - 4.7	N.D.	0.3 - 0.4
	Xylose	3.8	1.3 - 1.6	0.7	3.8
	Mannose	N.D	0.5 - 0.6	N.D.	0.6
	Fucose	0.2 - 0.3	0.3	N.D.	N.D.
	Rhamnose	0.7	1.1 - 1.3	N.D.	N.D.
Polarimetric	Starch	12.8 - 18.2	N.D.	N.D.	33.4 - 36.5
AOAC 996.11	Starch	12.3 - 19.4	0.5 - 1.1	N.D.	32.7 - 33.7
AOAC 995.16	Beta Glucans	N.T.	N.T.	N.T.	24.4 - 25.1
HPAEC-PAD	Inulin	N.T.	N.T	100	N.T.
Kjeldahal	Protein	7.91 - 10.6	5.7 - 7.5	N.D.	10.5 - 12.1
Acid Hydrolysis	Fat	0.86 - 0.94	2.0 - 3.0	N.D.	2.49 - 2.84
	Particle Size (um)				
Laser Diffraction	d10	9.3 - 11.8	59 - 107	10.1 - 12.7	78.3 - 130
	d50	40.9 - 53.2	306 - 329	37.6 - 45.2	219 - 289
	d90	110 - 128	686 - 712	122 - 125	470 -544
* = Fructose is unable to be detected with this methodology N.D. = Not Detected N.T. = Not Tested					

II. FOOD COMPOSITIONS

[0175] In another aspect, the present disclosure provides food compositions comprising one or more fiber preparation, each fiber preparation independently selected from the group consisting of a barley fiber preparation, a citrus fiber preparation, a citrus pectin preparation, a high molecular weight inulin preparation, a pea fiber preparation, a sugar beet fiber preparation, and glycan equivalents thereof. The glycan equivalent can be a compositional glycan equivalent or a functional glycan equivalent. Food compositions encompassed by the present disclosure may contain 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or more different fiber preparations independently

selected from the above group. Suitable fiber preparations are described in detail in **Section I**. Typically, each fiber preparation alone, or a combination of fiber preparations, is in an amount that increases the fiber degrading capacity of gut microbiota in a subject and/or promotes a healthy gut microbiota in a subject when administered to the subject on a daily basis for at least 5 days (e.g., 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 days or more). In some embodiments, a food composition is in a baked form. In some embodiments, a food composition is in an extruded or pressed form. Extruded foods can be shaped to limitless forms depending on the die. They also can be coated, filled, pressed into a bar (or other shape), or combinations thereof, with other ingredients using a binder. In some embodiments, a food composition is a bar; a drink; a gel, a gummy, a candy, or the like; a cookie, a cracker, a cake, or the like; a bread, a muffin, or the like; a dairy product (e.g., yogurt, ice cream or the like).

[0176] The term "wt% of the food composition" is the weight of an ingredient as a percentage of all ingredients in the food composition prior to processing (e.g., baking, extrusion, dehydration, etc.) into the final form (e.g., cookie, cracker, bar, extruded shape, gel, powder, etc.), but does not include any added water. Typically the ingredients are combined and then a suitable amount of water (e.g., about 15%) is added to make a dough for a baked product or a mix to go into an extrusion process. When combining the ingredients, all the ingredients may be added individually (inclusive of each fiber preparation), or various ingredients may be combined and then the combinations added. For instance, in some embodiments one or more fiber preparations may be first combined together to form a composition of fiber preparations, and then the composition of fiber preparations is combined with any other ingredients. In other embodiments, each fiber preparation may be added individually. The final moisture content of the baked, pressed or extruded product may vary, though typically the final moisture content may be around 2-5%, or more preferably 3%.

[0177] In some embodiments, the one or more fiber preparation, in total, is about 30 wt% to about 50 wt% of the food composition. In some embodiments, the one or more fiber preparation provides 50% of the food composition's total dietary fiber. In some embodiments, the one or more fiber preparation, in total, is about 30 wt% to about

50 wt% of the food composition and provides 50% of the food composition's total dietary fiber. In each of the above embodiments, the one or more fiber preparation, in total, may provide at least 3 g, at least 6 g, or at least 10 g of total dietary fiber per serving of the food composition. For instance, the one or more fiber preparations, in total, may provide 3 g, 4 g, 5 g, 6 g, 7 g, 8 g, 9 g, 10 g, or more of total dietary fiber per serving of the food composition. Serving size can vary, and may be about 20 g to about 50 g, about 25 g to about 40 g, about 30 g to about 40 g, or about 30 g to about 35 g. In some examples, the one or more fiber preparations, in total, may provide about 3 g to about 10 g of total dietary fiber per serving of the food composition. In other examples, the one or more fiber preparations, in total, may provide about 3 g to about 6 g of total dietary fiber per serving of the food composition, or about 6 g to about 10 g of total dietary fiber to the food composition. In preferred examples, the one or more fiber preparation comprises a pea fiber preparation and/or a glycan equivalent thereof, in particular, a pea fiber preparation of **Section I** and/or glycan equivalent.

[0178] As illustrated in the Examples, food compositions that differ in the number of barley fiber, citrus fiber, high molecular weight inulin and pea fiber preparations have overlapping and distinct effects on a subject's microbiome and on biomarkers of health, including biomarkers of cardiometabolic and immunoinflammatory state. Accordingly, the number of fiber preparations, and amounts of each, may be optimized to achieve a desired effect. Importantly, the Examples further illustrate the suitability of gnotobiotic mice colonized with gut microbial communities representing a human study population as a model system that can be used to select fiber preparations and define appropriate amounts. The Examples also identify health discriminatory biomarkers that can be measured in human blood samples that are linked to health discriminatory features of the gut microbiome (e.g.,CAZymes, PULs, etc.). Thus, the Examples demonstrate that the gut mcirobiome may be used as a read-out to evalute the effectivenes of a given food.

[0179] In a specific example, a food composition may comprise a first fiber preparation that is a pea fiber preparation or a glycan equivalent thereof, and a second fiber preparation that is a high molecular weight inulin preparation or a glycan equivalent

thereof, wherein the first and second fiber preparation, in total, provide about 3 g to about 10 g of total dietary fiber per serving of the food composition. In another specific example, a food composition may comprise a first fiber preparation that is a pea fiber preparation or a glycan equivalent thereof, a second fiber preparation that is a high molecular weight inulin preparation or a glycan equivalent thereof, a third fiber preparation that is a citrus fiber preparation or a glycan equivalent thereof, and fourth fiber preparation that is a barley fiber preparation or a glycan equivalent thereof, wherein the first, second, third and fourth fiber preparation, in total, provide about 3 g to about 10 g of total dietary fiber per serving of the food composition. In further examples, the food compositions above may have amounts of each fiber preparation as indicated in the table below.

Pea or GE	Citrus fiber or GE	HMW inulin or GE	Barley fiber or GE
about 25-40 wt%	about 5-15 wt%	about 30-40 wt%	about 10-30 wt%
about 30-40 wt%	about 10-15 wt%	about 30-40 wt%	about 15-25 wt%
about 60-70 wt%	0 wt%	30-40 wt%	0 wt%
about 55-65 wt%	0 wt%	35-45 wt%	0 wt%
about 60-65 wt%	0 wt%	35-40 wt%	0 wt%

GE = glycan equivalent

wt% = weight percentage, calculated as weight of individual fiber preparation / total weight of the four fiber preparations

In still further examples, the pea fiber preparation or glycan equivalent thereof may have a composition substantially similar to the pea fiber preparation of Table A or Table G, and/or a monosaccharide content substantially similar to the pea fiber preparation of Table B or Table G, and optionally glycosyl linkages substantially similar to the pea fiber preparation of Table C1 or C2. The high molecular weight inulin preparation or glycan equivalent thereof may have a composition substantially similar to the high molecular weight inulin preparation of Table A or Table G. The barley fiber preparation or glycan equivalent thereof may have a composition substantially similar to

the barley fiber preparation of Table A or Table G, and/or a monosaccharide content substantially similar to the barley fiber preparation of Table B or Table G, and optionally glycosyl linkages substantially similar to the barley fiber preparation of Table E. The citrus fiber preparation or glycan equivalent thereof may have a composition substantially similar to the citrus fiber preparation of Table A or Table G, and/or a monosaccharide content substantially similar to the citrus fiber preparation of Table B or Table G, and optionally glycosyl linkages substantially similar to the citrus fiber preparation of Table F1 or F2.

[0180] In addition to the selection of fiber preparation(s) in a food composition and its amount, a food composition may be processed in a manner such that the food increases the fiber degrading capacity of a gut microbiota in a subject and/or promotes a healthy gut microbiota in a subject when administered to the subject on a daily basis for at least 5 days (e.g., at least 6 days, at least 7 days, etc.). In particular, whereby such food composition effects an increase in the total or relative abundance of *Bacteroides* species measured in a fecal sample obtained from a subject after the subject has consumed the food composition at least once a day for at least 5 days (e.g., at least 6 days, at least 7 days, etc.).

[0181] Food compositions may further comprise one or more additional food ingredient. These additional ingredients may contribute favorable organoleptic properties (e.g., taste, texture, etc.) to the food, improve the processing and handling of the food, contribute additional nutritional value to the food, and the like. Non-limiting examples of additional food ingredient include flours, meals, sweeteners, preservatives, color additives, flavors, spices, flavor enhancers, fats, oils, fat replacers (including components of formulations used to replace fats), nutrients, vitamins, minerals, emulsifiers, stabilizers, thickeners, binders, texturizers, pH control agents, leavening agents, anti-caking agents, humectants, firming agents, probiotics, postbiotics, and enzyme preparations, as well as fruits, vegetables and grains. Non-limiting examples of food ingredients are further detailed in **Section II(i)**.

[0182] Example 11 illustrates how the selection of various forms of food and the use of additional food ingredients may influence organoleptic properties and/or nutritional values.

(a) exemplary embodiments

[0183] Each of the following embodiments contains a plurality of fiber preparations. In order to accurately describe the amount of each fiber preparation in each embodiment, the plurality of fiber preparations is referred to as "a composition." Use of the term "composition," in regards to a plurality of fiber preparations in a food composition (both in this section and elsewhere in this disclosure), encompasses embodiments where the plurality of fiber preparations are combined as one composition which is then added to other food ingredients, embodiments where the plurality of fiber preparations are combined into more than one composition which are then added to other food ingredients, and embodiments where each fiber preparation is individually added to other food ingredients. This is consistent with the disclosures above stating fiber preparations may be added individually in the amounts described in this section.

[0184] In one embodiment, the present disclosure provides a pressed, extruded or baked food composition, wherein a 30 g serving of the food composition has at least 3 g or at least 6 g of total dietary fiber, and wherein the food composition comprises about 40 wt% to about 95 wt% of a composition of fiber preparations, the composition of fiber preparations comprising about 25 wt% to about 40 wt% of a pea fiber preparation or a glycan equivalent thereof, about 5 wt% to about 15 wt% of a citrus fiber preparation or a glycan equivalent thereof, about 30 wt% to about 40 wt% of a high molecular weight inulin preparation or a glycan equivalent thereof, and about 10 wt% to about 30 wt% of a barley fiber preparation or a glycan equivalent thereof.

[0185] In another embodiment, the present disclosure provides a pressed, extruded or baked food composition, wherein a 30 g serving of the food composition has at least 3 g or at least 6 g of total dietary fiber, and wherein the food composition comprises about 40 wt% to about 95 wt% of a composition of fiber preparations, the composition of fiber preparations comprising about 15 wt% to about 32 wt% of a sugar beet fiber preparation or a glycan equivalent thereof, about 5 wt% to about 15 wt% of a

citrus fiber preparation or a glycan equivalent thereof, about 30 wt% to about 40 wt% of a high molecular weight inulin preparation or a glycan equivalent thereof, and about 10 wt% to about 30 wt% of a barley fiber preparation or a glycan equivalent thereof.

[0186] In another embodiment, the present disclosure provides a pressed, extruded or baked food composition, wherein a 30 g serving of the food composition has at least 3 g or at least 6 g of total dietary fiber, and wherein the food composition comprises about 40 wt% to about 95 wt% of a composition of fiber preparations, the composition of fiber preparations comprising 55 wt% to about 65 wt% of one or more pea fiber preparation or a glycan equivalent thereof and about 30 wt% to about 40 wt% of one or more high molecular weight inulin preparation or a glycan equivalent thereof.

[0187] In another embodiment, the present disclosure provides a pressed, extruded or baked food composition, wherein a 30 g serving of the food composition has at least 3 g or at least 6 g of total dietary fiber, and wherein the food composition comprises about 40 wt% to about 95 wt% of a composition of fiber preparations, the composition of fiber preparations comprising about 60 wt% to about 65 wt% of one or more pea fiber preparation or a glycan equivalent thereof and about 30 wt% to about 35 wt% of one or more high molecular weight inulin preparation or a glycan equivalent thereof.

[0188] In another embodiment, the present disclosure provides a pressed, extruded or baked food composition, wherein a 30 g serving of the food composition has at least 3 g or at least 6 g of total dietary fiber, and wherein the food composition comprises about 40 wt% to about 95 wt% of a composition of fiber preparations, the composition of fiber comprising of about 55 wt% to about 65 wt% of one or more sugar beet fiber preparation or a glycan equivalent thereof and about 30 wt% to about 40 wt% of one or more high molecular weight inulin preparation or a glycan equivalent thereof.

[0189] In another embodiment, the present disclosure provides a pressed, extruded or baked food composition, wherein a 30 g serving of the food composition has at least 3 g or at least 6 g of total dietary fiber, and wherein the food composition comprises about 40 wt% to about 95 wt% of a composition of fiber preparations, the composition of fiber preparations comprising of about 45 wt% to about 55 wt% of one or

more sugar beet preparation or a glycan equivalent thereof and about 30 wt% to about 50 wt% of one or more high molecular weight inulin preparation or a glycan equivalent thereof.

[0190] In another embodiment, the present disclosure provides a pressed, extruded or baked food composition, wherein a 30 g serving of the food composition has at least 3 g or at least 6 g of total dietary fiber, and wherein the food composition comprises about 40 wt% to about 95 wt% of a composition of fiber preparations, the composition of fiber preparations comprising 25 wt% to about 40 wt% of a pea fiber preparation or a glycan equivalent thereof, about 5 wt% to about 15 wt% of a citrus fiber *preparation* or a glycan equivalent thereof, about 30 wt% to about 40 wt% of a high molecular weight inulin preparation or a glycan equivalent thereof, and about 10 wt% to about 30 wt% of a barley fiber preparation or a glycan equivalent thereof.

[0191] In another embodiment, the present disclosure provides a pressed, extruded or baked food composition, wherein a 30 g serving of the food composition has at least 3 g or at least 6 g of total dietary fiber, and wherein the food composition comprises about 40 wt% to about 95 wt% of a composition of fiber preparations, the composition of fiber preparations consisting essentially of about 15 wt% to about 32 wt% of a sugar beet fiber preparation or a glycan equivalent thereof, about 5 wt% to about 15 wt% of a citrus fiber preparation or a glycan equivalent thereof, about 30 wt% to about 40 wt% of a high molecular weight inulin preparation or a glycan equivalent thereof, and about 10 wt% to about 30 wt% of a barley fiber preparation or a glycan equivalent thereof.

[0192] In another embodiment, the present disclosure provides a pressed, extruded or baked food composition, wherein a 30 g serving of the food composition has at least 6 g of total dietary fiber, and wherein the food composition comprises about 40 wt% to about 95 wt% of a composition of fiber preparations, the composition of fiber preparations consisting essentially of about 55 wt% to about 65 wt% of one or more pea fiber preparation or a glycan equivalent thereof and about 30 wt% to about 40 wt% of one or more high molecular weight inulin preparation or a glycan equivalent thereof.

[0193] In another embodiment, the present disclosure provides a pressed, extruded or baked food composition, wherein a 30 g serving of the food composition has at least 6 g of total dietary fiber, and wherein the food composition comprises about 40 wt% to about 95 wt% of a composition of fiber preparations, the composition of fiber preparations consisting essentially of about 60 wt% to about 65 wt% of one or more pea fiber preparation or a glycan equivalent thereof and about 30 wt% to about 35 wt% of one or more high molecular weight inulin preparation or a glycan equivalent thereof.

[0194] In another embodiment, the present disclosure provides a pressed, extruded or baked food composition, wherein a 30 g serving of the food composition has at least 6 g of total dietary fiber, and wherein the food composition about 40 wt% to about 95 wt% of a composition of fiber preparations, the composition of fiber preparations consisting essentially of about 55 wt% to about 65 wt% of one or more sugar beet fiber preparation or a glycan equivalent thereof and about 30 wt% to about 40 wt% of one or more high molecular weight inulin preparation or a glycan equivalent thereof.

[0195] In another embodiment, the present disclosure provides a pressed, extruded or baked food composition, wherein a 30 g serving of the food composition has at least 6 g of total dietary fiber, and wherein the food composition about 40 wt% to about 95 wt% of a composition of fiber preparations, the composition of fiber preparations consisting essentially of about 45 wt% to about 55 wt% of one or more sugar beet preparation or a glycan equivalent thereof and about 30 wt% to about 50 wt% of one or more high molecular weight inulin preparation or a glycan equivalent thereof.

[0196] In another embodiment, the present disclosure provides a pressed, extruded or baked food composition, wherein a 30 g serving of the food composition has at least 6 g of total dietary fiber and wherein the food composition about 40 wt% to about 95 wt% of a composition of fiber preparations, the composition of fiber preparations consisting essentially of about 45 wt% to about 55 wt% of one or more sugar beet preparation or a glycan equivalent thereof and about 30 wt% to about 50

wt% of one or more high molecular weight inulin preparation or a glycan equivalent thereof.

[0197] In some of the above embodiments, there may be about 30-40 wt% of one or more pea fiber preparation or a glycan equivalent thereof, about 9-11 wt% of one or more citrus fiber preparation or a glycan equivalent thereof, about 30-40 wt% of a high molecular weight inulin preparation or a glycan equivalent thereof, and about 18-22 wt% of a barley fiber preparation or a glycan equivalent thereof, in the composition of fiber preparations. In another example, there may be about 30-35 wt% of one or more pea fiber preparation or a glycan equivalent thereof, about 9-11 wt% of one or more citrus fiber preparation or a glycan equivalent thereof, about 35-40 wt% of one or more high molecular weight inulin preparation or a glycan equivalent thereof, and about 18-22 wt% of one or more barley fiber preparation or a glycan equivalent thereof, in the composition of fiber preparations. In still another example, there may be about 33 wt% of one or more pea fiber preparation or a glycan equivalent thereof, about 11 wt% of one or more citrus fiber preparation or a glycan equivalent thereof, about 36 wt% of one or more high molecular weight inulin preparation or a glycan equivalent thereof, and about 20 wt% of one or more barley fiber preparation or a glycan equivalent thereof, in the composition of fiber preparations.

[0198] In some of the above embodiments, there may be about 60 wt% to about 65 wt% of one more pea fiber preparation and about 35 wt% to about 40 wt% of one or more high molecular weight inulin preparation, in the composition of fiber preparations. In further embodiments, there may be about 65 wt% of one more pea fiber preparation and about 35 wt% of one or more high molecular weight inulin preparation, in the composition of fiber preparations.

[0199] In some of the above embodiments, there may be about 50 wt% to about 55 wt% of one more pea fiber preparation and about 35 wt% to about 40 wt% of one or more high molecular weight inulin preparation, in the composition of fiber preparations. In further embodiments, there may be about 55 wt% of one more pea fiber preparation and about 45 wt% of one or more high molecular weight inulin preparation, in the composition of fiber preparations.

[0200] In further embodiments, the composition of fiber preparation contains only one type of each fiber preparation. For instance, there may be about 55 wt% of one pea fiber preparation and about 45 wt% of one high molecular weight inulin preparation, in the composition of fiber preparations.

[0201] Suitable barley fiber preparations, citrus fiber preparations, citrus pectin preparations, high molecular weight inulin preparations, pea fiber preparations, and sugar beet fiber preparations are described above in **Section I**, as are compositional glycan equivalents and functional glycan equivalents of barley fiber preparations, citrus fiber preparations, citrus pectin preparations, high molecular weight inulin preparations, pea fiber preparations, and sugar beet fiber preparations. As non-limiting examples, the pea fiber preparation may have a composition substantially similar to the pea fiber preparation of Table A or Table G, and/or a monosaccharide content substantially similar to the pea fiber preparation of Table B or Table G,, and optionally glycosyl linkages substantially similar to the pea fiber preparation of Table C1 or C2; the high molecular weight inulin preparation may have a composition substantially similar to the high molecular weight inulin preparation of Table A or Table G; the barley fiber preparation may have a composition substantially similar to the barley fiber preparation of Table A or Table G, and/or a monosaccharide content substantially similar to the barley fiber preparation of Table B or Table G,, and optionally glycosyl linkages substantially similar to the barley fiber preparation of Table E; the citrus fiber preparation may have a composition substantially similar to the citrus fiber preparation of Table A or Table G, and/or a monosaccharide content substantially similar to the citrus fiber preparation of Table B or Table G, and optionally glycosyl linkages substantially similar to the citrus fiber preparation of Table F1 or F2.

[0202] When the food composition is a pressed or extruded food composition in the above embodiments, the composition of fiber preparations may comprise about 40 wt% to about 95 wt%, about 50 wt% to about 90 wt%, or about 60 wt % to about 80 wt% of the food. Alternatively, the composition of fiber preparations may comprise about 40 wt% to about 80 wt%, about 40 wt% to about 70 wt%, or about 40 wt % to about 60 wt% of the food composition. In still another alternative, the composition

of fiber preparations may comprise about 40 wt% to about 50 wt% of the food composition.

[0203] When the food composition is a baked food composition in the above embodiments, the composition of fiber preparations may comprise about 40 wt% to about 60 wt%, about 40 wt% to about 50 wt%, or about 50 wt% to about 60 wt% of the food composition. In still another alternative, the composition of fiber preparations may comprise about 40 wt% to about 50 wt% of the food composition.

[0204] In each of the above embodiments, the composition of fiber preparations may provide about 90% or more of the total dietary fiber in the food composition. For instance, the composition of fiber preparations may provide about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100% of the total dietary fiber in the food composition. In some embodiments, the composition of fiber preparations may provide about 95% or more of the total dietary fiber in the food composition. In some embodiments, the composition of fiber preparations may provide about 98% or more of the total dietary fiber in the food composition.

[0205] In each of the above embodiments, the baked, pressed or extruded food composition may further comprise one or more additional ingredient including, not limited to, flours, meals, sweeteners, preservatives, color additives, flavors, spices, flavor enhancers, fats, oils, fat replacers (including components of formulations used to replace fats), nutrients, vitamins, minerals, emulsifiers, stabilizers, thickeners, binders, texturizers, pH control agents, leavening agents, anti-caking agents, humectants, firming agents, and enzyme preparations. These additional ingredients may contribute favorable organoleptic properties (e.g., taste, texture, etc.) to the food and/or improve the processing and handling of the food.

[0206] In a specific embodiment, a baked, pressed or extruded food has a composition shown in **Table H** or **Table I**.

Table H

INGREDIENTS	**Composition 1**	**Composition 2**	**Composition 3**

Flours: Wheat, Rice, Corn or a blend	0 – 60 wt%	14.4 wt%	27.9 wt%
Sugar	0 – 30 wt%	5 wt%	5 wt%
Rice Starch (waxy variety)	0 – 40 wt%	30 wt%	20 wt%
Salt	0 – 5 wt%	0.5 wt%	0.5 wt%
Sodium Bicarbonate or Calcium carbonate	0 – 5 wt%	1 wt%	1 wt%
GMS (emulsifier)	0 – 2 wt%	0.2 wt%	0.2 wt%
Composition of Fiber Preparations	40 - 95 wt%	49.0 wt%	45.5 wt%
TOTAL	**100**	**100**	**100**

Table I

INGREDIENTS	Composition			
	1	**2**	**3**	**4**
Flours: Wheat, Rice, Corn or a blend	14.4 wt%	27.9 wt%	23.4 wt%	36.9 wt%
Sugar	5 wt%	5 wt%	5 wt%	5 wt%
Rice Starch (waxy variety)	30 wt%	20 wt%	30 wt%	20 wt%
Salt	0.5 wt%	0.5 wt%	0.5 wt%	0.5 wt%
Sodium Bicarbonate or Calcium carbonate	1 wt%	1 wt%	1 wt%	1 wt%
GMS (emulsifier)	0.2 wt%	0.2 wt%	0.2 wt%	0.2 wt%
Pea Fiber Preparation	16.2 wt%	29.1 wt%	0 wt%	0 wt%
Citrus Fiber Preparation	5.4 wt%	0 wt%	5.4 wt%	0 wt%
Inulin, HMW Preparation	17.6 wt%	16.4 wt%	17.6 wt%	16.4 wt%
Barley Fiber Preparation	9.8 wt%	0 wt%	9.8 wt%	0 wt%
Sugar Beet Fiber Preparation	0 wt%	0 wt%	7.2 wt%	20.1 wt%
TOTAL	**100**	**100**	**100**	**100**

(b) increases the fiber degrading capacity and/or promotes a healthy gut microbiota

[0207] The "fiber degrading capacity" of a subject's gut microbiota is defined by its compositional state, specifically the absence, presence and abundance of

primary and secondary consumers of dietary fiber. Microbes that are primary consumers initiate degradation of dietary fibers, while secondary consumers utilize glycans that are released by primary consumers. Increasing the fiber degrading capacity of a subject's gut microbiota may include, for example and without limitation, effecting an increase in the total and/or relative abundance of microorganisms with polysaccharide utilization loci (PULs) and/or genomic loci encoding CAZymes measured in a fecal sample obtained from a subject after the subject has consumed the food composition at least once a day for at least 5 days (e.g., at least 6 days, at least 7 days, etc.). In another example, increasing the fiber degrading capacity of a subject's gut microbiota may effect an increase in the total and/or relative abundance of a subset (one or more) of microorganisms with polysaccharide utilization loci (PULs) and/or genomic loci encoding CAZymes measured in a fecal sample obtained from a subject after the subject has consumed the food composition at least once a day for at least 5 days (e.g., at least 6 days, at least 7 days, etc.), the subset of microorganisms chosen from *Bacteroides ovatus*, *Bacteroides cellulosilyticus*, *Bacteroides thetaiotaomicron*, *Bacteroides vulgatus*, *Bacteroides caccae*, *Bacteroides finegoldii*, *Bacteroides massiliensis*, *Collinsella aerofaciens*, *Escherichia coli*, *Odoribacter splanchnicus*, *Parabacteroides distasonis*, a *Ruminococcaceae sp.,* or *Subdoligranulum variabile*. In another example, increasing the fiber degrading capacity of a subject's gut microbiota may effect an increase in the total or relative abundance of *Bacteroides* species measured in a fecal sample obtained from a subject after the subject has consumed the food composition at least once a day for at least 5 days (e.g., at least 6 days, at least 7 days, etc.). In another example, increasing the fiber degrading capacity of a subject's gut microbiota may effect an increase in the total or relative abundance of a subset (one or more) of *Bacteroides* species measured in a fecal sample obtained from a subject after the subject has consumed the food composition at least once a day for at least 5 days (e.g., at least 6 days, at least 7 days, etc.), the subset of *Bacteroides* species chosen from *B. caccae*, *B. cellulosilyticus*, *B. finegoldii*, *B. massiliensis*, *B. ovatus*, *B. thetaiotaomicron*, or *B. vulgatus*. Alternatively or in addition, increasing the fiber degrading capacity of a subject's gut microbiota may include effecting an increase in the

abundance or activity of one or more protein encoded by a PUL (with or without concomitant changes in microorganism abundance) and/or one or more CAZyme. In some examples, the one or more protein with an increased abundance or activity has α-L-arabinofuranosidase, β-galactosidase, N-acetylmuramidase, or endo-1,2,-α-mannanase enzymatic activities. In the above examples, the PULs may be chosen from PUL5, PUL6, PUL7, PUL27, PUL31, PUL34, PUL35, PUL38, PUL42, PUL43, PUL73, PUL75, PUL83, and PUL97, and/or the one or CAZymes may be chosen from GH5_1, GH5_4, GH5_5, GH5_46, GH43_1, GH43_2, GH43_3, GH43_8, GH43_9, GH43_12, GH43_16, GH43_17, GH43_18, GH43_19, GH43_28, GH43_29, GH43_31, GH43_33, GH43_34, GH43_35, GH43_38, GH99, GH108, GH116, and GH147.

[0208] In some embodiments, administration of a food composition described in this section, at least once daily for a minimum of five days, to a subject, increases the representation of members of one or more CAZyme family measured in a fecal sample obtained from the subject, wherein the one or more CAZyme family is selected from the group consisting of GH5_1, GH5_4, GH5_5, GH5_46, GH43_1, GH43_2, GH43_3, GH43_8, GH43_9, GH43_12, GH43_16, GH43_17, GH43_18, GH43_19, GH43_28, GH43_29, GH43_31, GH43_33, GH43_34, GH43_35, GH43_38, GH99, GH108, GH116, and GH147. In further embodiments, the one or more CAZyme family is selected from GH43_33, GH147, GH108, and GH99. As detailed in the Examples, increased representation of members of a CAZyme family may be an increase in genes encoding members of a CAZyme family. Increased representation of a CAZyme family may also be an increase in the abundance or activity of proteins in a CAZyme family. Methods for measuring protein abundance and enzyme activity are known in the art. Increasing the representation of one or more of these CAZyme families has a beneficial effect on or more aspects of a subject's health including but not limited to gut microbiota health, weight management, chronic inflammation, cardiovascular health, satiety, and glucose metabolism. In some examples, the subject is a healthy subject. In some examples, the subject is overweight or obese (e.g., as defined by a BMI outside the normal range for the subject's age, sex, and/or ethnicity). In some examples, the subject typically consumes a diet low in total dietary fiber (e.g.,

less than about 25 g per day). In some examples, the subject typically consumes a Western diet. A "Western diet" refers to a diet high in red meat, dairy products, processed and artificially sweetened foods and/or drinks, and salt, with minimal intake of fruits, vegetables, fish, legumes, and whole grains. An exemplary Western diet is the HiSF/LoFV diet detailed in the examples that is suitable for animals), and human equivalents thereof.

[0209] To "promote a healthy gut microbiota in a subject" means to change the feature of the microbiota or microbiome of the subject with the unhealthy gut microbiota in a manner towards the healthy subjects, and encompasses complete repair (i.e., the measure of gut microbiota health does not deviate by 1.5 standard deviation or more) and levels of repair that are less than complete. This may include, for example and without limitation, effecting an increase in the total abundance of *Bacteroides* species measured in a fecal sample obtained from a subject after the subject has consumed the food at least once a day for 5 days (e.g., at least 6 days, at least 7 days, etc.). Promoting a healthy gut microbiota in a subject also includes preventing the development of an unhealthy gut microbiota in a subject. In preferred embodiments, the microbiota of a subject is changed with regards to relative abundances of microbial community members and/or expression of proteins encoded by PULs, for instance as detailed in the Examples.

[0210] In still further embodiments, food compositions of the present disclosure have a beneficial effect on a subject's health after the subject has consumed the food composition for at least once a day for at least 5 days, or at least 7 days. For instance, administration for 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or more days may result in a beneficial effect. The improved aspect of the subject's health may be an improvement in weight management, chronic inflammation, cardiovascular health, satiety, and/or glucose metabolism. Non-limiting examples of measurable improvements in weight management may be a reduction in total body weight, a reduction in BMI, a reduction in weight gain, a reduction in fat mass gain, an increase in lean mass, a decrease in waist circumference, a decrease in waist to hip ratio, an increase in adiponectin levels, an increase in leptin levels, a decrease in resistin levels, or any combination thereof. Non-

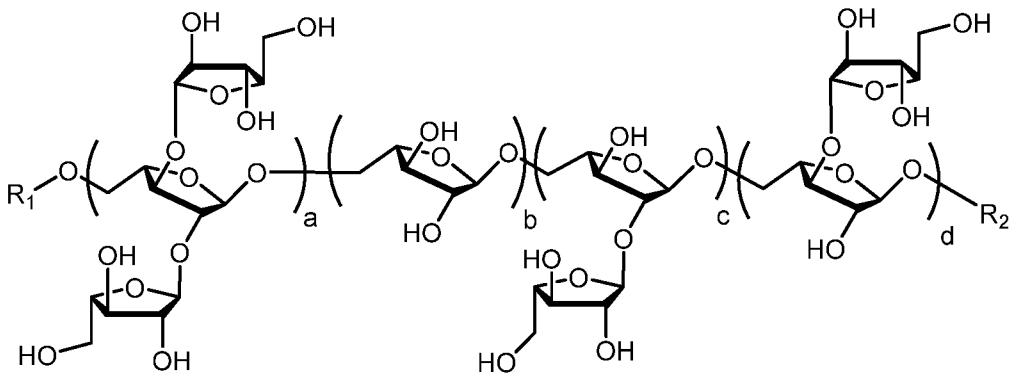
limiting examples of measurable improvements in chronic inflammation include a decrease in one or more plasma protein selected from CCL3, CRP, SPP1, F2, F3, VEGFA, PDGFRB, EFNA5, EPHA1, EPHA2, IL-6, IL-8, IL-1b, IL-1R1, IL-12, IL-17, IL-18, TNF-α, NF-kB, IFN-γ, and ceramides. Non-limiting examples of measurable improvements in cardiovascular health include a decrease in one or more plasma protein selected from C3, C1R, C4A/C4B, F3, SERPINE1, MASP1, PDGFRA, ICAM-1, VCAM-1, MCP-1, PAI-1, P-selectin, thromboxane-A2, F2a-isoprostanes, TBARS, MDA; as well as changes in LDL-cholesterol, HDL-cholesterol, total cholesterol, oxidized LDL, triglycerides, platelet aggregation and blood clotting. Non-limiting examples of measurable improvements in glucose metabolism include changes in fasting glucose, postprandial glucose, fasting insulin, postrprandial glucose, HOMAIR, HbA1c, glycated albumin, fructosamine, glucagon, QIUCKI, ISI, GIP, and GLP-1. Non-limiting examples of measurable improvements in satiety include improvements in AGRP, appetite VAS scores, food intake, GLP-1, PYY, GIP, ghrelin, cholecystokinin and leptin. In some examples, the improved aspect of the subject's health may be a reduction in total body weight, a reduction in BMI, a reduction in weight gain, a reduction in fat mass gain, an increase in fecal levels of succinate, a decrease in serum cholesterol, an increase in insulin sensitivity, a decrease in plasma markers of inflammation, an improvement in the relative abundances of health discriminatory plasma proteins, and/or an improvement in biomarkers/mediators of gut barrier function.

III. BIOACTIVE GLYCANS

[0211] Applicants have identified fiber preparations that promote a healthy gut microbiota in a subject, and further discovered that each fiber preparation has a number of bioactive glycans responsible for the observed beneficial effect(s). Thus, in another aspect, the present disclosure provides a composition comprising an enriched amount of one or more bioactive glycan, wherein "an enriched amount" refers to an amount of a bioactive glycan that is more than is found in a naturally occurring plant or plant part, and more than is found in commercially available fiber preparations, such as those used in Examples 2-6. A composition comprising an enriched amount of a bioactive glycan may be a purified (partially or completely) fraction from a commercially

available fiber preparation. Alternatively, a composition comprising an enriched amount of a bioactive glycan may comprise a chemically synthesized version of the bioactive glycan. The bioactive glycan may be enriched by about 10 wt% wt to about 50 wt%, about 50 wt% to about 100 wt% or more. For instance, the bioactive glycan may be enriched by about 2-fold, about 3-fold, about 4-fold, about 5-fold, about 6-fold, about 7-fold, about 8-fold, about 9-fold, about 10-fold or more. In another example, the bioactive glycan may be enriched by about 20-fold, about 30-fold, about 40-fold, about 50-fold, about 60-fold, about 70-fold, about 80-fold, about 90-fold, about 100-fold or more. In another example, the bioactive glycan may be enriched by about 500-fold, 1000-fold, or more.

[0212] Bioactive glycans of barley fiber, citrus fiber, citrus pectin, high molecular weight inulin, pea fiber, and sugar beet fiber can be identified as detailed herein. For instance, pea fiber includes one or more bioactive arabinan of formula (I)



wherein a is about 0.1 to about 0.3, b is about 0.4 to about 0.6, c is about 0.1 to about 0.4, d is about 0.04 to about 0.06 (calculated from the fractional abundance of arabinose linkages where the arabinose contained a 5-linkage, as determined by partially methylated alditol acetate GC-MS analysis); wherein R_1 and R_2 are each independently selected from H, a glycosyl, a sugar moiety (modified or not), an oligosaccharide (branched or not), or a polysaccharide (branched or not), and a polysaccharide containing galacturonic acid, galactose, and rhamnose. Example 10 describes methods for obtaining a composition that is enriched for this bioactive arabinan; however, alternative purification methods may also be used. Alternatively, a

chemically synthesized version may be used. An approach similar to the one detailed in Example 10 may be used to identify bioactive glycans in barley fiber, citrus fiber, citrus pectin, high molecular weight inulin, and sugar beet fiber.

[0213] The present disclosure also provides food compositions comprising a composition of this section. The food composition may further comprise one or more additional food ingredient including, not limited to, flours, meals, sweeteners, preservatives, color additives, flavors, spices, flavor enhancers, fats, oils, fat replacers (including components of formulations used to replace fats), nutrients, vitamins, minerals, emulsifiers, stabilizers, thickeners, binders, texturizers, pH control agents, leavening agents, anti-caking agents, humectants, firming agents, probiotics, and enzyme preparations.

[0214] The amount of a composition of this section in a food composition may vary. In some embodiments, a composition may be about 40 wt% to about 60 wt% of the ingredients used to make the food composition (excluding any added water). In some embodiments, a composition may be about 45 wt% to about 50 wt% of the ingredients used to make the food composition (excluding any added water).

[0215] In certain embodiments, the composition provides about 90% or more of the total dietary fiber in the food composition. For instance, the composition may provide about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100% of the dietary fiber in the food composition. In one example, the composition provides about 95% or more of the total dietary fiber in the food composition. In another example, the composition provides about 98% or more of the total dietary fiber in the food composition.

[0216] In further embodiments, the food composition provides at least 6 g of dietary fiber per serving. In some examples, the food composition may provide at least 7 grams, at least 8 grams, at least 9 grams, or at least 10 grams of dietary fiber per serving. In other examples, the food composition may provide about 6 g to about 20 g, about 6 g to about 15 g, or about 6 g to about 10 g of dietary fiber per serving.

[0217] In some embodiments, a food composition is in a baked form. In some embodiments, a food composition is in a pressed or extruded form. In some

embodiments, a food is in a powder form to be reconstituted. In some embodiments, a food is a bar; a drink; a gel, a gummy, a candy or the like; a cookie, a cracker, a cake, or the like; a dairy product (e.g., yogurt, ice cream or the like).

[0218] The present disclosure also provides other oral dosage forms comprising a composition of this section. Suitable dosage forms include a tablet, including a suspension tablet, a chewable tablet, an effervescent tablet or caplet; a pill; a powder such as a sterile packaged powder, a dispensable powder, and an effervescent powder; a capsule including both soft or hard gelatin capsules such as HPMC capsules; a lozenge; pellets; granules; liquids; suspensions; emulsions; or semisolids and gels. Capsule and tablet formulations may include, but are not limited to binders, lubricants, and diluents. Capsules and tablets may be coated according to methods well known in the art. Aqueous suspension formulations may include but are not limited to dispersants, flavor-modifying agents, taste-masking agents, and coloring agents.

IV. METHODS

[0219] In another aspect, the present disclosure provides methods for increasing the fiber degrading capacity of a subject's gut microbiota, promoting a healthy gut microbiota in a subject and/or improving a subject's health, the method comprising orally administering to a subject at least 3 grams or at least 6 grams of total dietary fiber per day in the form of a composition of **Section I** or **Section III**, or a food composition of **Section I, II,** or **III**. At least 3 grams of total dietary fiber per day includes 3 grams, 4 grams, 5 grams, 6 grams, 7 grams, or more of total dietary fiber per day. At least 6 grams of total dietary fiber per day includes 6 grams, 7 grams, 8 grams, 9 grams, 10 grams, 11 grams, 12 grams, 13 grams, 14, grams, 15 grams or more of total dietary fiber per day. In some embodiments, the method comprises orally administering to a subject at least 7 grams, at least 8 grams, at least 9 grams, or at least 10 grams of total dietary fiber per day in the form of a composition of **Section I** or **Section III**, or a food composition of **Section I, II,** or **III**. In some embodiments, the method comprises orally administering to a subject about 6 grams to about 10 grams of total dietary fiber per day in the form of a composition of **Section I** or **Section III**, or a food composition of

Section I, II, or **III**. If the composition of **Section I** or **Section III** does not contain at least 6 g of total dietary fiber, multiple doses of the composition can be administered. Similarly, the number of servings of the food composition of **Section I, II,** or **III** can be adjusted such that at least 6 g of dietary fiber is consumed by the subject.

[0220] In some examples, increasing the fiber degrading capacity of a subject's gut microbiota may include effecting an increase in the total and/or relative abundance of microorganisms with polysaccharide utilization loci (PULs) measured in a fecal sample obtained from a subject after the subject has consumed at least 3 grams or at least 6 grams of total dietary fiber per day in the form of a composition of **Section I** or **Section III**, or a food composition of **Section I, II,** or **III**. In another example, increasing the fiber degrading capacity of a subject's gut microbiota may effect an increase in the total and/or relative abundance of a subset (one or more) of microorganisms with polysaccharide utilization loci (PULs) measured in a fecal sample obtained from a subject after the subject has consumed at least 3 grams or at least 6 grams of total dietary fiber per day in the form of a composition of **Section I** or **Section III**, or a food composition of **Section I, II,** or **III**, the subset of microorganisms chosen from *Bacteroides ovatus*, *Bacteroides cellulosilyticus*, *Bacteroides thetaiotaomicron*, *Bacteroides vulgatus*, *Bacteroides caccae*, *Bacteroides finegoldii*, *Bacteroides massiliensis*, *Collinsella aerofaciens*, *Escherichia coli*, *Odoribacter splanchnicus*, *Parabacteroides distasonis*, a *Ruminococcaceae sp.,* or *Subdoligranulum variabile*. In another example, increasing the fiber degrading capacity of a subject's gut microbiota may effect an increase in the total or relative abundance of *Bacteroides* species measured in a fecal sample obtained from a subject after the subject has consumed at least 3 grams or at least 6 grams of total dietary fiber per day in the form of a composition of **Section I** or **Section III**, or a food composition of **Section I, II,** or **III**. In another example, increasing the fiber degrading capacity of a subject's gut microbiota may effect an increase in the total or relative abundance of a subset (one or more) of *Bacteroides* species measured in a fecal sample obtained from a subject after the subject has consumed at least 3 grams or at least 6 grams of total dietary fiber per day in the form of a composition of **Section I** or **Section III**, or a food composition of

Section I, II, or **III**, the subset of *Bacteroides* species chosen from *B. caccae*, *B. cellulosilyticus*, *B. finegoldii*, *B. massiliensis*, *B. ovatus*, *B. thetaiotaomicron*, or *B. vulgatus*. Alternatively or in addition, increasing the fiber degrading capacity of a subject's gut microbiota may include effecting an increase in the abundance or activity of one or more protein encoded by a PUL (with or without concomitant changes in microorganism abundance). In some examples, the one or more protein with an increased abundance or activity has α-L-arabinofuranosidase, β-galactosidase, N-acetylmuramidase, or endo-1,2,-α-mannanase enzymatic activities. In the above examples, the PULs are chosen from PUL5, PUL6, PUL7, PUL27, PUL31, PUL34, PUL35, PUL38, PUL42, PUL43, PUL73, PUL75, PUL83, and PUL97, and/or the one or CAZymes may be chosen from GH5_1, GH5_4, GH5_5, GH5_46, GH43_1, GH43_2, GH43_3, GH43_8, GH43_9, GH43_12, GH43_16, GH43_17, GH43_18, GH43_19, GH43_28, GH43_29, GH43_31, GH43_33, GH43_34, GH43_35, GH43_38, GH99, GH108, GH116, and GH147.

[0221] To "promote a healthy gut microbiota in a subject" means to change the feature of the microbiota or microbiome of the subject with the unhealthy gut microbiota in a manner towards the healthy subjects, and encompasses complete repair (i.e., the measure of gut microbiota health does not deviate by 1.5 standard deviation or more) and levels of repair that are less than complete.This may include, for example and without limitation, effecting an increase in the total abundance of *Bacteroides* species measured in a fecal sample obtained from a subject after the subject has consumed at least 3 grams or at least 6 grams of total dietary fiber per day in the form of a composition of **Section I** or **Section III**, or a food composition of **Section I, II,** or **III**. Promoting a healthy gut microbiota in a subject also includes preventing the development of an unhealthy gut microbiota in a subject. In preferred embodiments, the microbiota of a subject is changed with regards to relative abundances of microbial community members and/or expression of proteins encoded by PULs or members of CAZymes families, for instance as detailed in the Examples.

[0222] To "improve a subject's health" means to change one or more aspects of a subject's health in a manner towards healthy subjects with similar

environmental exposures, such as geography, diet, and age. The improved aspect of the subject's health may be an improvement in weight management, chronic inflammation, cardiovascular health, satiety, and/or glucose metabolism. Non-limiting examples of measurable improvements in weight management may be a reduction in total body weight, a reduction in BMI, a reduction in weight gain, a reduction in fat mass gain, an increase in lean mass, a decrease in waist circumference, a decrease in waist to hip ratio, an increase in adiponectin levels, an increase in leptin levels, a decrease in resistin levels, or any combination thereof. Non-limiting examples of measurable improvements in chronic inflammation include a decrease in one or more plasma protein selected from CCL3, CRP, SPP1, F2, F3, VEGFA, PDGFRB, EFNA5, EPHA1, EPHA2, IL-6, IL-8, IL-1b, IL-1R1, IL-12, IL-17, IL-18, TNF-α, NF-kB, IFN-γ, and ceramides. Non-limiting examples of measurable improvements in cardiovascular health include a decrease in one or more plasma protein selected from C3, C1R, C4A/C4B, F3, SERPINE1, MASP1, PDGFRA, ICAM-1, VCAM-1, MCP-1, PAI-1, P-selectin, thromboxane-A2, F2a-isoprostanes, TBARS, MDA, as well as changes in LDL-cholesterol, HDL-cholesterol, total cholesterol, oxidized LDL, triglycerides, platelet aggregation and blood clotting. Non-limiting examples of measurable improvements in glucose metabolism include changes in fasting glucose, postprandial glucose, fasting insulin, postrprandial glucose, HOMAIR, HbA1c, glycated albumin, fructosamine, glucagon, QIUCKI, ISI, GIP, and GLP-1. Non-limiting examples of measurable improvements in satiety include improvements in AGRP, appetite VAS scores, food intake, GLP-1, PYY, GIP, ghrelin, cholecystokinin and leptin. In some examples, the improved aspect of the subject's health may be a reduction in total body weight, a reduction in BMI, a reduction in weight gain, a reduction in fat mass gain, an increase in fecal levels of succinate, a decrease in serum cholesterol, an increase in insulin sensitivity, a decrease in plasma markers of inflammation, an improvement in the relative abundances of health discriminatory plasma proteins, and/or an improvement in biomarkers/mediators of gut barrier function.

[0223] In a specific embodiment, the present disclosure provides a method of decreasing weight gain of a subject on a Western diet, the method comprising

administering to the subject a composition comprising at least 15 wt% of one or more pea fiber preparation or a glycan equivalent thereof; and at least one additional fiber preparation chosen from (i) between 0 wt% and 28 wt% (inclusive) of one or more high molecular weight inulin preparation or a glycan equivalent thereof; (ii) between 0 wt% and 10 wt% (inclusive) of one or more citrus pectin preparation or a glycan equivalent thereof; (iii) between 0 wt% and 25 wt% (inclusive) of one or more citrus fiber preparation or a glycan equivalent thereof; or (iv) between 0 wt% and 45 wt% (inclusive) of one or more barley fiber preparation or a glycan equivalent thereof, wherein the administration is at least once a day, in conjunction with the Western diet, for at least 5 days, when weight gain is measured against a population of similar subjects on the same diet without administration of said composition.

[0224] In another specific embodiment, the present disclosure provides a method of decreasing the abundance of one or more plasma proteins involved in inflammation in a subject, the method comprising administering to the subject, at least once daily for at least five days, a composition comprising at least 15 wt% of one or more pea fiber preparation or a glycan equivalent thereof; and at least one additional fiber preparation chosen from (i) between 0 wt% and 28 wt% (inclusive) of one or more high molecular weight inulin preparation or a glycan equivalent thereof; (ii) between 0 wt% and 10 wt% (inclusive) of one or more citrus pectin preparation or a glycan equivalent thereof; (iii) between 0 wt% and 25 wt% (inclusive) of one or more citrus fiber preparation or a glycan equivalent thereof; or (iv) between 0 wt% and 45 wt% (inclusive) of one or more barley fiber preparation or a glycan equivalent thereof, wherein the one or more proteins are selected from the group consisting of CCL3, CRP, SPP1, F2, F3, VEGFA, PDGFRB, EFNA5, EPHA1, EPHA2, and IL1R1.

[0225] In another specific embodiment, the present disclosure provides a method of treating inflammation in a subject, the method comprising decreasing the abundance of one or more plasma proteins involved in inflammation by administering to the subject, at least once daily for at least five days, a composition comprising at least 15 wt% of one or more pea fiber preparation or a glycan equivalent thereof; and at least one additional fiber preparation chosen from (i) between 0 wt% and 28 wt% (inclusive)

of one or more high molecular weight inulin preparation or a glycan equivalent thereof; (ii) between 0 wt% and 10 wt% (inclusive) of one or more citrus pectin preparation or a glycan equivalent thereof; (iii) between 0 wt% and 25 wt% (inclusive) of one or more citrus fiber preparation or a glycan equivalent thereof; or (iv) between 0 wt% and 45 wt% (inclusive) of one or more barley fiber preparation or a glycan equivalent thereof, wherein the one or more proteins are selected from the group consisting of CCL3, CRP, SPP1, F2, F3, VEGFA, PDGFRB, EFNA5, EPHA1, EPHA2, and IL1R1.

[0226] In another specific embodiment, the present disclosure provides a method of increasing the representation of one or more CAZyme families in gut microbiome, wherein the one or more CAZyme families are selected from the group consisting of GH43_33, GH116, GH147, GH108, and GH99 activities, the method comprising administering to the subject, at least once daily for at least five days, a composition comprising at least 15 wt% of one or more pea fiber preparation or a glycan equivalent thereof; and at least one additional fiber preparation chosen from (i) between 0 wt% and 28 wt% (inclusive) of one or more high molecular weight inulin preparation or a glycan equivalent thereof; (ii) between 0 wt% and 10 wt% (inclusive) of one or more citrus pectin preparation or a glycan equivalent thereof; (iii) between 0 wt% and 25 wt% (inclusive) of one or more citrus fiber preparation or a glycan equivalent thereof; or (iv) between 0 wt% and 45 wt% (inclusive) of one or more barley fiber preparation or a glycan equivalent thereof.

[0227] In another specific embodiment, the present disclosure provides a method of decreasing the abundance of one or more plasma proteins involved in platelet activation and blood coagulation in a subject, the method comprising administering to the subject, at least once daily for at least five days, a composition comprising at least 15 wt% of one or more pea fiber preparation or a glycan equivalent thereof; and at least one additional fiber preparation chosen from (i) between 0 wt% and 28 wt% (inclusive) of one or more high molecular weight inulin preparation or a glycan equivalent thereof; (ii) between 0 wt% and 10 wt% (inclusive) of one or more citrus pectin preparation or a glycan equivalent thereof; (iii) between 0 wt% and 25 wt% (inclusive) of one or more citrus fiber preparation or a glycan equivalent thereof; or (iv)

between 0 wt% and 45 wt% (inclusive) of one or more barley fiber preparation or a glycan equivalent thereof, wherein the one or more plasma proteins are selected from the group consisting of C3, C1R, C4A/C4B, F3, SERPINE1, MASP1, and PDGFRA.

[0228] In another specific embodiment, the present disclosure provides a method of decreasing the abundance of appetite-stimulating agouti-related protein (AGRP) in a subject, the method comprising administering to the subject, at least once daily for at least five days, a composition comprising at least 15 wt% of one or more pea fiber preparation or a glycan equivalent thereof; and at least one additional fiber preparation chosen from (i) between 0 wt% and 28 wt% (inclusive) of one or more high molecular weight inulin preparation or a glycan equivalent thereof; (ii) between 0 wt% and 10 wt% (inclusive) of one or more citrus pectin preparation or a glycan equivalent thereof; (iii) between 0 wt% and 25 wt% (inclusive) of one or more citrus fiber preparation or a glycan equivalent thereof; or (iv) between 0 wt% and 45 wt% (inclusive) of one or more barley fiber preparation or a glycan equivalent thereof.

[0229] In another specific embodiment, the present disclosure provides a method of decreasing the abundance of one or more plasma proteins associated with inflammation and cardiovascular disease, wherein the proteins are selected from the group consisting of CCL3 and CRP, the method comprising administering to a subject, at least once daily for at least five days, a composition comprising at least 15 wt% of one or more pea fiber preparation or a glycan equivalent thereof; and at least one additional fiber preparation chosen from (i) between 0 wt% and 28 wt% (inclusive) of one or more high molecular weight inulin preparation or a glycan equivalent thereof; (ii) between 0 wt% and 10 wt% (inclusive) of one or more citrus pectin preparation or a glycan equivalent thereof; (iii) between 0 wt% and 25 wt% (inclusive) of one or more citrus fiber preparation or a glycan equivalent thereof; or (iv) between 0 wt% and 45 wt% (inclusive) of one or more barley fiber preparation or a glycan equivalent thereof.

[0230] In each of the above embodiments, the composition may comprise (i) about 25 wt% to about 40 wt% of one or more pea fiber preparation or a glycan equivalent thereof, about 5 wt% to about 15 wt% of one or more citrus fiber preparation or a glycan equivalent thereof, about 30 wt% to about 40 wt% of a high molecular

weight inulin preparation or glycan equivalent thereof, about 10 wt % to about 30 wt% of a barley fiber preparation or glycan equivalent thereof; or (ii) about 30 wt% to about 40 wt% of one or more pea fiber preparation or a glycan equivalent thereof, about 10 wt% to about 20 wt% of one or more citrus fiber preparation or a glycan equivalent thereof, about 30 wt% to about 40 wt% of a high molecular weight inulin preparation or glycan equivalent thereof, about 15 wt % to about 25 wt% of a barley fiber preparation or glycan equivalent thereof; or (iii) about 55 wt% to about 65 wt% of one or more pea fiber preparation or a glycan equivalent thereof and about 30 wt% to about 40 wt% of a high molecular weight inulin preparation or glycan equivalent thereof; or (iv) about 60 wt% to about 70 wt% of one or more pea fiber preparation or a glycan equivalent thereof and about 30 wt% to about 40 wt% of a high molecular weight inulin preparation or glycan equivalent thereof; or (v) about 60 wt% to about 65 wt% of one or more pea fiber preparation or a glycan equivalent thereof and about 35 wt% to about 40 wt% of a high molecular weight inulin preparation or glycan equivalent thereof.

[0231] In each of the above embodiments, the pea fiber preparation may have a composition substantially similar to the pea fiber preparation of Table A or Table G, and/or a monosaccharide content substantially similar to the pea fiber preparation of Table B or Table G,, and optionally glycosyl linkages substantially similar to the pea fiber preparation of Table C1 or Table C2; the high molecular weight inulin preparation has a composition substantially similar to the high molecular weight inulin preparation of Table A or Table G; the barley fiber preparation has a composition substantially similar to the barley fiber preparation of Table A or Table G, and/or a monosaccharide content substantially similar to the barley fiber preparation of Table B or Table G, and optionally glycosyl linkages substantially similar to the barley fiber preparation of Table E; the citrus fiber preparation has a composition substantially similar to the citrus fiber preparation of Table A or Table G, and/or a monosaccharide content substantially similar to the citrus fiber preparation of Table B or Table G, and optionally glycosyl linkages substantially similar to the citrus fiber preparation of Table F1 or Table F2.

[0232] In each of the above embodiments, the composition may be administered as part of a food composition. Alternatively, each of the fiber preparations

comprising the composition may be individual ingredients in a food composition and the food composition administered to a subject. The duration of administration may vary depending upon a variety of factors, including the severity of disrepair and/or the health of the subject. Typically, the duration of administration may be for at least one week, at least two weeks, at least three weeks, or at least four weeks. In some examples, a composition may be administered for about 1 month, about 2 months, about 3 months, about 4 months or more. In some examples, a composition or food composition may be administered for about 6 months, about 12 months, or more. In some examples, a composition or food composition may be administered for about 1 month to about 6 months. In some examples, a composition or food composition may be administered for about 6 months to about 12 months.

[0233] In some of the above embodiments, a subject is a healthy subject (e.g., a healthy BMI, adequate dietary fiber intake, no chronic or acute disease, etc.) looking to promote a healthy gut microbiota.

[0234] In some of the above embodiments, a subject has a diet that is high in saturated fats and/or low in fruits and vegetables, a total dietary fiber intake less than 30 grams a day, a total dietary fiber intake less than 25 grams a day, a total dietary fiber intake less than 20 grams a day, a total dietary fiber intake less than 15 grams a day, a total dietary fiber intake less than 10 grams a day, a BMI of 25 or greater, or any combination thereof.

[0235] In some of the above embodiments, a subject may have insulin insensitivity, insulin resistance, type I diabetes mellitus, type II diabetes mellitus, systemic inflammation, a chronic inflammatory disease, heart disease, cardiovascular disease, high cholesterol, high blood pressure, or any combination thereof. In some embodiments, a subject may have an increased risk of developing insulin insensitivity, insulin resistance, type I diabetes mellitus, type II diabetes mellitus, systemic inflammation, a chronic inflammatory disease, heart disease, cardiovascular disease, high cholesterol, high blood pressure, or any combination thereof, whether due to family history or lifestyle.

[0236] In some of the above embodiments, a subject is prone to having a gut microbiota in disrepair. Subjects prone to have a gut microbiota in disrepair may or may not have a measurable change in a measure of gut microbiota health as compared to reference healthy subjects, and confirmation of the health status of the subject's gut microbiota is not needed. Subjects prone to have a gut microbiota in disrepair include but are not limited to subjects that have a diet that is high in saturated fats and/or low in fruits and vegetables, a total dietary fiber intake less than 30 grams a day, a total dietary fiber intake less than 25 grams a day, a total dietary fiber intake less than 20 grams a day, a total dietary fiber intake less than 15 grams a day, a total dietary fiber intake less than 10 grams a day, a BMI of 25 or greater, insulin insensitivity, insulin resistance, type I diabetes mellitus, type II diabetes mellitus, systemic inflammation or a chronic inflammatory disease, heart disease, cardiovascular disease, high cholesterol, high blood pressure, or any combination thereof.

[0237] In some of the above embodiments, a subject has gut microbiota in disrepair. In further embodiments, the subject has a total dietary fiber intake less than 30 grams a day, a total dietary fiber intake less than 25 grams a day, a total dietary fiber intake less than 20 grams a day, a total dietary fiber intake less than 15 grams a day, a total dietary fiber intake less than 10 grams a day, a BMI of 25 or greater, insulin insensitivity, insulin resistance, type I diabetes mellitus, type II diabetes mellitus, systemic inflammation or a chronic inflammatory disease, heart disease, high cholesterol, high blood pressure, or any combination thereof.

[0238] In some of the above embodiments, the subject is overweight or obese (e.g., as defined by a BMI outside the normal range for the subject's age, sex, and/or ethnicity). In some of the above embodiments, the subject typically consumes a diet low in total dietary fiber (e.g., less than about 25 g per day). In some of the above embodiments, the subject typically consumes a Western diet. An exemplary Western diet is the HiSF/LoFV diet detailed in the examples that is suitable for animals), and human equivalents thereof.

NUMBERED EMBODIMENTS

[0239] 1. A composition comprising a plurality of fiber preparations, each fiber preparation independently selected from the group consisting of a barley fiber preparation, a citrus fiber preparation, a citrus pectin formulation, a high molecular weight inulin preparation, a pea fiber preparation, and a sugar beet fiber preparation, wherein the plurality of fiber preparations is at least 95 wt% of the composition.

[0240] 2. The composition of embodiment 1, wherein the composition comprises one or more citrus pectin preparation in an amount that does not exceed 10 wt%.

[0241] 3. The composition of embodiment 1, wherein the composition comprises one or more citrus fiber preparation in an amount that does not exceed 25 wt%.

[0242] 4. The composition of embodiment 1, wherein the composition comprises at least 15 wt% of one or more pea fiber preparation.

[0243] 5. The composition of embodiment 1, wherein the composition comprises at least 28 wt% of one or more high molecular weight inulin preparation.

[0244] 6. The composition of embodiment 1, wherein the composition comprises one or more barley fiber preparation in an amount that does not exceed 45 wt%.

[0245] 7. The composition of embodiment 1, wherein the composition comprises at least 15 wt% of one or more sugar beet fiber preparation.

[0246] 8. The composition of embodiment 1, wherein the composition comprises (a) at least 15 wt% of one or more pea fiber preparation and at least 28 wt% of one or more high molecular weight inulin preparation; (b) the total amount of citrus pectin preparations does not exceed 10 wt%, (c) the total amount of citrus fiber preparations does not exceed 25 wt%, and (d) the total amount of barley fiber preparations does not exceed 45 wt%.

[0247] 9. The composition of embodiment 1, wherein the composition comprises at least 15 wt% of one or more sugar beet fiber preparation and at least 28 wt% of one or more high molecular weight inulin preparation; the total amount of citrus

pectin preparations does not exceed 10 wt%, the total amount of citrus fiber preparations does not exceed 25 wt%, and the total amount of barley fiber preparations does not exceed 45 wt%.

[0248] 10. A composition comprising at least 15 wt% of one or more pea fiber preparation or a glycan equivalent thereof; and at least one additional fiber preparation chosen from (i) at least 28 wt% of one or more high molecular weight inulin preparation or a glycan equivalent thereof, (ii) 10 wt% or less of one or more citrus pectin preparation or a glycan equivalent thereof, (iii) 25 wt% or less of one or more citrus fiber preparation or a glycan equivalent thereof, or (iv) 45 wt% or less of one or more barley fiber preparation or a glycan equivalent thereof.

[0249] 11. The composition of embodiment 10, wherein there is at least 28 wt% of one or more pea fiber preparation, or a glycan equivalent thereof.

[0250] 12. The composition of embodiment 10, wherein there is at least 30 wt% of one or more pea fiber preparation, or a glycan equivalent thereof; and there is at least 30 wt% of one or more high molecular weight inulin preparation, or a glycan equivalent thereof.

[0251] 13. The composition of any of embodiments 10-12, wherein there is less than 1 wt% of one or more citrus pectin preparation, or a glycan equivalent thereof.

[0252] 14. The composition of any of embodiments 10-12, wherein there is no citrus pectin preparation, or a glycan equivalent thereof.

[0253] 15. The composition of any of embodiments 10-14, wherein there is 15 wt% or less of one or more citrus fiber preparation, or a glycan equivalent thereof.

[0254] 16. The composition of embodiment 15, wherein there is 12 wt% or less of one or more citrus fiber preparation, or a glycan equivalent thereof.

[0255] 17. The composition of any one of embodiments 10-16, wherein there is 25 wt% or less of one or more barley fiber preparation, or glycan equivalent thereof.

[0256] 18. The composition of embodiment 17, wherein there is 25 wt% or less of one or more barley fiber preparation, or glycan equivalent thereof.

[0257] 19. A composition comprising about 35 wt% of one or more pea fiber preparation or a glycan equivalent thereof, about 10 wt% of one or more citrus fiber preparation or a glycan equivalent thereof, about 35 wt% of one or more high molecular weight inulin preparation or a glycan equivalent thereof, and about 20 wt% of one or more barley fiber preparation or a glycan equivalent thereof; and wherein the pea fiber preparation(s), citrus fiber preparation(s), high molecular weight inulin preparation(s), and barley fiber preparation(s) are at least 95 wt% of the composition.

[0258] 20. A composition comprising about 30-40 wt% of one or more pea fiber preparation or a glycan equivalent thereof, about 9-11 wt% of one or more citrus fiber preparation or a glycan equivalent thereof, about 30-40 wt% of one or more high molecular weight inulin or a glycan equivalent thereof, and about 18-22 wt% of one or more barley fiber preparation or a glycan equivalent thereof; and wherein the pea fiber preparation(s), citrus fiber preparation(s), high molecular weight inulin preparation(s), and barley fiber preparation(s) are at least 95 wt% of the composition.

[0259] 21. A composition comprising about 30-35 wt% of one or more pea fiber preparation or a glycan equivalent thereof, about 9-11 wt% of one or more citrus fiber preparation or a glycan equivalent thereof, about 35-40 wt% of one or more high molecular weight inulin preparation or a glycan equivalent thereof, and about 18-22 wt% of one or more barley bran preparation or a glycan equivalent thereof; and wherein the pea fiber preparation(s), citrus fiber preparation(s), high molecular weight inulin preparation(s), and barley fiber preparation(s) are at least 95 wt% of the composition.

[0260] 22. A composition comprising about 33 wt% of one or more pea fiber preparation or a glycan equivalent thereof, about 11 wt% of one or more citrus fiber preparation or a glycan equivalent thereof, about 36 wt% of one or more high molecular weight inulin preparation or a glycan equivalent thereof, and about 20 wt% of one or more barley fiber preparation or a glycan equivalent thereof; and wherein the pea fiber preparation(s), citrus fiber preparation(s), high molecular weight inulin preparation(s), and barley fiber preparation(s) are at least 95 wt% of the composition.

[0261] 23. A composition comprising about 65 wt% pea fiber or a glycan equivalent thereof, and about 35 wt% high molecular weight inulin or a glycan

equivalent thereof; and wherein the pea fiber preparation(s) and high molecular weight inulin preparation(s) are at least 95 wt% of the composition.

[0262] 24. A food comprising a composition of any one of the preceding claims.

[0263] 25. A baked, pressed or extruded food comprising a composition of any one of embodiments 1 to 23.

[0264] 26. The food of embodiment 24 or 25, wherein the amount of the composition is about 40 wt% to about 50 wt% of the food.

[0265] 27. The food of embodiment 26, wherein the amount of the composition is about 45 wt% to about 50 wt% of the food.

[0266] 28. The food of embodiment 24, 25, 26, or 27, wherein the composition provides about 90% or more of the total dietary fibers in the food.

[0267] 29. The food of embodiment 28, wherein the dietary fiber blend provides about 95% or more of the total dietary fibers in the composition.

[0268] 30. The food of embodiment 29, wherein the dietary fiber blend provides about 98% or more of the total dietary fibers in the composition.

[0269] 31. A pressed, extruded or baked food, the food comprising about 40 wt% to about 95 wt% of a composition of fiber preparations, the composition of fiber preparations comprising (a) about 25 wt% to about 40 wt% of one or more pea fiber preparation, or a glycan equivalent thereof; about 5 wt% to about 15 wt% of one or more citrus fiber preparation, or a glycan equivalent thereof; about 30 wt% to about 40 wt% of one or more high molecular weight inulin preparation, or a glycan equivalent thereof; and about 10 wt% to about 30 wt% of one or more barley fiber preparation, or a glycan equivalent thereof; or (b) about 55 wt% to about 65 wt% of one or more pea fiber preparation, or a glycan equivalent thereof; and about 30 wt% to about 40 wt% of one or more high molecular weight inulin preparation, or a glycan equivalent thereof; wherein a 30 g serving of the food has at least 6 g of total dietary fiber; and wherein the food effects an increase in the fiber degrading capacity of a subject's gut microbiota and/or an improvement in the a subject's health, when the subject has consumed the food at least once a day for at least 7 days.

[0270] 32. The food of embodiment 31, wherein the composition of fiber preparations provides about 90% or more of the total dietary fiber in the food.

[0271] 33. The food of embodiment 31, wherein the composition of fiber preparations provides about 95% or more of the total dietary fiber in the composition.

[0272] 34. The food of embodiment 31, wherein the composition of fiber preparations provides about 98% or more of the total dietary fiber in the food.

[0273] 35. The food of any one of embodiments 31 to 34, wherein the composition of fiber preparations comprises (i) about 30 wt% to about 35 wt% of one or more pea fiber preparation, or a glycan equivalent thereof, (ii) about 9 wt% to about 11 wt% of one or more citrus fiber preparation, or a glycan equivalent thereof, (iii) about 35 wt% to about 40 wt% of one or more high molecular weight inulin preparation, or a glycan equivalent thereof, and about 18 wt% to about 22 wt% of one or more barley fiber preparation, or a glycan equivalent thereof; and wherein the pea fiber preparation(s), citrus fiber preparation(s), high molecular weight inulin preparation(s), and barley fiber preparation(s) are at least 95 wt% of the composition.

[0274] 36. The food of any one of embodiments 31 to 34, wherein the composition of fiber preparations comprises about 33 wt% of one or more pea fiber preparation, or a glycan equivalent thereof, about 11 wt% of one or more orange fiber preparation, or a glycan equivalent thereof, about 36 wt% of one or more high molecular weight inulin preparation, or a glycan equivalent thereof, and about 20 wt% of one or more barley fiber preparation, or a glycan equivalent thereof; and wherein the pea fiber preparation(s), citrus fiber preparation(s), high molecular weight inulin preparation(s), and barley fiber preparation(s) are at least 95 wt% of the composition.

[0275] 37. The food of any one of embodiments 31 to 34, wherein the composition of fiber preparations comprises about 30 wt% to about 35 wt% of one or more pea fiber preparation, about 9 wt% to about 11 wt% of one or more citrus fiber preparation, about 35 wt% to about 40 wt% of one or more high molecular weight inulin preparation, and about 18-22 wt% of one or more barley fiber preparation; and wherein the pea fiber preparation(s), citrus fiber preparation(s), high molecular weight inulin preparation(s), and barley fiber preparation(s) are at least 95 wt% of the composition.

[0276] 38. The food of any one of embodiments 31 to 34, wherein the composition of fiber preparations comprises about 33 wt% of one or more pea fiber preparation, about 11 wt% of one or more citrus fiber preparation, about 36 wt% of one or more high molecular weight inulin preparation, and about 20 wt% of one or more barley fiber preparation; and wherein the pea fiber preparation(s), citrus fiber preparation(s), high molecular weight inulin preparation(s), and barley fiber preparation(s) are at least 95 wt% of the composition.

[0277] 39. The food of any one of embodiments 31 to 34, wherein the composition of fiber preparations comprises about 60 wt% to about 65 wt% of one or more pea fiber preparation, or a glycan equivalent thereof; and about 30 wt% to about 35 wt% of one or more high molecular weight inulin preparation, or a glycan equivalent thereof; and wherein the pea fiber preparation(s), citrus fiber preparation(s), high molecular weight inulin preparation(s), and barley fiber preparation(s) are at least 95 wt% of the composition.

[0278] 40. The food of any one of embodiments 31 to 34, wherein the composition of fiber preparations comprises about 65 wt% of one or more pea fiber preparation, or a glycan equivalent thereof; and about 35 wt% of one or more high molecular weight inulin preparation, or a glycan equivalent thereof.

[0279] 41. The food of any one of embodiments 31 to 34, wherein the composition of fiber preparations comprises about 60 wt% to about 65 wt% of one or more pea fiber preparation; and about 30 wt% to about 35 wt% of one or more high molecular weight inulin preparation; and wherein the pea fiber preparation(s), citrus fiber preparation(s), high molecular weight inulin preparation(s), and barley fiber preparation(s) are at least 95 wt% of the composition.

[0280] 42. The food of any one of embodiments 31 to 34, wherein the composition of fiber preparations comprises about 65 wt% of one or more pea fiber preparation, and about 35 wt% of one or more high molecular weight inulin preparation; and wherein the pea fiber preparation(s), citrus fiber preparation(s), high molecular weight inulin preparation(s), and barley fiber preparation(s) are at least 95 wt% of the composition.

[0281] 43. The food of any one of embodiments 24 to 42, wherein the food further comprises flour(s), meal(s), oil(s), fat(s), inclusions, sweetener(s), starch(es), salt(s), emulsifier(s), leavening agent(s), preservative(s) or combinations thereof.

[0282] 44. The food of embodiment 43, wherein the food comprises one or more flour and/or meal in an amount that is about 10 wt% to about 60 wt% of the food.

[0283] 45. The food of embodiment 44, wherein the one or more flour is chosen from wheat four, rice flour, corn flour, or any combination thereof.

[0284] 46. The food of any one of embodiments 43 to 45, wherein the food comprises one or more sweetener in an amount that is about 0.005 wt% to about 40 wt% of the food.

[0285] 47. The food of embodiment 46, wherein the one or more sweetener is sugar.

[0286] 48. The food of any one of embodiments 43 to 47, wherein the food comprises one or more salt in an amount that is about 0.5 wt% to about 5 wt% of the food.

[0287] 49. The food of embodiment 48, wherein the one or more salt is sodium chloride.

[0288] 50. The food of any one of embodiments 43 to 49, wherein the food comprises one or more emulsifier in an amount that is about 0.1 wt% to about 2 wt% of the food.

[0289] 51. The food of embodiment 50, wherein the one or more emulsifier is chosen from glycerol monostearate, lecithin, polysorbate, or other mono or diglycerides.

[0290] 52. The food of any one of embodiment 43 to 49, wherein the food comprises one or more leavening agent in an amount that is about 0.1 wt% to about 5 wt% of the food.

[0291] 53. The food of embodiment 52, wherein the one or more leavening agent is chosen from sodium bicarbonate, monocalcium phosphate, or calcium carbonate, ammonium bicarbonate, mono calcium phosphate monohydrate, sodium acid pyrophosphate, sodium aluminum phosphate, organic acids, and yeast.

[0292] 54. The food of any one of embodiments 43 to 54, wherein the food further comprises a color additive, a flavor, a flavor enhancer, a stabilizer, a humectant, a firming agent, an enzyme, a probiotic, a spice, a binder, fruit, vegetables, grains, vitamins, minerals or combinations thereof.

[0293] 55. The food of any one of embodiments 43 to 54, wherein food is a baked food that has about 6 g to about 10 g of fiber in a 30 g serving.

[0294] 56. The baked food of embodiment 55, wherein the baked food has about 6 g of fiber in a 30 g serving.

[0295] 57. The baked food of embodiment 55, wherein the baked food has about 10 g of fiber in a 30 g serving.

[0296] 58. The baked food of any one embodiments 55 to 57, wherein the baked food is a cracker, a cookie, a cake, a bar, a bread, or a muffin.

[0297] 59. The food of any one of embodiments 43 to 54, wherein food is an extruded food that has about 6 g to about 10 g of fiber in a 30 g serving.

[0298] 60. The extruded food of embodiment 59, wherein the extruded food has about 6 g of fiber in a 30 g serving.

[0299] 61. The extruded food of embodiment 59, wherein the extruded food has about 10 g of fiber in a 30 g serving.

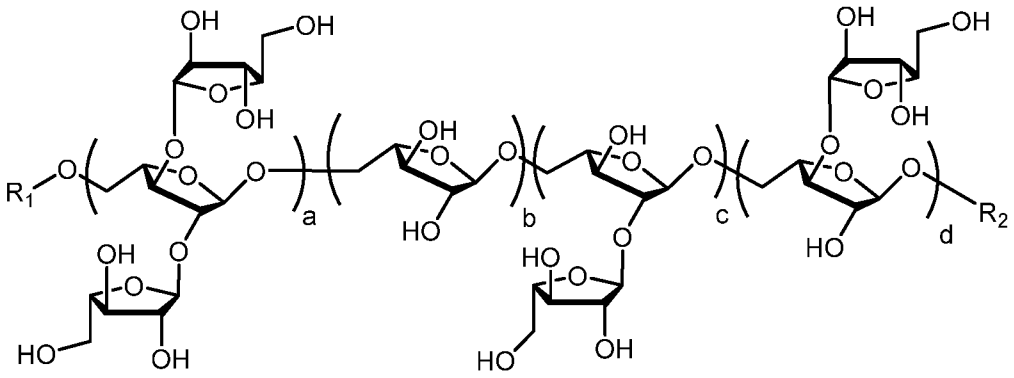
[0300] 62. The extruded food of any one of embodiments 59 to 61, wherein the extruded food is an extruded pillow or any other extruded shape.

[0301] 63. A pea fiber preparation for use in any one of embodiments 1 to 62, wherein about 55 wt% to about 65 wt% of the total dietary fiber in the pea fiber preparation is insoluble dietary fiber, and/or about 60 wt% to about 70 wt% of the total dietary fiber in the pea fiber preparation is high molecular dietary fiber.

[0302] 64. The pea fiber preparation of embodiment 63, wherein the pea fiber preparation has a monosaccharide content that is substantially similar to the preparation of **Table B**.

[0303] 65. The pea fiber preparation of embodiment 63 or 64, wherein the pea fiber preparation has glycosidic linkages substantially similar to the preparation of **Table D**, **Table 13**, **Table 14**, **Table 16**, or **Table 17**.

[0304] 66. The pea fiber preparation of embodiment 63, 64 or 65, wherein the pea fiber preparation comprises arabinan of formula (I)



(I), wherein a is about 0.1 to about 0.3, b is about 0.4 to about 0.6, c is about 0.1 to about 0.4, d is about 0.04 to about 0.06; and wherein R_1 and R_2 are each independently selected from H, a glycosyl, a sugar moiety (modified or not), an oligosaccharide (branched or not), or a polysaccharide (branched or not), and a polysaccharide containing galacturonic acid, galactose, and rhamnose.

[0305] 67. A glycan equivalent of a pea fiber preparation for use in any one of embodiments 1 to 62, wherein the glycan equivalent is a compositional glycan equivalent of a pea fiber preparation of any one of embodiments 63 to 66.

[0306] 68. A glycan equivalent of a pea fiber preparation for use in any one of embodiments 1 to 62, wherein the glycan equivalent is a functional glycan equivalent of a pea fiber preparation of any one of embodiments 63 to 66.

[0307] 69. A citrus fiber preparation for use in any one of embodiments 1 to 62, wherein about 30 wt% to about 40 wt% of the total dietary fiber in the citrus fiber preparation is insoluble dietary fiber, and/or about 65 wt% to about 75 wt% of the total dietary fiber in the citrus fiber preparation is high molecular dietary fiber.

[0308] 70. The citrus fiber preparation of embodiment 69, wherein the citrus fiber preparation has a monosaccharide content that is substantially similar to the preparation of **Table B**.

[0309] 71. The citrus fiber preparation of embodiment 69 or 70, wherein the citrus fiber preparation has glycosidic linkages substantially similar to the preparation of **Table F**.

[0310] 72. A citrus pectin preparation for use in any one of embodiments 1 to 62, wherein about 1 wt% to about 10 wt% of the total dietary fiber in the citrus pectin preparation is insoluble dietary fiber, and/or about 85 wt% to about 95 wt% of the total dietary fiber in the citrus pectin preparation is high molecular dietary fiber.

[0311] 73. The citrus pectin preparation of embodiment 72, wherein the citrus pectin preparation has a monosaccharide content that is substantially similar to the citrus pectin preparation of **Table B**.

[0312] 74. The citrus pectin preparation of embodiment 72 or 73, wherein the citrus pectin preparation has glycosidic linkages substantially similar to the preparation exemplified in **Table D**.

[0313] 75. A barley fiber preparation for use in any one of embodiments 1 to 62, wherein about 5 wt% to about 15 wt% of the total dietary fiber in the barley fiber preparation is insoluble dietary fiber, and/or about 40 wt% to about 45 wt% of the total dietary fiber in the barley fiber preparation is high molecular dietary fiber.

[0314] 76. The barley fiber preparation of embodiment 75, wherein the barley fiber preparation has a monosaccharide content that is substantially similar to the preparation of **Table B.**

[0315] 77. The barley fiber preparation of embodiment 75 or 76, wherein the barley fiber preparation has glycosidic linkages substantially similar to the preparation exemplified in **Table E**.

[0316] 78. A high molecular weight inulin preparation for use in any one of embodiments 1 to 62, wherein the total dietary fiber in the high molecular weight inulin preparation is about 85 wt% to about 99 wt%.

[0317] 79. The high molecular weight inulin preparation of embodiment 78, wherein the high molecular weight inulin preparation has a degree of polymerization greater than 5.

[0318] 80. A sugar beet fiber preparation for use in any one of embodiments 1 to 62, wherein about 55 wt% to about 65 wt% of the total dietary fiber in the sugar beet fiber preparation is insoluble dietary fiber, and/or about 75 wt% to about

85 wt% of the total dietary fiber in the sugar beet fiber preparation is high molecular dietary fiber.

[0319] 81. A composition of any one of embodiments 1 to 23, wherein the composition (i) effects an increase in the total abundance of *Bacteroides* species measured in a fecal sample obtained from a subject after the subject has consumed the composition at least once a day for at least 7 days, as compared to the total abundance of *Bacteroides* species measured in a fecal sample obtained from the subject prior to consumption of the composition, (ii) effects an increase in the relative abundance of *Bacteroides* species measured in a fecal sample obtained from a subject after the subject has consumed the composition at least once a day for at least 7 days, as compared to the relative abundance of *Bacteroides* species measured in a fecal sample obtained from the subject prior to consumption of the composition, or (iii) effects a health improvement in a subject after the subject has consumed the composition at least once a day for at least 7 days.

[0320] 82. The composition of embodiment 81, wherein the composition effects a health improvement in a subject after the subject has consumed the composition at least once a day for 7 days, the health improvement being selected from a reduction in total body weight, a reduction in BMI, a reduction in fat mass gain, an increase in fecal levels of succinate, a decrease in serum cholesterol, an increase in insulin sensitivity, a decrease in plasma markers of inflammation, an improvement in the relative abundances of health discriminatory plasma proteins, and/or an improvement in biomarkers/mediators of gut barrier function.

[0321] 83. The food of embodiment 31, wherein the food effects an increase in the total abundance of *Bacteroides* species measured in a fecal sample obtained from a subject after the subject has consumed the food at least once a day for at least 7 days.

[0322] 84. The food of embodiment 31, wherein the food effects an increase in the relative abundance of *Bacteroides* species measured in a fecal sample obtained from a subject after the subject has consumed the food at least once a day for at least 7 days.

[0323] 85. The food of embodiment 31, wherein the food effects an increase in the total abundance of *Bacteroides* species measured in a fecal sample obtained from a subject after the subject has consumed the food at least once a day for at least 14 days.

[0324] 86. The food of embodiment 31, wherein the food effects an increase in the relative abundance of *Bacteroides* species measured in a fecal sample obtained from a subject after the subject has consumed the food at least once a day for at least 14 days.

EXAMPLES

[0325] The following examples illustrate various iterations of the invention and in some instances demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples that follow represent techniques discovered by the inventors to function well in the practice of the invention. Those of skill in the art should, however, in light of the present disclosure, appreciate that changes may be made in the specific embodiments that are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention. Therefore, all matter set forth or shown in the accompanying drawings is to be interpreted as illustrative and not in a limiting sense.

[0326] The references listed below are cited in the Examples that follow.

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Example 1 – Glycan-coated magnetic beads

[0379] A food-grade, pea fiber preparation was purchased from a commercial supplier. The compositional analysis of the pea fiber preparation is found in **Table A**. Wheat Arabinoxylan and Icelandic Moss Lichenan were purchased from Megazyme (P-WAXYL, P-LICHN) and yeast alpha-mannan was purchased from Sigma-Aldrich (M7504). Polysaccharides were solubilized in water (at a concentration of 5mg/mL for pea fiber and 20 mg/mL for arabinoxylan and lichenan), sonicated and heated to 100°C for 1 minute, then centrifuged at 24,000 x g for 10 minutes to remove debris. TFPA-PEG3-biotin (Thermo Scientific), dissolved in DMSO (10 mg/mL) was added to the polysaccharide solution at a ratio of 1:5 (v/v). The sample was subjected to

UV irradiation for 10 minutes (UV-B 306 nm, 7844 mJ total), and then diluted 1:4 to facilitate desalting on 7 kD Zeba spin columns (Thermo Scientific).

[0380] Biotinylated polysaccharide was mixed with one of several biotinylated fluorophores (PF-505, PF-510LSS, PF-633, PF-415; all at a concentration of 50 ng/mL; all obtained from Promokine). A 500 µL aliquot of this preparation was incubated with 10^7 paramagnetic streptavidin-coated silica beads (LSKMAGT, Millipore Sigma) for 24 hours at room temperature. Beads were washed by centrifugation three times with 1 mL HNTB buffer (10mM HEPES, 150mM NaCl, 0.05% Tween-20, 0.1% BSA) followed by addition of 5 µg/mL streptavidin (Jackson Immunoresearch) in HNTB (30 min incubation at room temperature). Beads were washed as before and then incubated with 250 µL of the biotinylated polysaccharide preparation. The washing, streptavidin, and polysaccharide incubation steps were repeated three times.

[0381] Bead preparations were assessed using an Aria III cell sorter (BD Biosciences) to confirm adequate labeling. Beads were incubated with 70% ethanol for 1 minute in a biosafety cabinet, then washed three times with 1 mL sterile HNTB using a magnetic stand. The different bead types were combined, diluted, and aliquoted to 10^7 beads per 650 µL HNTB in sterile Eppendorf microcentrifuge tubes. The number of beads in each aliquot was counted using an Aria III cell sorter and CountBright fluorescent microspheres (BD Bioscience).

[0382] Bead preparations were analyzed by GC-MS to quantify the amount of carbohydrate bound. Beads were sorted back into their polysaccharide types based on fluorescence using an Aria III sorter (average sort purity, 96%). Sorted samples were centrifuged (500 x g for 5 minutes) to pellet beads and the beads were transferred to a 96-well plate. All bead samples were incubated with 1% SDS / 6M Urea / HNTB for 10 minutes at room temperature to remove exogenous components, washed three times with 200 µL HNTB using a magnetic plate rack, and then stored overnight at 4° C prior to monosaccharide analysis. The number and purity of beads in each sorted sample was determined by taking an aliquot for analysis on the Aria III cell sorter. Equal numbers of beads from each sample were transferred to a new 96-well plate and the supernatant was removed with a magnetic plate rack. For acid hydrolysis, 200 µL of 2M

trifluoroacetic acid and 250 ng/mL myo-inositol-D6 (CDN Isotopes; spike-in control) were added to each well, and the entire volume was transferred to 300 µL glass vials (ThermoFisher; catalog number C4008-632C). Another aliquot was taken to verify the final number of beads in each sample. Monosaccharide standards were included in separate wells and subjected to the hydrolysis protocol in parallel with the other samples. Vials were crimped with Teflon-lined silicone caps (ThermoFisher) and incubated at 100°C with rocking for 2 h. Vials were then cooled, spun to pellet beads, and their caps were removed. A 180 µL aliquot of the supernatant was collected and transferred to new 300 µL glass vials. Samples were dried in a SpeedVac for 4 hours, methoximated in 20 µL O-methoxyamine (15mg/mL pyridine) for 15 h at 37°C, followed by trimethylsilylation in 20 µL MSTFA/TMCS [N-Methyl-N-trimethylsilyltrifluoroacetamide/2,2,2-trifluoro-N-methyl-N-(trimethylsilyl)-acetamide, chlorotrimethylsilane] (ThermoFisher) for 1 h at 70° C. One half volume of heptane (20 µL) was added before loading the samples for injection onto a 7890B gas chromatography system coupled to a 5977B MS detector (Agilent). The mass of each monosaccharide detected in each sample of sorted beads was determined using monosaccharide standard curves. This mass was then divided by the final count of beads in each sample to produce a measurement of mass of recoverable monosaccharide per bead.

Table 1: Monosaccharide analysis of wheat arabinoxylan beads and pea fiber beads

		Mean (pg/bead)	sd
	Arabinoxylan beads	0.17	0.12
Xylose	Pea Fiber beads	0.06	0.07
	Uncoated beads	0.01	0.01
	Arabinoxylan beads	0.54	0.25
Arabinose	Pea Fiber beads	0.2	0.06
	Uncoated beads	0.06	0.02
	Arabinoxylan beads	0.02	0.02
Mannose	Pea Fiber beads	0.04	0.02
	Uncoated beads	0.06	0.03

	Arabinoxylan beads	0.02	0.01
Galactose	Pea Fiber beads	0.05	0.03
	Uncoated beads	0	0.01
	Arabinoxylan beads	0.02	0.04
Glucose	Pea Fiber beads	0.01	0.02
	Uncoated beads	0.01	0.01

Example 2 - *In vivo* screen for fiber preparations that target specific human gut microbes

[0383] In the present study, we describe an *in vivo* approach for identifying fibers and their bioactive components that selectively increase the fitness of a group of human gut *Bacteroides*, and the different mechanisms these organisms deploy when encountering these nutrient resources and one another. The bacterial targets for fiber-based manipulation originated from our previous study of twins stably discordant for obesity (Ridaura et al., 2013). Fecal microbiota from these twin pairs transmitted discordant adiposity and metabolic dysfunction phenotypes to recipient germ-free mice. Co-housing mice shortly after they received microbial communities from lean (Ln) or obese (Ob) co-twins prevented recipients of the Ob donor microbiota from developing obesity and associated metabolic abnormalities. Analysis of their gut communities revealed that invasion of *Bacteroides* species from Ln into Ob microbiota, notably *B. thetaiotaomicron, B. vulgatus*, *B. caccae*, and *B. cellulosilyticus*, correlated with protection from the increased adiposity and metabolic phenotypes that developed in co-housed Ob-Ob controls. Invasion was diet-dependent, occurring when animals consumed a human diet designed to represent the lower tertile of consumption of saturated fats and upper tertile of consumption of fruits and vegetables (high in fiber) in the USA, but not when they consumed a diet representing the upper tertile of saturated fat and lower tertile of fruit and vegetable consumption (Ridaura et al., 2013). Here we identify dietary fiber preparations and constituent bioactive components that increase the fitness of these targeted *Bacteroides* (*B. thetaiotaomicron, B. vulgatus*, *B. caccae*, and/or *B. cellulosilyticus*) *in vivo* in the high saturated fatty acid-low fruits and vegetables (HiSF-LoFV) diet context. To do so, we first colonized germ-free mice with a defined consortium of sequenced bacterial strains cultured from a Ln donor in an

obesity-discordant twin pair. Mice were fed 144 different diets generated by supplementing the HiSF-LoFV formulation with 34 different food-grade fiber preparations in different combinations at different concentrations. Armed with a consortium that contained targeted *Bacteroides* species, each in the form of a library of tens of thousands of transposon (Tn) mutant strains, and employing high resolution mass spectrometry, we subsequently characterized the effects of monotonous feeding of selected fiber preparations on the community's expressed proteome and on the fitness of Tn mutants. By identifying polysaccharide processing genes whose expression was increased and that functioned as key fitness determinants, we inferred which components of the fiber preparations were bioactive. Time series proteomic analyses of the complete community and derivatives lacking one or more *Bacteroides*, revealed nutrient harvesting strategies resulting in, as well as alleviating interspecies competition for fiber components. Finally, administering artificial food particles coated with dietary polysaccharides to gnotobiotic mice with deliberately varied community membership further established the contributions of individual *Bacteroides* species to glycan processing *in vivo*.

[0384] A schematic of the experimental design for screening 34 food grade fibers is shown in **FIG. 1A**. In total, three separate experiments were performed to complete an analysis of the effects of these fiber preparations on community structure. These fibers were obtained from diverse plant sources including fruits, vegetables, legumes, oilseeds, and cereals. Ten to 13 different fibers were tested per experiment (**Table 2**). Each mouse was colonized with a 20-member consortium of sequenced bacterial strains cultured from a single Ln co-twin donor. Each animal received a different fiber-supplemented diet each week for a total of four weeks. Each of the 144 unique diets tested contained one fiber type present at a concentration of 8% (w/w) and another fiber type at 2%. These two concentrations were systematically paired (*Methods*) to maximize the number of fiber preparations tested (**FIG. 1A**). Moreover, fiber types were presented in varying orders during the diet oscillation, mitigating potential hysteresis effects. Control groups were monotonously fed the unsupplemented HiSF-LoFV or LoSF-HiFV diet.

Table 2 – Food-grade dietary fiber preparations

(A) Experiment details

Fiber preparation	Screening experiment used	Fiber preparation	Screening experiment used
Citrus pectin	3	Oat beta-glucan	2
Pea fiber	2	Apple fiber	1
Citrus peel	3	Rye bran	1
Yellow mustard	3	Barley malted	1
Soy cotyledon	3	Wheat aleurone	1
Orange fiber (Coarse)	1	Wheat bran	2
Orange fiber (Fine)	1 and 3	Resistant maltodextrin	2
Orange peel	3	Psyllium	3
Tomato peel	2	Cocoa	3
Inulin, LMW	2	Citrus fiber	3
Potato Fiber	3	Tomato pomace	2
Apple pectin	1	Rice bran	2
Oat hull fiber	2	Chia seed	2
Acacia extract	1	Corn bran	2
Inulin, HMW	2 and 3	Soy fiber	2
Barley beta-glucan	1	Sugar cane fiber	3
Barley bran	1	Resistant starch 4	3

(B) Compositional analysis

	% TDF	% IDF	% SDF	HMW DF	LMW DF	% Prot	% Fat	% Carb	% Moisture	% Ash
Abbreviations: dietary fiber (DF), total dietary fiber (TDF), insoluble dietary fiber (IDF), soluble dietary fiber (SDF), high molecular weight (HMW), low molecular weight (LMW), protein (Prot), carbohydrate (Carb), not determined (ND)										
Citrus pectin*	78.9	1.4	75.5	76.9	2	3.34	0.56	86.82	7.97	1.31
Pea fiber*	67.2	61.36	4.94	66.3	0.8	9.49	0.93	79.75	7.37	2.46
Citrus peel	70.9	47.7	23.2	70.9	0.6	4.44	2.31	83.16	6.85	3.24
Yellow	41.8	40.7	0.47	40.8	1	25.34	10.68	50.86	8.12	5

mustard										
Soy cotyledon	62.9	54	7.5	61.5	1.4	24.49	1.48	60.78	8.41	4.84
Orange fiber (Coarse)*	68.5	33.2	29.5	68.5	0.6	7.47	2.16	80.92	5.69	1.96
Orange fiber (Fine)	68	28.2	28.1	66.8	1.1	9.92	4.13	78.39	4.74	1.17
Orange peel	60.1	42.9	17.2	60.1	0.6	6.19	4.03	79.49	7.36	2.93
Tomato peel	79.1	68.22	10.88	79.1	0.6	8.07	4.42	79.23	5.57	2.71
Inulin, LMW	98.5	<0.5	98.5	86	12.5	0.4	1.18	95.14	3.2	0.08
Potato Fiber	65.5	53.9	9.9	63.8	1.7	7.28	1.48	79.14	9.41	2.69
Apple pectin	60	0.47	58.65	59.3	0.7	12.04	0.98	70.61	10.76	5.61
Oat hull fiber	95.7	92.86	2.84	95.7	0.6	0.35	0.15	94.3	3.91	1.29
Acacia extract	72.4	0.47	72.4	72.4	0.6	0.79	0.65	84.11	9.89	4.56
Inulin, HMW*	90.9	ND	ND	59.5	31.3	0.28	3.71	91.44	4.28	0.29
Barley beta-glucan	84.6	0.47	74.4	81.6	3	3.08	1.56	88.45	5.85	1.06
Barley bran (barley fiber)*	46	11.1	20.8	45.2	0.9	18.72	4.13	69.28	5.69	1.96
Oat beta-glucan	46.6	25.6	20.3	45.5	1.1	21.64	4.98	65.45	4.07	3.86
Apple fiber	73.3	57.25	7.01	73.3	0.6	9.78	1.57	81.77	4.98	1.9
Rye bran	45.5	32.7	0.47	41.5	4	13.58	4.8	70.01	6.48	5.13
Barley	42.2	39.5	0.47	41.1	1.1	16.89	10.53	63.52	6.15	2.91

malted										
Wheat aleurone	43.7	39.89	0.47	42.3	1.5	13.64	9.05	63.55	7.14	6.62
Wheat bran	30.2	24.54	3.46	28	2.2	14.06	5.08	67.12	9.7	4.04
Resistant maltodextrin	72.3	0.47	1.8	1.8	70.5	0.71	0.08	95.52	3.77	0.04
Psyllium	95.6	87.8	3.6	91.4	4.2	1.63	0.74	88.08	6.98	2.57
Cocoa	31.6	21.5	9.3	30.8	0.9	27.81	12.61	50.29	2.67	6.62
Citrus fiber	91	85.3	4.7	91	0.6	0.61	1.23	90.07	3.51	4.58
Tomato pomace	56.7	49.1	7.6	56.7	0.6	15.63	14.37	62.26	4.76	2.98
Rice bran	23.5	22.19	0.61	22.8	0.7	15.13	21.62	49.88	5.21	8.16
Chia seed	40.8	39.17	1.63	40.8	0.6	22.07	36.91	30.67	5.62	4.73
Corn bran	76.8	72.34	4.46	76.8	0.6	4.97	4.08	83.9	6.09	0.96
Soy fiber	93.8	89.29	3.11	92.4	TBD	1.58	1.05	89.97	4.88	2.52
Sugar cane fiber	95.6	90.6	5	95.6	0.6	0.12	0.15	93.36	6.11	0.38
Resistant starch 4	90.7	70.3	20.4	90.7	0.6	0.12	0.08	86.48	11.72	1.8
* See Tables A-F for monosaccharide analysis and glycosyl linkage analysis										

Table 3 – Monosaccharide analysis of HiSF-LoFV diet

	F1 (390ug)		F2 (490ug)		F3 (480ug)	
Glycosyl residue	Mass (mg)	Mol %	Mass (mg)	Mol %	Mass (mg)	Mol %
Arabinose (Ara)	3.1	29.6	2	3.4	7.7	12.8
Rhamnose (Rha)	0.1	0.4	0.1	0.1	0.7	1
Fucose (Fuc)	n.d.	-	n.d.	-	0.1	0.1
Xylose (Xyl)	3.4	32.9	3.9	6.7	14.4	24
Galacturonic acid (GalA)	0.1	0.6	0.2	0.3	2.4	3.1
Mannose (Man)	1.8	14.1	6	8.6	13.9	19.4
Galactose (Gal)	0.9	7.2	0.7	1.1	4.9	6.8
Glucose (Glc)	1.9	15.2	55.8	79.8	23.5	32.7
Sum=	11.2		68.7		67.5	

Table 4 – Glycosyl-linkage analysis of HiSF-LoFV diet

		% detected linkage		
	Deduced Linkage	**F1**	**F2**	**F3**
Arabinose	(t-Araf)	19.7	5.2	3.9
	(2-Araf)	1.4	0.3	1.9
	(3-Araf)	0.7	0.2	0.8
	(4-Arap or 5-Araf)	2.4	0.5	1.6
Xylose	(t-Xyl)	0.9	0.4	2.4
	(4-Xyl)	17.8	8.4	9.1
	(2-Xyl)	1.8	0.7	2.3
	(2,4-Xyl)	0.7	0.8	0.4
	(3,4-Xyl)	5.9	2	2.1
	(2,3,4-Xyl)	6.6	-	-
Fucose	(t-Fuc)	-	-	0.3
Galactose	(t-Gal)	1.3	0.5	2.4
	(3-Gal)	0.9	-	-
	(2-Gal)	-	-	0.6
	(4-Gal)	2.6	0.9	4.8
	(6-Gal)	0.4	-	0.3
	(3,6-Gal)	4.6	1.2	0.4
Mannose	(t-Man)	5.2	5.4	6.3
	(2-Man)	2.9	1.5	1.5
	(3-Man)	0.9	0.3	0.2
	(4-Man)	1.4	1.4	13
	(2,4-Man)	0.2	-	-
	(4,6-Man)	-	-	1
	(3,6-Man)	1.2	-	0.5
	(2,6-Man)	1.1	0.6	0.9
Glucose	(t-Glc)	2.9	4.6	2.4
	(3-Glc)	4.8	1.1	1.5
	(6-Glc)	0.8	0.7	0.6

(4-Glc)	10	58	29.9
(3,4-Glc)	0.5	1.2	0.3
(2,4-Glc)	0.3	0.5	0.5
(4,6-Glc)	-	3.6	7.8

[0385] We analyzed the relative abundance of each member of the defined community at two time points at the end of each diet treatment by collecting fecal samples and performing 16S rRNA gene sequencing. Binning the data according to the fiber preparation present at 8% concentration revealed potent and specific effects on distinct taxa (**FIG. 1A**). To analyze the independent effects of the two fiber preparations administered during each diet treatment, we generated a linear mixed-effects model for each bacterial taxon using the data from the last two days of consumption of each diet. The coefficient estimates in these models describe the slope of the predicted dose response curve for each fiber preparation's effect on each community member (**Tables 5A, 6A, 7A**). Twenty-one fiber preparations had significant estimated coefficients of >1 (with a coefficient of 1 indicating a 1% increase in the relative abundance of a bacterial species for every 1% increase in the concentration of the fiber preparation added to the HiSF-LoFV diet) (**FIG. 1B**). Large coefficients were observed in the *B. thetaiotaomicron* models for citrus pectin (2.6) and pea fiber (2.1). The *B. ovatus* models revealed pronounced effects of barley beta-glucan (3.9) and barley bran (3.1). Estimated coefficients for high molecular weight inulin (4.5, *B. caccae* model), resistant maltodextrin (3.8, *P. distasonis* model), and psyllium (3.4, *E. coli* model) were notable with 8% fiber administration driving the relative abundance of these community members from 10-20% to nearly 50%. Two of the fiber preparations tested (rice bran and corn bran) either had no detectable effect on the abundance of community members or produced estimated coefficients <0.5. High molecular weight inulin and an orange fiber preparation were tested across two separate experiments; the results established that the effects on the relative abundances of community members were reproducible (coefficients were highly correlated between these independent experiments, R^2=0.96; **Tables 5A, 6A, 7A**). The even distributions of residuals around the fitted values in the models indicated that there were no pronounced threshold or

saturation effects of these fiber preparations at the concentrations tested. For bacterial species that exhibited notable responses to fiber (at least one coefficient >1), the average R^2 value of the models was 0.82. We repeated our analyses using DNA yield from each fecal sample to estimate the absolute abundance of each organism as a function of fiber preparation. The estimated coefficients obtained from these two measures were highly correlated (R^2 = 0.88) (**Tables 5B, 6B, 7B**). Together, results obtained from this screen illustrate the specificity of the effects of different types of dietary fiber on community configuration.

Table 5 - Screening Experiment 1

(A) Estimated Coefficients from linear mixed effect models generated using relative abundance

		1	2	3	4	5	6	7	8	9	10
Taxonomy	**OTU no.**										
Bacteroides thetaiotaomicron	848236	0.31	0.83	1.33	1.62	-	-0.48	-	-	-	0.61
Bacteroides cellulosilyticus	539126	-	-	-	-	-0.3	-0.8	-	-	-	-0.37
Bacteroides vulgatus	850870	-	-	0.42	0.61	-0.7	-0.71	-	-	-0.49	0.89
Bacteroides caccae	579112	-	-0.5	-0.73	-0.35	-0.63	-0.88	-0.4	-0.33	-	-0.94
Bacteroides ovatus	844958	2.13	1.83	0.84	-	3.12	3.89	1.41	1.32	1.78	0.81
Parabacteroides distasonis	846317	0.24	-0.65	-0.45	-0.37	-0.22	-0.34	-	-0.23	-0.15	-
Escherichia coli	1111717	-1	-	-	-0.9	-0.95	-0.48	-0.84	-0.8	-1.05	-0.84
Ruminococcaceae sp.	360801	-	0.2	-	0.14	-	-	-	-	-	-
Subdoligranulum variabile	364609	-0.34	-0.29	-	-	-	-0.2	-	-	-	-
Collinsella aerofaciens	1110606	-0.18	-0.16	-0.19	-0.16	-0.15	-0.09	-0.14	-0.12	-0.12	-0.13
Bacteroides massiliensis	840832										
Odoribacter splanchnicus	210303	-0.04	-0.04	-0.02	-0.02	-0.03	-0.05	-0.03	-0.02	-0.04	-0.04
Bacteroides finegoldii	de novo OTU	0.03	0.04	0.05	0.03	-	-	-	-	0.02	-
Peptococcus niger	1135793	-0.35	-0.21	-0.25	-0.27	-0.23	-0.34	-0.15	-0.14	-0.23	-
Dorea longicatena	de novo	-0.47	-0.66	-0.59	-0.57	-	0.31	-	-	-	-0.32

	OTU										
1 – apple fiber, 2- apple pectin, 3 orange fiber fine, 4- orange fiber course, 5 barley bran, 5- barley beta glucan, 7- barley malted, 8- wheat aleurone, 9- rye bran, 10- acacia extract											
"-" indicates estimate was not statistically significant (ANOVA, P < 0.05)											
blank cells indicate that the organism was not detected above the 0.05% relative abundance cut-off in the experiment											

(B) Estimated Coefficients from linear mixed effect models generated using DNA-scaled abundance.

		1	2	3	4	5	6	7	8	9	10
Taxonomy	**OTU no.**										
Bacteroides thetaiotaomicron	848236	-	1.15	1.64	1.54	-	-	-	-	-	-
Bacteroides cellulosilyticus	539126	-	-	-	-	-	-	-	-	-	-0.55
Bacteroides vulgatus	850870	-	-	0.78	0.63	-	-	-	-	-	-
Bacteroides caccae	579112	-	-	-0.45	-	-	-0.55	-	-	-	-0.9
Bacteroides ovatus	844958	1.54	2.05	1.35	-	3.72	4.9	0.97	1.06	1.64	-
Parabacteroides distasonis	846317	-	-0.4	-	-	-	-	-	-	-	-0.37
Escherichia coli	1111717	-0.84	-	-	-0.55	-0.42	-	-0.62	-0.63	-0.72	-1
Ruminococcaceae sp.	360801	-	0.24	-	0.14	-	-	-	-	-	-
Subdoligranulum variabile	364609	-0.28	-	0.26	-	-	-	-	-	-	-0.27
Collinsella aerofaciens	1110606	-0.16	-0.11	-0.12	-0.12	-0.09	-	-0.13	-0.09	-0.09	-0.15
Bacteroides massiliensis	840832										
Odoribacter splanchnicus	210303	-0.03	-	-	-	-	-0.03	-0.02	-	-0.03	-0.05
Bacteroides finegoldii	de novo OTU	0.02	0.04	0.07	0.03	0.02	-	-	-	0.02	-
Peptococcus niger	1135793	-0.29	-	-	-0.18	-0.1	-0.19	-0.13	-0.12	-0.15	-0.19
Dorea longicatena	de novo OTU	-0.47	-0.46	-0.32	-0.38	-	0.83	-	-	-	-0.51
1 – apple fiber, 2- apple pectin, 3 orange fiber fine, 4- orange fiber course, 5 barley bran, 5- barley beta glucan, 7- barley malted, 8- wheat aleurone, 9- rye bran, 10- acacia extract											
"-" indicates estimate was not statistically significant (ANOVA, P < 0.05)											
blank cells indicate that the organism was not detected above the 0.05% relative abundance cut-off in the experiment											

Table 6 - Screening Experiment 2

(A) Estimated Coefficients from linear mixed effect models generated using relative abundance

		1	2	3	4	5	6	7	8	9	10	11	12	13
Taxonomy	**OTU no.**													
Bacteroides thetaiotaomicron	848236	0.87	-	-	0.89	-	-	-	-	-	-	2.09	-	-
Bacteroides cellulosilyticus	539126	-	-0.69	-	0.47	-	-	-	-0.63	-	0.83	-	-	0.51
Bacteroides vulgatus	850870	0.9	-	-	-	-	-	-	-	-	-	-	-	-
Bacteroides caccae	579112	-	4.54	-	-	-	-	-	-	-	-	-	-	-
Bacteroides ovatus	844958	0.35	-0.55	-	-	-	0.52	0.91	-	2.99	0.68	0.34	-	-
Parabacteroides distasonis	846317	-1.06	-0.95	-	-	-	-	-	3.75	-	-	-	-	-
Escherichia coli	1111717	-	-	-0.92	-1.16	-	-	-	-	-0.89	-1.03	-1.09	-0.85	-1.11
Ruminococcaceae sp.	360801	-0.03	-	0.03	0.09	-	-	-	-	-	-	-	-	-
Subdoligranulum variabile	364609	-0.28	-0.4	-	-	-	-	-	-0.26	-	-	-0.33	-	-
Collinsella aerofaciens	1110606	-	-0.3	-0.32	-0.33	-	-0.27	-	-0.34	-	-	-0.34	-	-0.3
Bacteroides massiliensis	840832	-0.07	-0.08	-0.09	-0.08	-0.06	-	-0.09	-0.09	-0.09	-	-0.1	-	-0.09
Odoribacter splanchnicus	210303	-	-0.04	-	-	-	-	-	-0.05	-	-0.04	-0.04	-	-
Bacteroides finegoldii	de novo OTU													
Peptococcus niger	1135793	-0.21	-0.19	-0.18	-0.18	-	-	-	-0.27	-	-0.18	-0.28	-0.21	-
1, inulin LMW, 2, inulin HMW, 3- tomato pomace, 4- tomato peel, 5- rice bran, 6- chia see, 7- wheat bran, 8- resistant maltodextrin, 9- oat beta glucan, 10- oat hull fiber, 11- pea fiber, 12- corn bran														
"-" indicates estimate was not statistically significant (ANOVA, $P < 0.05$)														
blank cells indicate that the organism was not detected above the 0.05% relative abundance cut-off in the experiment														

(B) Estimated Coefficients from linear mixed effect models generated using DNA-scaled abundance.

		Screening experiment 2												
		1	2	3	4	5	6	7	8	9	10	11	12	13
Taxonomy	**OTU no.**													
Bacteroides thetaiotaomicron	848236	0.73	-	-	-	-	-	-	-	-	-	1.24	-	-
Bacteroides cellulosilyticus	539126	-	-0.42	-	-	-	-	-	-0.36	-	-	-	-	-
Bacteroides vulgatus	850870	0.67	-	-	-	-	-	-	-	-	-	-	-	-
Bacteroides caccae	579112	-	1.96	-	-	-	-	-	-	-	-	-	-	-
Bacteroides ovatus	844958	0.39	-0.36	-	-	-	-	0.5	-	2.01	-	0.27	-	-
Parabacteroides distasonis	846317	-0.55	-0.53	-	-	-	-	-	1.72	-	-	-	-	-
Escherichia coli	1111717	0.59	-0.57	-0.69	-0.86	-0.91	-	-	-0.52	-	-0.93	-0.57	-0.71	-0.87
Ruminococcaceae sp.	360801	-0.02	-	-	0.04	-	-	-	-	-	-	-	-	-
Subdoligranulum variabile	364609	-	-0.22	-	-0.14	-0.19	-	-	-0.17	-	-0.18	-0.16	-	-0.17
Collinsella aerofaciens	1110606	-	-0.19	-0.25	-0.23	-0.26	-0.23	-	-0.22	-	-0.18	-0.2	-0.2	-0.27
Bacteroides massiliensis	840832	-0.04	-0.06	-0.06	-0.05	-0.05	-0.04	-0.05	-0.06	-0.06	-0.04	-0.06	-0.04	-0.06
Odoribacter splanchnicus	210303	-	-0.03	-	-	-	-	-	-0.03	-	-0.03	-0.02	-	-0.02
Bacteroides finegoldii	de novo OTU													
Peptococcus niger	1135793	-0.11	-0.13	-0.14	-0.13	-0.15	-	-	-0.17	-	-0.15	-0.16	-0.15	-0.14
1, inulin LMW, 2, inulin HMW, 3- tomato pomace, 4- tomato peel, 5- rice bran, 6- chia see, 7- wheat bran, 8- resistant maltodextrin, 9- oat beta glucan, 10- oat hull fiber, 11- pea fiber, 12- corn bran														
"-" indicates estimate was not statistically significant (ANOVA, $P < 0.05$)														
blank cells indicate that the organism was not detected above the 0.05% relative abundance cut-off in the experiment														

Table 7 – Screening Experiment 3

(A) Estimated Coefficients from linear mixed effect models generated using relative abundance

		Screening experiment 3											
		1	2	3	4	5	6	7	8	9	10	11	12
Taxonomy	**OTU no.**												
Bacteroides thetaiotaomicron	848236	2	-	-	1	-	1.72	0.4	1.67	1.31	-0.52	2.55	0.87
Bacteroides cellulosilyticus	539126	-	0.57	-	-	-	-	-	-	-	-0.88	-	-
Bacteroides vulgatus	850870	0.6	0.73	-	1.09	0.55	0.81	0.65	-	0.82	-	-	-
Bacteroides caccae	579112	-0.54	-	-	-	-0.91	-0.57	-	-	-0.78	4.85	-0.67	0.79
Bacteroides ovatus	844958	0.59	-	0.64	0.52	-	-	0.72	1.17	0.93	-0.46	0.34	1.05
Parabacteroides distasonis	846317	-0.95	-0.47	-0.32	-0.8	-0.78	-0.63	-0.38	-0.88	-0.94	-1.02	-1.15	-0.8
Escherichia coli	1111717	-0.85	-	-0.71	-1.03	3.43	-0.56	-0.84	-0.59	-0.56	-0.55	-	-1.33
Ruminococcaceae sp.	360801	0.17	-	-	0.13	-0.08	-	-	0.21	0.07	-	0.45	-
Subdoligranulum variabile	364609	-0.39	-0.29	-	-	-0.79	-0.45	-	-	-0.35	-0.65	-0.66	-0.28
Collinsella aerofaciens	1110606	-0.26	-	-	-0.25	-0.3	-	-	-	-0.25	-	-0.26	-
Bacteroides massiliensis	840832	-0.11	-0.06	-	-0.1	-0.11	-0.06	-0.09	-0.12	-0.1	-0.1	-0.11	-0.1
Odoribacter splanchnicus	210303	-0.04	-0.04	-0.02	-0.03	-0.07	-	-0.04	-0.04	-	-0.05	-0.04	-0.02
Bacteroides finegoldii	de novo OTU	0.05	-	-	0.04	-	0.06	0.04	0.07	0.07	-	0.06	0.07
Peptococcus niger	1135793	-0.42	-0.34	-0.37	-0.41	-0.58	-0.38	-0.24	-0.53	-0.37	-0.5	-0.49	-0.48

1- citrus peel, 2- sugar cane fiber, 3- resistant starch 4, 4- orange peel, 5- psyllium, 6- yellow mustard bran, 7- citrus fiber, 8- soy cotyledon, 9- orange fiber fine, 10- inulin HMW, 11- citrus pectin, 12- potato fiber

"-" indicates estimate was not statistically significant (ANOVA, $P < 0.05$)

blank cells indicate that the organism was not detected above the 0.05% relative abundance cut-off in the experiment

(B) Estimated Coefficients from linear mixed effect models generated using DNA-scaled abundance.

		1	2	3	4	5	6	7	8	9	10	11	12	13
Taxonomy	**OTU no.**													
Bacteroides thetaiotaomicron	848236	2.02	-	-	1.21	-	1.52	-	2.47	1.39	-0.57	3.22	0.73	2.02
Bacteroides cellulosilyticus	539126	-	-	-	-	-0.47	-	-0.52	-	-	-0.83	0.49	-	-
Bacteroides vulgatus	850870	0.62	-	-	1.26	-	0.64	-	-	0.91	-	-	-	0.62
Bacteroides caccae	579112	-	-	-	-	-0.92	-0.51	-0.51	-	-0.57	4.22	-	0.76	-
Bacteroides ovatus	844958	0.67	-	-	0.66	-	-	-	1.76	1.01	-0.48	0.65	0.94	0.67
Parabacteroides distasonis	846317	-0.76	-0.6	-0.36	-0.64	-0.82	-0.54	-0.67	-0.51	-0.73	-0.9	-0.89	-0.65	-0.76
Escherichia coli	1111717	-0.69	-0.75	-0.73	-0.74	1.24	-	-1.16	-	-	-	-	-0.98	-0.69
Ruminococcaceae sp.	360801	0.17	-	-	0.15	-0.1	-	-	0.28	0.08	-	0.53	-	0.17
Subdoligranulum variabile	364609	-0.25	-0.34	-	-	-0.7	-0.37	-0.32	-	-	-0.56	-0.46	-	-0.25
Collinsella aerofaciens	1110606	-0.21	-	-	-0.2	-0.29	-	-0.19	-	-0.2	-	-	-	-0.21
Bacteroides massiliensis	840832	-0.09	-0.06	-	-0.09	-0.09	-	-0.09	-0.1	-0.09	-0.09	-0.1	-0.09	-0.09
Odoribacter splanchnicus	210303	-0.03	-0.05	-0.02	-0.02	-0.07	-	-0.05	-	-	-0.04	-	-	-0.03
Bacteroides finegoldii	de novo OTU	0.04	-	-	0.05	-	0.05	-	0.09	0.07	-	0.08	0.07	0.04
Peptococcus niger	1135793	-0.32	-0.35	-0.34	-0.33	-0.52	-0.32	-0.36	-0.38	-0.27	-0.43	-0.34	-0.4	-0.32

1, inulin LMW, 2, inulin HMW, 3- tomato pomace, 4- tomato peel, 5- rice bran, 6- chia see, 7- wheat bran, 8- resistant maltodextrin, 9- oat beta glucan, 10- oat hull fiber, 11- pea fiber, 12- corn bran

"-" indicates estimate was not statistically significant (ANOVA, $P < 0.05$)

blank cells indicate that the organism was not detected above the 0.05% relative abundance cut-off in the experiment

Example 3: Proteomics and forward genetics identify bioactive polysaccharides in fiber preparations

[0386] Several possible mechanisms could account for the increase of a target *Bacteroides* in response to fiber administration, including indirect effects involving other species. Therefore, we sought to determine which polysaccharides in the fiber preparations caused the target species to expand and whether they acted directly on those species by serving as nutrient sources for their growth. To do so, we simultaneously quantified community-wide protein expression and assessed the contributions of proteins to bacterial fitness using a forward genetic screen. The screen was based on genome-wide transposon (Tn) mutagenesis and a method known as multi-taxon INsertion Sequencing (INSeq), which allows simultaneous analysis of Tn mutant libraries generated from different *Bacteroides* species in the same recipient gnotobiotic mouse. We employed five INSeq libraries constructed using type strains corresponding to four *Bacteroides* species present in the Ln co-twin donor culture collection. The quality and performance of these libraries had been characterized previously *in vitro* and *in vivo* (30,300-167,000 isogenic Tn mutants/library; single site of Tn insertion/strain; 11-26 Tn insertions/gene; 71-92% genes covered/genome; (Hibberd et al., 2017; Wu et al., 2015)). Additionally, we simplified the community used in these experiments by omitting six strains from the original 20 member consortium that were not robust colonizers in the HiSF-LoFV diet context (Faith et al., 2014; Ridaura et al., 2013). All mice were colonized with the resulting 15-member community see Table S4 of Patnode et al., Cell, 2019, 179(1): 59-73) while consuming the base (unsupplemented) HiSF-LoFV diet. Animals were divided into five groups (n=6 animals/group) and were either continued on the base HiSF-LoFV diet or, two days after gavage, switched to the HiSF-LoFV diet supplemented with one of the fibers identified in the screen. We tested pea fiber, citrus pectin, orange peel, and tomato peel, each at a concentration of 10% (w/w), based on their ability to increase the representation of one or more of the targeted *Bacteroides* (**FIG. 1B**). All diets were administered *ad libitum* and given monotonously for the duration of the experiment (**FIG. 11**, see also

Tables S4A-S4C of Patnode et al., Cell, 2019, 179(1): 59-73). DNA isolated from fecal samples was subjected to short read shotgun DNA sequencing (COmmunity PROfiling by Sequencing, COPRO-Seq; (Hibberd et al., 2017; McNulty et al., 2013) to quantify the representation of each community member as a function of fiber treatment, including the combined abundance of all INSeq mutants for a given species. Our previous studies had established that in aggregate, a population of INSeq mutants behaves similarly to the corresponding wild-type parental strain (Hibberd et al., 2017; Wu et al., 2015).

[0387] Consistent with results obtained from seven days of fiber administration in the screening experiments, we observed a statistically significant expansion of *B. thetaiotaomicron* VPI-5482 in mice consuming pea fiber (ANOVA, P <0.05; **FIG. 2B**, see also Tables S4A-S4C of Patnode et al., Cell, 2019, 179(1): 59-73). Also in accordance with observations made in the screen, the relative abundance of *B. ovatus* ATCC-8483 was significantly greater in the pea fiber-treated group (**FIG. 2C**), while *B. cellulosilyticus* WH2 and *B. vulgatus* ATCC-8482 did not exhibit significant changes during this time period (**FIG. 2D and FIG. 2E**). Citrus pectin induced significant expansion of three species (*B. cellulosilyticus*, *Bacteroides finegoldii*, and a member of the *Ruminococcaceae*) that was distinct from the set affected by pea fiber (**FIG. 7D**, see also Tables S4A, B, and D of Patnode et al., Cell, 2019, 179(1): 59-73). Although the fiber screen predicted an increase in the abundance of *B. thetaiotaomicron* in response to citrus pectin, this was not observed during monotonous feeding until later in the time course, indicating a difference between the strains employed or the effect of different community context (**FIG. 7B**). Orange peel significantly increased the representation of *B. vulgatus*, but otherwise had a minimal effect on community structure (see Table S4A of Patnode et al., Cell, 2019, 179(1): 59-73). Tomato peel did not significantly increase any members of this community, which may indicate the strain-dependency of a given species' response to a certain fiber when the effect size of a given fiber preparation is low (see Table S4A of Patnode et al., Cell, 2019, 179(1): 59-73). Since both pea fiber and citrus pectin had pronounced effects on distinct sets of taxa, we selected these preparations for more detailed functional studies of their utilization by community members.

[0388] *Structural analyses of lead fibers* - We used permethylation and gas-chromatography-mass spectrometry to analyze the monosaccharide composition and glycosidic linkages of polysaccharides present in pea fiber and citrus pectin. After accounting for starch (typically degraded and absorbed by the host) and cellulose (not metabolized by the target *Bacteroides*; (McNulty et al., 2013)), the most abundant polysaccharide in pea fiber was arabinan, consisting of a linear 1,5-linked arabinose backbone with arabinose residues as side chains at position 2 or 3 (**FIG. 2A, Table C**). Linear xylan (4-linked xylose), homogalacturonan (4-linked galacturonic acid) and rhamnogalacturonan I (2- and 2,4-linked rhamnose) were also detected as structural features of the polysaccharides in pea fiber. Homogalacturonan with a high degree of methyl esterification was the main structural component of citrus pectin (88.6% galacturonic acid), with arabinan, 1,4-linked galactan and RGI present as minor components (**FIG. 7A, Table D**).

[0389] *High-resolution proteomic analysis of community gene expression* - The results of these biochemical analyses raised the possibility that metabolism of arabinan in pea fiber and methylated homogalacturonan in citrus pectin were involved in the responses of target *Bacteroides*. To test this hypothesis, we turned to high-resolution shotgun proteomic analysis, focusing on fecal samples obtained on day 6 of the monotonous feeding experiment. After considering only peptides that uniquely mapped to a single seed protein, 11,493 proteins were advanced to quantitative analysis (summed abundances; 59% from community members, 36% from mouse and 2% from diet; *see Methods*). We calculated a z-score for each expressed protein from each bacterial species using the abundances of all proteins assigned to that individual species in a given sample. This allowed us to determine changes in the abundance of each protein irrespective of changes in the abundance of that species in the community. In the case of the *Bacteroides* species represented by INSeq libraries, we considered the measured abundance of a given protein to reflect the summed contributions of all the mutant strains of that species (thus representing the level of expression we would expect from a corresponding wild-type strain). Linear models were constructed using limma (Smyth, 2004; Ting et al., 2009) and significant effects were identified between bacterial protein

abundances and supplementation of the control diet with pea fiber and citrus pectin (245 and 450 proteins, respectively; |fold-change| > log2(1.2), $P < 0.05$, FDR corrected). *Bacteroides* contain multiple polysaccharide utilization loci (PULs) in their genomes. PULs provide a fitness advantage by endowing a species with the ability to sense, import, and process complex glycans using their encoded carbohydrate-responsive transcription factors, SusC/SusD-like transporters, and carbohydrate active enzymes (CAZymes) (Glenwright et al., 2017; Kotarski and Salyers, 1984; Martens et al., 2011; McNulty et al., 2013; Shepherd et al., 2018). Eighty-five of the proteins whose levels were significantly altered by pea fiber and 134 that were significantly affected by citrus pectin were encoded by PULs (Terrapon et al., 2018).

[0390] Ranking proteins by the pea-fiber induced increase in their abundance disclosed that in *B. thetaiotaomicron*, 6 of the top 10 were encoded by PULs 7, 73, and 75. PUL7 is known to be involved in arabinan metabolism (Lynch and Sonnenburg, 2012; Schwalm et al., 2016), and encodes characterized and predicted arabinofuranosidases in glycoside hydrolase (GH) family 43, GH51, and GH146. PUL75 carries out the degradation of rhamnogalacturonan I (RGI) (Luis et al., 2018), but its expression is also triggered by exposure to purified arabinan *in vitro* (Martens et al., 2011). PUL73 processes homogalacturonan (Luis et al., 2018) and encodes CAZymes that cleave linked galacturonic acid residues and remove methyl and acetyl esters from galacturonic acid [polysaccharide lyase (PL)1, GH105, GH28, CE8, CE12 family members]. *B. ovatus* proteins encoded by predicted RGI-processing PULs (PUL97) (Luis et al., 2018) were among the most increased by pea fiber administration. Supplementation of the HiSF-LoFV diet with citrus pectin resulted in increased abundance of proteins encoded by a *B. cellulosilyticus* PUL that is induced by homogalacturonan *in vitro* (PUL83). In addition, citrus pectin induced expression of proteins in several *B. finegoldii* PULs (PUL34, 35, 42, and 43) that encode galacturonan-processing enzymes (GH28, GH105, GH106, PL11 subfamily 1, CE8 and CE12). This latter finding correlates with the organism's citrus pectin-driven expansion (see Tables S4A-B of Patnode et al., Cell, 2019, 179(1): 59-73).

[0391] *Combining proteomic and INSeq analyses* - As noted above, we colonized mice with INSeq libraries and then fed them the base HiSF-LoFV diet for two days before switching the experimental groups to fiber-supplemented diets. We measured the abundances of Tn mutant strains, and calculated log ratios between fecal samples collected on experimental day 6 (posttreatment) and day 2 (pre-treatment); results were compared to the reference HiSF-LoFV treatment arm to focus on genes that had significant fitness effects in the context of these fibers ($P < 0.05$, FDR corrected; see *Methods*; 223 genes, 24% in PULs; see also Table S6A of Patnode et al., Cell, 2019, 179(1): 59-73). Genes exhibiting a significant positive fold-change in protein abundance and negative effect on fitness when mutated appear in the bottom right quadrant of the orthogonal protein-fitness plots shown in **FIG. 2F- FIG. 2I**.

[0392] Genes in PULs were ranked by the magnitude of pea-fiber-dependent increases in the abundances their protein products and decreases in strain fitness when they were disrupted by a Tn insertion. The results revealed genes in three PULs (PUL7 in *B. thetaiotaomicron*, PUL5 in *B. cellulosilyticus*, and PUL27 in *B. vulgatus*; **FIG. 2F, FIG. 2H, and FIG. 2I**) that were affected by pea fiber. These three PULs are homologous as judged by a BLASTp comparison of their encoded proteins against the genomes of other community members (**FIG. 2J**). Genes in a highly-conserved arabinose utilization operon, present within the *B. thetaiotaomicron* and *B. cellulosilyticus* PULs, but at a site distant from PUL27 in *B. vulgatus,* had the greatest effect on *B. vulgatus* fitness of any genes represented in the mutant library (**FIG. 2I**; see also Table S6A of Patnode et al., Cell, 2019, 179(1): 59-73). We subsequently compared the genomes of five strains of *B. thetaiotaomicron*, and found that PUL7 was highly conserved with the exception of a single gene of unknown function (BT_0352) that was present in two of the strains (**FIG. 27**). PUL27 in *B. vulgatus* was also well conserved across 6 strains with the exception of some variability in the gene lengths of the hybrid two-component system and SusC-like transporter.

[0393] The increased fitness cost of mutations in *B. ovatus* RGI-processing PUL97, but not the *B. thetaiotaomicron* RGI-processing PUL75, indicated that these species utilize different carbohydrates in the pea fiber-supplemented diet

(RGI and arabinan, respectively; **FIG. 2G**; see also Table S6A of Patnode et al., Cell, 2019, 179(1): 59-73). In contrast, the overlapping reliance on arabinan degradation pathways in *B. thetaiotaomicron*, *B. vulgatus,* and *B. cellulosilyticus* raised the possibility that these species were engaged in competition with one another for arabinan in pea fiber.

[0394] A parallel analysis of mice monotonously fed citrus pectin revealed that five genes encoded by galacturonan-processing PUL83 in *B. cellulosilyticus* were among the most abundantly expressed and most important for fitness compared to the base diet condition (**FIG. 7H**). *B. vulgatus* did not expand with citrus pectin supplementation (**FIG. 7E**), nevertheless, it contained galacturonan-processing PULs (PUL5/6, PUL31, and PUL42/43) with genes involved in hexuronate metabolism whose protein products increased in abundance and, when mutated, conveyed decreased fitness when exposed to this fiber preparation (**FIG. 7I**). Consistent with increased reliance on citrus pectin, the abundance of *B. vulgatus* proteins involved in starch utilization (PUL38) was decreased in the presence of this fiber.

[0395] Together, our proteomic and INSeq datasets revealed the microbial genes required during fiber-driven expansion, highlighted the polysaccharides that contributed to the fitness effects of these fibers and provided evidence for functional overlap in the nutrient harvesting strategies of *B. cellulosilyticus* and *B. vulgatus*, in two distinct fiber conditions. The dominance of *B. cellulosilyticus* in diverse diet contexts led us to ask whether (and how) this species directly competes with other community members for polysaccharides.

Example 4 - Interspecies competition controls the outcomes of fiber-based microbiota manipulation

[0396] We performed a direct test for interactions between *B. cellulosilyticus* and other species by comparing the defined 15-member community, to the derivative 14-member community lacking *B. cellulosilyticus*. Using an experimental design that mimicked the monotonous feeding study described above, groups of germ-free mice were colonized with these two communities and fed the HiSF-LoFV diet with or without 10% (w/w) pea fiber or citrus pectin (see Tables S4B-S4C of Patnode et al.,

Cell, 2019, 179(1): 59-73). COPRO-Seq analysis was used to determine the abundance of each strain as a proportion of all strains other than *B. cellulosilyticus,* thereby controlling for the compositional effect of removing this species. Defined this way, the abundance of *B. thetaiotaomicron* did not increase upon omission of *B. cellulosilyticus* in the presence of pea fiber, suggesting minimal competition between these two species for arabinan (**FIG. 3A**; see also Tables S4B, C of Patnode et al., Cell, 2019, 179(1): 59-73). Proteomic analysis of fecal samples collected on experimental days 6, 12, 19, and 25 demonstrated that the proteins in *B. thetaiotaomicron* PUL7 whose abundances were increased by pea fiber in the complete community context, were not further increased in the absence of *B. cellulosilyticus* (**FIG. 3B**). *B. vulgatus* was the only species that expanded with pea fiber administration in the absence of *B. cellulosilyticus* ($P < 0.05$, ANOVA, FDR corrected; **FIG. 3C**; see also Tables S4B, D of Patnode et al., Cell, 2019, 179(1): 59-73). Proteomic analysis of serially collected fecal samples disclosed that the abundances of proteins encoded by *B. vulgatus* PUL27, as well as its arabinose operon, were persistently increased during exposure to pea fiber, regardless of whether *B. cellulosilyticus* was included in the community (**FIG. 3D**). Citrus pectin provided a second example of fiber-driven expansion of *B. vulgatus* in the absence of *B. cellulosilyticus* (**FIG. 8B**; see also Tables S4B, D of Patnode et al., Cell, 2019, 179(1): 59-73). Expression of proteins encoded by *B. vulgatus'* galacturonan-processing PULs 5, 6, 31, 42, and 43, were also induced by citrus pectin, irrespective of *B. cellulosilyticus* (**FIG. 8C and FIG. 8D**). *Odoribacter splanchnicus* expanded in the absence of *B. cellulosilyticus*; this effect was repressed by both pea fiber and citrus pectin administration.

[0397] These results demonstrate negative interactions between *B. vulgatus* and *B. cellulosilyticus* and suggest that the suppression of *B. vulgatus* when *B. cellulosilyticus* is present occurs due to the persistent competition between these organisms for arabinan in pea fiber and homogalacturonan in citrus pectin.

Example 5 - Artificial food particles as biosensors of community glycan degradative activities

[0398] To directly test the capacity of competing *Bacteroides* to process the same nutrient substrate *in vivo*, a bead-based glycan degradation assay was developed (**FIG. 4A**).Two polysaccharides of interest were selected: (i) a soluble, starch-depleted fraction of pea fiber polysaccharides composed predominantly of arabinose (83% of monosaccharides) with little xylose (4%), and (ii) wheat arabinoxylan (38% arabinose/62% xylose). The latter was used as a control given its established ability to support growth (*in vitro*) of *B. cellulosilyticus* (McNulty et al., 2013) but not *B. vulgatus* (Tauzin et al., 2016). These polysaccharides were biotinylated and each product was attached to a distinct population of microscopic (20μm diameter) streptavidin-coated paramagnetic glass beads, generating carbohydrate-coated artificial 'food particles' that could be recovered from mouse intestinal contents using a magnetic field. Each population of beads was also labeled with a distinct biotinylated fluorophore so that several types of polysaccharide-beads could be pooled, administered at the same time to the same mouse, recovered from the gut lumen or feces and then sorted into their original groups using a flow cytometer (**FIG. 4B**). 'Empty' beads that had not been incubated with polysaccharides, but were labeled with a unique biotinylated fluorophore, served as negative controls. The sorted beads were subjected to acid hydrolysis and the hydrolysis products were assayed by gas chromatography-mass spectrometry (GC-MS) to quantify the levels of bead-bound carbohydrate present before and after transit through the mouse gut. Alternative methods to quantify the levels of bead-bound carbohydrate present before and after administration can also be used.

[0399] Germ-free mice were colonized with either *B. cellulosilyticus* or *B. vulgatus* alone and fed a HiSF-LoFV diet supplemented with 10% (w/w) pea fiber. Seven days after colonization, all mice were gavaged with an equal mixture of the three bead types ($5x10^6$ of each type/animal, n=5-6 animals). Mice were euthanized 4 h later, beads were recovered from their cecum and colon, and the mass of monosaccharides on the different purified bead types was quantified. The fluorescent signal present on all

bead types persisted after intestinal transit, confirming that the biotin-streptavidin interactions were stable under these conditions (**FIG. 4B**). Pea fiber-beads recovered from both groups of mice had significantly reduced arabinose [26.1±3.4% (mean±SD) and 29.1±0.7% of levels in input beads, respectively]. In contrast, levels of arabinose were only significantly decreased on arabinoxylan-coated beads recovered from mice colonized with *B. cellulosilyticus* (**FIG. 4C**; **Table 9**).

[0400] A follow-up experiment of identical design was performed except that animals fed HiSF-LoFV supplemented with pea fiber were gavaged 12 days rather than seven days after colonization with a collection of four rather than three types of beads. These beads were either empty (no glycan bound) or coated with (i) the soluble, starch-depleted fraction of pea fiber, or wheat arabinoxylan, or lichenan from Icelandic moss, a control glycan low in arabinose (81% glucose / 8% mannose / 6% galactose / 2% arabinose). Beads were recovered, purified by flow cytometry and analyzed using GC-MS. The degradation of bead-bound pea fiber and arabinoxylan was similar to that observed on day 7.

[0401] To control for microbe-independent polysaccharide degradation, germ-free mice were given a gavage of arabinoxylan-coated, pea-fiber coated, lichenan-coated, and empty beads (n=13 animals). We collected all fecal samples produced during an 8 h period (from 4 to 12 hours after gavage). Assays of the arabinoxylan-, pea fiber-, and lichenan-coated beads purified from fecal samples obtained from each germ-free animal revealed no significant degradation of these polysaccharides after passage through their intestines (**FIG. 9C and FIG. 9D**;**Tables 8 and 9**). Together, these results provide a direct, in vivo demonstration of the overlapping capacities of competing *Bacteroides* species to degrade arabinan present in pea fiber.

[0402] Given the observation that several species can metabolize pea fiber arabinan *in vivo*, whether the absence of *B. cellulosilyticus* would compromise the efficiency with which the community carried out this function was assessed. Mice consuming the unsupplemented HiSF-LoFV diet were given pea fiber-coated, arabinoxylan-coated, lichenan-coated, and empty beads 12 days after colonization with

(i) the 15-member consortium or (ii) the derivative 14-member community lacking *B. cellulosilyticus*. Analysis of beads recovered from the cecal and colonic contents of these mice disclosed that the level of pea fiber degradation was not affected by the absence of *B. cellulosilyticus* (**FIG. 4D**). In a separate group of mice fed a pea-fiber supplemented HiSF-LoFV diet, degradation of bead-bound pea-fiber was also the same regardless of the presence of *B. cellulosilyticus* (**FIG. 9E and FIG. 9F**).

[0403] Thus, these artificial food particles provide a way to conduct *in vivo* assessments of dietary nutrient degradation by microbes as a function of community composition. Consistent with our detection of multiple species exploiting pea fiber arabinan as a nutrient source (**FIG. 2 and FIG. 3**), this community can compensate for the loss of *B. cellulosilyticus*-mediated arabinan degradation. In contrast, the breakdown of dietary arabinoxylan represents a non-redundant function provided by *B. cellulosilyticus*.

Table 8

		Input		*B. vulgatus* HiSF-LoFV		*B. cellulosilyticus* HiSF-LoFV	
	Monosaccharide	Mean (pg/bead)	sd	Mean (pg/bead)	sd	Mean (pg/bead)	sd
	Arabinoxylan beads	0.17	0.12	0.11	0.12	0.05	0.05
Xy	Pea Fiber beads	0.06	0.07	0.01	0.01	0.01	0.02
	Uncoated beads	0.01	0.01	0.01	0.01	0.02	0.03
	Arabinoxylan beads	0.54	0.25	0.38	0.15	0.15	0.07
Ara	Pea Fiber beads	0.2	0.06	0.12	0.06	0.1	0.02
	Uncoated beads	0.06	0.02	0.13	0.06	0.09	0.02
	Arabinoxylan beads	0.02	0.02	0.04	0.03	0.05	0.03
Man	Pea Fiber beads	0.04	0.02	0.06	0.04	0.06	0.04
	Uncoated beads	0.06	0.03	0.14	0.07	0.1	0.06
	Arabinoxylan beads	0.02	0.01	0.06	0.05	0.02	0.01
Gal	Pea Fiber beads	0.05	0.03	0.07	0.05	0.05	0.02
	Uncoated beads	0	0.01	0.05	0.03	0.04	0.2

	Arabinoxylan beads	0.02	0.04	0	0.01	0.01	0.01
Glc	Pea Fiber beads	0.01	0.02	0	0.01	0	0
	Uncoated beads	0.01	0.01	0	0.01	0.01	0.01

Abbreviations: xylose (Xyl), arabinose (Ara), mannose (Man), galactose (Gal), glucose (Glc)

Table 9A

		Mean (sd) pg/bead				
	BEAD TYPE	Input	A	B	C	D
Xyl	Arabinoxylan	2.1 (0.55)	0.44 (0.21)	0.85 (0.24)	0.23 (0.18)	0.36 (0.11)
	Pea Fiber	0.2 (0.29)	0.09 (0.07)	0.06 (0.03)	0.03 (0.02)	0.08 (0.04)
	Lichenan	0.06 (0.02)	0.06 (0.05)	0.11 (0.09)	0.04 (0.02)	0.08 (0.04)
	Uncoated	0.07 (0.02)	0.21 (0.37)	0.07 (0.07)	0.1 (0.14)	0.43 (0.52)
Ara	Arabinoxylan	1.11 (0.31)	0.21 (0.08)	0.69 (0.28)	0.15 (0.05)	0.28 (0.1)
	Pea Fiber	0.7 (0.21)	0.2 (0.09)	0.23 (0.04)	0.16 (0.06)	0.24 (0.04)
	Lichenan	0.14 (0.07)	0.12 (0.02)	0.17 (0.12)	0.11 (0.04)	0.1 (0.04)
	Uncoated	0.06 (0.01)	0.08 (0.03)	0.15 (0.16)	0.09 (0.07)	0.09 (0.03)
Man	Arabinoxylan	0 (0.01)	0.02 (0.01)	0.02 (0.01)	0.02 (0.01)	0.02 (0.01)
	Pea Fiber	0.05 (0.04)	0.04 (0.01)	0.09 (0.04)	0.02 (0.01)	0.03 (0.01)
	Lichenan	0.12 (0.05)	0.14 (0.06)	0.17 (0.13)	0.09 (0.03)	0.07 (0.03)
	Uncoated	0 (0.01)	0.04 (0.01)	0.04 (0.02)	0.02 (0.01)	0.03 (0.01)
Gal	Arabinoxylan	0.02 (0.01)	0.09 (0.02)	0.11 (0.06)	0.09 (0.02)	0.07 (0.01)
	Pea Fiber	0.22 (0.29)	0.21 (0.04)	0.35 (0.14)	0.13 (0.04)	0.18 (0.05)
	Lichenan	0.23 (0.1)	0.2 (0.14)	0.44 (0.29)	0.21 (0.1)	0.29 (0.09)
	Uncoated	0.02 (0.01)	0.21 (0.08)	0.27 (0.13)	0.12 (0.03)	0.11 (0.04)
Glc	Arabinoxylan	9.15 (7.95)	11.53 (7.18)	14.21 (11.64)	8 (3.43)	23.27 (17.41)
	Pea Fiber	38.2 (33.13)	12.81 (3.85)	22.32 (8.36)	6.91 (1.14)	20.48 (10.25)
	Lichenan	193.16 (71.35)	23.41 (4.76)	37.74 (26.01)	24.89 (8.33)	19.62 (7.81)
	Uncoated	8.41 (4.65)	11.3 (5.51)	14.49 (10.38)	7.12 (2.65)	11.68 (1.3)

A = 15-member, HiSF-LoFV; B= 14-member (No B.c.), HiSF-LoFV; C= 15-member HiSF-LoFV + Pea Fiber; D=14-member (No B.c.), HiSF-LoFV+ Pea Fiber

Abbreviations: xylose (Xyl), arabinose (Ara), mannose (Man), galactose (Gal), glucose (Glc)

Table 9B

		Mean (sd) pg/bead	
	BEAD TYPE	E	F
Xyl	Arabinoxylan	0.22 0.18	1.46 0.24
	Pea Fiber	0.14 0.15	0.06 0.01
	Lichenan	0.04 0.02	0.14 0.09
	Uncoated	0.16 0.04	0.1 0.04
Ara	Arabinoxylan	0.1 0.02	1.19 0.12
	Pea Fiber	0.18 0.02	0.2 0.01
	Lichenan	0.08 0.08	0.14 0.01
	Uncoated	0.04 0.03	0.07 0.01
Man	Arabinoxylan	0.03 0.03	0.01 0.01
	Pea Fiber	0.03 0.02	0.02 0.01
	Lichenan	0.1 0.04	0.1 0.02
	Uncoated	0.06 0.03	0.02 0.01
Gal	Arabinoxylan	0.15 0.14	0.1 0.02
	Pea Fiber	0.25 0.12	0.22 0.02
	Lichenan	0.36 0.15	0.46 0.18
	Uncoated	0.27 0.13	0.2 0.06
Glc	Arabinoxylan	7.13 5.46	8.39 0.81
	Pea Fiber	8.35 3.84	8.34 2.2
	Lichenan	23.52 3.84	69.59 15.85
	Uncoated	14.47 2.24	17.82 5.76

E = B. cellulosilyticus, HiSF-LoFV+ Pea Fiber; F = B. vulgatus, HiSF-LoFV+ Pea Fiber

Abbreviations: xylose (Xyl), arabinose (Ara), mannose (Man), galactose (Gal), glucose (Glc)

Table 10

		Input		Germ-free HiSF-LoFV	
	Monosaccharide	Mean (pg/bead)	sd	Mean (pg/bead)	sd
Xyl	Arabinoxylan beads	1.18	0.16	1.35	0.41

	Pea Fiber beads	0.11	0.08	0.22	0.26
	Lichenan beads	0.16	0.22	0.14	0.08
	Uncoated beads	0.11	0.03	0.18	0.06
	Arabinoxylan beads	0.56	0.11	0.77	0.3
Ara	Pea Fiber beads	0.2	0.02	0.3	0.14
	Lichenan beads	0.04	0.02	0.1	0.04
	Uncoated beads	0.05	0.03	0.11	0.04
	Arabinoxylan beads	0.01	0.01	0.05	0.05
Man	Pea Fiber beads	0.03	0.02	0.05	0.03
	Lichenan beads	0.12	0.07	0.23	0.14
	Uncoated beads	0.02	0.01	0.03	0.01
	Arabinoxylan beads	0.03	0.01	0.2	0.07
Gal	Pea Fiber beads	0.06	0.03	0.37	0.14
	Lichenan beads	0.2	0.1	0.59	0.23
	Uncoated beads	0.07	0.08	0.26	0.11
	Arabinoxylan beads	13.72	3.3	13.78	5.2
Glc	Pea Fiber beads	7.27	4.32	25.47	22.97
	Lichenan beads	79.39	40.38	92.09	57.16
	Uncoated beads	22.58	25	22.24	19.15

Abbreviations: xylose (Xyl), arabinose (Ara), mannose (Man), galactose (Gal), glucose (Glc)

Table 11

			Mean (sd) pg/bead			
	BEAD TYPE	Input	A	B	C	D
Xyl	Arabinoxylan	0.15 (0.09)	0.1 (0.02)	0.13 (0.07)	0.22 (0.33)	0.11 (0.07)
	Mannan	0.38 (0.16)	0.31(0.07)	0.23 (0.17)	0.26 (0.12)	0.32 (0.25)
	Uncoated	0.05 (0.05)	0.08 (0.04)	0.44 (0.66)	0.09 (0.05)	0.08 (0.01)
	Arabinoxylan (spike-in control)	0.1 (0.03)	0.17 (0.21)	0.43 (0.75)	0.17 (0.14)	0.09 (0.05)
Ara	Arabinoxylan	9.02 (2.84)	3.07 (0.68)	3.73 (1.57)	4.41 (4.08)	8.45 (3.53)
	Mannan	1.09 (2.46)	0.09 (0.05)	0.04 (0.02)	0.07 (0.06)	0.1 (0.05)

	Uncoated	0.29 (0.42)	0.1 (0.04)	0.06 (0.03)	0.1 (0.06)	0.24 (0.1)
	Arabinoxylan (spike-in control)	7.44 (3.09)	8.61 (4.43)	8.02 (2.38)	10.52 (1.95)	8.67 (6.21)
	Arabinoxylan	7.15 (2.24)	2.07 (0.49)	3.61 (1.86)	2.83 (2.48)	6.43 (2.87)
	Mannan	1.18 (1.93)	0.45 (0.39)	0.27 (0.16)	0.55 (0.18)	0.34 (0.19)
Man	Uncoated	0.26 (0.4)	0.1 (0.05)	0.12 (0.07)	0.1 (0.02)	0.17 (0.02)
	Arabinoxylan (spike-in control)	5.82 (2.39)	5.46 (2.11)	7.06 (2.31)	6.16 (1.72)	5.78 (3.89)
	Arabinoxylan	0.17 (0.1)	0.12 (0.02)	0.16 (0.05)	0.17 (0.1)	0.15 (0.07)
	Mannan	0.23 (0.29)	0.11 (0.1)	0.06 (0.05)	0.1 (0.06)	0.08 (0.06)
Gal	Uncoated	0.02 (0.01)	0.06 (0.02)	0.06 (0.03)	0.08 (0.02)	0.07 (0.02)
	Arabinoxylan (spike-in control)	0.14 (0.04)	0.17 (0.09)	0.14 (0.02)	0.8 (0.05)	0.12 (0.08)
	Arabinoxylan	0.06 (0.03)	0.03 (0.03)	0.05 (0.02)	0.06 (0.09)	0.05 (0.04)
	Mannan	1.08 (0.49)	1.09 (0.53)	0.85 (0.83)	0.87 (0.6)	1.15 (0.8)
Glc	Uncoated	0.02 (0.02)	0.04 (0.03)	0.05 (0.04)	0.04 (0.03)	0.07 (0.06)
	Arabinoxylan (spike-in control)	0.04 (0.02)	0.03 (0.02)	0.04 (0.03)	0.02 (0.02)	0.03 (0.03)

A = 15-member, HiSF-LoFV; B= 14-member (No B.c.), HiSF-LoFV; C= 14-member (No B.o.), HiSFmBO; D=13-member (No B.c., No B.o.), HiSF-LoFVmBO

Abbreviations: xylose (Xyl), arabinose (Ara), mannose (Man), galactose (Gal), glucose (Glc)

Example 6 – Acclimation to the presence of a potential competitor alleviates resource conflict

[0404] The *in vivo* bead-based glycan degradation assays revealed that in contrast to arabinan, the capacity of the community to process arabinoxylan was not rescued by other species in the absence of *B. cellulosilyticus* (**FIG. 4D**; **Tables 8-11**). This was unexpected, given that *B. cellulosilyticus* omission resulted in a significant increase in the relative abundance of *B. ovatus* (**FIG. 5**; see also Table S4 of Patnode et al., Cell, 2019, 179(1): 59-73), which encodes PULs capable of arabinoxylan breakdown (Martens et al., 2011; Rogowski et al., 2015). We examined whether these

results could arise from a type of interspecies relationship between *B. cellulosilyticus* and *B. ovatus* distinct from that observed between *B. cellulosilyticus* and B. *vulgatus*.

[0405] As discussed above, the abundances of *B. vulgatus* proteins involved in pea fiber or citrus pectin degradation were unchanged upon removal of its competitor *B. cellulosilyticus*. In contrast, *B. ovatus* exhibited metabolic flexibility, with proteins encoded by two arabinoxylan-processing PULs (PUL26 and PUL81) predominating among those whose abundances were increased when *B. cellulosilyticus* was absent versus present (**FIG. 5C and FIG.**). This effect was apparent regardless of whether mice were fed the pea fiber-supplemented, citrus pectin- supplemented, or control unsupplemented HiSF-LoFV diets, consistent with the presence of arabinoxylan in the HiSF-LoFV diet. When we analyzed the contributions of genes to the fitness of *B. ovatus* (by calculating the changes in the abundance of Tn mutant strains from day 2 to day 6), those in these two arabinoxylan PULs were the most affected by omission of *B. cellulosilyticus* (**FIG. 5D and FIG. 5F**; see also Table S4B of Patnode et al., Cell, 2019, 179(1): 59-73). This result indicates that *B. ovatus* exhibits a marked decrease in its reliance on arabinoxylan in the full 15-member community context. Examining another group of mice that received a 14-member community lacking *B. vulgatus* revealed that its absence did not induce changes in *B. ovatus* at the level of its relative abundance, the abundances of proteins encoded by its PULs involved in arabinoxylan processing or by other PULs, or in the fitness cost associated with mutations in its arabinoxylan-processing PULs or in other PULs (**FIG. 5A and FIG. 5C, and FIG. 5D**; see also Tables S4D, A5F, and S6B of Patnode et al., Cell, 2019, 179(1): 59-73).

[0406] Monosaccharide and linkage analysis verified that arabinoxylan was present in the HiSF-LoFV diet; this conclusion was based on finding abundant 4-linked xylose with branching 4,3-linked xylose, and terminal arabinose (**Tables 3-4**). We also detected small amounts of 3-linked glucose (indicative of hemicellulose beta-glucans), galacturonic acid and rhamnose. The presence of these structures in the base HiSF-LoFV diet are consistent with the observed increase in abundance of proteins in *B. ovatus* PULs shown or predicted to process beta-glucan, rhamnogalacturonan, and host glycan when *B. cellulosilyticus* is present (**FIG. 28**).

[0407] Based on these results, we reasoned that metabolic flexibility allows *B. ovatus* to acclimate to the presence of *B. cellulosilyticus* by shifting its nutrient harvesting strategies, de-emphasizing arabinoxylan degradation, thus mitigating competition between the two species. To test this notion further, we performed an experiment omitting B. *cellulosilyticus*, *B. ovatus*, or both species from the 15-member consortium introduced into mice (see Tables S4E of Patnode et al., Cell, 2019, 179(1): 59-73). Animals were fed the base HiSF-LoFV diet for 12 days and fecal samples were collected as in previous experiments. Confirming our earlier results, COPRO-Seq revealed that the abundance of *B. ovatus* was increased in the absence of *B. cellulosilyticus* (**FIG. 6B**; see also Tables S4B-E of Patnode et al., Cell, 2019, 179(1): 59-73). Proteomics analysis of fecal samples obtained on day 6 of this experiment also revealed an increase in the abundance of 16 proteins encoded by arabinoxylan-processing PULs 26 and 81 in *B. ovatus* when *B. cellulosilyticus* was removed (**FIG. 6D**). In contrast, the abundance of *B. cellulosilyticus* as a proportion of the remaining strains did not increase (**FIG. 6C**; see also Table S4E of Patnode et al., Cell, 2019, 179(1): 59-73), with just one protein specified by each of its arabinoxylan-processing PULs in *B. cellulosilyticus* (PULs 86 and 87) significantly increasing in abundance when *B. ovatus* was absent (**FIG. 6E**). These results, combined with the observation that arabinoxylan-processing genes are important for fitness of *B. ovatus* only when *B. cellulosilyticus* is absent (**FIG. 5E**), indicate that the metabolic flexibility of *B. ovatus* mitigates competition between two species with the capacity to process the same dietary fiber resource.

[0408] We sought to directly measure the functional outcome of metabolic flexibility in *B. ovatus* and establish that this species degraded arabinoxylan in the community lacking *B. cellulosilyticus*. Therefore, arabinoxylan-beads, as well as empty and yeast alpha-mannan coated control beads, were administered to the four groups of mice described above, with all mice consuming the base HiSF-LoFV diet. In the absence of *B. cellulosilyticus*, significant degradation of arabinoxylan was still detected (**FIG. 6F**), consistent with our previous observations (**Tables 8-11**). Omission of *B. ovatus* was also associated with persistent degradation (**FIG. 6F**), as expected based

on the expression of arabinoxylan PULs by *B. cellulosilyticus*. However, arabinoxylan-coated beads recovered from mice lacking *B. ovatus* and *B. cellulosilyticus* were indistinguishable from input beads (**FIG. 6F**). In addition, omission of both *B. cellulosilyticus* and *B. ovatus* did not produce significant increases in the proportions of the remaining strains relative to one another (see Table S4E of Patnode et al., Cell, 2019, 179(1): 59-73), suggesting that these other species were unable to take advantage of the available arabinoxylan resources in the diet. None of the community contexts examined produced significant decreases in bead-bound mannan, controlling for non-specific polysaccharide degradation (**FIG. 6G**). As an additional 'spike-in' control, we added arabinoxylan beads to cecal and fecal samples obtained from all groups of mice immediately after they were euthanized and recovered and processed them in parallel with the orally administered beads. The preservation of carbohydrate on spike-in beads established that *B. cellulosilyticus/B. ovatus*-dependent degradation occurred during intestinal transit and not sample processing (**FIG. 6F**).

[0409] Together, these experiments show that, in contrast to the persistent competition for arabinan and homogalacturonan exhibited by *B. vulgatus*, *B. ovatus* avoids competition for arabinoxylan via acclimation to the presence of its potential competitor, *B. cellulosilyticus.* This conclusion is based on several observations; (i) the HiSF-LoFV diet contains arabinoxylan polysaccharides, which can be metabolized by both species in question, (ii) omission of *B. ovatus* did not cause detectable expansion of *B. cellulosilyticus*, (iii) proteins encoded by *B. ovatus* arabinoxylan PULs were significantly increased when *B. cellulosilyticus* was absent, (iv) genes in *B. ovatus* arabinoxylan PULs were more important for fitness when *B. cellulosilyticus* was absent, and (v) *B. ovatus* was responsible for the residual arabinoxylan degradation that took place in the absence of *B. cellulosilyticus*.

Example 7 – Discussion for Examples 2-6

[0410] Together, Examples 2-6 show that, in contrast to the persistent competition for arabinan and homogalacturonan exhibited by *B. vulgatus*, *B. ovatus* avoids competition via acclimation to the presence of its potential competitor, *B. cellulosilyticus*. This conclusion is based on the observations that (i) omission of *B.*

ovatus did not cause detectable expansion of *B. cellulosilyticus*, (ii) proteins encoded by *B. ovatus* arabinoxylan PULs were significantly increased when *B. cellulosilyticus* was absent, (iii) genes in *B. ovatus* arabinoxylan PULs were significantly more important for fitness when *B. cellulosilyticus* was absent, and (iv) *B. ovatus* was responsible for the residual arabinoxylan degradation that took place in the absence of *B. cellulosilyticus*.

[0411] Combining (i) high resolution proteomics, (ii) forward genetic screens for fitness determinants, (iii) a collection of glycan-coated artificial food particles, and (iv) deliberate manipulations of community membership in gnotobiotic mice fed 'representative' high-fat, low-fiber USA diet led to the direct characterization of how human gut *Bacteroides* with distinct, as well as overlapping, nutrient harvesting capacities respond to different food-grade fibers. Our approach allowed us to identify bioactive components in compositionally complex fibers that impact specific members of the microbiota. Obtaining this type of information can inform food manufacturing practices by directing efforts to seek sources of and enrich for these active components; e.g., through judicious selection of cultivars of a given food staple, food processing methods or an existing waste stream from food manufacturing to mine for these components.

[0412] Deliberately manipulating membership of a consortium of cultured, sequenced human-donor derived microbes prior to their introduction into gnotobiotic mice fed a human diet, with or without fiber supplementation, provides an opportunity to determine whether and how organisms compete and what mechanisms they use to avoid competition. Simultaneous harvest of a particular dietary resource by two species is theoretically possible whenever they both contain a genetic apparatus sufficient for metabolism of that resource. We provide evidence that competition for particular glycans in fiber preparations is realized in such a model community, since glycan-degrading genes were expressed and required for fitness in both species, and negative interactions were observed in strain omission experiments. These omission experiments disclosed distinct relationships between *B. vulgatus*, *B. ovatus* and *B. cellulosilyticus*; namely, the ability of *B. ovatus* to acclimate to the presence of a competitor (*B. cellulosilyticus*) as opposed to the persistent competition between *B. vulgatus* and *B.*

cellulosilyticus for the same resource. A healthy human gut microbiota has great strain-level diversity. Determining which strains representing a given species to select as a lead candidate probiotic agent, or for incorporation into synbiotic (prebiotic plus probiotic) formulations, is a central challenge for those seeking to develop next generation microbiota-directed therapeutics. Identifying organisms with metabolic flexibility, as opposed to those that are more prone to competing with other community members, could contribute to understanding how certain strains are capable of coexisting with the residents of diverse human gut communities.

[0413] Particles present in foods prior to consumption, or generated by physical and biochemical/enzymatic processing of foods during their transit through the gut, provide community members with opportunities to attach to their surfaces, and harvest surface-exposed nutrient resources. The ability of organisms to adhere to such particles, the carrying capacity of particles (size relative to nutrient content), and the physical partitioning their component nutrients can be envisioned as affecting competition, conflict avoidance, and cooperation. The ability of a given gut microbial community to degrade different fiber components was quantified in our studies using artificial food particles composed of fluorescently labeled, paramagnetic microscopic beads coated with different polysaccharides. This approach provides an additional dimension for characterizing the functional properties of a microbial community, and has a number of advantages. First, the measurement of polysaccharides coupled to magnetic beads is not confounded by the presence in the gut of structurally similar (or even identical) dietary or microbial polysaccharides. Second, this technology, when applied to gnotobiotic mice, permits simultaneous testing of multiple glycans in the same animal, allowing a direct comparison of the degradative capabilities of different assemblages of human gut microbes *in vivo*. For example, we were able to demonstrate non-redundant arabinoxylan degradation carried out by *B. cellulosilyticus* in this community, despite the presence of another arabinoxylan degrader, *B. ovatus*. Third, applied directly to humans, these diagnostic 'biosensors' could be used to quantify functional differences between their gut microbiota, and physical associations between carbohydrates and strains of interest, as a function of host health status,

nutritional status/interventions, or other perturbations. As such, results obtained with these biosensors could facilitate ongoing efforts to use machine learning algorithms that integrate a variety of parameters, including biomarkers of host physiologic state and features of the microbiota, to develop more personalized nutritional recommendations (Zeevi et al., 2015). Lastly, this technology could be used to advance food science. The bead coating strategy employed was successful with over 30 commercially available polysaccharide preparations and the assay has been extended to measure the degradation of other biomolecules, including proteins. Particles carrying components of food that have been subjected to different processing methods, or particles bearing combinations of nutrients designed to attract different sets of primary (and secondary) microbial consumers could also be employed in preclinical models to develop and test food prototypes optimized for processing by the microbiota representative of different targeted human consumer populations.

Example 8– Methods for Examples 2-6

[0414] *Gnotobiotic mice* – All experiments involving mice were carried out in accordance with protocols approved by the Animal Studies Committee of Washington University in St. Louis. For screening different fiber preparations, germ-free male C57BL/6J mice (10-16 weeks-old) were singly housed in cages located within flexible plastic isolators. Cages contained paper houses for environmental enrichment. Animals were maintained on a strict light cycle (lights on at 0600 h, off at 1900 h). Mice were fed a LoSF-HiFV diet for five days prior to colonization. After colonization, the community was allowed to stabilize on the LoSF-HiFV diet for an additional five days. One group of control mice remained on this diet for the rest of the experiment and a second control group was switched to the HiSF-LoFV diet for the rest of the experiment.

[0415] Mice in the experimental group first received an introductory diet containing equal parts of all fiber preparations employed in a given screen (totaling 10% of the diet by weight), and then received a series of diets containing different fiber preparations as described in **FIG. 1A**. A 10 g aliquot of a given diet/fiber mixture was hydrated with 5 mL sterile water in a gnotobiotic isolator; the resulting paste was pressed into a feeding dish and placed on the cage floor. Food levels were monitored

nightly, and a freshly hydrated aliquot of that diet was supplied every two days (preventing levels from dropping below roughly one third of the original volume). Bedding (Aspen Woodchips; Northeastern Products) was replaced after each 7-day diet period to prevent any spilled food from being consumed during the next diet exposure. Fresh fecal samples were collected from each animal within seconds of being produced on days 1, 3, 6, and 7 of every diet period, and placed in liquid nitrogen within 45 min. Pre-colonization fecal samples were collected to verify the germ-free status of mice.

[0416] For monotonous feeding experiments, mice were fed the control HiSF-LoFV diet in its pelleted form for two weeks prior to colonization. Two days after colonization, mice were switched to paste diets containing 10% of the powdered fiber preparation mixed into the base diet (or the base diet in paste form without added fiber) for the remainder of the experiment. As noted above, these diets were delivered in freshly hydrated aliquots every two days. Fecal samples, including those obtained prior to colonization, were collected on the days indicated in **FIG. 11**.

[0417] *Defined microbial communities* –The screening experiments used cultured, sequenced bacterial strains obtained from a fecal sample that had been collected from a lean co-twin in an obesity-discordant twin-pair [Twin Pair 1 in (Ridaura et al., 2013); also known as F60T2 in (Faith et al., 2013)]. Isolates were grown to stationary phase in TYGS medium (Goodman et al., 2009) in an anaerobic chamber (atmosphere; 75% N2, 20% CO2, 5% H2). Equivalent numbers of organisms were pooled (based on OD600 measurements). The pool was divided into aliquots that were frozen in TYGS/15% glycerol, and maintained at -80°C until use. On experimental day 0, aliquots were thawed, the outer surface of their tubes were sterilized with Clidox (Pharmacal) and the tubes were introduced into gnotobiotic isolators. The bacterial consortium was administered through a plastic tipped oral gavage needle (total volume, 400μL per mouse). Based on inconsistent colonization observed in screening experiment 1 (see Table S1A of Patnode et al., Cell, 2019, 179(1): 59-73), one isolate (*Enterococcus fecalis*; average relative abundance, 2.1%) was not included in screening experiments 2 and 3.

[0418] *Model communities containing INSeq libraries* – Ten strains selected from the human donor-derived community described above were colony purified, and each frozen in 15% glycerol and TYGS medium. Recoverable CFUs/mL were quantified by plating on brain-heart-infusion (BHI) blood agar. The identity of strains was verified by sequencing full-length 16S rRNA amplicons. On the day of gavage, stocks of these strains were thawed in an anaerobic chamber and mixed together along with each of five multi-taxon INSeq libraries (*B. thetaiotaomicron* VPI-5482, *B. thetaiotaomicron* 7330, *B. cellulosilyticus* WH2, *B. vulgatus* ATCC-8482, *B. ovatus* ATCC-8483) whose generation and characterization have been described in earlier publications (Hibberd et al., 2017; Wu et al., 2015). An aliquot of this mixture was administered by oral gavage to germ-free mice housed in gnotobiotic isolators ($2x10^6$ CFUs of each donor organism plus an OD600 0.5 of each INSeq library per mouse recipient; total gavage volume, 400 µL). For *B. cellulosilyticus, B. vulgatus, B. ovatus,* or *B. cellulosilyticus and B. ovatus* omission experiments, gavage mixtures were prepared in parallel without these organisms. The absence of one or both of these strains was verified by COPRO-Seq analysis of both the gavage mixture and fecal samples collected throughout the experiment from recipient mice.

[0419] *Fiber-rich food ingredient mixtures* – HiSF-LoFV and LoSF-HiFV diets were produced using human foods, selected based on consumption patterns from the National Health and Nutrition Examination Survey (NHANES) database (Ridaura et al., 2013). Diets were milled to powder (D90 particle size, 980 µm), and mixed with pairs of powdered fiber preparations [one preparation at 8% (w/w) and the other preparation at 2% (w/w)]. Fiber content was defined for each preparation [Association of Official Agricultural Chemists (AOAC) 2009.01], as was protein, fat, total carbohydrate, ash, and water content [protein AOAC 920.123; fat AOAC 933.05; ash AOAC 935.42; moisture AOAC 926.08; total carbohydrate (100 - (Protein + Fat + Ash + Moisture)]. The powdered mixtures were sealed in containers and sterilized by gamma irradiation (20-50 kilogreys, Steris, Mentor, OH). Sterility was confirmed by culturing the diet under aerobic and anaerobic conditions (atmosphere, 75% N_2, 20% CO_2, 5% H_2) at 37°C in

TYG medium, and by feeding the diets to germ-free mice followed by COPRO-Seq analysis of their fecal DNA.

[0420] *Monosaccharide and linkage analysis of fiber preparations* – For fiber preparations, uronic acid (as GalA) was measured using the *m*-hydroxybiphenyl method (Thibault, 1979). Sodium tetraborate was used to distinguish GlcA and GalA (Filisetti-Cozzi and Carpita, 1991). The degree of methylation of galacturonic acid (pectins) in the sample was estimated as previously described (Levigne et al., 2002). Samples were hydrolyzed with 1M H_2SO_4 for 2h at 100°C and individual neutral sugars were analyzed as their alditol acetate derivatives (Englyst and Cummings, 1988) by gas chromatography. To fully release glucose from cellulose, a pre-hydrolysis step was carried out by incubation in 72% H_2SO_4 for 30 minutes at 25°C prior to the hydrolysis step. Linkage analysis was performed after carboxyl reduction of uronic acid with NaBD4/NaBH4 according to a previously published procedure (Pettolino et al., 2012) with minor modifications (this procedure allows galactose, galacturonic acid and methylesterified galacturonic acid to be distinguished). Methylation of carboxyl-reduced samples was performed as described in (Buffetto et al., 2015).

[0421] Polysaccharides from the HiSF-LoFV diet were isolated by sequential alkaline extractions (Pattathil et. al., 2012). Briefly, lipids were removed from a sample of powdered HiSF-LoFV by sequential incubation in 80% ethanol, 100% ethanol, and acetone. The dried precipitate was suspended in 1M KOH containing 0.5%(w/w) NaBH4 and stirred overnight. The solution was neutralized and the supernatant was collected by centrifugation (this material is referred to as fraction 1 (F1)). The insoluble material was suspended in 1M KOH/ 0.5%(w/w) NaBH4 overnight, and the supernatant was collected (referred to as F2). The insoluble material was suspended in 4M KOH/0.5%(w/w) NaBH4 overnight and the supernatant was collected (referred to as F3). Each fraction was dialyzed (SnakeSkin 3.5K MWCO, Thermo Scientific) in water, lyophilized, and then treated for 4 hours at 37° C with amyloglucosidase (36 units/mg) and alpha-amylase (100 units/mg; both enzymes from Megazyme). Enzymes were inactivated by boiling and samples were dialyzed and lyophilized. Measurement of the dry mass of each fraction before and digestion revealed that the total starch content of

the base HiSF-LoFV diet was 22% (w/w) (note a comparable analysis the pea fiber yielded a value of 3.6%, meaning that HiSF-LoFV diet supplemented with 10% pea fiber contains a total starch content of 20% by weight).

[0422] HiSF-LoFV diet polysaccharides were analyzed by the Center for Complex Carbohydrate Research at the University of Georgia in Athens. Glycosyl composition analysis was performed by combined GC-MS of the per-O-trimethylsilyl (TMS) derivatives of the monosaccharide methyl glycosides produced from the sample by acidic methanolysis (Santander et al., 2013). Briefly, samples (300-500 µg) were heated with methanolic HCl in a sealed screw-top glass test tube for 17 h at 80 °C. After cooling and removal of the solvent under a stream of nitrogen, samples were derivatized with Tri-Sil® (Pierce) at 80 °C for 30 min. GC-MS analysis of the TMS methyl glycosides was performed on an Agilent 7890A GC interfaced to a 5975C mass selective detector (MSD), using a Supelco Equity-1 fused silica capillary column (30 m × 0.25 mm ID).

[0423] Glycosyl-linkage analysis of HiSF-LoFV diet polysaccharides was performed as previously described with slight modification (Heiss et. al., 2009). Samples were permethylated, depolymerized, reduced and acetylated, and the resulting partially methylated alditol acetates (PMAAs) were analyzed by GC-MS. About 1 mg of the sample was used for linkage analysis. The sample was suspended in 200 µL of dimethyl sulfoxide and left to stir for 1 day. Permethylation of the sample was affected by two rounds of treatment with sodium hydroxide (15 minutes) and methyl iodide (45 minutes). The permethylated material was hydrolyzed using 2 M TFA (2 hours in sealed tube at 121 °C), reduced with NaBD4, and acetylated using acetic anhydride/TFA. The resulting PMAAs were analyzed on an Agilent 7890A GC interfaced to a 5975C MSD (electron impact ionization mode); separation was performed on a 30 m Supelco SP-2331 bonded phase fused silica capillary column.

[0424] *V4-16S rRNA gene sequencing* – DNA was isolated from fecal samples by first bead-beating the sample with 0.15mm-diameter zirconium oxide beads and a 5mm-diameter steel ball in 2X buffer A (200 mM NaCl, 200 mM Tris, 20 mM EDTA), followed by extraction in phenol:chloroform:isoamyl alcohol, and further purification (QiaQuick 96 purification kit; Qiagen, Valencia, CA). PCR amplification of the

V4 region of bacterial 16S rRNA genes was performed as described (Bokulich et al., 2013). Amplicons with sample-specific barcodes were pooled for multiplex sequencing using an Illumina MiSeq instrument. Reads were demultiplexed and rarefied to 5000 reads per sample. Reads sharing ≥99% nucleotide sequence identity [99% ID operational taxonomic units (OTUs)], that mapped to a reference OTU in the GreenGenes 16S rRNA gene database (McDonald et al., 2012) were assigned to that OTU. The 16S rRNA gene could not be amplified in multiple fecal DNA samples from mice fed 8% cocoa fiber. A small subset of reads (<5%) representing additional V4-16S rDNA amplicon sequences produced from colony-purified stocks of *Bacteroides ovatus*, *Parabacteroides distasonis*, *Dorea longicatena, and Collinsella aerofaciens* were omitted from our analyses of fecal DNA samples. *Streptococcus thermophilus*, an organism heavily used in cheese processing, was also omitted based on its detection in DNA isolated from samples of the sterile HiSF-LoFV diet.

[0425] *COPRO-Seq analyses of bacterial species abundances* – Libraries were prepared from fecal DNA using sonication and addition of paired-end barcoded adaptors (McNulty et al., 2013) or by tagmentation using the Nextera DNA Library Prep Kit (Illumina) and combinations of custom barcoded primers (Adey et al., 2010). Libraries were sequenced using an Illumina NextSeq instrument [1,011,017± 314,473 reads/sample (mean ± SD) across experiments]. Reads were mapped to bacterial genomes with previously published custom Perl scripts (see below) adapted to use Bowtie II for genome alignments (Hibberd et al., 2017); samples represented by less than 150,000 uniquely mapped reads were omitted from the analysis.

[0426] *Community-wide quantitative proteomics* - Lysates were prepared from fecal samples by bead beating in SDS buffer (4% SDS, 100 mM Tris-HCl, 10 mM dithiothreitol, pH 8.0) using 0.15 mm diameter zirconium oxide beads, followed by centrifugation at 21,000 x *g* for 10 minutes. Pre-cleared protein lysates were further denatured by incubation at 85°C for 10 minutes, and adjusted to 30 mM iodoacetamide to alkylate reduced cysteines. After incubation in the dark for 20 minutes at room temperature, protein was isolated by chloroform-methanol extraction. Protein pellets were then washed with methanol, air dried, and re-solubilized in 4% sodium

deoxycholate (SDC) in 100 mM ammonium bicarbonate (ABC) buffer, pH 8.0. Protein concentrations were measured using the BCA (bicinchoninic acid) assay (Pierce). Protein samples (250 □g) were then transferred to a 10 kDa MWCO spin filter (Vivaspin 500, Sartorius), concentrated, rinsed with ABC buffer, and digested *in situ* with sequencing-grade trypsin (Clarkson et al., 2017). The tryptic peptide solution was then passed through the spin-filter membrane, adjusted to 1% formic acid to precipitate the remaining SDC, and the precipitate removed from the peptide solution with water-saturated ethyl acetate. Peptide samples were concentrated using a SpeedVac, measured by BCA assay and analyzed by automated 2D LC-MS/MS using a Vanquish UHPLC with autosampler plumbed directly in-line with a Q Exactive Plus mass spectrometer (Thermo Scientific) outfitted with a 100 µm ID triphasic back column [RP-SCX-RP; reversed-phase (5 µm Kinetex C18) and strong-cation exchange (5 µm Luna SCX) chromatographic resins; Phenomenex] coupled to an in-house pulled, 75 µm ID nanospray emitter packed with 30 cm Kinetex C18 resin. For each sample, 12 µg of peptides were autoloaded, desalted, separated and analyzed across four successive salt cuts of ammonium acetate (35, 50, 100 and 500 mM), each followed by a 105-minute organic gradient. Eluting peptides were measured and sequenced by data-dependent acquisition on the Q Exactive Plus (Clarkson et al., 2017).

[0427] MS/MS spectra were searched with MyriMatch v.2.2 (Tabb et al., 2007) against a proteome database derived from the genomes of the strains in the defined model community concatenated with major dietary protein sequences, common protein contaminants, and reversed entries to estimate false-discovery rates (FDR). Since the relative abundance of *B. thetaiotaomicron* 7330 was low on day 6 [0.05% ± 0.041% (mean ± SD) for all groups], we chose to analyze all peptides that mapped to the *B. thetaiotaomicron* VPI-5482 proteome, regardless of whether they also mapped to *B. thetaiotaomicron* 7330. Peptide spectrum matches (PSM) were required to be fully tryptic with any number of missed cleavages, and contain a static modification of 57.0214 Da on cysteine and a dynamic modification of 15.9949 Da on methionine. PSMs were filtered using IDPicker v.3.0 (Ma et al., 2009) with an experiment-wide FDR <1% at the peptide-level. Peptide intensities were assessed by chromatographic area-under-the-

curve (label-free quantification option in IDPicker). To remove cases of extreme sequence redundancy, the community meta-proteome was clustered at 100% sequence identity post-database search [UCLUST; (Edgar, 2010)] and peptide intensities were summed to their respective protein groups/seeds to estimate overall protein abundance. Proteins were included in the analysis only if they were detected in more than 3 biological replicates in at least one experimental group. Missing values were imputed to simulate the limit of detection of the mass spectrometer, using mean minus 2.2 x standard deviation with a width of 0.3 x standard deviation. Four additional imputed distributions produced results that were in general agreement with this approach in terms of fold-abundance change induced by fiber treatment and statistical significance.

[0428] *Multi-taxon INSeq* – Multi-taxon INSeq allows simultaneous analysis of multiple mutant libraries in the same recipient gnotobiotic mouse owing to the fact that the *mariner* Tn vector contains MmeI sites at each end plus taxon-specific barcodes. MmeI digestion cleaves genomic DNA at a site 20-21 bp distal to the restriction enzyme's recognition site so that the site of Tn insertion and the relative abundance of each Tn mutant can be defined in given diet/community contexts by sequencing the flanking genomic sequence and taxon-specific barcode (Wu et al., 2015). Purified fecal DNA was processed as described previously (Wu et al., 2015). DNA was digested with MmeI and the products were ligated to sample-specific barcoded adaptors. Sequencing was performed on an Illumina HiSeq 2500 instrument, with a custom indexing primer providing the strain-specific barcode for the insertion. Analysis of mutant strain frequencies was carried out using custom software. Log ratios of the abundances of Tn mutant strains on experimental days 6 and 2 (corresponding to the period of fiber treatment compared to just prior to fiber exposure) were calculated for each mouse.

[0429] *PUL nomenclature and homology* – All PUL assignments were made based on "new assembly" genomes present in the CAZy PUL database (www.cazy.org/PULDB) (Terrapon et al., 2018). All boundaries of PULs were algorithmically defined (listed as 'predicted PUL' in PULDB). The algorithmically defined boundaries of *B. thetaiotaomicron* PUL7 were extended to include the adjacent arabinose operon based on previously published experimental datasets (Schwalm et al.,

2016). A cluster of three or more adjacent CAZymes was defined as a 'polysaccharide utilization complement'. Homology between genes in PULs was determined using a reciprocal BLASTp approach with an E-value threshold of 1 x 10^{-9}, querying each protein product contained within a CAZy-annotated PUL against reference genomes from other species in the community.

[0430] *Generation of glycan-coated magnetic beads* – Wheat Arabinoxylan and Icelandic Moss Lichenan were purchased from Megazyme (P-WAXYL, P-LICHN) and yeast alpha-mannan was purchased from Sigma-Aldrich (M7504). Polysaccharides were solubilized in water (at a concentration of 5mg/mL for pea fiber and 20 mg/mL for arabinoxylan and lichenan), sonicated and heated to 100°C for 1 minute, then centrifuged at 24,000 x g for 10 minutes to remove debris. TFPA-PEG3-biotin (Thermo Scientific), dissolved in DMSO (10 mg/mL) was added to the polysaccharide solution at a ratio of 1:5 (v/v). The sample was subjected to UV irradiation for 10 minutes (UV-B 306 nm, 7844 mJ total), and then diluted 1:4 to facilitate desalting on 7 kD Zeba spin columns (Thermo Scientific).

[0431] Biotinylated polysaccharide was mixed with one of several biotinylated fluorophores (PF-505, PF-510LSS, PF-633, PF-415; all at a concentration of 50 ng/mL; all obtained from Promokine). A 500 µL aliquot of this preparation was incubated with 10^7 paramagnetic streptavidin-coated silica beads (LSKMAGT, Millipore Sigma) for 24 hours at room temperature. Beads were washed by centrifugation three times with 1 mL HNTB buffer (10mM HEPES, 150mM NaCl, 0.05% Tween-20, 0.1% BSA) followed by addition of 5 tg/mL streptavidin (Jackson Immunoresearch) in HNTB (30 min incubation at room temperature). Beads were washed as before and then incubated with 250 µL of the biotinylated polysaccharide preparation. The washing, streptavidin, and polysaccharide incubation steps were repeated three times. Bead preparations were assessed using an Aria III cell sorter (BD Biosciences) to confirm adequate labeling, and then analyzed by GC-MS (see below) to quantify the amount of carbohydrate bound.

[0432] *Administration and recovery of beads* – Beads were incubated with 70% ethanol for 1 minute in a biosafety cabinet, then washed three times with 1 mL sterile

HNTB using a magnetic stand. The different bead types were combined, diluted, and aliquoted to 10^7 beads per 650 µL HNTB insterile Eppendorf microcentrifuge tubes. The number of beads in each aliquot was counted using an Aria III cell sorter and CountBright fluorescent microspheres (BD Bioscience). Tubes containing beads were introduced into gnotobiotic isolators and the beads were administered by oral gavage (600 µL per mouse). Separate aliquots of control beads, used to establish input carbohydrate content were stored in the dark at 37°C until collection of experimental beads from mouse fecal or cecal samples had been completed.

[0433] For germ-free mouse experiments, animals were fed the HiSF-LoFV diet for two weeks and then gavaged with beads; all fecal pellets were collected during the 4- to 12-hour interval that followed gavage. During this time period, bedding was removed and mice were placed on grated cage bottoms (with access to food and water); cage bottoms were placed just above a 0.5 cm deep layer of sterile water on the floor of the cage, to prevent pellets from drying. For colonized animals, cecal and colonic contents were collected four hours after administration of beads at the time of euthanasia. Recovered samples were immediately placed in sterile water on ice.

[0434] Fecal, cecal, and input samples were vortexed and filtered through nylon mesh (100 µm pore-diameter). The resulting suspension of luminal contents was layered over sterile Percoll Plus (GE Health Care) and centrifuged for 5 minutes at 500 x g. Beads were collected from underneath the Percoll layer and washed four times using a magnetic stand, each time with 1 mL fresh HNTB. Recovered beads were counted by flow cytometry as before, filtered through nylon mesh (40 µm pore diameter, BD Biosciences) and stored at 4°C overnight. Beads were sorted back into their polysaccharide types based on fluorescence using an Aria III sorter (average sort purity, 96%). Sorted samples were centrifuged (500 x g for 5 minutes) to pellet beads and the beads were transferred to a 96-well plate. All bead samples were incubated with 1% SDS / 6M Urea / HNTB for 10 minutes at room temperature to remove exogenous components, washed three times with 200 µL HNTB using a magnetic plate rack, and then stored overnight at 4° C prior to monosaccharide analysis.

[0435] *Analysis of bead-bound glycan by GC-MS* – The number and purity of beads in each sorted sample was determined by taking an aliquot for analysis on the Aria III cell sorter. Equal numbers of beads from each sample were transferred to a new 96-well plate and the supernatant was removed with a magnetic plate rack. For acid hydrolysis, 200 µL of 2M trifluoroacetic acid and 250 ng/mL myo-inositol-D6 (CDN Isotopes; spike-in control) were added to each well, and the entire volume was transferred to 300 µL glass vials (ThermoFisher; catalog number C4008-632C). Another aliquot was taken to verify the final number of beads in each sample. Monosaccharide standards were included in separate wells and subjected to the hydrolysis protocol in parallel with the other samples. Vials were crimped with Teflon-lined silicone caps (ThermoFisher) and incubated at 100°C with rocking for 2 h. Vials were then cooled, spun to pellet beads, and their caps were removed. A 180 µL aliquot of the supernatant was collected and transferred to new 300 µL glass vials. Samples were dried in a SpeedVac for 4 hours, methoximated in 20 µL O-methoxyamine (15mg/mL pyridine) for 15 h at 37°C, followed by trimethylsilylation in 20 µL MSTFA/TMCS [N-Methyl-N-trimethylsilyltrifluoroacetamide/2,2,2-trifluoro-N-methyl-N-(trimethylsilyl)-acetamide, chlorotrimethylsilane] (ThermoFisher) for 1 h at 70° C. One half volume of heptane (20 µL) was added before loading the samples for injection onto a 7890B gas chromatography system coupled to a 5977B MS detector (Agilent). The mass of each monosaccharide detected in each sample of sorted beads was determined using monosaccharide standard curves. This mass was then divided by the final count of beads in each sample to produce a measurement of mass of recoverable monosaccharide per bead.

[0436] *Quantification and Statistical Analysis* – Using data from days 6 and 7 of each diet treatment, a mixed effects model was generated in the R programming environment for each species in each of three fiber screening experiments. The relative abundance of that species in feces (or the relative abundance scaled by fecal DNA yield) was used as the dependent variable, and the concentration of administered fiber (10 to 13 fibers tested per experiment), as well as experimental day were used as independent variables. Mixed effects models incorporated terms to describe repeated measures of

individual mice. In rare cases where *B. cellulosilyticus* failed to colonize (5 of 60 mice), the animals were not considered biological replicates since they harbored a distinct microbiota; they were omitted from the models. ANOVA (with Satterthwaite approximation for degrees of freedom) was performed to evaluate the significance of individual terms in models (FDR corrected *P* value cutoff of 0.01). Models were evaluated based on conditional R^2 values (incorporating random factors) and plots of the residuals and Cook's distance (no samples were excluded based on these assessments).

[0437] For COPRO-Seq analyses, differences between groups were assessed using mixed-effect models with time as a categorical variable, including day 2 as a pre-treatment time point. For omission experiments, the abundance of each strain as a proportion of all other strains except the omitted strain or strains was used for statistical tests. Significant terms in models were identified using ANOVA (FDR corrected *P* value cutoff of 0.05). Mann-Whitney *U* test was used for analyses of individual time-points of interest.

[0438] For quantitative proteomics, significant differences in protein abundance were determined using limma (Ting et al., 2009). For multi-taxon INSeq analyses, mutant strain abundances were analyzed using limma-voom (Law et al., 2014) after quantile normalization. The general linear model framework in limma-voom allowed us to perform moderated t-tests to determine the statistical significance ($P < 0.05$, FDR corrected) of differences in fitness in the context of the control versus fiber-supplemented diets. A Mann-Whitney *U* test was used to calculate significant differences in monosaccharide abundance between bead samples. All tests were two-tailed.

[0439] *Data and Software Availability* – Datasets of V4-16S rRNA sequences in raw format prior to post-processing and data analysis, plus COPRO-Seq and INSeq datasets have been deposited at the European Nucleotide Archive under study accession PRJEB26564. All LC-MS/MS proteomic data have been deposited into the MassIVE data repository under accession numbers MSV000082287 (MassIVE) and PXD009535 (ProteomeXchange). INSeq software: github.com/mengwu1002/Multi-taxon_analysis_pipeline. COPRO-Seq software: github.com/nmcnulty/COPRO-Seq.

Example 9 – Sugar beet arabinan degradation

[0440] This example describes an alternative method used to attach polysaccharides to paramagnetic glass beads. To covalently immobilize polysaccharides onto paramagnetic glass beads for use as biosensors of gut microbiota biochemical function, a bead with unique chemical functionality was developed. Amine functional groups were added to the bead surface as a chemical handle because of their nucleophilic nature at neutral pH and their utility in multiple bioconjugation reactions (Koniev et al., 2015). It was hypothesized that the amine functional group could be used for two critical functions: 1) addition of a fluorophore for the multiplexed analysis of multiple bead types within a single animal or subject, and 2) the covalent immobilization of an activated polysaccharide (**FIG. 12**).

[0441] To install amines on the bead surface, the activated amine-silyl reagent (3-aminopropyl)triethoxysilane (ATPS) was reacted with bead in the presence of water. Under the same reaction conditions, a zwitterionic surface could be generated with 3-(trihydroxysilyl)propyl methylphosphonate (THPMP) to an ATPS containing reaction. The additional phosphonate functionality was important to reduce nonspecific binding to the bead surface (Bagwe et al., 2006). The zeta potential of surface modified paramagnetic silica beads was used to monitor the addition of both amine and phosphonate functional groups onto the bead surface (**FIG. 13A**).

[0442] With fluorescent amine-phosphonate paramagnetic glass beads in hand, we next sought to covalently immobilize polysaccharides of interest of the bead surface. Strategies for bioconjugation with polysaccharides are lacking compared to proteins, peptide, and nucleic acids due to the limited chemical functionality naturally occurring within polysaccharides. We chose to activate polysaccharides using a cyano (CN-) donor to generate a cyano-ester. Suitable cyano-donors include, but are not limited to, cyanogen bromide (CNBr) (Glabe et al., 1983) and the organic nitrile donor 1-cyano-4-dimethylaminopyridinium tetrafluoroborate (CDAP) (Lees et al., 1996). Both donors have been used for the generation of affinity matrixes on agarose beads and the synthesis of polysaccharide-conjugate vaccines; specifically, CDAP activation and conjugation was used for the development of the pneumococcal-conjugate vaccines

(Lees et al., 1996; Ridaura et al., 2013). We chose CDAP because of its solubility in DMSO and the fact that it is less pH sensitive and less toxic than CNBr. CDAP was dissolved in DMSO and added to a solution of polysaccharide in the presence of catalytic triethylamine. CDAP nonspecifically generates cyano-ester electrophiles from the hydroxyls naturally present within a polysaccharide (**FIG. 14**). After activation, fluorescent amine-phosphonate beads were added. The solution was allowed to react overnight. Reaction of bead surface amine and the cyano-ester group of the activated polysaccharide yields a liable isourea bond that is reduced to a stable covalent bond with the addition of a hydride donor. We chose 2-methylpyridine borane although harsher donors such as sodium borohydride or sodium cyanoborohydride will also work. Immobilization of polysaccharide on the bead surface and reduction of the isourea bond has little to no effect on bead fluorescence.

[0443] Polysaccharide immobilization on the bead surface was quantified via acid hydrolysis of surface-immobilized polysaccharide and quantification of the liberated monosaccharides using gas chromatography mass spectrometry (GC-MS). Polysaccharide was hydrolyzed using 2 M trifluoroacetic acid and liberated monosaccharide were quantified on as silylated methoxyamine-reduced monosaccharides using free monosaccharides as standards. Beads were enumerated with flow cytometry and an equal number of each bead type were assayed in parallel. Beads lacking surface amines, or beads reacted with polysaccharides not activated with CDAP lacked surface-immobilized polysaccharide (**FIG. 15**). Typical bead yields are 5-25 pg of immobilized polysaccharide per bead.

[0444] Multiple types of polysaccharide-coated beads labeled with distinct fluorophores were pooled and gavaged into gnotobiotic mouse models as biosensors of gut community biochemical function. Polysaccharide degradation was measured as a function of 1) community composition, and 2) diet. Pooled beads were gavaged into germ-free mice 4 hours prior to animals were euthanized; beads were subsequently isolated from the mouse cecum based on their density and magnetic properties. Polysaccharide degradation was quantified as the amount of polysaccharide remaining

covalently bound to the bead after passage through the gut and recovery from the cecum (**FIG. 16**).

[0445] The ability of a microbiota to degrade a commercially available preparation of sugar beet arabinan (Megazyme; cat. no.: P-ARAB) was determined by comparing amine phosphonate beads coated with the carbohydrate to control beads whose surface amines were acetylated. Sugar beet arabinan is a polymer containing the monosaccharides arabinose, galactose, rhamnose, and galacturonic acid. Neutral monosaccharides were quantified after hydrolysis of bead-bound polysaccharide. Arabinose liberated during acid hydrolysis of sugar beet arabinan-coated beads was used as a marker of arabinan degradation. Comparison of input beads to beads passed through germ-free animals demonstrates that sugar beet arabinan is not digested by host enzymes during passage through a mouse (**FIG. 17**). However, beads gavaged into colonized mice exhibited reduced levels of arabinan remaining on the surface, and the levels of degradation changed as a function of mouse diet. The microbiota of mice fed a diet high in saturated fat and low in fruits and vegetables (HiSF-LoFV) or mice fed a HiSF-LoFV diet supplemented with 100 mg/mouse/day sugar beet arabinan degraded a significant amount of sugar beet arbainan when compared to input beads that were not gavaged into mice colonized with a defined 14-member consortium composed of human gut microbiota that had been cultured and their genomes sequenced (**Table 12**) (Ridaura et al., 2013; Wu et al., 2015). Additionally, colonized mice fed HiSF-LoFV diet supplemented sugar beet arabinan showed increased degradation capacity as compared to colonized mice fed the unsupplemented HiSF-LoFV diet ($p = 0.086$; pairwise Welch's t-test). These results demonstrate that 1) the defined model human microbiota was required for sugar beet arabinan degradation and 2) dietary supplementation with sugar beet arabinan changed the functional capacity of the microbiota to degrade this glycan.

Table 12. Bacterial strains comprising the model defined human gut community.

Bacteria	Strain	Citation
Bacteroides ovatus	ATCC 8483 INSeq	(Wu et al., 2015)

Bacteroides cellulosilyticus	WH2 INSeq	(Wu et al., 2015)
Bacteroides thetaiotaomicron	ATCC 7330 INSeq	(Wu et al., 2015)
Bacteroides thetaiotaomicron	VPI-5482 INSeq	(Wu et al., 2015)
Bacteroides vulgatus	ATCC 8482 INSeq	(Wu et al., 2015)
Bacteroides caccae	TSDC17.2	(Ridaura et al., 2013)
Bacteroides finegoldii	TSDC17.2	(Ridaura et al., 2013)
Bacteroides massiliensis	TSDC17.2	(Ridaura et al., 2013)
Collinsella aerofaciens	TSDC17.2	(Ridaura et al., 2013)
Escherichia coli	TSDC17.2	(Ridaura et al., 2013)
Odoribacter splanchnicus	TSDC17.2	(Ridaura et al., 2013)
Parabacteroides distasonis	TSDC17.2	(Ridaura et al., 2013)
Ruminococcaceae sp.	TSDC17.2	(Ridaura et al., 2013)
Subdoligranulum variabile	TSDC17.2	(Ridaura et al., 2013)

[0446] Further details are provided below for the materials and methods used in the above experiments.

[0447] *Synthesis of amine phosphonate beads:* To a solution of microscopic (10 μm) paramagnetic silica beads (Millipore Sigma; Cat no: LSKMAGN01) in water, equal molar amounts of (3-aminopropyl)triethoxysilane (ATPS) (Sigma Aldrich) and 3-(trihydroxysilyl)propyl methylphosphonate (THPMP) (Sigma Aldrich) were added (Bagwe et al., 2006; Soto-Cantu et al., 2012). The reaction was allowed to proceed for 5 hours at 50°C with shaking. The reaction was terminated with repeated washing of beads with water using a magnet.

[0448] *Zeta potential measurement:* Zeta potential was measured to track modification of the bead surface. Zeta potential measurements were obtained on a Malvern ZEN3600 using disposable Malvern zeta potential cuvettes. Measurements were obtained with the default settings of the instrument, using the refractive index of SiO_2 as the material, and water as the dispersant. Beads were resuspended to a concentration of 5 x 10^5/mL in 10 mM (4-(2-hydroxyethyl)-1-piperazineethanesulfonic

acid) (HEPES; pH 7.2) and analyzed in triplicate. Zeta potential of starting beads and beads monofunctionalized with ATPS or THPMP were used as standards.

[0449] *Fluorophore labeling of amine phosphonate beads*: Fluorophores were covalently bound to the bead surface to facilitate the multiplexed analysis of multiple bead types within a single animal. N-Hydroxysuccinimide ester (NHS)-activated fluorophores were dissolved in dimethyl sulfoxide (DMSO) at 1 mM. Resuspended fluorophore was diluted into a solution of 20 mM HEPES (pH 7.2) and 50 mM NaCl to a final concentration of 100 nM and incubated with amine phosphonate beads for 50 minutes at 22°C. Beads were washed repeatedly with water to terminate the reaction. The extent of fluorophore labeling was assessed on each bead type using flow cytometry. The concentration of fluorophore used was the lowest at which the bead populations could be reliably and easily distinguished via flow cytometry. Fluorophores and their sources: Alexa Fluor 488 NHS ester (Life Technologies; cat. no.: A20000), Promofluor 415 NHS ester (PromoKine; cat. no.: PK-PF415-1-01), Promofluor 633P NHS ester (PromoKine; cat. no.: PK-PF633P-1-01), and Promofluor 510-LSS NHS ester (PromoKine; cat. no.: PK-PF510LSS-1-01).

[0450] *Amine phosphonate bead acetylation*: Acetylation of bead surface amines was used to confirm the specific linkage of both fluorophore and polysaccharides to the bead surface. Acetylated beads were also used as an empty bead control when gavaged into mice. Bead surface amines were acetylated using acetic anhydride under anhydrous conditions. Amine phosphonate beads were washed repeatedly with multiple solvents with the goal of resuspending the beads in anhydrous methanol; beads were washed in water, then methanol, then anhydrous methanol. Pyridine (0.5 volume equivalents) was then added as a base followed by acetic anhydride (0.5 volume equivalents). The reaction was allowed to proceed for 3 hours at 22°C and then quenched with repeated washing with water. The described acetylation conditions had no effect on the fluorescence of any of the four fluorophores tested.

[0451] *Polysaccharide conjugation to amine phosphonate beads*: Polysaccharides were dissolved at 3-10 mg/mL in 50 mM HEPES (pH 8) with heat and sonication. To a solution of polysaccharide (5 mg/mL) containing trimethylamine (0.5

equivalent), 1-cyano-4-dimethylaminopyridinium tetrafluoroborate (CDAP; Sigma Aldrich; 1 eq.) dissolved in DMSO was added. The optimal concentration of CDAP was found to be 0.2 mg of CDAP per mg of polysaccharide. The polysaccharide/CDAP solution was mixed for 5 minutes at 22°C to allow for polysaccharide activation. Amine phosphonate beads resuspended in 50 mM HEPES (pH 8) were added to the activated polysaccharide solution and the reaction was allowed to proceed for 15 hours at 22°C. Any aggregated beads were resuspended with light sonication. The resulting isourea linkage between the bead and polysaccharide was reduced by addition of 2-picoline borane dissolved in DMSO (10% wt:wt) and incubation for 40 minutes at 40°C. The reaction was terminated with repeated washing in water and then 20 mM HEPES (pH 7.2) 50 mM NaCl. The described reaction conditions for polysaccharide conjugation or reduction had little or no effect on the fluorescence of any of the four fluorophores tested.

[0452] *Bead counting*: The absolute number of beads in a solution was determined with flow cytometry using CountBright Absolute Counting Beads (ThermoFisher Scientific; cat. no.: C36950) according to the manufacturer's suggested protocol.

[0453] *Bead pooling and gavage into gnotobiotic mice*: Pools of equal number of each bead type were prepared from fluorophore-labeled polysaccharide-coated amine phosphonate beads. The required number of a given bead type was sterilized with 70% ethanol for 10 minutes before washing with sterile water and 20 mM HEPES (pH 7.2), 50 mM NaCl, 0.01% bovine serum albumin, and 0.01% Tween-20. The different bead types were then pooled into a single mixture.

[0454] Pooled bead mixtures (10-15 x 10^6 beads) were gavaged into gnotobiotic mice 4-6 hours prior to sacrifice. Beads were harvested from cecal contents using bead density and magnetism. Beads were sorted back into the original bead type using fluorescence-activated cell sorting (FACS; BD FACSAria III).

[0455] *Quantitation of polysaccharide degradation*: Polysaccharide degradation was determined by quantifying the amount of monosaccharide hydrolyzed from bead-bound polysaccharide after bead passage through a mouse. To do so, an

equal number of beads were placed in crimp-top glass vials and hydrolyzed using 2 M trifluoroacetic acid for 2 hours at 95°C. The solution was reduced to dryness under reduced pressure. Liberated monosaccharides were reduced with methoxyamine (15 mg/mL in pyridine) for 15 hours at 37°C. Hydroxyl groups were silylated using N-Methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) + 1% 2,2,2-Trifluoro-N-methyl-N-(trimethylsilyl)-acetamide, chlorotrimethylsilane (TCMS) (ThermoFisher Scientific; Cat. no.: TS-48915) for 1 hour at 60°C. Samples were diluted with heptane and analyzed by GC-MS on Agilent 7890A gas chromatography system, coupled with a 5975C mass spectrometer detector (Agilent). Monosaccharide composition and quantitation were determined using chemical standards simultaneously derivatized.

Example 10

[0456] This example describes experiments to determine if there was a bioactive component of the pea fiber preparation used in Examples 2-6 that was responsible for increasing the representation of targeted *Bacteroides* represented in a model human gut community installed in gnotobiotic mice. The pea fiber preparation was subjected to extraction under increasingly harsh conditions with aqueous solutions to differentially solubilize constituents (Pattathil et al.) (**FIG. 18**). In total, 8 fractions were isolated and characterized for protein content (BCA assay), total carbohydrate content (phenol-sulfuric acid assay (Masuko et al.), and molecular size (high performance liquid chromatography-size exclusion chromatography with an evaporative light scattering detector). The monosaccharide composition of each fraction was determined (polysaccharide methanolysis followed by gas chromatography mass spectrometry (GC-MS; (Doco et al.)) (**FIG. 19**). Carbohydrate linkages were determined as partially methylated alditol acetates (PMAA) (Doares et al.).

[0457] Fraction 8, obtained using the harshest conditions (4 M KOH for 24 hours at 22°C) and containing high relative content of arabinose and galactose, was selected for further evaluation. Based on its monosaccharide composition and the results obtained from PMAA linkage analysis (**Tables 13, 14**), it appears that (i) fraction 8 is largely composed of arabinan that is predominately branched at the 2-, or doubly

branched at the 2- and 3-positions of a linear α1-5 L-arabinofuranose backbone (**FIG. 20**) and (ii) the arabinan is covalently attached to small pectic fragments containing galacturonic acid, galactose, and rhamnose. The structure of the pea fiber arabinan is more highly branched and sterically encumbered than the more commonly observed arabinan structure, exemplified by commercially available sugar beet arabinan which is branched almost exclusively at the 3-position (Megazyme; cat. no.: P-ARAB) (**Tables 13, 14**). In addition to arabinan, fraction 8 contains lesser amounts of two additional plant polysaccharides that are not covalently bound to the arabinan: a small amount of xylan (linear β1-4 xylose) and a small amount of starch (α1-4 glucose).

[0458] The method for Fraction 8 isolation was scaled up using a procedure similar to what was employed in the initial fractionation to supply sufficient quantities for studies in gnotobiotic mice (yield 22% ± 2% wt:wt) (**FIG. 21**) . Briefly, 50 grams of the pea fiber preparation was first treated with 1 M KOH + 0.5 wt.% sodium borohydride at room temperature for 24 hours to dissolve starch, proteins, free oligosaccharides and other smaller compounds. The mixtures were then centrifuged at 3,900 g for 20 minutes. The pellets were collected and resuspended in 4 M KOH + 0.5 wt.% sodium borohydride and stirred at room temperature for 24 hours. The mixture was centrifuged at 3,900 g for 20 minutes again. The supernatant containing the targeted polysaccharides was then neutralized with 4 M acetic in cold bath. The extracted polysaccharides were then precipitated after adding ethanol to the mixture at the ratio of 3.75 : 1 and cooled down to -20 °C. The precipitated polysaccharides were then collected by centrifuging the mixtures at 3,900 g at 4 °C for 20 minutes. The collected pellets were then crushed and washed in 80% ethanol at 4 °C to remove organics such as polyphenols. The latter step was repeated three times. The final pellets were then dried under dry nitrogen overnight to yield “Fraction 8”.

[0459] Next Fraction 8 (150 mg) was solubilized in 50 mM sodium malate (pH 6) + 2 mM calcium chloride (30 mL) via incubation in a 95°C water bath and sonication to yield a 5 mg/mL solution. To this, 3.5 mg of amyloglucoside (Megazyme; cat. no.: E-AMGFR) and 1.25 mg of alpha-amylase (Megazyme, cat. no.:E-PANAA) were added as 3 mg/mL stock solutions in 50 mM sodium malate (pH 6) + 2 mM

calcium chloride. Starch was digested via incubation at 37°C for 4 hours. The digestion was terminated via enzyme denaturation by incubation at 90°C for 30 min. The glucose product resulting from starch digestion was removed with extensive dialysis against ddH20 using 3.5 kDa molecular weight cut off Snakeskin dialysis tubing (ThermoFisher, cat. no,: 88244). The sample was dried via lyophilization to yield enzymatically destarched Fraction 8. Monosaccharide analysis and glycosyl linkage analysis was performed as described above (**Table 16** and **Table 17**).The enzymatically destarched Fraction 8 was then used in the following animal experiment.

[0460] Four groups of adult C57BL/6J male mice fed the HiSF-LoFV diet were colonized with a defined community comprising 14 cultured, sequenced human gut bacterial strains (Ridaura et al.) (n=5 mice/arm; **Table 15**, **FIG. 22**). Two days after colonization, mice in three experimental groups were switched to the HiSF-LoFV diet supplemented with (i) 10% (wt:wt) the pea fiber preparation (calculated consumption 16.6 g/kg mouse weight/day), (ii) 100 mg/mouse/day enzymatically destarched Fraction 8 (3.3 g/kg/day), or (iii) 100 mg/mouse/day sugar beet arabinan (3.3 g/kg/day). A fourth control arm received the unsupplemented HiSF-LoFV diet.

[0461] Mice were given *ad libitum* access to the diets for 10 days at which point all animals were gavaged with polysaccharide-coated paramagnetic fluorescent beads. Animals were sacrificed 4 hours after gavage of the beads. Bacterial community composition was assessed via short read shotgun sequencing (COPRO-Seq) of DNA purified from serially-collected fecal samples and from cecal contents harvested at the conclusion of the experiment (McNulty et al.).

[0462] Principal components analysis of the relative abundances of community members in fecal samples collected on day 11 post-colonization revealed that all 3 experimental diets produced microbial community configurations that were distinct from those in mice consuming the control unsupplemented HiSF-LoFV diet (**FIG. 23**). Of note the microbial communities of mice supplemented with enzymatically destarched Fraction 8 were compositionally similar to that of mice whose diets were supplemented with the pea fiber preparation from which it was derived, but distinct from those consuming the sugar beet arabinan-supplemented HiSF-LoFV diet.

[0463] A time series analysis of the effects of the different glycans on the representation of community members in the fecal microbiota of mice belonging to the four treatment groups is presented in **FIG. 24**. Supplementaion with both enzymatically destarched Fraction 8 and the pea fiber preparation enhanced the fitness (relative abundance) of *B. ovatus* ATCC 8483 and *B. thetaiotaomicron* VPI-5482 compared to the unsupplemented HiSF-LoFV diet. In general, the responses of all *Bacteroides* to the pea fiber preparation and the enzymatically destarched Fraction 8 were similar (as judged by their relative abundances), the one exception being *B. cellulosilyticus* WH2, which achieved a higher representation in the community in the presence of the pea fiber preparation. In contrast, sugar beet arabinan differed from both the pea fiber preparation and the enzymatically destarched Fraction 8 in increasing the fractional abundance of *B. vulgatus* ATCC 8482 while having no significant effect on *B. ovatus*. Collectively, these results reveal that the enzymatically destarched Fraction 8 is able to recapitulate the majority of the effects on community composition of the pea fiber preparation from which it was derived, and also highlight the structural specificity of responses by different *Bacteroides* species to arabinan prepared from different plant sources.

[0464] We next sought to quantify how the *in vivo* degradative capacity of each individual mouse's microbiota changed with dietary fiber supplementation. To do so, we employed microscopic paramagnetic silica beads (average diameter = 10 μm) with covalently bound glycans from the enzymatically destarched Fraction 8 or with purified sugar beet arabinan. Each bead type could be distinguished based on its distinct covalently linked fluorophore. Empty control beads contained no bound glycan. Beads were pooled and gavaged into mice colonized with the defined community and fed either the unsupplemented HiSF-LoFV, or the HiSF-LoFV supplemented with the pea fiber preparation, the enzymatically destarched Fraction 8 or the purified sugar beet arabinan. A separate group of animals that were maintained as germ-free fed the enzymatically destarched Fraction 8 supplemented HiSF-LoFV served as controls (n=5 mice/treatment group)

[0465] Animals from all groups were euthanized 4 hours after gavage of the bead mixture. Beads were then separated from cecal contents based on their density and magnetism, and each bead type was purified using fluorescence activated cell sorting (FACS) (**FIG. 25**). To compare the *in vivo* degradative capacities of each diet-exposed microbiota, recovered sorted beads were subjected to acid hydrolysis to release all residual bead-bound polysaccharide as free monosaccharides which were then quantified using GC-MS.

[0466] Comparison of germ-free controls to animals containing the defined consortium of human gut bacteria established that removal of arabinan from the different bead types was colonization- dependent. Moreover, no arabinose was detected in the empty beads that were administered to germ-free or colonized animals (**FIG. 26**). When colonized mice were fed the HiSF-LoFV diet supplemented with the pea fiber preparation, arabinose removal from beads with bound Fraction 8 glycans or sugar beet arabinan was significantly ($p = 0.018$ and 0.025, respectively; unpaired t-test) enhanced compared to mice consuming the unsupplemented diet (**FIG. 26**). These results indicate that the pea fiber preparation has the capacity to change the functional configuration of the defined community to a state of enhanced capacity to process arabinan-containing polysaccharides. Mice fed the HiSF-LoFV diet supplemented with either enzymatically destarched Fraction 8 or sugar beet arabinan demonstrated a trend toward enhanced arabinose removal in both bead contexts compared to that in observed in mice fed the unsupplemented HiSF-LoFV diet (**FIG. 26**). These results might suggest that the purified ('free') forms of arabinan prepared from pea fiber (fraction 8), or sugar beet arabinan, compete with bead-bound arabinan for degradation/consumption by members of the community more effectively than the structurally bound, compositionally more complex pea fiber preparation, i.e., this more complex form requires additional processing by CAZymes before they are available to arabinan consumers represented in the model human gut microbiota.

Table 13. Percent fractional abundance of each detected linkage in the purified sugar beet and fraction 8 preparations.

Residue	% Fractional abundance

	Sugar beet arabinan	Fraction 8
Terminal Rhamnopyranosyl residue (t-Rha)		0.2
Terminal Arabinofuranosyl residue (t-Ara(f))	21.3	20.5
Terminal Fucopyranosyl residue (t-Fuc)	-	0.7
Terminal Arabinopyranosyl residue (t-Ara)	-	-
Terminal Xylopyranosyl residue (t-Xyl)	-	3.8
2 linked Rhamnopyranosyl residue (2-Rha)	0.8	0.2
2 linked Arabinofuranosyl residue (2-Ara(f))	0.6	0.3
Terminal Glucuronic Acid residue (t-Glc A)	0.7	
Terminal Glucopyranosyl residue (t-Glc)	-	0.8
3 linked Arabinofuranosyl residue (3-Ara(f))	0.7	0.1
Terminal Galactopyranosyl residue (t-Gal)	2.9	2.9
4 linked Arabinopyranosyl residue or 5 linked Arabinofuranosyl residue (4-Ara(p) or 5-Ara(f))	29.3	20.7
4 linked Xylopyranosyl residue (4-Xyl)	-	3.8
2 linked Xylopyranosyl residue (2-Xyl)	-	1.5
2,4 linked Rhamnopyranosyl residue (2,4-Rha)	1.7	1.0
2 linked Glucopyranosyl residue (2-Glc)	0.3	
3 linked Galactopyranosyl residue (3-Gal)	1.5	1.5
2 linked Galactopyranosyl residue (2-Gal)	-	0.7
3,4 linked Arabinopyranosyl residue or 3,5 linked Arabinofuranosyl residue (3,4-Ara(p) or 3,5-Ara(f))	24.5	2.3
4 linked Galactopyranosyl residue (4-Gal)	6.0	3.1
4 linked Galacturonic Acid residue (4-Gal A)	0.4	
4 linked Glucopyranosyl residue (4-Glc)	-	19.1[a]
6 linked Galactopyranosyl residue (6-Gal)	1.5	-
2,4 linked Arabinopyranosyl residue or 2,5 linked Arabinofuranosyl residue (2,4-Ara(p) or 2,5-Ara(f))	1.7	6.0[a]
2,3,4 linked Arabinopyranosyl residue or 2,3,5 linked Arabinofuranosyl residue (2,3,4-Ara(p) or 2,3,5-Ara(f))	4.2	7.8
3,4 linked Glucopyranosyl residue (3,4-Glc)	-	-
2,4 linked Glucopyranosyl residue (2,4-Glc)	-	-
3,6 linked Galactopyranosyl residue (3,6-Gal)	1.7	
4,6 linked Glucopyranosyl residue (4,6-Glc)	-	3.1

[a] These 2 peaks overlapped; percentages were estimated based on MS fragmentation

Table 14 – Percent fractional abundance of each detected arabinose linkage relative to the total arabinose linkages in purified sugar beet and Fraction 8.

Residue	% Fractional abundance	
	Sugar beet arabinan	Fraction 8
Terminal Arabinofuranosyl residue (t-Ara(f))	25.9	35.5
2 linked Arabinofuranosyl residue (2-Ara(f))	0.7	0.5

3 linked Arabinofuranosyl residue (3-Ara(f))	0.9	0.2
4 linked Arabinopyranosyl residue or 5 linked Arabinofuranosyl residue (4-Ara(p) or 5-Ara(f))	35.6	25.9
3,4 linked Arabinopyranosyl residue or 3,5 linked Arabinofuranosyl residue (3,4-Ara(p) or 3,5-Ara(f))	29.8	4.0
2,4 linked Arabinopyranosyl residue or 2,5 linked Arabinofuranosyl residue (2,4-Ara(p) or 2,5-Ara(f))	2.1	10.4[a]
2,3,4 linked Arabinopyranosyl residue or 2,3,5 linked Arabinofuranosyl residue (2,3,4-Ara(p) or 2,3,5-Ara(f))	5.1	13.5

[a] Peak overlapped with another peak; percentage estimated based on MS fragmentation

Table 15 – Bacterial strains comprising the model defined human gut community.

Bacteria	**Strain**	**Citation**
Bacteroides ovatus	ATCC 8483 INSeq	Ridaura et al.
Bacteroides cellulosilyticus	WH2 INSeq	Ridaura et al.
Bacteroides thetaiotaomicron	ATCC 7330 INSeq	Ridaura et al.
Bacteroides thetaiotaomicron	VPI-5482 INSeq	Ridaura et al.
Bacteroides vulgatus	ATCC 8482 INSeq	Ridaura et al.
Bacteroides caccae	TSDC17.2	Wu et al.
Bacteroides finegoldii	TSDC17.2	Wu et al.
Bacteroides massiliensis	TSDC17.2	Wu et al.
Collinsella aerofaciens	TSDC17.2	Wu et al.

Table 16 – Percent fractional abundance of linkages in the enzymatically destarched Fraction 8

Glycosyl linkage	% Fractional abundance
t-Rha(p)	0.15%
t-Ara(f)	19.37%
t-Fuc(p)	0.46%
t-Ara(p)	0.13%
t-Xyl(p)	2.66%
2-Rha(p)	1.30%
t-Man(p)	0.36%
3-Rha(p)	0.10%
t-Glc(p)	0.16%

3-Ara(f)	0.34%
t-Gal(p)	2.93%
4-Ara(p)/5-Ara(f)	21.78%
3'-Api(f)	0.17%
4-Xyl(p)	6.52%
2,3-Rha(p)	0.00%
2,4-Rha(p)	2.71%
2,3,4-Rha(p)	0.34%
3-Gal(p)	1.91%
2-Gal(p)	0.66%
3,4-Ara(p)/3,5-Ara(f)	2.21%
2,4-Ara(p)/2,5Ara(f)	13.07%
4-Gal(p)	7.22%
2,3,4-Ara(p)/2,3,5-Ara(f)	9.44%
4-Glc(p)	0.07%
3,4-Xyl(p)	0.91%
2,4-Glc(p)	0.34%
2,3,4-Xyl(p)	0.22%
3,6-Man(p)	0.00%
2,6-Man(p)	0.03%
4,6-Glc(p)	0.05%
4,6-Gal(p)	4.38%
3,6-Gal(p)	0.54%
3,4,6-Gal(p)	0.29%
2,3,6-Gal(p)	0.02%
	100.84%

Table 17 – Fractional abundance of arabinose monosaccharides. Abundance is relative to total arabinose content. Data was generated from enzymatically destarched fraction #8 as partially methylated alditol acetate via GC-MS analysis which was supported by the Chemical Sciences, Geosciences and Biosciences Division, Office of Basic Energy Sciences, U.S. Department of Energy grant (DE-SC0015662) to DOE - Center for Plant and Microbial Complex Carbohydrates at the Complex Carbohydrate Research Center.

Monosaccharide	% fractional abundance of arabinose monosaccharides (relative to total arabinose)

t-Ara(f)	29.20%
t-Ara(p)	0.20%
3-Ara(f)	0.51%
4-Ara(p)/5-Ara(f)	32.83%
3,4-Ara(p)/3,5-Ara(f)	3.33%
2,4-Ara(p)/2,5Ara(f)	19.70%
2,3,4-Ara(p)/2,3,5-Ara(f)	14.23%
	100.00%

Example 11

[0467] Fiber preparations were evaluated in various product formats for a number of attributes relating to production (e.g., dough processability, etc.) and organoleptic qualities (e.g., taste, texture, etc.). An "acceptable" product (A) was determined to have suitable processability, taste and texture. An "unacceptable" product (U) was deficient in processability, taste and/or texture.

[0468] **Table 18** summarizes the findings from tests of three fiber compositions. In each product format, the indicated fiber composition provided 3 g, 6 g, or 10 g of dietary fiber. The remaining ingredients contributed additional dietary fiber. The "Pea" composition consisted of 100 wt% pea fiber. The "2 Fiber" composition consisted of 33 wt% pea fiber, 36 wt% high molecular weight inulin, 11 wt% orange fiber, and 20 wt% barley fiber. Attributes of the various fiber preparations are provided in **Table A**, **Table B**, **Table C1**, **Table E**, and **Table F1**.

Table 18

Product Format	3 g Dietary Fiber* per serving			6 g Dietary Fiber* per serving			10 g Dietary Fiber* per serving		
Fiber Comp.	Pea	2 Fiber	4 Fiber	Pea	2 Fiber	4 Fiber	Pea	2 Fiber	4 Fiber
Cracker	A	A	A	A	NT	NT	U	U	U
Cookie, Sweet Bites	A	A	A	NT	NT	NT	U	U	U
Bars	A	A	A	A	A	A	NT	NT	NT
Extruded	A	A	A	A	A	A	A	A	A
Extruded Filled	A	A	A	NT	NT	NT	U	U	U

* Amount of Dietary Fiber in the product contributed by the Fiber Composition.
NT = not tested

[0469] Based on the above testing, additional work was done to further improve overall sensory attributes by optimizing the additional ingredients in a given product format. Table 19 contains several representative products.

Table 19

Product Type	%					%		mg
	Moisture	Ash	Protein	Fat	Carb.	Total Dietary Fiber	Dietary Fiber from fiber composition	Sodium
Cracker w/mushrooms	1.6	2.0	7.4	19.5	69.5	14.9	9.3	425
Cracker w/inclusions	1.7	1.6	7.4	19.3	70.0	16.1	9.9	328
Cookie w/yoghurt	2.6	1.4	5.7	20.1	70.1	10.3	5.7	224
Ginger cookie	8.6	1.3	3.0	13.4	73.8	8.8	7.8	221
Extruded pillow w/almond filling	1.8	2.9	13.7	35.5	46.2	14.7	12.3	735
Extruded pillow w/probiotic	2.9	1.7	3.3	13.4	78.6	29.8	28.8	512
Protein, fat, ash, and moisture content were measured by methods established by Association of Official Analytical Chemists (AOAC) 2009.01, AOAC 920.123, AOAC 933.05, AOAC 935.42, and AOAC 926.08, respectively. Carbohydrate is calculated as (100 - (Protein + Fat + Ash + Moisture). Total dietary fiber was measured by AOAC method 2009.01.								

Introduction to Examples 12 – 17

[0470] Examples 12-17 describe and execute an approach for developing microbiome-directed foods (MDF) that reconfigure the gut community in ways that improve nutritional status. Gnotobiotic mice, colonized with microbiomes from nine obese adults, were fed a prototypical Western diet, high in saturated fats and low in fruits and vegetables (HiSF-LoFV) supplemented with different plant fiber preparations. Fiber-discriminating responses of bacterial taxa, carbohydrate-active enzyme genes

(CAZymes) and metabolic pathways in the microbiome were identified using feature reduction methods. Snack food prototypes containing one, two or four fiber preparations were administered for 2-3-week-long periods to overweight or obese adults consuming a controlled HiSF-LoFV diet. Analyses of serially sampled microbiomes and ~1300 plasma proteins identified fiber-specific changes in the representation of CAZymes that correlated with alterations in the proteome indicative of improved health status.

Example 12. Effects of a HiSF-LoFV diet in gnotobiotic mice

[0471] Three plant fiber preparations to be included in a fiber-supplemented HiSF-LoFV (high in saturated fats and low in fruits and vegetables) diet were selected based on their affordability, reliable sourcing, predicted/known sensory properties, and postulated feasibility for incorporation into food prototypes. Fibers isolated from the pea *Pisum sativum*, the vesicular pulp of the orange *Citrus sinensis*, and the bran of barley (*Hordeum vulgare*) all contain a diverse set of glycan constituents. Arabinan and galacturonan are the most abundant glycans in pea fiber as defined by monosaccharide composition (22.4% arabinose [Ara] and 13.9% galacturonic acid [GalA]), and detection of α-1,5-Ara and α-1,4-GalA linkages by permethylation analysis (**Table 26**). In gnotobiotic mice colonized with a 20-member consortium of gut bacterial strains cultured from a single Ln donor revealed that pea fiber induced a marked increase in the abundance of *Bacteroides thetaiotaomicron* (*15*). Forward genetic screens and high-resolution mass spectrometry analysis of their fecal meta-proteomes identified pea-fiber-dependent increases in the expression of genes that were important fitness factors; they encode members of glycoside hydrolase (GH) families GH51, GH43_4, and GH146, that cleave linear α-1,5-Ara linkages, and α-1,2- and α-1,3-Ara branching linkages (*15*). Orange fiber also contains arabinan and galacturonan, but in contrast to pea fiber, galacturonan dominates (13.9% Ara, 42.9% GalA) (**Table 26**). Orange fiber administration also resulted in a pronounced increase in the abundance of *Bacteroides thetaiotaomicron* (*15*). Barley bran contains 17% mixed-linkage β-glucans; arabinose and xylose (7.1% and 9.9%) are represented in arabinoxylans (linear β-1,4-linked xylose with terminal α-1,2- and α-1,3-linked arabinose substitutions) (**Table 26**). Barley bran was one of the most active fibers screened in our

previous study in gnotobiotic mice, producing a 3% increase in the relative abundance of *B. ovatus* for every 1% w/w increase in the fiber (*15*). Forward genetic and proteomic analyses were not performed in mice consuming orange fiber- or barley bran-supplemented HiSF-LoFV diets.

[0472] Nine groups of 12-to-16-week-old gnotobiotic mice were each colonized with a fecal sample obtained from one of nine 32-41-year-old women with obesity. Each mouse in each treatment group was subjected to the diet oscillation protocol summarized in **FIG. 30A**. The base HiSF-LoFV diet was supplemented with 10% (w/w) of one of the three types of food-grade fibers (see **FIG. 34**, **Table 20,** and **Table 26**). Mice consumed each fiber-supplemented HiSF-LoFV diet monotonously for 10 days. The unsupplemented HiSF-LoFV diet was given for 10 days between each period of fiber-supplemented diet consumption with fecal samples obtained on the day preceding and on the last day of each 10-day cycle. DNA was prepared from fecal samples, amplicons were generated by PCR of variable region 4 (V4) of bacterial 16S rDNA genes to identify bacterial taxa in the microbiota, and whole community DNA was subjected to shotgun sequencing to identify genes in the microbiome. A total of 381 taxa [amplicon sequence variants (ASVs)] were identified (threshold for inclusion in the analysis: present at a relative abundance of ≥0.1% in at least five samples collected from the 69 microbiota transplant recipients). Shotgun sequencing reads of fecal microbiomes were assembled and annotated. The annotation focused on (i) the representation of carbohydrate-active enzymes represented in the Carbohydrate-Active enZYmes (CAZy) database [includes glycoside hydrolases, polysaccharide lyases, carbohydrate-binding modules, and glycosyltransferases (*16*), and (ii) metabolic pathways involved in carbohydrate utilization and fermentation, biosynthesis of amino acids, and B-vitamins/cofactors (the latter play a critical role in myriad metabolic reactions). Metabolic pathway annotations were based on the RAST/SEED platform; this platform combines homology- and genome context-based evidence with known sets of enzymatic reactions and nutrient transporters to group genes into 'microbial community (mc) subsystems' (mcSEED subsystems) that capture and project variations

in particular metabolic pathways/modules across thousands of microbial genomes (*17-19*).

Table 20

Nutritional composition	HiSF-LoFV diet (unsupplemented) per 100g	HiSF-LoFV diet + 10% (w/w) pea fiber per 100g	HiSF-LoFV diet + 10% (w/w) orange fiber per 100g	HiSF-LoFV diet + 10% (w/w) barley bran per 100g
Total energy (kcal)	469.0	422.1	422.1	422.1
Fat (% kcal)	39.0	39.0	39.0	39.0
Saturated fat (% kcal)	16.0	16.0	16.0	16.0
Monounsaturated fat (% kcal)	11.0	11.0	11.0	11.0
Polyunsaturated fat (% kcal)	7.0	7.0	7.0	7.0
Cholesterol (mg/100 g)	74.0	66.6	66.6	66.6
Protein (% kcal)	18.0	18.0	18.0	18.0
Carbohydrate (% kcal)	43.0	43.0	43.0	43.0
Sugars (% kcal)	22.0	22.0	22.0	22.0
Total dietary fiber (g)	3.1	13.1	13.1	13.1
Pea fiber (g)	—	10.0	—	—
Orange fiber (coarse) (g)	—	—	10.0	—
Barley bran (g)	—	—	—	10.0
Nutritional composition per fiber	—	Pea fiber	Orange fiber (coarse)	Barley bran
% Total dietary fiber	—	67.2	68.5	46.0
% Protein	—	9.5	7.5	18.7
% Fat	—	0.9	2.2	4.1
% Carbohydrate	—	79.8	80.9	69.3
% Moisture	—	7.4	5.7	5.7
% Ash	—	2.5	2.0	2.0

Example 13. Effects of dietary fiber on microbial community configuration

[0473] Singular Value Decomposition (SVD) is a method used for dimension reduction where substantial compression of information is often sought (**FIG. 30B**). However, a substantial limitation of SVD is that it can only be used on datasets comprising two feature types (e.g., samples as rows, microbial genes or taxa as columns). In cases where a third feature type is used (temporal data or different conditions) a generalization of SVD to higher dimensions, termed 'Higher-Order

Singular Value Decomposition' (HO-SVD), can be used. Briefly, this technique relates variation among all dimensions included in the data and can be extended to an arbitrary number of dimensions **(FIG. 30B** and *Methods* for details).

[0474] HO-SVD was used to evaluate the response to pea fiber by considering the initial three dietary phases (unsupplemented HiSF-LoFV on day 14, HiSF-LoFV plus pea fiber at day 24, and return to unsupplemented HiSF-LoFV on day 34) (**FIG. 30C**, **FIG. 35**. Fractional abundance values for ASVs were transformed by computing the $\log_2$ fold-change from a reference time point (experimental day 14 when animals were consuming the unsupplemented HiSF-LoFV diet). HO-SVD was employed to identify a set of Tensor Components (TCs) that signified taxonomic variation due to pea fiber consumption. A 'randomized tensor' was generated by shuffling the rows (each mouse), columns (each ASV "taxon"), and z-axis (timepoints) of the tensor. The results disclosed that two tensor components encompassed non-random covariation between mice, taxa, and dietary condition. Each of the 57 mice included in the analysis, each of the 381 ASVs detected, and each of the three dietary phases (conditions) applied contribute towards, or 'project on', each TC. Plotting the ASV response to pea fiber revealed a pronounced effect on community structure (**FIG. 35A**). Ten days after withdrawal of pea fiber, the configuration of the microbiota had not fully returned to the pre-intervention state seen on day 14 (**FIG. 35A**). The projections of the three dietary phases on TC1 to TC2 illustrated that both tensors capture diet-dependent variation in the fractional abundance of bacterial taxa (**FIG. 35A**). **FIG. 35B** displays a histogram of taxonomic projections on TC1. The major drivers underlying this response were members of *Bacteroides,* including *Bacteroides thetaiotaomicron* and *Bacteroides vulgatus* (**FIG. 35B**). The heatmap in **FIG. 35C-E** shows the increases in their abundance upon exposure to pea fiber, with the extent of change varying between mice gavaged with the different human donor microbiota.

[0475] A comparable HO-SVD-based study of genes encoding CAZymes disclosed pronounced configurational changes in transplanted donor microbiomes after pea fiber supplementation (**FIG. 30C**), including increases in the representation of genes encoding arabinosidases belonging to glycoside hydrolase family 43 (GH43_2,

GH43_9, GH43_17, GH43_18 and GH43_19 subfamilies contain alpha-L-arabinofuranosidases) (**FIG. 30D,E**). This finding suggested that arabinan is a predominant polysaccharide in pea fiber utilized by several human gut *Bacteroides* species (*15*). In addition, pea fiber-associated increases in the representation of genes encoding galactosidases (GH43_3, GH43_8, GH43_31 families contain beta-D-galactofuranosidases) and beta-1,4-glucanases belonging to GH family 5 (GH5_1, GH5_4, GH5_5, and GH5_38) were identified (**FIG. 30E**). HO-SVD also disclosed alterations in the abundances of mcSEED pathways for utilization of monosaccharides that are prominently represented in pea fiber (arabinose, xylose, rhamnose, and galacturonic acid; see **FIG. 35F-I**). Notably, the degree of interpersonal variation in the response to pea fiber when defined at the level of the microbiome (CAZymes and mcSEED metabolic pathways) was less than that defined at the level of the microbiota (ASVs) (compare **FIG. 30E** with **FIG. 35C-E,H, I**).

[0476] **FIG. 36-39** present the results of a comparable HO-SVD study of the effects of orange fiber and barley bran. Consistent with the similarities in polysaccharide composition between pea fiber and orange fiber, fecal microbiomes sampled during orange fiber administration revealed increased representation of genes involved in the processing of arabinan (GH43_2, GH43_17, GH43_18), galactan (GH43_3, GH43_8, GH43_31), and galacturonans (PL1, PL9, PL10) (**FIG. 36B,C**), as well as genes involved in the mcSEED pathway for rhamnose utilization (**FIG. 37G,H**). As was the case with pea fiber, *B. thetaiotaomicron* and *B. vulgatus* were the major drivers underlying the orange fiber response, in addition to *Bacteroides nordii* (**FIG. 37B ,C,D,E**). Similar to pea fiber, the degree of interpersonal variation in community response to orange fiber was less in CAZyme, and mcSEED metabolic pathway feature space than ASV-feature space (compare **FIG. 36C,D,E,F** and **FIG. 37CD, E, H**).

[0477] Barley bran produced increases in the representation of genes involved in the processing of beta-glucans (GH5_5, GH5_46), arabinoxylans (GH43_1, GH43_12, GH43_16, GH43_35), and galacturonans (PL1, PL10, PL11) (**FIG. 38B,C,D,E,F**), as well as genes involved mcSEED pathways for arabinose and arabinosides utilization (**FIG. 39E-G**). In contrast to pea and orange fibers, *Blautia*

faecis, *Ruminococcus bicirculans*, *Bacteroides uniformis,* and *Bacteroides ovatus*, were the major drivers of the response to barley bran consumption (**FIG. 39B,C,D**).

Example 14. Testing fiber-containing snack food in overweight or obese adults

[0478] To assess the degree to which results obtained from gnotobiotic mice were translatable to humans, we performed a controlled diet study involving 12 participants who were overweight or obese and a food prototype containing pea fiber (see **Table 21A** for the snack formulation and **Table 28** for a description of the subjects). Each participant provided a fecal sample while on their normal diet during the first four days of the study. Participants then followed a 45-day regimen where their normal diet was replaced with the equivalent of the HiSF-LoFV diet (**Table 21B**). Each 35 gram snack (**Table 21A**) contained 8.1 grams of extruded pea fiber (see **Table 26**or the monosaccharide and glycosidic linkage composition of the extruded fiber preparation). Energy intake from the HiSF-LoFV diet was reduced to account for the energy provided by the snacks, so that overall energy intake was constant. Participants were followed for 14 days after stopping snack consumption, while still continuing the HiSF-LoFV diet (post-intervention 'washout phase'). Daily body weight was monitored using "smart scales" which used cellular networks to send the data to the research team. No additional adjustments in the amount of the HiSF-LoFV diet consumed were needed to maintain a constant body weight during or after the period of treatment with the fiber snack. At various time points during the study, blood samples were obtained for clinical chemistry and plasma proteomic analyses, while fecal samples (n=202) were collected for V4-16S rDNA amplicon and whole community shotgun sequencing (**FIG. 31A**).

[0479] HO-SVD of the representation of CAZymes and mcSEED metabolic pathway components was performed to characterize the response of each subject (**FIG. 31A,C,F,G**, **FIG. 40**, **FIG. 41**. Two tensors were created where rows were subjects, columns were microbiome genes (either CAZymes or mcSEED metabolic pathways), and the third dimension was the three dietary conditions [HiSF-LoFV alone (pre-intervention phase), supplementation with the pea fiber snacks (3 snacks/ day), and

return to HiSF-LoFV (post-intervention phase) for a total of nine time points]. **FIG. 31C** demonstrates that gut microbiome CAZyme gene representation changed upon initiation of consumption of the pea fiber snack and moved towards the pre-treatment state when the intervention ceased. There was remarkable conservation of the treatment-discriminating CAZymes in these subjects and in pea fiber-treated gnotobiotic mice colonized with microbiota from distinct human donors; they include members of GH43 subfamily arabinofuranosidases and arabinanases (GH43_2, GH43_19), members of GH5 subfamily glucanases and glucosidases (GH5_1, GH5_4, GH5_5), pectin/ pectate lyases (PL1, PL9), rhamnogalacturonan lyase (PL11), alginate lyase (PL6), and heparin lyase (PL13) [compare **FIG. 31F,G** (human) and **FIG. 30E** (mouse)]. The heatmaps in **FIG. 31F,G**, which plot the $\log_2$ fold-change of CAZyme gene abundances in the human subjects' microbiomes relative to the time of initiation of pea fiber snack consumption (day 14), disclosed variations in a pea fiber CAZyme response across subjects. CAZymes were ranked based on their projections along TC4; only those within the top 20^{th} percentile of positive projections are shown. Hierarchical clustering of this discriminatory CAZyme dataset allowed us to group participants with similar microbiome responses to pea fiber snack consumption.

[0480] To examine the biotransformation of pea fiber by the participants' microbiota, we used liquid chromatography triple quadrupole mass spectrometry (LC-QQQ-MS) under dynamic multiple reaction monitoring (dMRM) to quantify the absolute concentrations of monosaccharides and the relative abundances of glycosidic linkages in fecal samples collected at the end of the pre-intervention phase (day 14), at peak dose of the fiber snack (days 29 and 35), and during the post-intervention period (days 45 and 49). A Spearman-rank cross-correlation analysis was performed between the $\log_2$ fold-change of HO-SVD-defined discriminatory CAZyme gene abundances (top 20^{th} percentile; matched by time and subject) and the $\log_2$ fold-change in levels of monosaccharides and glycosidic linkages normalized to day 14. Monosaccharides abundant in pea fiber (arabinose, xylose and galacturonic acid) significantly positively correlated with discriminatory CAZymes whose abundances increased during pea fiber supplementation (see areas in the green box in **FIG. 42A**). Cross-correlation analysis of

fecal glycosidic linkages with discriminatory CAZyme gene abundances revealed a specific cleavage pattern of 1,2-arabinofuranose and 1,3-arabinofuranose linkages found in the branches of pea fiber arabinan (*15*) (**FIG. 42B**). Levels of these linkages, in addition to 3,4,6-galactose found in arabinogalactan branches, *negatively* correlated with discriminatory CAZymes that increased during pea fiber supplementation (including GH43_1, GH43_2, GH43_19, and GH43_29 which have known α-L-arabinofuranosidase activity). In contrast, levels of 1,5-arabinofuranose, a linkage primarily found in the backbone of pea fiber arabinan, *positively* correlated with discriminatory CAZymes that increased during pea fiber supplementation (**FIG. 42B**). Data suggest that the microbiomes of these participants are equipped with CAZymes capable of recognizing and cleaving multiple branches of pea fiber arabinan, leaving the backbone (1,5-arabinofuranose) to concentrate in feces.

Table 21A: Nutritional composition of fiber snack prototypes for human studies

Nutritional values	Extruded pea fiber bars		Extruded snack pillows with pea fiber + inulin		Extruded snack pillows with pea fiber + inulin + orange fiber + barley bran	
	Per 100g	Per portion (35g)	Per 100g	Per portion (30g)	Per 100g	Per portion (30g)
Energy (kcal)	370.0	130.0	304.0	91.0	311.0	93.0
Protein (g)	9.0	3.2	3.0	0.9	3.2	1.0
Lipid (g)	10.0	3.5	5.9	1.8	6.4	1.9
Total carbohydrate (g)	59.0	20.7	83.8	25.1	83.6	25.1
Sugars (g)	20.0	7.0	5.0	1.5	4.5	1.3
Starch (g)	16.0	5.6	48.1	14.4	47.0	14.0
Total dietary fibers (g)	23.0	8.1	33.6	10.1	34.9	10.5
% of fiber on the snack						
Pea fiber (extruded)	100%	100%	64%	64%	33%	33%
Inulin (HMW)	—	—	36%	36%	36%	36%
Orange fiber (extruded)	—	—	—	—	11%	11%
Barley bran	—	—	—	—	20%	20%

HMW = high-molecular weight

Table 21B: Nutritional composition of HiSF-LoFV meals for human studies.

	Meal option	Energy (kcal)	Protein (%)	Lipid (%)	Carbohydrate (%)	Fiber (g)
Breakfast	Three meat biscuit pocket	565.0	19.7	44.7	35.6	1.6

	Dreamsicle™ smoothie	570.0	15.8	44.5	39.6	1.2
	Pancake with sausage & bacon	569.0	15.0	45.3	39.8	0.6
	Chips Ahoy! smoothie	571.0	15.6	44.0	40.5	1.3
	Biscuit & gravy	571.0	15.8	44.7	39.7	1.4
	French toast with sausage & bacon	571.0	15.0	44.9	40.1	1.2
	Ham & cheese bagel	570.0	20.2	44.2	35.5	1.4
	Average per breakfast meal	570.0	16.7	44.6	38.7	1.2
Lunch/ dinner	Toasted cheese and bacon sandwich (with soda)	570.0	19.7	45.0	35.5	1.4
	Sloppy Joe on bun (with soda)	570.0	20.0	40.5	39.6	1.1
	Roast beef & salami sammies (with soda)	570.0	20.0	40.6	39.7	1.5
	Cheeseburger sliders (with soda)	570.0	19.3	40.6	40.2	1.5
	Bacon mac & cheese (with soda)	571.0	19.5	44.4	36.3	1.4
	Bacon cheddar BBQ hotdog (with soda)	570.0	15.2	45.8	39.1	1.0
	Spaghetti with meat sauce (with soda)	571.0	19.9	42.9	37.5	1.5
	Quesadilla (with soda)	570.0	18.9	42.9	38.4	1.5
	Pig in a blanket (with soda)	568.0	14.9	45.0	40.3	1.1
	Pepperoni pizza (with soda)	573.0	15.0	45.4	39.9	1.3
	Nachos (with soda)	570.0	17.7	44.3	38.2	1.5
	Hamburger & sausage pizza (with soda)	570.0	16.9	43.8	39.6	1.3
	Ham & cheese rollup with potato chips (with soda)	569.0	19.8	42.7	37.8	1.5
	Chili mac (with soda)	569.0	17.2	42.9	40.0	1.3
	Average per lunch/ dinner meal	570.0	18.1	43.3	38.7	1.3
Snacks	Chocolate fudge bite	100.0	16.2	44.2	39.6	0.3
	Oreo® cream cheese bite	100.0	15.9	44.5	39.7	0.2
	Saltines with Easy Cheese™	101.0	19.0	43.9	37.1	0.3
	Frozen orange soda smoothie	100.0	16.1	44.3	16.1	0.1
	Hard boiled egg & sour patch kids snack pack	103.0	23.4	40.9	35.5	0
	String cheese & M&Ms®	100.0	15.4	47.6	37.1	0.3
	Chocolate chip cookie bites & milk	100.0	15.5	44.3	39.9	0.2
	Average per snack	101.0	17.4	44.2	35.0	0.2
Fiber bar substitute	Peanut butter rice krispies	130.0	8.6	22.5	68.8	0.9

Table 22: Effects of consumption of the pea fiber snack prototype on clinical meta-data values.

Parameter	Mean ± standard deviation			P value (ANOVA of linear mixed-effects model)	FDR-adjusted P value (least-squares means of linear mixed-effects model)		
	Day 14	Day 35	Day 49		Day 14 vs 35	Day 14 vs 49	Day 35 vs 49
BMI (kg/m^2)	30.2 ± 3.3	30.3 ± 3.2	31.2 ± 5.1	0.365	0.882	0.378	0.378
Fasting insulin (pmol/L)	17.5 ± 7.6	17.4 ± 9.4	14.7 ± 8.2	0.175	0.951	0.169	0.169
Fasting glucose (mg/dL)	100.7 ± 12.9	106.8 ± 12.8	103.9 ± 13.7	0.066	0.064	0.261	0.261
HOMA-IR	4.5 ± 2.3	4.8 ± 3.1	4.0 ± 2.7	0.271	0.498	0.498	0.335
Triglyceride (mg/dL)	122.7 ± 62.6	126.8 ± 81.7	125.3 ± 84.4	0.927	0.891	0.891	0.891
Total cholesterol (mg/dL)	194.1 ± 26.2	190.8 ± 33.6	185.8 ± 30.5	0.243	0.509	0.297	0.457
HDL cholesterol (mg/dL)	47.9 ± 10.8	47.2 ± 9.8	46.4 ± 9.3	0.656	0.647	0.647	0.647
LDL cholesterol (mg/dL)	121.6 ± 21.8	118.3 ± 29.8	114.3 ± 25.7	0.264	0.453	0.321	0.453
Hemoglobin (g/dL)	14.2 ± 1.2	14.0 ± 1.4	13.8 ± 1.5	**0.032**	0.275	**0.029**	0.152
Hematocrit (%)	42.4 ± 3.0	42.2 ± 3.6	41.5 ± 4.1	0.106	0.739	0.143	0.143
Red blood count (x 10^{12}/L)	5.0 ± 0.6	4.9 ± 0.5	4.8 ± 0.6	0.118	0.676	0.155	0.175
Platelet count (x 10^9/L)	267.7 ± 79.4	256.6 ± 74.8	257.7 ± 67.7	0.128	0.144	0.144	0.852
Mean platelet volume (fL)	8.7 ± 1.0	8.8 ± 0.8	8.8 ± 0.9	0.268	0.269	0.269	0.909
White blood count (x 10^9/L)	5.8 ± 1.3	5.9 ± 1.4	5.7 ± 1.3	0.870	0.917	0.917	0.917
Neutrophil (%)	53.2 ± 9.4	55.1 ± 7.7	53.7 ± 7.8	0.488	0.589	0.766	0.589
Lymphocyte (%)	34.9 ± 7.9	33.3 ± 6.5	34.9 ± 6.9	0.432	0.398	1.000	0.398
Monocyte (%)	8.5 ± 2.6	8.3 ± 2.1	7.7 ± 1.5	0.240	0.652	0.317	0.347
Eosinophil (%)	2.6 ± 1.3	2.7 ± 1.4	2.9 ± 1.5	0.542	0.668	0.668	0.668
Basophil (%)	0.8 ± 0.3	0.7 ± 0.3	0.8 ± 0.3	0.183	0.242	0.721	0.242
Absolute neutrophil (x 10^9/L)	3.1 ± 1.1	3.3 ± 1.1	3.1 ± 0.9	0.670	0.717	0.915	0.717
Absolute lymphocyte (x 10^9/L)	3.4 ± 4.9	1.9 ± 0.5	2.0 ± 0.5	0.373	0.353	0.353	0.978
Absolute monocyte (x 10^9/L)	0.5 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.177	0.532	0.327	0.211
Absolute eosinophil (x 10^9/L)	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.3	0.446	0.897	0.458	0.458
Absolute basophil (x 10^9/L)	0.0 ± 0.1	0.0 ± 0.1	0.0 ± 0.1	0.468	0.434	0.434	1.000
Total protein (g/dL)	7.5 ± 0.6	7.5 ± 0.5	7.4 ± 0.6	0.566	0.879	0.607	0.607
Albumin (g/L)	4.5 ± 0.3	4.4 ± 0.2	4.4 ± 0.2	0.105	0.270	0.112	0.415
Calcium (mmol/L)	9.3 ± 0.4	9.2 ± 0.4	9.2 ± 0.4	0.263	0.327	0.473	0.527
Total bilirubin (µmol/L)	0.5 ± 0.3	0.5 ± 0.3	0.5 ± 0.3	0.813	0.885	0.885	0.885
SGOT (AST) (U/L)	19.5 ± 7.5	20.3 ± 7.4	20.8 ± 5.7	0.516	0.679	0.679	0.706
SGPT (ALT) (U/L)	21.6 ± 18.7	23.4 ± 17.6	20.8 ± 12.8	0.493	0.638	0.716	0.638
Alk. phos, total (IU/L)	64.3 ± 16.0	64.8 ± 16.0	62.3 ± 14.5	0.075	0.700	0.112	0.102
Urea nitrogen (mg/dL)	15.8 ± 3.3	14.0 ± 4.2	14.7 ± 3.9	**0.009**	**0.007**	0.069	0.206
Creatinine (mg/dL)	1.0 ± 0.2	1.0 ± 0.1	1.0 ± 0.2	0.691	0.705	0.705	0.705
Sodium (mmol/L)	138.8 ± 2.1	138.6 ± 1.6	138.2 ± 1.6	0.306	0.563	0.395	0.508
Potassium (mmol/L)	4.2 ± 0.3	4.2 ± 0.3	4.1 ± 0.3	0.067	0.866	0.078	0.078
Chloride (mEq/L)	102.4 ± 2.4	102.8 ± 2.1	102.8 ± 2.4	0.640	0.717	0.717	0.858
CO_2 content (mEq/L)	24.8 ± 1.8	24.3 ± 2.3	23.6 ± 2.0	**0.027**	0.258	**0.025**	0.144

Example 15. Effects of snack food containing mixtures of two and four fibers in overweight or obese adults

[0481] To determine whether combining pea fiber with the other fibers characterized in our gnotobiotic mouse experiments would have greater effects on the microbiome and host than those obtained with pea fiber alone, we performed a second controlled diet study involving 14 people who were overweight or obese, nine of whom had also participated in the pea fiber study (see **Table 29** for a description of subjects enrolled). Two multi-fiber snack prototypes were tested; one contained pea fiber and inulin (10.1 gram (g) fiber/30 g snack; 64% pea fiber and 36% inulin) and the other a combination of pea fiber, inulin, orange fiber and barley bran (10.5 g fiber/30 g snack; 33% pea fiber, 36% inulin, 11% orange fiber, and 20% barley bran; **Table 21A**). Inulin, isolated from the root of chicory *Cichorium intybus*, is a beta-2-1-linked fructose polymer with limited degree of polymerization relative to many other dietary plant polysaccharides (*20*). In our previously published gnotobiotic mouse fiber screening experiment involving the 20-member consortium of cultured human gut bacterial strains (*15*), each 1% w/w increase in the amount of inulin added to the HiSF-LoFV diet resulted in a pronounced 4.5% increase in the relative abundance of another target *Bacteroides*, *B. caccae*, which possessed GH2 enzymes involved in beta-2-1-linked fructan metabolism as well as a GH91 inulin lyase (*15*).

[0482] The study design is summarized in **FIG. 31B**. On the first day, each participant provided a fecal sample produced while on their normal diet; they then followed a 48-day controlled diet protocol where their normal diet was replaced with the same HiSF-LoFV diet used for the pea fiber study (**Table 21B**). Subjects were provided with the pea fiber/inulin snack food prototype to supplement their HiSF-LoFV diet for 14 days starting on study day 12. The dosage escalation protocol consisted of one snack/day for one day (day 12, with lunch), followed by two snacks/day for an additional day (day 13; lunch and dinner), and three snacks a day for the following 12 days (breakfast, lunch and dinner). Participants continued on the HiSF-LoFV diet for 10 additional days after stopping consumption of the two-fiber snack food prototype and then started on the four-fiber blend for 14 days. The dosage escalation protocol for this

phase consisted of one snack/day for one day (day 36), followed by two snacks/day for an additional day (day 37), and three snacks a day for the following 12 days (with total caloric intake maintained constant throughout). During the course of the study, no additional caloric adjustments had to be made in the amount of HiSF-LoFV diet consumed to maintain a constant body weight in all subjects. Blood (plasma) and fecal samples were collected at the time points shown in **FIG. 31B;** fecal samples were used to generate shotgun sequencing and V4-16S rDNA amplicon datasets.

[0483] We constructed two tensors to distinguish the effects of each type of snack prototype on the CAZyme composition of participant microbiomes. In both tensors, rows represented subjects and columns were genes (log_2 fold-change of CAZymes normalized to day 9 when subjects were on the HiSF-LoFV base diet). The third dimension represented study days corresponding to the different diet conditions. The results of HO-SVD analyses are provided in **FIG. 31**, **FIG. 40**, **FIG. 43** and **FIG. 44**.

[0484] Changes in microbiome CAZyme gene composition in response to the dietary interventions with both fiber blend preparations were represented by changes in projection along TC1 (**FIG. 31D,E**). The heatmaps in **FIG. 31H-K** display the log_2 fold-change in CAZyme gene abundances normalized to the last day of the pre-intervention phase (day 11). CAZymes were ranked based on their projections along TC1; only those within the top 20^{th} percentile of positive projections are shown. Increased representation of genes encoding arabinan-processing enzymes, including the arabinase/ arabinofuranosidases GH43_1 and GH43_19, occurs with both the two- and four-fiber blends (**FIG. 35G,H**) as well as with the pea fiber alone formulation (**FIG. 35H, I**), whereas GH43, GH43_9, GH43_18, GH43_28, GH43_33, and GH43_34 CAZymes increased with the two- and four-fiber snack food prototypes (**FIG. 35G,H**). CAZymes that process galacturonic acid and xylose increased in response to all three fiber snacks including the xylanase/ xylosidase GH30, pectin/pectate lyases PL1, PL9, and the rhamnogalacturonan lyase PL11 (**FIG. 35F-H**). The inulin-processing GH91 and inulin-binding protein CBM38 were part of the response to the four-fiber combination, with CBM38 but not GH91 increasing after exposure to the pea fiber plus inulin combination (**FIG. 35G,H**). While galacturonan and arabinan are abundant in pea fiber

and orange fiber, beta-glucans and arabinoxylans are prominent in barley bran (*15*). The representation of rhamnogalacturonan lyase PL26, the α-galacturonidase GH138, the α-L-arabinofuranosidase GH43_16, and the endo-1,2-α-mannanase GH99 were only found to change after consumption of the four-fiber blend snack, as did the β-glucosidase GH116 (**FIG. 35H**), consistent with the targeting of beta-glucans that were distinctly represented in barley bran. Hierarchical clustering (Canberra distance) of changes in discriminatory CAZyme gene abundances provided an informative way to compare and group the microbiome responses of participants to each of the snack prototypes (**FIG. 35F-H**).

[0485] Comparable HO-SVD analyses of the effects of the two-fiber and four-fiber blends on mcSEED pathway and ASV composition are presented in **FIG. 43** and **FIG. 44**. With both fiber blends, there was an increase in the representation of pathways for rhamnose and rhamnogalacturonan utilization, and galacturonate, glucuronate and glucuronides utilization (**FIG. 43B,C; FIG. 44B,C**). The four-fiber blend snack produced significant increases in the abundance of a broader range of targeted *Bacteroides* species, including *B. vulgatus, B. uniformis*, *B. xylanisolvens*, *B. ovatus* and *B. caccae*, than the two-fiber blend or pea fiber alone (**FIG. 41E,F**; **FIG. 43E,F,G,H,I**; **FIG. 44E,F,G,H,I**).

Table 23: Effects of consumption of the snack fiber prototypes on clinical meta-data values.

(A)

Parameter	Mean ± standard deviation				P value (ANOVA of linear mixed-effects model)
	Day 11	Day 25	Day 35	Day 49	
BMI (kg/m^2)	29.6 ± 3.6	29.6 ± 3.7	29.5 ± 3.8	29.5 ± 3.7	0.406
Fasting insulin (pmol/L)	12.1 ± 6.0	14.3 ± 8.0	14.7 ± 8.2	12.4 ± 5.9	0.050
Fasting glucose (mg/dL)	93.0 ± 6.9	95.1 ± 6.1	97.6 ± 8.5	96.9 ± 6.1	**0.002**
HOMA-IR	2.8 ± 1.5	3.4 ± 2.1	3.6 ± 2.3	3.0 ± 1.6	**0.027**
Triglyceride (mg/dL)	93.1 ± 40.6	96.8 ± 45.1	104.6 ± 46.4	96.3 ± 46.7	0.309
Total cholesterol (mg/dL)	188.9 ± 20.4	187.5 ± 26.2	187.9 ± 27.4	185.7 ± 29.4	0.913
HDL cholesterol (mg/dL)	52.3 ± 9.9	51.6 ± 9.8	51.7 ± 9.9	51.4 ± 9.4	0.895
LDL cholesterol (mg/dL)	117.8 ± 19.8	116.5 ± 24.2	115.2 ± 27.0	115.0 ± 27.1	0.878
Hemoglobin (g/dL)	13.7 ± 0.8	13.6 ± 0.8	13.8 ± 0.9	13.7 ± 1.1	0.724
Hematocrit (%)	41.0 ± 2.2	40.6 ± 1.9	40.9 ± 2.3	41.1 ± 2.9	0.753
Red blood count (x 10^{12}/L)	4.7 ± 0.6	4.7 ± 0.5	4.7 ± 0.6	4.7 ± 0.6	0.625

Platelet count (x 10^9/L)	260.7 ± 70.6	259.9 ± 66.1	258.1 ± 70.7	263.4 ± 58.0	0.780
Mean platelet volume (fL)	8.9 ± 0.9	8.9 ± 0.8	8.9 ± 0.9	8.9 ± 0.8	0.962
White blood count (x 10^9/L)	5.4 ± 1.0	5.2 ± 1.1	5.3 ± 1.2	5.5 ± 1.4	0.707
Neutrophil (%)	50.4 ± 7.4	50.4 ± 7.2	51.7 ± 8.9	53.1 ± 8.1	0.218
Lymphocyte (%)	37.9 ± 7.0	38.3 ± 6.9	36.8 ± 7.0	35.7 ± 7.7	0.290
Monocyte (%)	8.3 ± 1.8	7.9 ± 1.8	8.1 ± 1.9	7.8 ± 1.7	0.413
Eosinophil (%)	2.6 ± 1.8	2.5 ± 1.5	2.4 ± 1.4	2.5 ± 1.9	0.929
Basophil (%)	0.9 ± 0.6	0.9 ± 0.3	1.0 ± 0.4	0.9 ± 0.4	0.750
Absolute neutrophil (x 10^9/L)	2.7 ± 0.7	2.7 ± 0.8	2.8 ± 0.9	3.0 ± 1.1	0.513
Absolute lymphocyte (x 10^9/L)	2.0 ± 0.4	2.0 ± 0.5	1.9 ± 0.5	1.9 ± 0.5	0.776
Absolute monocyte (x 10^9/L)	0.4 ± 0.1	0.5 ± 0.5	0.4 ± 0.1	0.4 ± 0.1	0.650
Absolute eosinophil (x 10^9/L)	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.325
Absolute basophil (x 10^9/L)	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.1	0.0 ± 0.1	0.403
Total protein (g/dL)	7.5 ± 0.3	7.4 ± 0.2	7.4 ± 0.3	7.5 ± 0.4	0.729
Albumin (g/L)	4.5 ± 0.2	4.4 ± 0.2	4.4 ± 0.2	4.4 ± 0.2	**0.016**
Calcium (mmol/L)	9.5 ± 0.3	9.4 ± 0.2	9.4 ± 0.4	9.5 ± 0.3	0.493
Total bilirubin (µmol/L)	0.5 ± 0.2	0.5 ± 0.2	0.6 ± 0.3	0.5 ± 0.2	0.208
SGOT (AST) (U/L)	19.2 ± 4.8	19.1 ± 4.0	18.6 ± 3.9	20.8 ± 6.3	0.404
SGPT (ALT) (U/L)	17.0 ± 8.3	16.8 ± 8.7	16.5 ± 8.4	18.1 ± 9.1	0.334
Alk. phos, total (IU/L)	61.5 ± 15.7	63.8 ± 17.6	63.1 ± 15.1	62.8 ± 15.1	0.202
Urea nitrogen (mg/dL)	13.6 ± 3.7	12.4 ± 2.7	13.1 ± 2.5	12.9 ± 3.1	0.236
Creatinine (mg/dL)	0.9 ± 0.2	0.9 ± 0.2	0.9 ± 0.2	0.9 ± 0.2	0.782
Sodium (mmol/L)	140.2 ± 1.6	140.8 ± 1.9	141.1 ± 1.3	139.5 ± 2.0	0.054
Potassium (mmol/L)	4.2 ± 0.5	4.2 ± 0.4	4.3 ± 0.4	4.3 ± 0.5	0.438
Chloride (mEq/L)	103.4 ± 2.2	103.1 ± 1.9	103.7 ± 2.0	103.1 ± 2.6	0.683
CO_2 content (mEq/L)	24.8 ± 2.2	24.7 ± 1.8	24.1 ± 2.2	24.1 ± 2.3	0.145

(B)

Parameter	FDR-adjusted P value (least-squares means of linear mixed-effects model)					
	Day 11 vs 25	Day 11 vs 35	Day 11 vs 49	Day 25 vs 35	Day 25 vs 49	Day 35 vs 49
BMI (kg/m^2)	0.930	0.577	0.489	0.577	0.489	0.577
Fasting insulin (pmol/L)	0.102	0.102	0.808	0.808	0.127	0.102
Fasting glucose (mg/dL)	0.131	**0.003**	**0.008**	0.095	0.183	0.562
HOMA-IR	0.078	**0.045**	0.531	0.531	0.210	0.078
Triglyceride (mg/dL)	0.732	0.430	0.732	0.432	0.937	0.432
Total cholesterol (mg/dL)	0.938	0.938	0.938	0.938	0.938	0.938
HDL cholesterol (mg/dL)	0.954	0.954	0.954	0.954	0.954	0.954
LDL cholesterol (mg/dL)	0.887	0.887	0.887	0.887	0.887	0.956
Hemoglobin (g/dL)	0.810	0.988	1.000	0.810	0.810	0.988
Hematocrit (%)	0.817	0.817	0.817	0.817	0.817	0.817

Red blood count (x 10^{12}/L)	0.634	0.978	0.634	0.634	0.634	0.634
Platelet count (x 10^9/L)	0.881	0.869	0.869	0.869	0.869	0.869
Mean platelet volume (fL)	1.000	1.000	1.000	1.000	1.000	1.000
White blood count (x 10^9/L)	0.853	0.853	0.853	0.853	0.853	0.853
Neutrophil (%)	0.988	0.458	0.223	0.458	0.223	0.458
Lymphocyte (%)	0.776	0.552	0.420	0.552	0.420	0.552
Monocyte (%)	0.591	0.698	0.591	0.680	0.841	0.680
Eosinophil (%)	0.913	0.913	0.913	0.913	0.978	0.913
Basophil (%)	0.883	0.883	0.883	0.883	0.883	0.883
Absolute neutrophil (x 10^9/L)	0.754	0.754	0.732	0.732	0.732	0.732
Absolute lymphocyte (x 10^9/L)	0.888	0.888	0.888	0.888	0.888	0.888
Absolute monocyte (x 10^9/L)	0.772	0.885	0.885	0.772	0.772	0.885
Absolute eosinophil (x 10^9/L)	0.553	0.553	0.478	0.478	0.478	0.553
Absolute basophil (x 10^9/L)	0.560	0.560	0.587	0.587	0.560	0.587
Total protein (g/dL)	0.918	0.918	0.918	0.926	0.926	0.926
Albumin (g/L)	**0.023**	**0.023**	**0.023**	1.000	0.890	0.890
Calcium (mmol/L)	0.706	0.706	0.706	0.706	0.706	0.745
Total bilirubin (µmol/L)	0.585	0.233	0.585	0.352	0.938	0.352
SGOT (AST) (U/L)	0.957	0.847	0.478	0.847	0.478	0.478
SGPT (ALT) (U/L)	0.821	0.821	0.465	0.821	0.465	0.465
Alk. phos, total (IU/L)	0.226	0.442	0.467	0.606	0.529	0.789
Urea nitrogen (mg/dL)	0.259	0.430	0.430	0.430	0.430	0.818
Creatinine (mg/dL)	0.854	0.854	0.854	0.854	0.854	0.854
Sodium (mmol/L)	0.406	0.308	0.349	0.630	0.106	0.067
Potassium (mmol/L)	0.683	0.619	0.683	0.619	0.619	0.683
Chloride (mEq/L)	0.778	0.778	0.778	0.778	1.000	0.778
CO_2 content (mEq/L)	1.000	0.178	0.178	0.178	0.178	1.000

Example 16. Effects of fiber supplementation on plasma proteome

[0486] An aptamer-based platform was used to perform a quantitative multiplex proteomic analysis of the abundances of 1305 plasma proteins in subjects enrolled in both studies described in the Examples. These proteins include biomarkers and regulators of a range of physiologic, metabolic, and immunologic functions and thus provide a broad view of the effects of consuming the different snack food prototypes. A tensor comprised of subjects (rows), protein abundances (columns), and timepoints (third dimension) was created for each type of fiber intervention. For human study 1, the first tensor was made using the $\log_2$ fold-change of plasma protein markers on day 14 (pre-intervention), day 29 (pea fiber snack at highest dose) and day 49 (post-

intervention), normalized to day 14. For human study 2, second and third tensors were made using the log_2 fold-change of plasma protein markers on (i) days 11 (pre-intervention), 25 (the two-fiber snack at the highest dose) and 35 (washout phase) normalized to day 11, and (ii) days 11 (pre-intervention), 35 (washout phase) and 49 (four-fiber snack at the highest dose) normalized to day 11. [Note that because human study 2 tested the effects of two weeks of treatment with each of the snack prototypes, we used days 14, 29, and 49 (not day 35) to analyze the responses to the pea fiber snack]. The results of HO-SVD analysis of the resulting plasma proteomic datasets are described in **FIG. 32**.

[0487] For the first human study, TC1 distinguishes consumption of the HiSF-LoFV base diet and consumption of the maximum dose of the pea fiber snack; it also shows a return to baseline 14 days after stopping the intervention (**FIG. 32A**). For the second study, TC2 distinguishes the plasma proteome sampled during the pre-intervention phase on day 11 from the proteome sampled at the end of the period of consumption of the maximum dose of the two-fiber snack (**FIG. 32C**) and the end of the period of maximum consumption of four-fiber snack (**FIG. 32E**). Unlike the snack food prototype containing pea fiber alone, the plasma proteomes of participants enrolled in the multi-fiber snack study did not completely return to baseline during the washout phase (**FIG. 32,E**) suggesting longer-lived effects of the fiber blends.

[0488] Proteins within the 20^{th} percentile of most positive and negative projections along TC1 for human study 1 and TC2 for human study 2 were defined as most discriminatory for host responses to the different snack prototypes. Using the total number of measured plasma proteins that passed quality control (see *Methods*), we identified KEGG pathways significantly enriched within the set of proteins that discriminate the responses to the different fiber snack prototypes; 24 of these pathways were enriched in all three dietary interventions, including insulin signaling pathway, glucagon signaling pathway, glycolysis/ gluconeogenesis, carbon metabolism, carbohydrate digestion/absorption, platelet activation, and B- and T-cell receptor signaling.

[0489] During the 2-3 week-long period of consumption of the three different snack prototypes, the four-fiber blend produced the greatest reduction in HOMA-IR (**Table 22** and **Table 23)** although the decrease after the short 14-day period of supplementation did not achieve statistical significance ($p=0.078$). The $\log_2$ fold-changes in the abundances of 25 plasma proteins in the insulin and glucagon signaling pathways for each subject are shown for all diet interventions in **FIG. 32B,D,F** (changes normalized to day 14 and day 11 for the first and second human studies, respectively). Hierarchical clustering (Canberra distances) of the subjects' responses revealed two distinct groups for each treatment. The direction of change in the abundance of each of these proteins that is indicative of movement towards a healthier state (based on a literature evidence; **Table 24**) was denoted by the bar on right side of the heatmaps (increase in red; decrease in blue). Responders were defined as those subjects with an aggregate change of ≥ 50% of the protein markers towards a healthier state. Combining this definition of response to a healthier state in plasma proteins markers belonging to insulin and glucagon signaling pathways with the hierarchical clustering results, we classified three of the 12 subjects in Study 1 as being responsive to pea fiber snacks (**FIG. 32B**), 8/14 and 7/14 subjects to the two- and four-fiber snack formulations (**FIG. 32D,E**).

[0490] Non-targeted liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) of fecal samples collected from mice colonized with obese donor TP01-01 microbiota in the study shown in **FIG. 30A**, and fed the orange fiber-supplemented HiSF-LoFV diet, revealed an analyte with m/z of 274.1442. The m/z of 274.1442 analyte was also detected in their orange-fiber treated germ-free counterparts, but not in TP-01-01 colonized mice or germ-free controls fed the unsupplemented or pea fiber supplemented HiSF-LoFV diets (**FIG. 45A**). We reasoned that this m/z of 274.1442 analyte would be a useful biomarker of consumption of the four-fiber snack prototype. LC-QTOF-MS of fecal samples collected on days 25 and 49 from participants in study 2 during the time of consumption of the maximum dose of the two- and four-fiber snacks respectively, revealed significantly higher levels of the m/z of 274.1442 analyte in those consuming the orange-fiber containing snack. Interestingly,

four of the eight participants who were classified as hypo-responders based on the plasma proteome response in the insulin/glucagon signaling pathways had the lowest fecal levels of the m/z 274.1442 analyte (**FIG. 45B,C**).

Table 24

Somamer ID	Protein name	Entrez Gene ID	Desired change
BAD.5870.23.2	BAD (Bcl2-associated agonist of cell death)	572	Decrease
CAMK2A.3350.53.2	CAMK2A (Calcium/calmodulin-dependent protein kinase type II subunit alpha)	815	Decrease
CAMK2B.3351.1.1	CAMK2B (Calcium/calmodulin-dependent protein kinase type II subunit beta)	816	Decrease
CAMK2D.3419.49.2	CAMK2D (Calcium/calmodulin-dependent protein kinase type II subunit delta)	817	Decrease
CRK.4976.57.1	CRK (Adapter molecule crk)	1398	Decrease
GCG.4891.50.1	GCG (Glucagon)	2641	Decrease
GRB2.5464.52.3	GRB2 (Growth factor receptor-bound protein 2)	2885	Decrease
INS.4883.56.2	INS (Insulin)	3630	Decrease
MAPK1.3115.64.2	MAPK1 (Mitogen-activated protein kinase 1)	5594	Decrease
MAPK8.3825.18.2	MAPK8 (Mitogen-activated protein kinase 8)	5599	Decrease
PDPK1.4460.8.2	PDPK1 (3-phosphoinositide-dependent protein kinase 1)	5170	Decrease
PIK3CA.PIK3R1.3390.72.2	PIK3CA, PIK3R1 (PIK3CA/PIK3R1)	5290, 5295	Decrease
PKM2.4240.31.2	PKM2 (Pyruvate kinase PKM)	5315	Decrease
PPP3CA.PPP3R1.4903.72.1	PPP3CA, PPP3R1 (Calcineurin)	5530, 5534	Decrease
PRKACA.3466.8.2	PRKACA (cAMP-dependent protein kinase catalytic subunit alpha)	5566	Decrease
PRKCI.3379.29.1	PRKCI (Protein kinase C iota type)	5584	Decrease
PTPN1.3005.5.2	PTPN1 (Tyrosine-protein phosphatase non-receptor type 1)	5770	Decrease
SHC1.5272.55.2	SHC1 (SHC-transforming protein 1)	6464	Decrease
AKT1.AKT2.AKT3.3392.68.2	AKT1, AKT2, AKT3 (RAC-alpha/beta/gamma serine/threonine-protein kinase)	207, 208, 10000	Increase
AKT2.5360.9.2	AKT2 (RAC-beta serine/threonine-protein kinase)	208	Increase
HK2.13130.150.3	HK2 (Hexokinase-2)	3099	Increase
INSR.3448.13.2	INSR (Insulin receptor)	3643	Increase
PGAM1.3896.5.2	PGAM1 (Phosphoglycerate mutase 1)	5223	Increase
PRKAA2.PRKAB2. PRKAG1.5245.40.5	PRKAA2, PRKAB2, PRKAG1 (AMP Kinase (alpha2beta2gamma1))	5563, 5565, 5571	Increase
PRKCZ.2645.54.1	PRKCZ (Protein kinase C zeta type)	5590	Increase

Example 17. Relating features of the gut microbiome to features of the plasma proteome as a function of fiber-snack consumption

[0491] Cross-correlation singular value decomposition (CC-SVD) is a method for correlating variation in disparate feature-sets. To relate changes in the microbiome in response to different snack food prototypes and host biological status, we performed CC-SVD by creating a cross-correlation matrix where columns comprised the discriminatory plasma proteins identified by HO-SVD (i.e., the top 20th percentile of most positive and negative projections along the selected TC), rows comprised the fiber-responsive CAZymes identified by HO-SVD (top 20th percentile of most positive projections along the selected TC), and each element of the matrix measured the Spearman correlation between plasma protein *i* and CAZyme *j* over time. SVD was then performed on this matrix to delineate the plasma proteins and CAZymes whose variances in abundance are positively and negatively correlated. The resulting analysis provided a way to relate microbiome responses during fiber snack consumption with host responses for each subject and to discern whether there are shared features of the responses across individuals. Details of the method are provided in *Methods* and in **FIG. 33A,B**. We focused on the first singular vector (SV1) because it explained the highest percentage of the cross-correlation variance for responses to each of the snacks.

[0492] As noted above, consumption of the four-fiber snack prototype increased the abundances of genes encoding CAZymes with α-L-arabinofuranosidase (GH43_33), beta-galactosidase (GH147), endo-1,2-α-mannanase (GH99) and beta-glucosidase (GH116) activities; increases in the latter two GHs are a discriminatory feature of this multi-fiber formulation whereas both the two- and four-fiber snack prototypes increase the former two groups of CAZyme genes. CC-SVD revealed that in participants who received the four-fiber blend, these four GH families are negatively correlated with plasma proteins whose reduced abundances signal improvement to a healthier state. These included proteins involved in acute and chronic inflammation [chemokine ligand 3 (CCL3) and C-reactive protein (CRP) which are known markers of cardiovascular disease risk (*21,22*), secreted phosphoprotein 1 (SPP1), thrombin (F2),

tissue factor (F3), vascular endothelial growth factor-A (VEGFA), platelet-derived growth factor receptor beta (PDGFRB), ephrin A5 (EFNA5), ephrin type-A receptor 1 precursor (EPHA1), ephrin type A receptor 2 precursor (EPHA2), and interleukin 1 receptor type 1 (IL1R1)]. They also included proteins involved in platelet activation and blood coagulation [complement component 3 (C3), complement receptor type 1 (C1R), complement component 4 (C4A/C4B), plasminogen activator inhibitor 1 (SERPINE1), mannan-binding lectin serine protease 1 (MASP1), and platelet-derived growth factor receptor A (PDGFRA)] (**Table 25**). The four GH family members showed a more coordinated negative association profile with these inflammatory proteins after supplementation with the four-fiber blend compared to supplementation with the other two snack prototypes (**FIG. 33C-D**).

Table 25: Annotated KEGG orthologies of top correlated discriminatory plasma proteins with top discriminatory CAZymes.

(A) Study 1: pea fiber snack food prototype.			
Protein name	Entrez Gene ID	KEGG-associated function	KEGG Orthology (KO)
SLPI (Antileukoproteinase)	6590	Proteolysis	01002 Peptidases and inhibitors
IL2 (Interleukin-2)	3558	Lectins, Intestinal immune network, Adaptive immune response	04625 C-type lectin receptor signaling pathway
			04660 T cell receptor signaling pathway
			04658 Th1 and Th2 cell differentiation
			04659 Th17 cell differentiation
			04672 Intestinal immune network for IgA production
MFGE8 (Lactadherin)	4240	Signal transduction	04147 Exosome
TYMS (Thymidylate synthase)	7298	Nucleotide metabolism, Metabolism of cofactors and vitamins	00240 Pyrimidine metabolism
			00670 One carbon pool by folate
KPNA2 (Importin subunit alpha-1)	3838	Genetic information processing	03036 Chromosome and associated proteins
S100A7 (Protein S100-A7)	6278	Acute and chronic inflammation	04657 IL-17 signaling pathway
TNFRSF12A (Tumor necrosis	51330	Acute and chronic	04050 Cytokine receptors

factor receptor superfamily member 12A)		inflammation	
SPP1 (Osteopontin)	6696	Signal transduction, Acute and chronic inflammation, Innate immune response	04371 Apelin signaling pathway
			04151 PI3K-Akt signaling pathway
			04512 ECM-receptor interaction
			04510 Focal adhesion
			04620 Toll-like receptor signaling pathway
			04929 GnRH secretion
JAG1 (Protein jagged-1)	182	Signal transduction, Acute and chronic inflammation, Adaptive immune response	04330 Notch signaling pathway
			04371 Apelin signaling pathway
			04668 TNF signaling pathway
			04658 Th1 and Th2 cell differentiation
ENG (Endoglin)	2022	Signal transduction	09183 Signaling and cellular processes
F2 (Thrombin)	2147	Signal transduction, Platelet activation and blood coagulation, Acute and chronic inflammation	04072 Phospholipase D signaling pathway
			04080 Neuroactive ligand-receptor interaction
			04810 Regulation of actin cytoskeleton
			04610 Complement and coagulation cascades
			04611 Platelet activation
CST2 (Cystatin-SA)	1470	Salivary secretion	04970 Salivary secretion
CCL23 (Ck-beta-8-1)	6368	Signal transduction, Acute and chronic inflammation	04060 Cytokine-cytokine receptor interaction
			04061 Viral protein interaction with cytokine and cytokine receptor
			04062 Chemokine signaling pathway
			04052 Cytokines and growth factors
APOM (Apolipoprotein M)	55937	—	—
PLA2G1B (Phospholipase A2)	5319	Carbon and glycan biosynthesis metabolism, Signal transduction, Blood circulation, Fat digestion	00564 Glycerophospholipid metabolism
			00565 Ether lipid metabolism
			00590 Arachidonic acid metabolism

		and absorption	00591 Linoleic acid metabolism
			00592 alpha-Linolenic acid metabolism
			04014 Ras signaling pathway
			04270 Vascular smooth muscle contraction
			04972 Pancreatic secretion
			04975 Fat digestion and absorption
MB (Myoglobin)	4151	Signal transduction	02000 Transporters
CXCL13 (C-X-C motif chemokine 13)	10563	Acute and chronic inflammation	04060 Cytokine-cytokine receptor interaction
			04061 Viral protein interaction with cytokine and cytokine receptor
			04062 Chemokine signaling pathway
			04052 Cytokines and growth factors
IGFBP1 (Insulin-like growth factor-binding protein 1)	3484	Genetic information processing	04131 Membrane trafficking
KLK5 (Kallikrein-5)	25818	Proteolysis	01002 Peptidases and inhibitors
PIGR (Polymeric immunoglobulin receptor)	5284	Intestinal immune network	04672 Intestinal immune network for IgA production
GPD1 (Glycerol-3-phosphate dehydrogenase [NAD(+)], cytoplasmic)	2819	Fat digestion and absorption	00564 Glycerophospholipid metabolism
FABP1 (Fatty acid-binding protein, liver)	2168	Fat digestion and absorption, Signal transduction	03320 PPAR signaling pathway
			04975 Fat digestion and absorption
EPHA1 (Ephrin type-A receptor 1)	2041	Development and regeneration	04360 Axon guidance
			01001 Protein kinases
CST1 (Cystatin-SN)	1469	Salivary secretion, Proteolysis	04970 Salivary secretion
			01002 Peptidases and inhibitors
PLA2G2A (Phospholipase A2, membrane associated)	5320	Carbon and glycan biosynthesis metabolism, Fat digestion and absorption, Signal transduction, Genetic information processing, Blood circulation	00564 Glycerophospholipid metabolism
			00565 Ether lipid metabolism
			00590 Arachidonic acid metabolism
			00591 Linoleic acid metabolism
			00592 alpha-Linolenic acid metabolism

			04014 Ras signaling pathway
			04270 Vascular smooth muscle contraction
			04972 Pancreatic secretion
			04975 Fat digestion and absorption
			03036 Chromosome and associated proteins
LTA LTB (Lymphotoxin alpha1:beta2)	4049, 4050	Acute and chronic inflammation, Signal transduction	04064 NF-kappa B signaling pathway
			04668 TNF signaling pathway
			04060 Cytokine-cytokine receptor interaction
			04061 Viral protein interaction with cytokine and cytokine receptor
			04052 Cytokines and growth factors
TNFRSF18 (Tumor necrosis factor receptor superfamily member 18)	8784	Signal transduction, Acute and chronic inflammation	04060 Cytokine-cytokine receptor interaction
			04050 Cytokine receptors
			04090 CD molecules
TGM3 (Protein-glutamine gamma-glutamyltransferase E)	7053	—	09191 Unclassified: metabolism

(B) Study 2: two-fiber snack food prototype.			
Protein name	Entrez Gene ID	KEGG-associated function	KEGG Orthology (KO)
TGFBI (Transforming growth factor-beta-induced protein ig-h3)	7045	Signal transduction	99995 Signaling proteins
PLAU (Urokinase-type plasminogen activator)	5328	Signal transduction, Platelet activation and blood coagulation, Acute and chronic inflammation, Proteolysis	04064 NF-kappa B signaling pathway
			04610 Complement and coagulation cascades
			01002 Peptidases and inhibitors
			00536 Glycosaminoglycan binding proteins
CCL23 (Ck-beta-8-1)	6368	Signal transduction, Acute and chronic inflammation	04060 Cytokine-cytokine receptor interaction
			04061 Viral protein interaction with

			cytokine and cytokine receptor
			04062 Chemokine signaling pathway
			04052 Cytokines and growth factors
SLAMF7 (SLAM family member 7)	57823	Signal transduction	04090 CD molecules
ERAP1 (Endoplasmic reticulum aminopeptidase 1)	51752	Proteolysis	01002 Peptidases and inhibitors
FGF19 (Fibroblast growth factor 19)	9965	Signal transduction	04014 Ras signaling pathway
			04015 Rap1 signaling pathway
			04010 MAPK signaling pathway
			04151 PI3K-Akt signaling pathway
			04810 Regulation of actin cytoskeleton
			04052 Cytokines and growth factors
			00536 Glycosaminoglycan binding proteins
PTH (Parathyroid hormone)	5741	Signal transduction	04080 Neuroactive ligand-receptor interaction
			04928 Parathyroid hormone synthesis, secretion and action
			04961 Endocrine and other factor-regulated calcium reabsorption
THBS2 (Thrombospondin-2)	7058	Signal transduction	04151 PI3K-Akt signaling pathway
			04512 ECM-receptor interaction
			04145 Phagosome
			04510 Focal adhesion
			04131 Membrane trafficking
			00536 Glycosaminoglycan binding proteins
S100A7 (Protein S100-A7)	6278	Acute and chronic inflammation	04657 IL-17 signaling pathway
SPINT1 (Kunitz-type protease inhibitor 1)	6692	Proteolysis	01002 Peptidases and inhibitors
EPO (Erythropoietin)	2056	Signal transduction, Anti-inflammatory	04630 Jak-STAT signaling pathway
			04066 HIF-1 signaling pathway
			04151 PI3K-Akt signaling pathway

			04060 Cytokine-cytokine receptor interaction
			04640 Hematopoietic cell lineage
			04052 Cytokines and growth factors
IL18BP (Interleukin-18-binding protein)	10068	Anti-inflammatory	—
Human-virus (gp41 C34 peptide, HIV)	—	—	—
PCSK7 (Proprotein convertase subtilisin/kexin type 7)	9159	Proteolysis	01002 Peptidases and inhibitors
			03110 Chaperones and folding catalysts
ISLR2 (Immunoglobulin superfamily containing leucine-rich repeat protein 2)	57611	—	—
BST1 (ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase 2)	683	Metabolism of cofactors and vitamins, Salivary secretion, Signal transduction	00760 Nicotinate and nicotinamide metabolism
			04970 Salivary secretion
			04972 Pancreatic secretion
			04090 CD molecules
			00537 Glycosylphosphatidylinositol
EPHB2 (Ephrin type-B receptor 2)	2048	Development and regeneration	04360 Axon guidance
			01001 Protein kinases
LRP8 (Low-density lipoprotein receptor-related protein 8)	7804	Genetic information processing	04131 Membrane trafficking
KIT (Mast/stem cell growth factor receptor Kit)	3815	Signal transduction	04014 Ras signaling pathway
			04015 Rap1 signaling pathway
			04010 MAPK signaling pathway
			04072 Phospholipase D signaling pathway
			04151 PI3K-Akt signaling pathway
			04640 Hematopoietic cell lineage
			04916 Melanogenesis
HSPA1A (Heat shock 70 kDa protein 1A)	3303	Genetic information processing, Signal transduction, Proteolysis	03040 Spliceosome
			04141 Protein processing in endoplasmic reticulum

			04010 MAPK signaling pathway
			04144 Endocytosis
			04612 Antigen processing and presentation
			04915 Estrogen signaling pathway
			04213 Longevity regulating pathway - multiple species
			01009 Protein phosphatases and associated proteins
			03009 Ribosome biogenesis
			03110 Chaperones and folding catalysts
			04131 Membrane trafficking
			03051 Proteasome
			03029 Mitochondrial biogenesis
			04147 Exosome
KLK8 (Kallikrein-8)	11202	Proteolysis	01002 Peptidases and inhibitors
SFRP1 (Secreted frizzled-related protein 1)	6422	Signal transduction, Development and regeneration	04310 Wnt signaling pathway
TPSG1 (Tryptase gamma)	25823	Proteolysis	01002 Peptidases and inhibitors
NRCAM (Neuronal cell adhesion molecule)	4897	Signal transduction	04514 Cell adhesion molecules (CAMs)
IL18R1 (Interleukin-18 receptor 1)	8809	Signal transduction, Acute and chronic inflammation	04668 TNF signaling pathway
			04060 Cytokine-cytokine receptor interaction
			04061 Viral protein interaction with cytokine and cytokine receptor
			04050 Cytokine receptors
			04090 CD molecules
SHH (Sonic hedgehog protein)	6469	Signal transduction, Development and regeneration, Proteolysis	04340 Hedgehog signaling pathway
			04360 Axon guidance
			01002 Peptidases and inhibitors
			00536 Glycosaminoglycan binding proteins

ANGPT2 (Angiopoietin-2)	285	Signal transduction	04014 Ras signaling pathway
			04015 Rap1 signaling pathway
			04010 MAPK signaling pathway
			04066 HIF-1 signaling pathway
			04151 PI3K-Akt signaling pathway
FLRT2 (Leucine-rich repeat transmembrane protein FLRT2)	23768	Signal transduction	99995 Signaling proteins
CD36 (Platelet glycoprotein 4)	948	Signal transduction, Development and regeneration, Fat digestion and absorption	04152 AMPK signaling pathway
			04512 ECM-receptor interaction
			04145 Phagosome
			04640 Hematopoietic cell lineage
			04920 Adipocytokine signaling pathway
			03320 PPAR signaling pathway
			04975 Fat digestion and absorption
			04979 Cholesterol metabolism
			04131 Membrane trafficking
			04147 Exosome
			04090 CD molecules
MMP12 (Macrophage metalloelastase)	4321	Proteolysis	01002 Peptidases and inhibitors
EDA (Ectodysplasin-A, secreted form)	1896	Signal transduction	04064 NF-kappa B signaling pathway
			04060 Cytokine-cytokine receptor interaction
			04052 Cytokines and growth factors
PYY (Peptide YY)	5697	Feeding behavior, Signal transduction	04080 Neuroactive ligand-receptor interaction
LRP1 (Low-density lipoprotein receptor-related protein 1, soluble)	4035	Fat digestion and absorption	04979 Cholesterol metabolism
			04131 Membrane trafficking
			04090 CD molecules
LRRTM1 (Leucine-rich repeat transmembrane neuronal protein 1)	347730	Signal transduction	99995 Signaling proteins

LIFR (Leukemia inhibitory factor receptor)	3977	Signal transduction, Anti-inflammatory	04630 Jak-STAT signaling pathway
			04060 Cytokine-cytokine receptor interaction
			04550 Signaling pathways regulating pluripotency of stem cells
			04050 Cytokine receptors
			04090 CD molecules
PDGFRA (Platelet-derived growth factor receptor alpha)	5156	Signal transduction, Platelet activation and blood coagulation	04014 Ras signaling pathway
			04015 Rap1 signaling pathway
			04010 MAPK signaling pathway
			04630 Jak-STAT signaling pathway
			04020 Calcium signaling pathway
			04072 Phospholipase D signaling pathway
			04151 PI3K-Akt signaling pathway
			04144 Endocytosis
			04510 Focal adhesion
			04540 Gap junction
			04810 Regulation of actin cytoskeleton
			01001 Protein kinases
			04090 CD molecules
TNFSF13B (Tumor necrosis factor ligand superfamily member 13B)	10673	Signal transduction, Intestinal immune network	04064 NF-kappa B signaling pathway
			04060 Cytokine-cytokine receptor interaction
			04672 Intestinal immune network for IgA production
			04052 Cytokines and growth factors
			04090 CD molecules
INSR (Insulin receptor)	3643	Signal transduction, Insulin signaling pathway, Fat digestion and absorption	04014 Ras signaling pathway
			04015 Rap1 signaling pathway
			04010 MAPK signaling pathway
			04066 HIF-1 signaling pathway

			04068 FoxO signaling pathway
			04072 Phospholipase D signaling pathway
			04022 cGMP-PKG signaling pathway
			04151 PI3K-Akt signaling pathway
			04152 AMPK signaling pathway
			04150 mTOR signaling pathway
			04520 Adherens junction
			04910 Insulin signaling pathway
			04923 Regulation of lipolysis in adipocytes
			04913 Ovarian steroidogenesis
			04960 Aldosterone-regulated sodium reabsorption
			04211 Longevity regulating pathway
			04213 Longevity regulating pathway - multiple species
			01001 Protein kinases
			04090 CD molecules
TEK (Angiopoietin-1 receptor, soluble)	7010	Signal transduction	04014 Ras signaling pathway
			04015 Rap1 signaling pathway
			04010 MAPK signaling pathway
			04066 HIF-1 signaling pathway
			04151 PI3K-Akt signaling pathway
			01001 Protein kinases
			04090 CD molecules
LRIG3 (Leucine-rich repeats and immunoglobulin-like domains protein 3)	121227	—	—
WFIKKN2 (WAP, Kazal, immunoglobulin, Kunitz and NTR domain-containing protein 2)	124857	Proteolysis	01002 Peptidases and inhibitors
TFF1 (Trefoil factor 1)	7031	Signal transduction	04915 Estrogen signaling pathway

MDH1 (Malate dehydrogenase, cytoplasmic)	4190	Carbon and glycan biosynthesis metabolism	00020 Citrate cycle (TCA cycle)
			00620 Pyruvate metabolism
			00630 Glyoxylate and dicarboxylate metabolism
			00270 Cysteine and methionine metabolism
			04964 Proximal tubule bicarbonate reclamation
SFTPD (Pulmonary surfactant-associated protein D)	6441	Genetic information processing, Lectins	04145 Phagosome
			04131 Membrane trafficking
			04091 Lectins
BCL2 (Apoptosis regulator Bcl-2)	596	Signal transduction, Genetic information processing, Proteolysis, AGE-RAGE signaling pathway in diabetic complications	04141 Protein processing in endoplasmic reticulum
			04340 Hedgehog signaling pathway
			04630 Jak-STAT signaling pathway
			04064 NF-kappa B signaling pathway
			04066 HIF-1 signaling pathway
			04071 Sphingolipid signaling pathway
			04151 PI3K-Akt signaling pathway
			04140 Autophagy - animal
			04210 Apoptosis
			04217 Necroptosis
			04115 p53 signaling pathway
			04510 Focal adhesion
			04621 NOD-like receptor signaling pathway
			04915 Estrogen signaling pathway
			04928 Parathyroid hormone synthesis, secretion and action
			04261 Adrenergic signaling in cardiomyocytes
			04725 Cholinergic synapse
			04722 Neurotrophin signaling pathway

			01009 Protein phosphatases and associated proteins
ICOS (Inducible T-cell costimulator)	29851	Adaptive immune response, Intestinal immune network	04514 Cell adhesion molecules (CAMs)
			04660 T cell receptor signaling pathway
			04672 Intestinal immune network for IgA production
			04090 CD molecules
HIST1H1C (Histone H1.2)	3006	Genetic information processing	03036 Chromosome and associated proteins
SERPINE2 (Glia-derived nexin)	5270	Proteolysis	01002 Peptidases and inhibitors
			00536 Glycosaminoglycan binding proteins
IL16 (Interleukin-16)	3603	Acute and chronic inflammation	04060 Cytokine-cytokine receptor interaction
			04052 Cytokines and growth factors
KIR2DL4 (Killer cell immunoglobulin-like receptor 2DL4)	3805	Innate immune response	04218 Cellular senescence
			04650 Natural killer cell mediated cytotoxicity
			04612 Antigen processing and presentation
			04090 CD molecules
CXCL3 CXCL2 (Gro-beta/gamma)	2921	Acute and chronic inflammation	04064 NF-kappa B signaling pathway
			04668 TNF signaling pathway
			04060 Cytokine-cytokine receptor interaction
			04061 Viral protein interaction with cytokine and cytokine receptor
			04621 NOD-like receptor signaling pathway
			04657 IL-17 signaling pathway
			04062 Chemokine signaling pathway
			04052 Cytokines and growth factors
PRDX1 (Peroxiredoxin-1)	5052	Transport and catabolism, Signal transduction,	04146 Peroxisome
			04147 Exosome
CMA1 (Chymase)	1215	Blood circulation, Proteolysis	04614 Renin-angiotensin system

			01002 Peptidases and inhibitors
CCL13 (C-C motif chemokine 13)	6357	Acute and chronic inflammation	04064 NF-kappa B signaling pathway
			04060 Cytokine-cytokine receptor interaction
			04061 Viral protein interaction with cytokine and cytokine receptor
			04062 Chemokine signaling pathway
			04052 Cytokines and growth factors
			00536 Glycosaminoglycan binding proteins
RSPO2 (R-spondin-2)	340419	Signal transduction, Development and regeneration	04310 Wnt signaling pathway
RPS3A (40S ribosomal protein S3a)	6189	Genetic information processing	03010 Ribosome
HNRNPA2B1 (Heterogeneous nuclear ribonucleoproteins A2/B1)	3181	Genetic information processing, Signal transduction	03019 Messenger RNA biogenesis
			03041 Spliceosome
			04147 Exosome
BPI (Bactericidal permeability-increasing protein)	671	—	—
EPB41 (Protein 4.1)	2035	Signal transduction	04812 Cytoskeleton proteins
TBP (TATA-box-binding protein)	6908	Genetic information processing	03022 Basal transcription factors
RBM39 (RNA-binding protein 39)	9584	Genetic information processing	03041 Spliceosome
BSG (Basigin)	682	Signal transduction	04147 Exosome
			04090 CD molecules
SOD1 (Superoxide dismutase [Cu-Zn])	6647	Transport and catabolism	04146 Peroxisome
ACVR1B (Activin receptor type-1B)	91	Aging	04213 Longevity regulating pathway - multiple species
S100A9 (Protein S100-A9)	6280	Acute and chronic inflammation	04657 IL-17 signaling pathway
SH2D1A (SH2 domain-containing protein 1A)	4068	Innate immune response	04650 Natural killer cell mediated cytotoxicity
SMPDL3A (Acid sphingomyelinase-like phosphodiesterase 3a)	10924	—	09191 Unclassified: metabolism

(C) Study 2: four-fiber snack food prototype.			
Protein name	Entrez Gene ID	KEGG-associated function	KEGG Orthology (KO)
CCL3 (C-C motif chemokine 3)	6348	Acute and chronic inflammation	04060 Cytokine-cytokine receptor interaction
			04061 Viral protein interaction with cytokine and cytokine receptor
			04620 Toll-like receptor signaling pathway
			04062 Chemokine signaling pathway
			04052 Cytokines and growth factors
			00536 Glycosaminoglycan binding proteins
C3 (C3a anaphylatoxin)	718	Innate immune response, Platelet activation and blood coagulation	04080 Neuroactive ligand-receptor interaction
			04145 Phagosome
			04610 Complement and coagulation cascades
			01002 Peptidases and inhibitors
			04131 Membrane trafficking
			04147 Exosome
IL2 (Interleukin-2)	3558	Signal transduction, Anti-inflammatory, Intestinal immune network, Adaptive immune response	04625 C-type lectin receptor signaling pathway
			04660 T cell receptor signaling pathway
			04658 Th1 and Th2 cell differentiation
			04659 Th17 cell differentiation
			04672 Intestinal immune network for IgA production
HIST2H2BE (Histone H2B type 2-E)	8349	Genetic information processing	03036 Chromosome and associated proteins
			04147 Exosome
PTH (Parathyroid hormone)	5741	Signal transduction	04080 Neuroactive ligand-receptor interaction
			04928 Parathyroid hormone synthesis, secretion and action
			04961 Endocrine and other factor-regulated calcium reabsorption

PIGR (Polymeric immunoglobulin receptor)	5284	Intestinal immune network	04672 Intestinal immune network for IgA production
EPO (Erythropoietin)	2056	Signal transduction, Anti-inflammatory	04630 Jak-STAT signaling pathway
			04066 HIF-1 signaling pathway
			04151 PI3K-Akt signaling pathway
			04060 Cytokine-cytokine receptor interaction
			04640 Hematopoietic cell lineage
			04052 Cytokines and growth factors
CRP (C-reactive protein)	1401	AGE-RAGE signaling pathway in diabetic complications, Acute and chronic inflammation	09193 Unclassified: signaling and cellular processes
CST2 (Cystatin-SA)	1470	Salivary secretion	04970 Salivary secretion
INHBA (Inhibin beta A chain)	3624	Signal transduction	04350 TGF-beta signaling pathway
			04060 Cytokine-cytokine receptor interaction
			04550 Signaling pathways regulating pluripotency of stem cells
			04052 Cytokines and growth factors
SPP1 (Osteopontin)	6696	Signal transduction, Acute and chronic inflammation, Innate immune response	04371 Apelin signaling pathway
			04151 PI3K-Akt signaling pathway
			04512 ECM-receptor interaction
			04510 Focal adhesion
			04620 Toll-like receptor signaling pathway
			04929 GnRH secretion
FUT5 (Alpha-(1,3)-fucosyltransferase 5)	2527	Carbon and glycan biosynthesis metabolism	00601 Glycosphingolipid biosynthesis - lacto and neolacto series
			01003 Glycosyltransferases
KLK11 (Kallikrein-11)	11012	Proteolysis	01002 Peptidases and inhibitors
FCN1 (Ficolin-1)	2219	Lectins	04091 Lectins
C1R (Complement C1r subcomponent)	715	Platelet activation and blood coagulation, Proteolysis	04145 Phagosome
			04610 Complement and coagulation cascades

			01002 Peptidases and inhibitors
			04131 Membrane trafficking
MFGE8 (Lactadherin)	4240	Signal transduction	04147 Exosome
PIANP (PILR alpha-associated neural protein)	196500	—	—
C4A C4B (Complement C4b)	720, 721	Platelet activation and blood coagulation, Proteolysis	04610 Complement and coagulation cascades
			01002 Peptidases and inhibitors
			04147 Exosome
F2 (Thrombin)	2147	Signal transduction, Platelet activation and blood coagulation, Acute and chronic inflammation	04072 Phospholipase D signaling pathway
			04080 Neuroactive ligand-receptor interaction
			04810 Regulation of actin cytoskeleton
			04610 Complement and coagulation cascades
			04611 Platelet activation
TGM3 (Protein-glutamine gamma-glutamyltransferase E)	7053	—	09191 Unclassified: metabolism
F3 (Tissue Factor)	2152	Platelet activation and blood coagulation, Acute and chronic inflammation	04610 Complement and coagulation cascades
			04090 CD molecules
			00537 Glycosylphosphatidylinositol (GPI)-anchored proteins
IL5RA (Interleukin-5 receptor subunit alpha)	3568	Signal transduction, Innate immune response	04630 Jak-STAT signaling pathway
			04060 Cytokine-cytokine receptor interaction
			04640 Hematopoietic cell lineage
			04050 Cytokine receptors
			04090 CD molecules
ENPP7 (Ectonucleotide pyrophosphatase/phosphodiesterase family member 7)	339221	Carbon and glycan biosynthesis metabolism	00600 Sphingolipid metabolism
IBSP (Bone sialoprotein 2)	3381	Signal transduction	04151 PI3K-Akt signaling pathway
			04512 ECM-receptor interaction
			04510 Focal adhesion

TFF2 (Trefoil factor 2)	7032	--	99992 Structural proteins
VEGFA (Vascular endothelial growth factor A)	7422	Signal transduction, Acute and chronic inflammation	04014 Ras signaling pathway
			04015 Rap1 signaling pathway
			04010 MAPK signaling pathway
			04370 VEGF signaling pathway
			04066 HIF-1 signaling pathway
			04151 PI3K-Akt signaling pathway
			04510 Focal adhesion
			04926 Relaxin signaling pathway
			04052 Cytokines and growth factors
			00536 Glycosaminoglycan binding proteins
SLITRK5 (SLIT and NTRK-like protein 5)	26050	--	—
LAG3 (Lymphocyte activation gene 3 protein)	3902	Signal transduction	04090 CD molecules
PDGFRB (Platelet-derived growth factor receptor beta)	5159	Signal transduction, Acute and chronic inflammation	04014 Ras signaling pathway
			04015 Rap1 signaling pathway
			04010 MAPK signaling pathway
			04630 Jak-STAT signaling pathway
			04020 Calcium signaling pathway
			04072 Phospholipase D signaling pathway
			04151 PI3K-Akt signaling pathway
			04510 Focal adhesion
			04540 Gap junction
			04810 Regulation of actin cytoskeleton
			01001 Protein kinases
			04090 CD molecules
IDS (Iduronate 2-sulfatase)	3423	Carbon and glycan biosynthesis metabolism	00531 Glycosaminoglycan degradation
			04142 Lysosome

CNTFR (Ciliary neurotrophic factor receptor subunit alpha)	1271	Signal transduction	04630 Jak-STAT signaling pathway
			04060 Cytokine-cytokine receptor interaction
			04050 Cytokine receptors
			00537 Glycosylphosphatidylinositol (GPI)-anchored proteins
NRCAM (Neuronal cell adhesion molecule)	4897	Signal transduction	04514 Cell adhesion molecules (CAMs)
LPO (Lactoperoxidase)	4025	Salivary secretion	04970 Salivary secretion
LRP1 (Low-density lipoprotein receptor-related protein 1, soluble)	4035	Fat digestion and absorption	04979 Cholesterol metabolism
			04131 Membrane trafficking
			04090 CD molecules
AGRP (Agouti-related protein)	181	Feeding behavior, Signal transduction	04920 Adipocytokine signaling pathway
GCG (Glucagon)	2641	Feeding behavior, Glucagon signaling pathway	04024 cAMP signaling pathway
			04080 Neuroactive ligand-receptor interaction
			04911 Insulin secretion
			04922 Glucagon signaling pathway
			04714 Thermogenesis
BST1 (ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase 2)	683	Metabolism of cofactors and vitamins, Salivary secretion, Signal transduction	00760 Nicotinate and nicotinamide metabolism
			04970 Salivary secretion
			04972 Pancreatic secretion
			04090 CD molecules
			00537 Glycosylphosphatidylinositol
EFNA5 (Ephrin-A5)	1946	Signal transduction, Development and regeneration, Acute and chronic inflammation	04014 Ras signaling pathway
			04015 Rap1 signaling pathway
			04010 MAPK signaling pathway
			04151 PI3K-Akt signaling pathway
			04360 Axon guidance
			04052 Cytokines and growth factors
			00536 Glycosaminoglycan binding

			proteins
NRP1 (Neuropilin-1)	8829	Development and regeneration, Signal transduction	04360 Axon guidance
			04090 CD molecules
EPHA1 (Ephrin type-A receptor 1)	2041	Development and regeneration, Acute and chronic inflammation	04360 Axon guidance
			01001 Protein kinases
HIST1H1C (Histone H1.2)	3006	Genetic information processing	03036 Chromosome and associated proteins
CTLA4 (Cytotoxic T-lymphocyte protein 4)	1493	Signal transduction, Adaptive immune response	04514 Cell adhesion molecules (CAMs)
			04660 T cell receptor signaling pathway
			04090 CD molecules
DCTPP1 (dCTP pyrophosphatase 1)	79077	Nucleotide metabolism	00240 Pyrimidine metabolism
EPHA2 (Ephrin type-A receptor 2)	1969	Signal transduction, Development and regeneration, Acute and chronic inflammation	04014 Ras signaling pathway
			04015 Rap1 signaling pathway
			04010 MAPK signaling pathway
			04151 PI3K-Akt signaling pathway
			04360 Axon guidance
			01001 Protein kinases
SERPINE1 (Plasminogen activator inhibitor 1)	5054	Signal transduction, Platelet activation and blood coagulation, Proteolysis	04390 Hippo signaling pathway
			04371 Apelin signaling pathway
			04066 HIF-1 signaling pathway
			04115 p53 signaling pathway
			04218 Cellular senescence
			04610 Complement and coagulation cascades
			01002 Peptidases and inhibitors
			04147 Exosome
			00536 Glycosaminoglycan binding proteins
MASP1 (Mannan-binding lectin serine protease 1)	5648	Platelet activation and blood coagulation, Proteolysis	04610 Complement and coagulation cascades

			01002 Peptidases and inhibitors
NAAA (N-acylethanolamine-hydrolyzing acid amidase)	27163	Proteolysis	01002 Peptidases and inhibitors
PDGFRA (Platelet-derived growth factor receptor alpha)	5156	Signal transduction, Platelet activation and blood coagulation	04014 Ras signaling pathway
			04015 Rap1 signaling pathway
			04010 MAPK signaling pathway
			04630 Jak-STAT signaling pathway
			04020 Calcium signaling pathway
			04072 Phospholipase D signaling pathway
			04151 PI3K-Akt signaling pathway
			04144 Endocytosis
			04510 Focal adhesion
			04540 Gap junction
			04810 Regulation of actin cytoskeleton
			01001 Protein kinases
			04090 CD molecules
CD200 (OX-2 membrane glycoprotein)	4345	Signal transduction	04090 CD molecules
DCN (Decorin)	1634	Signal transduction	04350 TGF-beta signaling pathway
			00535 Proteoglycans
IL1R1 (Interleukin-1 receptor type 1)	3554	Signal transduction, Platelet activation and blood coagulation, Acute and chronic inflammation	04010 MAPK signaling pathway
			04064 NF-kappa B signaling pathway
			04060 Cytokine-cytokine receptor interaction
			04640 Hematopoietic cell lineage
			04659 Th17 cell differentiation
			04750 Inflammatory mediator regulation of TRP channels
			04380 Osteoclast differentiation
			04050 Cytokine receptors
			04090 CD molecules

ENO2 (Gamma-enolase)	2026	Carbon and glycan biosynthesis metabolism	00010 Glycolysis / Gluconeogenesis
			03018 RNA degradation
			04066 HIF-1 signaling pathway
			03019 Messenger RNA biogenesis
			04147 Exosome
CTSH (Cathepsin H)	1512	Proteolysis	04142 Lysosome
			04210 Apoptosis
			01002 Peptidases and inhibitors
ESD (S-formylglutathione hydrolase)	2098	Carbon and glycan biosynthesis metabolism	hsa01200 Carbon metabolism
RPS27A (Ubiquitin+1, truncated mutation for UbB)	6233	Genetic information processing	03010 Ribosome
			04147 Exosome
UBE2N (Ubiquitin-conjugating enzyme E2 N)	7334	Proteolysis	04120 Ubiquitin mediated proteolysis
			04121 Ubiquitin system
			03400 DNA repair and recombination proteins
GAPDH (Glyceraldehyde-3-phosphate dehydrogenase)	2597	Carbon and glycan biosynthesis metabolism	00010 Glycolysis / Gluconeogenesis
			04066 HIF-1 signaling pathway
			04131 Membrane trafficking
			04147 Exosome
MMP8 (Neutrophil collagenase)	4317	Proteolysis	01002 Peptidases and inhibitors
IFNL1 (Interferon lambda-1)	282618	Signal transduction	04630 Jak-STAT signaling pathway
			04060 Cytokine-cytokine receptor interaction
			04052 Cytokines and growth factors
KIF23 (Kinesin-like protein KIF23)	9493	Genetic information processing	04131 Membrane trafficking
			03036 Chromosome and associated proteins
			04812 Cytoskeleton proteins
IL3RA (Interleukin-3 receptor subunit alpha)	3563	Signal transduction, Development and regeneration	04630 Jak-STAT signaling pathway
			04151 PI3K-Akt signaling pathway

			04060 Cytokine-cytokine receptor interaction
			04210 Apoptosis
			04640 Hematopoietic cell lineage
			04050 Cytokine receptors
			04090 CD molecules
VIP (Vasoactive Intestinal Peptide)	7432	Signal transduction, Feeding behavior	04024 cAMP signaling pathway
			04080 Neuroactive ligand-receptor interaction
FLRT1 (Leucine-rich repeat transmembrane protein FLRT1)	23769	Signal transduction	99995 Signaling proteins
DNAJC19 (Mitochondrial import inner membrane translocase subunit TIM14)	131118	Genetic information processing	03110 Chaperones and folding catalysts
			03029 Mitochondrial biogenesis
FGF8 (Fibroblast growth factor 8 isoform A)	2253	Signal transduction	04014 Ras signaling pathway
			04015 Rap1 signaling pathway
			04010 MAPK signaling pathway
			04151 PI3K-Akt signaling pathway
			04810 Regulation of actin cytoskeleton
			04052 Cytokines and growth factors
			00536 Glycosaminoglycan binding proteins
PRKCI (Protein kinase C iota type)	5584	Signal transduction, Platelet activation and blood coagulation, Insulin signaling pathway	04015 Rap1 signaling pathway
			04390 Hippo signaling pathway
			04144 Endocytosis
			04530 Tight junction
			04611 Platelet activation
			04910 Insulin signaling pathway
			01001 Protein kinases
PSMA6 (Proteasome subunit alpha type-6)	5687	Proteolysis	03050 Proteasome
			01002 Peptidases and inhibitors

HDGFRP2 (Hepatoma-derived growth factor-related protein 2)	84717	—	—
RPS6KA5 (Ribosomal protein S6 kinase alpha-5)	9252	Signal transduction	04010 MAPK signaling pathway
			04668 TNF signaling pathway
			04261 Adrenergic signaling in cardiomyocytes
			04722 Neurotrophin signaling pathway
			04713 Circadian entrainment
			01001 Protein kinases
FGF6 (Fibroblast growth factor 6)	2251	Signal transduction	04014 Ras signaling pathway
			04015 Rap1 signaling pathway
			04010 MAPK signaling pathway
			04151 PI3K-Akt signaling pathway
			04810 Regulation of actin cytoskeleton
			04052 Cytokines and growth factors
			00536 Glycosaminoglycan binding proteins
CAMKK1 (Calcium/calmodulin-dependent protein kinase kinase 1)	84254	Signal transduction	05034 Alcoholism
			01001 Protein kinases
CRTAM (Cytotoxic and regulatory T-cell molecule)	56253	Signal transduction	04090 CD molecules
ULBP3 (NKG2D ligand 3)	79465	Innate immune response	04650 Natural killer cell mediated cytotoxicity
DHH (Desert hedgehog protein N-product)	50846	Signal transduction, Proteolysis, Development and regeneration	04340 Hedgehog signaling pathway
			01002 Peptidases and inhibitors
LGALS4 (Galectin-4)	3960	Lectins	04091 Lectins
RAN (GTP-binding nuclear protein Ran)	5901	Genetic information processing	03013 RNA transport
			03008 Ribosome biogenesis in eukaryotes
			03019 Messenger RNA biogenesis
			03009 Ribosome biogenesis
			03016 Transfer RNA biogenesis

			03036 Chromosome and associated proteins
			04147 Exosome
			04031 GTP-binding proteins
ACE2 (Angiotensin-converting enzyme 2)	59272	Proteolysis	04614 Renin-angiotensin system
			04974 Protein digestion and absorption
			01002 Peptidases and inhibitors [BR:hsa01002]
			04147 Exosome
AK1 (Adenylate kinase isoenzyme 1)	203	Nucleotide metabolism	00230 Purine metabolism
			00730 Thiamine metabolism
			04147 Exosome
MBD4 (Methyl-CpG-binding domain protein 4)	8930	Genetic information processing	03410 Base excision repair
			03036 Chromosome and associated proteins
			03400 DNA repair and recombination proteins
S100A6 (Protein S100-A6)	6277	—	—
FCRL3 (Fc receptor-like protein 3)	115352	Signal transduction	04090 CD molecules

Table 26: Monosaccharide and glycosidic linkage composition of fiber preparations

(A) Monosaccharide content (%/Σ sugars)

Purified fiber preparation	Monosaccharide content (%/Σ sugars)							
	Glc	GalA	Ara	Xyl	Gal	Man	Rha	Fuc
Pea fiber	51.5	13.9	22.4	6.2	3.3	0.6	1.9	0.1
Orange fiber (coarse)	26.4	42.9	13.9	3.5	6.5	4.1	2.3	0.4
Barley bran	80.6	0	7.1	9.9	0.7	1.2	0.5	0
Pea fiber (extruded)	60.1	11.9	18.3	4.4	3.0	0.8	1.5	0
Orange fiber (extruded)	40.7	35.4	9.8	3.0	4.8	3.9	2.1	0.3
Abbreviations: glucose (Glc), galacturonic acid (GalA), arabinose (Ara), xylose (Xyl), galactose (Gal), mannose (Man), rhamnose (Rha), fucose (Fuc)								

(B) Deduced glycosyl-linkage (%/Σ linked-sugars)

	Pea fiber	Orange fiber (coarse)	Barley bran	Pea fiber (extruded)	Orange fiber (extruded
T-Glc(p)	1.3	0	3.0	2.6	1.5
3-Glc(p)	0	0	5.2	0	0
4-Glc(p)	46.5	13.0	71.3	51.7	49.4
3,4-Glc(p)	0.4	0	0	0.6	0.3
4,6-Glc(p)	1.7	0	3.4	3.2	1.9
2,3,4,6-Glc(p)	0.2	0	1.5	0	0.1
T-GalA(p)	0	0.1	0	0	0
T-GalA(p)-methyl ester	0	0.4	0	0	0
4-GalA(p)	6.8	24.0	0	3.7	6.3
4-GalA(p)-methyl ester	0.7	31.5	0	0.5	11.1
3,4-GalA(p)	0.9	0.4	0	0.5	0
3,4-GalA(p)-methyl ester	0	0.2	0	0.2	0
4,6-GalA(p)	0	0.1	0	0	0
4,6-GalA(p)-methyl ester	0	0.4	0	0	0
T-Ara(f)	9.4	2.4	1.6	13.3	8.8
5-Ara(f)	12.2	8.3	0	10.4	8.5
2,5-Ara(f)	3.5	0	0	2.8	0
3,5-Ara(f)	1.4	4.7	0	0.7	2.8
T-Xyl(p)	1.3	0	0	2.8	0
4-Xyl(p)	6.9	2.4	3.0	2.8	3.2
3,4-Xyl(p)	0	0	0.9	0	0
2,3,4-Xyl(p)	0	0	2.4	0	0
T-Gal(p)	0.5	0.9	0	0.4	0.5
3-Gal(p)	1.5	1.2	0	0.5	0.7
4-Gal(p)	3.0	8.1	1.0	2.3	3.7
2,3,4-Gal(p)	0.1	0	0	0	0
4,6-Gal(p)	0	0.2	0	0	0
4,6-Man(p)	0	0	0	0	0.2
T-Rha(p)	0.1	0	0	0	0
2-Rha(p)	0.4	0.9	0	0.5	0.7
2,4-Rha(p)	0.8	0	0	0.5	0
2,4-Hex	0	0	1.1	0	0
3,4-Hex	0	0	2.9	0	0
2,3,4-Hex	0	0	0.9	0	0
3,4,6-Hex	0	0	1.7	0	0
Abbreviations: glucose (Glc), galacturonic acid (GalA), arabinose (Ara), xylose (Xyl), galactose (Gal), mannose (Man), rhamnose (Rha), fucose (Fuc), hexose (Hex), terminal (T), pyranose (p), furanose (f)					

Table 27: Description of humans with obesity whose fecal microbial community samples were used in the gnotobiotic animal experiments

Subject ID	Race/ ethnicity	Age	H (m)	W (kg)	BMI	Insulin Basal	Glc Basal	H-IR	LDL	HDL	Total chol.	TG	hs-CRP
TP01-01	White (Caucasian)	41	1.6	104.6	38.8	25.3	5.2	5.9	138.0	34.0	213.0	204.0	5.2
TP02-01	White (Caucasian)	38	1.6	83.2	34.6	16.0	3.6	2.5	88.0	53.0	189.0	241.0	3.9
TP03-02	Black or African American	36	1.5	111.9	46.6	13.1	5.0	2.9	77.0	66.0	163.0	101.0	16.0
TP04-01	White (Caucasian)	33	1.7	114.6	38.7	13.9	5.0	3.1	135.0	44.0	203.0	122.0	4.6
TP05-02	White (Caucasian)	32	1.8	104.3	34.0	12.1	4.1	2.2	97.0	47.0	164.0	101.0	3.4
TP06-01	White (Caucasian)	37	1.7	119.0	41.9	20.2	4.2	3.8	77.0	40.0	135.0	88.0	9.9
TP07-02	White (Caucasian)	37	1.6	82.6	32.7	7.3	4.6	1.5	108.0	80.0	199.0	56.0	1.6
TP08-02	White (Caucasian)	41	1.7	92.4	32.6	9.9	4.5	2.0	103.0	45.0	165.0	87.0	6.0
TP09-02	White (Caucasian)	38	1.6	104.8	39.3	21.9	5.1	5.0	97.0	33.0	145.0	74.0	2.8

Abbreviations: height (H), weight (W), cholesterol (chol), triglycerides (TG), Glucose (Glc), Body mass index (BMI), Homeostatic model asssessment of insulin resistance (H-IR), low-density lipoproteins (LDL), high-density lipoproteins (HDL), high-sensitivity C-reactive protein (hs-CRP)

BMI values are kg/m^2

LDL, HDL, Total chol, and TG values are in mg/dL

hs-CRP values are in mg/L

Insulin values are in µU/mL, and Glucose values are in mmol/L.

Table 28: Description of subjects enrolled human study 1 (pea fiber snack food prototype)

Subject ID	Sex	Race/ ethnicity	Age	Height (cm)	Weight (kg)	BMI (kg/m^2)	HOMA-IR
S.01	M	Asian	31	167.9	74.6	26.5	2.6
S.02	F	White (Caucasian)	38	161.3	85.5	32.9	2.0
S.03	M	White (Caucasian)	38	174.9	86.8	28.4	3.5
S.05	M	Black or African American	47	172.5	98.3	33.0	1.6
S.06	F	Black or African American	47	158.5	68.3	27.2	1.1
S.07	M	White (Caucasian)	34	179.0	102.0	31.8	2.0
S.09	F	Black or African American	38	163.4	89.1	33.4	7.3
S.11	F	Black or African American	51	158.5	72.5	28.9	3.0

S.12	F	Black or African American	34	154.0	65.3	27.5	3.6
S.13	M	Black or African American	32	177.0	102.3	32.7	3.6
S.14	M	White (Caucasian)	48	182.5	113.9	34.2	2.2
S.15	M	Black or African American	55	188.5	98.4	27.7	0.3

Abbreviations: body mass index (BMI), homeostatic model assessment of insulin resistance (HOMA-IR)

Table 29: Description of subjects enrolled in enrolled in human study 2 (pea fiber plus inulin snack prototype, and pea fiber, inulin, orange fiber and barley bran snack prototype)

Subject ID	Sex	Race/ ethnicity	Age	Height (cm)	Weight (kg)	BMI (kg/m^2)	HOMA-IR
S.02	F	White (Caucasian)	39	162.5	80.0	30.3	2.5
S.03	M	White (Caucasian)	39	175.6	80.7	26.2	1.0
S.05	M	Black or African American	47	172.1	99.4	33.6	3.0
S.06	F	Black or African American	48	158.7	67.6	26.8	0.9
S.09	F	Black or African American	38	162.0	90.1	34.3	7.3
S.11	F	Black or African American	51	158.6	71.6	28.5	2.0
S.12	F	Black or African American	34	155.0	65.5	27.3	2.5
S.13	M	Black or African American	32	177.8	106.2	33.6	6.7
S.14	M	White (Caucasian)	48	181.5	115.7	35.1	2.8
S.110	M	White (Caucasian)	34	182.8	87.3	26.1	1.8
S.113	F	White (Caucasian)	38	164.0	69.7	25.9	0.8
S.114	F	White (Caucasian)	59	159.8	85.8	33.6	4.4
S.122	F	White (Caucasian)	27	167.5	76.3	27.2	1.7
S.123	F	White (Caucasian)	26	170.0	75.1	26.0	2.7

Abbreviations: body mass index (BMI), homeostatic model assessment of insulin resistance (HOMA-IR)

References for Examples 12 –17

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Methods for Examples 12 – 17

(a) Gnotobiotic Mouse Studies

[0493] *Husbandry* - To test the effects of different fiber preparations on uncultured human fecal microbial communities, adult germ-free male C57BL/6J mice (12-16-weeks-old) were dually-housed in plastic cages located in plastic flexible film gnotobiotic isolators (Class Biologically Clean Ltd., Madison, WI). Mice were maintained at 23 ºC under a strict 12 h light cycle (lights on at 0600 h). Cages contained autoclaved paper 'shepherd shacks' to facilitate their natural nesting behaviors and to provide environmental enrichment.

[0494] *Diets* - The HiSF-LoFV diet was milled to powder (D90 particle size, 980 µm), and mixed with powdered fiber preparations [10% (w/w)]. Fiber content was defined for each preparation [Association of Official Agricultural Chemists (AOAC) 2009.01]. Similarly, protein, fat, total carbohydrate, ash, and water content were measured [protein AOAC 920.123; fat AOAC 933.05; ash AOAC 935.42; moisture AOAC 926.08; total carbohydrate (100 - (Protein + Fat + Ash + Moisture)]. The powdered food mixtures were vacuum-packed in sterile plastic containers and sterilized by gamma irradiation (20-50 kilograys, Steris, Mentor, OH). Sterility was confirmed by culturing the diets under aerobic and anaerobic conditions (atmosphere, 75% N_2, 20% CO_2, 5% H_2) at 37 ºC in TYG medium.

[0495] *Transplantation of human fecal microbiota into germ-free mice* - A 500 mg aliquot of a pulverized frozen fecal sample that had been obtained from nine unrelated obese adult female members of the Missouri Adolescent Female Twin Study

(MOAFTS) cohort (*25*) listed in **Table 27** was diluted in 5 mL of reduced PBS [1X PBS supplemented with 0.1% Resazurin (w/v), 0.05% L-cysteine-HCl] in an anaerobic Coy chamber (atmosphere, 75% N_2, 20% CO_2, 5% H_2). The sample was vortexed for 2 minutes at room temperature in 5 mL of 2 mm-diameter autoclaved borosilicate glass beads in order to disrupt clumps of bacterial cells trapped within the fecal matrix. The resulting suspension was filtered through a sterile nylon mesh cell strainer (100 μM pore diameter; BD Falcon). The filtrate was then mixed with 5 mL of sterile PBS containing 0.1% Resazurin (w/v), 0.05% L-cysteine-HCl, and 30% (v/v) glycerol, transferred to a sterile glass crimped tube and stored at -80 ºC until further use. Aliquots of the stored filtrate were transported in a frozen state to the gnotobiotic mouse facility. The outer surface of the tube was sterilized by a 30-minute exposure to chlorine dioxide in the transfer sleeve attached to the gnotobiotic isolator, and then introduced into the isolator.

[0496] *Diet oscillation studies* - Germ-free C57BL/6J mice were weaned onto and subsequently maintained on an autoclaved, low-fat, high-plant polysaccharide chow (catalog number 2018S, Envigo) that was administered *ad libitum*. Four days prior to colonization, mice were switched to a diet low in saturated fats and high in fruits and vegetables (LoSF-HiFV) that was formulated based on the National Health and Nutrition Examination Survey of US dietary practices (*14*). A 300 μL aliquot of a clarified suspension of a given fecal microbiota sample was introduced into the stomachs of 12-16-week-old male mice using an oral gavage needle. Recipients were maintained in separate gnotobiotic isolators dedicated to animals colonized with the same donor microbiota. Four days after gavage, mice were switched to the HiSF-LoFV diet (*14,15*).

[0497] Mice in the experimental groups completed a 64-day multi-phase diet-oscillation feeding protocol. On day 4 after colonization, animals were fed a pelleted version of the HiSF-LoFV diet (*14,15*) for 10 days. Beginning on experimental day 14, mice were fed 20-30 g aliquots of a dough-like diet/fiber mixture that was made from the milled HiSF-LoFV diet supplemented with 10% (w/w) raw pea fiber (Pea fiber EF 100; J. Rettenmaier & Söhne GmbH & Co. KG) and hydrated with 10-15 mL sterile water (mixing of the sterile powdered diet and sterile water occurred within the gnotobiotic isolator). The resulting dough-like mixture was pressed into a plastic feeding dish and

placed on the cage floor for feeding *ad libitum*. Food supply was monitored daily, and a freshly hydrated aliquot of the diet was supplied every 3 days to prevent food levels from dropping below roughly one third of the original volume. The HiSF-LoFV/pea fiber mixture was administered for 10 d after which time mice were returned to the unsupplemented pelleted HiSF-LoFV diet for 10 d ('wash out period'). On day 34, mice were given 20-30 g aliquots of a diet/fiber mixture made from the milled HiSF-LoFV diet supplemented with 10% (w/w) coarse orange fiber (CitriFi 100; Fiber Star, Inc.). The HiSF-LoFV/orange fiber mixture was administered for 10 days. Mice were then returned to the unsupplemented pelleted HiSF-LoFV diet for 10 days. On experimental day 54, mice began receiving 20-30 g aliquots of the diet/fiber mixture made from the powdered HiSF-LoFV diet supplemented with 10% (w/w) raw barley bran fiber (Barley Balance - concentrated (1-3)(1-4) β-glucan; PolyCell Technologies, LLC). The HiSF-LoFV/barley bran fiber mixture was administered for 10 days. All animals were euthanized by cervical dislocation without prior fasting on experimental day 64.

[0498] Bedding (Aspen Woodchips; Northeastern Products) was replaced after each 10-day diet oscillation period to prevent any leftover food or fecal matter from being ingested during the following diet change. Fresh fecal samples were collected from each animal into sterile cryo-resistant polypropylene tubes within seconds of being produced on day 4 after initial colonization while consuming the LoSF-HiFV diet, and on days 5 and 10 of each 10-day oscillation period. Samples were placed in liquid nitrogen 45-60 min after they were collected. Pre-colonization fecal samples were also collected to verify the germ-free status of mice (by culture and by bacterial V4-16S rDNA amplicon sequencing).

(b) Human Studies

[0499] Subjects provided written, informed consent before participating in these studies. The first study (ClinicalTrials.gov NCT04159259) was performed between February and July, 2019. The second study (ClinicalTrials.gov NCT04101344) was conducted between August and December, 2019.

[0500] *Study 1 Design* - A total of 18 men and women who were overweight or obese (BMI ≥25.0 and ≤35.0 kg/m^2), aged ≥18 and ≤60 years, were

screened for potential participation in this study. Subjects completed a comprehensive medical evaluation, including a medical history, physical examination, assessment of food preferences and aversions, and standard blood tests. A fecal sample was collected during the medical evaluation phase and used to determine whether *Bacteroides* species were present in their microbiota (*B. vulgatus*, *B. thetaiotaomicron*, *B. cellulosilyticus*, *B. uniformis*, and/or *B. ovatus*). Subjects whose fecal microbiota contained less than 0.1% relative abundance of *B. vulgatus* (defined by V4 16S rDNA amplicon sequencing), and less than 0.1% relative abundance of at least one of the other *Bacteroides*. were excluded from the study. Additional exclusion criteria included: (i) history of previous bariatric surgery; (ii) significant organ system dysfunction (e.g., diabetes, severe pulmonary, kidney, liver or cardiovascular disease); (iii) history of inflammatory gastrointestinal disease; (iv) pregnant or lactating; (v) use of medications known to affect the study outcome measures that could not be temporarily discontinued; (vi) use during the month prior to screening of medications known to affect the composition of the gut microbiota (e.g., antibiotics); (vii) bowel movements <3 times per week; (viii) vegans, vegetarians, those with lactose intolerance and/or severe allergies/aversions/sensitivities to foods and ingredients included in the prescribed meal plan; and (ix) individuals who were not able to grant voluntary informed consent. Of 18 participants who were screened, four were excluded based on the *Bacteroides* criterion and two were excluded based on the screening assessment. Each of the 12 subjects who participated in the study completed the study per protocol.

[0501] The study design is described in **FIG. 31A**. Participants who met the enrollment criteria were asked to maintain their usual eating habits for 4 days with fecal samples collected at home on days 1, 2, 3 and 4. On days 5 through day 14, participants consumed only HiSF-LoFV meals (see below) that were provided by the study team in the form of packed-out meals. Fecal samples were collected at home on study days 6, 8, 10, 12 and 14 and a fasting blood sample was collected on day 14. Ten days after starting the HiSF-LoFV diet, subjects supplemented their diet with the pea fiber-containing snack (see below and **Table 21**), starting with 1 bar per day on day 15 and 16 (with lunch), 2 bars per day on day 17 and 18 (one at lunch and one at dinner)

and 3 bars per day from day 19 through 35 (at breakfast, lunch and dinner). All at-home fecal samples during the ramp up period (i.e., days 15-20) were collected and subsequently, at-home fecal samples every two days (i.e., on study days 21, 23, 25, 27, 29, 31, 33 and 35), with fasting blood collected on day 29 and day 35. After completing 17 consecutive days consuming 3 snacks per day (24.3g of pea fiber), participants returned to consuming the unsupplemented HiSF-LoFV diet for an additional 14 days, with fecal samples collected every two days (study days 37, 39, 41, 43, 45, 47 and 49) and fasting blood collected on day 49.

[0502] *Study 2 Design* – A total of 23 men and women who were overweight or obese were screened for potential participation in this study by using the same exclusion/exclusion criteria as Study 1 with the exception that there was no prescreen for the representation of *Bacteroides* species in subjects' fecal samples. Among the 23 participants who completed screening, 19 were enrolled and 14 completed the study protocol. Five participants did not complete the intervention for personal reasons unrelated to the study intervention. The study design is described in **FIG. 35B**. On day 1, participants provided a fecal sample prior to entering the controlled diet phase of the study. From day 2-11, participants consumed HiSF-LoFV meals provided by the study team in the form of packed-out meals and snacks; they collected at-home fecal samples on study days 5, 9,10 and 11. A fasting blood sample was obtained on day 11. On day 12, subjects began supplementing their diet with the two-fiber snack prototype [1 snack serving per day on day 12 (with lunch), 2 bars per day on day 13 (one at lunch and one at dinner) and 3 servings per day on day 14 through 25 (at breakfast, lunch and dinner)]. Participants collected all at home fecal samples during the ramp up period (i.e., days 12-14) and subsequently, at-home fecal samples on days 18, 23, 24 and 25. A fasting blood sample was collected on day 25 just prior to returning to the unsupplemented HiSF-LoFV diet (days 26-35). During this 'wash-out' phase of the study, fecal samples were collected on days 28, 33, 34 and 35 and a fasting blood sample on day 35. Consumption of the four-fiber snack prototype began on day 36 [1 snack serving per day on day 36 (with lunch), 2 snacks per day on day 37 (one at lunch and one at dinner) and 3 snacks per day on day 38 through 49 (at breakfast, lunch and

dinner)]. Subjects collected all at-home fecal samples during the ramp-up period (days 36-38) and subsequently, at-home fecal samples on days 41, 47, 48 and 49. A fasting blood sample was obtained on day 49 (last day of study).

[0503] *Fiber snacks* – Snack food prototypes were prepared by Mondelēz International, Inc., tested to confirm the absence of microbial contamination/pathogens, and then shipped to and stored at Washington University. Research participants received weekly shipments of the snack food prototypes. The composition of these prototypes is described in **Table 21A**. Their organoleptic properties were designed based on common USA consumer preferences.

[0504] *Design, manufacture and distribution of HiSF-LoFV diets* – Participants consumed a diet composed of approximately 40% fat, 20% protein, and 40% carbohydrate that was high in saturated fat and low in fruits and vegetables during the dietary intervention. The HiSF-LoFV diet is high in refined grains (white bread and pasta, bagels, and corn cereals), added sugars (sugar-sweetened beverages, candies, and desserts), vegetables sourced primarily from potatoes and tomatoes, and protein and fat derived from animals. Representative diets are shown in **table 6B**. Each participant's estimated energy requirements were calculated using the Mifflin St. Jeor equation (*26*) multiplied by an appropriate physical activity level (PAL). To ensure consistent intake of nutrients across all participants and ensure weight stability, a registered dietitian designed a seven-day cycle menu specific to the participant's energy needs and instructed each participant to consume only foods prescribed by the study team during the dietary intervention. The energy provided was adjusted, as needed, to ensure subjects remained weight stable throughout. All food was provided in the form of packed-out meals and snacks prepared by the metabolic kitchen in the Clinical Translational Research Unit (CTRU) at Washington University.

[0505] *Collection of clinical meta-data* – Subjects were provided with electronic smart scales (BodyTrace, Inc.) to enable weight monitoring between study visits. At enrollment, habitual dietary patterns were assessed using the National Cancer Institute Diet History Questionnaire III (DHQIII) food frequency questionnaire (*27*). Subjects visited the CTRU on a weekly basis to pick up packed-out meals (using

insulated bags and rolling coolers), have their body weight measured, and any changes to their health and medications reviewed. During the study, participants recorded all food and beverage intake using a web-based food diary during all diet phases. An experienced study dietitian trained study participants on how to complete the food records and reviewed these records with the participants at each study visit to ensure the accuracy of self-reported data. In addition, a member of the study team contacted participants regularly to (i) check on study progress, (ii) discuss prescribed and non-prescribed foods and beverages consumed, (iii) discuss weight changes, and (iv) ensure participants have sufficient fecal collection kits.

[0506] *Preparation of blood samples* - Fasting blood samples were obtained in the CTRU. Conventional blood chemistry tests were performed by the Clinical Laboratory Improvement Amendments (CLIA)-certified Core Laboratory for Clinical Studies (CLCS) at Washington University School of Medicine. To prepare plasma for SOMAscan proteomics analysis (SomaLogic, Boulder, CO), blood samples (10-20 mL) were aliquoted into EDTA-K2 treated tubes and centrifuged at 2,000 x g for 10 minutes at 4 ºC. Following centrifugation, plasma was immediately transferred into cryo-resistant polypropylene tubes (0.5 mL aliquots), de-identified, and stored at -80 ºC prior to analysis according to manufacturer's recommendations.

(c) Fecal sample collection, processing and culture-independent analyses

[0507] *Sample collection and processing* - Participants collected fecal samples using small medically approved collection containers. Participants were provided with a freezer (-20 ºC) at the beginning of the study for temporary storage of fecal samples. Containers were labeled with a unique study identifier to protect subject confidentiality, and the collection date and time. Each sample was frozen immediately at -20 ºC and shipped in a frozen state (using frozen gel packs). Samples were shipped on a regular basis to a biospecimen repository in Washington University in St. Louis where they were stored at -80 ºC until the time of processing. Fecal samples were homogenized with a porcelain mortar (4 L) and pestle while submerged in liquid nitrogen. Multiple 500 mg aliquots of the pulverized frozen material were prepared and stored at -80 ºC.

b. DNA was extracted from an aliquot of each pulverized human fecal sample (~50-100 mg) or mouse fecal pellets (~20-50 mg) by first bead-beating (BioSpec Mini-beadbeater-96,) for 4 minutes in 250 µL of 0.1 mm-diameter zirconium oxide beads and a 3.97 mm-diameter steel ball in a solution consisting of 500 µL buffer A (200 mM NaCl, 200 mM Trizma base, 20 mM EDTA), 210 µL of 20% SDS, and 500 µL phenol:chloroform:isoamyl alcohol (25:24:1), followed by centrifugation at 3,220 x g for 4 minutes. DNA was purified (QiaQuick 96 purification kit; Qiagen, Valencia, CA), eluted in 130 µL of 10 mM Tris-HCl pH 8.5 (buffer EB, Qiagen), and quantified (Quant-iT dsDNA broad range kit; Invitrogen). Purified DNA was stored at -20 ºC for further processing.

[0508] *16S rDNA amplicon sequencing and identification of ASVs* - Purified DNA samples were adjusted to a concentration of 1 ng/µL and subjected to PCR using barcoded primers directed against variable region 4 of the bacterial 16S rRNA gene (*28*). PCR amplification was performed using the following cycling conditions: denaturation (94 ºC for 2 minutes), 26 cycles of 94 ºC for 15 seconds, 50 ºC for 30 seconds and 68 ºC for 30 seconds, and incubation at 68 ºC for 2 minutes. Amplicons with sample-specific barcodes were quantified, pooled and sequenced (Illumina MiSeq instrument, paired-end 250 nt reads).

[0509] Paired-end reads were demultiplexed, trimmed to 200 nucleotides, merged, and chimeras removed using the 1.13.0 version of the DADA2 pipeline (*29*) in R (v. 3.6.1). Amplicon sequence variants (ASV) generated from DADA2 were aligned against the GreenGenes 2016 (v. 13.8) reference database to 97% sequence identity, followed by taxonomic and species assignment with RDP 16 (release 11.5) and SILVA (v. 128). The resulting ASV table was filtered to include only ASVs with ≥0.1% relative abundance in at least five samples and rarefied to 15,000 reads/sample.

[0510] Results were also obtained with another approach to taxonomic assignment. In this procedure, each representative sequence is aligned (NCBI BLAST toolkit version 2.10.0) to a 16S rRNA gene reference database compiled by joining unique sequences from Ribosomal Database Project (RDP) version 11.5 and the NCBI 16S ribosomal RNA Project. Alignment results are sorted based on percentage of

sequence identity, with maximum values denoted as "*M*". Hits are selected with identities in the range [*M*] to [*M*– *(1–M)/S*] where "*S*" is scaling parameter that controls the maximum number of taxonomic descriptors accepted for a 'multi-taxonomic assignment' (MTA) based on 16S rDNA sequence identity (in this study, set to 4) (*30*).

[0511] *Shotgun sequencing and annotation of microbiomes* - Purified DNA samples were adjusted to a concentration of 0.75 ng/μL. Sequencing libraries were generated from each DNA sample using the Nextera DNA Library Prep Kit (Illumina) with the reaction volume scaled down 10-fold to 2.5 μL (*31*). Samples were pooled and sequenced with Illumina NextSeq 550 instrument in the case of all mouse samples [10.7 ± 0.6 x 10^6 paired-end 150 nucleotide-long reads/sample (mean ± s.d.)] and all human samples in Study 2 (12.8 ± 1.2 x 10^6 paired-end 150 nucleotide-long reads/ sample), while an Illumina NovaSeq Model 6000 instrument was used to sequence human samples collected during the course of Study 1 (28.0 ± 4.2 10^6 paired-end 150 nucleotide-long reads/ sample).

[0512] After sequencing, reads were demultiplexed (bcl2fastq, Illumina), adapter sequences were trimmed using cutadapt (*32*) and reads were quality filtered with Sickle (*33*). Human and mouse DNA sequences were identified, and removed using Bowtie2 (*34*) and either the hg19 build of the *H. sapiens* genome or the *Mus musculus* C57BL/6J strain genome (UCSC mm10), depending on sample type, prior to further processing. Host-filtered reads were assembled using IDBA-UD (*35*) and annotated with prokka (*36*) Gene counts were generated by mapping quality-controlled, paired-end reads generated from each sample to the corresponding assembled contigs. Duplicate reads (optical- and PCR-generated) were identified and removed from mapped data using the Picard MarkDuplicates tool (v 2.9.3). Mapping results were processed to generate count data (featureCounts; Subread v. 1.5.3 package) (*37*) and normalized (transcripts per kilobase million reads, TPM) in R (v. 3.4.1; *38*).

[0513] The genomic integration platform SEED, which is a growing repertoire of complete and nearly complete microbial genomes with draft annotations performed by the RAST server (*39*), was used for additional annotations of fecal microbiomes. Functional profiles for each fecal microbiome were generated by

assigning microbiome-encoded proteins to microbial community SEED (mcSEED) metabolic pathways/modules that capture core metabolism of nutrients/metabolites in four major categories (amino acids, sugars, fermentation products and vitamins) projected over ~2,600 reference bacterial genomes (*19*). Protein sequences from prokka-annotated (*36*) fecal DNA assemblies were queried against representative protein sequences from the mcSEED subsystems/pathway modules using DIAMOND (*40*) with a threshold of ≥80% identity for best hits. Microbiome-encoded proteins were assigned the best-hit annotation of the representative mcSEED protein.

[0514] CAZymes annotations were performed for the full set of open reading frames identified by prokka. Assignment to CAZyme families was performed using a custom script which, in a first step, compared each amino acid sequence to the full-length sequences listed in the CAZy database (download date April 21, 2020) using Blastp (version 2.3.0+) (*41*). Sequences giving e-values worse than 10^{-4} were discarded while sequences showing 100% coverage with an e-value of at least 10^{-6} and more than 50% identity with a sequence already in the CAZy database were directly assigned to the same family (or families in the case of modular proteins) as the subject sequence. All other sequences were subjected to a second, parallel, similarity search using two methods; (i) Blastp against a library of sequences corresponding to the individual modules in the CAZy database, and (ii) HMMER3 (*42*) using a collection of custom-made HMMs built after the CAZy families (and subfamilies for families GH5, 13, 16, 30 and 43). Assignments were kept when the two methods gave the same results with >90% overlap and an e-value better than 10^{-4} for all families except for carbohydrate esterases (threshold 10^{-20}), and non-LPMO auxiliary activities (threshold 10^{-25}). These various thresholds were designed to eliminate as much as possible oxidoreductases or esterases not specific for carbohydrates and to give results more consistent with the manual procedure used for updates of the CAZy database.

(d) Quantitative proteomics of human plasma samples

[0515] Levels of 1305 proteins were quantified in a 50 mL aliquot of plasma using the SOMAscan 1.3K Proteomic Assay plasma/serum kit (SomaLogic, Boulder, CO). Procedures used for quality control filtering and analysis of differential

protein abundances are described in ref 43. Briefly, microarrays were scanned with an Agilent SureScan instrument at 5 □m resolution and the Cy3 fluorescence readout was quantified. Raw fluorescence signal values from each SOMAmer reagent were processed using standardization procedures that are recommended by the manufacturer (i.e., datasets were normalized to remove hybridization variation within a run followed by median normalization across all samples to remove other assay biases). The final .adat file was log_2-transformed, quantile-normalized and then filtered to remove non-human SOMAmer reagents. A total of 1205 and 1170 proteins were then used for downstream analyses of participants in human studies 1 and 2, respectively.

(e) Higher-order singular value decomposition

[0516] **FIG. 30B** illustrates SVD. The result of performing SVD on matrix **M** is creation of three matrices; **U**, **E**, and **V**. **U** is a matrix of 'left singular vectors' (LSVs), **V** is a matrix of 'right singular vectors' (RSVs), and **E** is a matrix only of diagonal values ('singular values'). The k^{th} element of **E** relates the k^{th} left and right singular vectors. A new matrix can be created that considers the information contained within a single singular value. For example, **FIG. 30B** shows that multiplying left singular vector 1 (LSV1), singular value 1 (SV1), and right singular vector 1 (RSV1) creates a new matrix $\mathbf{M}^1$ that has the same dimensions as matrix **M** but exclusively contains information within singular vector 1. Higher-order (HO)-SVD is used when the input matrix has more than two degrees of freedom (**FIG. 30B**). Mathematically, these types of matrices are called 'tensors.' Unlike SVD, HO-SVD is not a technique with an analytical solution; i.e., a tensor of rank N cannot be written as a product of N+1 tensors as in the case with SVD. As a consequence, several methods of approximation exist to deconstruct higher-order tensors for feature-reduction purposes. 'Canonical Polyadic decomposition' (CP decomposition), deconstructs a tensor into a sum of rank-1 tensors (arrays) related to each other through a 'core tensor'. **FIG. 30B** shows the result of CP decomposition on a three-dimensional tensor O. The core tensor, G, is a three-dimensional tensor comprised of only diagonal elements, each of which specifies the amount of variance carried by a 'tensor component' analogous to the singular values computed by SVD. Each tensor component (TC) relates the rows, columns, and third-dimensional entries of

O. The number of tensor components is determined by creating a tensor that is randomized with respect to the rows, columns, and third dimension entries and performing CP decomposition over 100 trials. The randomization process scrambles the correlations between each dimension of the tensor; therefore, the resulting CP decomposition reflects a random distribution of tensor component values. We used the alternating least squares algorithm for the CP decomposition (CP-ALS) to iteratively improve the matrix factorization. The lowest tensor component whose variance is above that of tensor component 1 of the scrambled tensor defines the number of tensor components considered.

(f) *Over representation analysis of HO-SVD discriminatory plasma proteins*

[0517] A list of discriminatory plasma proteins, defined as being in the 20th percentile of the most positive and negative projections along HO-SVD-defined tensor components was mapped to the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database (*44-46*) and tested for functional enrichment analysis using clusterProfiler in R (*47*). An over-representation analysis employing a hypergeometric test was used to identify KEGG pathways enriched during consumption of each fiber snack prototype. A list of all plasma proteins measured by SOMAscan that passed quality control criteria (1205 for human study 1 and 1170 for human study 2) was used as the background list of plasma proteins for the over representation pathway analysis (parameters: organism = "hsa", keyType = "kegg", pAdjustMethod = "BH", minGSSize = 5, maxGSSize = 500). A combined list of plasma proteins identified as significantly enriched in insulin and glucagon signaling pathways for all three fiber snack prototypes was used in the heatmaps shown in **FIG. 33**.

(g) Cross-correlation singular value decomposition analysis

[0518] CC-SVD begins by computing the cross-correlation matrix between two feature types. Given two matrices of dimensions $N^{m \times n}$ (with elements $N_{i,j}$) and $P^{m \times p}$ (with elements $P_{k,l}$) where m is the number of samples and n and p are the number of features of each feature type, a cross-correlation matrix is calculated by taking each feature in the $m \times n$ matrix N and correlating them with each feature in the $m \times p$ matrix

P. The resulting matrix is a *n x p* cross-correlation matrix $C^{n \times p}$, where each element $C_{j,l}$ contains the correlation between feature $N_{1:m,j}$ from the first matrix and feature $P_{1:m,l}$ from the second matrix (note that these starting matrices contain abundance information whereas the resulting cross-correlation matrix contains correlations between features). Next, SVD is used to decompose the cross-correlation matrix *C* into left and right singular matrices which contain left and right singular vectors (SVs), respectively; the left SVs correspond to the features of *N* and the right SVs correspond to the features of *P*. An SV represents a module of cross-correlated features with a unique correlation profile, and the projections of each feature onto an SV represents the module membership of that feature (e.g., how similar a feature's correlation profile is to the overall module's correlation profile). To define a module, a user-defined threshold truncates the leading and trailing tails of the distribution of projections along an SV, and the features above and below the truncation are considered module members. Note that SVD determines a projection for all features along each SV, providing a continuous measure of module membership. Because the input matrix decomposed by SVD is a correlation matrix, features with large positive projections on a left SV will be strongly correlated with features with large positive projections on the matching right SV and negatively correlated with features with large negative projections on the matching left SV. Concordantly, features with large negative projections on a left SV will be strongly correlated with features with large negative projections on the right SV and negatively correlated with features with large positive projections on the left SV. The number of SVs that should be considered modules is determined using a random-matrix approximation described elsewhere (*48*). Module members are selected from the original cross-correlation matrix *C* and plotted using the 'corrplot' function in R (*49*) in rank order by their projections onto an SV, with larger magnitude projection values indicating a correlation pattern similar to the module's overall correlation profile. The continuous nature of projection values enabled us to rank-order proteins by their projections along SV1 and to use the KEGG database (*44-46*) to identify and relate biological processes to CAZyme-associated proteins.

(h) Mass spectrometry-based carbohydrate analys is of fibers, diets and fecal samples

[0519] *Monosaccharide and linkage analysis of fiber preparations* – Methods described in ref. 15 were used to define the carbohydrate composition of the pea, orange and barley bran fiber preparations. Following a pre-hydrolysis step (incubation in concentrated sulfuric acid (72%) for 30 minutes at 30 °C to release glucose from cellulose), the fibers were treated with 1 M sulfuric acid for 6 hours at 100 °C. Individual neutral sugars were analyzed by gas chromatography as their alditol acetate derivatives (*50,51*). The metahydroxydiphenyl colorimetric acid method was used to measure uronic acid (as galacturonic acid) (*52,53*); sodium tetraborate was used to differentiate glucuronic acid from galacturonic acid (*54*). Galacturonic acid (pectins) methylation was estimated according to ref. 55.

[0520] Linkage analysis of fibers followed procedures detailed in ref. 56 with minor modifications that allowed for discrimination of galactose, galacturonic acid, and methyl-esterified galacturonic acid. Briefly, reduction of carboxymethyl ester groups of uronic acids was performed with $NaBD_4$ and imidazole-HCl, followed by activation of carboxylic acid groups with carbodiimide and a second reduction with imidazole-HCl, $NaBH_4$ (D/H) and $NaBD_4$ (D/D). Samples were dialyzed, freeze-dried and then solubilized in DMSO before methylation with iodomethane of the accessible hydroxyl groups of reduced polysaccharides. Acid hydrolysis with trifluoroacetic acid and a subsequent reduction with $NaBD_4$ of partially methylated sugars was performed. Lastly, samples were acetylated, extracted as partially methylated alditol acetates (PMAA) into dichloromethane, and analyzed by gas chromatography-mass spectrometry (GC-MS) (*57*).

[0521] *Homogenization of mouse diets and fecal biospecimens* - For homogenization of HiSF-LoFV diet with and without fiber supplementation, a 10 mg/mL stock solution was prepared from frozen starting material. Pre-weighed mouse and human fecal samples were diluted 10-fold in Nanopure water (Thermo Fisher) and homogenized overnight. Samples were then centrifuged and a 200 µL aliquot of the supernatant was taken for metabolomic analysis, while the remaining material was

lyophilized to complete dryness and diluted to create a stock solution (10 mg/mL water). Stock solutions were bullet-blended using 1.4 mm stainless steel beads followed by incubation at 100°C for 1 h. Lastly, samples were subjected to another bullet blend process and aliquots were taken for monosaccharide and linkage analysis.

[0522] *Monosaccharide analysis* - Methods for monosaccharide analysis of diets and fecal samples were adapted from ref. 58. Briefly, three 10 µL aliquots were taken from each bullet-blended 'stock', transferred to a 96-well plate and subjected to acid hydrolysis (4 M trifluoroacetic acid for 1 h at 121°C). The reaction was quenched with 855 µL of ice-cold Nanopure water. Hydrolyzed samples were derivatized with 1-phenyl-3-methyl-5-pyrazolone (PMP) according to conditions described in ref. 59. Samples and 14 monosaccharide standards (0.001-100 µg/mL) were reacted in 0.2 M PMP (prepared in methanol) and 28% NH_4OH at 70 °C for 30 minutes. Derivatized glycosides were then dried to completion (vacuum centrifuge) and reconstituted in Nanopure water. Excess PMP was removed (chloroform extraction) and a 1µL aliquot of the aqueous layer was injected into an Agilent 1290 infinity II UHPLC coupled to an Agilent 6495A triple quadrupole mass spectrometer under dMRM mode. Monosaccharides were quantified using an external calibration curve.

[0523] *Linkage analysis* - The procedure for linkage analysis was adapted from previously described protocols. In short, three replicate 5 µL aliquots of each bullet-blended stock solution were incubated in saturated NaOH and iodomethane (in DMSO) to achieve methylation of free hydroxyl groups. Excess NaOH and DMSO were removed by extraction with dichloromethane and water. Permethylated samples were subsequently hydrolyzed and derivatized (using the same procedure employed for monosaccharide analysis). Derivatized samples were subjected for ultra-high-performance liquid chromatography-multiple reaction monitoring-mass spectrometry. Glycosidic linkages present in samples were identified using a pool of oligosaccharide standards and a comprehensive linkage library described elsewhere (*60,61*).

[0524] *LC-QTOF-MS identification of a fecal biomarker of orange fiber consumption* – Methods for preparing samples and performing LC-QTOF-MS using an Agilent 1290 LC system coupled to an Agilent 6545 Q-TOF mass spectrometer are

detailed in an earlier publication (*62*). Five µL of each prepared fecal sample for positive ESI ionization were injected into a BEH C18 column (2.1 × 150 mm, 1.7 µm, Waters Corp.) that was heated to 35 °C. The mobile phase was 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The following gradient was applied at a flow rate of 0.3 ml/min over 14 minutes; 95% A/5% B to 100% B, followed by 3 minutes at 100% B.

References for Methods for Examples 1 – 6

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CLAIMS

What is claimed is:

1. A fiber blend comprising

at least 15 wt% of one or more pea fiber preparation or a glycan equivalent thereof; and

at least one additional fiber preparation chosen from

- at least 28 wt% of one or more high molecular weight inulin preparation or a glycan equivalent thereof,
- between 0 wt% and 10 wt%, inclusive, of one or more citrus pectin preparation or a glycan equivalent thereof,
- between 0 wt% and 25 wt%, inclusive, of one or more citrus fiber preparation or a glycan equivalent thereof, or
- between 0 wt% and 45 wt%, inclusive, of one or more barley fiber preparation or a glycan equivalent thereof.

2. The fiber blend of claim 1, which comprises at least 28 wt% of one or more pea fiber preparation, or a glycan equivalent thereof.

3. The fiber blend of claim 2, which comprises at least 30 wt% of one or more pea fiber preparation, or a glycan equivalent thereof; and there is at least 30 wt% of one or more high molecular weight inulin preparation, or a glycan equivalent thereof.

4. The fiber blend of any of claims 1-3, which comprises less than 1 wt% of one or more citrus pectin preparation, or a glycan equivalent thereof.

5. The fiber blend of any of claims 1-4, which comprises no citrus pectin preparation, or a glycan equivalent thereof.

6. The fiber blend of any of claims 1-5, which comprises 15 wt% or less of one or more citrus fiber preparation or a glycan equivalent thereof, or 12 wt% or less of one or more citrus fiber preparation or a glycan equivalent thereof

7. The fiber blend of claim 1, 5, or 6, wherein the citrus fiber preparation is an orange fiber preparation.

8. The fiber blend of any one of claims 1-7, which comprises is 25 wt% or less of one or more barley fiber preparation or glycan equivalent thereof, or 20 wt% or less of one or more barley fiber preparation, or glycan equivalent thereof.

9. The fiber blend of any one of the preceding claims, wherein

the pea fiber preparation has a composition substantially similar to the pea fiber preparation of Table A or Table G, and/or a monosaccharide content substantially similar to the pea fiber preparation of Table B or Table G, and optionally glycosyl linkages substantially similar to the pea fiber preparation of Table C1 or Table C2;

the high molecular weight inulin preparation has a composition substantially similar to the high molecular weight inulin preparation of Table A or Table G;

the barley fiber preparation has a composition substantially similar to the barley fiber preparation of Table A or Table G, and/or a monosaccharide content substantially similar to the barley fiber preparation of Table B or Table G, and optionally glycosyl linkages substantially similar to the barley fiber preparation of Table E;

the citrus fiber preparation has a composition substantially similar to the citrus fiber preparation of Table A or Table G, and/or a monosaccharide content substantially similar to the citrus fiber preparation of Table B or Table G, and optionally glycosyl linkages substantially similar to the citrus fiber preparation of Table F1 or Table F2.

10. The fiber blend of claim 1`, which comprises

about 25 wt% to about 40 wt% of one or more pea fiber preparation or a glycan equivalent thereof, about 5 wt% to about 15 wt% of one or more citrus fiber preparation or a glycan equivalent thereof, about 30 wt% to about 40 wt% of a high molecular weight inulin preparation or glycan equivalent thereof, about 10 wt % to about 30 wt% of a barley fiber preparation or glycan equivalent thereof; or

about 30 wt% to about 40 wt% of one or more pea fiber preparation or a glycan equivalent thereof, about 10 wt% to about 20 wt% of one or more citrus fiber preparation or a glycan equivalent thereof, about 30 wt% to about 40 wt% of a high molecular weight inulin preparation or glycan equivalent thereof, about 15 wt % to about 25 wt% of a barley fiber preparation or glycan equivalent thereof; or

about 55 wt% to about 65 wt% of one or more pea fiber preparation or a glycan equivalent thereof and about 30 wt% to about 40 wt% of a high molecular weight inulin preparation or glycan equivalent thereof; or

about 60 wt% to about 70 wt% of one or more pea fiber preparation or a glycan equivalent thereof and about 30 wt% to about 40 wt% of a high molecular weight inulin preparation or glycan equivalent thereof; or

about 60 wt% to about 65 wt% of one or more pea fiber preparation or a glycan equivalent thereof and about 35 wt% to about 40 wt% of a high molecular weight inulin preparation or glycan equivalent thereof.

11. The fiber blend of claim 10, which comprises less than 1 wt% of one or more citrus pectin preparation, or a glycan equivalent thereof.

12. The fiber blend of claim 10 or 11, wherein the citrus fiber preparation is an orange fiber preparation.

13. The fiber blend of claim 10, 11 or 12, wherein

the pea fiber preparation has a composition substantially similar to the pea fiber preparation of Table A or Table G, and/or a monosaccharide content substantially similar to the pea fiber preparation of Table B or Table G, and optionally glycosyl linkages substantially similar to the pea fiber preparation of Table C1 or Table C2;

the high molecular weight inulin preparation has a composition substantially similar to the high molecular weight inulin preparation of Table A or Table G;

the barley fiber preparation has a composition substantially similar to the barley fiber preparation of Table A or Table G, and/or a monosaccharide content

substantially similar to the barley fiber preparation of Table B or Table G, and optionally glycosyl linkages substantially similar to the barley fiber preparation of Table E;

the citrus fiber preparation has a composition substantially similar to the citrus fiber preparation of Table A or Table G, and/or a monosaccharide content substantially similar to the citrus fiber preparation of Table B or Table G, and optionally glycosyl linkages substantially similar to the citrus fiber preparation of Table F1 or Table F2.

14. A food composition comprising a fiber blend of any one of the preceding claims.

15. A baked, pressed or extruded food composition comprising a fiber blend of any one claims 1 to 13.

16. The food composition of claim 14 or 15, wherein the fiber blend is about 30 wt% to about 50 wt% of the food composition.

17. The food composition of any one of claims 14, 15, or 16, wherein the fiber blend provides about 30% or more of the total dietary fiber in the food composition or about 50% or more of the total dietary fiber in the food composition.

18. The food composition of any one of claims 14 to 17, wherein the fiber blend is in an amount that provides about 3 g to about 10 g of total dietary fiber.

19. The food composition of any one of claims 14 to 18, wherein the food composition further comprises flour(s), meal(s), oil(s), fat(s), inclusions, sweetener(s), starch(es), salt(s), emulsifier(s), leavening agent(s), preservative(s) or combinations thereof.

20. The food composition of claim 19, wherein the food composition comprises one or more flour and/or meal in an amount that is about 10 wt% of the food composition to about 60 wt% of the food composition.

21. The food composition of claim 20, wherein the one or more flour is chosen from wheat four, rice flour, corn flour, or any combination thereof.

22. The food composition of any one of claims 19 to 21, wherein the food composition comprises one or more sweetener in an amount that is about 0.005 wt% of the food composition to about 40 wt% of the food composition.

23. The food composition of claim 22, wherein the one or more sweetener is sugar.

24. The food composition of any one of claims 19 to 23, wherein the food composition comprises one or more salt in an amount that is about 0.5 wt% of the food composition to about 5 wt% of the food composition.

25. The food composition of claim 24, wherein the one or more salt is a sodium salt.

26. The food composition of any one of claims 19 to 25, wherein the food composition comprises one or more emulsifier in an amount that is about 0.1 wt% of the food composition to about 2 wt% of the food composition.

27. The food composition of claim 26, wherein the one or more emulsifier is chosen from glycerol monostearate, lecithin, polysorbate, or other mono or diglycerides.

28. The food composition of any one of claim 19 to 27, wherein the food composition comprises one or more leavening agent in an amount that is about 0.1 wt% of the food composition to about 5 wt% of the food composition.

29. The food composition of claim 28, wherein the one or more leavening agent is chosen from sodium bicarbonate, monocalcium phosphate, or calcium carbonate, ammonium bicarbonate, mono calcium phosphate monohydrate, sodium acid pyrophosphate, sodium aluminum phosphate, organic acids, and yeast.

30. The food composition of any one of claims 19 to 27, wherein the food composition further comprises a color additive, a flavor, a flavor enhancer, a stabilizer, a humectant, a firming agent, an enzyme, a probiotic, a spice, a binder, fruit, vegetables, grains, vitamins, minerals or combinations thereof.

31. The food composition of any one of claims 19 to 30, wherein food composition is a baked food composition that has about 3 g to about 10 g of total dietary fiber in a 30 g serving.

32. The baked food composition of claim 31, wherein the baked food composition has about 3 g, about 6 g, or about 10 g of total dietary fiber in a 30 g serving.

33. The baked food composition of claim 31 or 32, wherein the baked food composition is a cracker, a cookie, a cake, a bar, a bread, or a muffin.

34. The food composition of any one of claims 19 to 30, wherein food composition is an extruded food composition that has about 3 g to about 10 g of total dietar fiber in a 30 g serving.

35. The extruded food composition of claim 34, wherein the extruded food composition has about 3 g, about 6 g, or about 10 g of total dietary fiber in a 30 g serving.

36. The extruded food composition of claim 35, wherein the extruded food composition has about 10 g of total dietary fiber in a 30 g serving.

37. The extruded food composition of claim 35 or 36, wherein the extruded food composition is an extruded pillow or any other extruded shape.

38. A food composition of any one of claims 1-37, wherein administration of the food composition at least once daily for a minimum of five days to a subject increases the abundance of one or more member of at least one CAZyme family measured in a fecal sample obtained from the subject.

39. A food composition of claim 38, wherein the one or more member of at least one CAZyme family is selected from the group consisting of α-L-arabinofuranosidase (GH43_33), β-galactosidase (GH147), N-acetylmuramidase (GH108), endo-1,2,-α-mannanase (GH99), and β-glucosidase (GH116).

40. A food composition of any one of claims 1-37, wherein administration of the food composition at least once daily for a minimum of five days to a subject changes the abundance of one or more proteins measured in a blood sample obtained from the subject toward a healthier state.

41. A food composition of claim 40, wherein administration of the food composition at least once daily for a minimum of five days to a subject decreases the abundance of one or more proteins selected from the group consisting of CCL3, CRP, SPP1, F2, F3, VEGFA, PDGFRB, EFNA5, EPHA1, EPHA2, and IL1R1.

42. A food composition of claim 40, wherein administration of the food composition at least once daily for a minimum of five days to a subject decreases the abundance of one or more proteins selected from the group consisting of C3, C1R, C4A/C4B, SERPINE1, MASP1, and PDGFRA.

43. A food composition of claim 40, wherein administration of the food composition at least once daily for a minimum of five days to a subject decreases the abundance of AGRP.

44. A food composition of any one of claims 1-37, wherein administration of the food composition at least once daily for a minimum of five days to a subject decreases fasting blood glucose, fasting insulin, and HOMA-IR.

45. The food composition of any one of claims 38 to 44, wherein the subject is on a Western diet.

46. A food composition of any one of claims 1-37, wherein administration of the food composition at least once daily for a minimum of five days to a subject consuming

a Western diet reduces weight gain in the subject, as measured against a population of similar subjects consuming a Western diet without administration of the food composition.

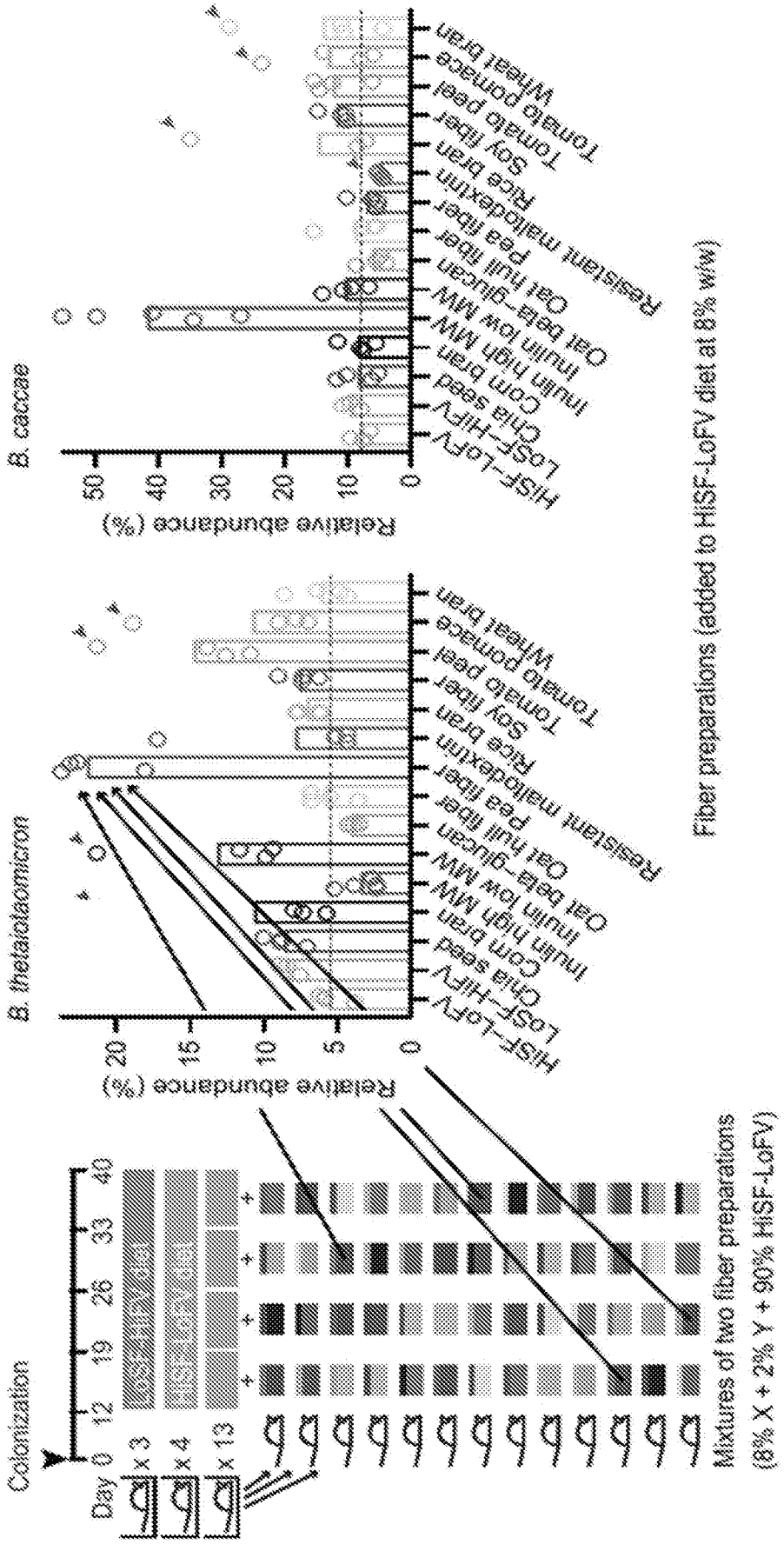

Colonization
Day
0
12
19
26
33
40
x 3
x 4
x 13
Mixtures of two fiber preparations
(8% X + 2% Y + 90% HiSF-LoFV)
B. thetaiotaomicron
Relative abundance (%)
B. caccae
Relative abundance (%)
HiSF–LoFV
LoSF–HiFV
Chia seed
Corn bran
Inulin high MW
Inulin low MW
Oat beta–glucan
Oat hull fiber
Pea fiber
Rice bran
Soy fiber
Tomato pomace
Tomato peel
Wheat bran
Resistant maltodextrin
Fiber preparations (added to HiSF-LoFV diet at 8% w/w)

FIG. 1A

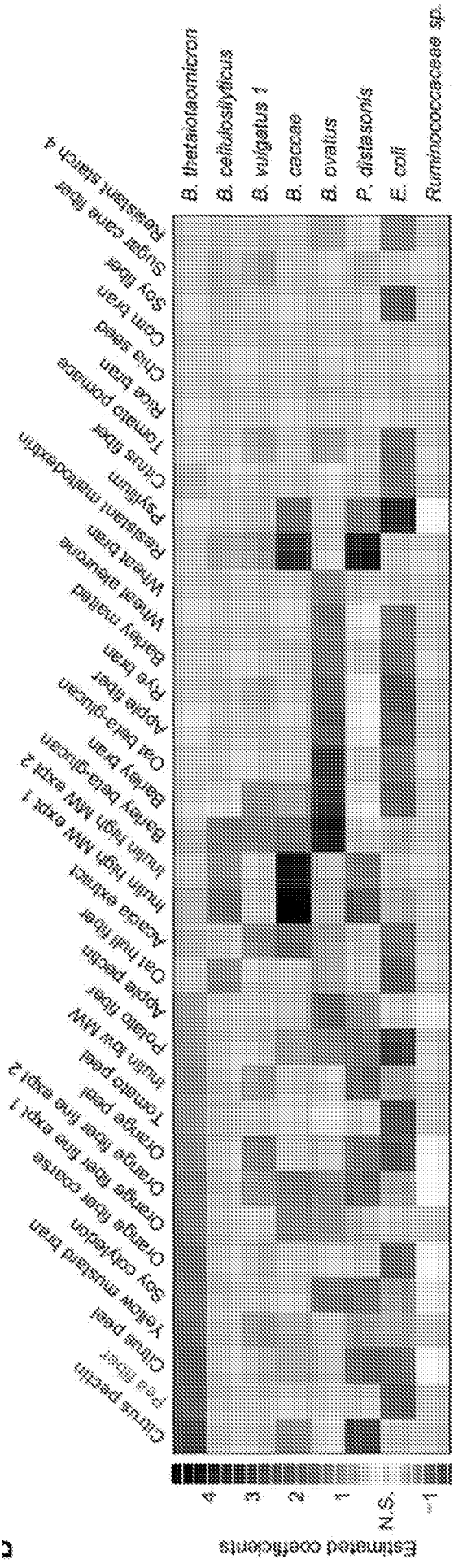

B. thetaiotaomicron
B. cellulosilyticus
B. vulgatus 1
B. caccae
B. ovatus
P. distasonis
E. coli
Ruminococcaceae sp.
Estimated coefficients
4
3
2
1
N.S.
-1
Citrus pectin
Pea fiber
Citrus peel
Yellow mustard bran
Soy cotyledon
Orange fiber coarse
Orange fiber fine expt 1
Orange fiber fine expt 2
Tomato peel
Inulin low MW
Potato fiber
Apple pectin
Oat hull fiber
Acacia extract
Inulin high MW expt 1
Barley beta-glucan high MW expt 2
Barley bran
Oat beta-glucan
Apple fiber
Rye bran
Barley rolled
Wheat aleurone
Wheat bran
Resistant maltodextrin
Psyllium
Citrus fiber
Tomato pomace
Rice bran
Chia seed
Corn bran
Soy fiber
Sugar cane fiber
Resistant starch 4

FIG. 1B

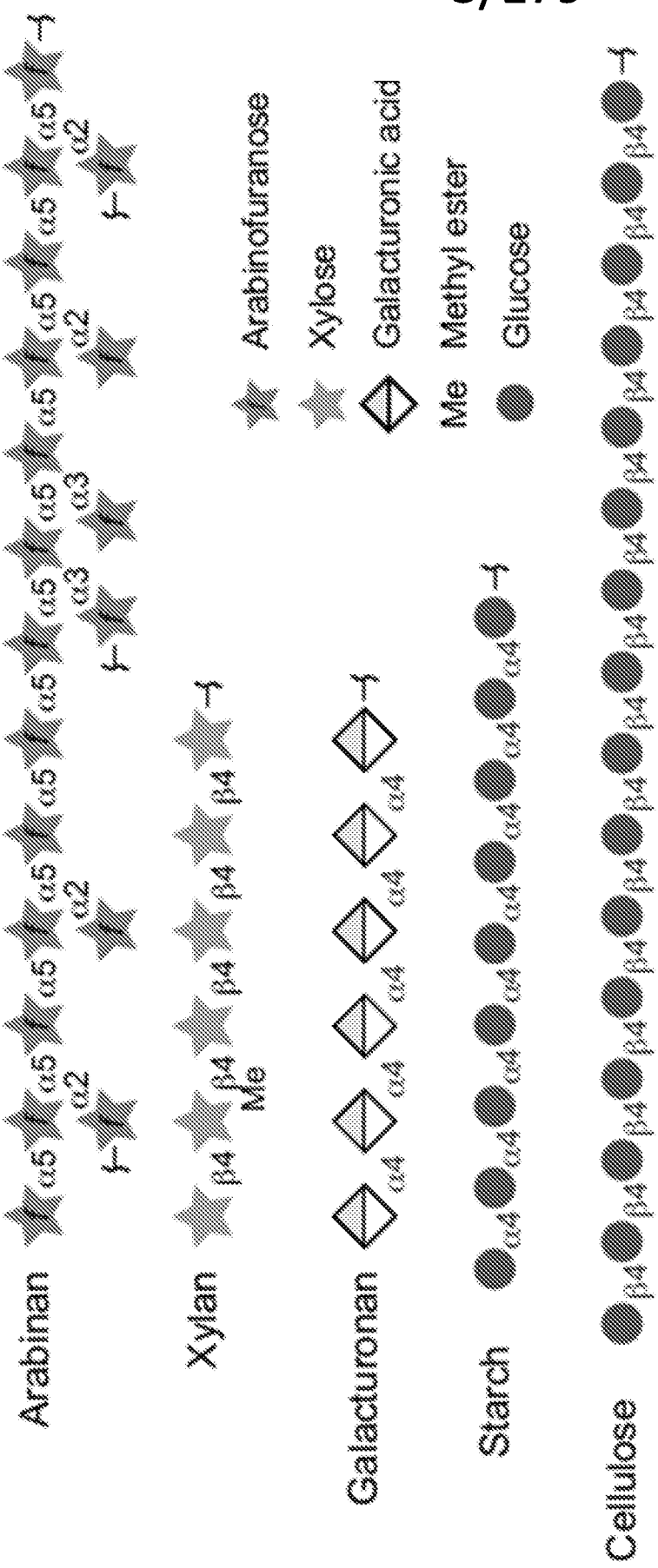

Arabinan
Xylan
Galacturonan
Starch
Cellulose
Arabinofuranose
Xylose
Galacturonic acid
Me Methyl ester
Glucose

FIG. 2A

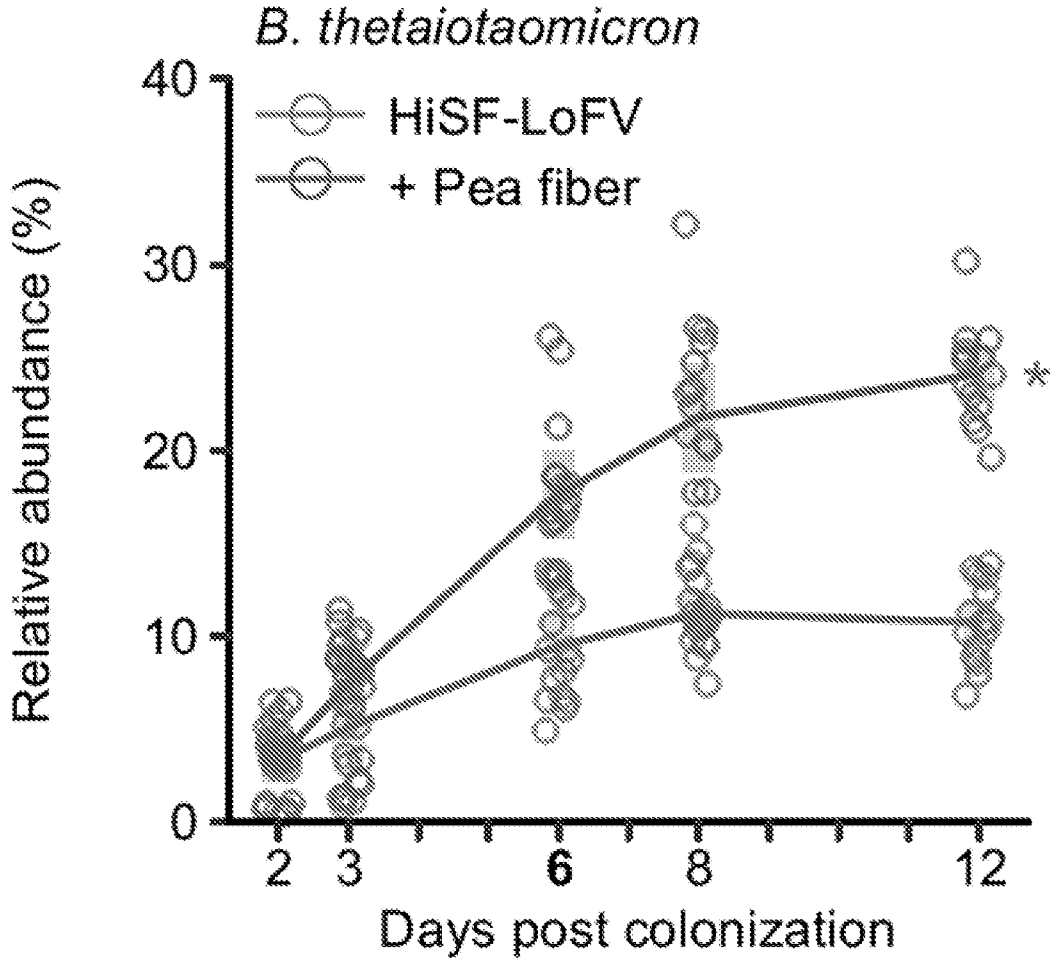

B. thetaiotaomicron
HiSF-LoFV
+ Pea fiber
Relative abundance (%)
40
30
20
10
0
2
3
6
8
12
Days post colonization
*

FIG. 2B

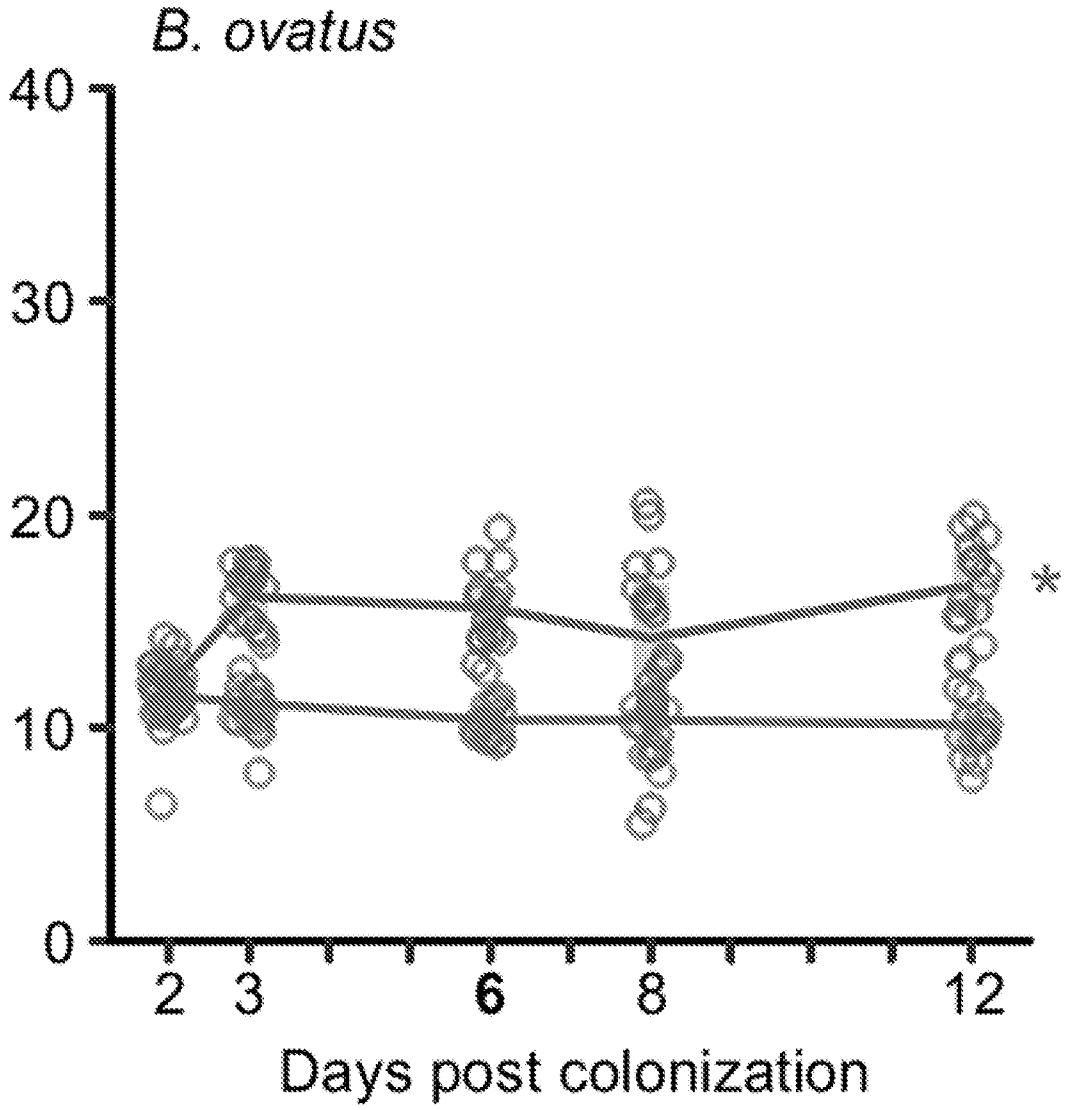


FIG. 2C

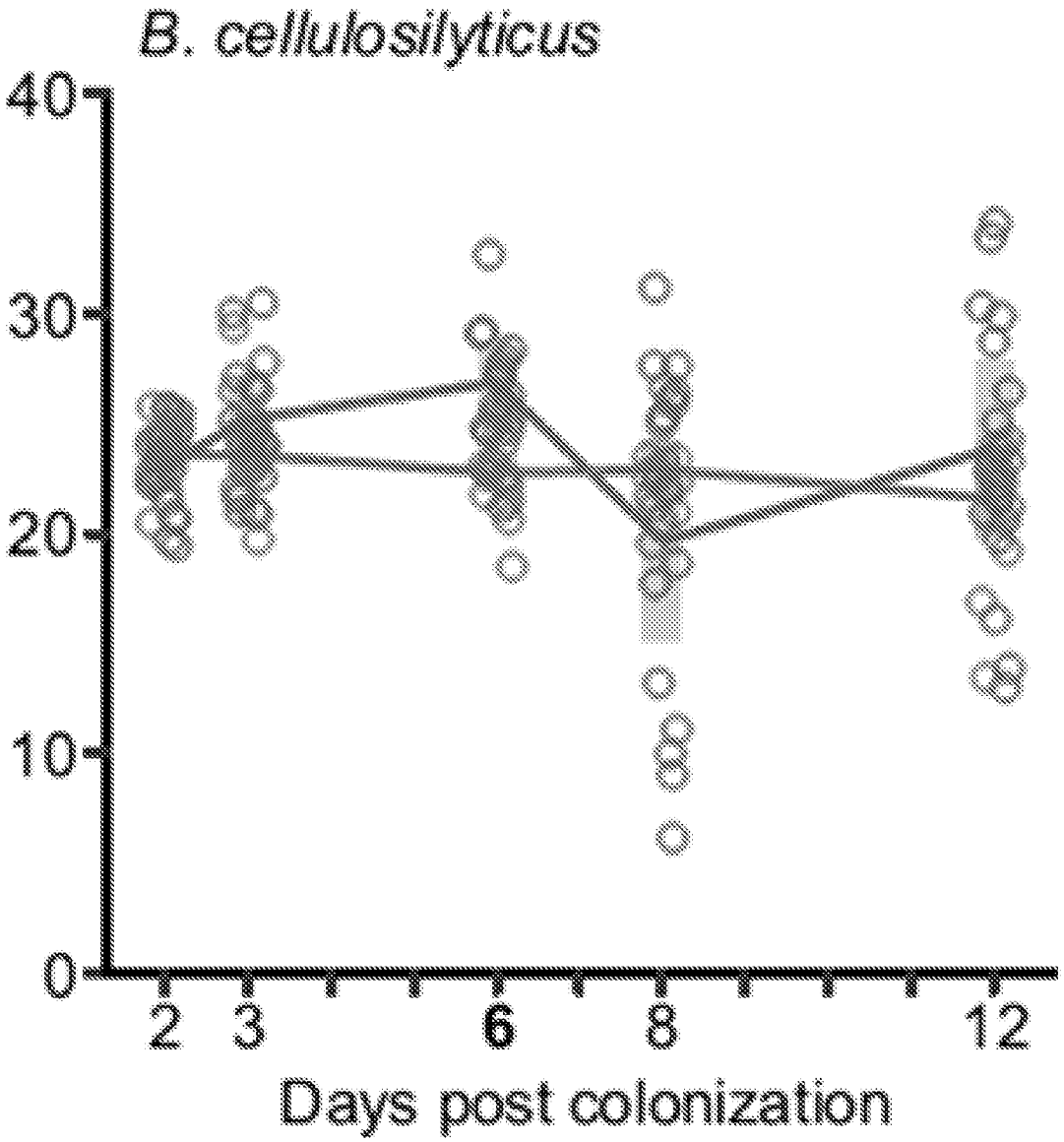

B. cellulosilyticus
40
30
20
10
0
2
3
6
8
12
Days post colonization

FIG. 2D

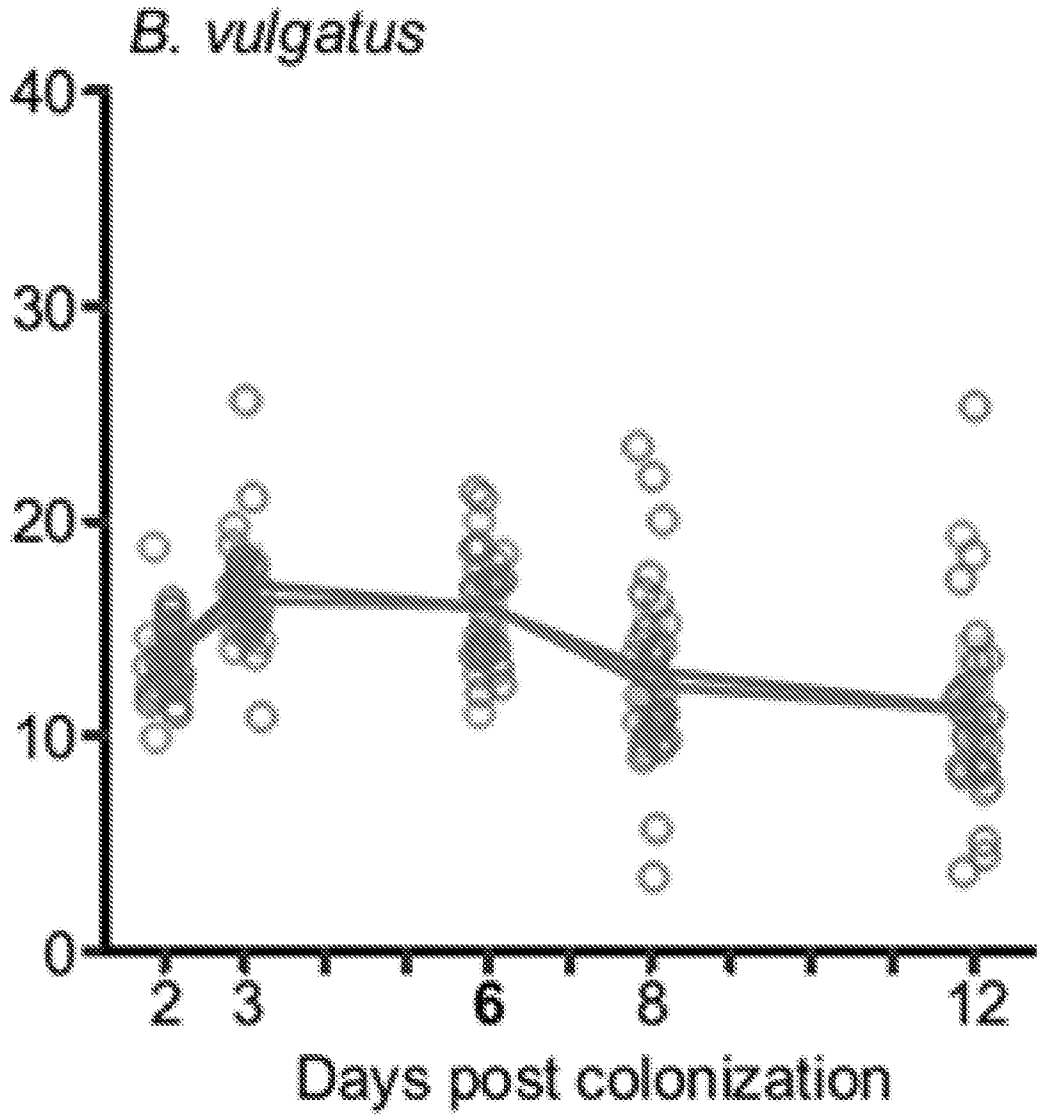


FIG. 2E

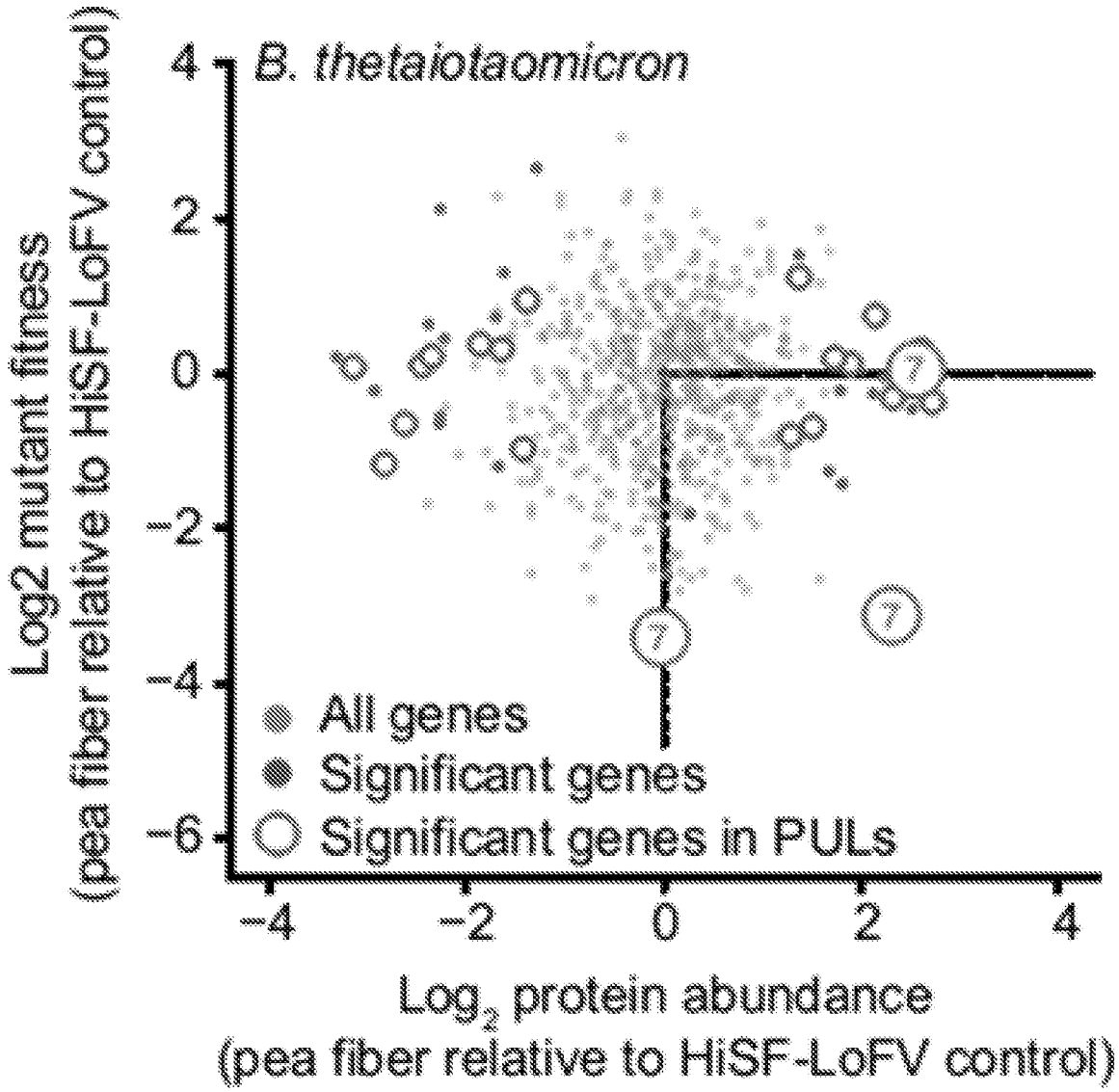

B. thetaiotaomicron
Log2 mutant fitness
(pea fiber relative to HiSF-LoFV control)
4
2
0
−2
−4
−6
All genes
Significant genes
Significant genes in PULs
−4
−2
0
2
4
Log2 protein abundance
(pea fiber relative to HiSF-LoFV control)

FIG. 2F

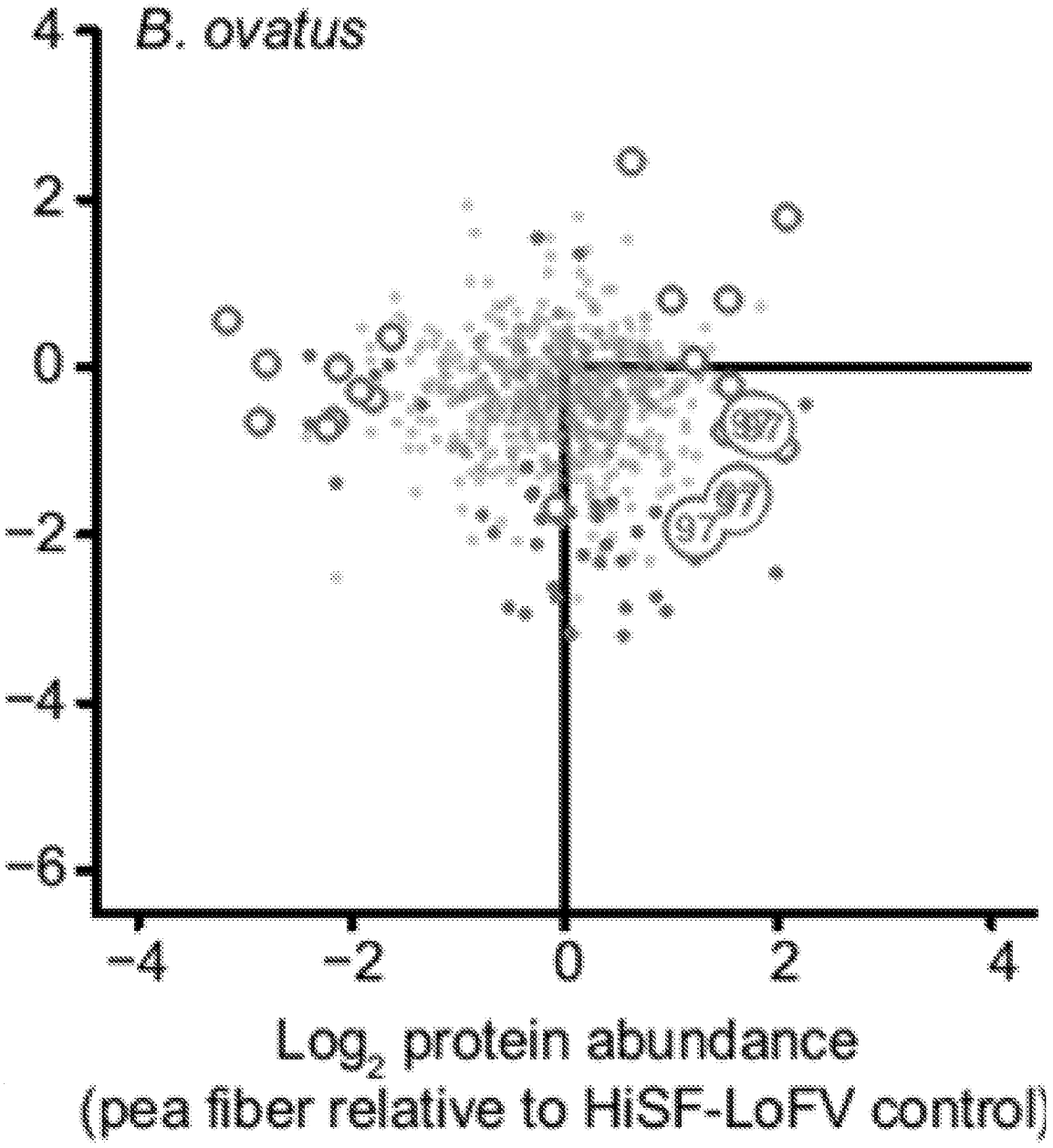

B. ovatus
4
2
0
−2
−4
−6
−4
−2
0
2
4
Log2 protein abundance
(pea fiber relative to HiSF-LoFV control)

FIG. 2G

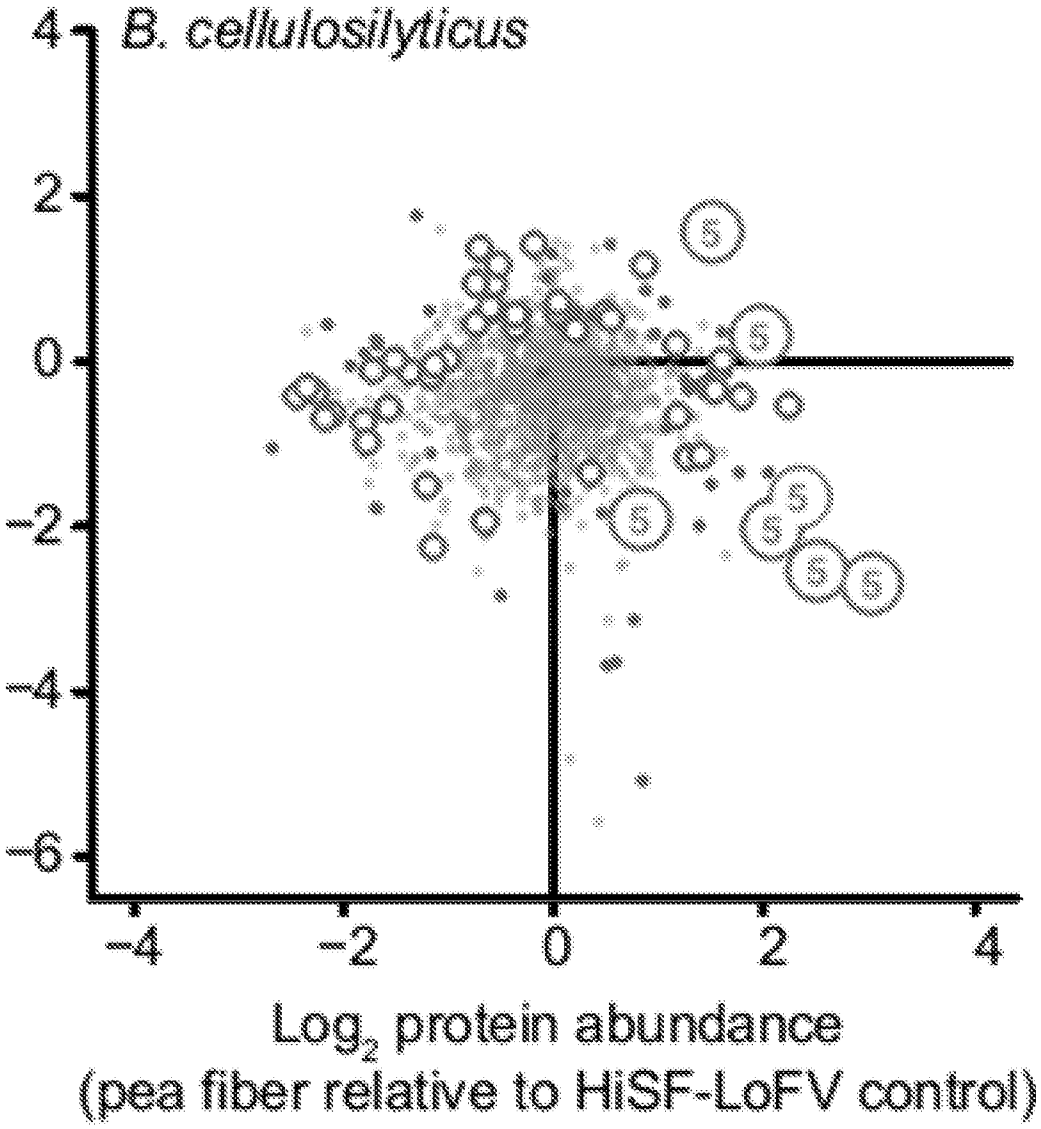

B. cellulosilyticus
4
2
0
−2
−4
−6
−4
−2
0
2
4
Log2 protein abundance
(pea fiber relative to HiSF-LoFV control)

FIG. 2H

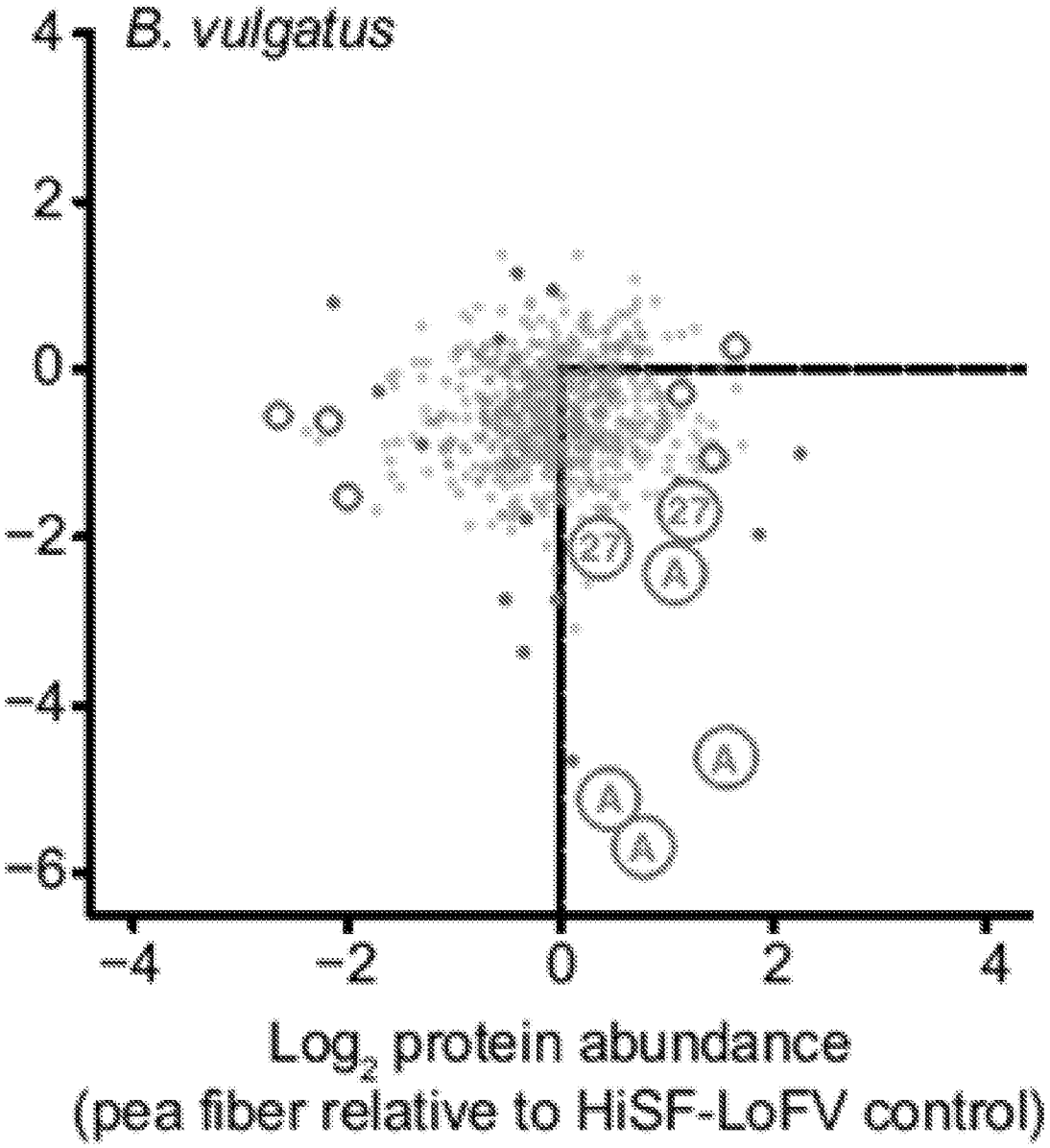

B. vulgatus
4
2
0
−2
−4
−6
−4
−2
0
2
4
Log2 protein abundance
(pea fiber relative to HiSF-LoFV control)

FIG. 2I

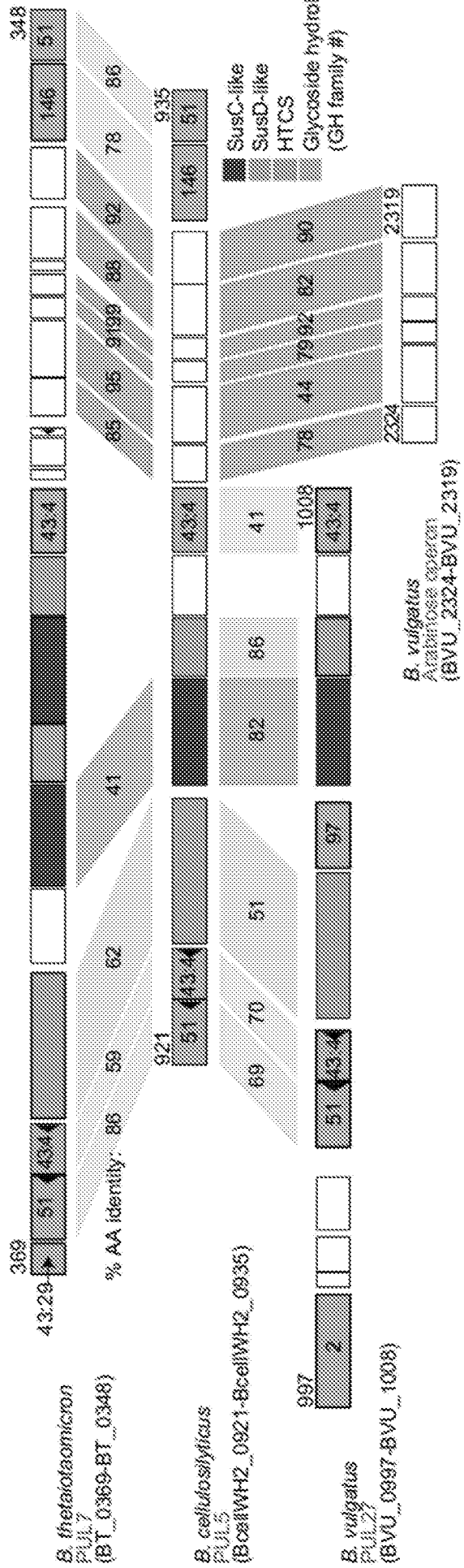

B. thetaiotaomicron
PUL7
(BT_0369-BT_0348)
B. cellulosilyticus
PUL5
(BcellWH2_0921-BcellWH2_0935)
B. vulgatus
PUL27
(BVU_0997-BVU_1008)
B. vulgatus
Arabinose operon
(BVU_2324-BVU_2319)
SusC-like
SusD-like
HTCS
Glycoside hydrolase
(GH family #)
% AA identity:

FIG. 2J

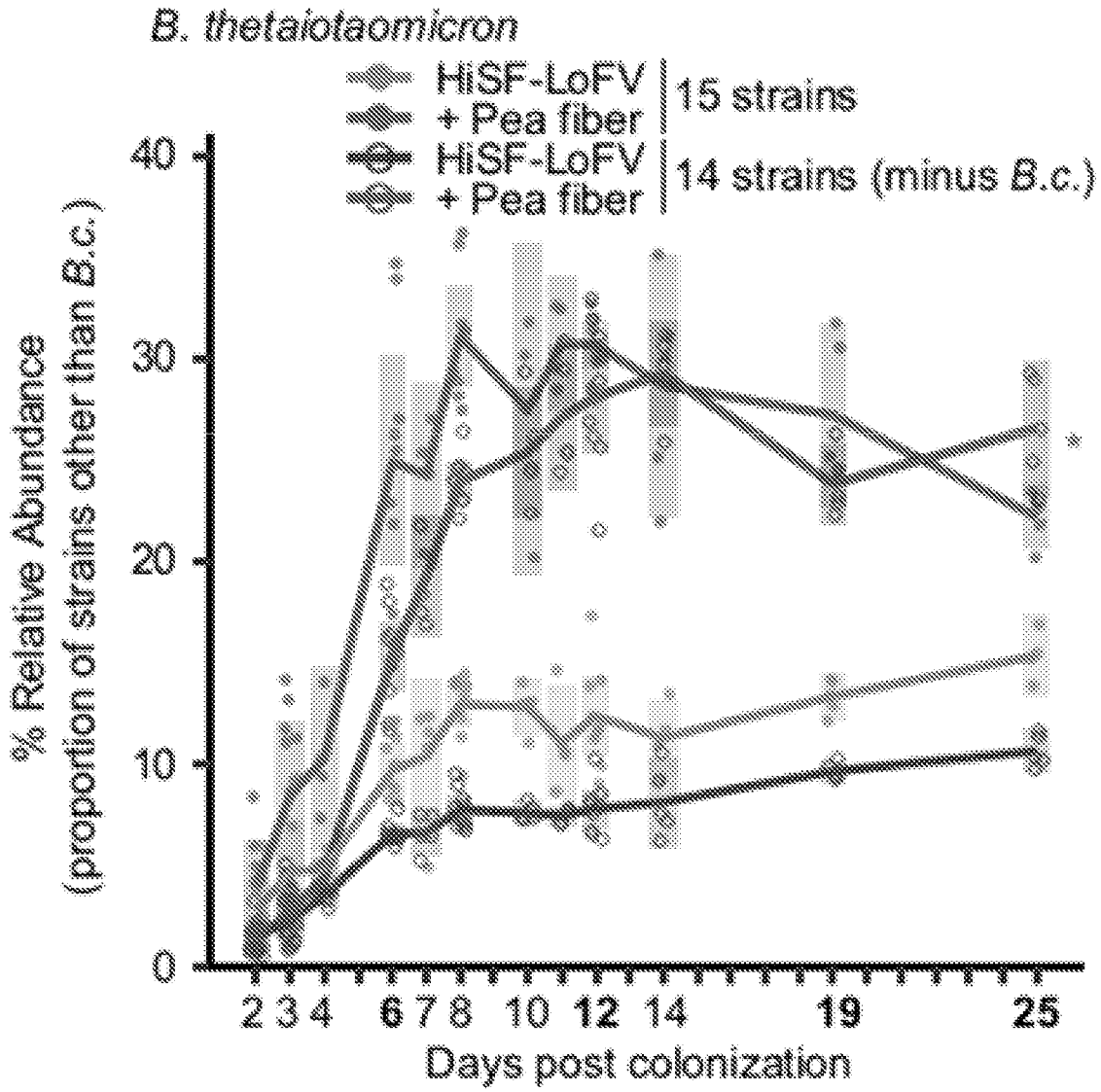

B. thetaiotaomicron
HiSF-LoFV
+ Pea fiber
15 strains
HiSF-LoFV
+ Pea fiber
14 strains (minus B.c.)
% Relative Abundance
(proportion of strains other than B.c.)
0
10
20
30
40
2 3 4 6 7 8 10 12 14 19 25
Days post colonization

FIG. 3A

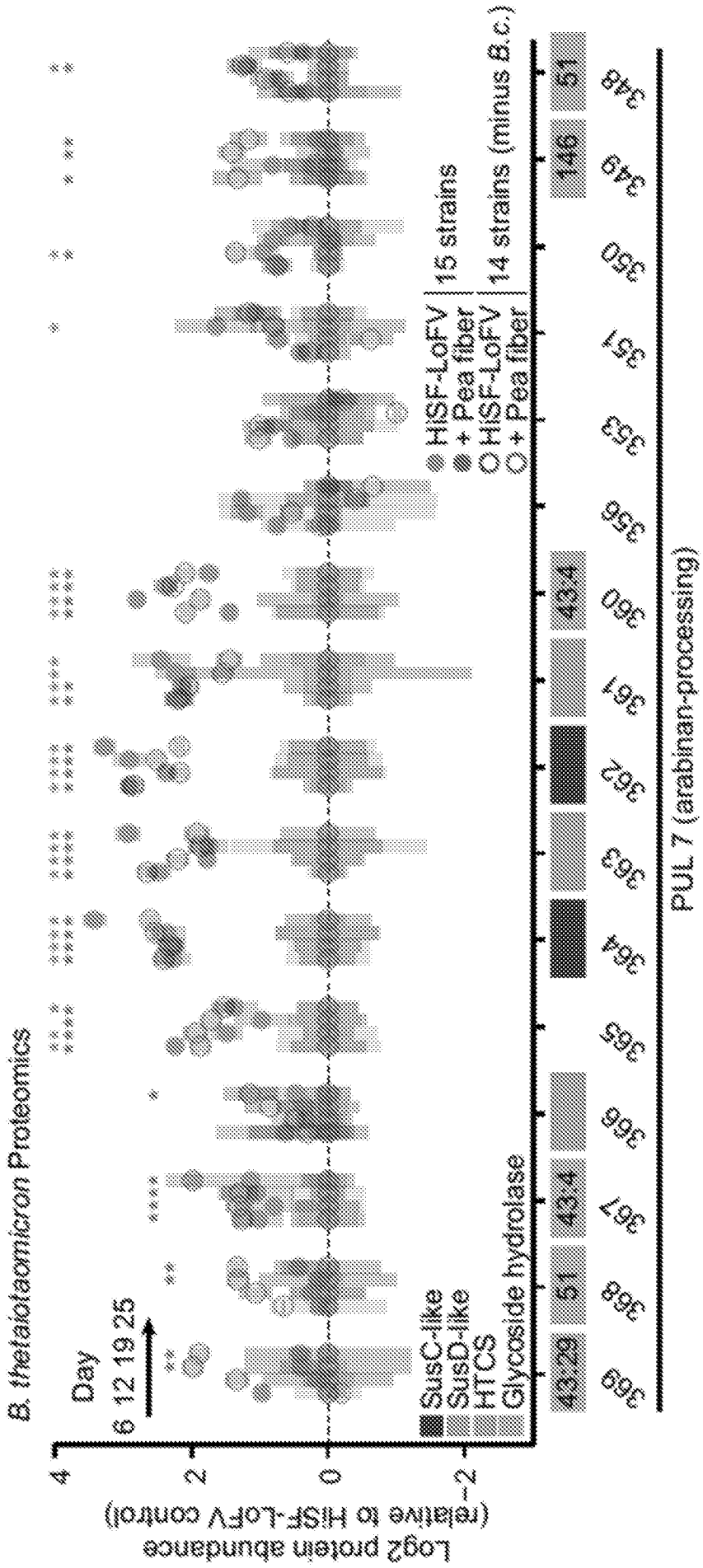

B. thetaiotaomicron Proteomics
Day
6 12 19 25
SusC-like
SusD-like
HTCS
Glycoside hydrolase
HISF-LoFV | 15 strains
+ Pea fiber
HISF-LoFV | 14 strains (minus B.c.)
+ Pea fiber
Log2 protein abundance
(relative to HISF-LoFV control)
PUL 7 (arabinan-processing)

FIG. 3B

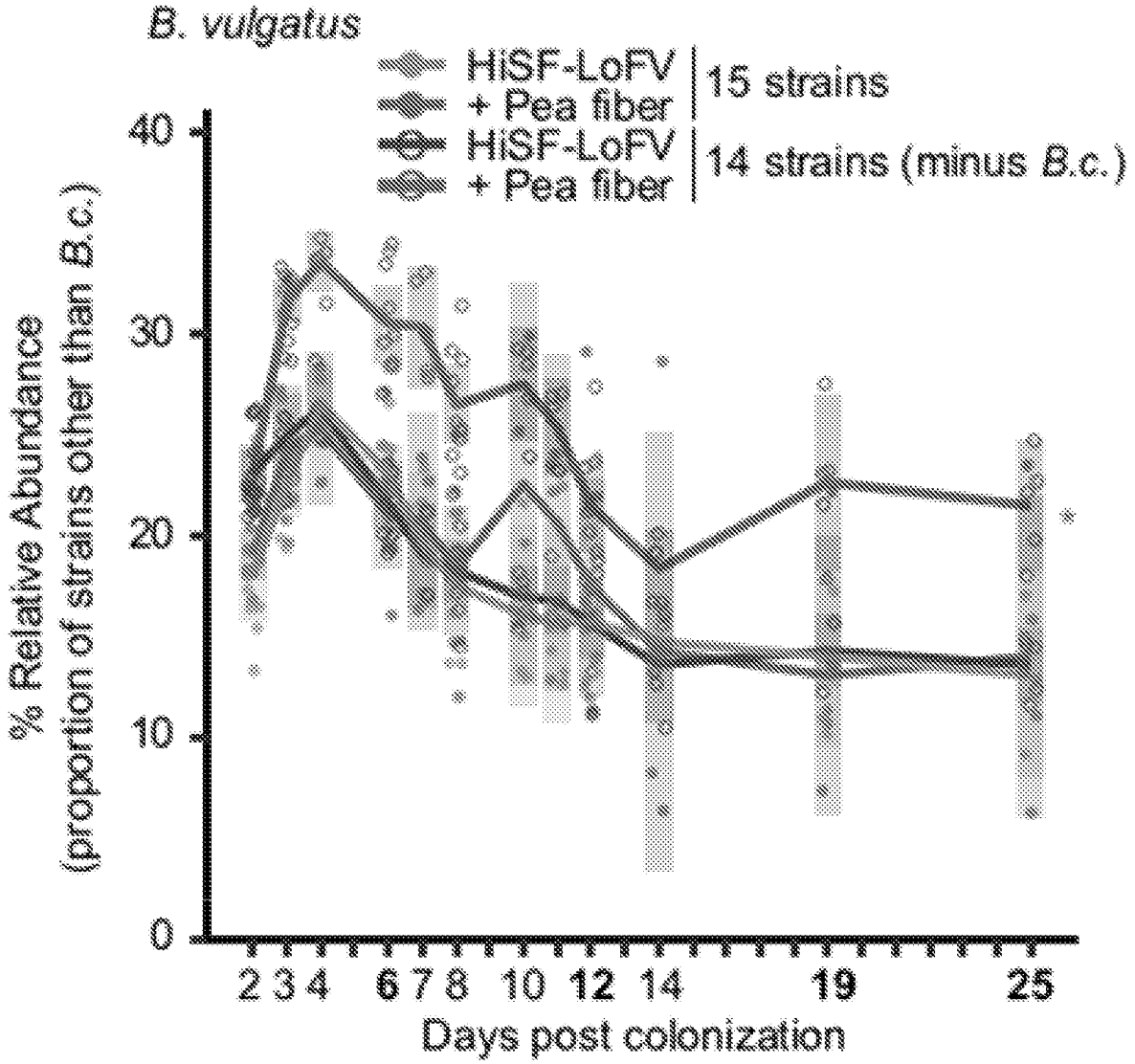

B. vulgatus
HiSF-LoFV
+ Pea fiber
15 strains
HiSF-LoFV
+ Pea fiber
14 strains (minus B.c.)
% Relative Abundance
(proportion of strains other than B.c.)
40
30
20
10
0
2 3 4 6 7 8 10 12 14 19 25
Days post colonization

FIG. 3C

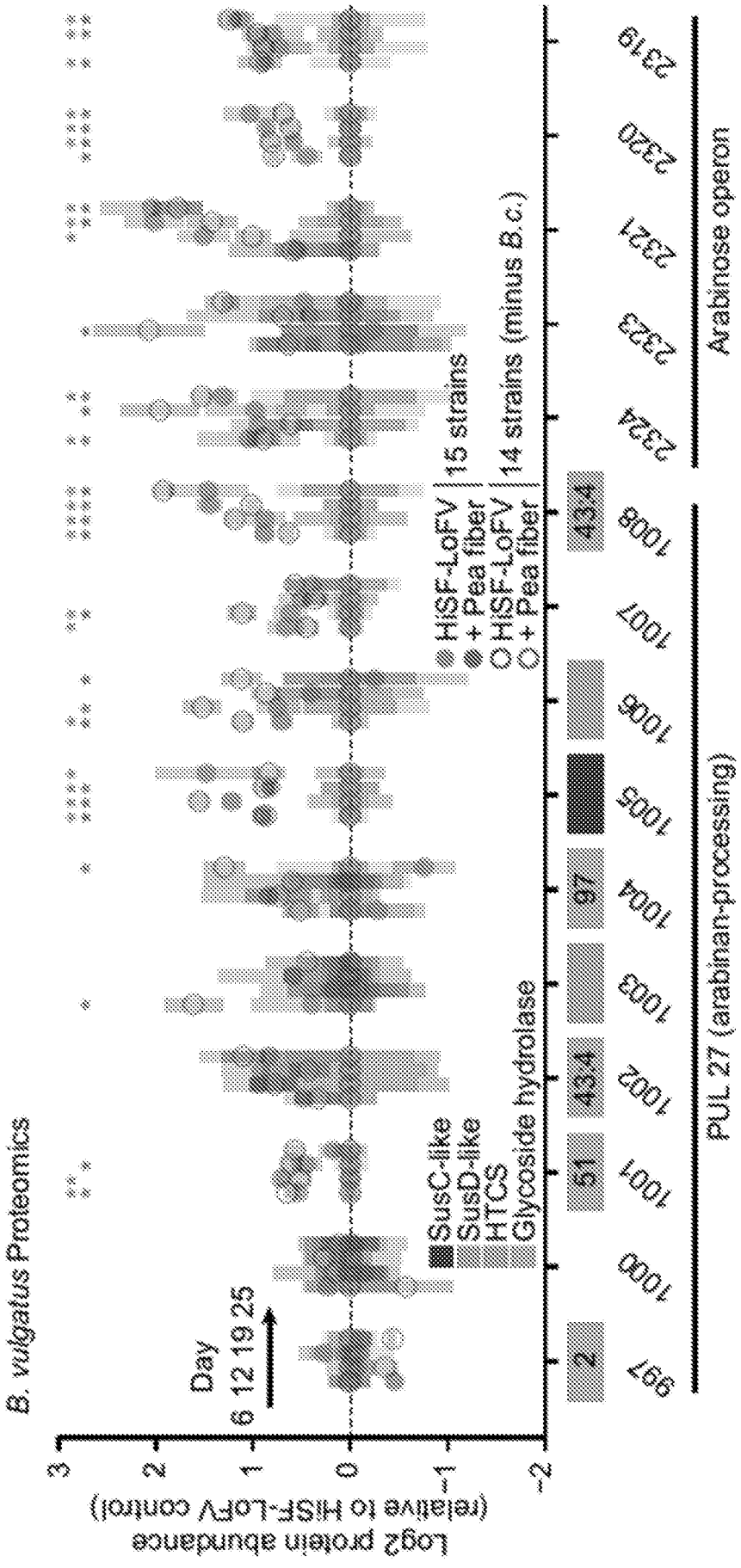

B. vulgatus Proteomics
Log2 protein abundance (relative to HiSF-LoFV control)
3
2
1
0
−1
−2
Day
6 12 19 25
SusC-like
SusD-like
HTCS
Glycoside hydrolase
HiSF-LoFV | 15 strains
+ Pea fiber
HiSF-LoFV | 14 strains (minus B.c.)
+ Pea fiber
2
51
434
97
434
PUL 27 (arabinan-processing)
997
1000
1001
1002
1003
1004
1005
1006
1007
1008
2324
2323
2321
2320
2319
Arabinose operon

FIG. 3D

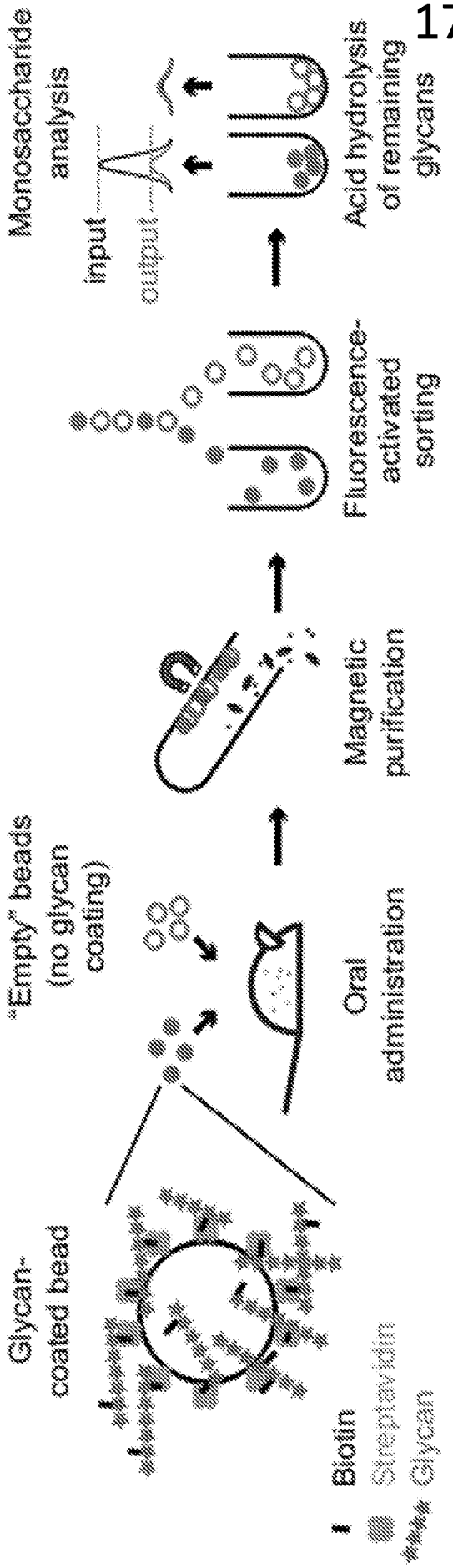

Glycan-coated bead
"Empty" beads (no glycan coating)
Oral administration
Magnetic purification
Fluorescence-activated sorting
Acid hydrolysis of remaining glycans
Monosaccharide analysis
input
output
Biotin
Streptavidin
Glycan

FIG. 4A

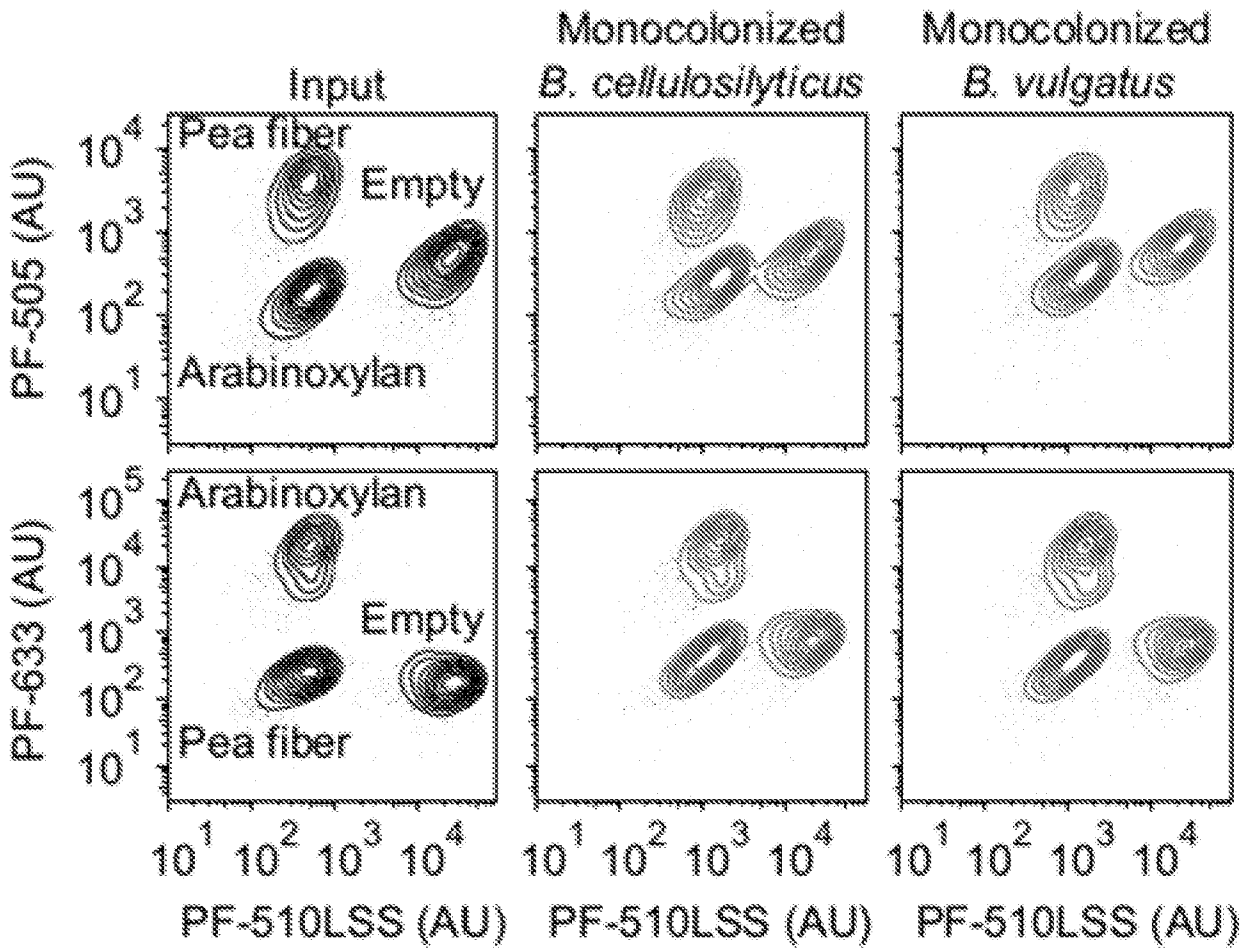

Input
Monocolonized
B. cellulosilyticus
Monocolonized
B. vulgatus
Pea fiber
Empty
Arabinoxylan
Arabinoxylan
Empty
Pea fiber
PF-505 (AU)
PF-633 (AU)
PF-510LSS (AU)
PF-510LSS (AU)
PF-510LSS (AU)

FIG. 4B

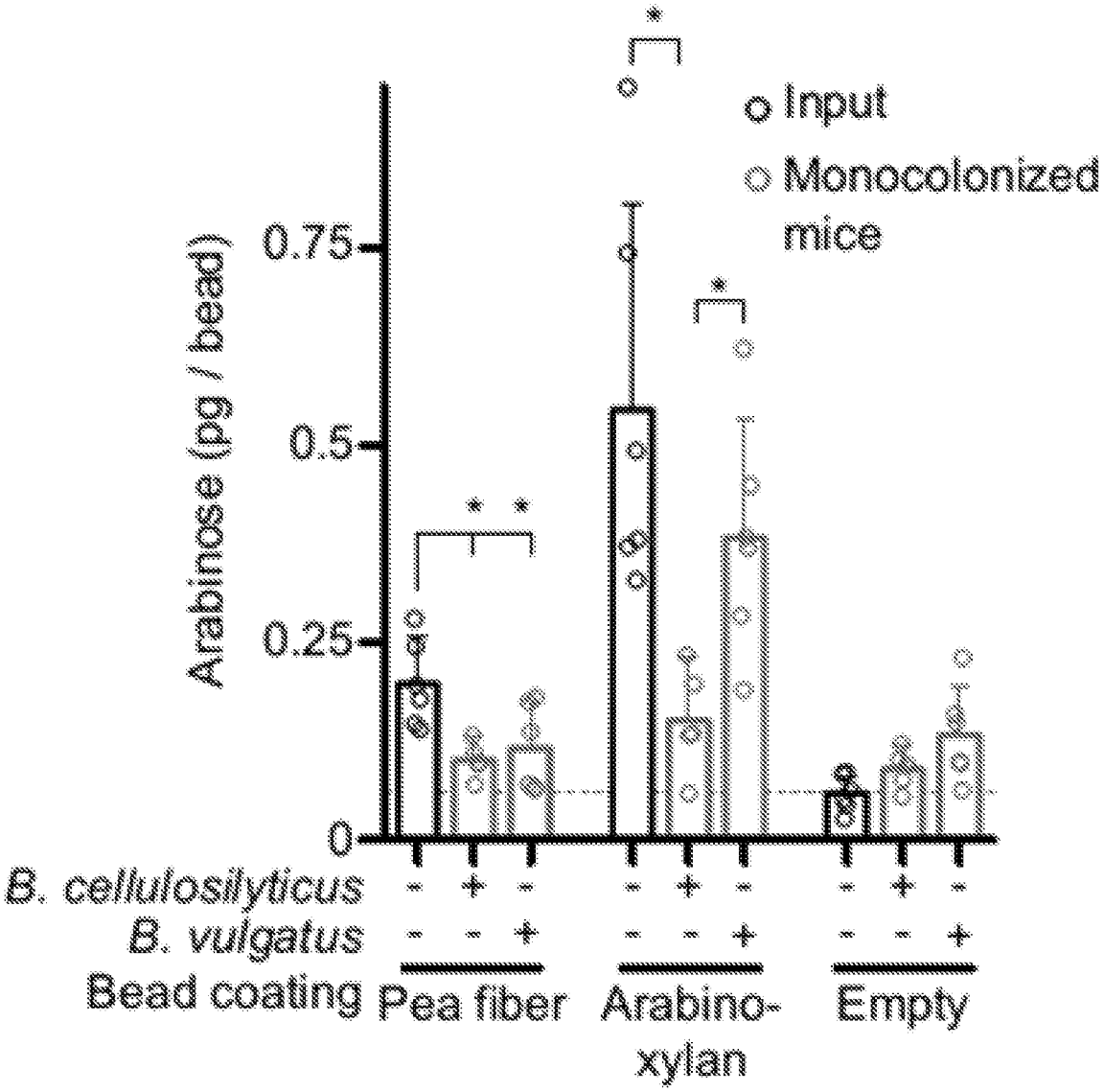

Input
Monocolonized mice
Arabinose (pg / bead)
0.75
0.5
0.25
0
B. cellulosilyticus
B. vulgatus
Bead coating
Pea fiber
Arabino-xylan
Empty

FIG. 4C

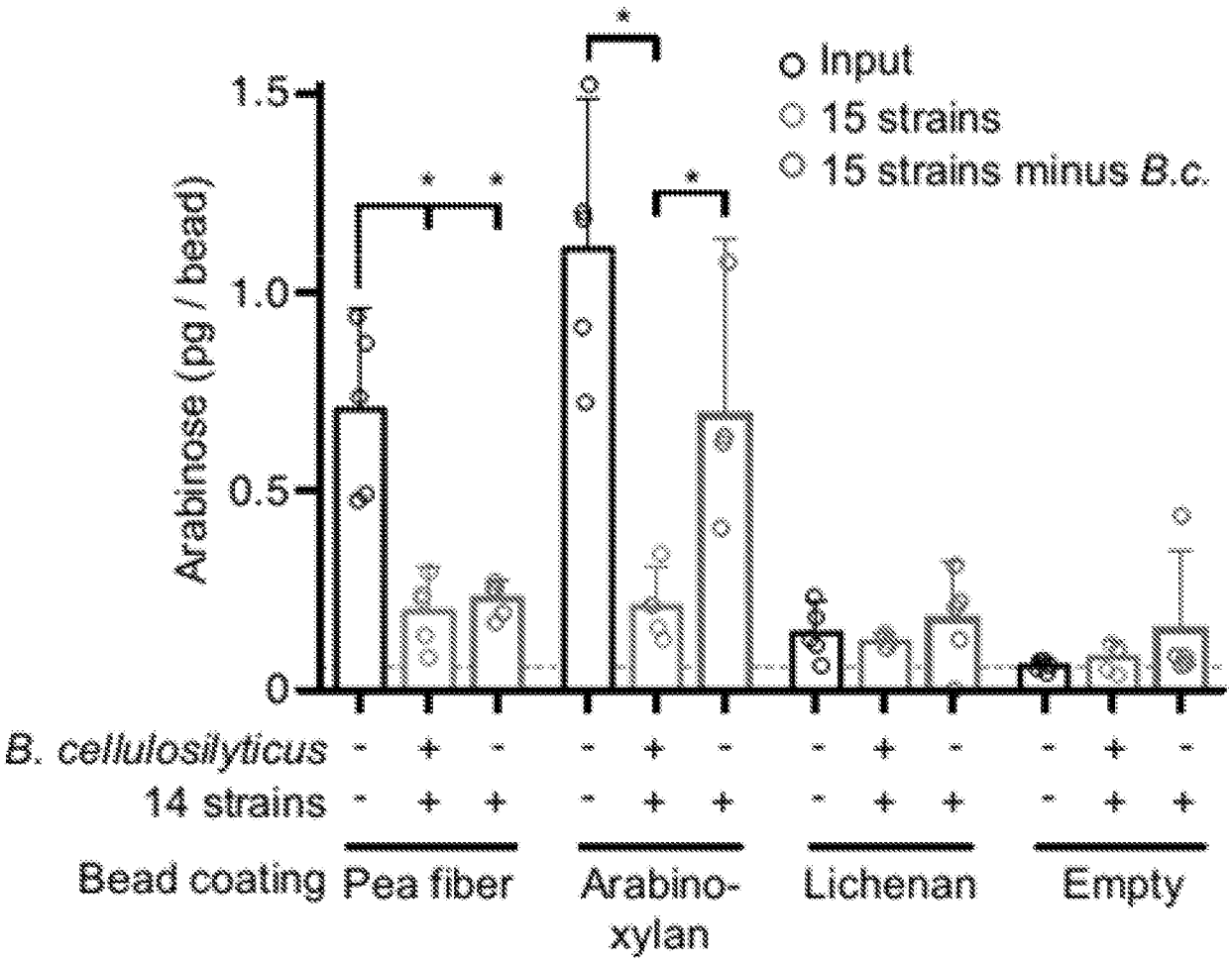

Input
15 strains
15 strains minus B.c.
Arabinose (pg / bead)
1.5
1.0
0.5
0
*
*
*
*
B. cellulosilyticus - + - - + - - + - - + -
14 strains - + + - + + - + + - + +
Bead coating
Pea fiber
Arabino-xylan
Lichenan
Empty

FIG. 4D

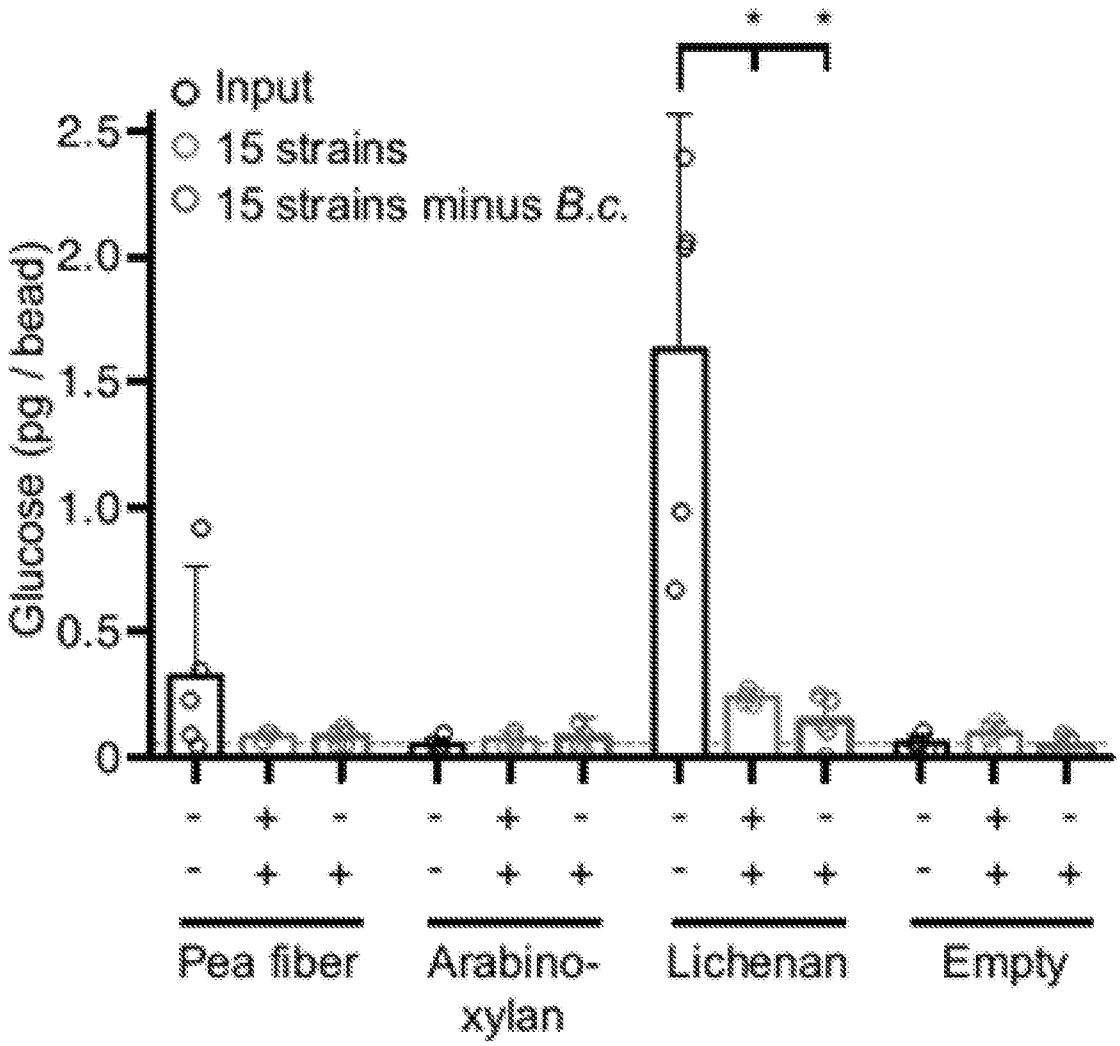

Input
15 strains
15 strains minus B.c.
Glucose (pg / bead)
2.5
2.0
1.5
1.0
0.5
0
* *
- + - - + - - + - - + -
- + + - + + - + + - + +
Pea fiber
Arabino-xylan
Lichenan
Empty

FIG. 4E

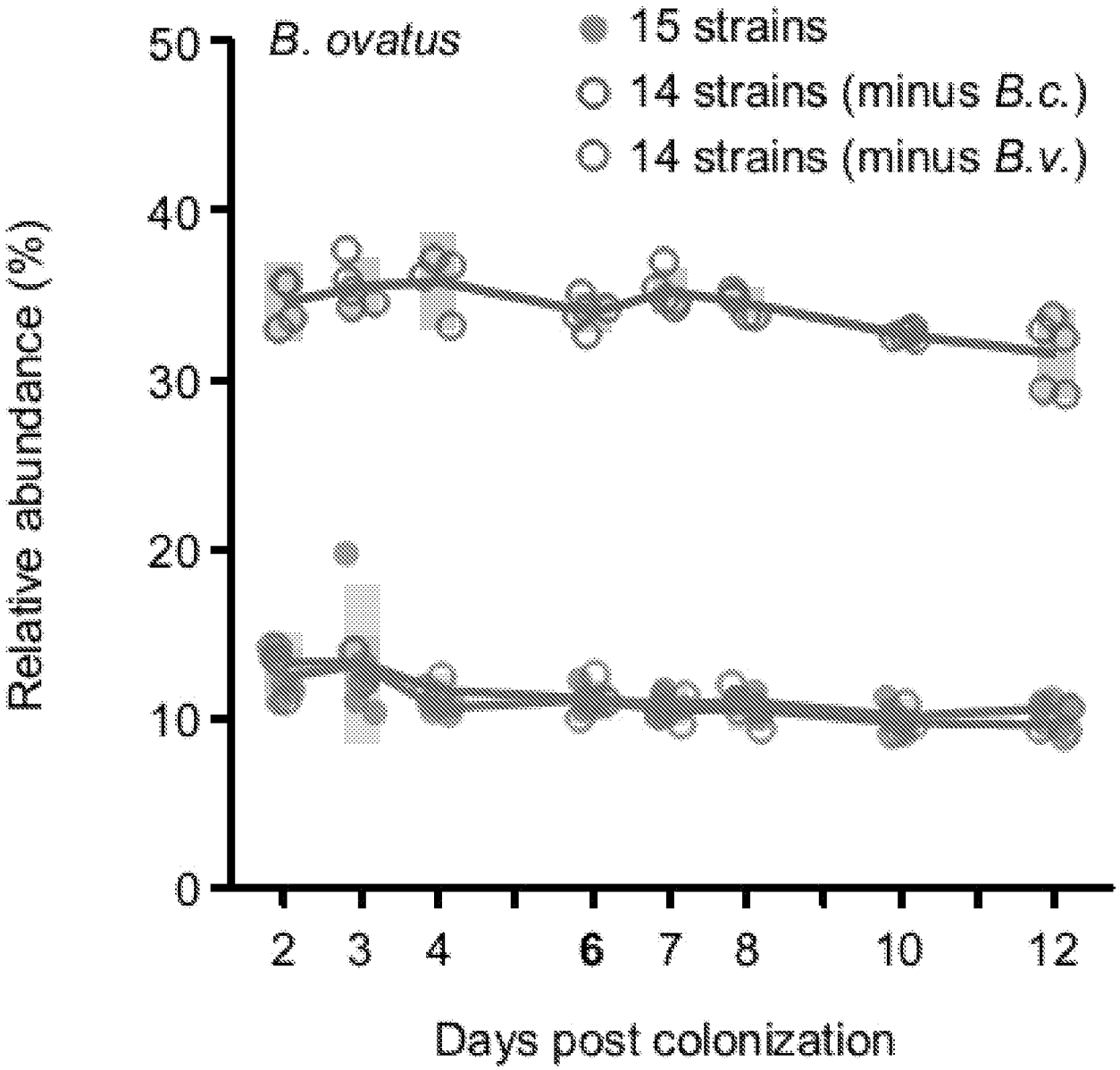

B. ovatus
15 strains
14 strains (minus B.c.)
14 strains (minus B.v.)
Relative abundance (%)
50
40
30
20
10
0
2
3
4
6
7
8
10
12
Days post colonization

FIG. 5A

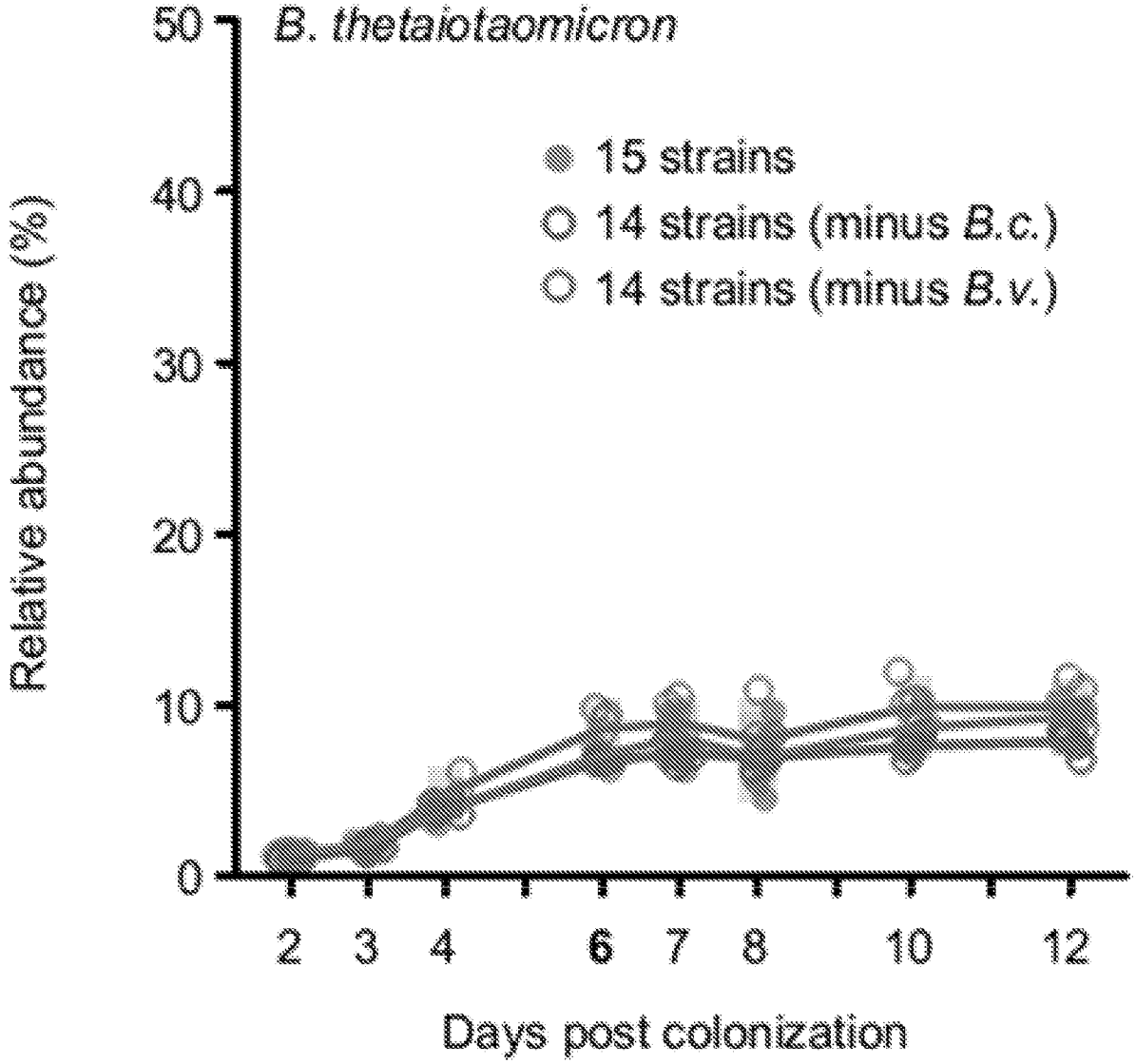

B. thetaiotaomicron
15 strains
14 strains (minus B.c.)
14 strains (minus B.v.)
Relative abundance (%)
50
40
30
20
10
0
2
3
4
6
7
8
10
12
Days post colonization

FIG. 5B

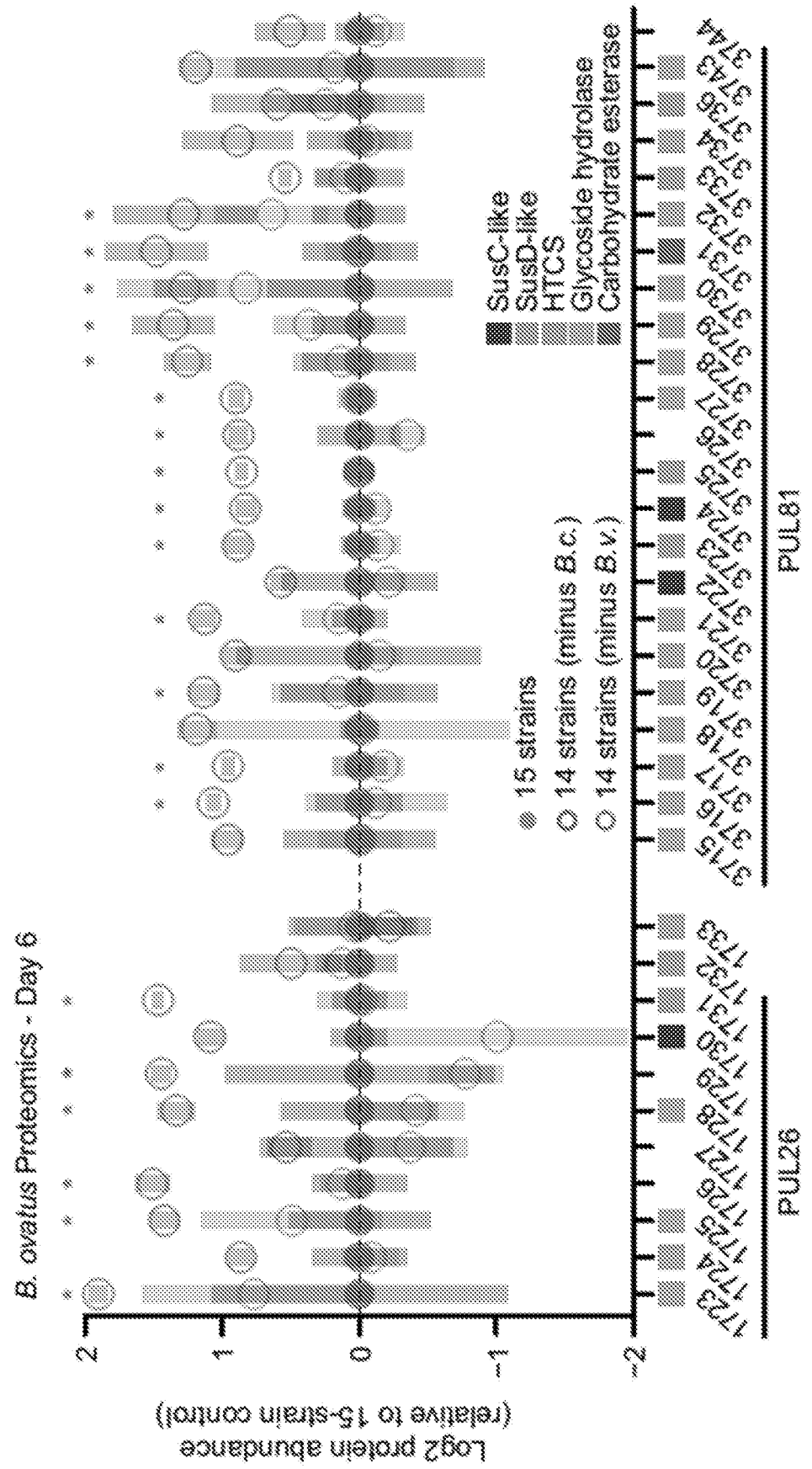

B. ovatus Proteomics - Day 6
Log2 protein abundance
(relative to 15-strain control)
2
1
0
-1
-2
15 strains
14 strains (minus B.c.)
14 strains (minus B.v.)
SusC-like
SusD-like
HTCS
Glycoside hydrolase
Carbohydrate esterase
PUL29
PUL81

FIG. 5C

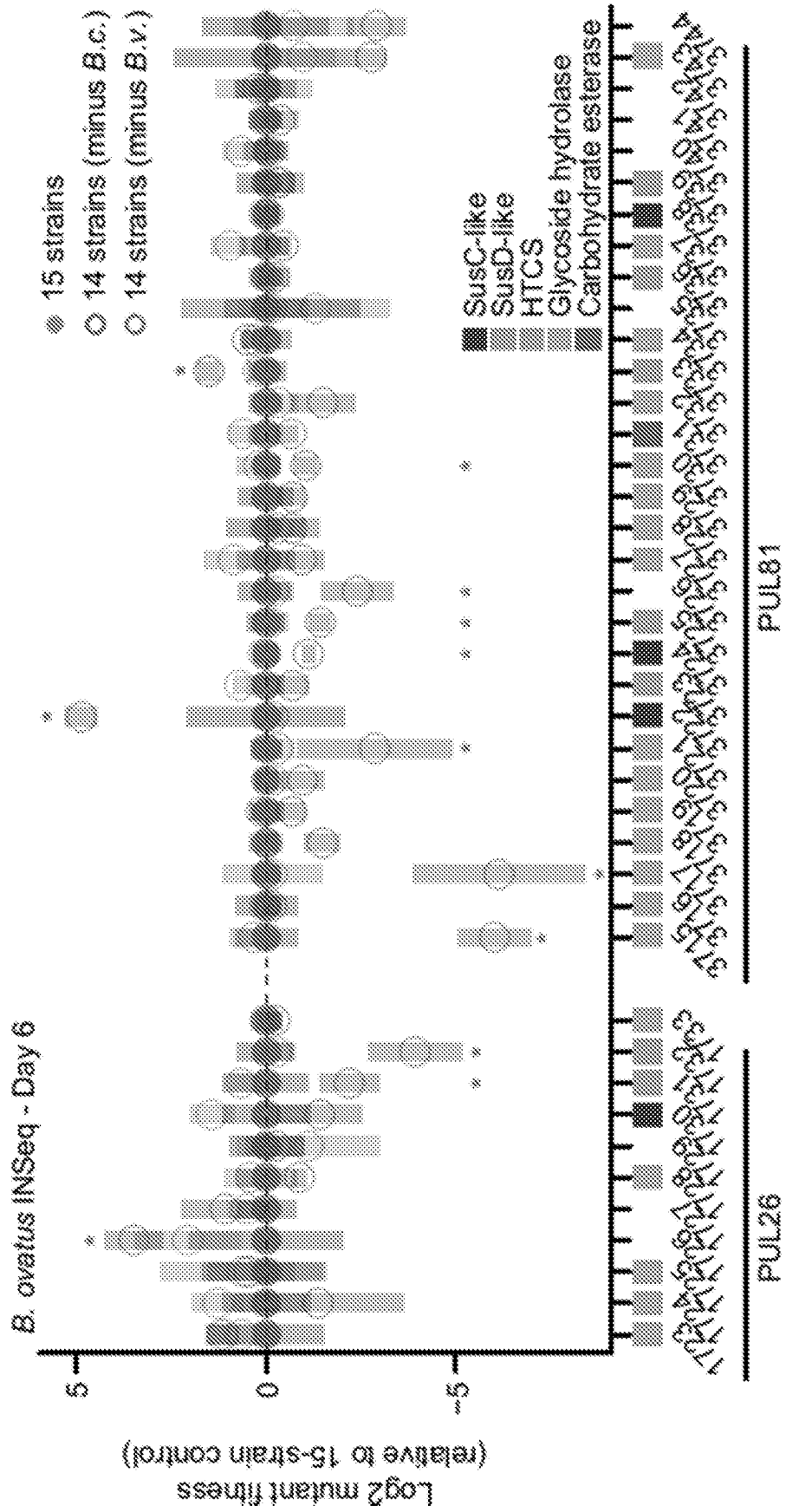

B. ovatus INSeq - Day 6
Log2 mutant fitness
(relative to 15-strain control)
5
0
-5
15 strains
14 strains (minus B.c.)
14 strains (minus B.v.)
SusC-like
SusD-like
HTCS
Glycoside hydrolase
Carbohydrate esterase
PUL26
PUL81

FIG. 5D

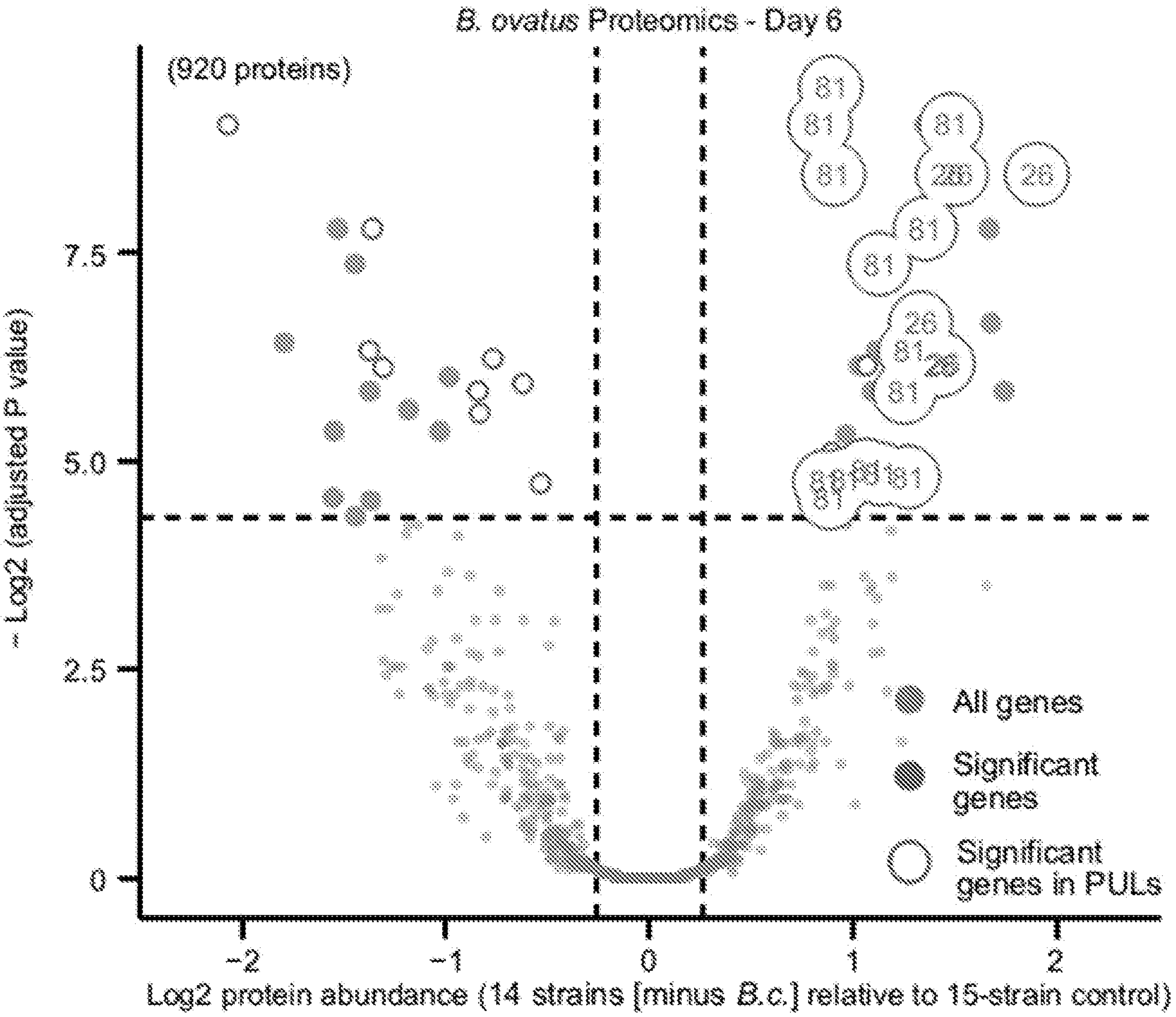

B. ovatus Proteomics - Day 6
(920 proteins)
- Log2 (adjusted P value)
Log2 protein abundance (14 strains [minus B.c.] relative to 15-strain control)
All genes
Significant genes
Significant genes in PULs

FIG. 5E

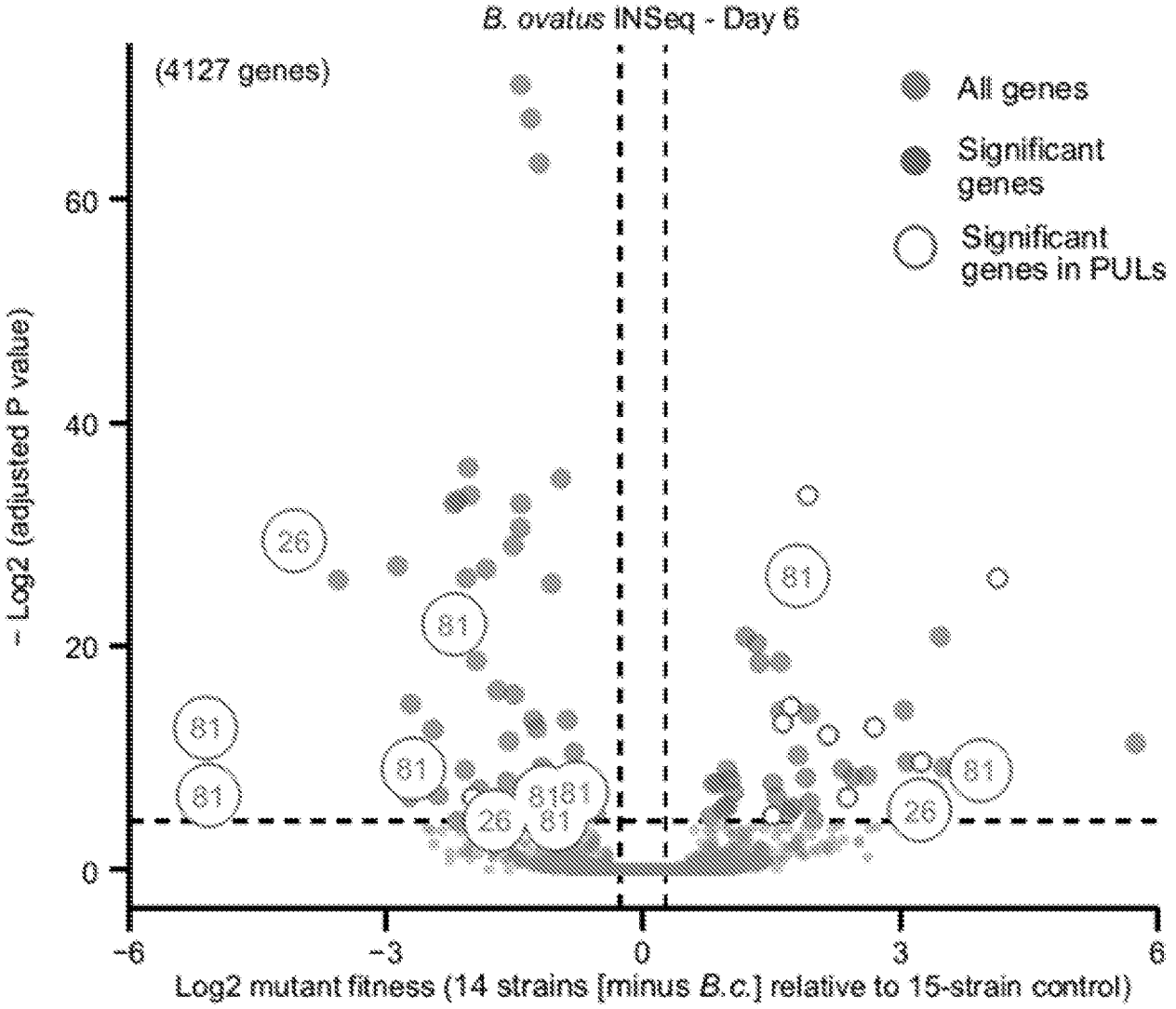

B. ovatus INSeq - Day 6
(4127 genes)
All genes
Significant genes
Significant genes in PULs
60
40
20
0
~ Log2 (adjusted P value)
26
81
81
81
81
81
8181
81
26
26
81
-6
-3
0
3
6
Log2 mutant fitness (14 strains [minus B.c.] relative to 15-strain control)

FIG. 5F

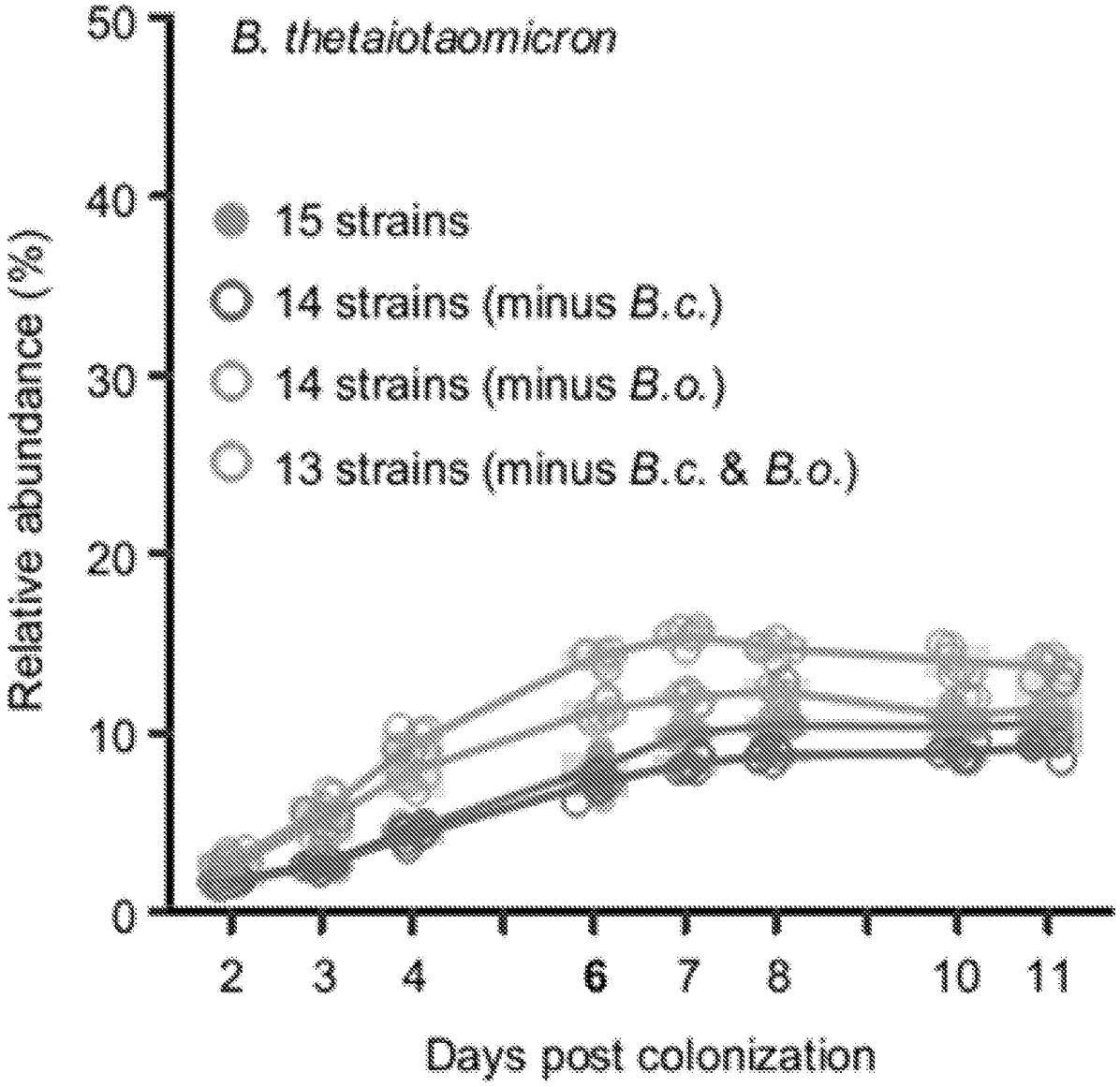

B. thetaiotaomicron
15 strains
14 strains (minus B.c.)
14 strains (minus B.o.)
13 strains (minus B.c. & B.o.)
Relative abundance (%)
50
40
30
20
10
0
2
3
4
6
7
8
10
11
Days post colonization

FIG. 6A

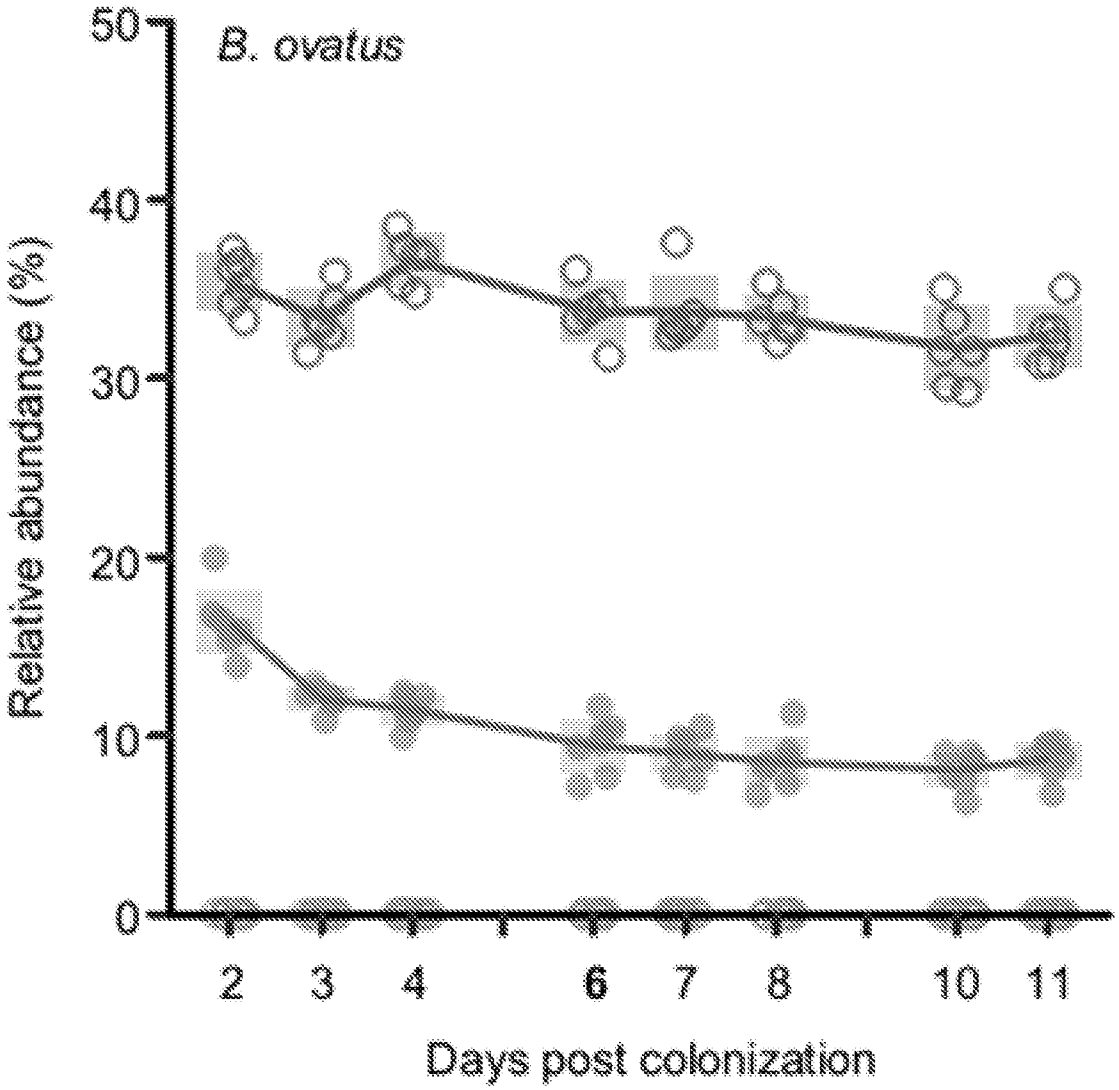

B. ovatus
Relative abundance (%)
50
40
30
20
10
0
2
3
4
6
7
8
10
11
Days post colonization

FIG. 6B

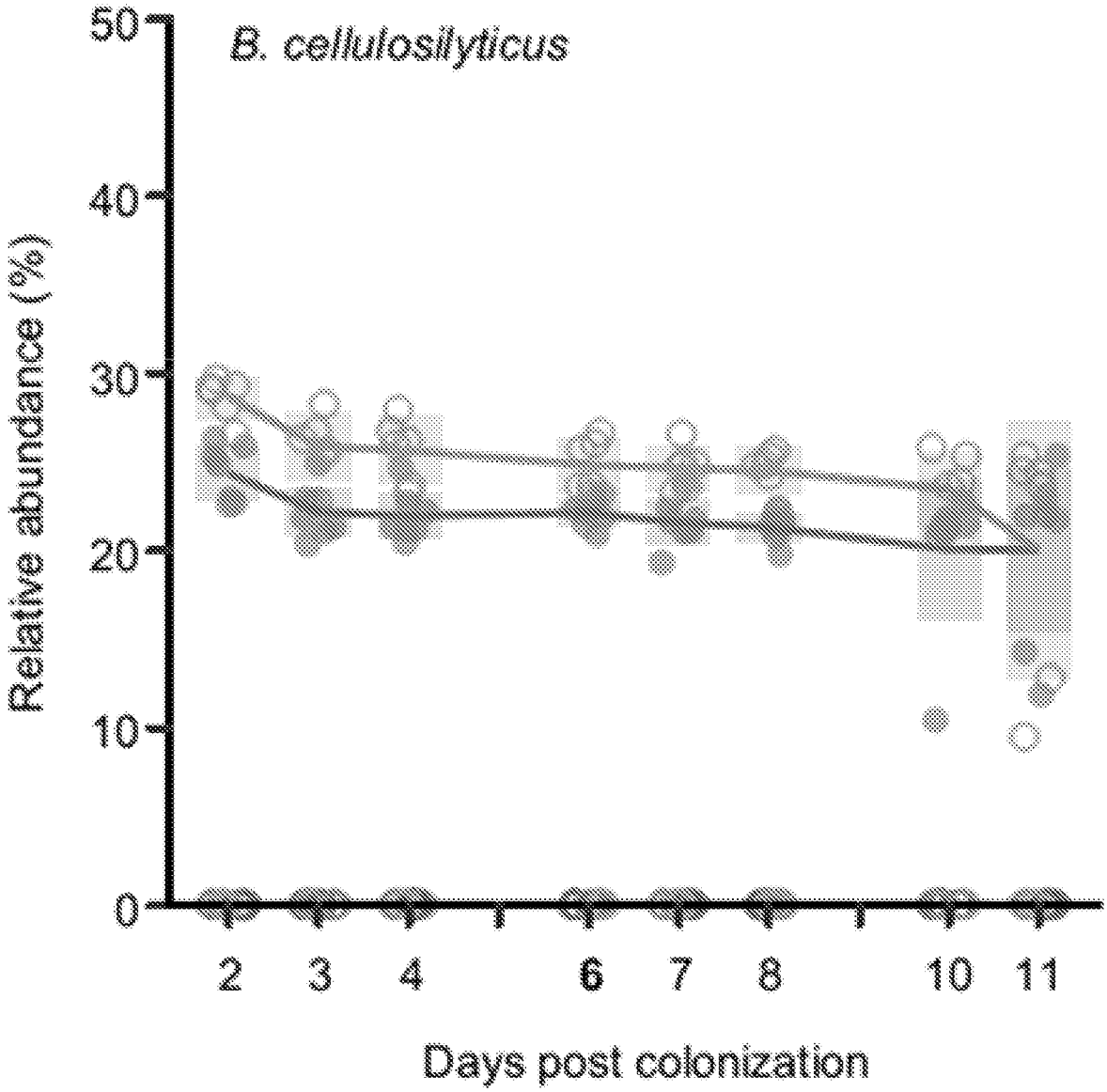

B. cellulosilyticus
Relative abundance (%)
50
40
30
20
10
0
2
3
4
6
7
8
10
11
Days post colonization

FIG. 6C

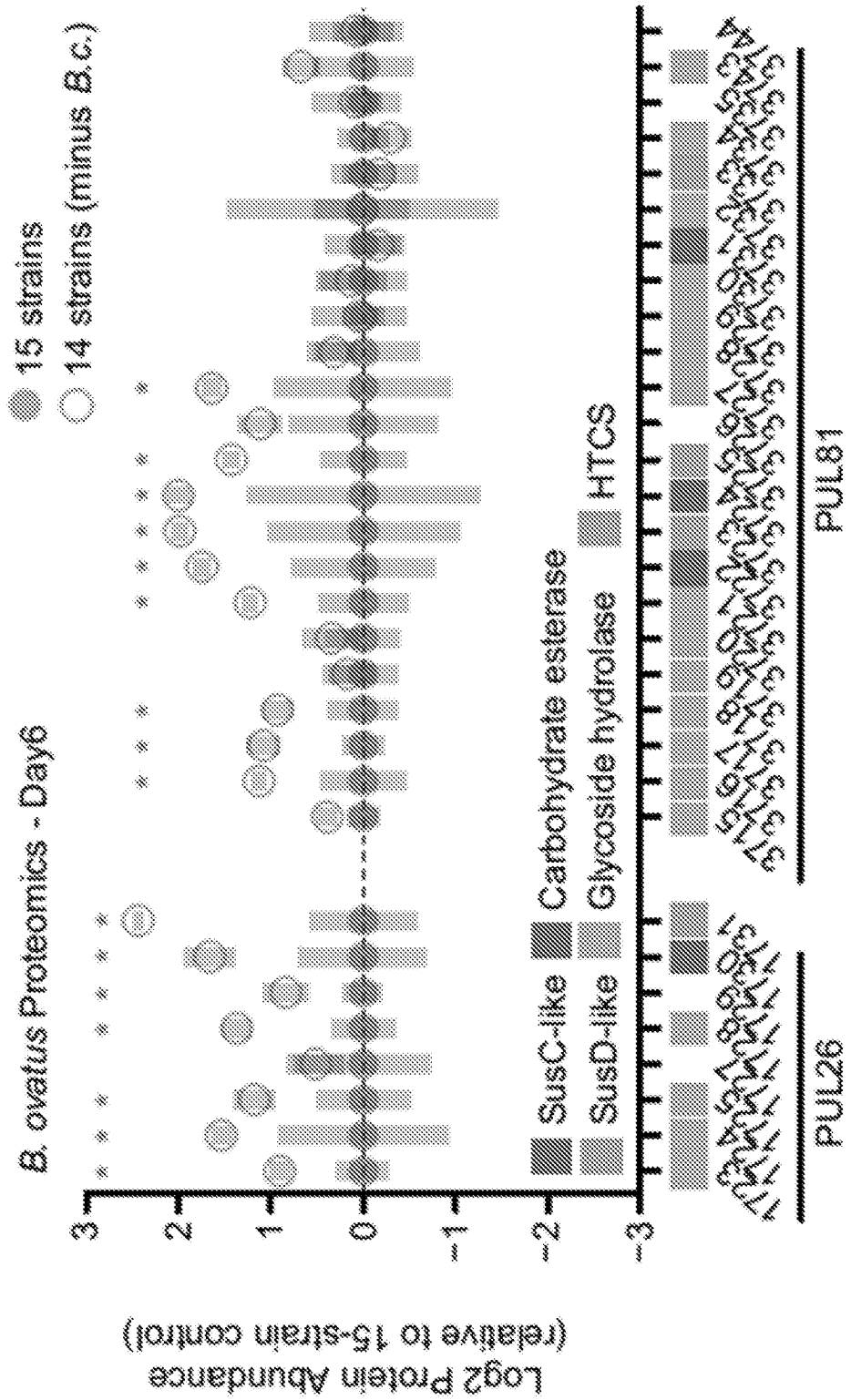

B. ovatus Proteomics - Day6
15 strains
14 strains (minus B.c.)
Log2 Protein Abundance
(relative to 15-strain control)
3
2
1
0
−1
−2
−3
SusC-like
SusD-like
Carbohydrate esterase
Glycoside hydrolase
HTCS
PUL26
PUL81

FIG. 6D

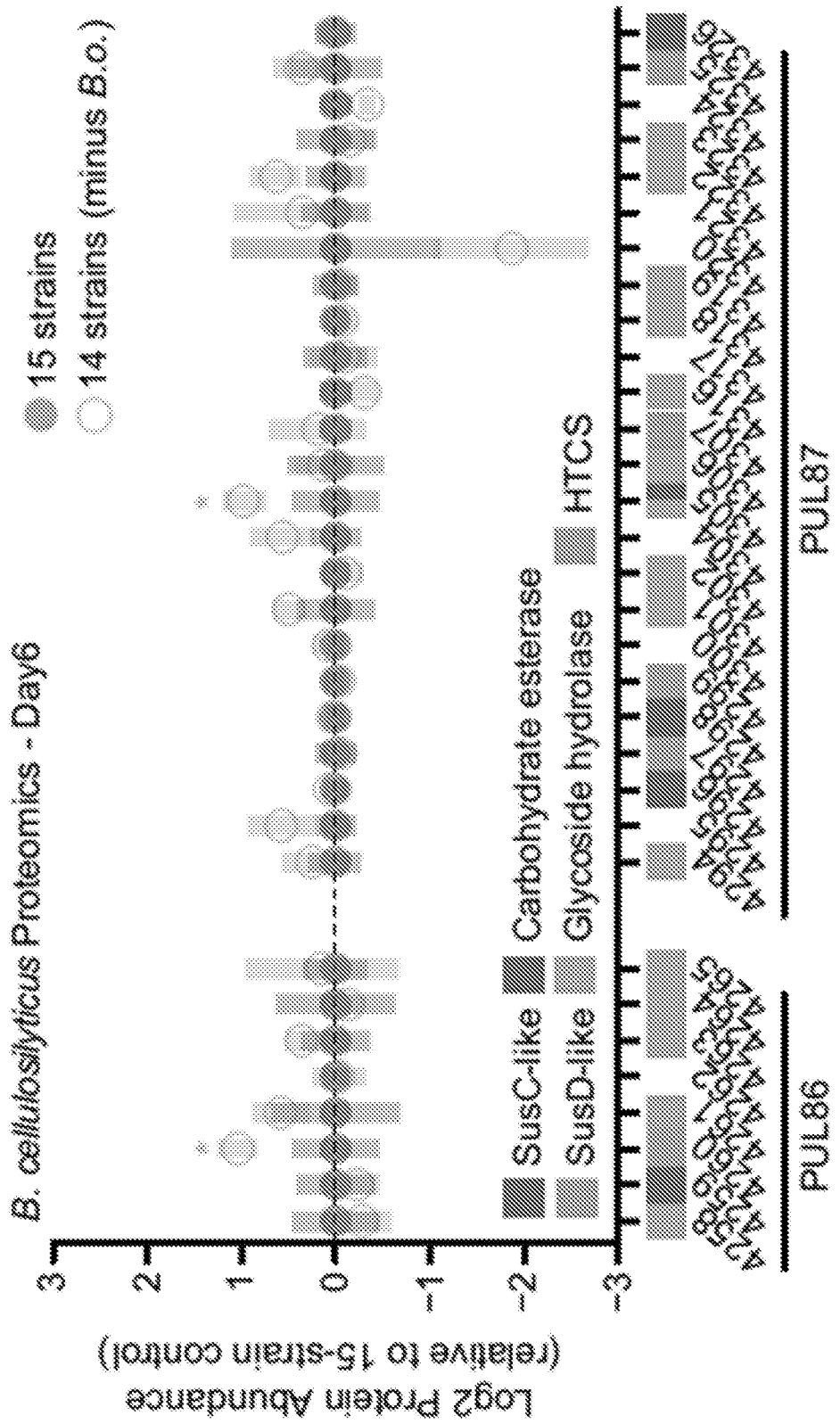

B. cellulosilyticus Proteomics - Day6
15 strains
14 strains (minus B.o.)
Log2 Protein Abundance
(relative to 15-strain control)
3
2
1
0
−1
−2
−3
SusC-like
SusD-like
Carbohydrate esterase
Glycoside hydrolase
HTCS
PUL86
PUL87

FIG. 6E

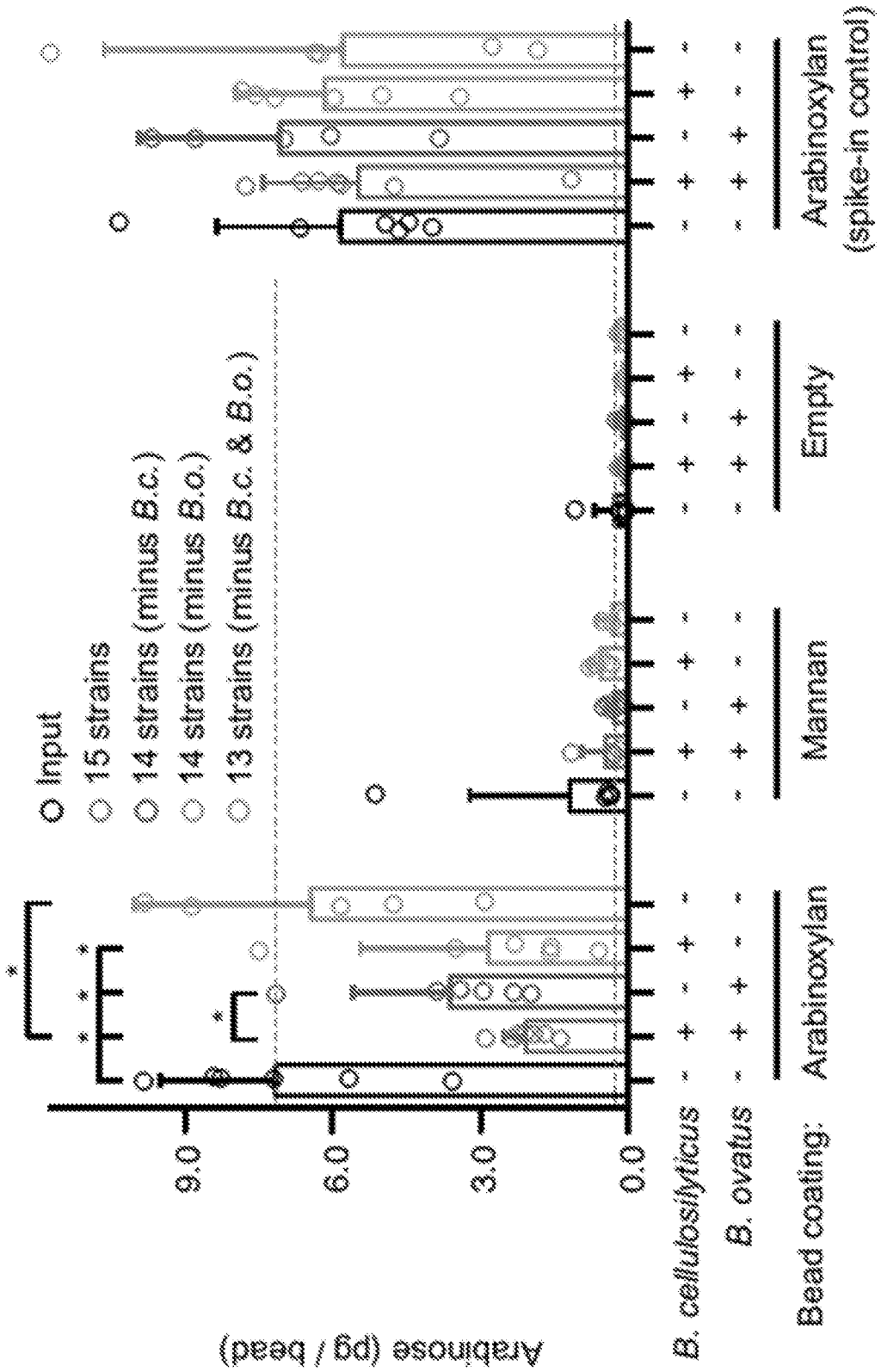

Input
15 strains
14 strains (minus B.c.)
14 strains (minus B.o.)
13 strains (minus B.c. & B.o.)
Arabinose (pg / bead)
9.0
6.0
3.0
0.0
B. cellulosilyticus
B. ovatus
Bead coating:
Arabinoxylan
Mannan
Empty
Arabinoxylan
(spike-in control)

FIG. 6F

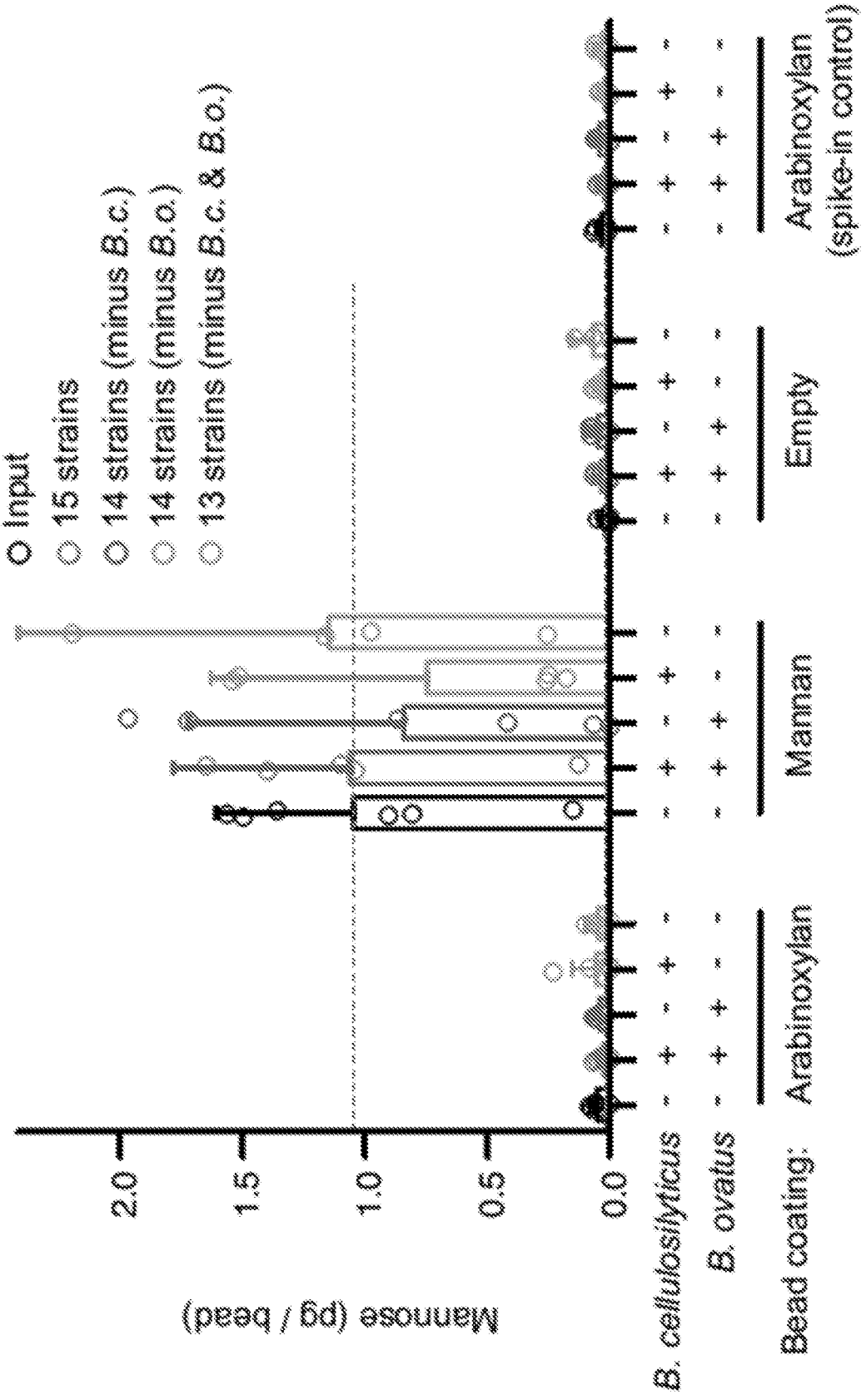

Input
15 strains
14 strains (minus B.c.)
14 strains (minus B.o.)
13 strains (minus B.c. & B.o.)
Mannose (pg / bead)
2.0
1.5
1.0
0.5
0.0
B. cellulosilyticus
B. ovatus
Bead coating:
Arabinoxylan
Maman
Empty
Arabinoxylan
(spike-in control)

FIG. 6G

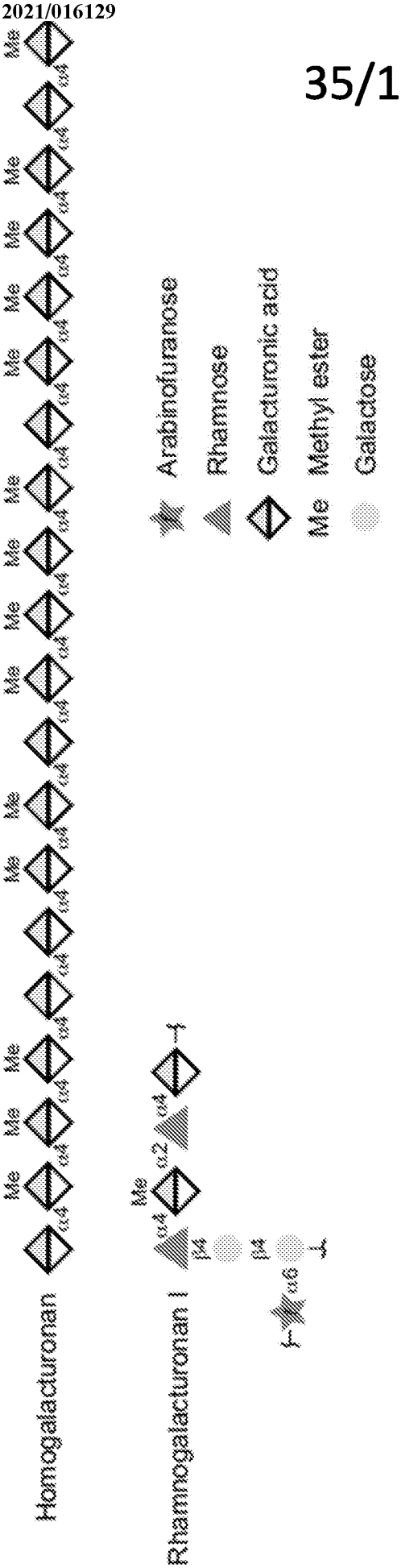

Homogalacturonan
Rhamnogalacturonan I
Me
α4
α2
β4
α6
Arabinofuranose
Rhamnose
Galacturonic acid
Methyl ester
Galactose

FIG. 7A

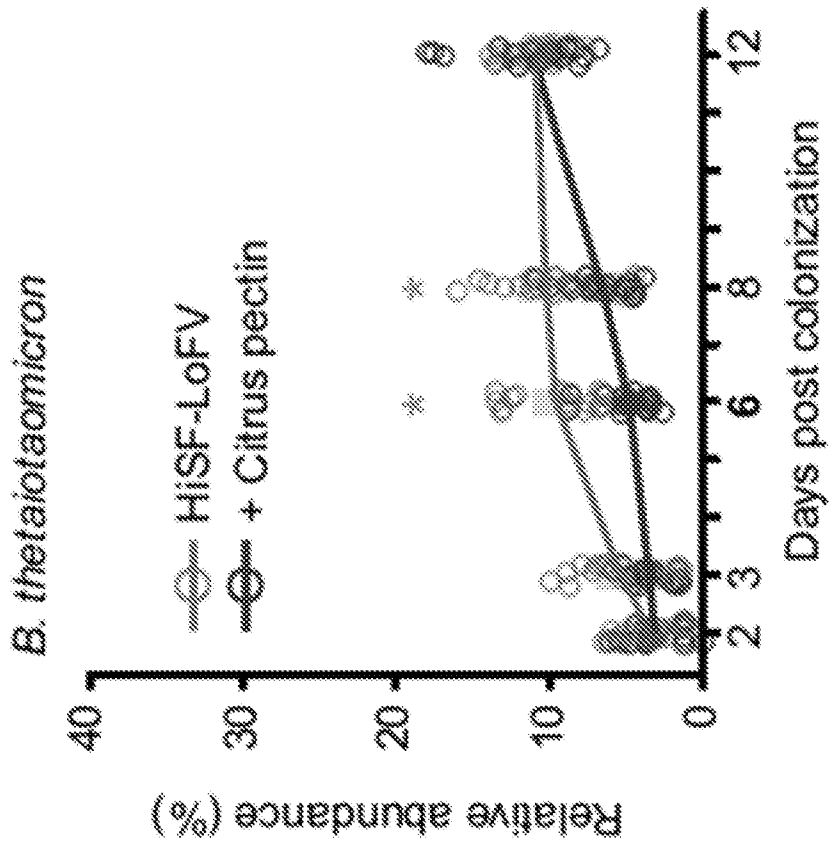

B. thetaiotaomicron
HiSF-LoFV
+ Citrus pectin
Relative abundance (%)
40
30
20
10
0
2
3
6
8
12
Days post colonization

FIG. 7B

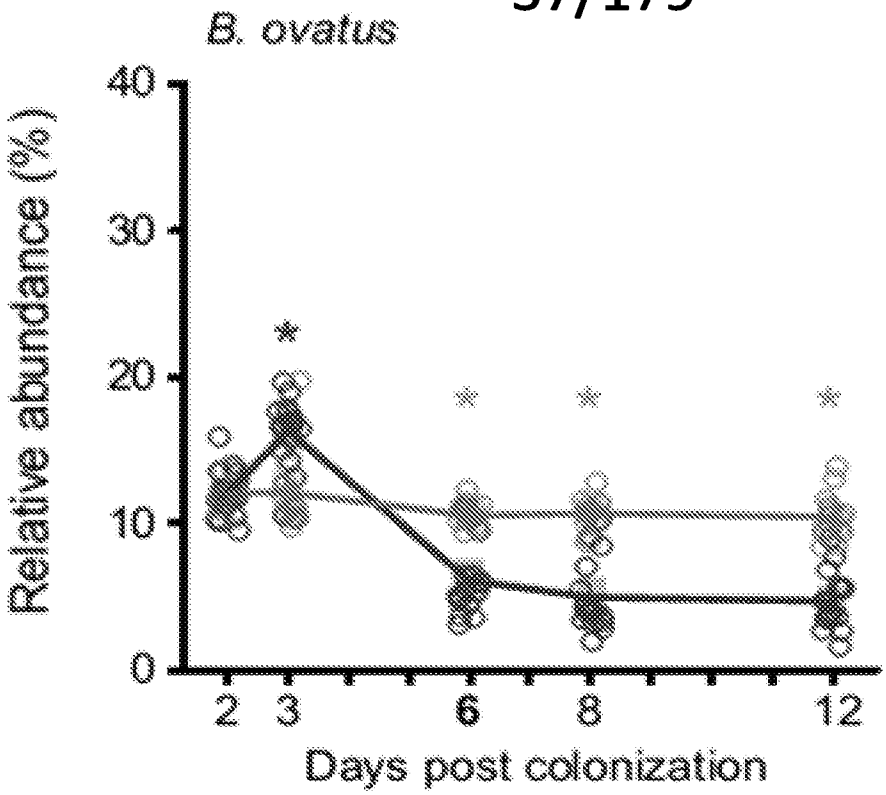

B. ovatus
Relative abundance (%)
40
30
20
10
0
2
3
6
8
12
Days post colonization

FIG. 7C

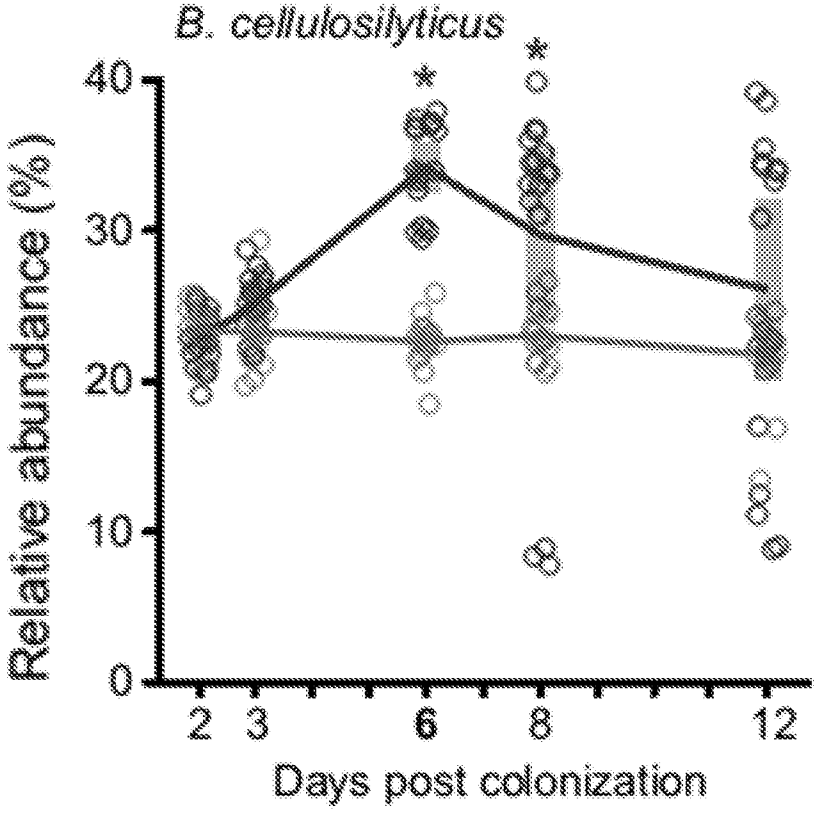

B. cellulosilyticus
Relative abundance (%)
40
30
20
10
0
2
3
6
8
12
Days post colonization

FIG. 7D

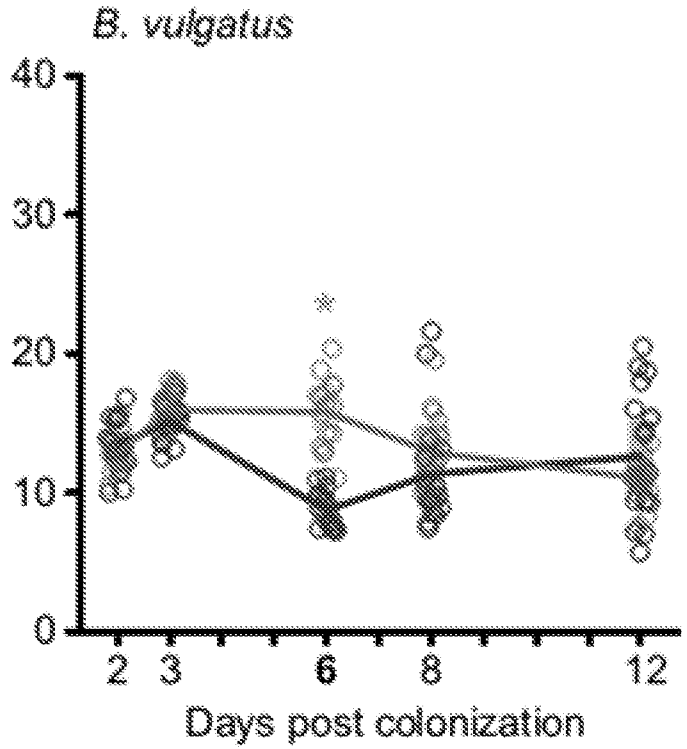

B. vulgatus
40
30
20
10
0
2
3
6
8
12
Days post colonization

FIG. 7E

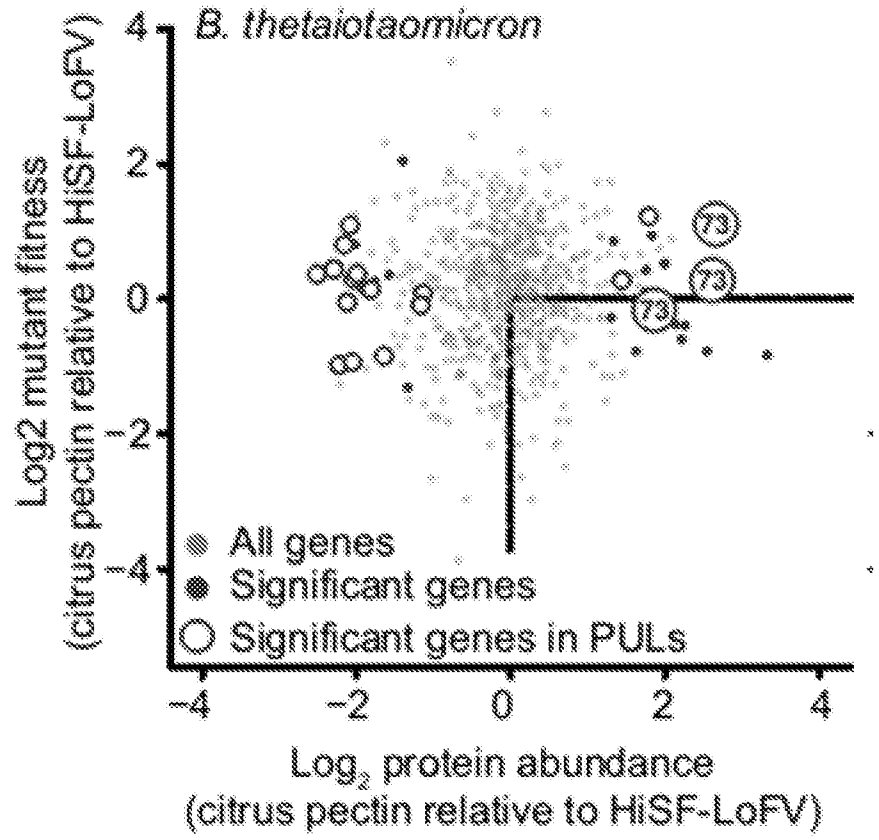

B. thetaiotaomicron
Log2 mutant fitness
(citrus pectin relative to HiSF-LoFV)
4
2
0
−2
−4
All genes
Significant genes
Significant genes in PULs
−4
−2
0
2
4
Log2 protein abundance
(citrus pectin relative to HiSF-LoFV)

FIG. 7F

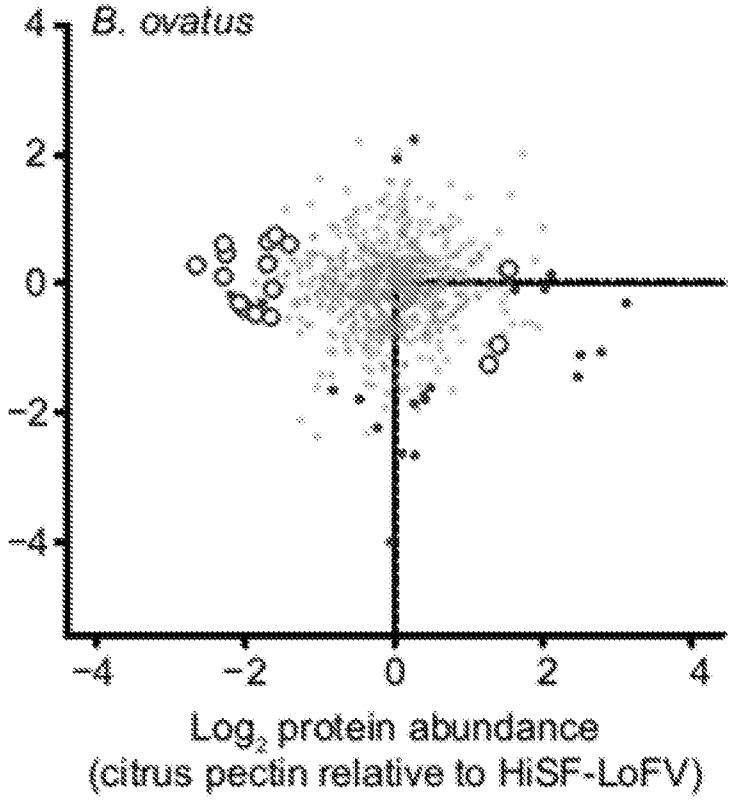

B. ovatus
4
2
0
−2
−4
−4
−2
0
2
4
Log2 protein abundance
(citrus pectin relative to HiSF-LoFV)

FIG. 7G

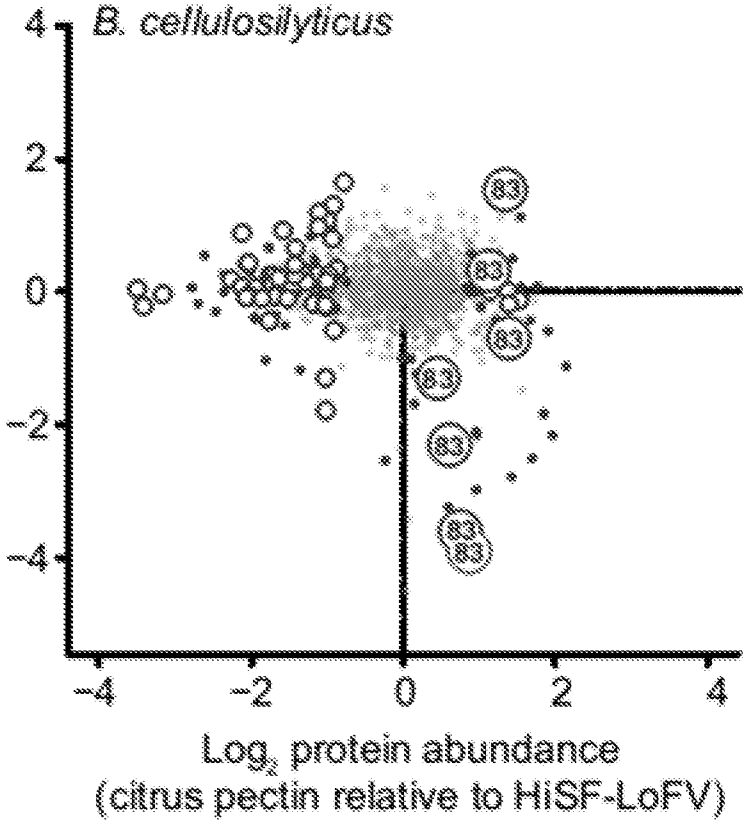

B. cellulosilyticus
4
2
0
−2
−4
−4
−2
0
2
4
Log2 protein abundance
(citrus pectin relative to HiSF-LoFV)

FIG. 7H

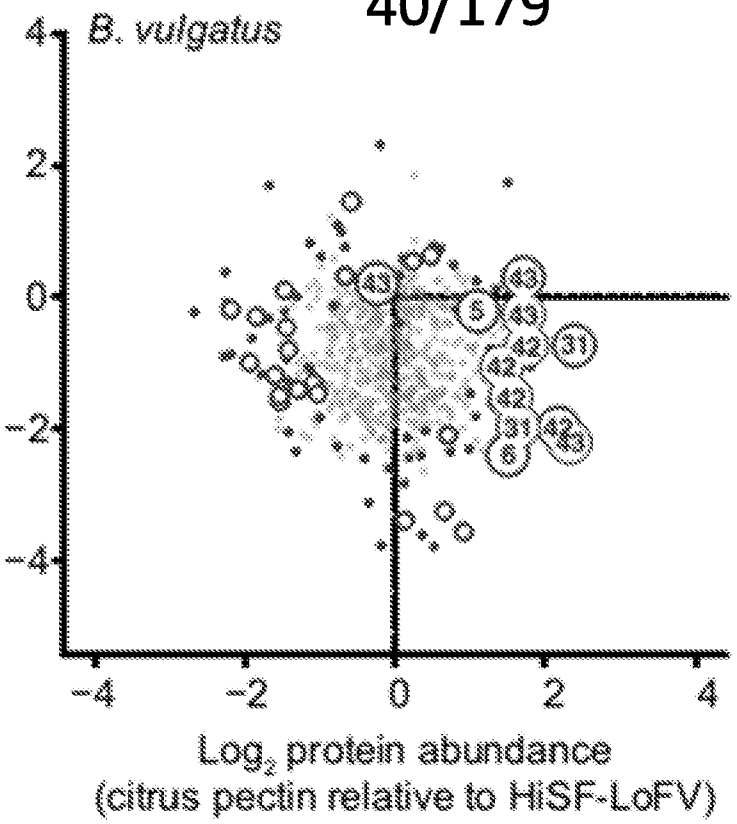

B. vulgatus
4
2
0
−2
−4
−4
−2
0
2
4
Log2 protein abundance
(citrus pectin relative to HiSF-LoFV)

FIG. 7I

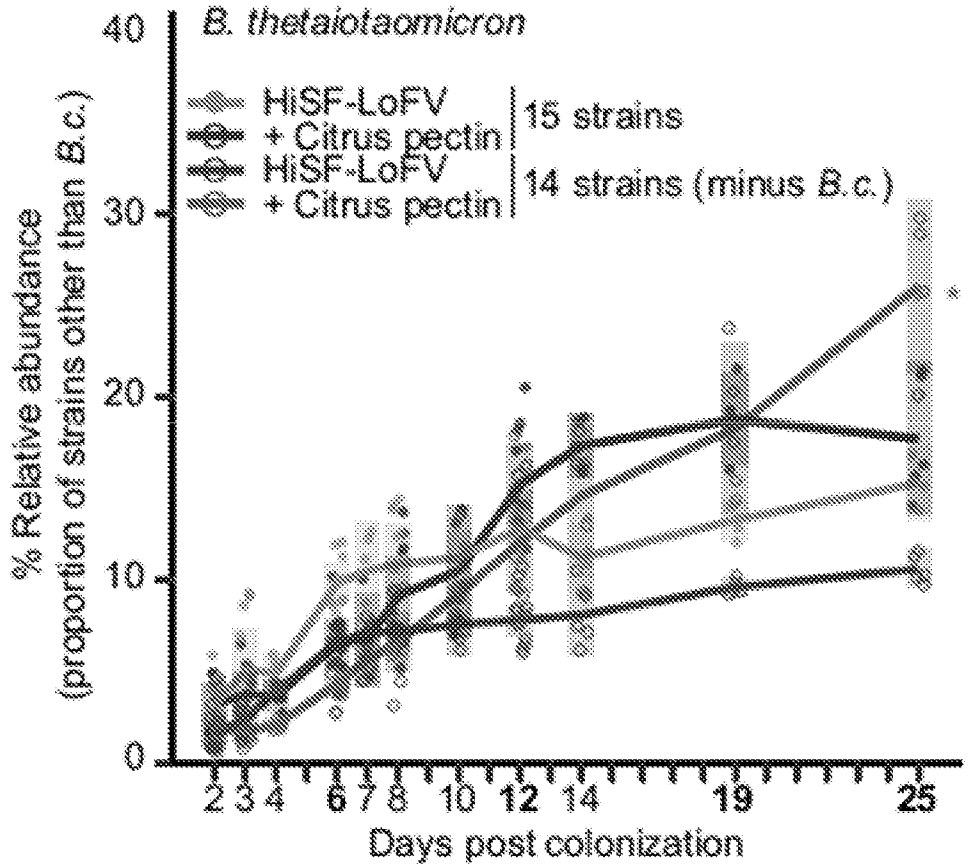

B. thetaiotaomicron
HiSF-LoFV
+ Citrus pectin
15 strains
HiSF-LoFV
+ Citrus pectin
14 strains (minus B.c.)
% Relative abundance
(proportion of strains other than B.c.)
40
30
20
10
0
2 3 4 6 7 8 10 12 14 19 25
Days post colonization

FIG. 8A

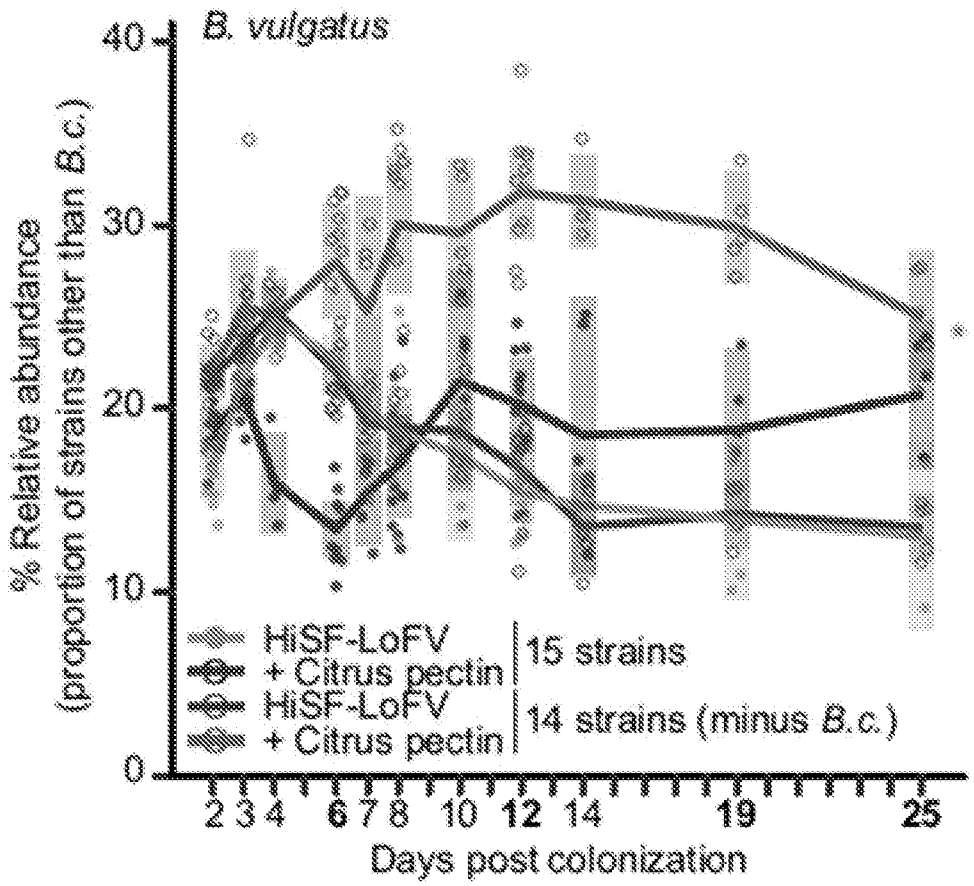

B. vulgatus
% Relative abundance
(proportion of strains other than B.c.)
40
30
20
10
0
HiSF-LoFV
+ Citrus pectin
15 strains
HiSF-LoFV
+ Citrus pectin
14 strains (minus B.c.)
2 3 4 6 7 8 10 12 14 19 25
Days post colonization

FIG. 8B

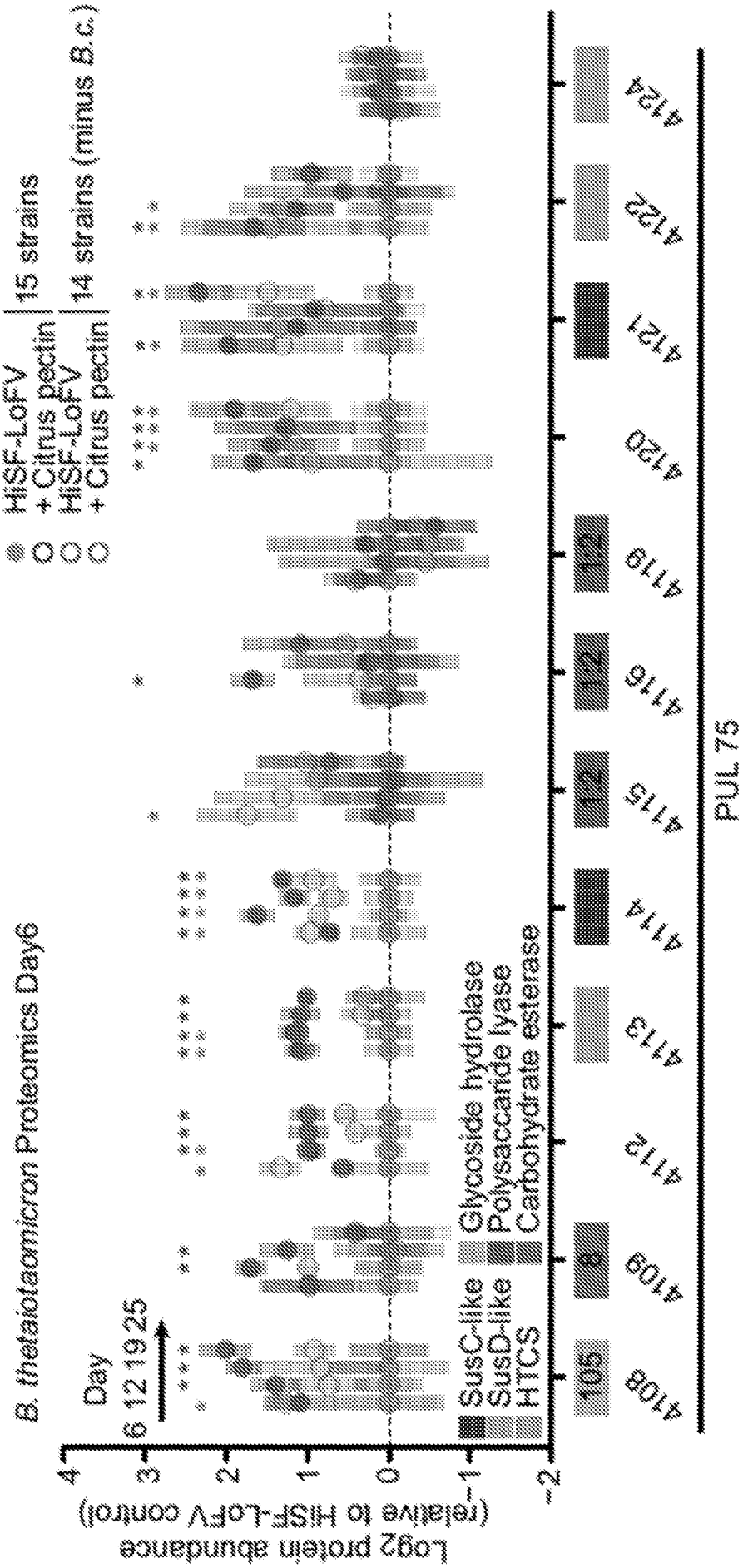

B. thetaiotaomicron Proteomics Day6
HiSF-LoFV | 15 strains
+ Citrus pectin
HiSF-LoFV | 14 strains (minus B.c.)
+ Citrus pectin
Day
6 12 19 25
Log2 protein abundance
(relative to HiSF-LoFV control)
SusC-like
SusD-like
HTCS
Glycoside hydrolase
Polysaccaride lyase
Carbohydrate esterase
PUL 75

FIG. 8C

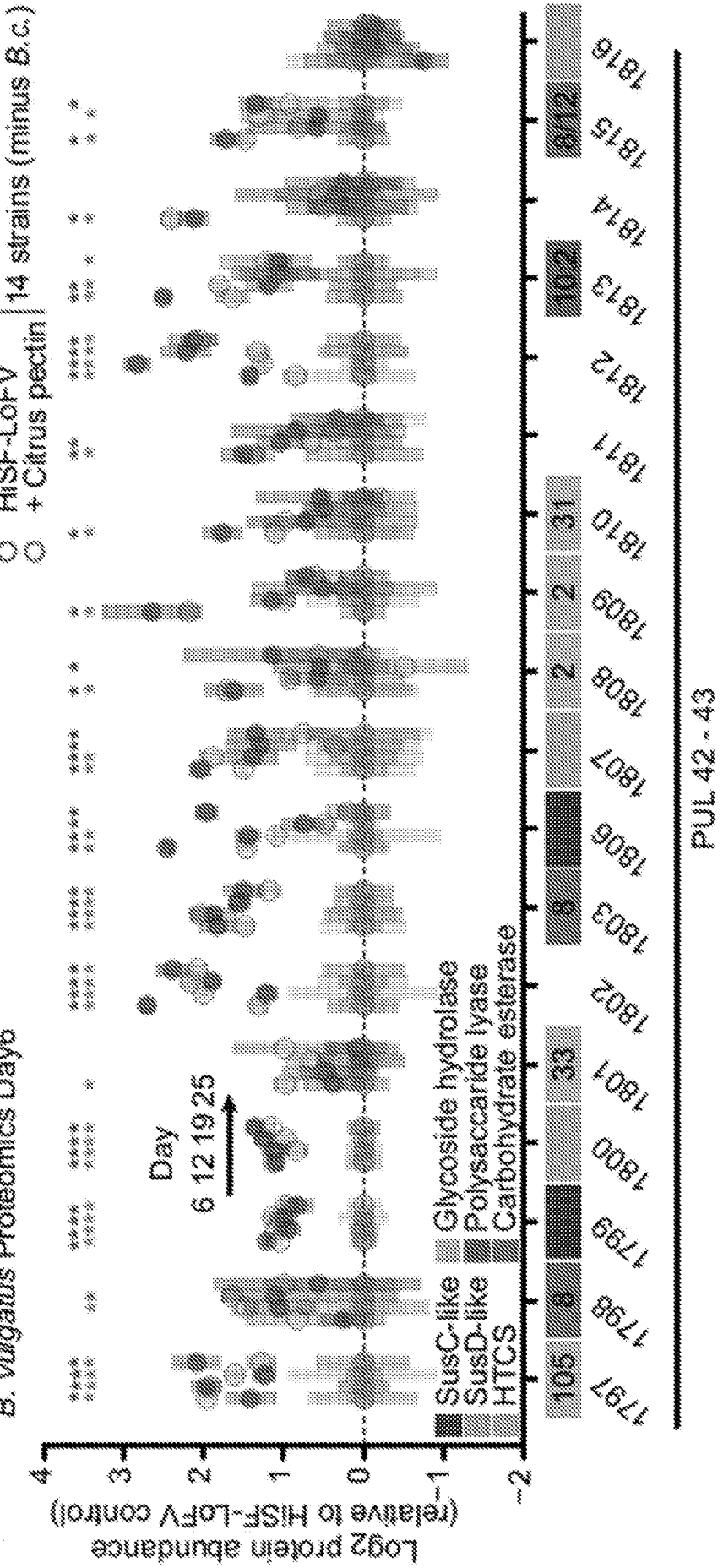

B. vulgatus Proteomics Day6
HiSF-LoFV | 15 strains
+ Citrus pectin
HiSF-LoFV | 14 strains (minus B.c.)
+ Citrus pectin
Day
6 12 19 25
Log2 protein abundance
(relative to HiSF-LoFV control)
SusC-like
SusD-like
HTCS
Glycoside hydrolase
Polysaccaride lyase
Carbohydrate esterase
PUL 42 - 43

FIG. 8D

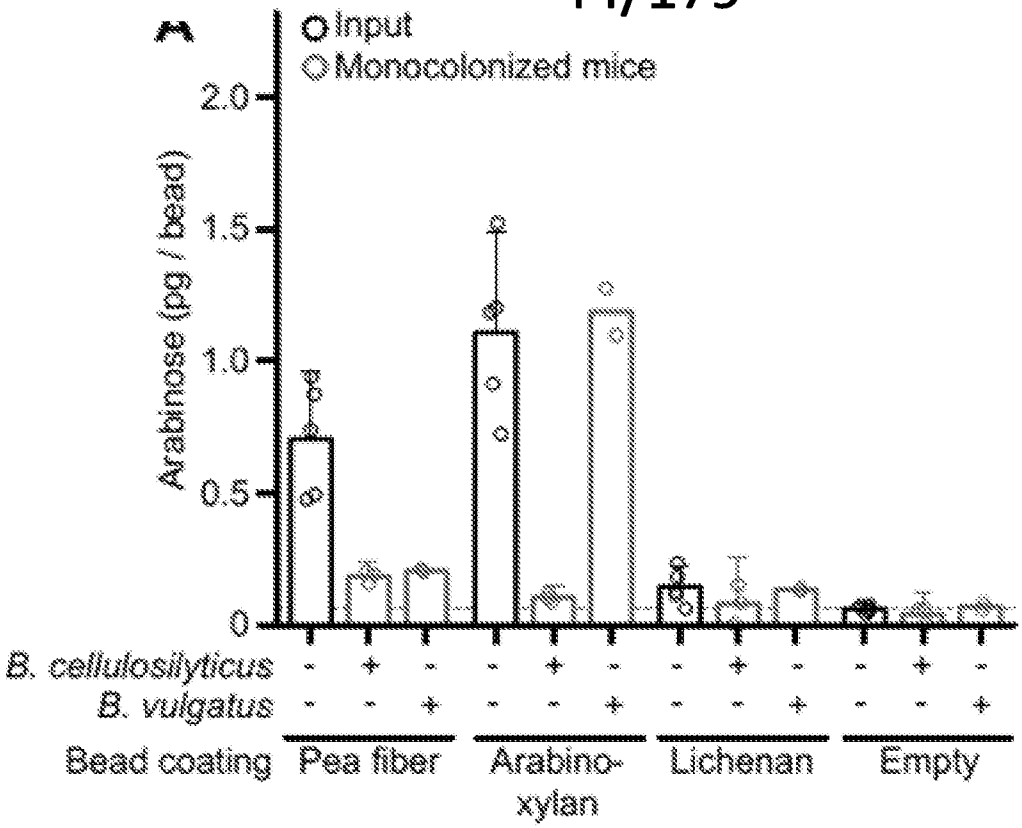

A
Input
Monocolonized mice
Arabinose (pg / bead)
2.0
1.5
1.0
0.5
0
B. cellulosilyticus - + - - + - - + - - + -
B. vulgatus - - + - - + - - + - - +
Bead coating
Pea fiber
Arabino-xylan
Lichenan
Empty

FIG. 9A

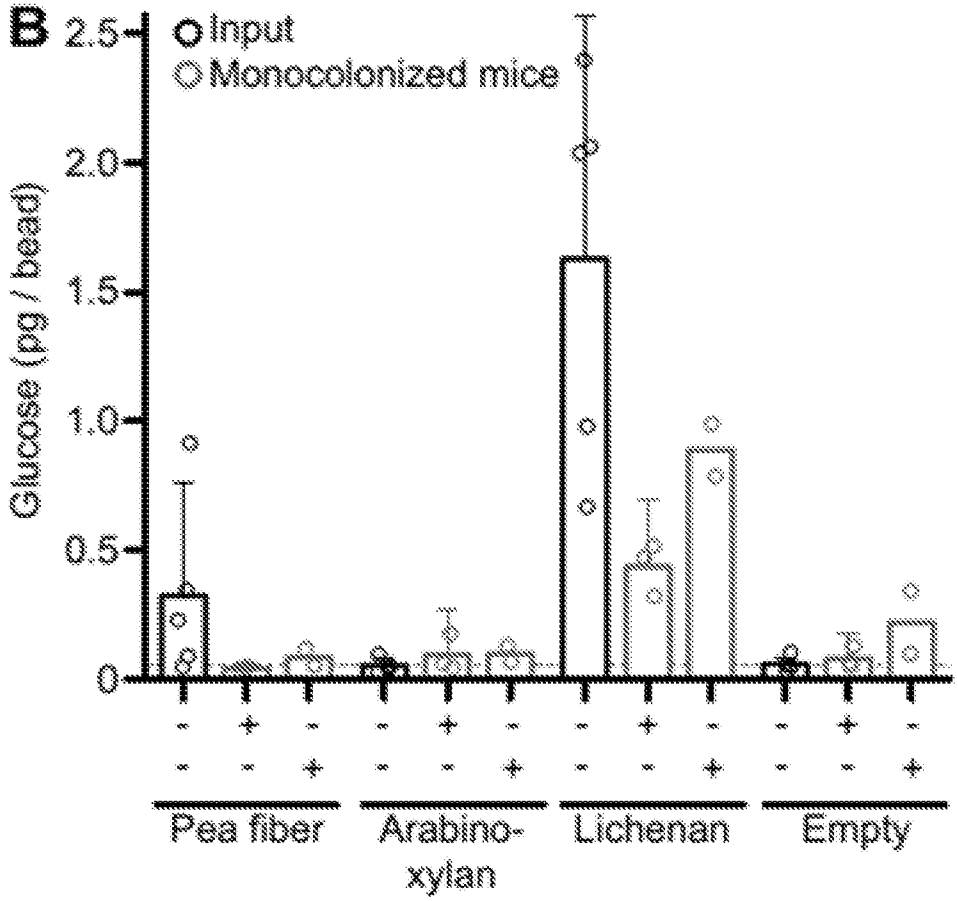

B
Input
Monocolonized mice
Glucose (pg / bead)
2.5
2.0
1.5
1.0
0.5
0
Pea fiber
Arabino-xylan
Lichenan
Empty

FIG. 9B

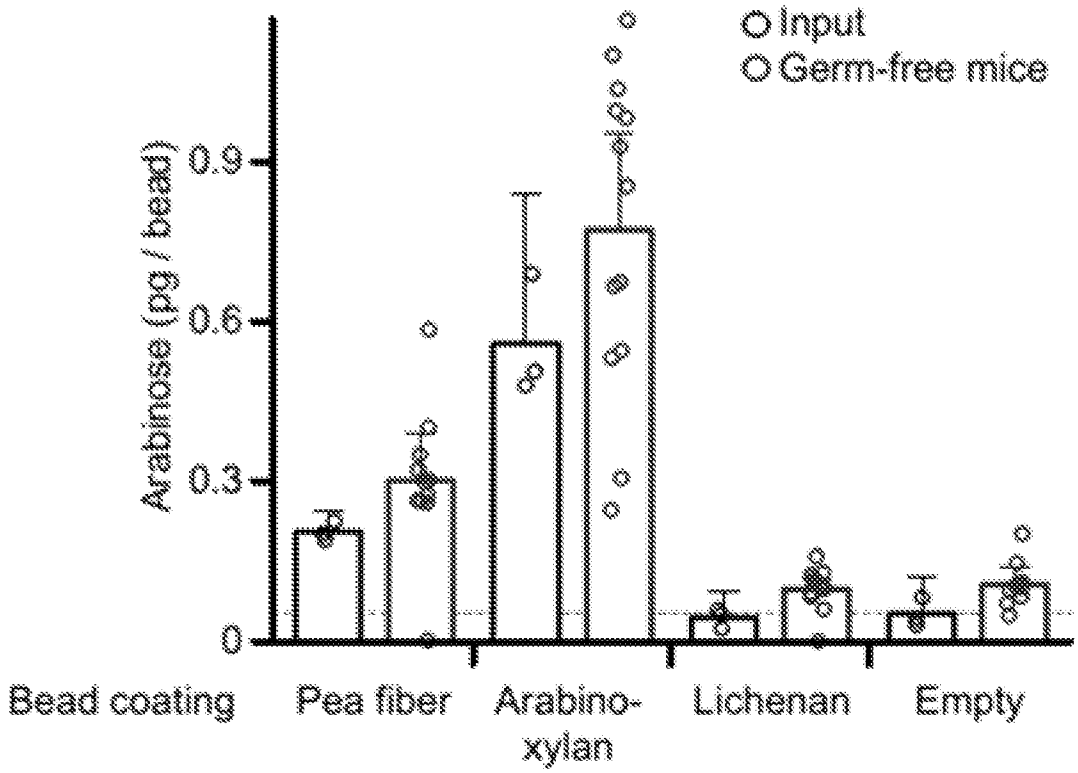

Input
Germ-free mice
Arabinose (pg / bead)
0.9
0.6
0.3
0
Bead coating
Pea fiber
Arabino-xylan
Lichenan
Empty

FIG. 9C

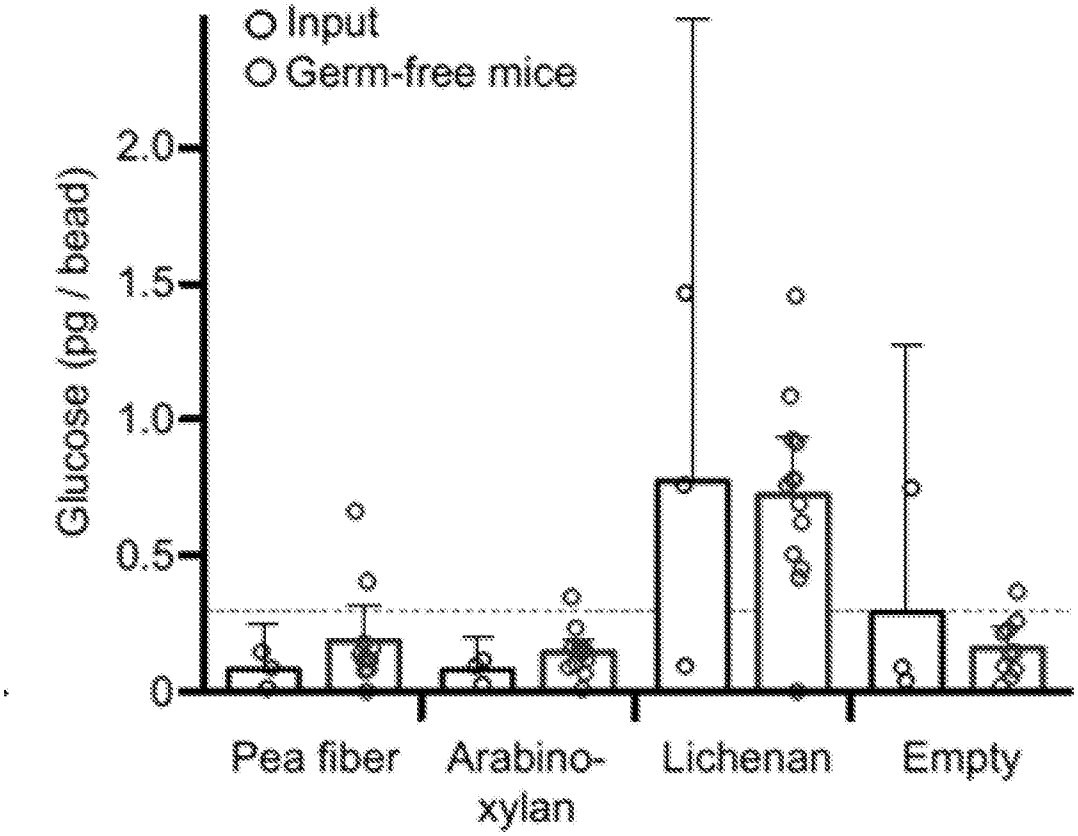

Input
Germ-free mice
Glucose (pg / bead)
2.0
1.5
1.0
0.5
0
Pea fiber
Arabino-xylan
Lichenan
Empty

FIG. 9D

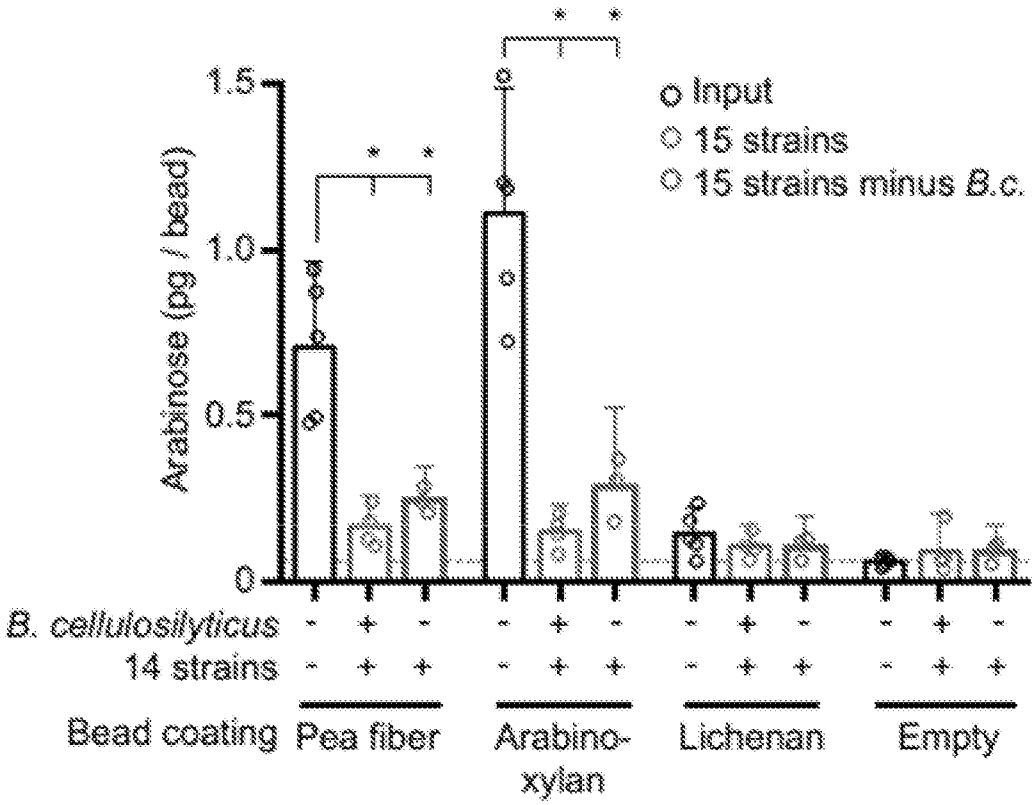

Arabinose (pg / bead)
1.5
1.0
0.5
0
Input
15 strains
15 strains minus B.c.
* *
* *
B. cellulosilyticus
14 strains
Bead coating
Pea fiber
Arabino-xylan
Lichenan
Empty

FIG. 9E

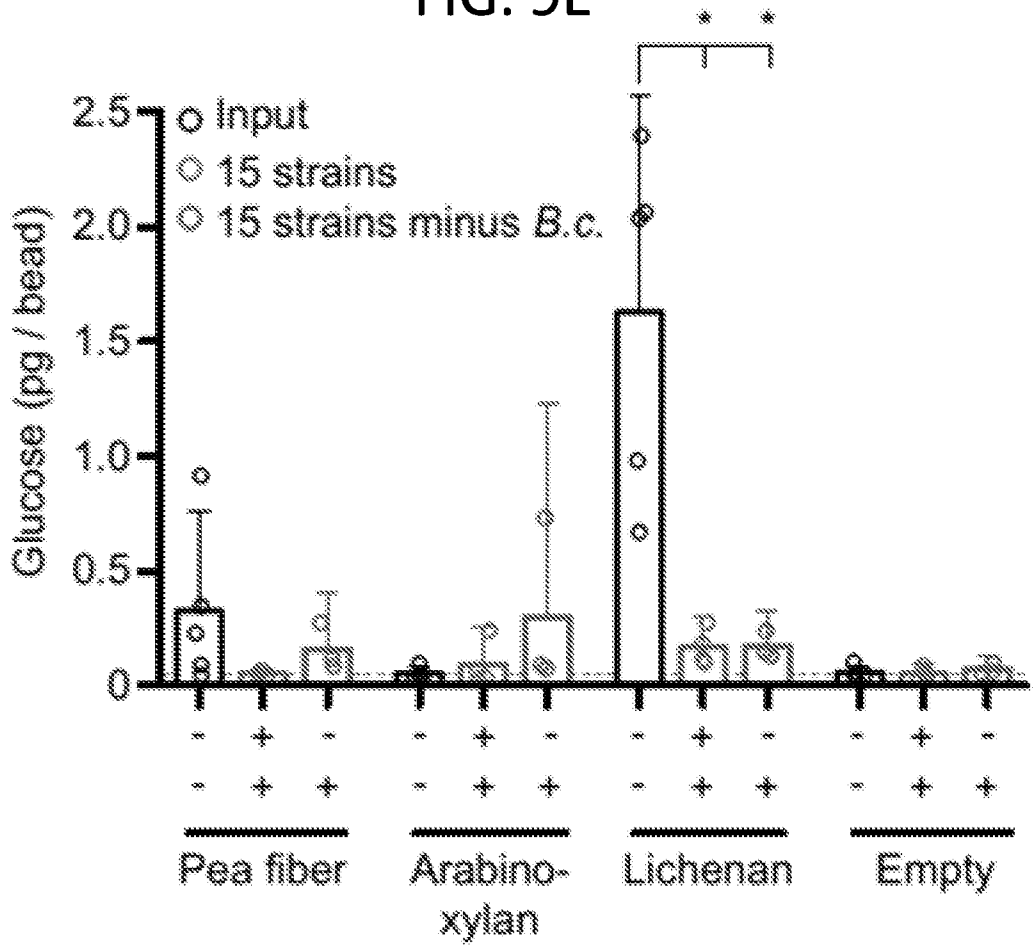

Glucose (pg / bead)
2.5
2.0
1.5
1.0
0.5
0
Input
15 strains
15 strains minus B.c.
* *
Pea fiber
Arabino-xylan
Lichenan
Empty

FIG. 9F

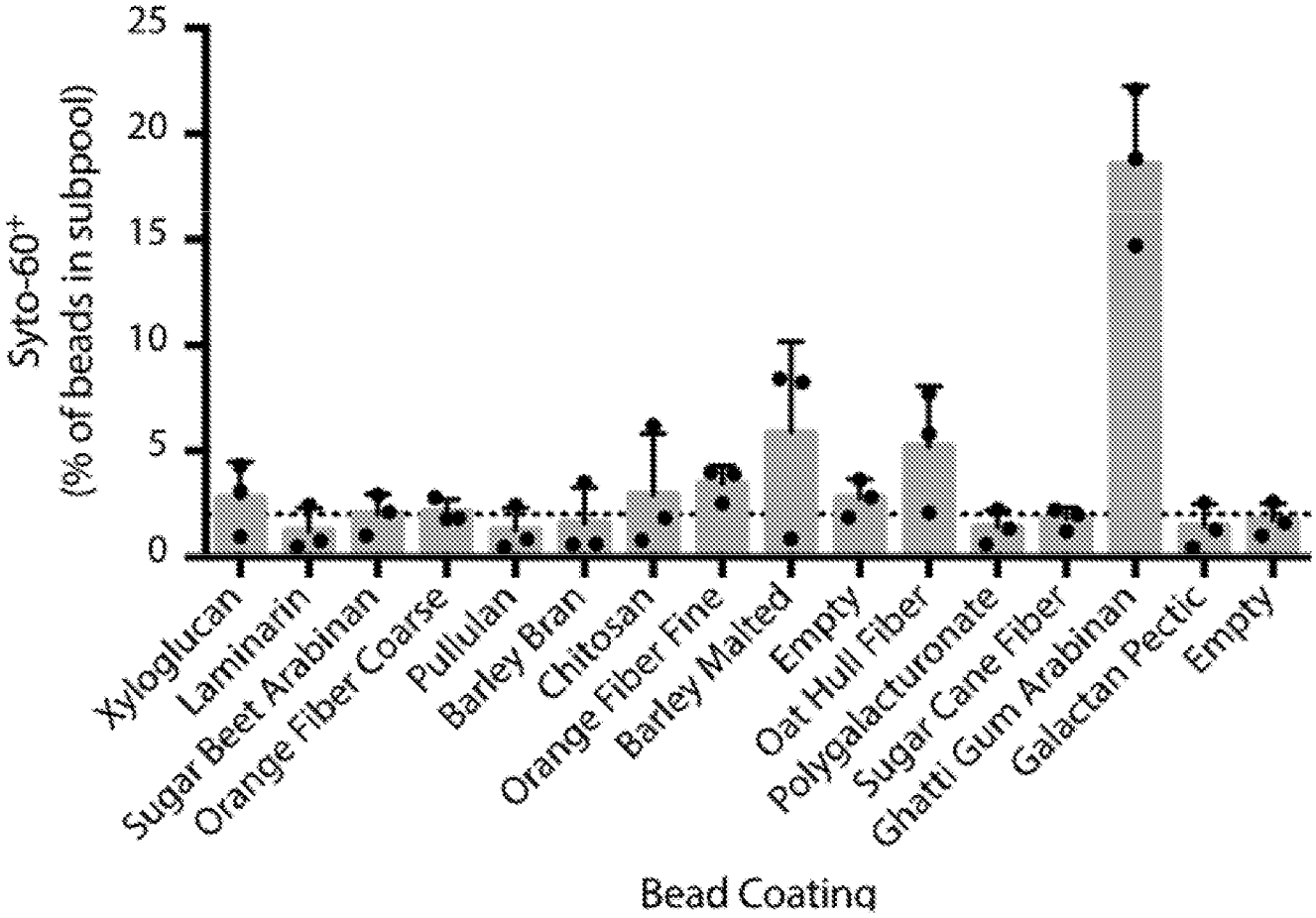

Syto-60+
(% of beads in subpool)
25
20
15
10
5
0
Xyloglucan
Laminarin
Sugar Beet Arabinan
Orange Fiber Coarse
Pullulan
Barley Bran
Chitosan
Orange Fiber Fine
Barley Malted
Empty
Oat Hull Fiber
Polygalacturonate
Sugar Cane Fiber
Ghatti Gum Arabinan
Galactan Pectic
Empty
Bead Coating

FIG. 10

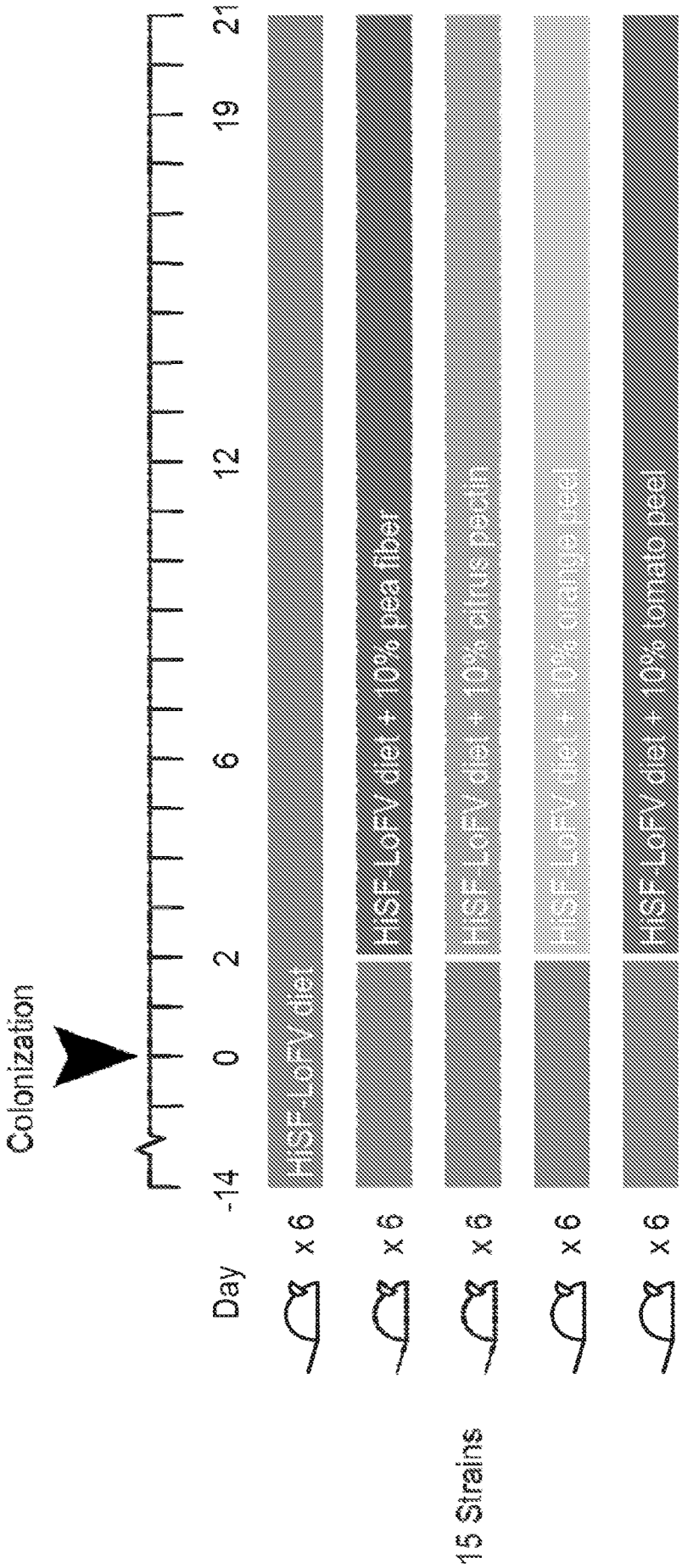

Colonization
Day -14 0 2 6 12 19 21
HSF LoFV diet
HSF LoFV diet + 10% pea fiber
HSF LoFV diet + 10% citrus pectin
HSF LoFV diet + 10% orange peel
HSF LoFV diet + 10% tomato peel
x 6
x 6
x 6
x 6
x 6
15 Strains

FIG. 11A

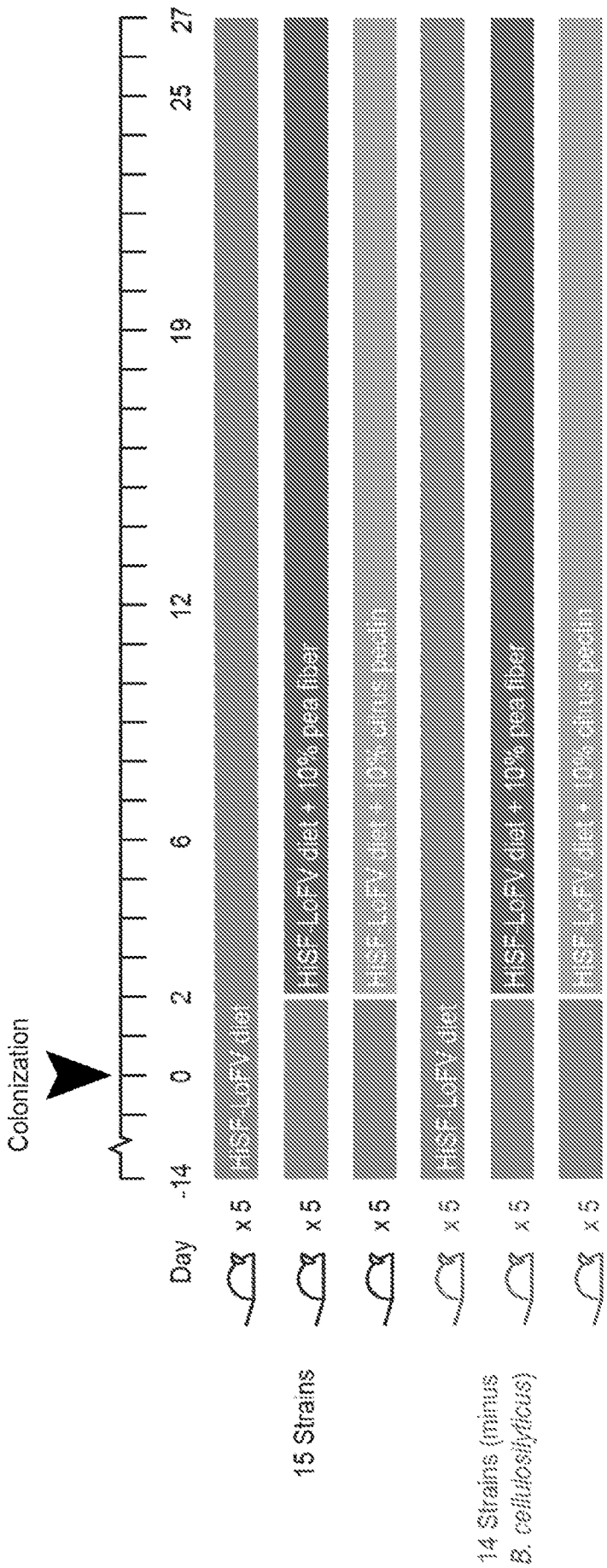

Colonization
Day
-14
0
2
6
12
19
25
27
15 Strains
14 Strains (minus B. cellulosilyticus)
x 5
HSF LoFV diet
HSF LoFV diet + 10% pea fiber
HSF LoFV diet + 10% pea protein
HSF LoFV diet + 10% soy protein

FIG. 11B

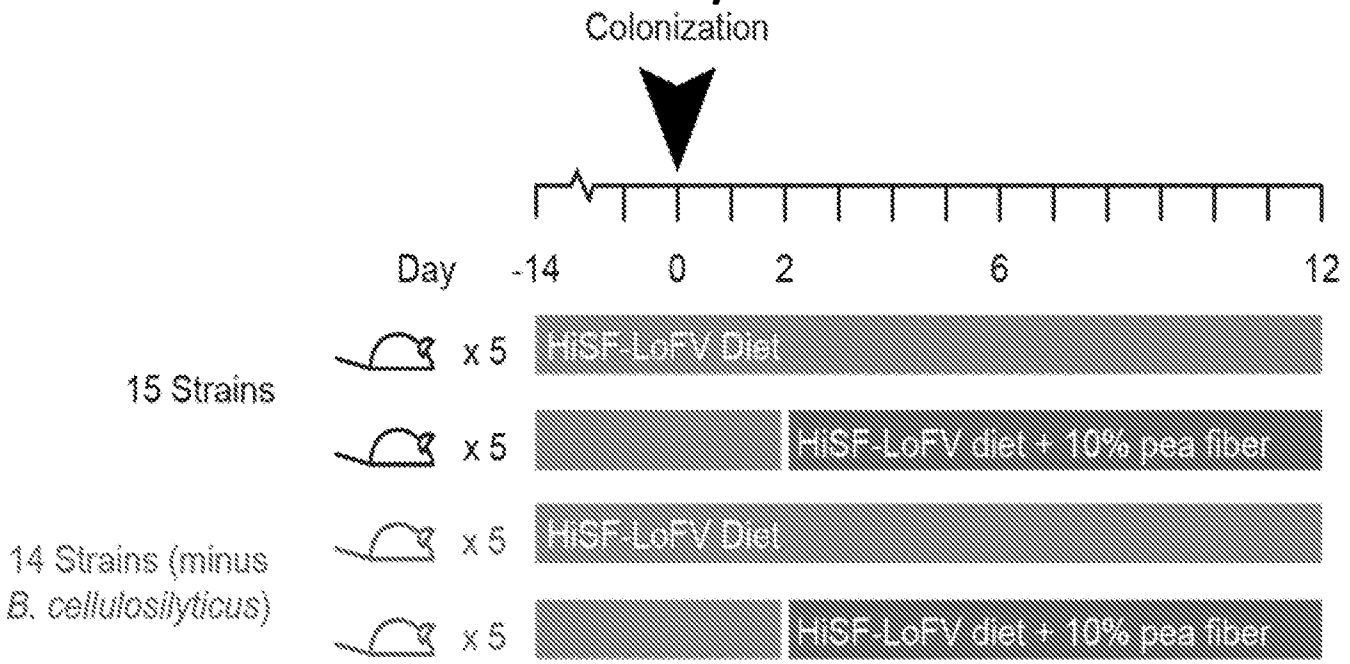


FIG. 11C

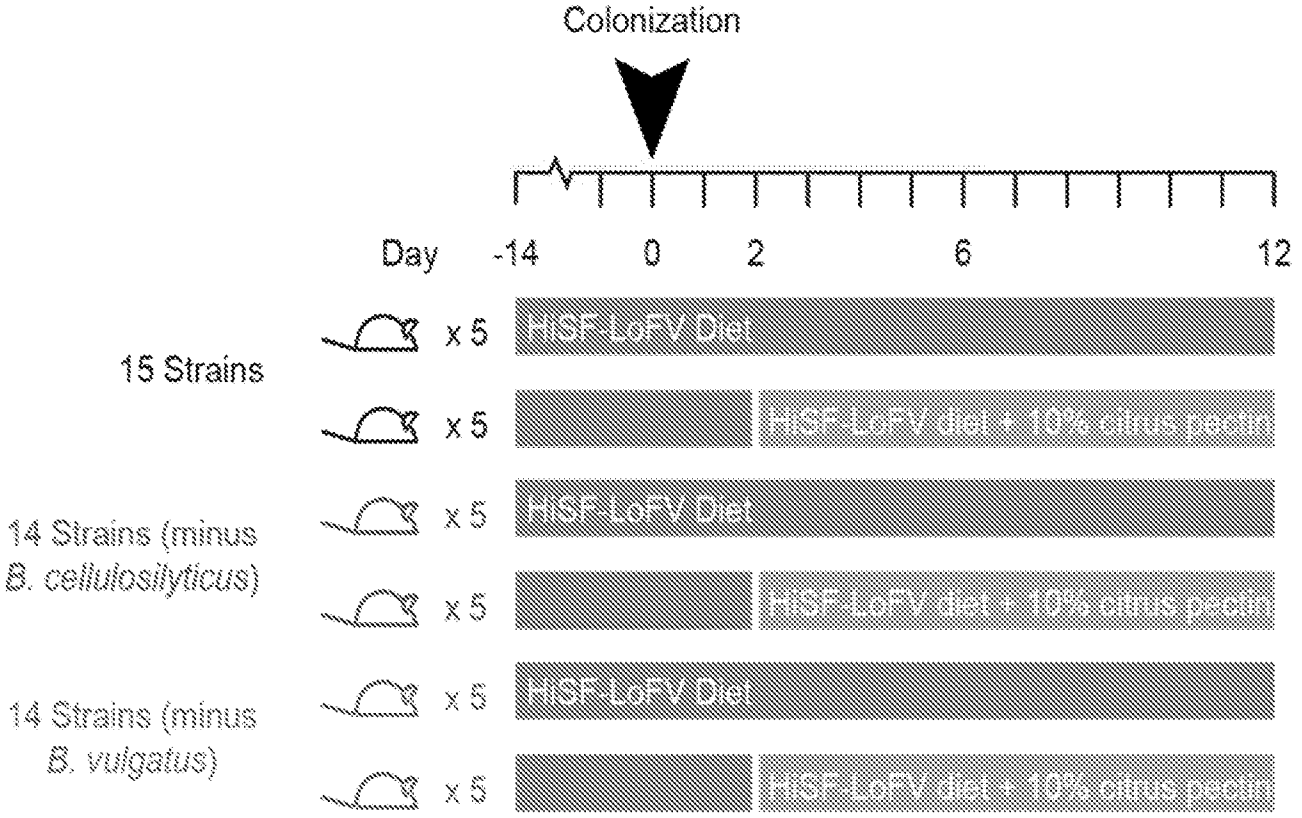


FIG. 11D

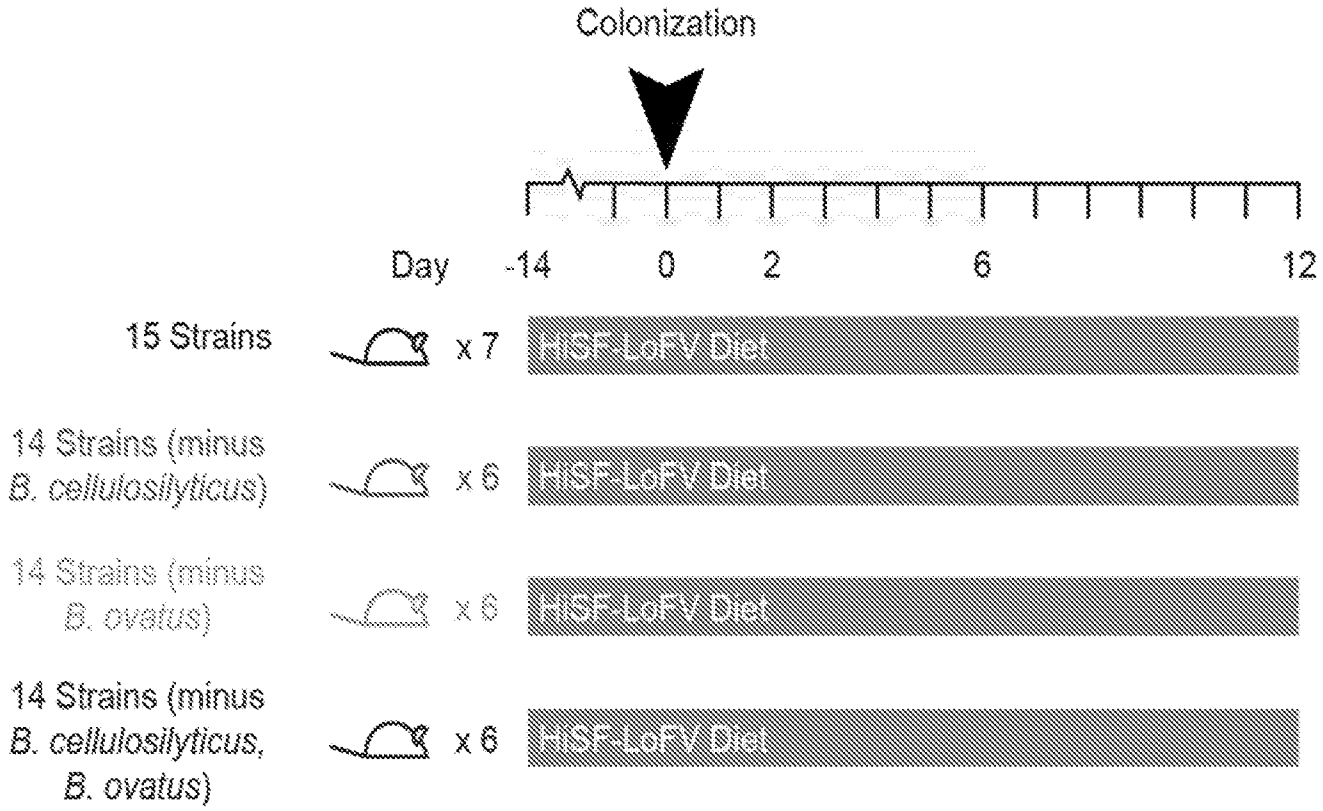

Colonization
Day
-14
0
2
6
12
15 Strains
x 7
HISF-LoFV Diet
14 Strains (minus B. cellulosilyticus)
x 6
HISF-LoFV Diet
14 Strains (minus B. ovatus)
x 6
HISF-LoFV Diet
14 Strains (minus B. cellulosilyticus, B. ovatus)
x 6
HISF-LoFV Diet

FIG. 11E

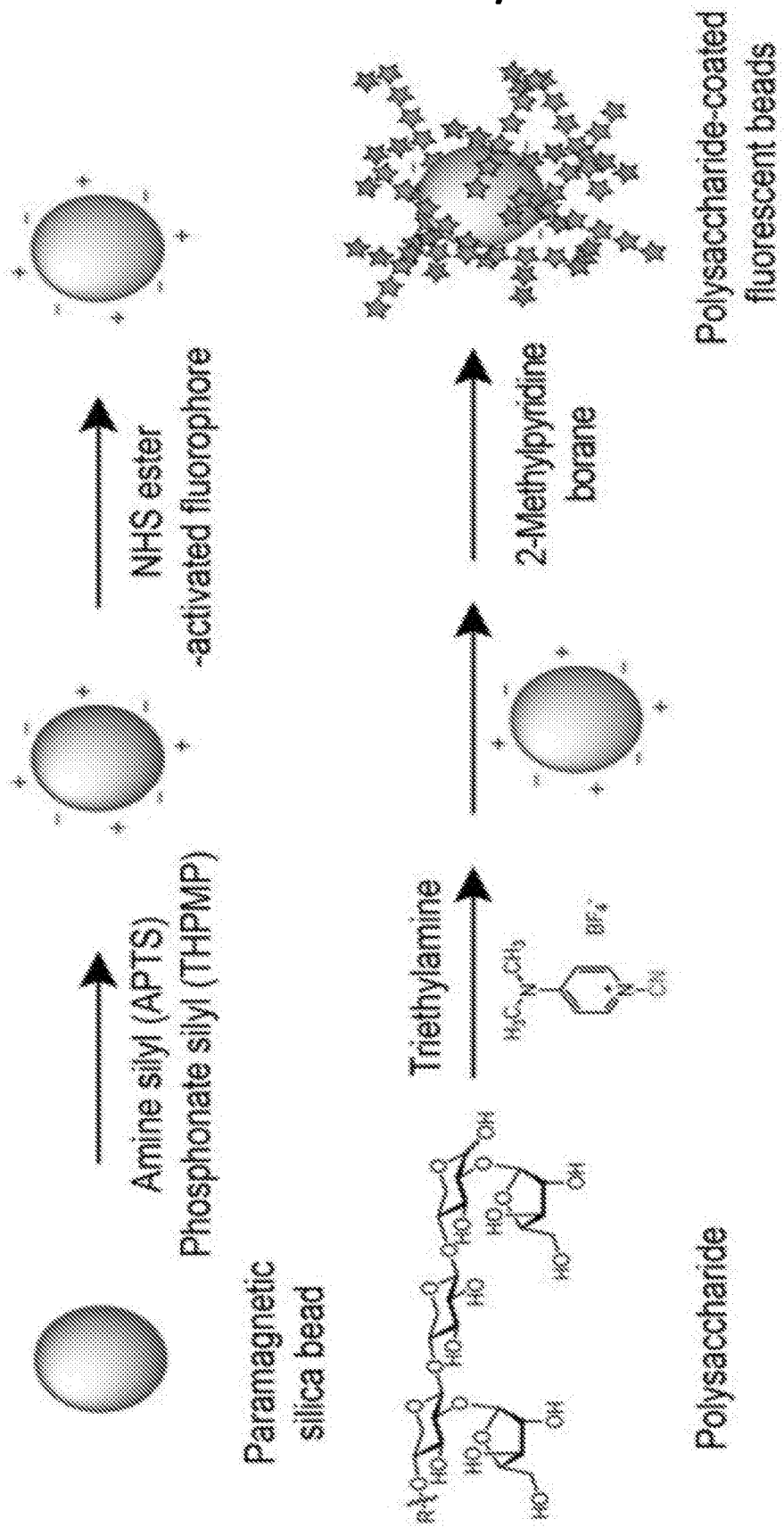

Paramagnetic
silica bead
Amine silyl (APTS)
Phosphonate silyl (THPMP)
NHS ester
-activated fluorophore
Polysaccharide
Triethylamine
2-Methylpyridine
borane
Polysaccharide-coated
fluorescent beads

FIG. 12

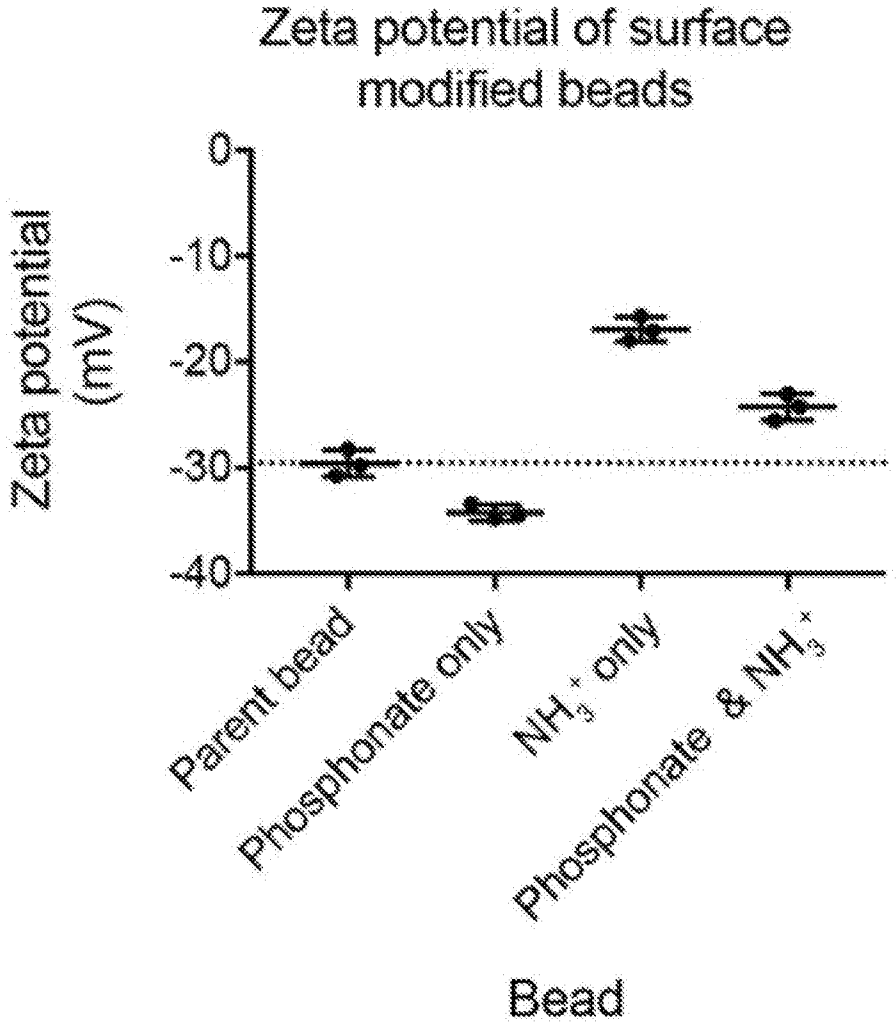

Zeta potential of surface modified beads
Zeta potential (mV)
0
-10
-20
-30
-40
Parent bead
Phosphonate only
NH_3^+ only
Phosphonate & NH_3^+
Bead

FIG. 13A

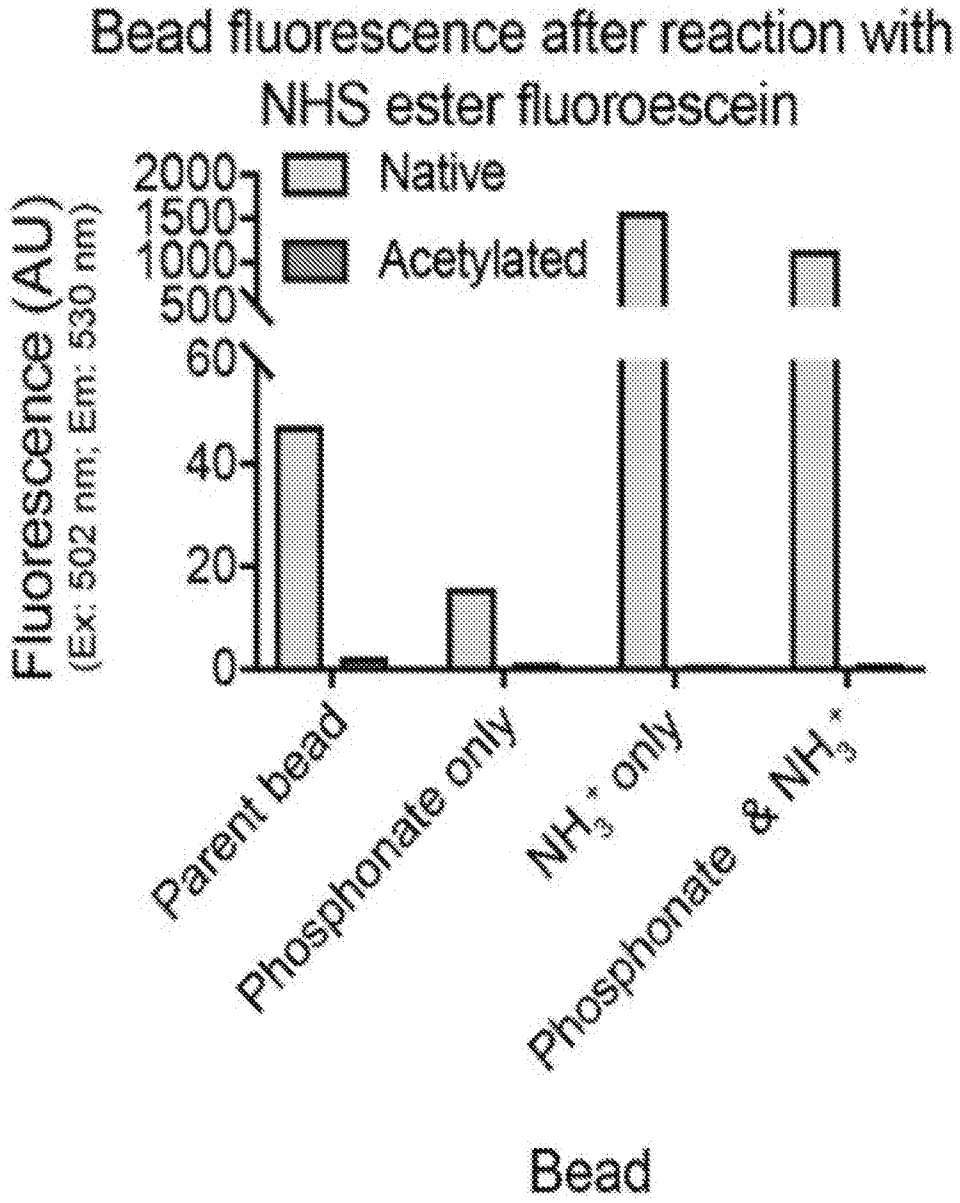

Bead fluorescence after reaction with NHS ester fluoroescein
Native
Acetylated
2000
1500
1000
500
60
40
20
0
Fluorescence (AU)
(Ex: 502 nm; Em: 530 nm)
Parent bead
Phosphonate only
NH3* only
Phosphonate & NH3*
Bead

FIG. 13B

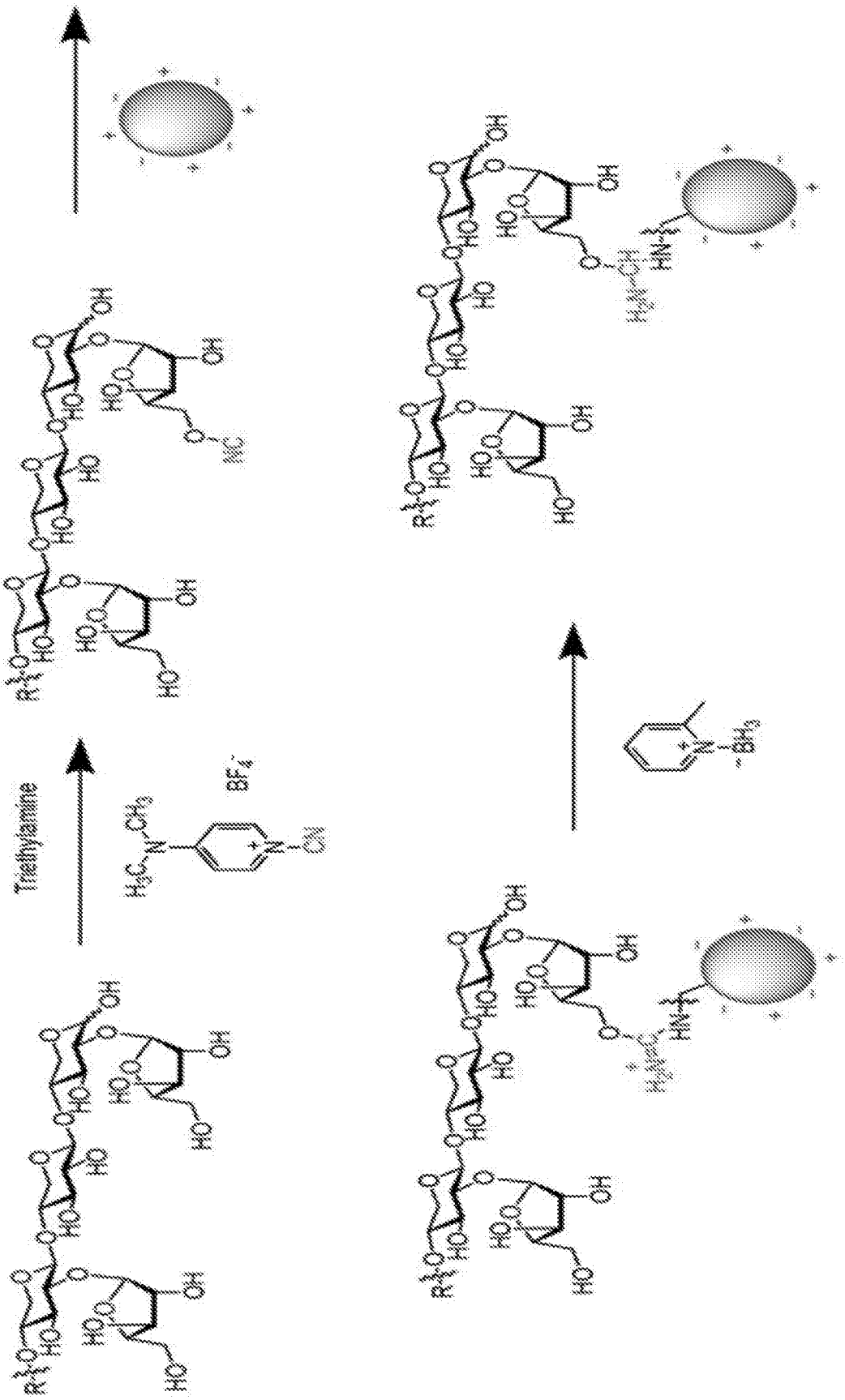


FIG. 14

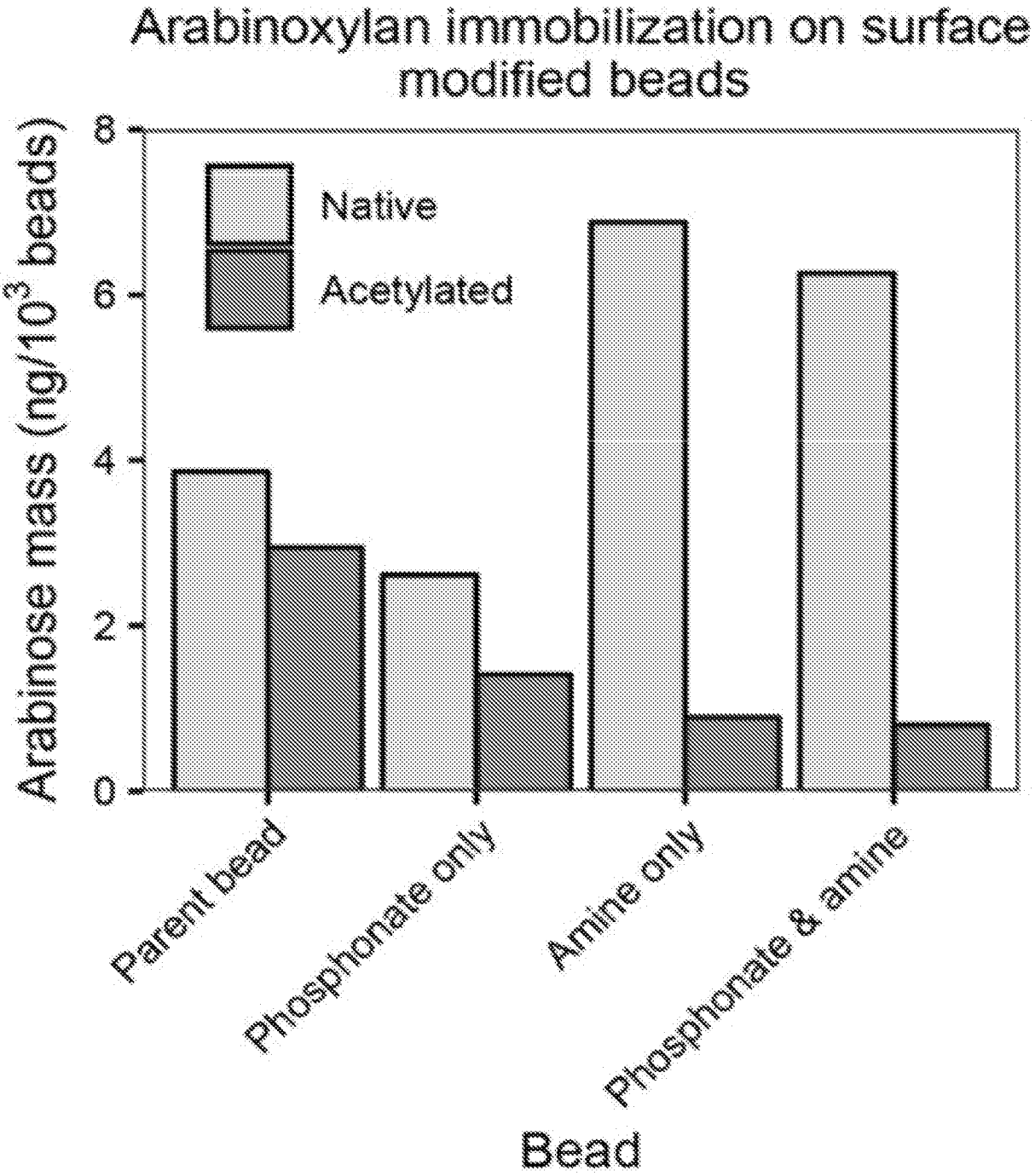

Arabinoxylan immobilization on surface modified beads
Arabinose mass (ng/10^3 beads)
0
2
4
6
8
Native
Acetylated
Parent bead
Phosphonate only
Amine only
Phosphonate & amine
Bead

FIG. 15

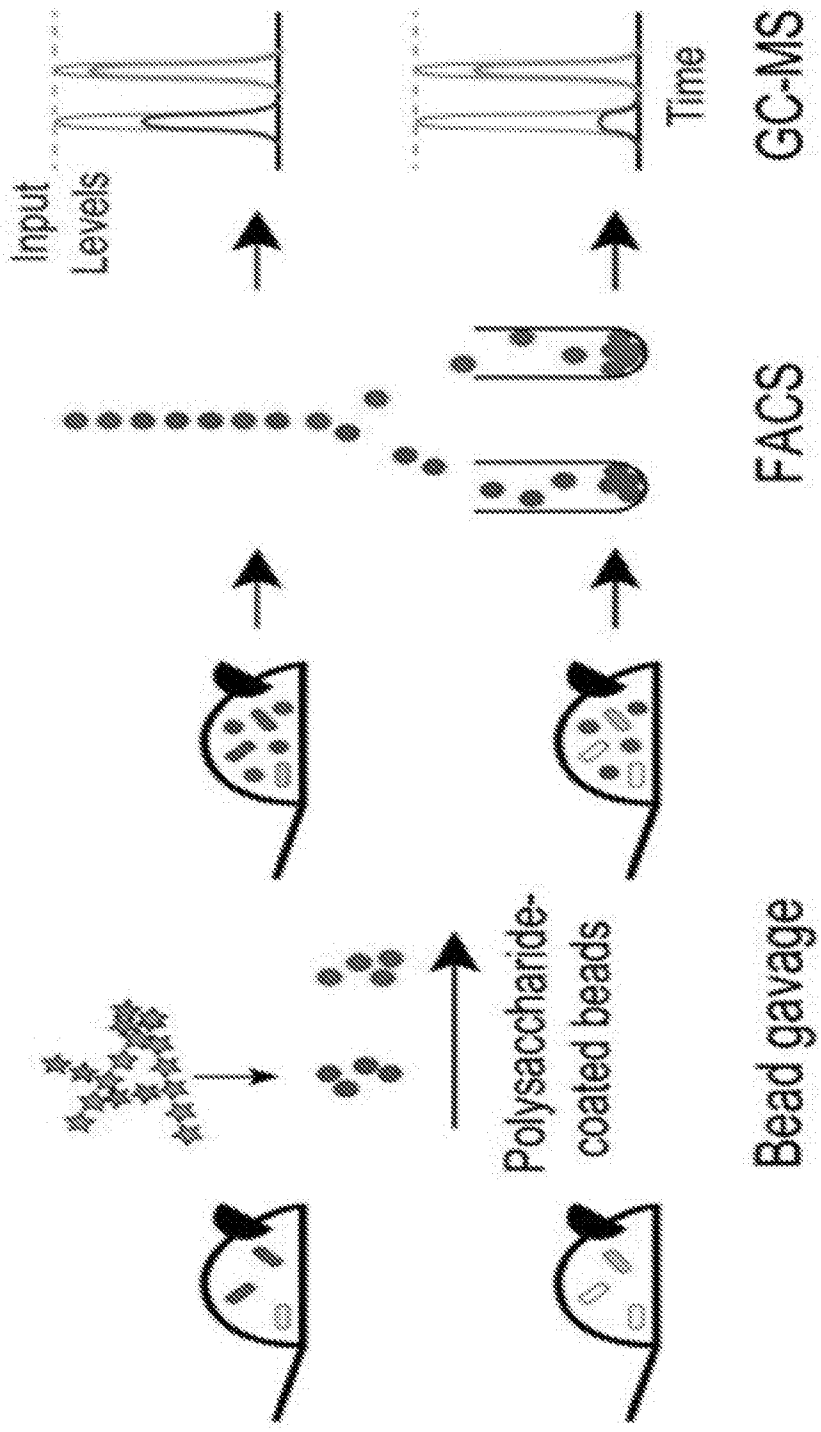

Input
Levels
Time
GC-MS
FACS
Polysaccharide-
coated beads
Bead gavage

FIG. 16

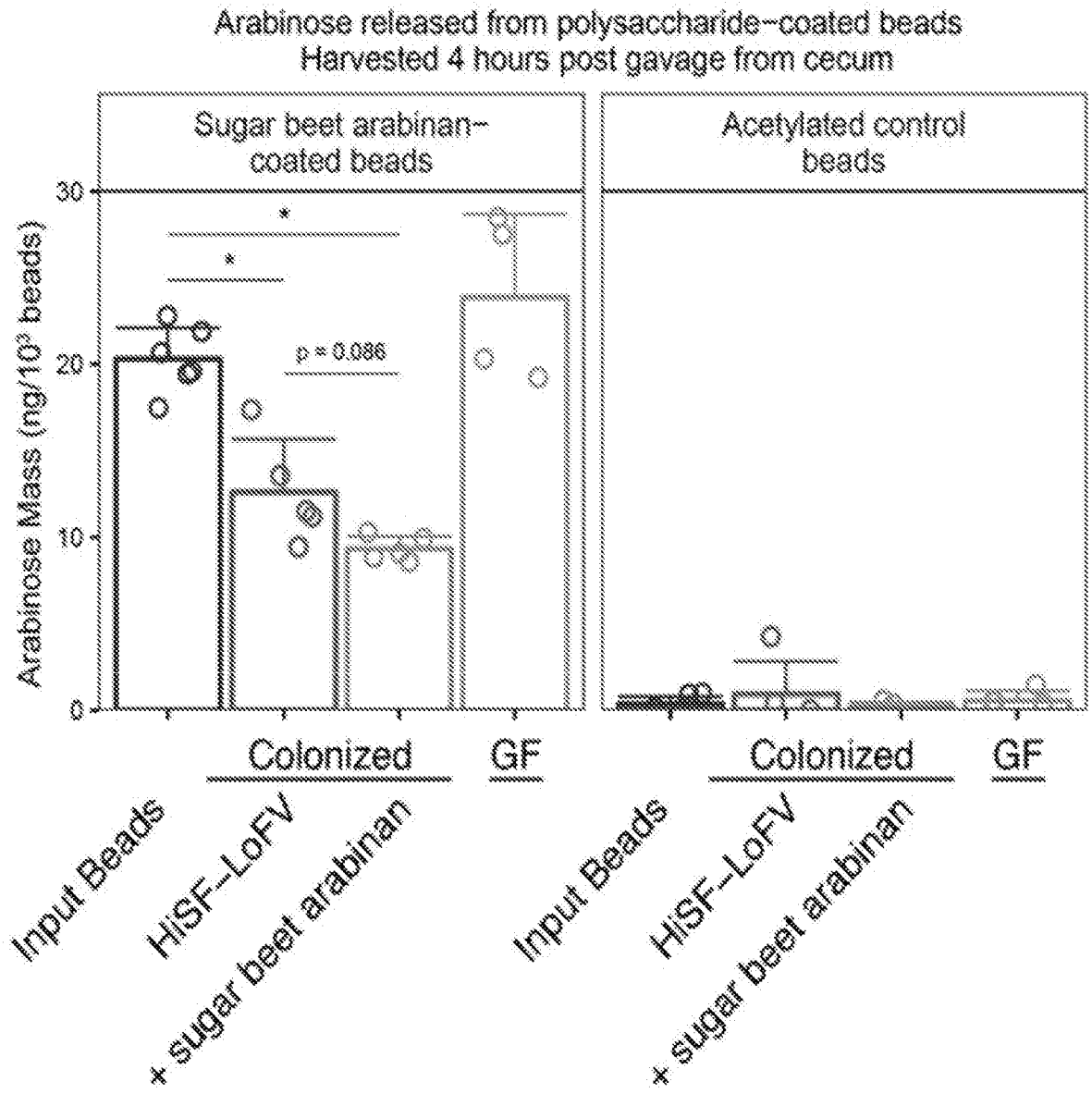

Arabinose released from polysaccharide-coated beads
Harvested 4 hours post gavage from cecum
Sugar beet arabinan-coated beads
Acetylated control beads
Arabinose Mass (ng/10³ beads)
30
20
10
0
*
*
p = 0.086
Colonized
GF
Colonized
GF
Input Beads
HiSF-LoFV
+ sugar beet arabinan
Input Beads
HiSF-LoFV
+ sugar beet arabinan

FIG. 17

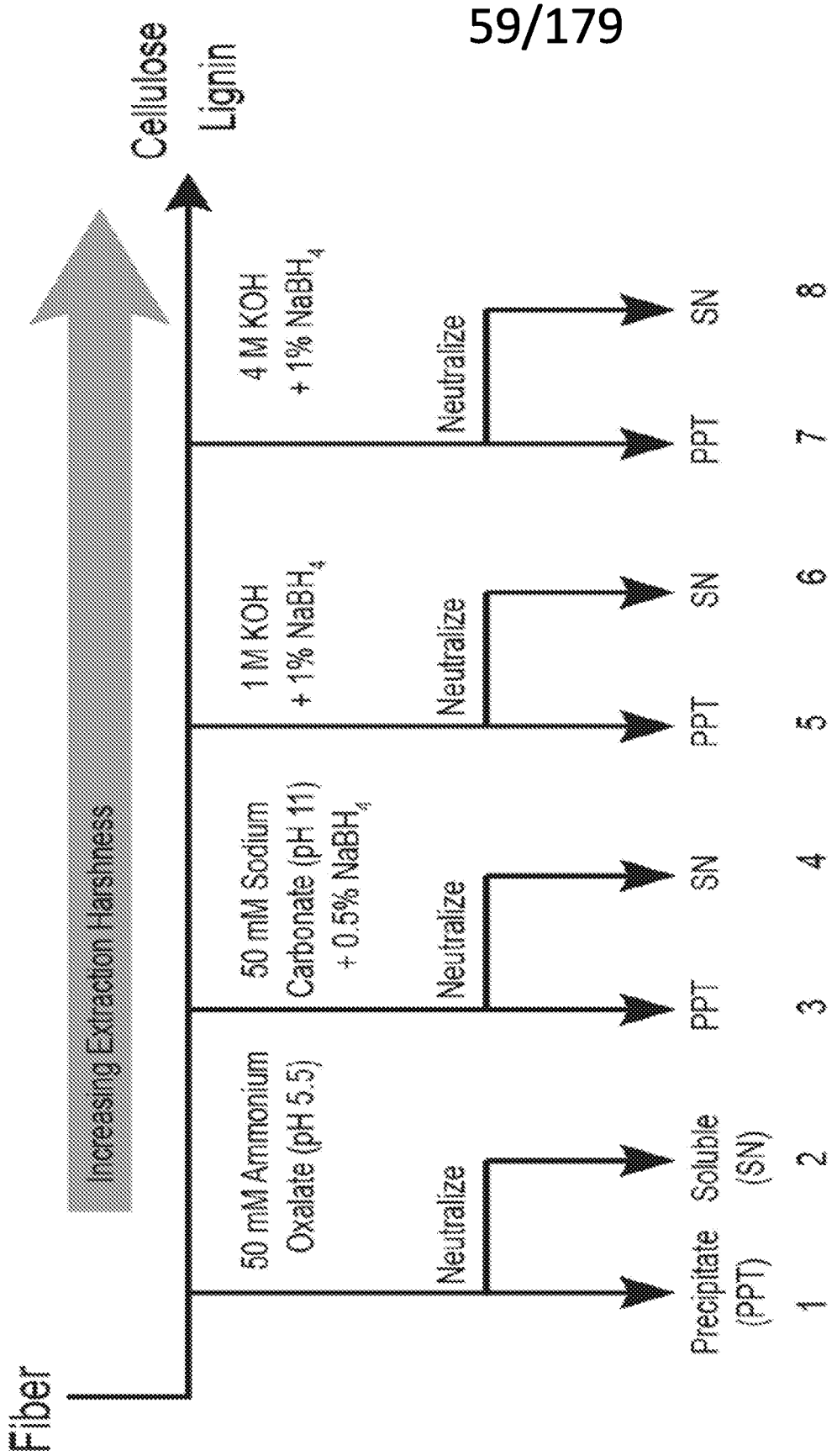

Fiber
Cellulose
Lignin
Increasing Extraction Harshness
50 mM Ammonium Oxalate (pH 5.5)
50 mM Sodium Carbonate (pH 11) + 0.5% NaBH4
1 M KOH + 1% NaBH4
4 M KOH + 1% NaBH4
Neutralize
Precipitate (PPT)
Soluble (SN)
PPT
SN
1
2
3
4
5
6
7
8
Fraction

FIG. 18

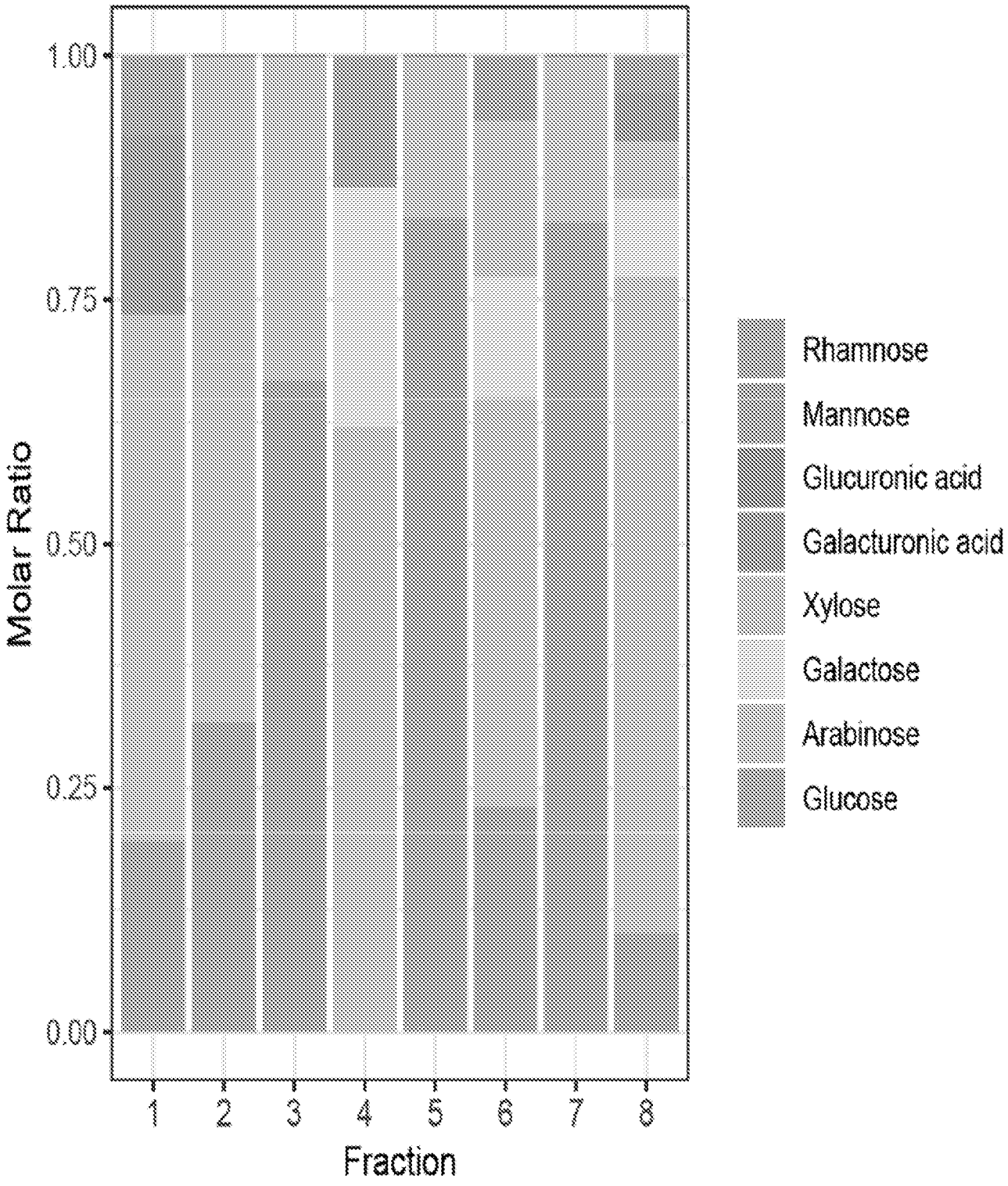

1.00
0.75
0.50
0.25
0.00
Molar Ratio
1
2
3
4
5
6
7
8
Fraction
Rhamnose
Mannose
Glucuronic acid
Galacturonic acid
Xylose
Galactose
Arabinose
Glucose

FIG. 19

Pea fiber arabinan (Fraction 8)

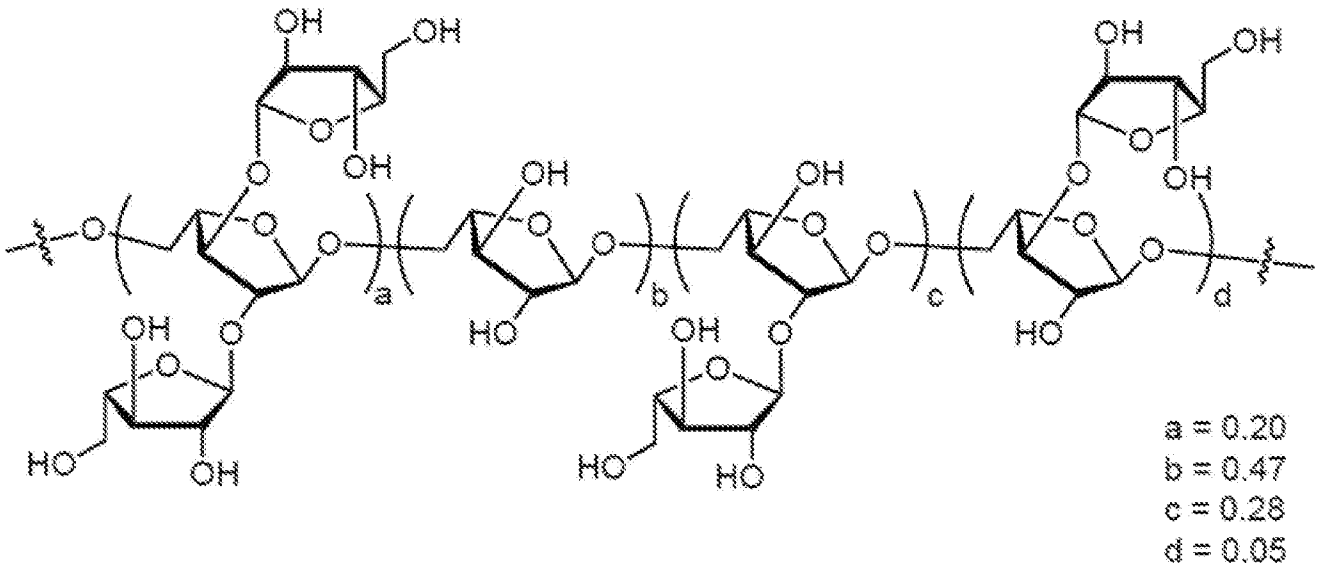


FIG. 20A

Sugar beet arabinan

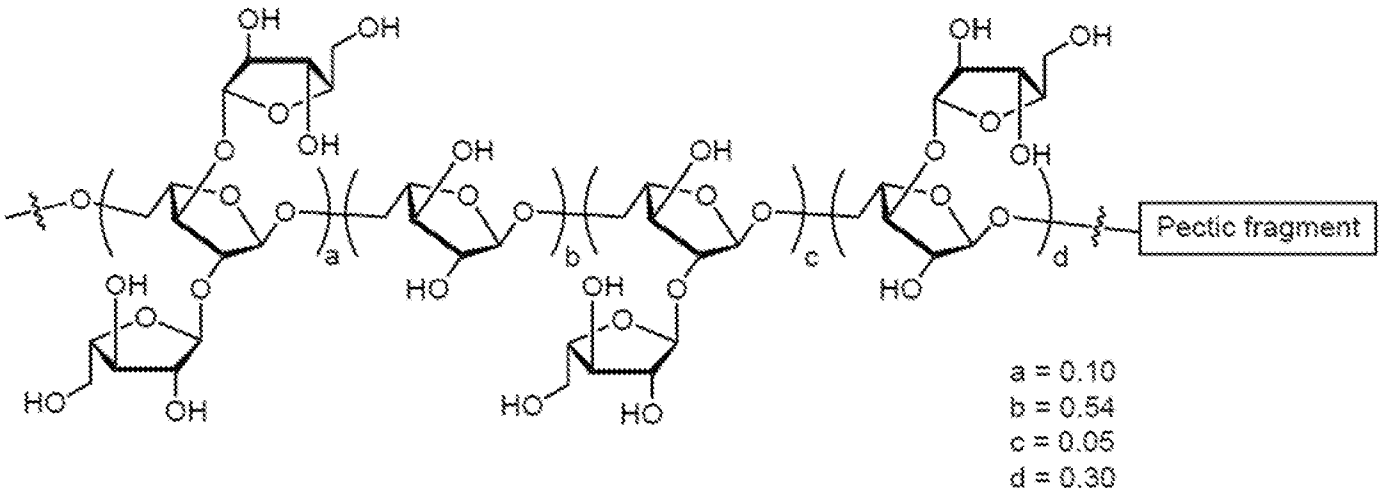


FIG. 20B

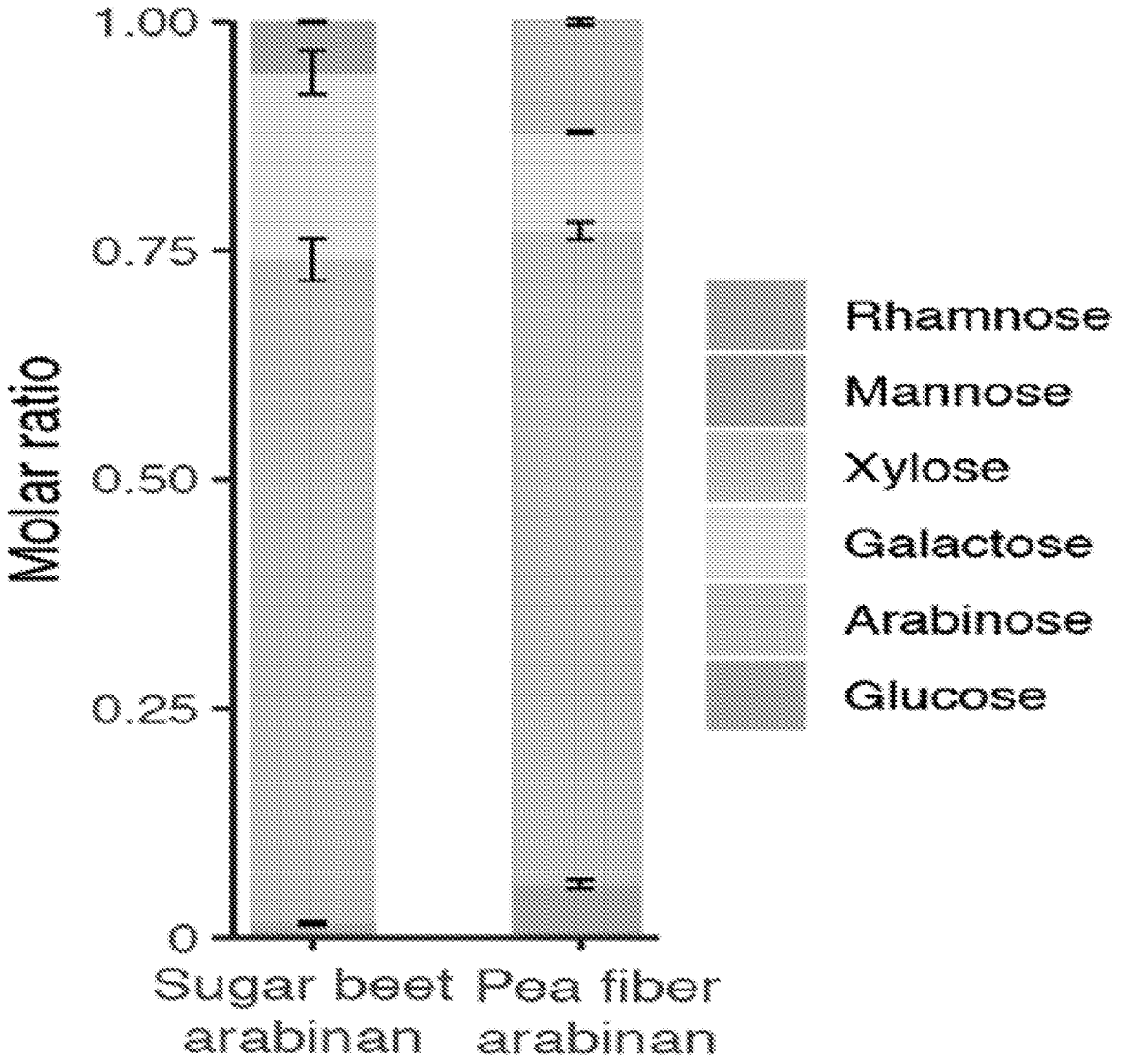

1.00
0.75
0.50
0.25
0
Molar ratio
Sugar beet arabinan
Pea fiber arabinan
Rhamnose
Mannose
Xylose
Galactose
Arabinose
Glucose

FIG. 21

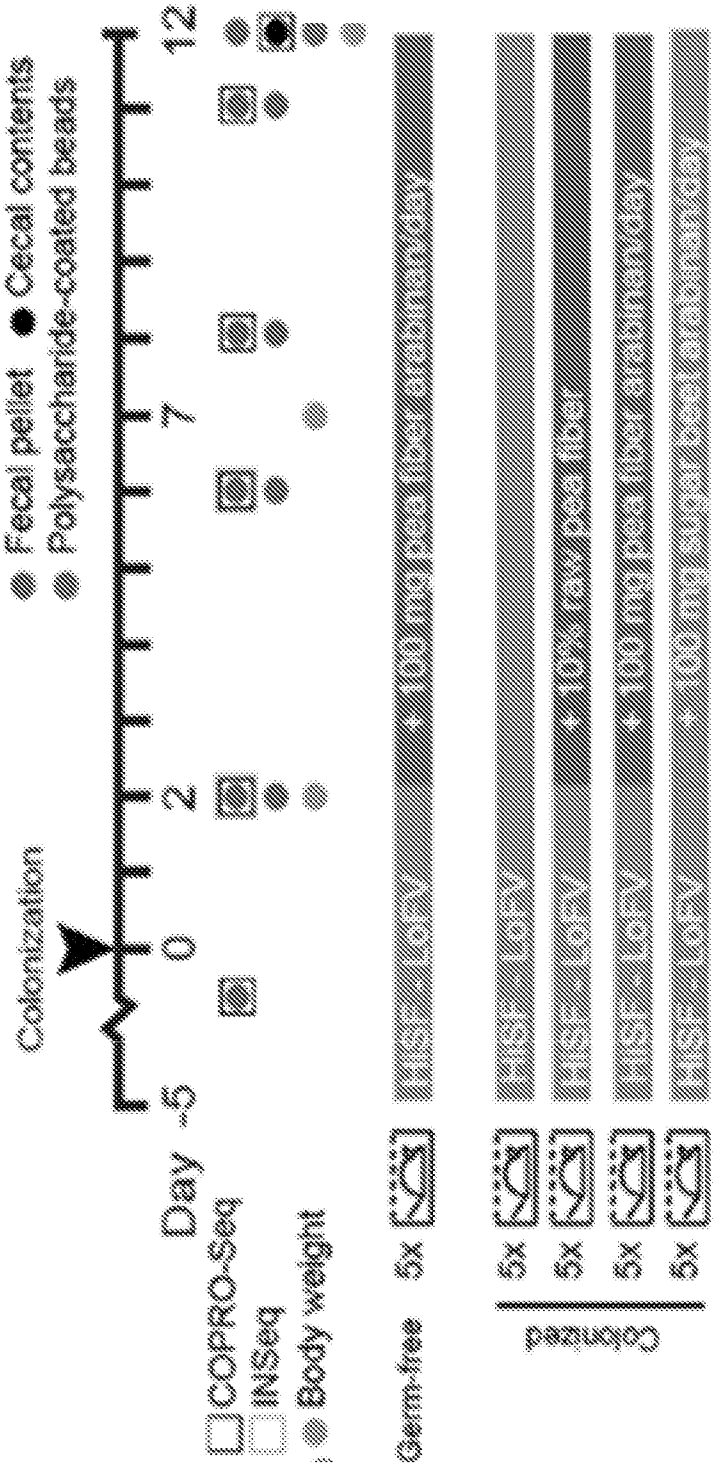

Fecal pellet
Cecal contents
Polysaccharide-coated beads
Colonization
Day -5
0
2
7
12
COPRO-Seq
INSeq
Body weight
Germ-free
5x
HiSeq 100PE
100 mg per liver contralateral
Colonized
5x
HiSeq 100PE
1 lobe raw ceca liver
5x
HiSeq 100PE
100 mg per liver contralateral
5x
HiSeq 100PE
100 mg cecal content contralateral

FIG. 22

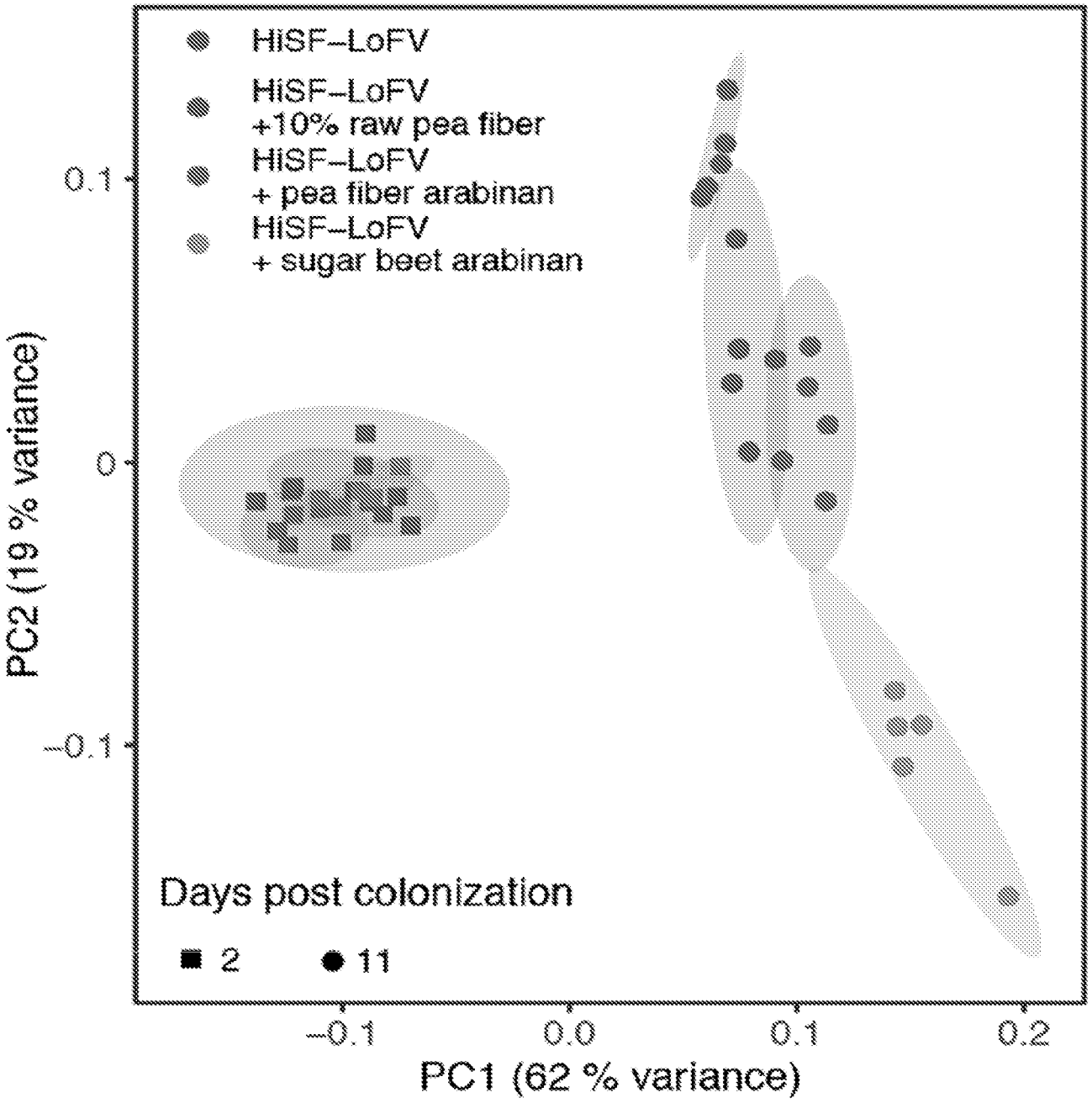

HiSF–LoFV
HiSF–LoFV
+10% raw pea fiber
HiSF–LoFV
+ pea fiber arabinan
HiSF–LoFV
+ sugar beet arabinan
0.1
0
–0.1
PC2 (19 % variance)
Days post colonization
2
11
–0.1
0.0
0.1
0.2
PC1 (62 % variance)

FIG. 23

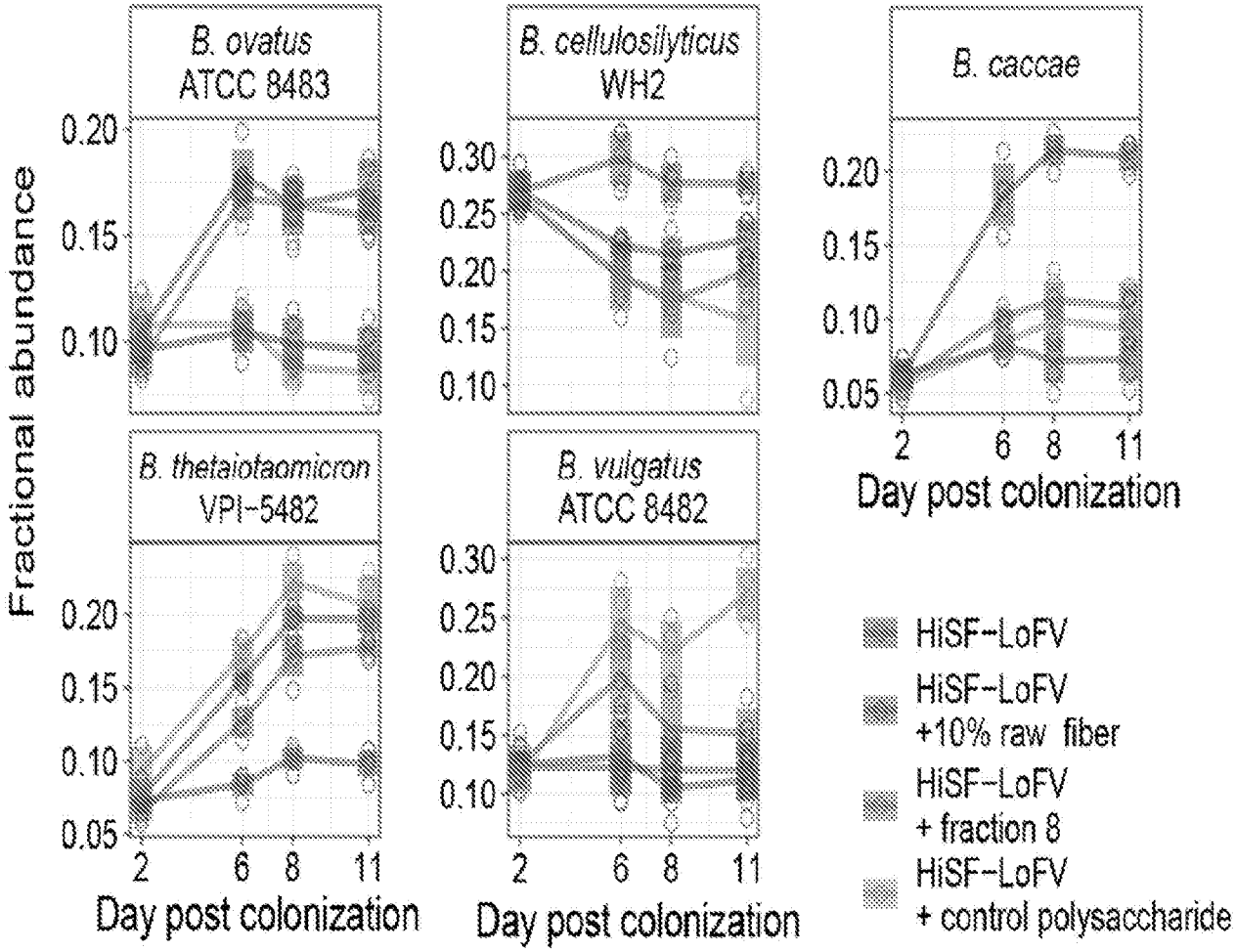

B. ovatus
ATCC 8483
B. cellulosilyticus
WH2
B. caccae
B. thetaiotaomicron
VPI-5482
B. vulgatus
ATCC 8482
Fractional abundance
Day post colonization
HiSF-LoFV
HiSF-LoFV
+10% raw fiber
HiSF-LoFV
+ fraction 8
HiSF-LoFV
+ control polysaccharide

FIG. 24

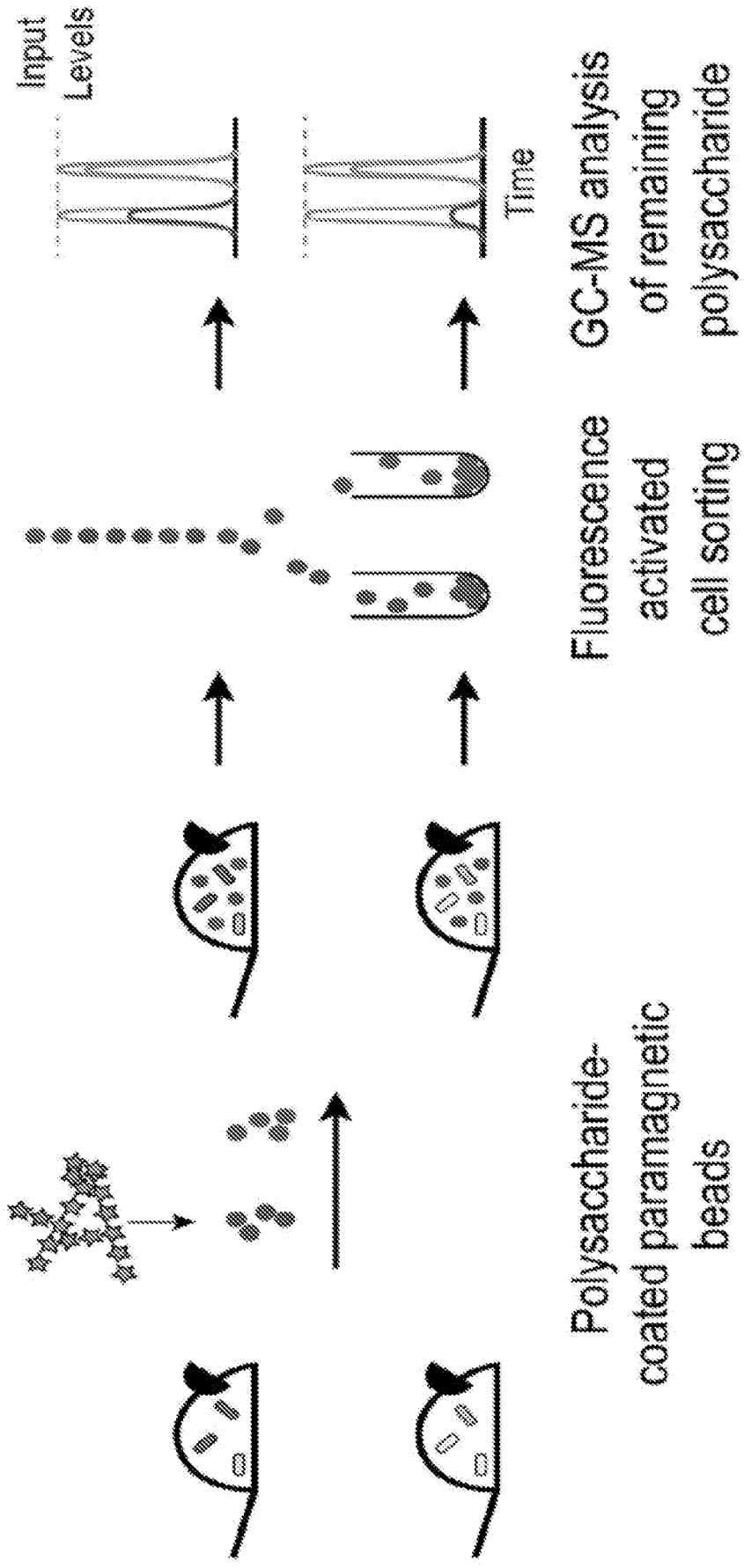

Input
Levels
Time
Polysaccharide-
coated paramagnetic
beads
Fluorescence
activated
cell sorting
GC-MS analysis
of remaining
polysaccharide

FIG. 25

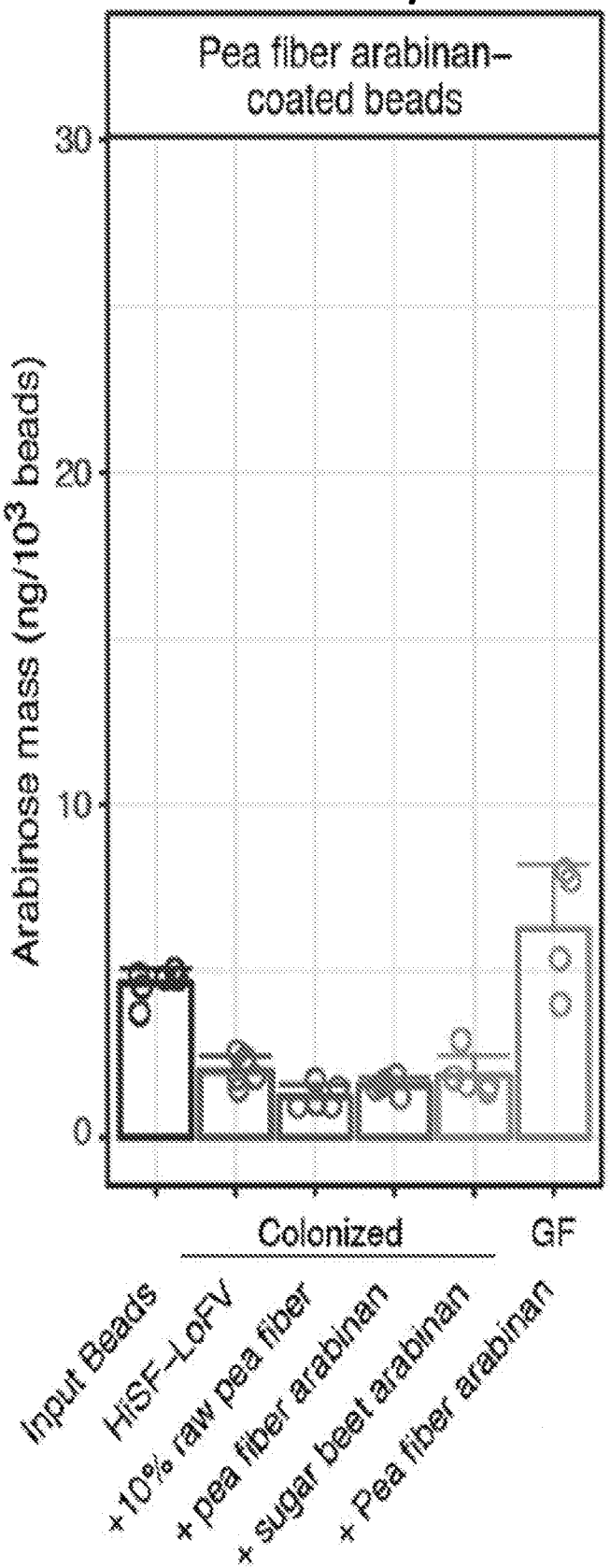

Pea fiber arabinan–
coated beads
30
20
10
0
Arabinose mass (ng/10^3 beads)
Colonized
GF
Input Beads
HiSF–LoFV
+10% raw pea fiber
+ pea fiber arabinan
+ sugar beet arabinan
+ Pea fiber arabinan

FIG. 26A

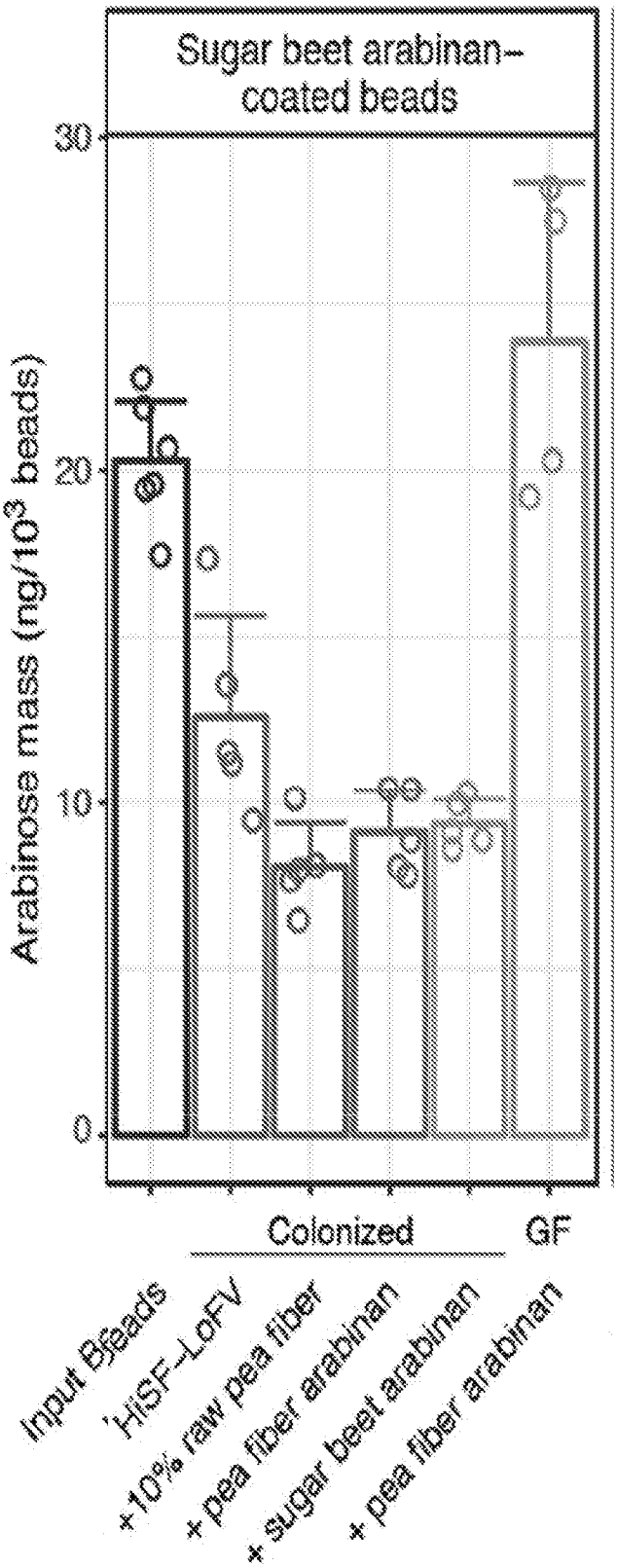

Sugar beet arabinan–
coated beads
Arabinose mass (mg/10^3 beads)
30
20
10
0
Colonized
GF
Input Bfeads
'HiSF–LoFV
+10% raw pea fiber
+ pea fiber arabinan
+ sugar beat arabinan
+ pea fiber arabinan

FIG. 26B

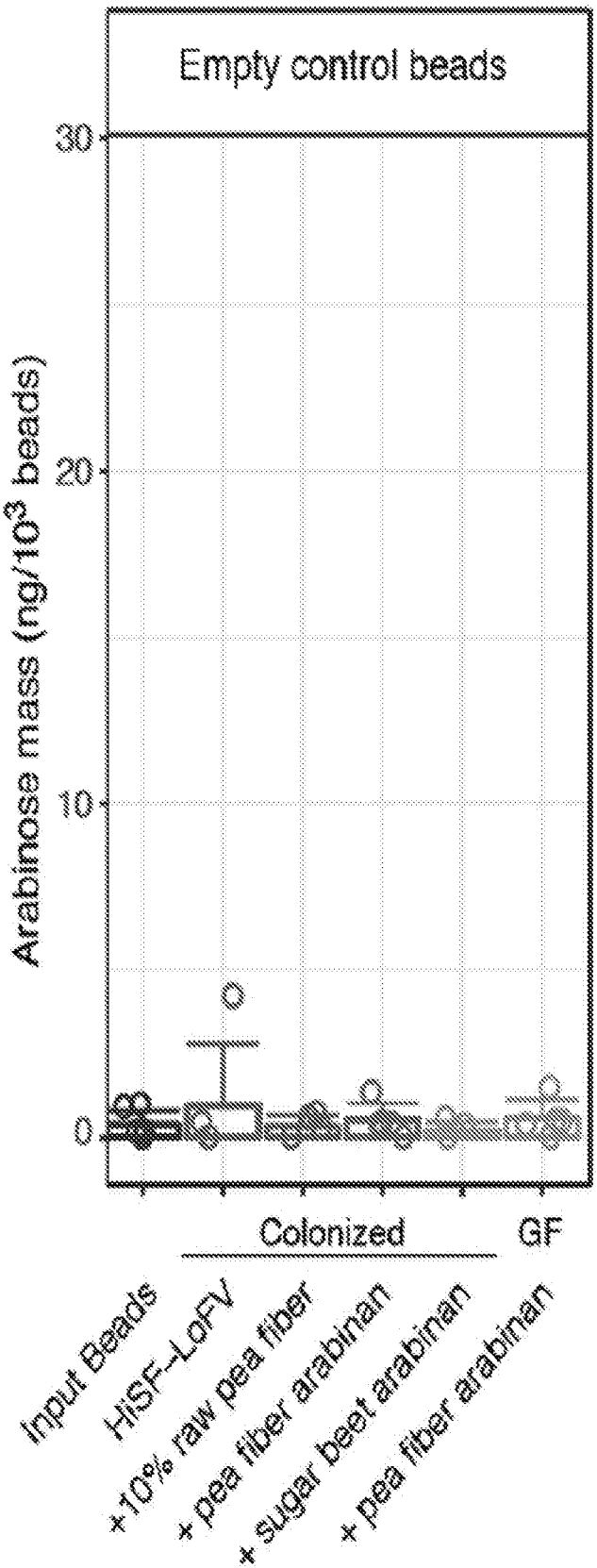

Empty control beads
Arabinose mass (ng/10^3 beads)
30
20
10
0
Colonized
GF
Input Beads
HISF-LOPV
+10% raw pea fiber
+ pea fiber arabinan
+ sugar beet arabinan
+ pea fiber arabinan

FIG. 26C

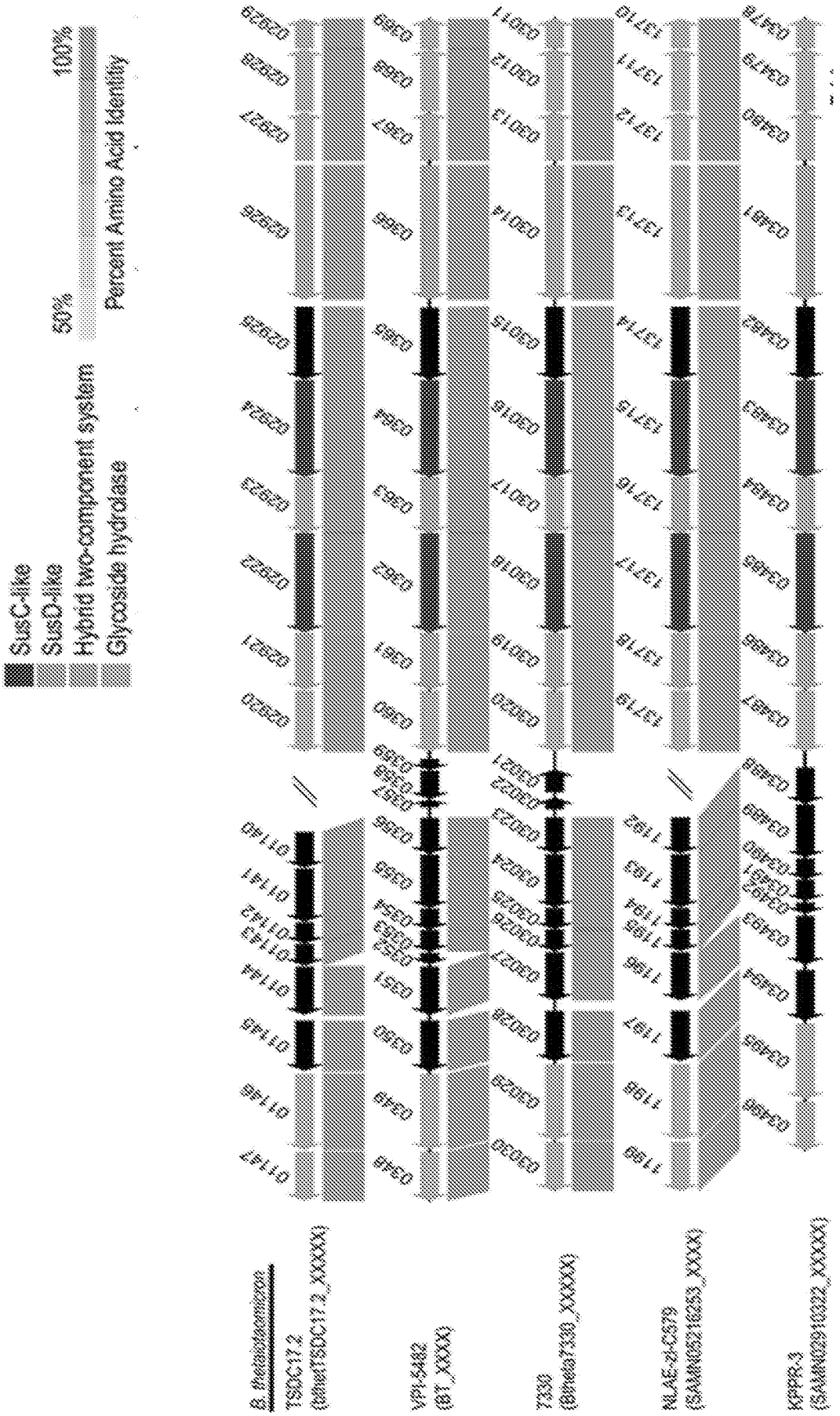

SusC-like
SusD-like
Hybrid two-component system
Glycoside hydrolase
50%
100%
Percent Amino Acid Identity
B. wexlerae
TSDC17.2
(bmmTSDC17.2_XXXXX)
VPI-5482
(BT_XXXX)
7330
(Bmeta7330_XXXXX)
NLAE-zl-C579
(SAMN05216353_XXXX)
KPPR-3
(SAMN02910322_XXXXX)

FIG. 27A

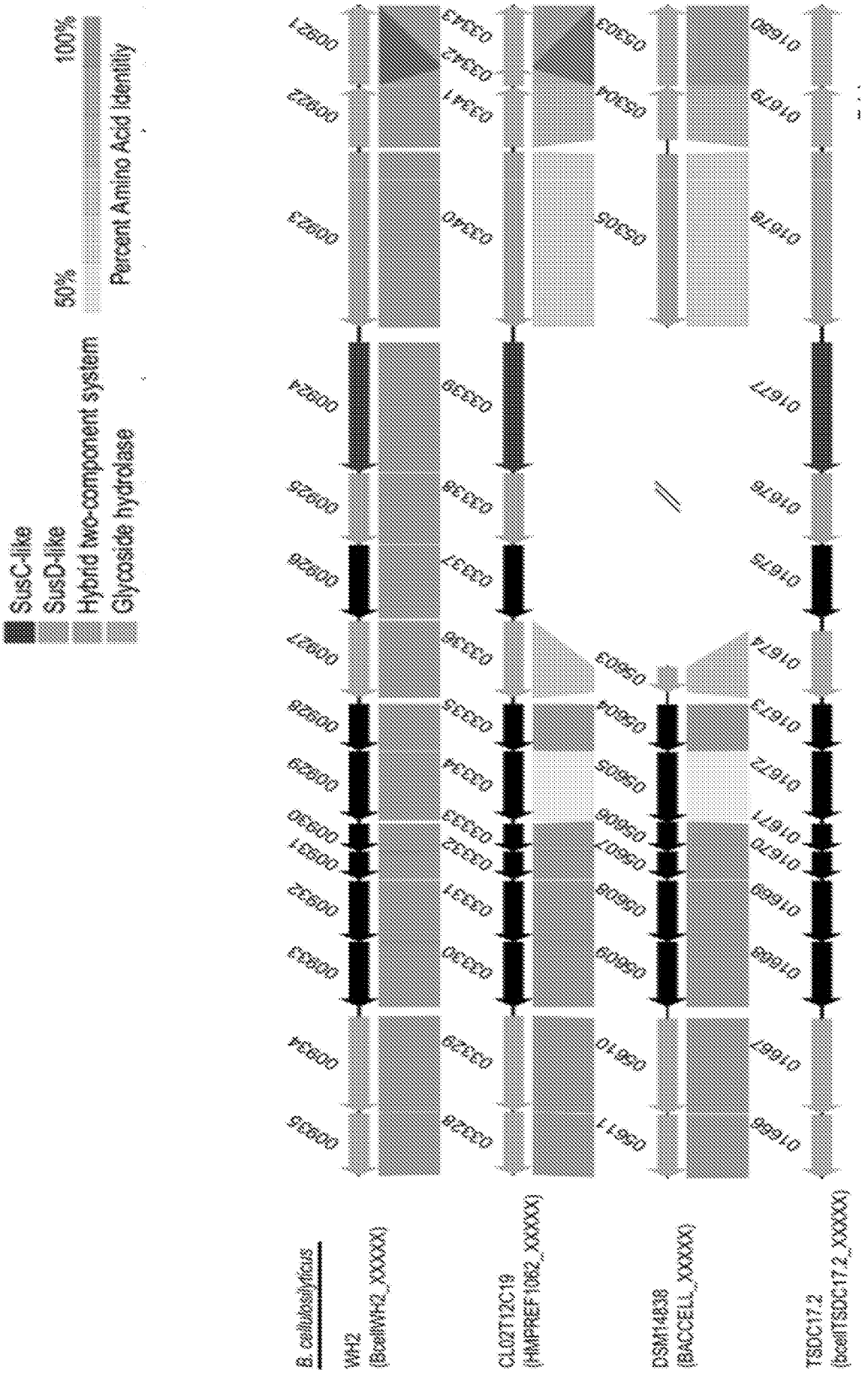

SusC-like
SusD-like
Hybrid two-component system
Glycoside hydrolase
50%
100%
Percent Amino Acid Identity
B. cellulosilyticus
WH2
(BcellWH2_XXXXX)
CL02T12C19
(HMPREF1062_XXXXX)
DSM14838
(BACCELL_XXXXX)
TSDC17.2
(bcelTSDC17.2_XXXXX)

FIG. 27B

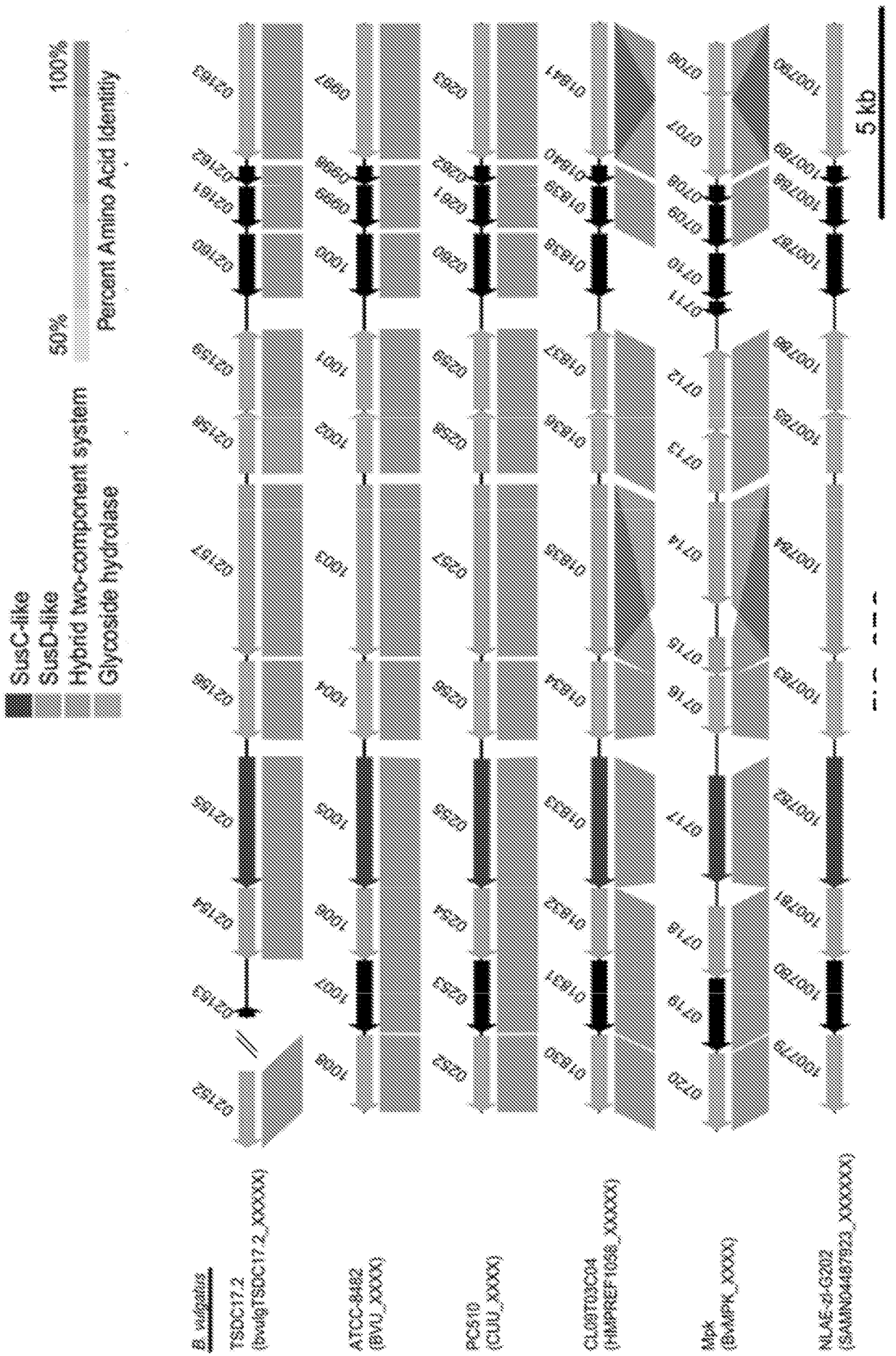

SusC-like
SusD-like
Hybrid two-component system
Glycoside hydrolase
50%
100%
Percent Amino Acid Identity
B. vulgatus
TSDC17.2
(bvulgTSDC17.2_XXXXX)
ATCC-8482
(BVU_XXXX)
PC510
(CIU_XXXX)
CL09T03C04
(HMPREF1058_XXXXX)
MpK
(BVMPK_XXXX)
KLAE-2-G03
(SAMN04487823_XXXXX)
5 kb

FIG. 27C

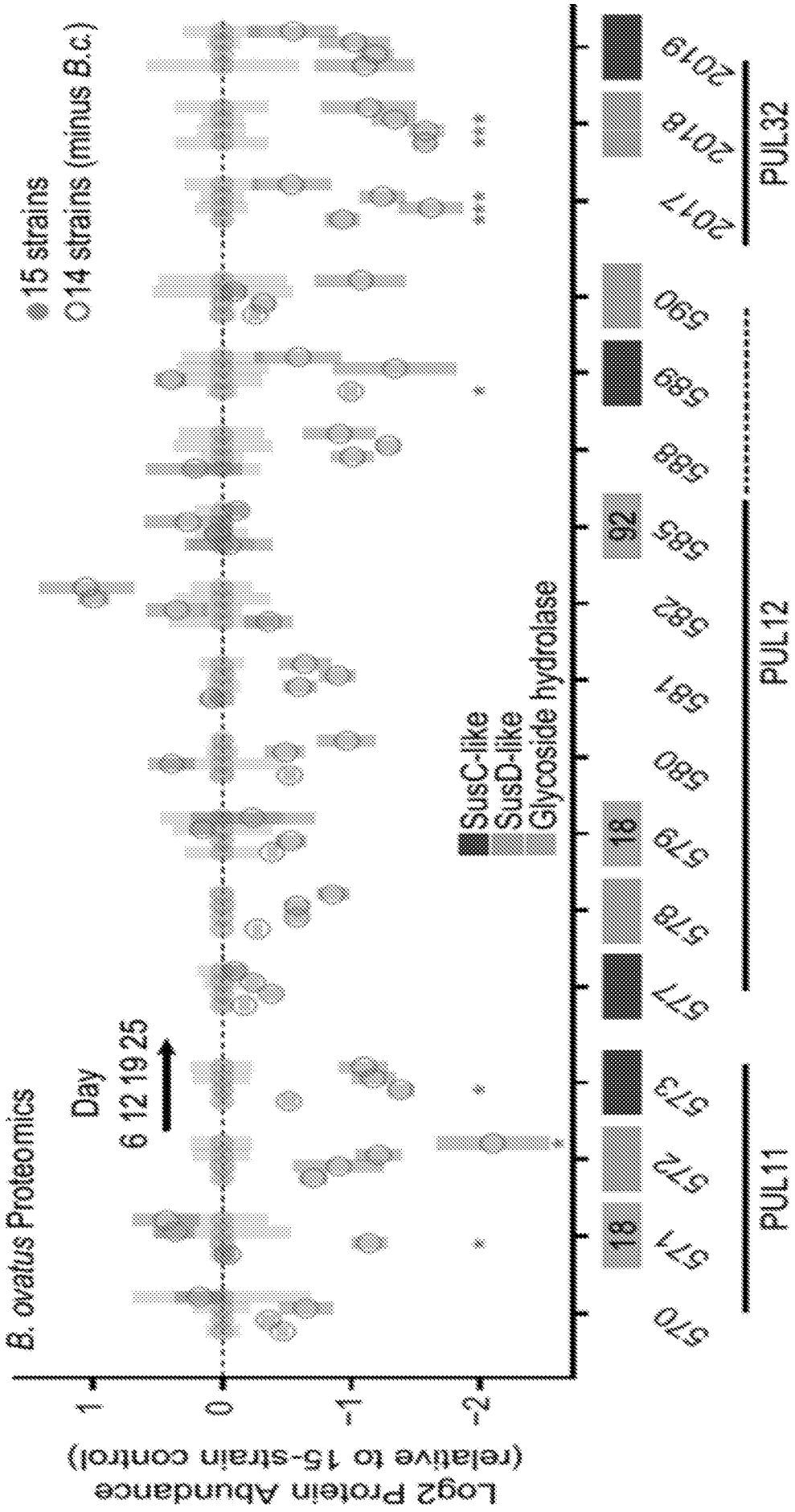

B. ovatus Proteomics
15 strains
14 strains (minus B.c.)
Day
6 12 19 25
Log2 Protein Abundance
(relative to 15-strain control)
1
0
-1
-2
SusC-like
SusD-like
Glycoside hydrolase
PUL11
PUL12
PUL32
570
571
572
573
577
578
579
580
581
582
585
588
589
590
2017
2018
2019
18
92

FIG. 28A

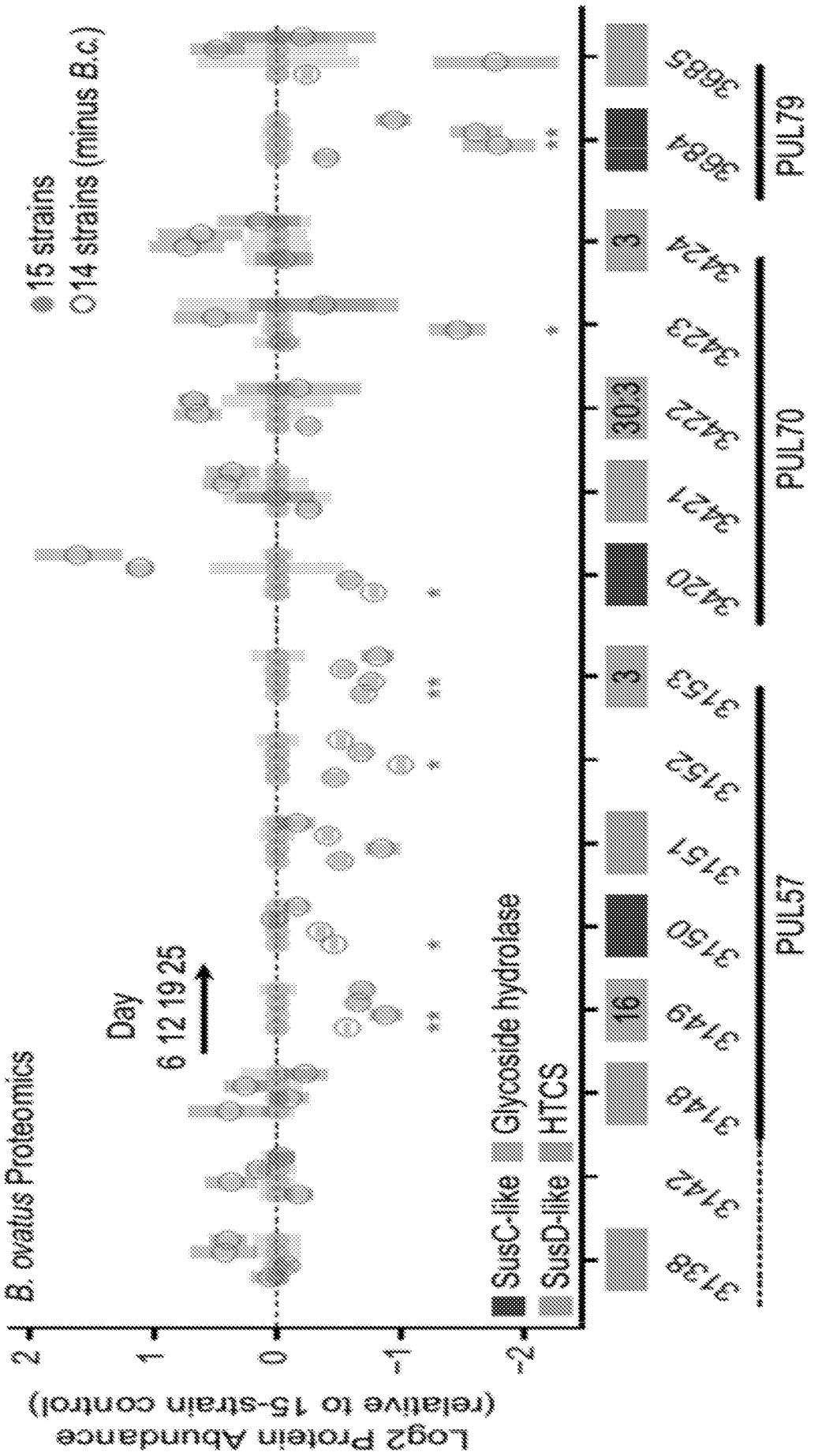

B. ovatus Proteomics
15 strains
14 strains (minus B.c.)
Day
6 12 19 25
SusC-like
SusD-like
Glycoside hydrolase
HTCS
Log2 Protein Abundance
(relative to 15-strain control)
2
1
0
-1
-2
PUL57
PUL70
PUL79

FIG. 28B

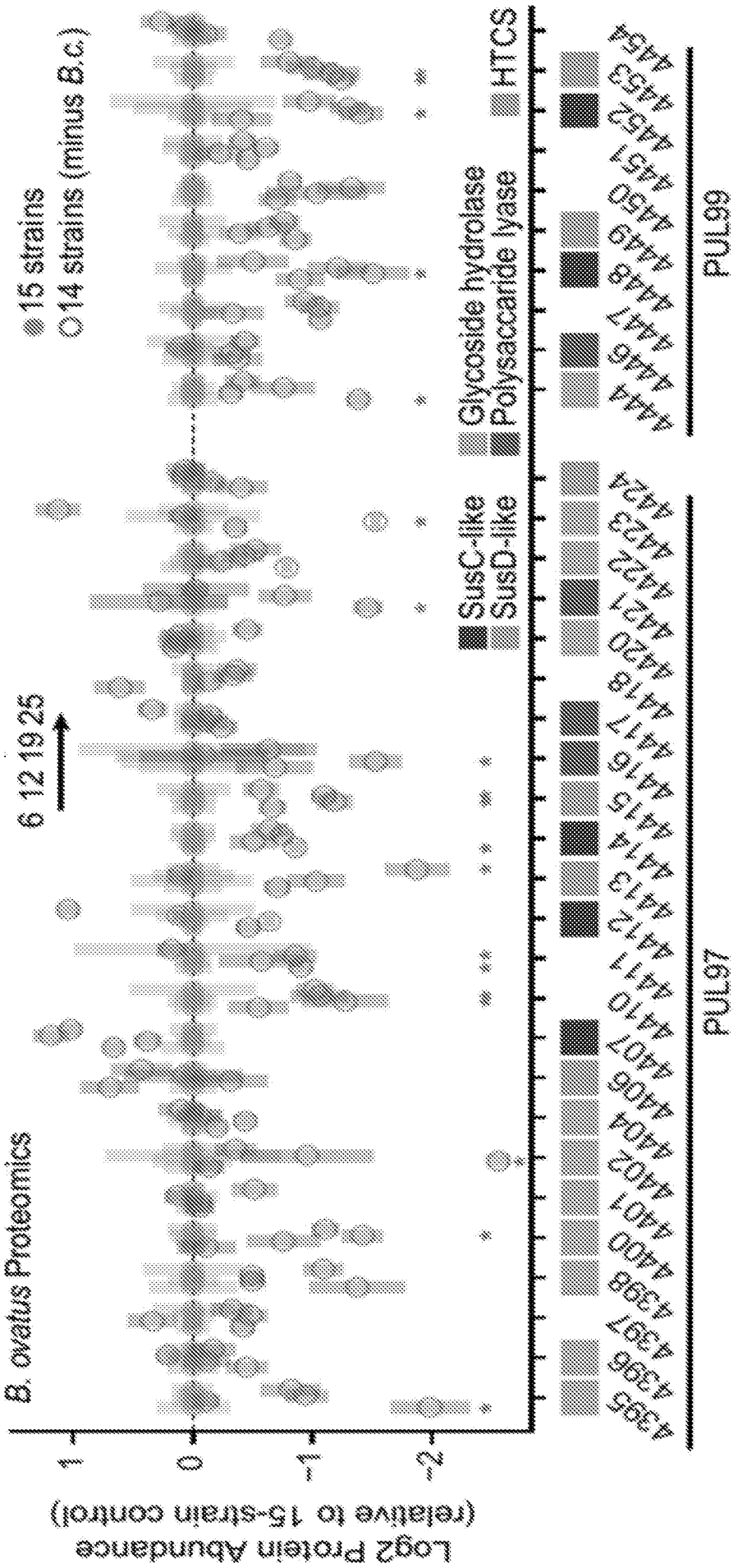

B. ovatus Proteomics
15 strains
14 strains (minus B.c.)
6 12 19 25
SusC-like
SusD-like
Glycoside hydrolase
Polysaccaride lyase
HTCS
PUL97
PUL99
Log2 Protein Abundance
(relative to 15-strain control)
1
0
-1
-2

FIG. 28C

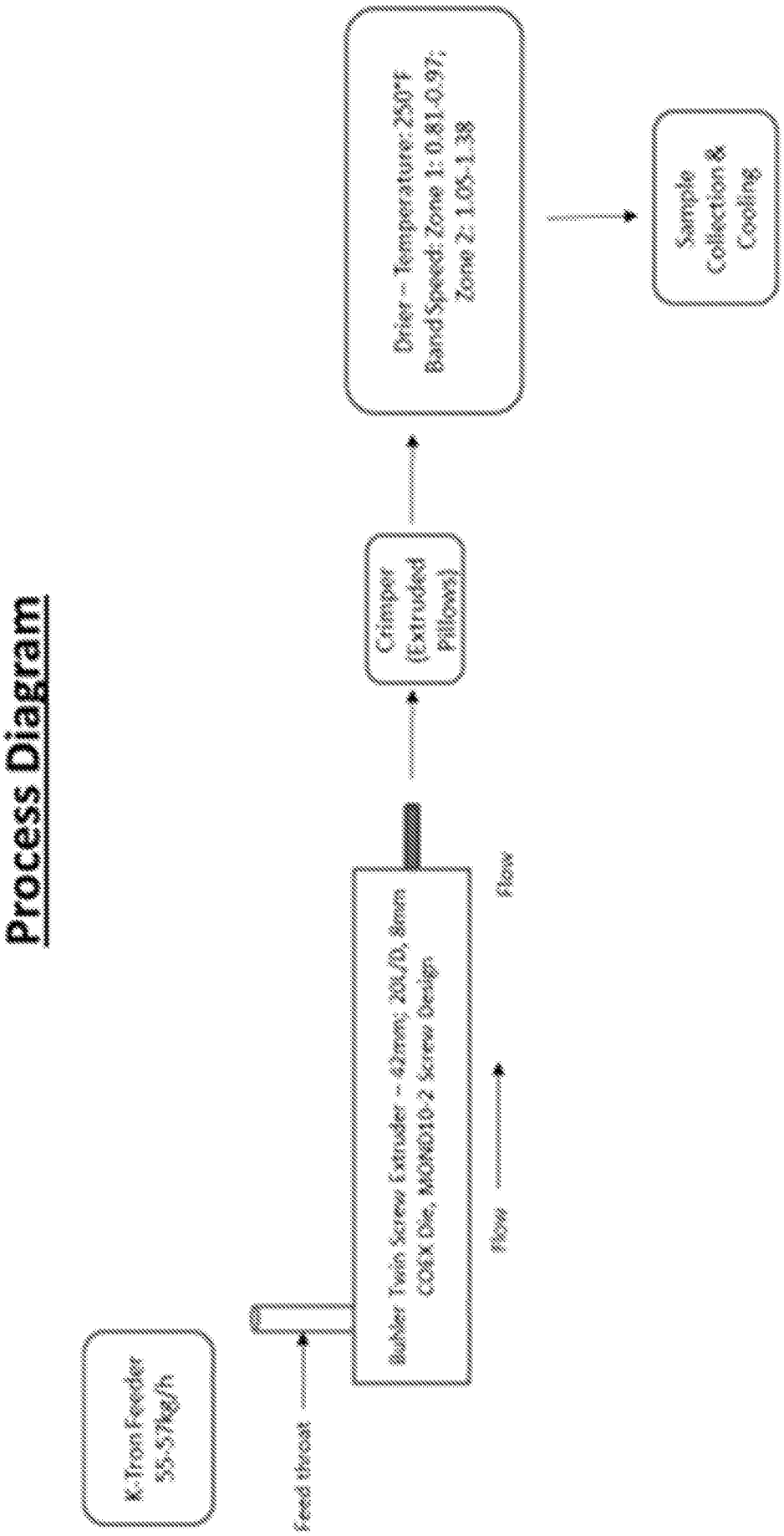

Process Diagram
K-Tron Feeder
55-5 Kg/h
Feed Stream
Buhler Twin Screw Extruder – 42mm; 20:D; 8mm
CCDX Die; MDN010-2 Screw Design
Flow
Flow
Crimper
(Extruded
Pellets)
Dryer – Temperature: 250°F
Band Speed: Zone 1: 0.81-0.97;
Zone 2: 1.05-1.38
Sample
Collection &
Cooling

FIG. 29

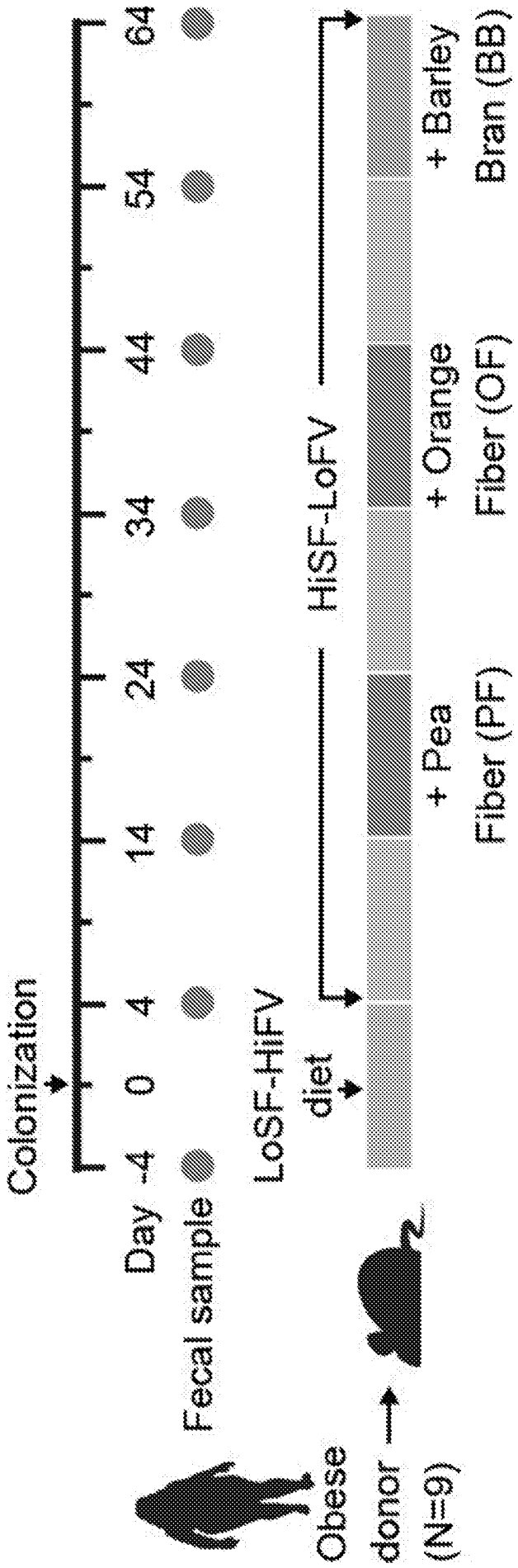

Colonization
Day -4 0 4 14 24 34 44 54 64
Fecal sample
LoSF-HiFV diet
HiSF-LoFV
+ Pea Fiber (PF)
+ Orange Fiber (OF)
+ Barley Bran (BB)
Obese donor (N=9)

FIG. 30A

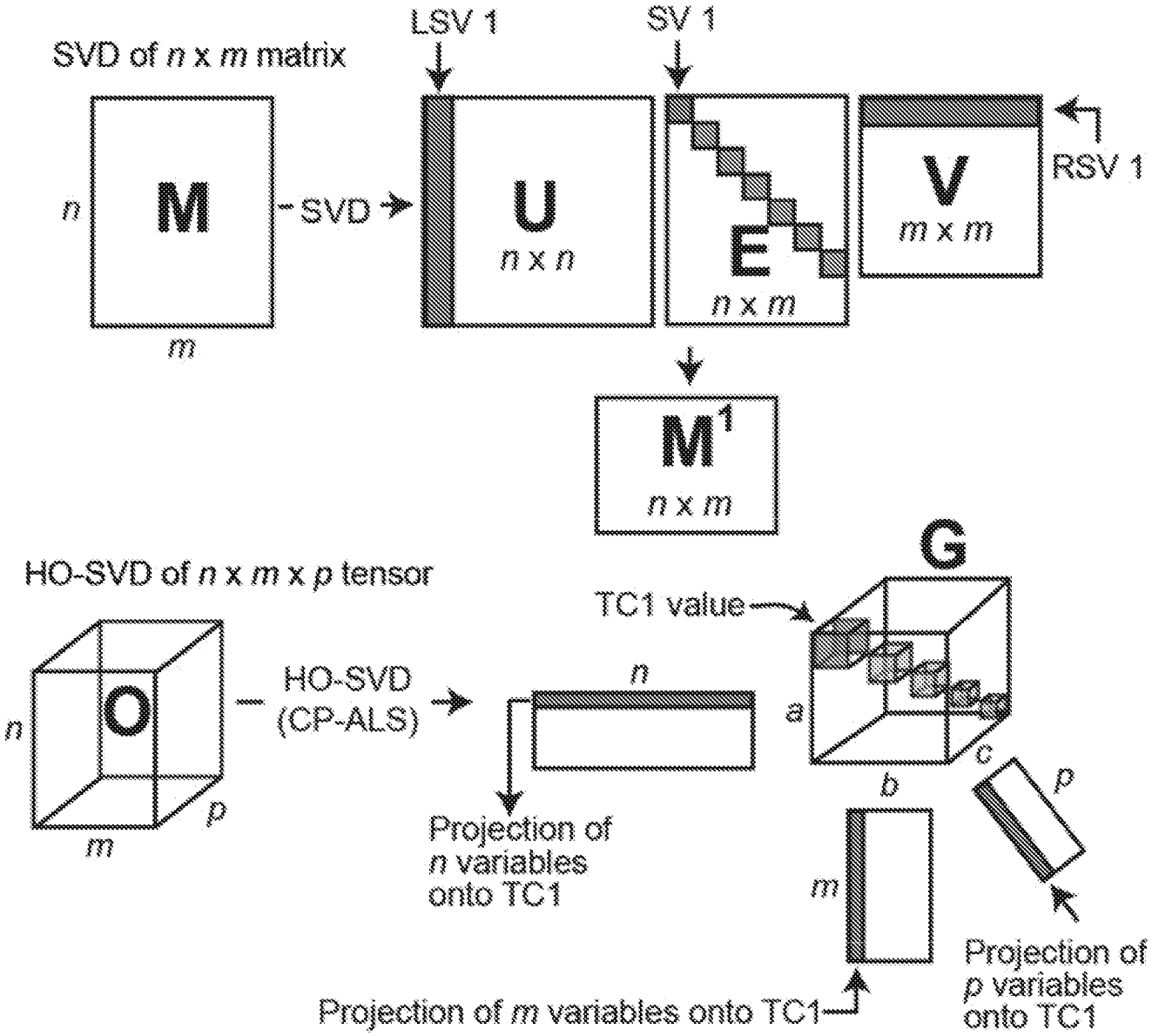

SVD of n x m matrix
LSV 1
SV 1
RSV 1
M
n
m
SVD
U
n x n
E
n x m
V
m x m
M1
n x m
HO-SVD of n x m x p tensor
O
n
m
p
HO-SVD
(CP-ALS)
n
Projection of
n variables
onto TC1
G
TC1 value
a
b
c
m
p
Projection of m variables onto TC1
Projection of
p variables
onto TC1

FIG. 30B

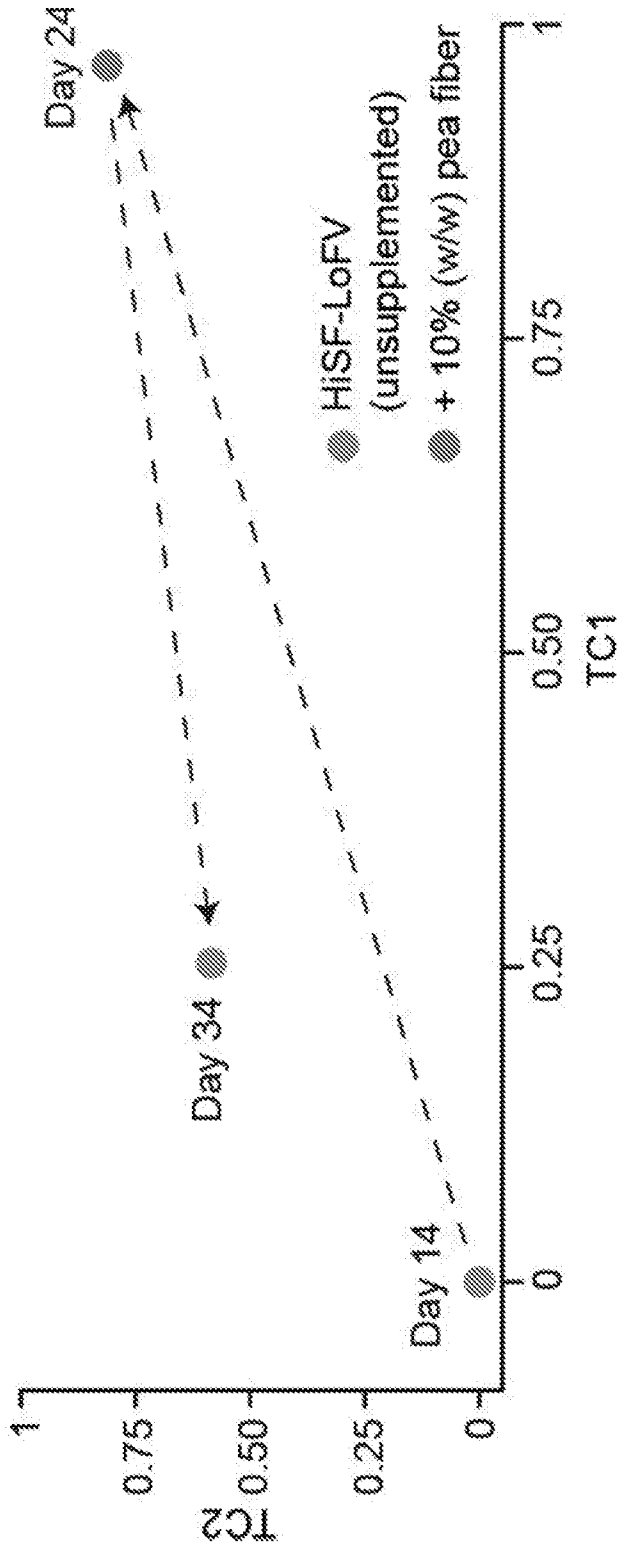

Day 24
Day 34
Day 14
HiSF-LoFV
(unsupplemented)
+ 10% (w/w) pea fiber
TC1
TC2
0
0.25
0.50
0.75
1

FIG. 30C

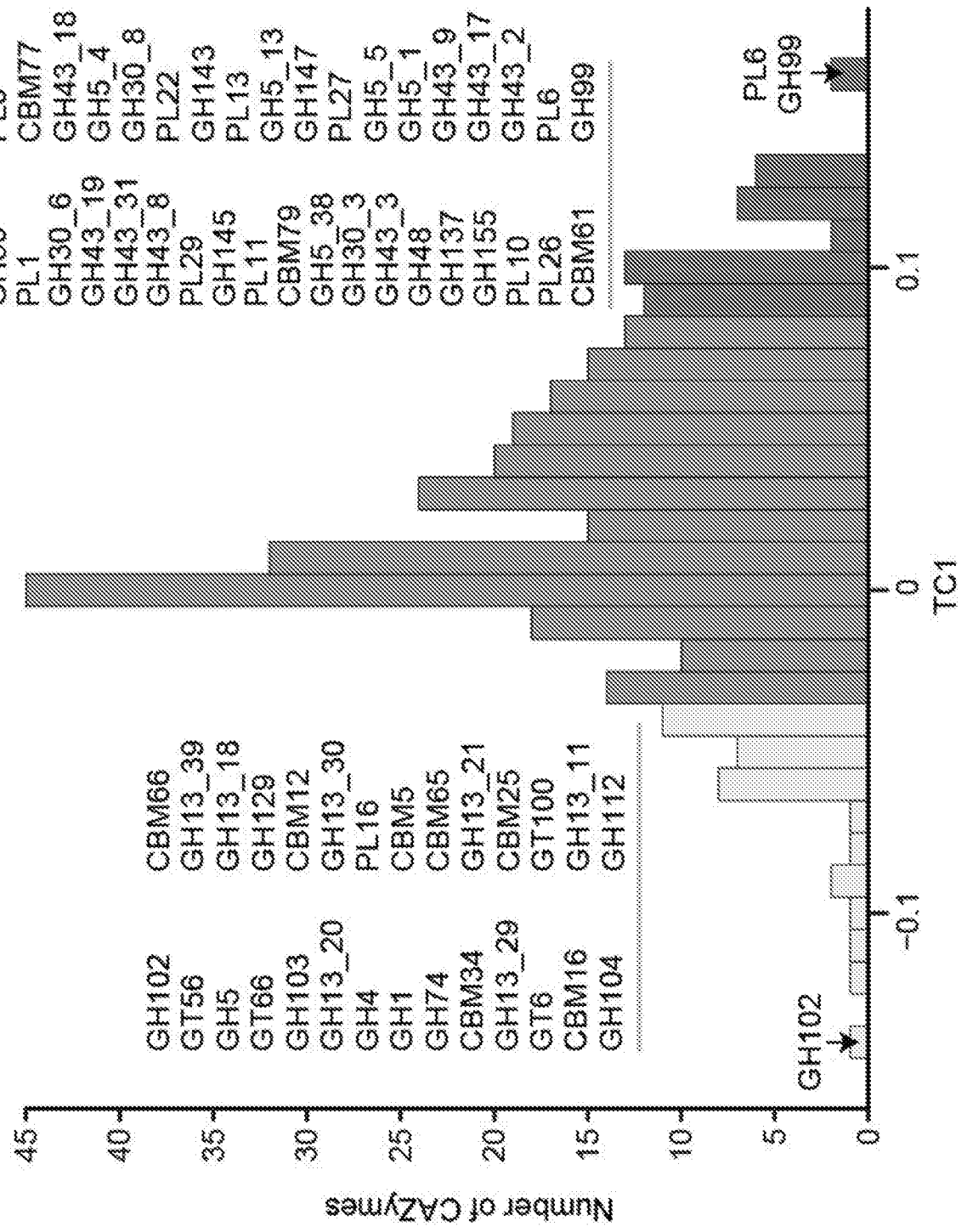

Number of CAZymes
45
40
35
30
25
20
15
10
5
0
TC1
−0.1
0
0.1
GH102
GT56
GH5
GT66
GH103
GH13_20
GH4
GH1
GH74
CBM34
GH13_29
GT6
CBM16
GH104
CBM66
GH13_39
GH13_18
GH129
CBM12
GH13_30
PL16
CBM5
CBM65
GH13_21
CBM25
GT100
GH13_11
GH112
GH50
PL1
GH30_6
GH43_19
GH43_31
GH43_8
PL29
GH145
PL11
CBM79
GH5_38
GH30_3
GH43_3
GH48
GH137
GH155
PL10
PL26
CBM61
PL9
CBM77
GH43_18
GH5_4
GH30_8
PL22
GH143
PL13
GH5_13
GH147
PL27
GH5_5
GH5_1
GH43_9
GH43_17
GH43_2
PL6
GH99
PL6
GH99

FIG. 30D

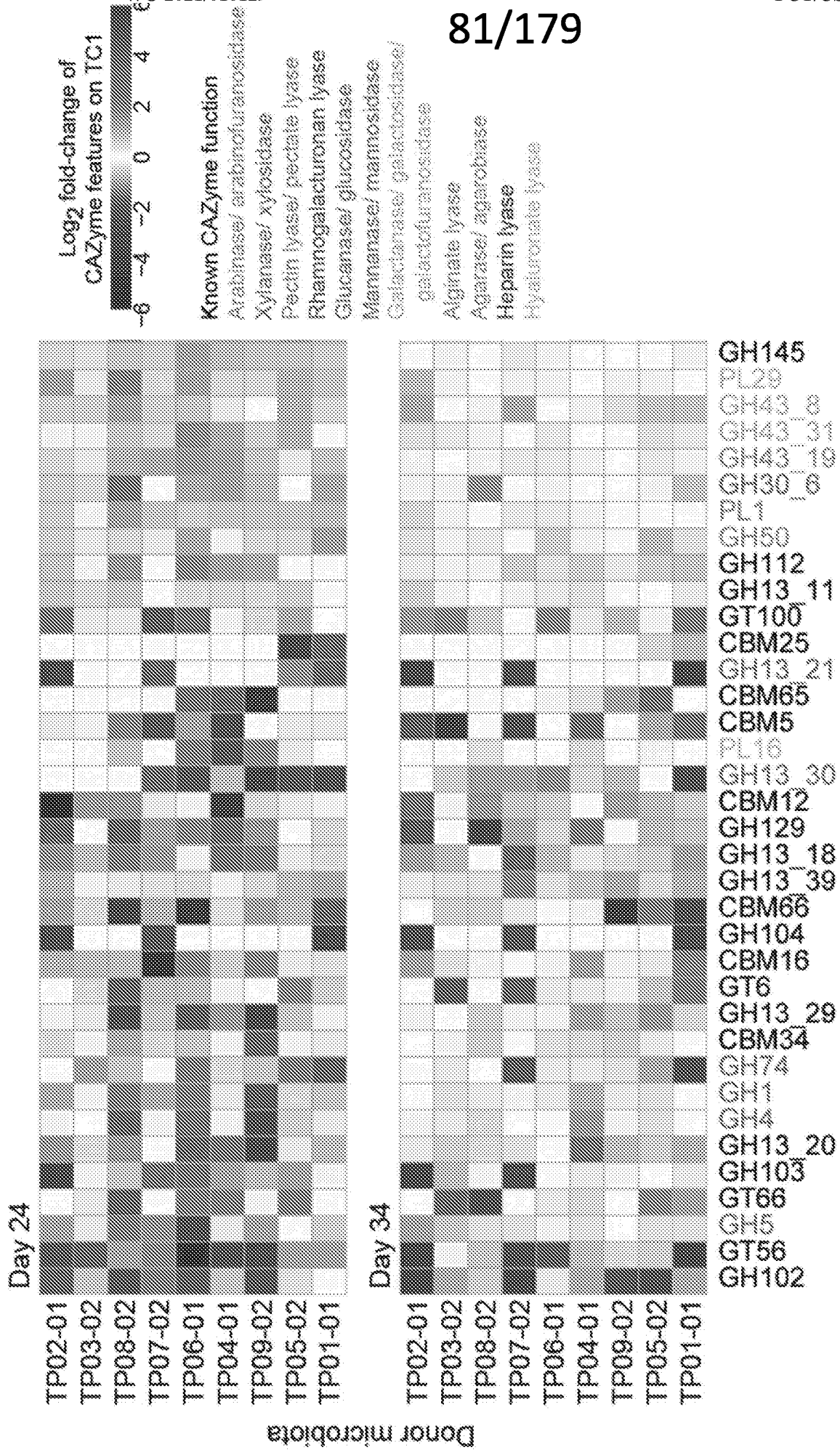

Log2 fold-change of CAZyme features on TC1
−6 −4 −2 0 2 4
Known CAZyme function
Arabinase/ arabinofuranosidase
Xylanase/ xylosidase
Pectin lyase/ pectate lyase
Rhamnogalacturonan lyase
Glucanase/ glucosidase
Mannanase/ mannosidase
Galactanase/ galactosidase/ galactofuranosidase
Alginate lyase
Agarase/ agarobiase
Heparin lyase
Hyaluronate lyase
Day 24
Day 34
Donor microbiota
TP02-01
TP03-02
TP08-02
TP07-02
TP06-01
TP04-01
TP09-02
TP05-02
TP01-01
GH145
PL29
GH43_8
GH43_31
GH43_19
GH30_6
PL1
GH50
GH112
GH13_11
GT100
CBM25
GH13_21
CBM65
CBM5
PL16
GH13_30
CBM12
GH129
GH13_18
GH13_39
CBM66
GH104
CBM16
GT6
GH13_29
CBM34
GH74
GH1
GH4
GH13_20
GH103
GT66
GH5
GT56
GH102

FIG. 30E

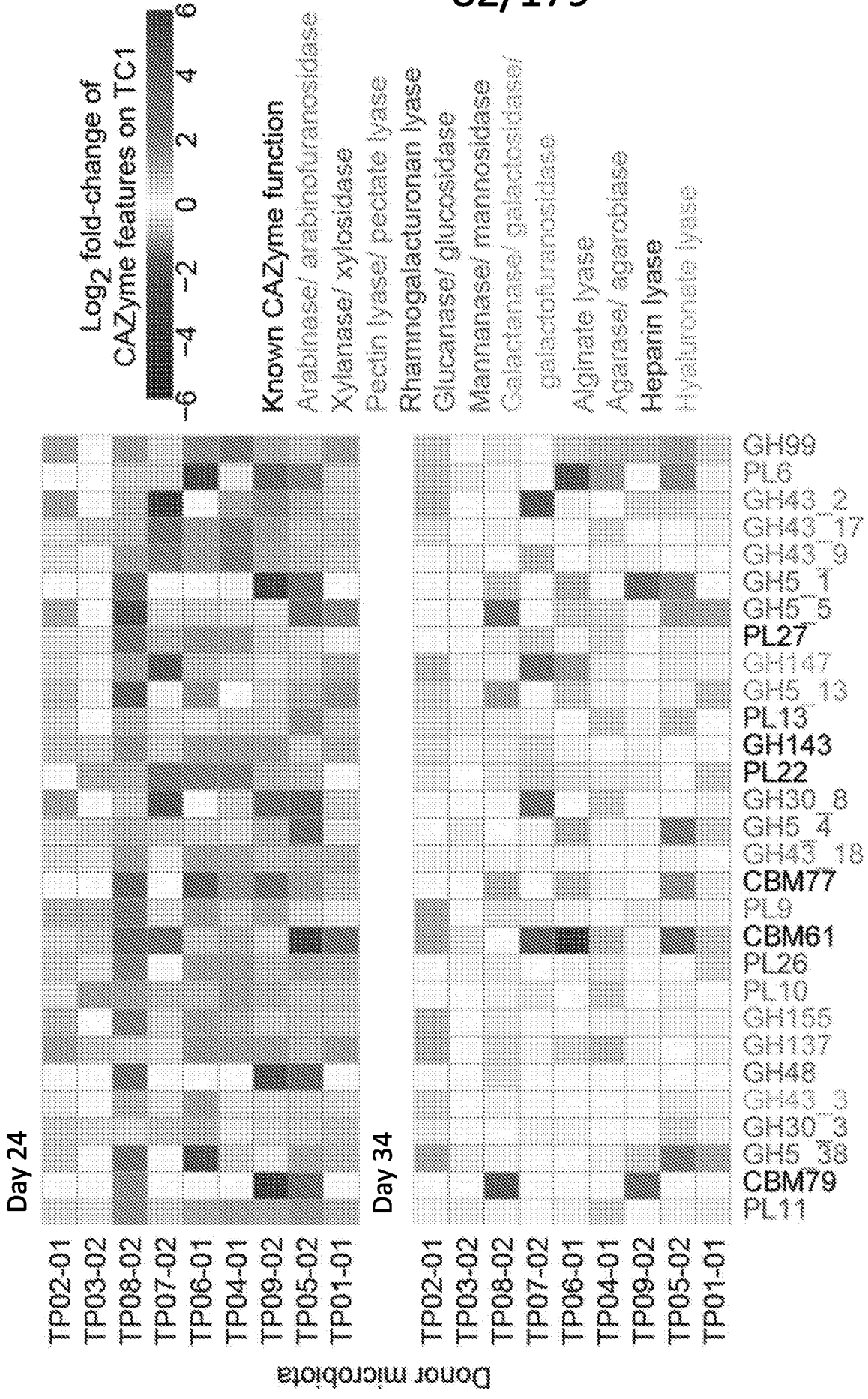

Log2 fold-change of CAZyme features on TC1
−6 −4 −2 0 2 4 6
Known CAZyme function
Arabinase/ arabinofuranosidase
Xylanase/ xylosidase
Pectin lyase/ pectate lyase
Rhamnogalacturonan lyase
Glucanase/ glucosidase
Mannanase/ mannosidase
Galactanase/ galactosidase/ galactofuranosidase
Alginate lyase
Agarase/ agarobiase
Heparin lyase
Hyaluronate lyase
Day 24
Day 34
Donor microbiota
TP02-01
TP03-02
TP08-02
TP07-02
TP06-01
TP04-01
TP09-02
TP05-02
TP01-01
GH99
PL6
GH43_2
GH43_17
GH43_9
GH5_1
GH5_5
PL27
GH147
GH5_13
PL13
GH143
PL22
GH30_8
GH5_4
GH43_18
CBM77
PL9
CBM61
PL26
PL10
GH155
GH137
GH48
GH43_3
GH30_3
GH5_38
CBM79
PL11

FIG. 30F

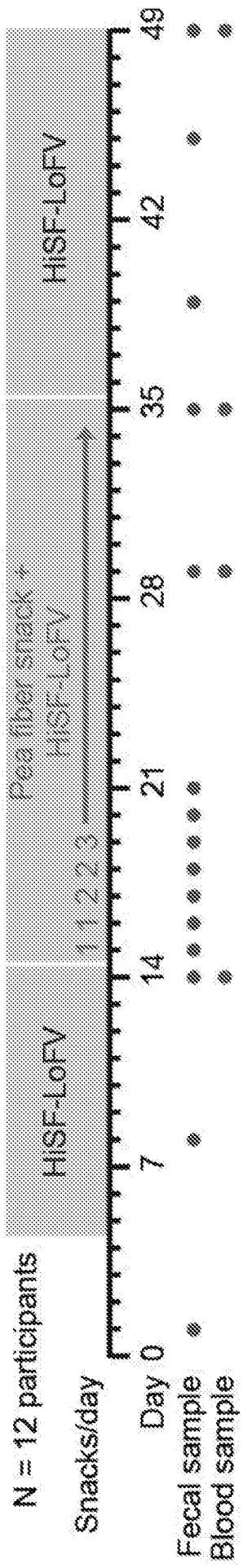

N = 12 participants
HiSF-LoFV
Pea fiber snack +
HiSF-LoFV
HiSF-LoFV
Snacks/day
1 1 2 2 3
Day 0
7
14
21
28
35
42
49
Fecal sample
Blood sample

FIG. 31A

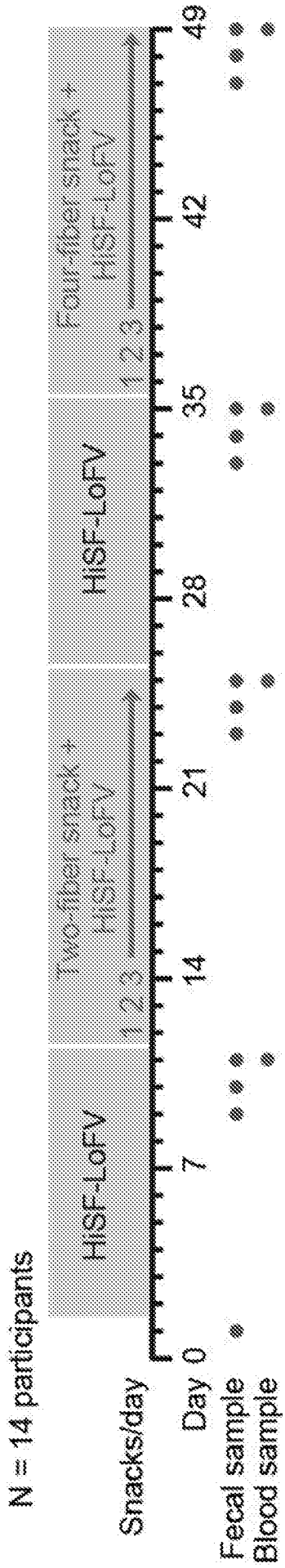

N = 14 participants
Snacks/day
HiSF-LoFV
Two-fiber snack + HiSF-LoFV
HiSF-LoFV
Four-fiber snack + HiSF-LoFV
1 2 3
1 2 3
Day 0
7
14
21
28
35
42
49
Fecal sample
Blood sample

FIG. 31B

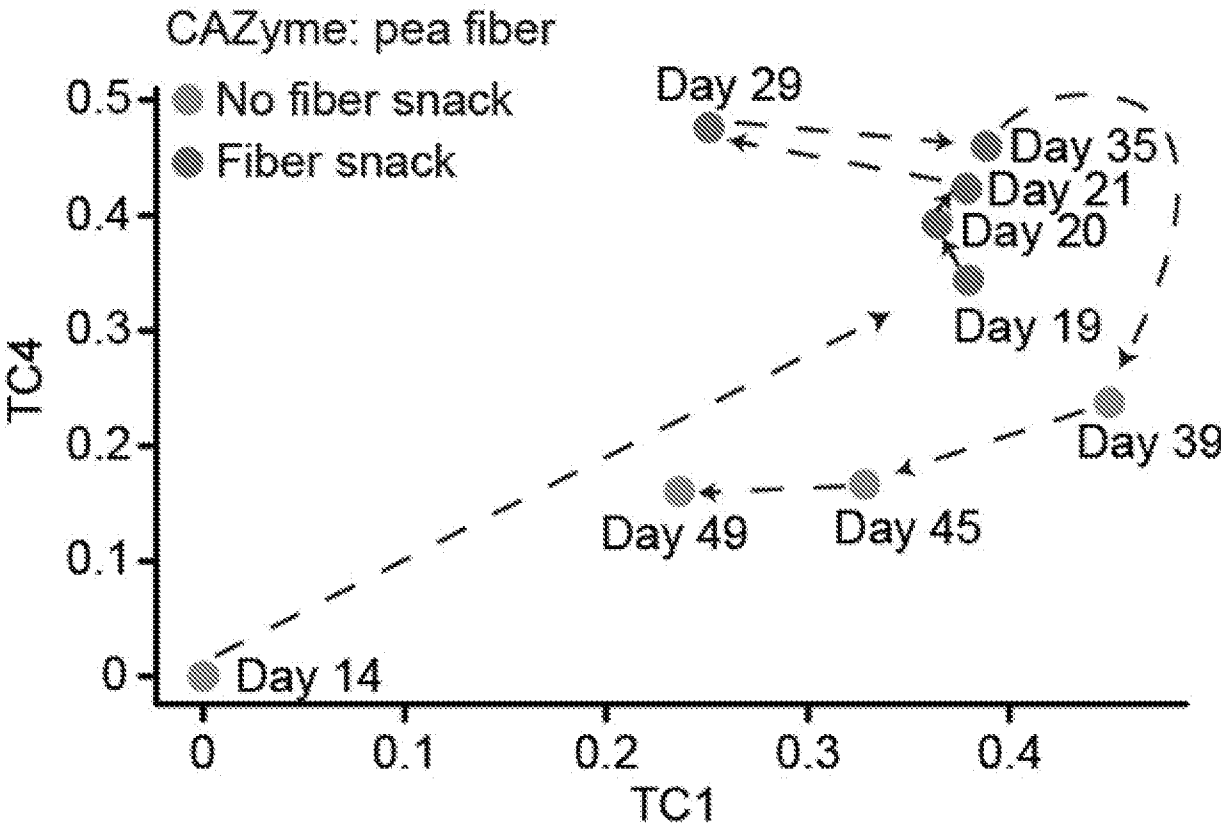

CAZyme: pea fiber
No fiber snack
Fiber snack
Day 29
Day 35
Day 21
Day 20
Day 19
Day 39
Day 49
Day 45
Day 14
TC4
TC1
0.5
0.4
0.3
0.2
0.1
0
0
0.1
0.2
0.3
0.4

FIG. 31C

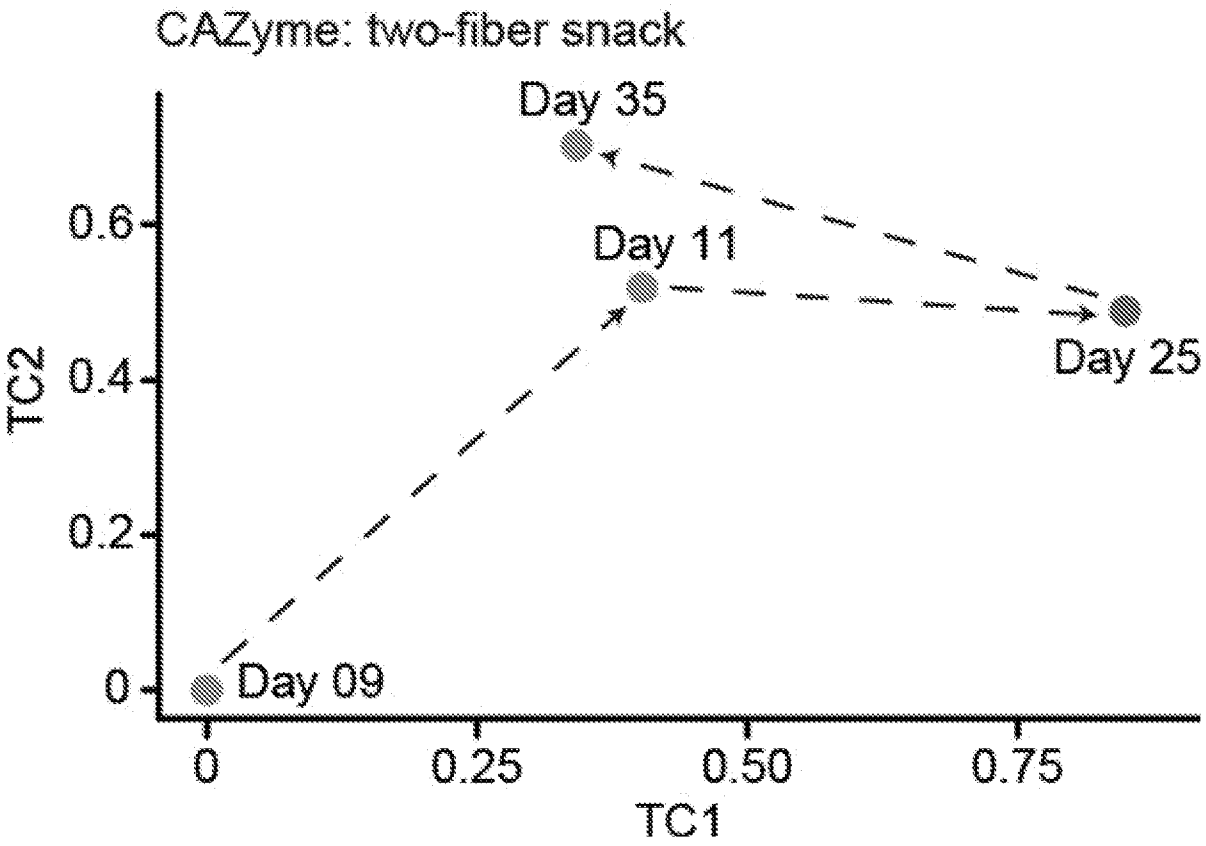

CAZyme: two-fiber snack
Day 35
Day 11
Day 25
Day 09
0.6
0.4
0.2
0
TC2
0
0.25
0.50
0.75
TC1

FIG. 31D

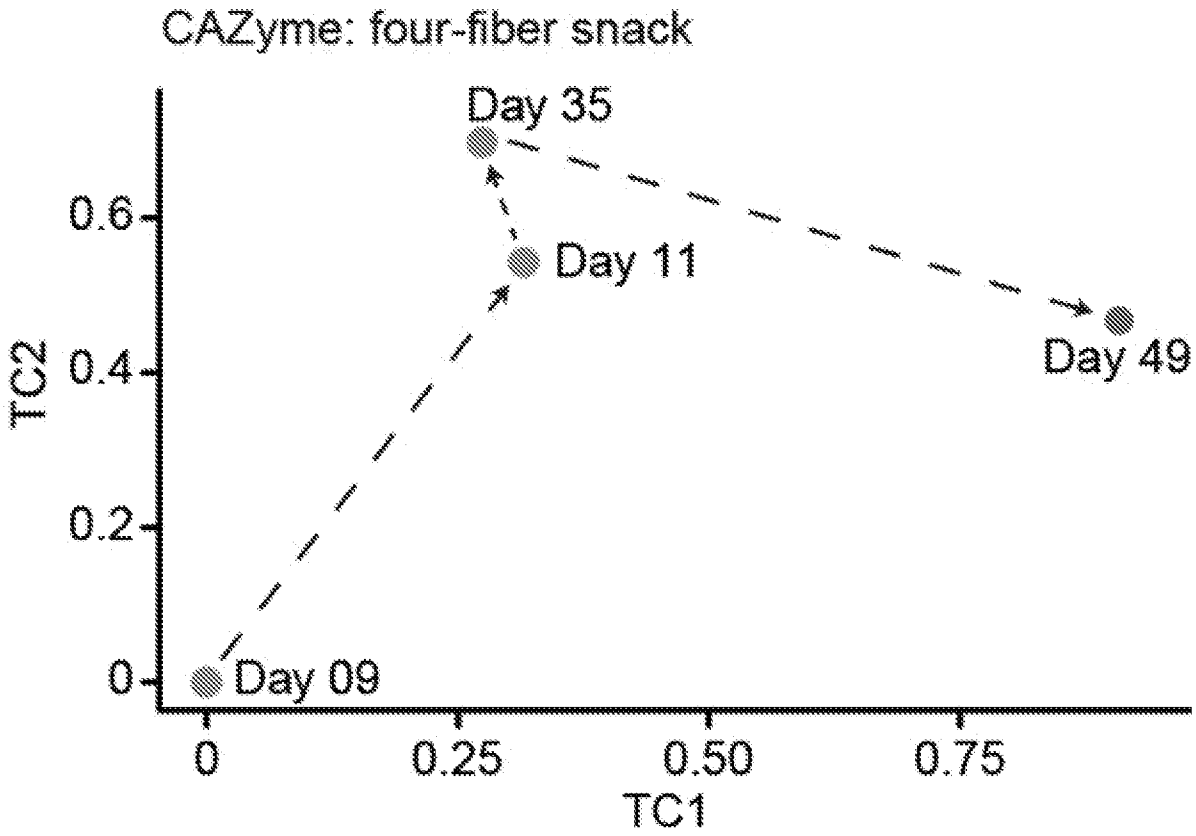

CAZyme: four-fiber snack
Day 35
Day 11
Day 49
Day 09
0.6
0.4
0.2
0
TC2
0
0.25
0.50
0.75
TC1

FIG. 31E

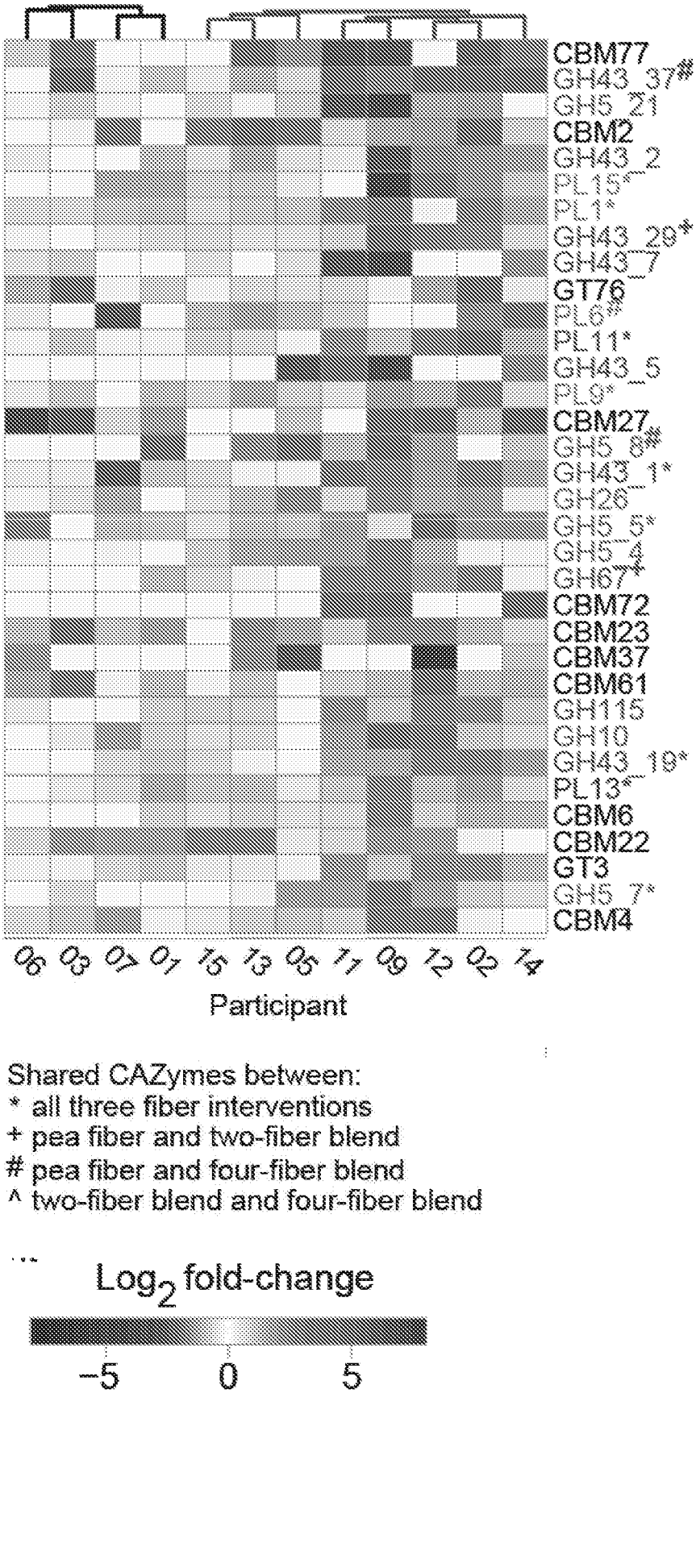
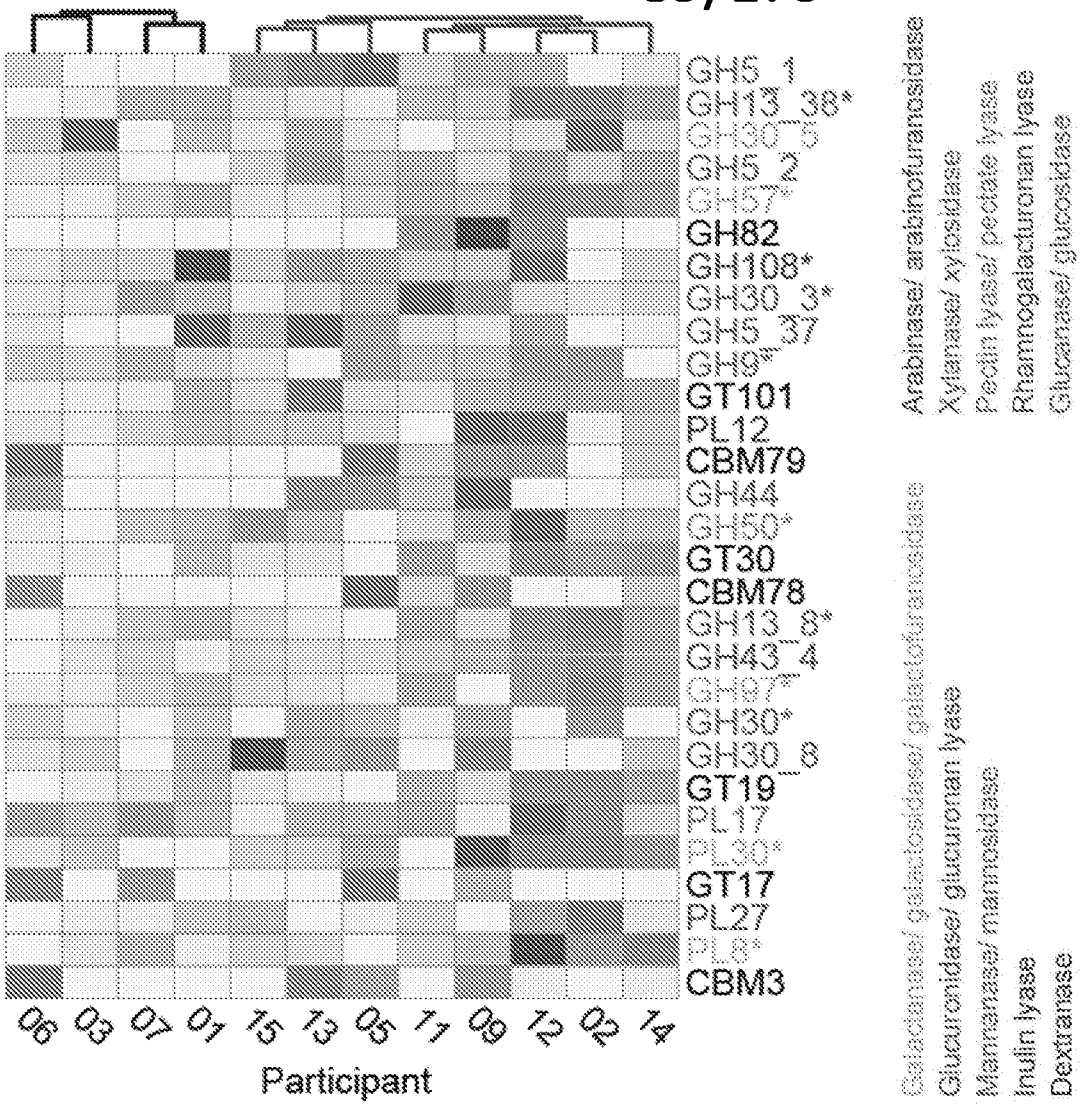

CBM77
GH43_37#
GH5_21
CBM2
GH43_2
PL15*
PL1*
GH43_29*
GH43_7
GT76
PL6#
PL11*
GH43_5
PL9*
CBM27
GH5_8#
GH43_1*
GH26
GH5_5*
GH5_4
GH67+
CBM72
CBM23
CBM37
CBM61
GH115
GH10
GH43_19*
PL13*
CBM6
CBM22
GT3
GH5_7*
CBM4
06 03 01 01 15 13 05 11 09 12 02 14
Participant
Shared CAZymes between:
* all three fiber interventions
+ pea fiber and two-fiber blend
# pea fiber and four-fiber blend
^ two-fiber blend and four-fiber blend
Log2 fold-change
−5 0 5
Hyaluronate lyase
Chondroitin lyase/ chondroitinase
N-acetylglucosaminidase
N-acetylmuramidase
Alginate lyase
Agarase/ agarobiase
Heparin lyase
Galactanase/ galactosidase/ galactofuranosidase
Glucuronidase/ glucuronan lyase
Mannanase/ mannosidase
Inulin lyase
Dextranase
Arabinase/ arabinofuranosidase
Xylanase/ xylosidase
Pectin lyase/ pectate lyase
Rhamnogalacturonan lyase
Glucanase/ glucosidase

FIG. 31F



Shared CAZymes between:
* all three fiber interventions
+ pea fiber and two-fiber blend
# pea fiber and four-fiber blend
^ two-fiber blend and four-fiber blend

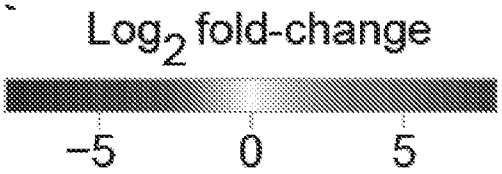


FIG. 31G

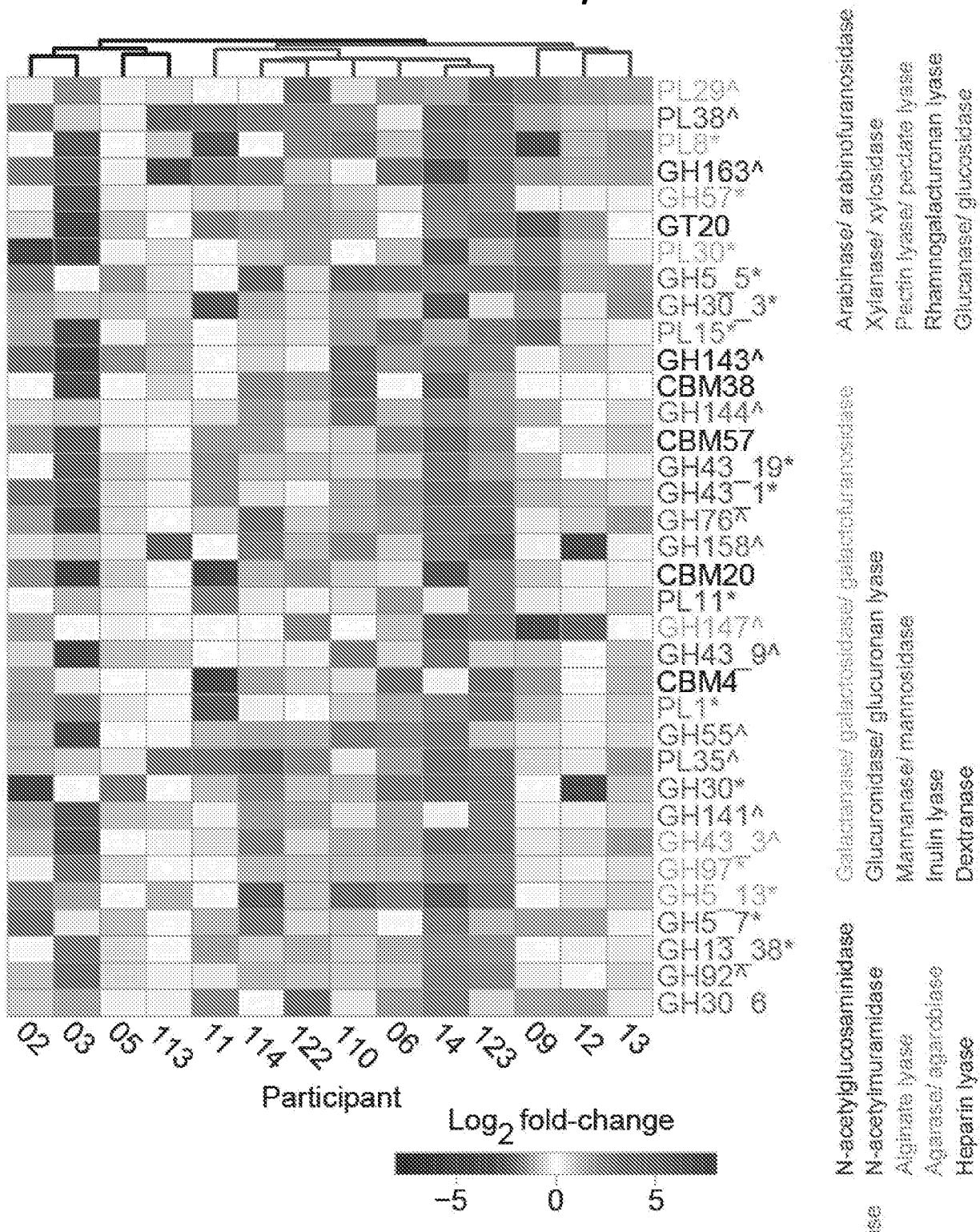


FIG. 31H

Shared CAZymes between:
* all three fiber interventions
+ pea fiber and two-fiber blend
# pea fiber and four-fiber blend
^ two-fiber blend and four-fiber blend

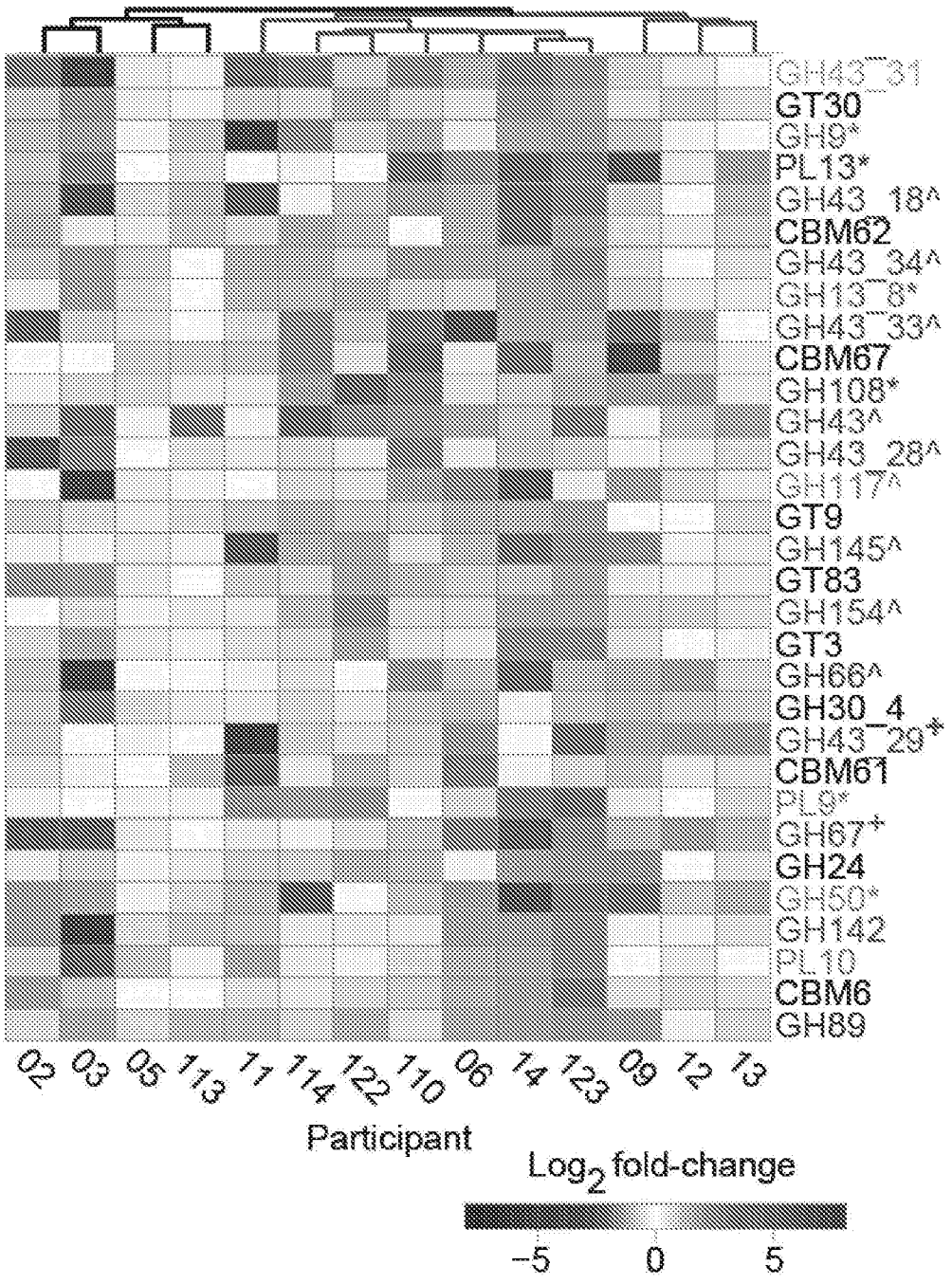

GH43_31
GT30
GH9*
PL13*
GH43_18^
CBM62
GH43_34^
GH13_8*
GH43_33^
CBM67
GH108*
GH43^
GH43_28^
GH117^
GT9
GH145^
GT83
GH154^
GT3
GH66^
GH30_4
GH43_29*
CBM61
PL8*
GH67+
GH24
GH50*
GH142
PL10
CBM6
GH89
02 03 05 113 11 114 122 110 06 14 123 09 12 13
Participant
Log2 fold-change
−5 0 5
Shared CAZymes between:
* all three fiber interventions
+ pea fiber and two-fiber blend
# pea fiber and four-fiber blend
^ two-fiber blend and four-fiber blend
Hyaluronate lyase
Chondroitin lyase/ chondroitinase
N-acetylglucosaminidase
N-acetylmuramidase
Alginate lyase
Agarase/ agarobiase
Heparin lyase
Galactanase/ galactosidase/ galacturonanase
Glucuronidase/ glucuronan lyase
Mannanase/ mannosidase
Inulin lyase
Dextranase
Arabinase/ arabinofuranosidase
Xylanase/ xylosidase
Pectin lyase/ pectate lyase
Rhamnogalacturonan lyase
Glucanase/ glucosidase

FIG. 31I

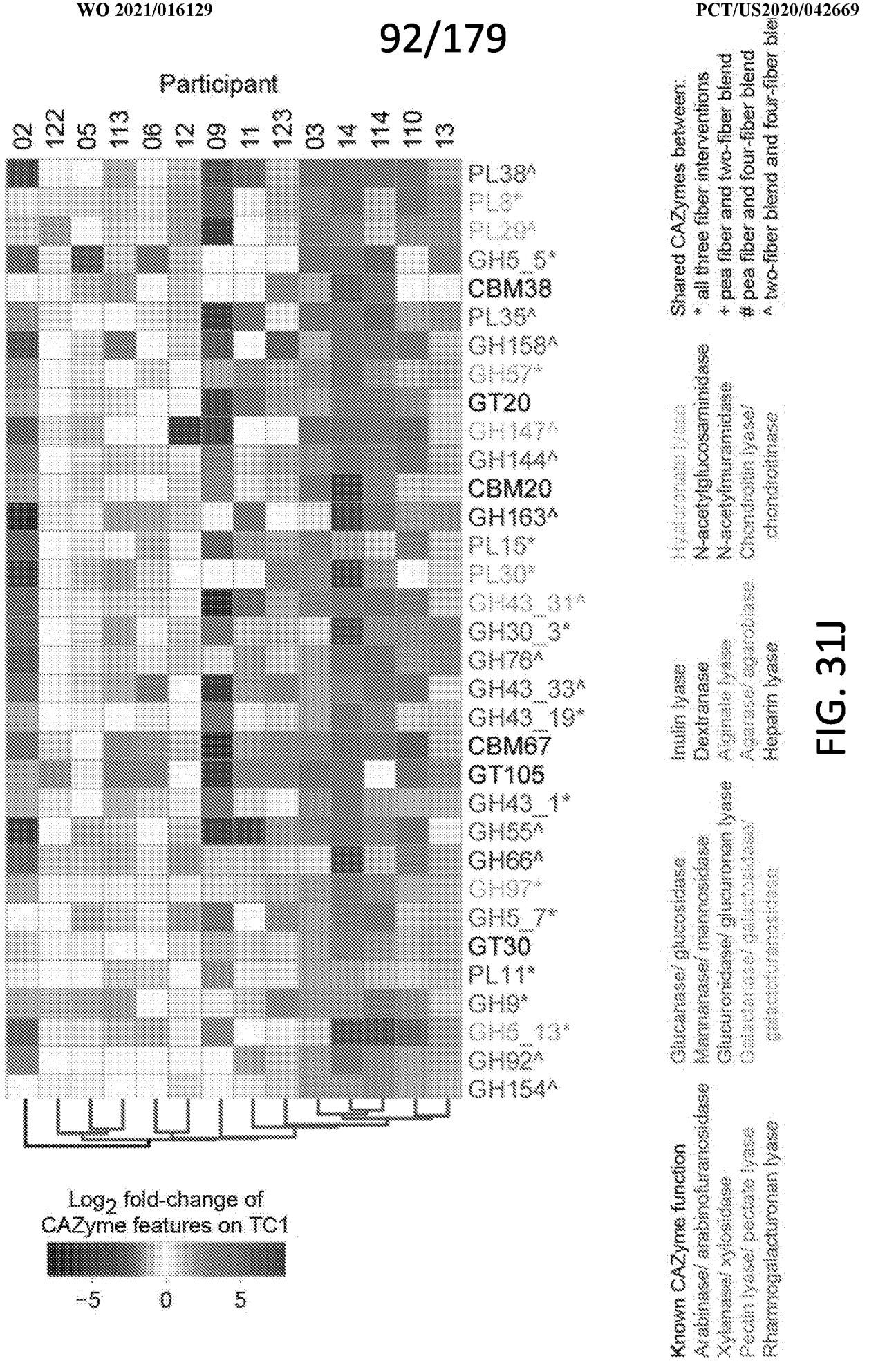

Participant
02
122
05
113
06
12
09
11
123
03
14
114
110
13
PL38^
PL8*
PL29^
GH5_5*
CBM38
PL35^
GH158^
GH57*
GT20
GH147^
GH144^
CBM20
GH163^
PL15*
PL30*
GH43_31^
GH30_3*
GH76^
GH43_33^
GH43_19*
CBM67
GT105
GH43_1*
GH55^
GH66^
GH97*
GH5_7*
GT30
PL11*
GH9*
GH5_13*
GH92^
GH154^
Log2 fold-change of CAZyme features on TC1
−5
0
5
Known CAZyme function
Arabinase/ arabinofuranosidase
Xylanase/ xylosidase
Pectin lyase/ pectate lyase
Rhamnogalacturonan lyase
Glucanase/ glucosidase
Mannanase/ mannosidase
Glucuronidase/ glucuronan lyase
Galactanase/ galactosidase/ galactofuranosidase
Inulin lyase
Dextranase
Alginate lyase
Agarase/ agarobiase
Heparin lyase
Hyaluronate lyase
N-acetylglucosaminidase
N-acetylmuramidase
Chondroitin lyase/ chondroitinase
Shared CAZymes between:
* all three fiber interventions
+ pea fiber and two-fiber blend
# pea fiber and four-fiber blend
^ two-fiber blend and four-fiber ble

FIG. 31J

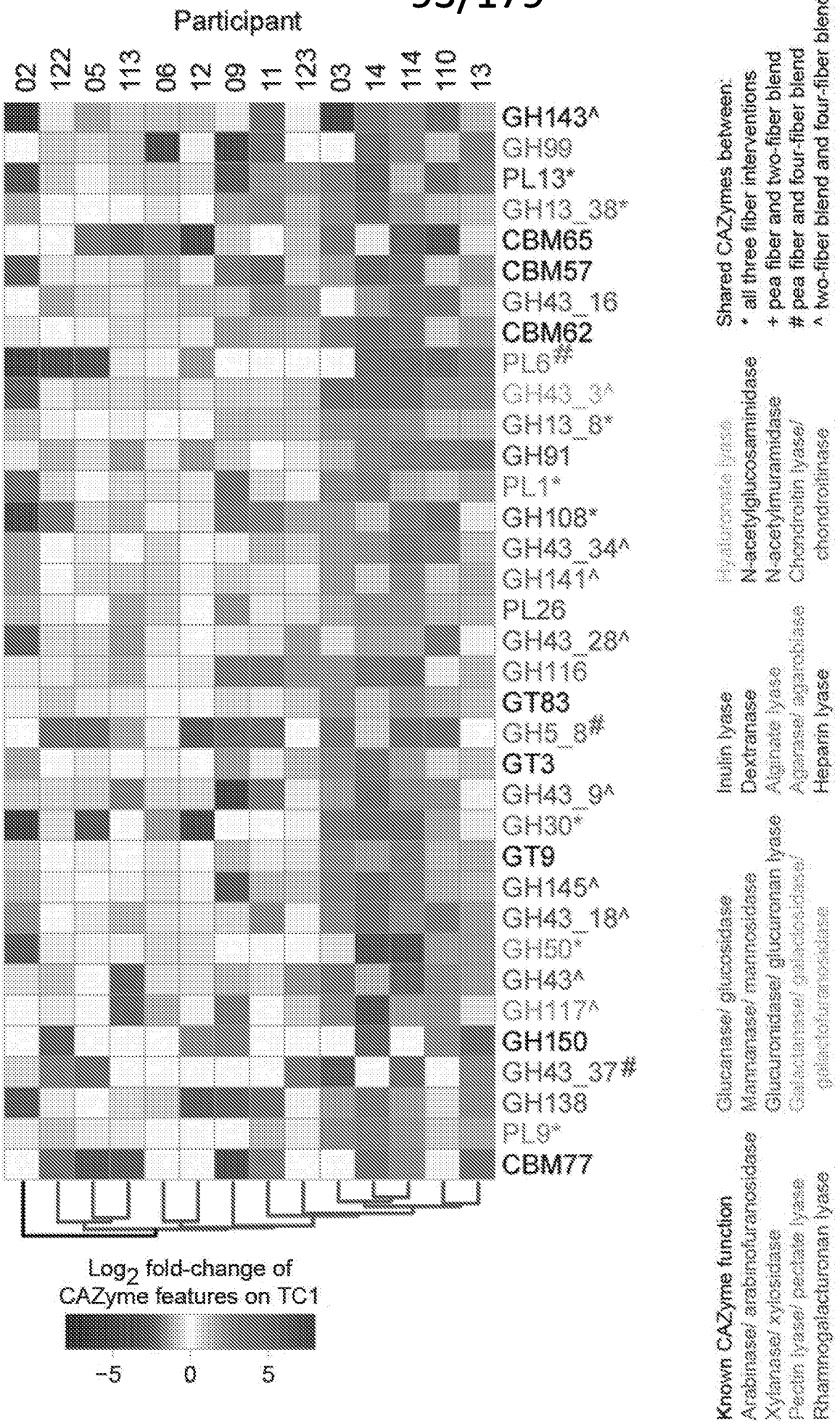

Participant
02
122
05
113
06
12
09
11
123
03
14
114
110
13
GH143^
GH99
PL13*
GH13_38*
CBM65
CBM57
GH43_16
CBM62
PL6#
GH43_3^
GH13_8*
GH91
PL1*
GH108*
GH43_34^
GH141^
PL26
GH43_28^
GH116
GT83
GH5_8#
GT3
GH43_9^
GH30*
GT9
GH145^
GH43_18^
GH50*
GH43^
GH117^
GH150
GH43_37#
GH138
PL9*
CBM77
Log2 fold-change of CAZyme features on TC1
−5
0
5
Known CAZyme function
Arabinase/ arabinofuranosidase
Xylanase/ xylosidase
Pectin lyase/ pectate lyase
Rhamnogalacturonan lyase
Glucanase/ glucosidase
Mannanase/ mannosidase
Glucuronidase/ glucuronan lyase
Galactanase/ galactosidase/ galactofuranosidase
Inulin lyase
Dextranase
Alginate lyase
Agarase/ agarobiase
Heparin lyase
Hyaluronate lyase
N-acetylglucosaminidase
N-acetylmuramidase
Chondroitin lyase/ chondroitinase
Shared CAZymes between:
* all three fiber interventions
+ pea fiber and two-fiber blend
# pea fiber and four-fiber blend
^ two-fiber blend and four-fiber blend

FIG. 31K

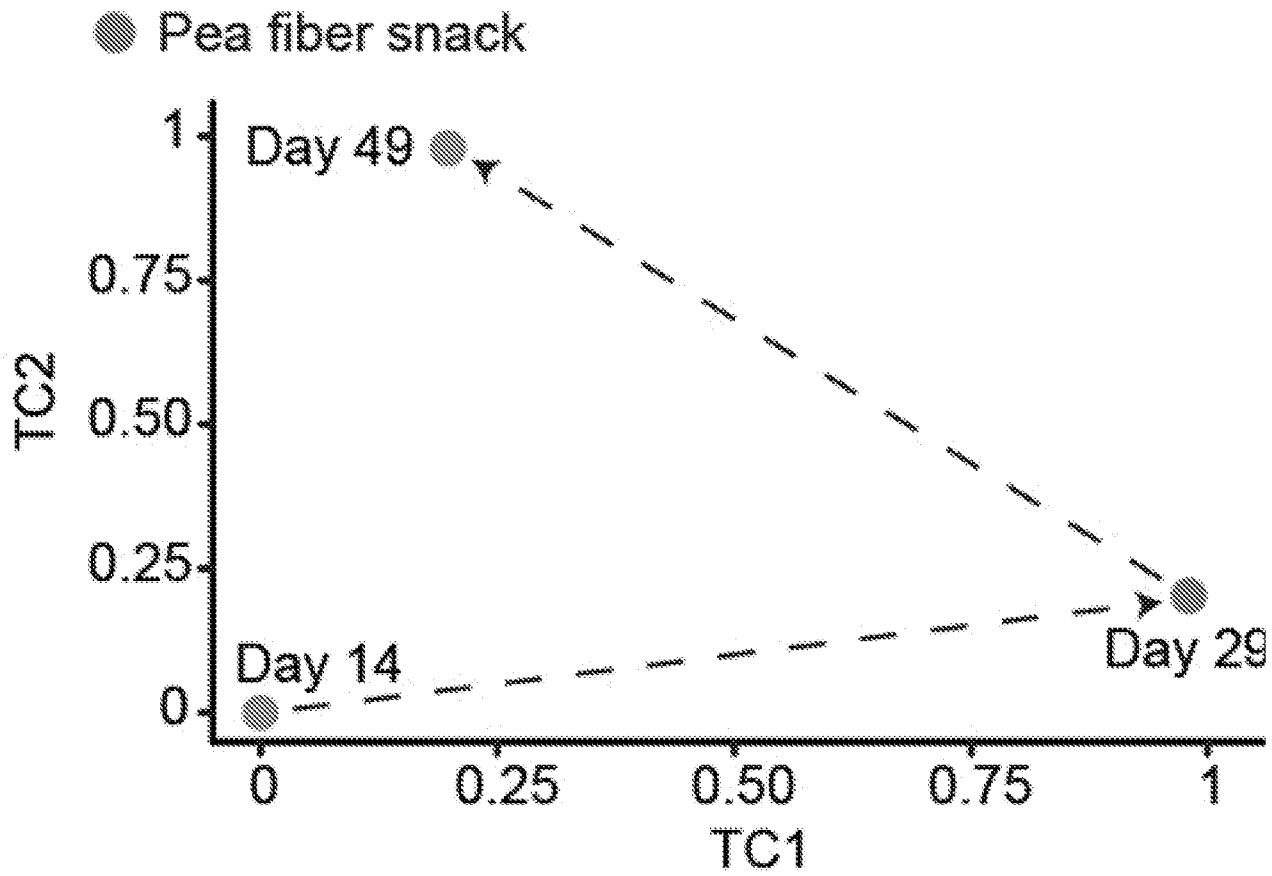


FIG. 32A

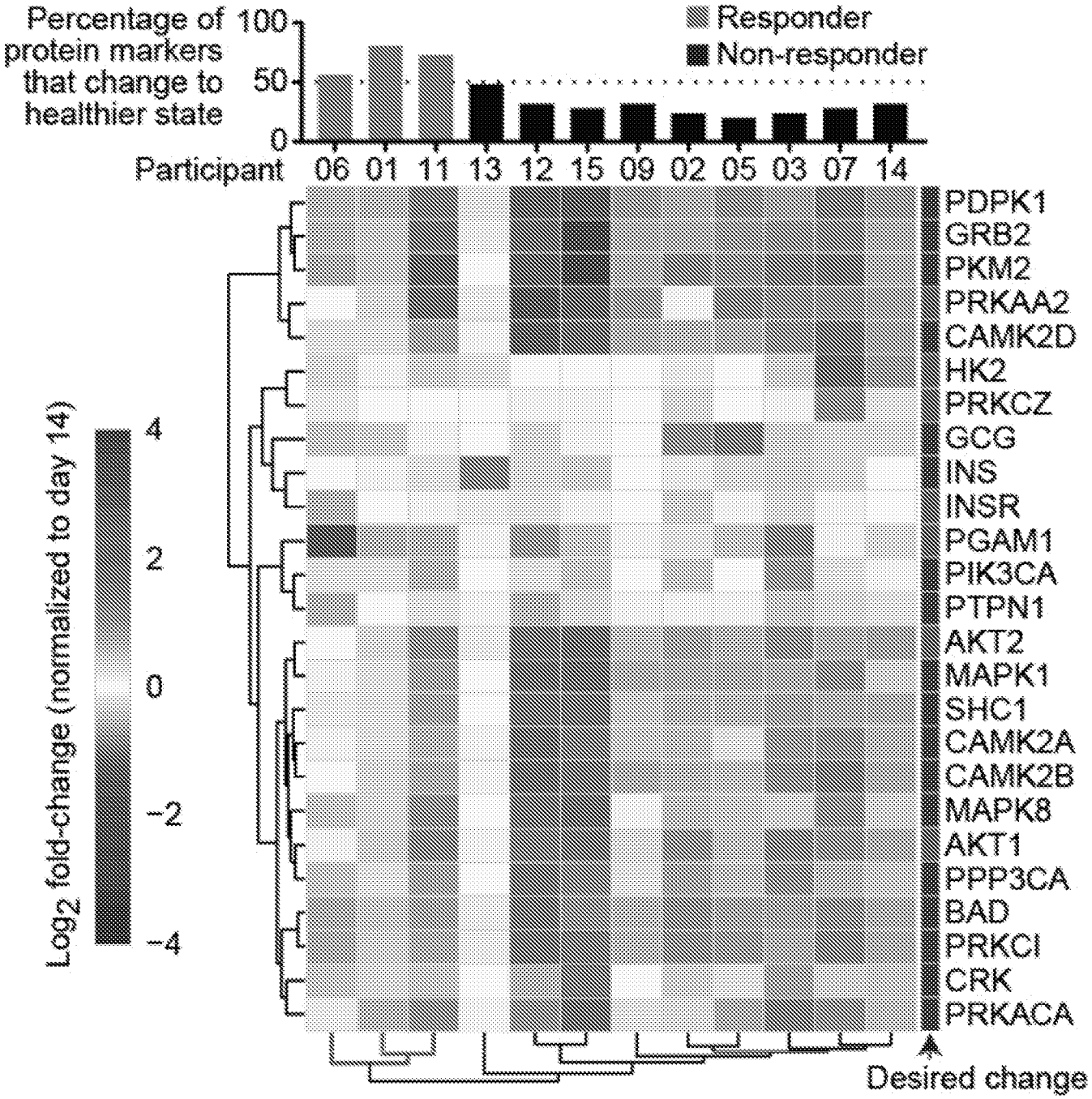

Percentage of protein markers that change to healthier state
100
50
0
Responder
Non-responder
Participant
06 01 11 13 12 15 09 02 05 03 07 14
PDPK1
GRB2
PKM2
PRKAA2
CAMK2D
HK2
PRKCZ
GCG
INS
INSR
PGAM1
PIK3CA
PTPN1
AKT2
MAPK1
SHC1
CAMK2A
CAMK2B
MAPK8
AKT1
PPP3CA
BAD
PRKCI
CRK
PRKACA
Log2 fold-change (normalized to day 14)
4
2
0
−2
−4
Desired change

FIG. 32B

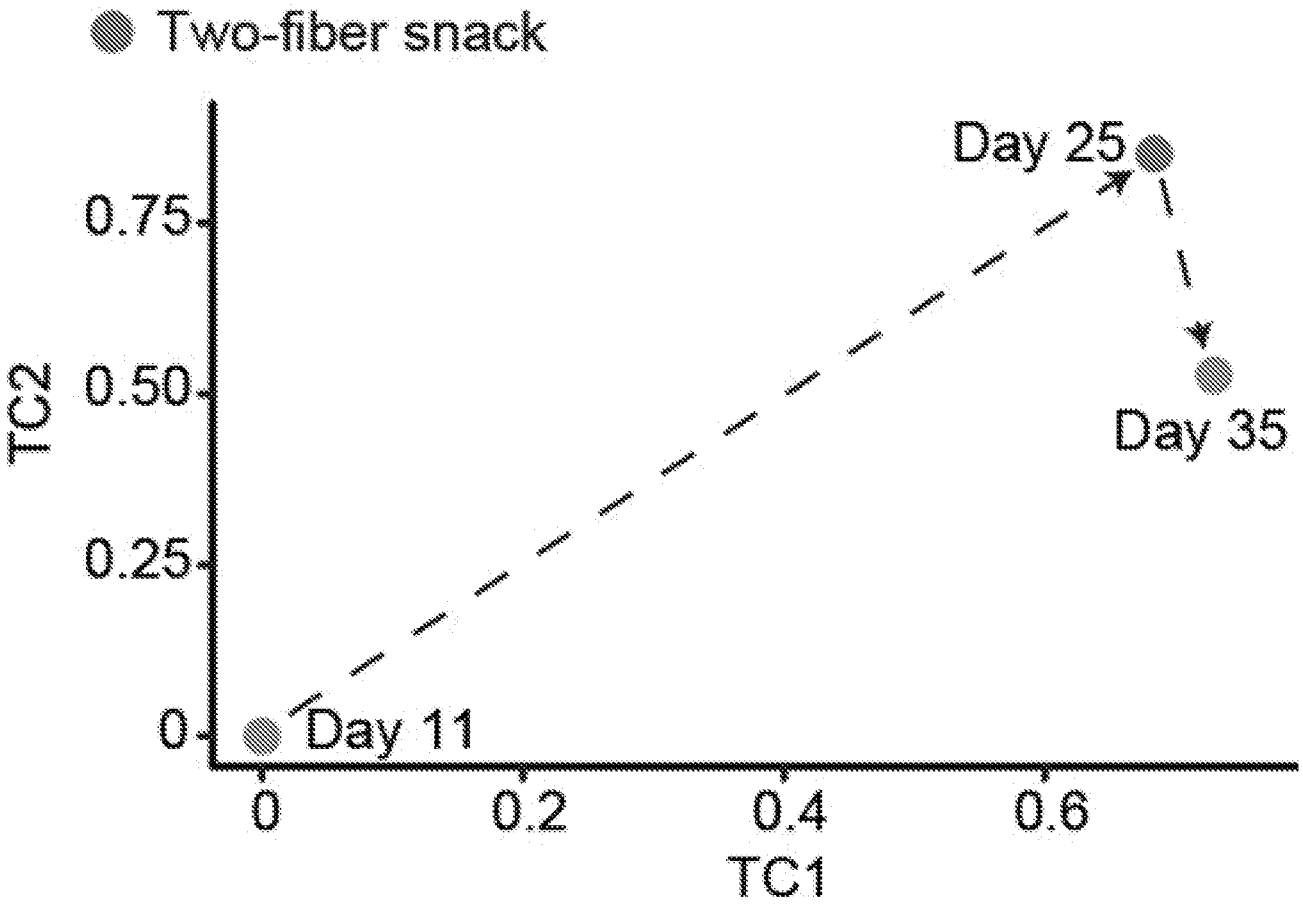

Two-fiber snack
Day 25
Day 35
Day 11
0.75
0.50
0.25
0
TC2
0
0.2
0.4
0.6
TC1

FIG. 32C

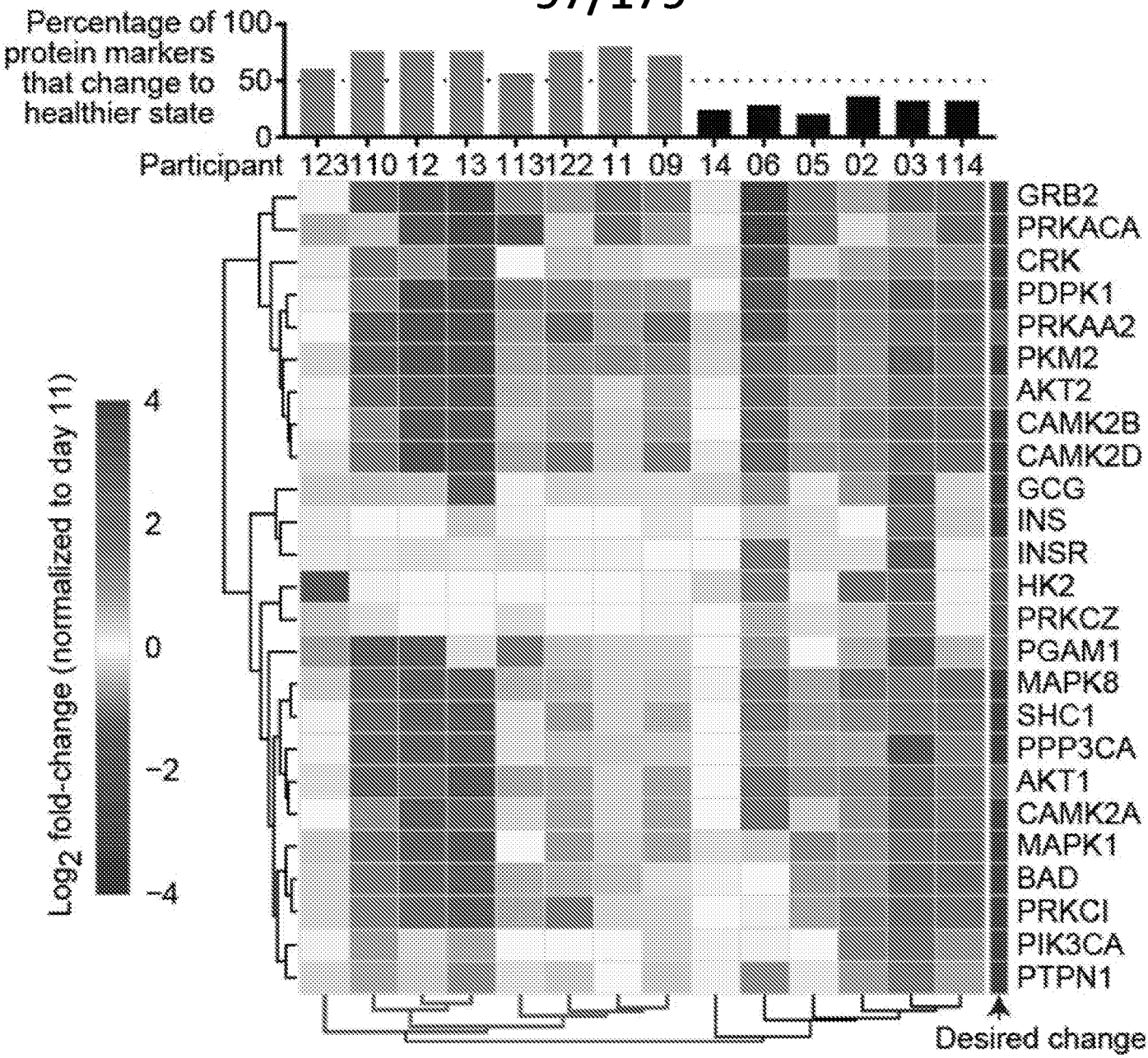

Percentage of protein markers that change to healthier state
100
50
0
Participant 123110 12 13 113122 11 09 14 06 05 02 03 114
GRB2
PRKACA
CRK
PDPK1
PRKAA2
PKM2
AKT2
CAMK2B
CAMK2D
GCG
INS
INSR
HK2
PRKCZ
PGAM1
MAPK8
SHC1
PPP3CA
AKT1
CAMK2A
MAPK1
BAD
PRKCI
PIK3CA
PTPN1
Log2 fold-change (normalized to day 11)
4
2
0
−2
−4
Desired change

FIG. 32D

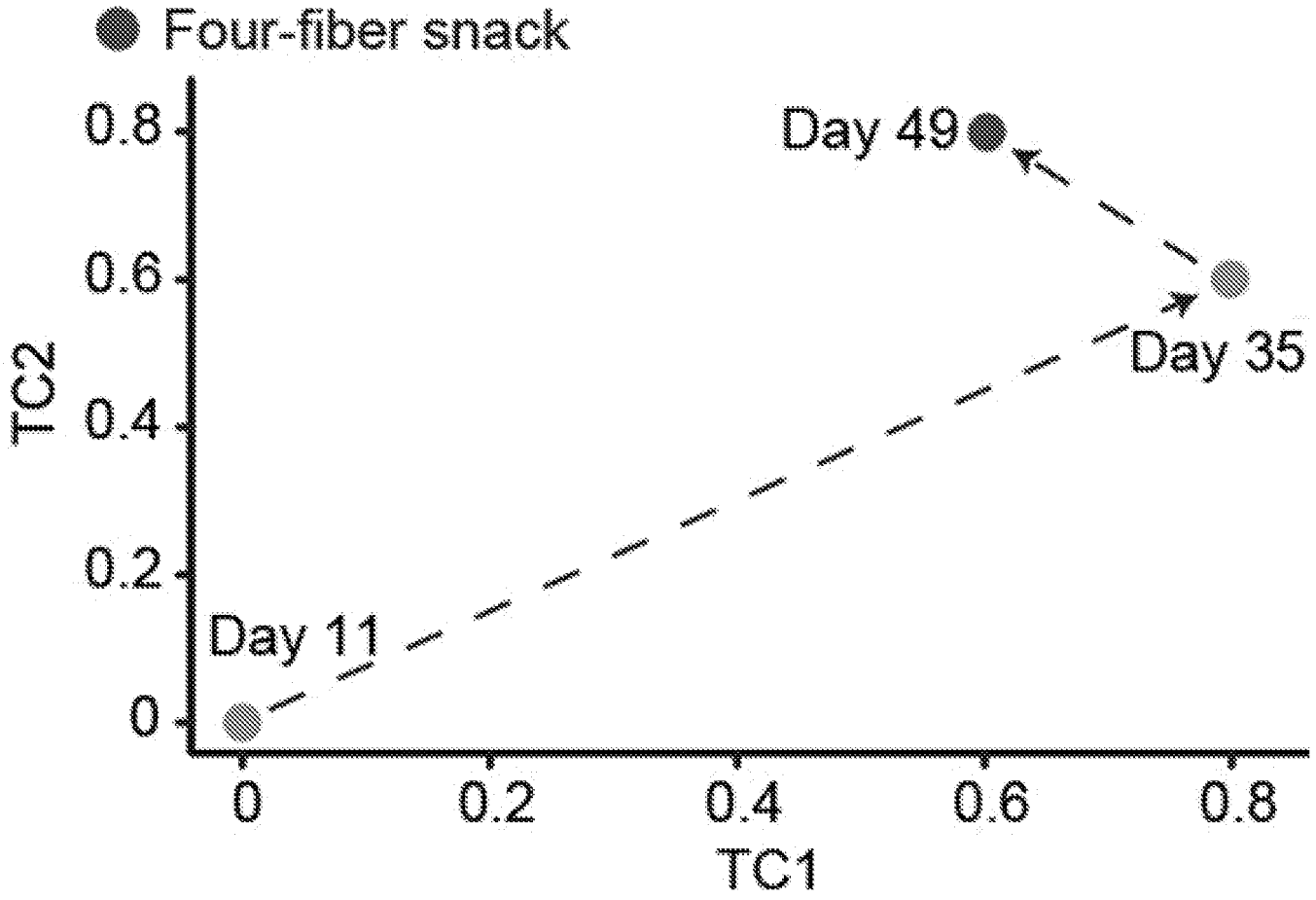

Four-fiber snack
0.8
0.6
0.4
0.2
0
TC2
Day 49
Day 35
Day 11
0
0.2
0.4
0.6
0.8
TC1

FIG. 32E

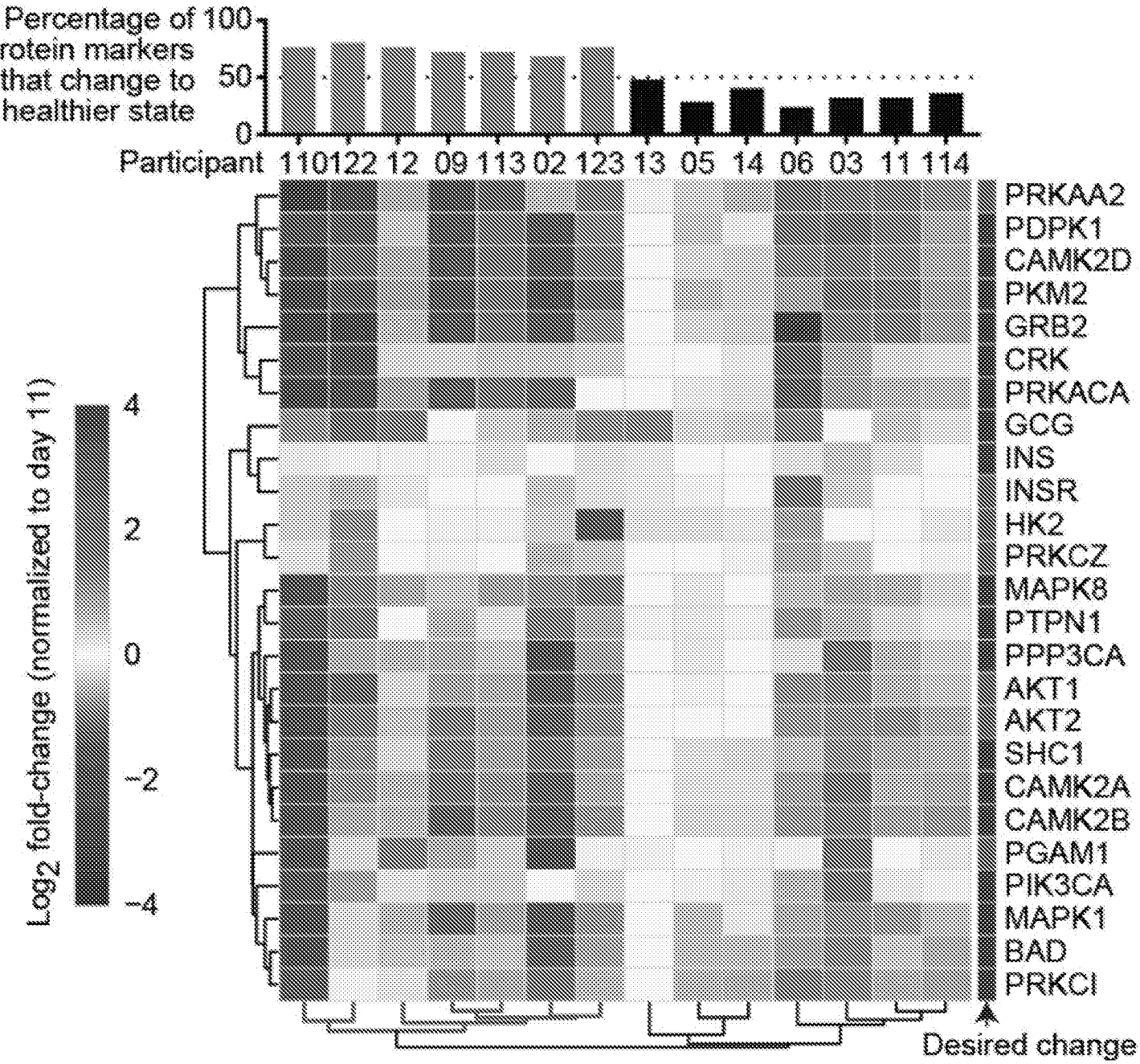

Percentage of protein markers that change to healthier state
100
50
0
Participant 110122 12 09 113 02 123 13 05 14 06 03 11 114
PRKAA2
PDPK1
CAMK2D
PKM2
GRB2
CRK
PRKACA
GCG
INS
INSR
HK2
PRKCZ
MAPK8
PTPN1
PPP3CA
AKT1
AKT2
SHC1
CAMK2A
CAMK2B
PGAM1
PIK3CA
MAPK1
BAD
PRKCI
Log2 fold-change (normalized to day 11)
4
2
0
−2
−4
Desired change

FIG. 32F

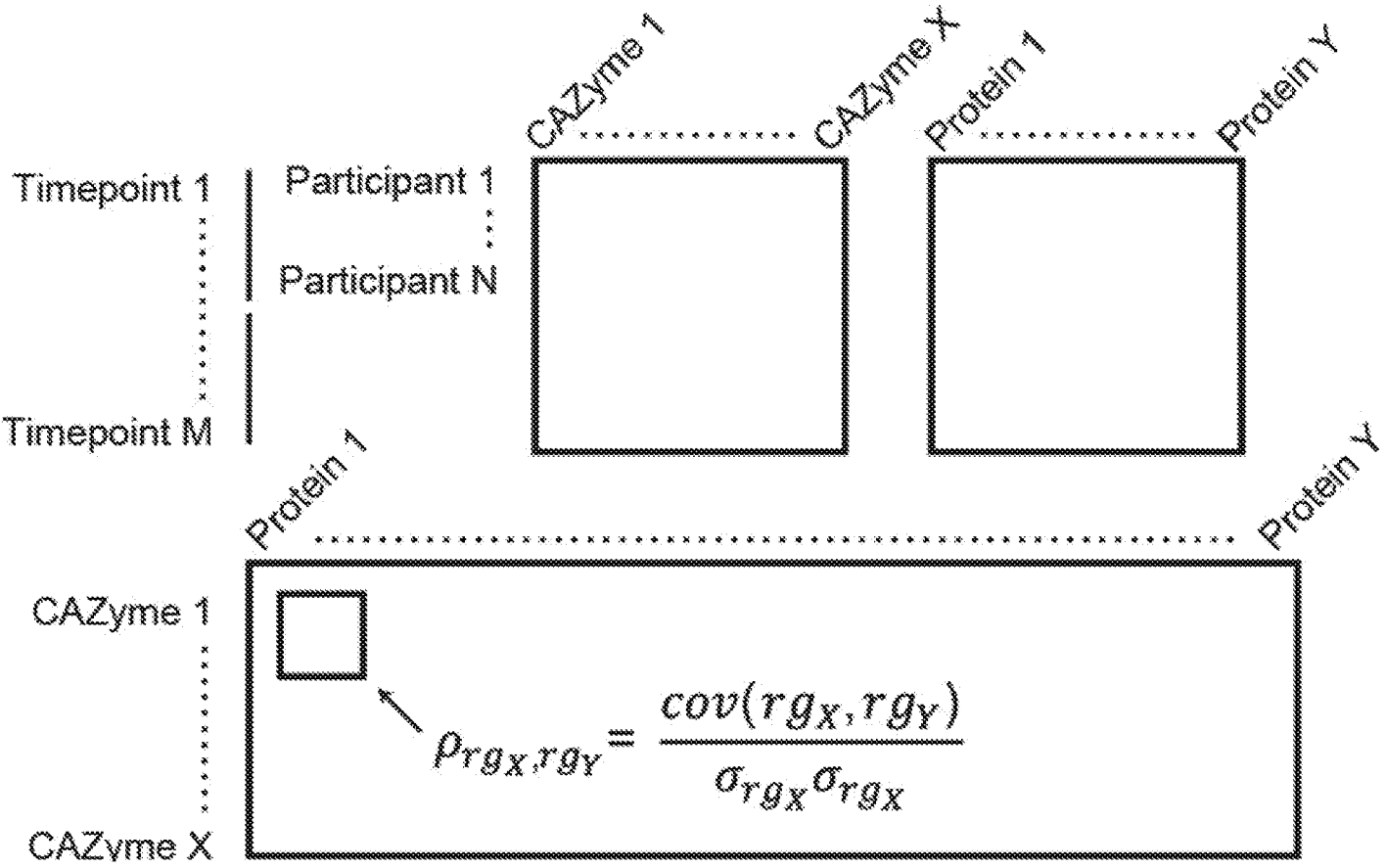

CAZyme 1
CAZyme X
Protein 1
Protein Y
Timepoint 1
Participant 1
Participant N
Timepoint M
Protein 1
Protein Y
CAZyme 1
CAZyme X
$\rho_{rg_X,rg_Y} = \frac{cov(rg_X, rg_Y)}{\sigma_{rg_X}\sigma_{rg_X}}$

FIG. 33A

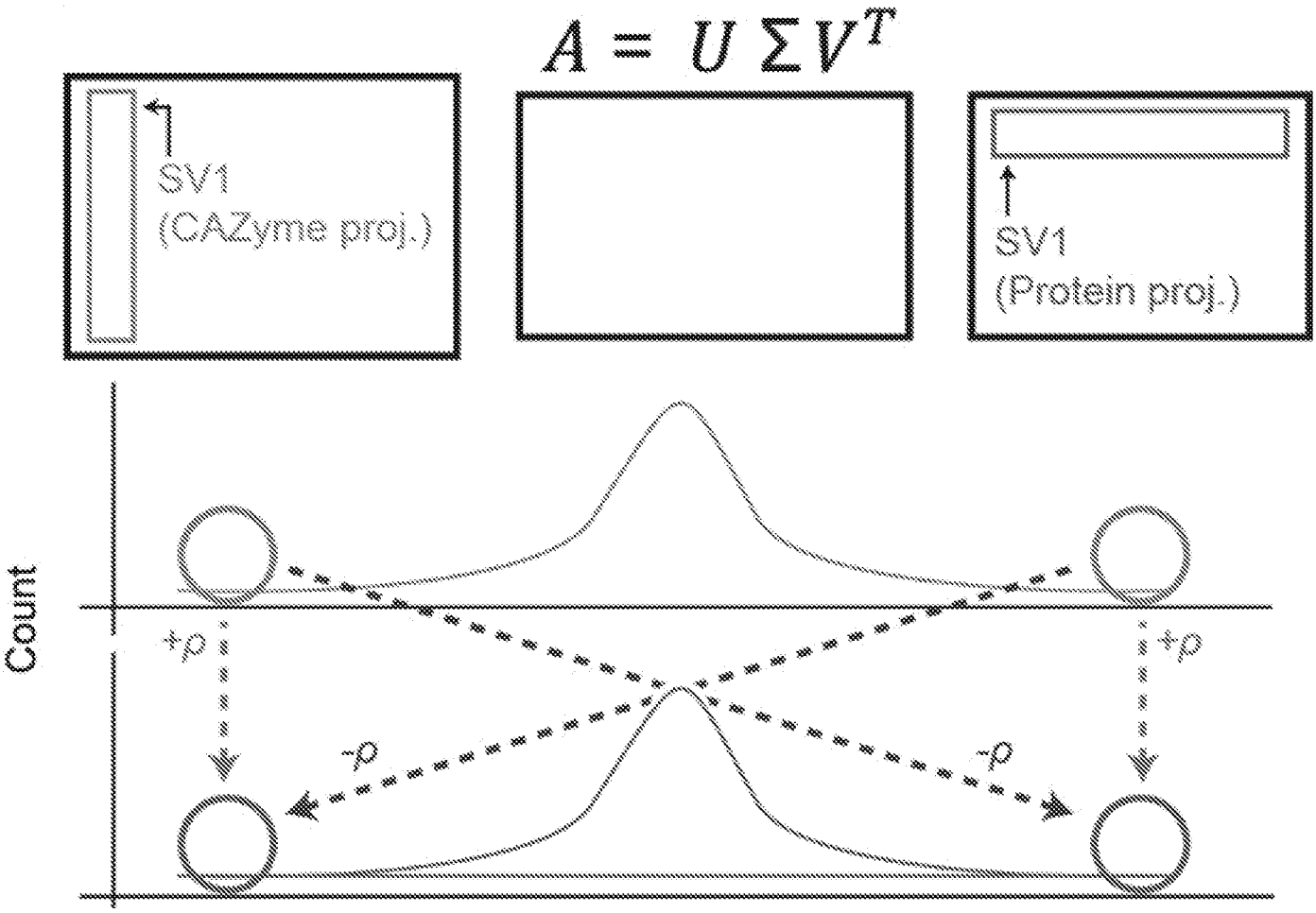


FIG. 33B

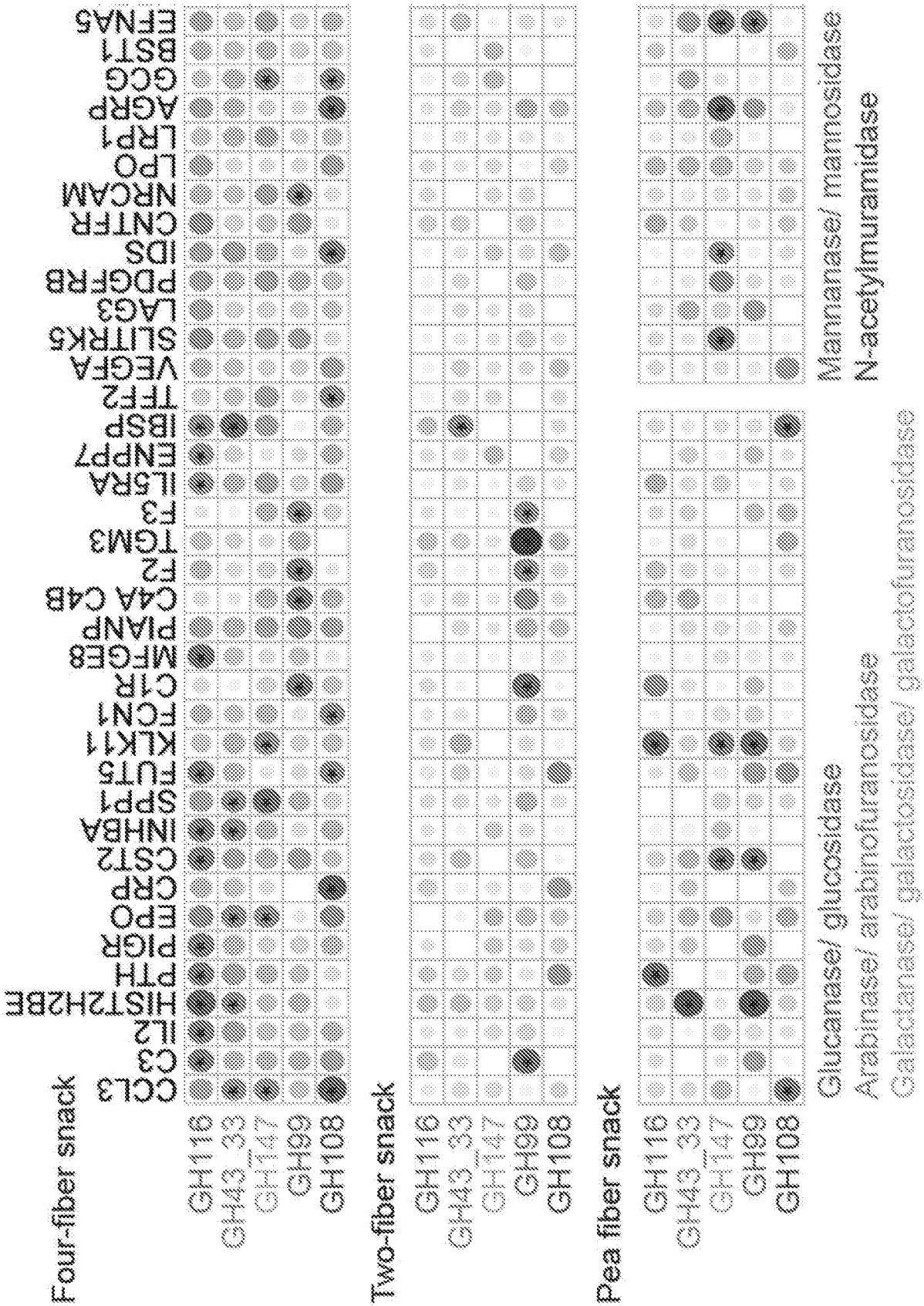

Four-fiber snack
Two-fiber snack
Pea fiber snack
CCL3
C3
IL2
HIST2H2BE
PTH
PIGR
EPO
CRP
CST2
INHBA
SPP1
FUT5
KLK11
FCN1
C1R
MFGE8
PIANP
C4A C4B
F2
TGM3
F3
IL5RA
ENPP7
IBSP
TFF2
VEGFA
SLITRK5
LAG3
PDGFRB
IDS
CNTFR
NRCAM
LPO
LRP1
AGRP
GCG
BST1
FNA5
GH116
GH43_33
GH147
GH99
GH108
Glucanase/ glucosidase
Arabinase/ arabinofuranosidase
Galactanase/ galactosidase/ galactofuranosidase
Mannanase/ mannosidase
N-acetylmuramidase

FIG. 33C

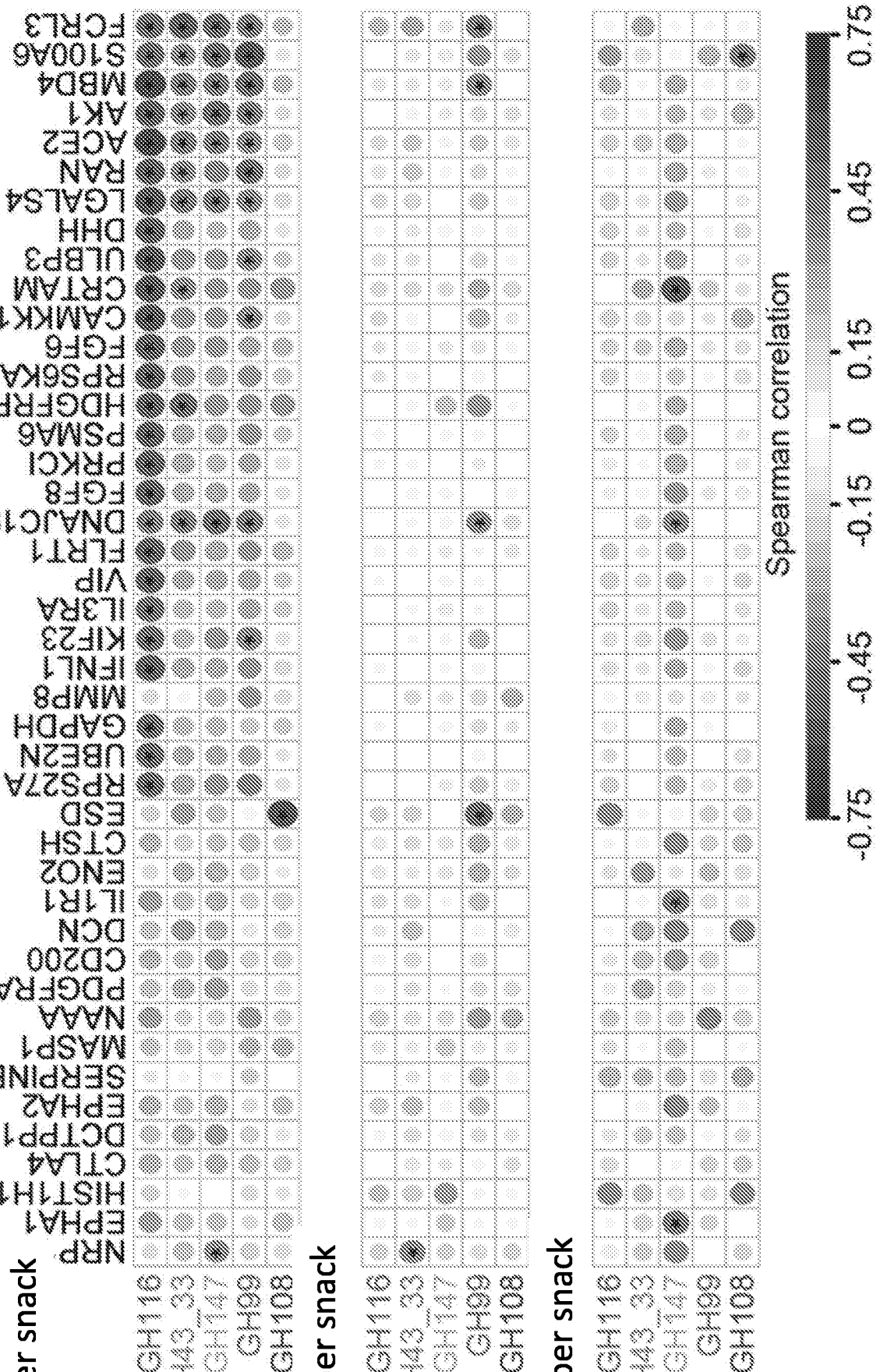

Four fiber snack
Two fiber snack
Pea fiber snack
GH116
GH43_33
GH147
GH99
GH108
NRP
EPHA1
HIST1H1C
CTLA4
DCTPP1
EPHA2
SERPINE1
MASP1
NAAA
PDGFRA
CD200
DCN
IL1R1
ENO2
CTSH
ESD
RPS27A
UBE2N
GAPDH
MMP8
IFNL1
KIF23
IL3RA
VIP
FLRT1
DNAJC19
FGF8
PRKCI
PSMA6
HDGFRP2
RPS6KA5
FGF6
CAMKK1
CRTAM
CLBP3
DHH
LGALS4
RAN
ACE2
AK1
MBD4
S100A6
FCRL3
Spearman correlation
-0.75
-0.45
-0.15
0
0.15
0.45
0.75

FIG. 33D

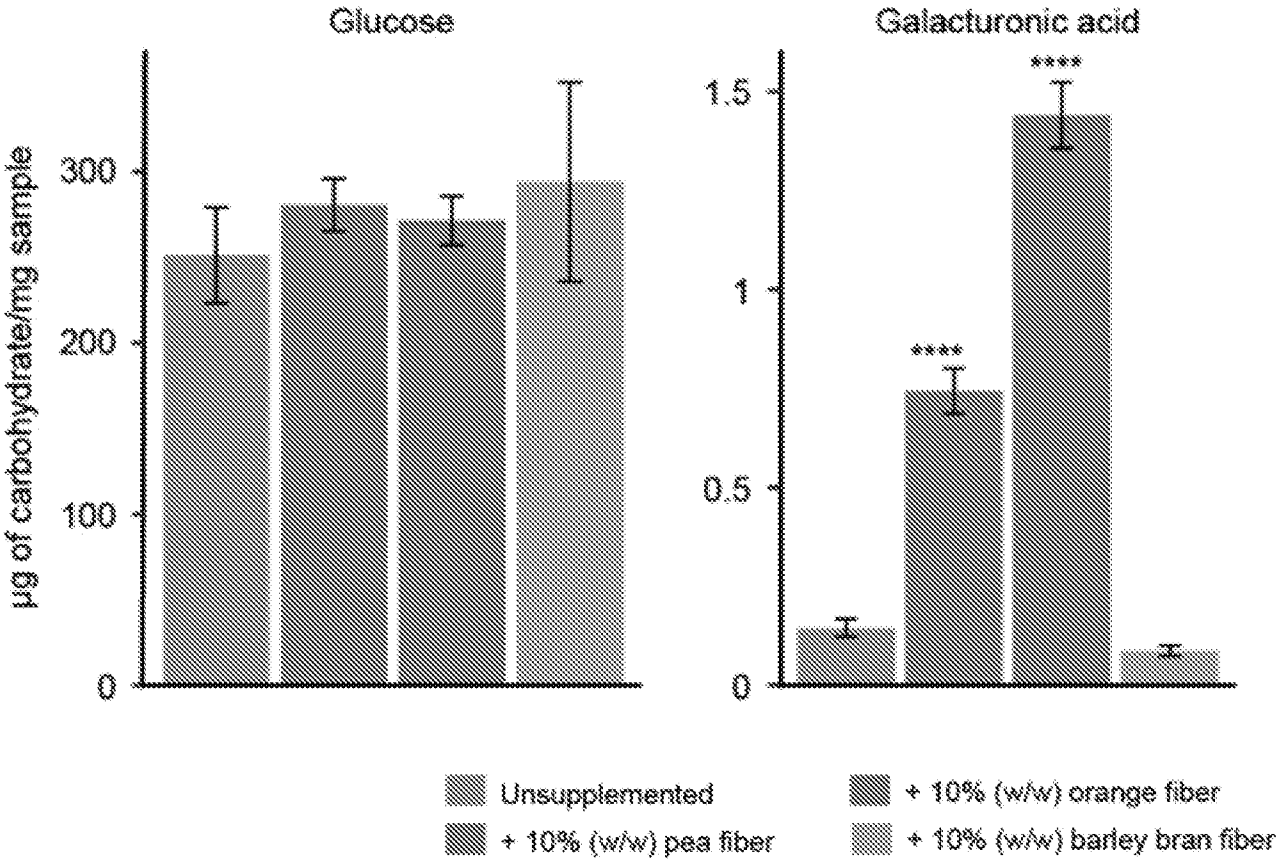

Glucose
Galacturonic acid
µg of carbohydrate/mg sample
0
100
200
300
0
0.5
1
1.5
****
****
Unsupplemented
+ 10% (w/w) pea fiber
+ 10% (w/w) orange fiber
+ 10% (w/w) barley bran fiber

FIG. 34A

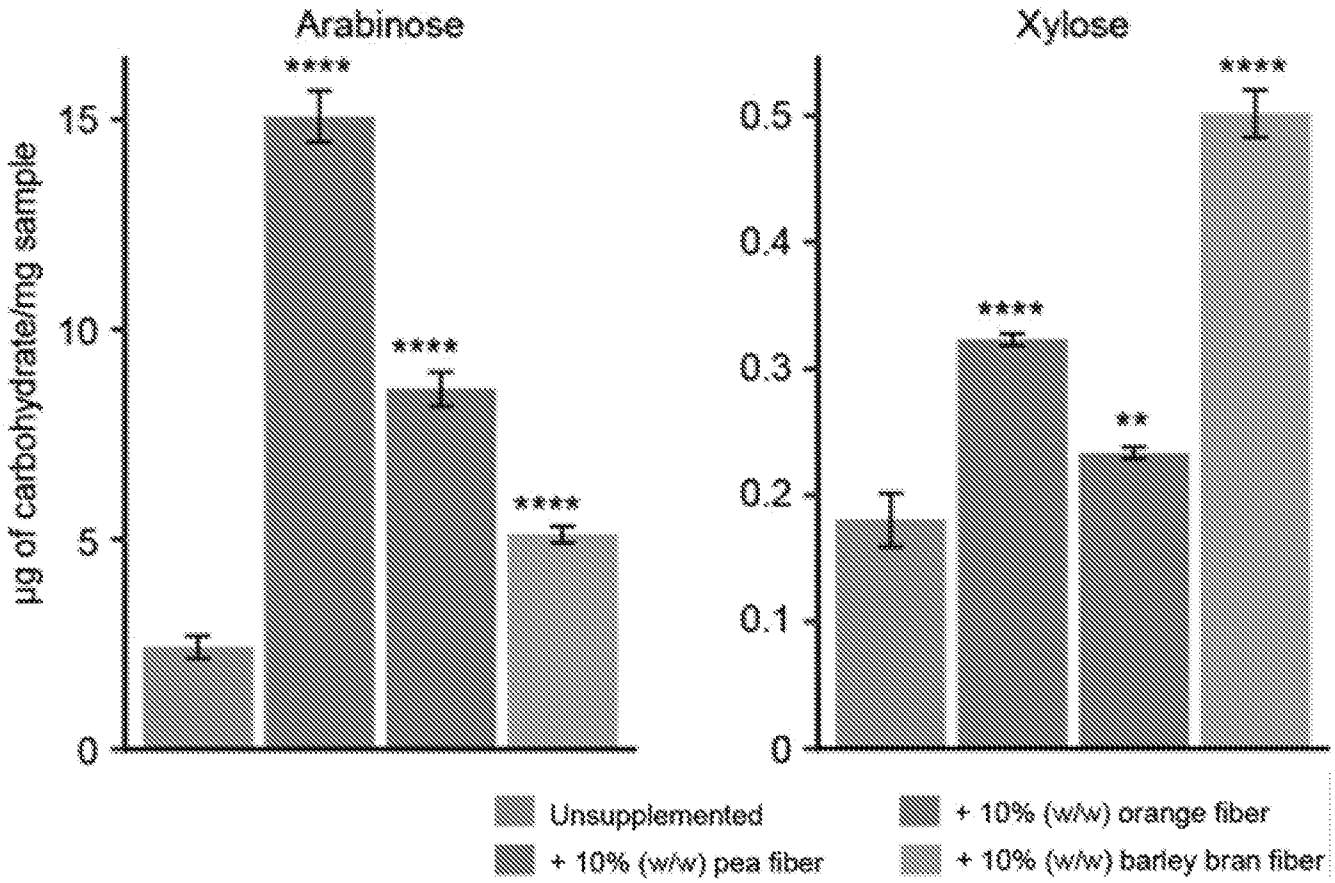

Arabinose
Xylose
µg of carbohydrate/mg sample
15
10
5
0
0.5
0.4
0.3
0.2
0.1
0
****
****
****
****
****
**
Unsupplemented
+ 10% (w/w) pea fiber
+ 10% (w/w) orange fiber
+ 10% (w/w) barley bran fiber

FIG. 34B

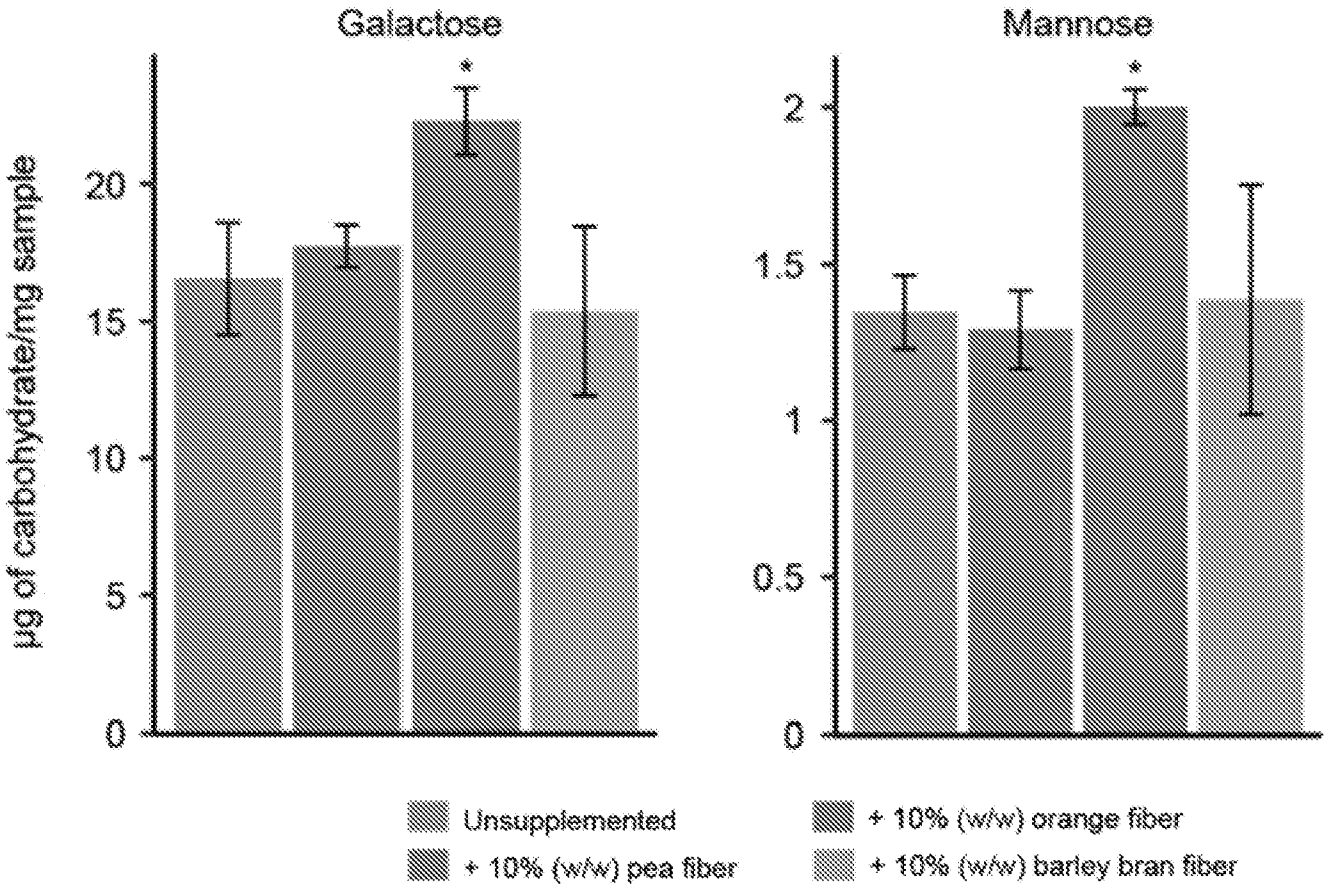

Galactose
Mannose
µg of carbohydrate/mg sample
0
5
10
15
20
0
0.5
1
1.5
2
*
*
Unsupplemented
+ 10% (w/w) pea fiber
+ 10% (w/w) orange fiber
+ 10% (w/w) barley bran fiber

FIG. 34C

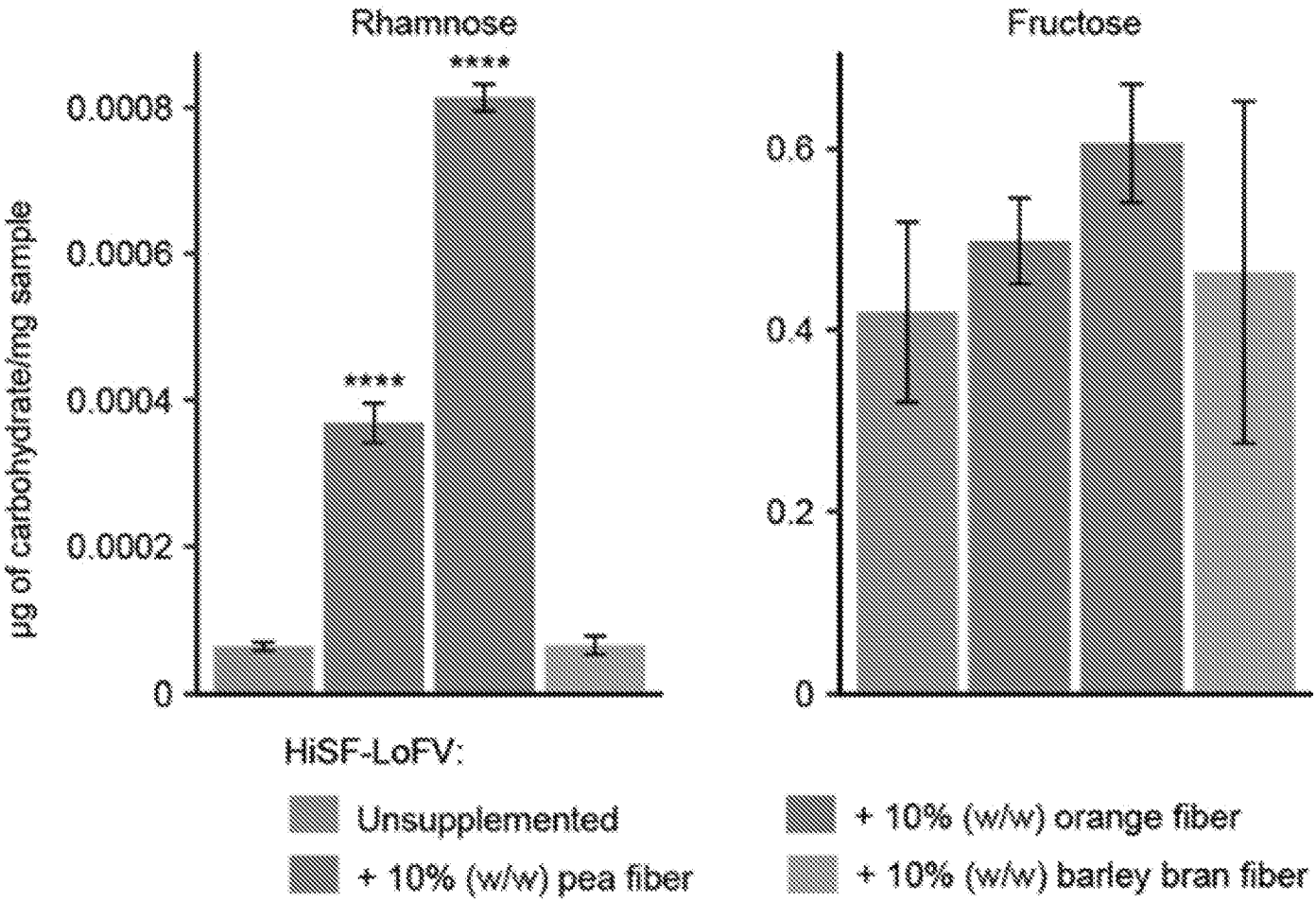

Rhamnose
Fructose
μg of carbohydrate/mg sample
0.0008
0.0006
0.0004
0.0002
0
****
****
0.6
0.4
0.2
0
HiSF-LoFV:
Unsupplemented
+ 10% (w/w) pea fiber
+ 10% (w/w) orange fiber
+ 10% (w/w) barley bran fiber

FIG. 34D

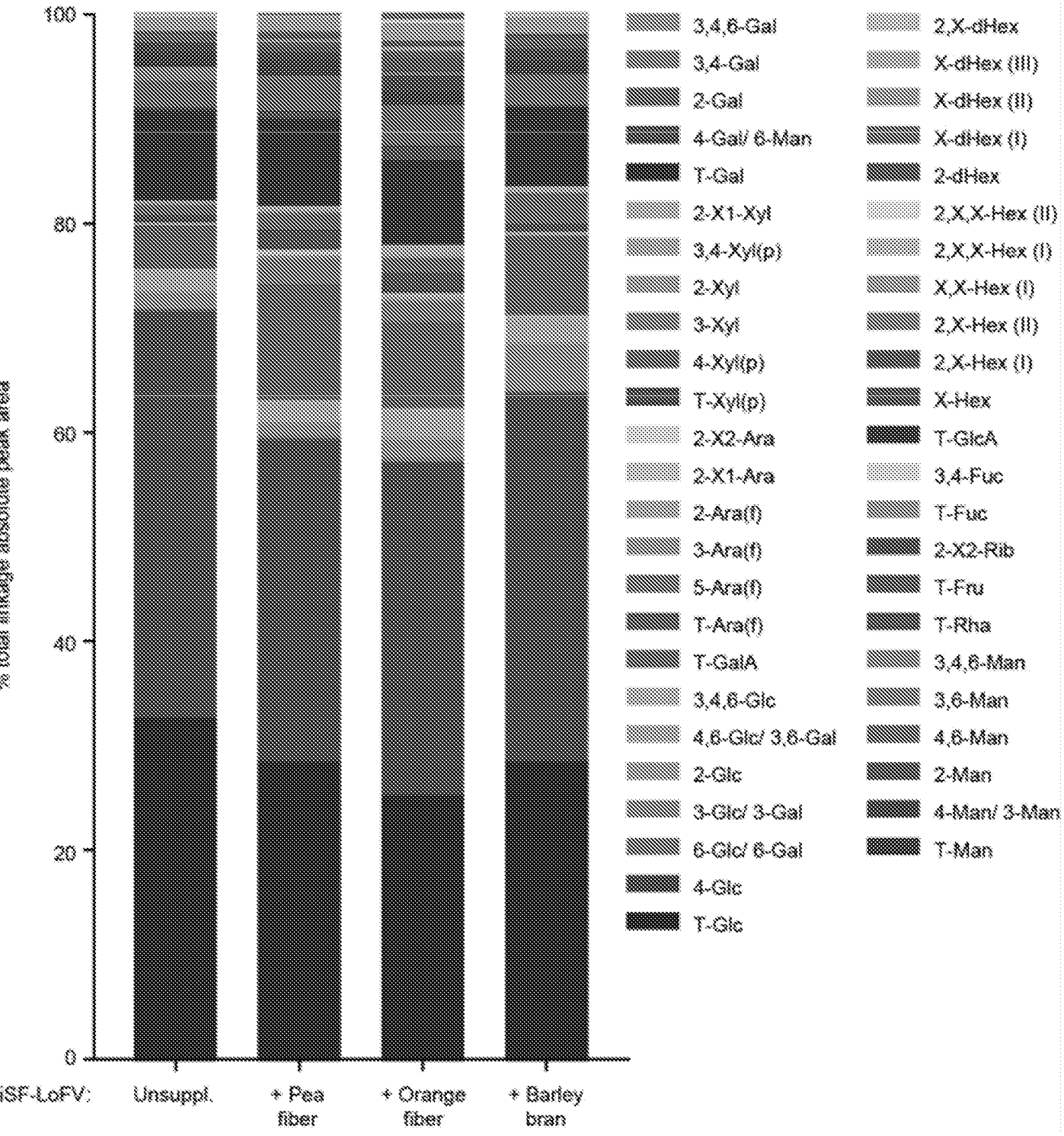

100
80
60
40
20
0
Unsuppl.
+ Pea fiber
+ Orange fiber
+ Barley bran
3,4,6-Gal
3,4-Gal
2-Gal
4-Gal/ 6-Man
T-Gal
2-X1-Xyl
3,4-Xyl(p)
2-Xyl
3-Xyl
4-Xyl(p)
T-Xyl(p)
2-X2-Ara
2-X1-Ara
2-Ara(f)
3-Ara(f)
5-Ara(f)
T-Ara(f)
T-GalA
3,4,6-Glc
4,6-Glc/ 3,6-Gal
2-Glc
3-Glc/ 3-Gal
6-Glc/ 6-Gal
4-Glc
T-Glc
2,X-dHex
X-dHex (III)
X-dHex (II)
X-dHex (I)
2-dHex
2,X,X-Hex (II)
2,X,X-Hex (I)
X,X-Hex (I)
2,X-Hex (II)
2,X-Hex (I)
X-Hex
T-GlcA
3,4-Fuc
T-Fuc
2-X2-Rib
T-Fru
T-Rha
3,4,6-Man
3,6-Man
4,6-Man
2-Man
4-Man/ 3-Man
T-Man

FIG. 34E

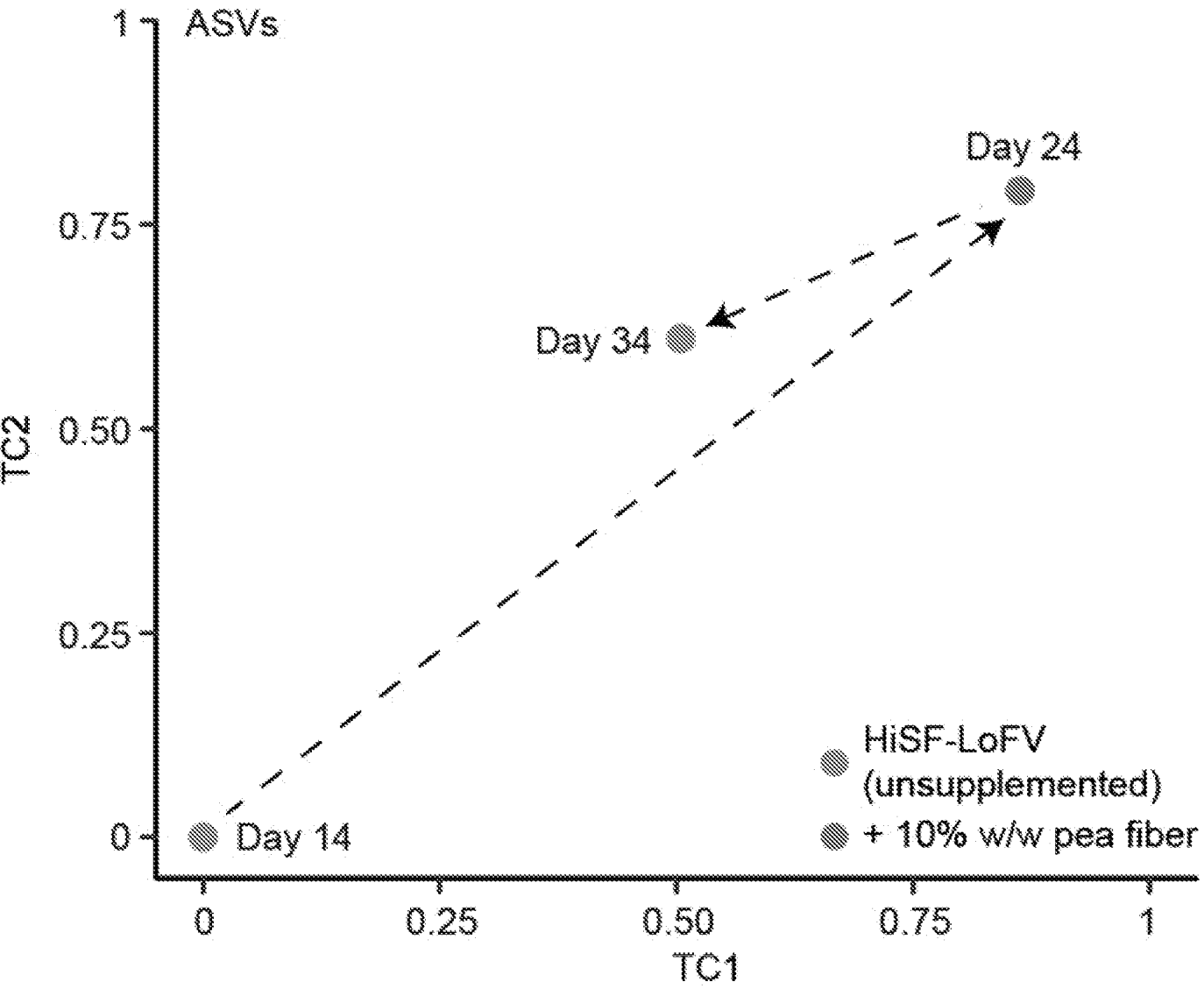

ASVs
1
0.75
0.50
0.25
0
TC2
Day 24
Day 34
Day 14
HiSF-LoFV
(unsupplemented)
+ 10% w/w pea fiber
0
0.25
0.50
0.75
1
TC1

FIG. 35A

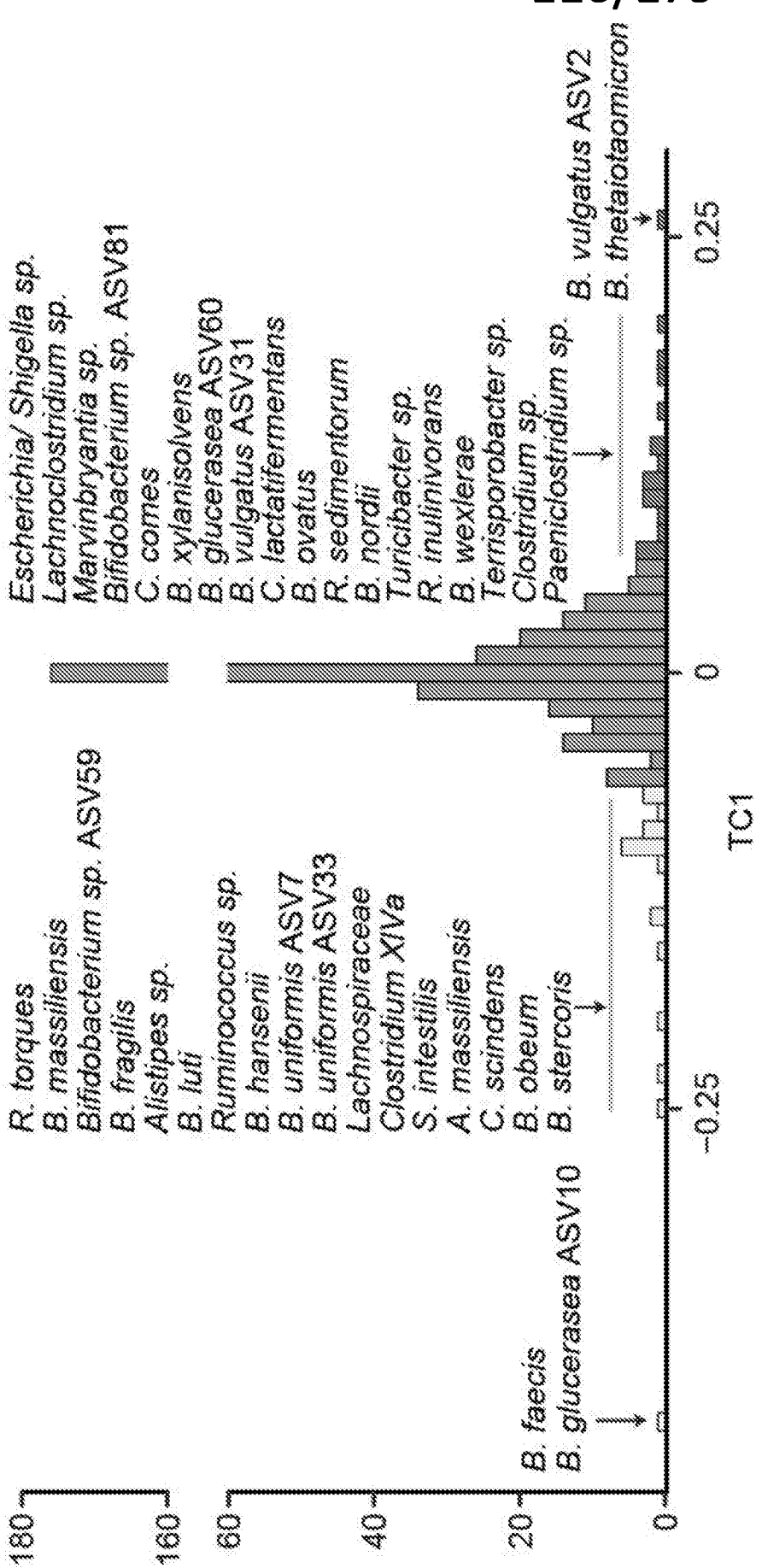
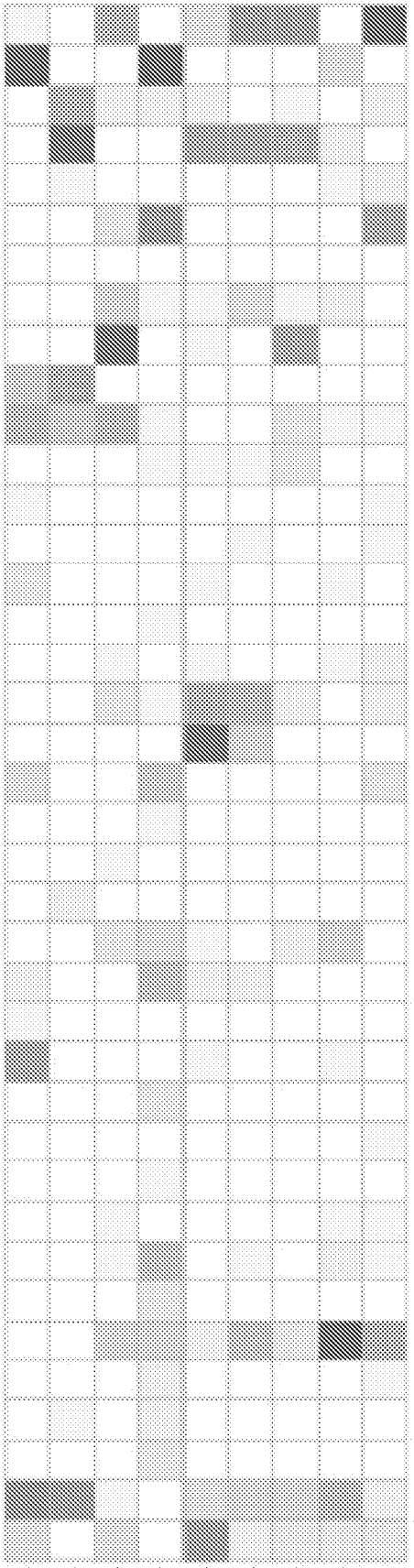

Escherichia/ Shigella sp.
Lachnoclostridium sp.
Marvinbryantia sp.
Bifidobacterium sp. ASV81
C. comes
B. xylanisolvens
B. glucerasea ASV60
B. vulgatus ASV31
C. lactatifermentans
B. ovatus
R. sedimentorum
B. nordii
Turicibacter sp.
R. inulinivorans
B. wexlerae
Terrisporobacter sp.
Clostridium sp.
Paeniclostridium sp.
B. vulgatus ASV2
B. thetaiotaomicron
R. torques
B. massiliensis
Bifidobacterium sp. ASV59
B. fragilis
Alistipes sp.
B. luti
Ruminococcus sp.
B. hansenii
B. uniformis ASV7
B. uniformis ASV33
Lachnospiraceae
Clostridium XIVa
S. intestilis
A. massiliensis
C. scindens
B. obeum
B. stercoris
B. faecis
B. glucerasea ASV10
180
160
60
40
20
0
Number of taxa
−0.25
0
0.25
TC1

FIG. 35B

Day 14



B. faecis
B. glucerasea ASV10
R. torques
B. massiliensis
Bifidobacterium sp. ASV59
B. fragilis
Alistipes sp.
B. luti
Ruminococcus sp.
B. hansenii
B. uniformis ASV7
B. uniformis ASV33
Lachnospiraceae
Clostridium XlVa
S. intestilis
A. massiliensis
C. scindens
B. obeum
B. stercoris
Escherichia/ Shigella sp.
Lachnoclostridium sp.
Marvinbryantia sp.
Bifidobacterium sp. ASV81
C. comes
B. xylanisolvens
B. glucerasea ASV60
B. vulgatus ASV31
C. lactatifermentans
B. ovatus
R. sedimentorum
B. nordii
Turicibacter sp.
R. inulinivorans
B. wexlerae
Terrisporobacter sp.
Clostridium sp.
Paeniclostridium sp.
B. vulgatus ASV2
B. thetaiotaomicron

TP02-01
TP03-02
TP08-02
TP07-02
TP06-01
TP04-01
TP09-02
TP05-02
TP01-01

Fractional abundance of taxa on TC1

0 0.1 0.2 0.3 0.4

FIG. 35C

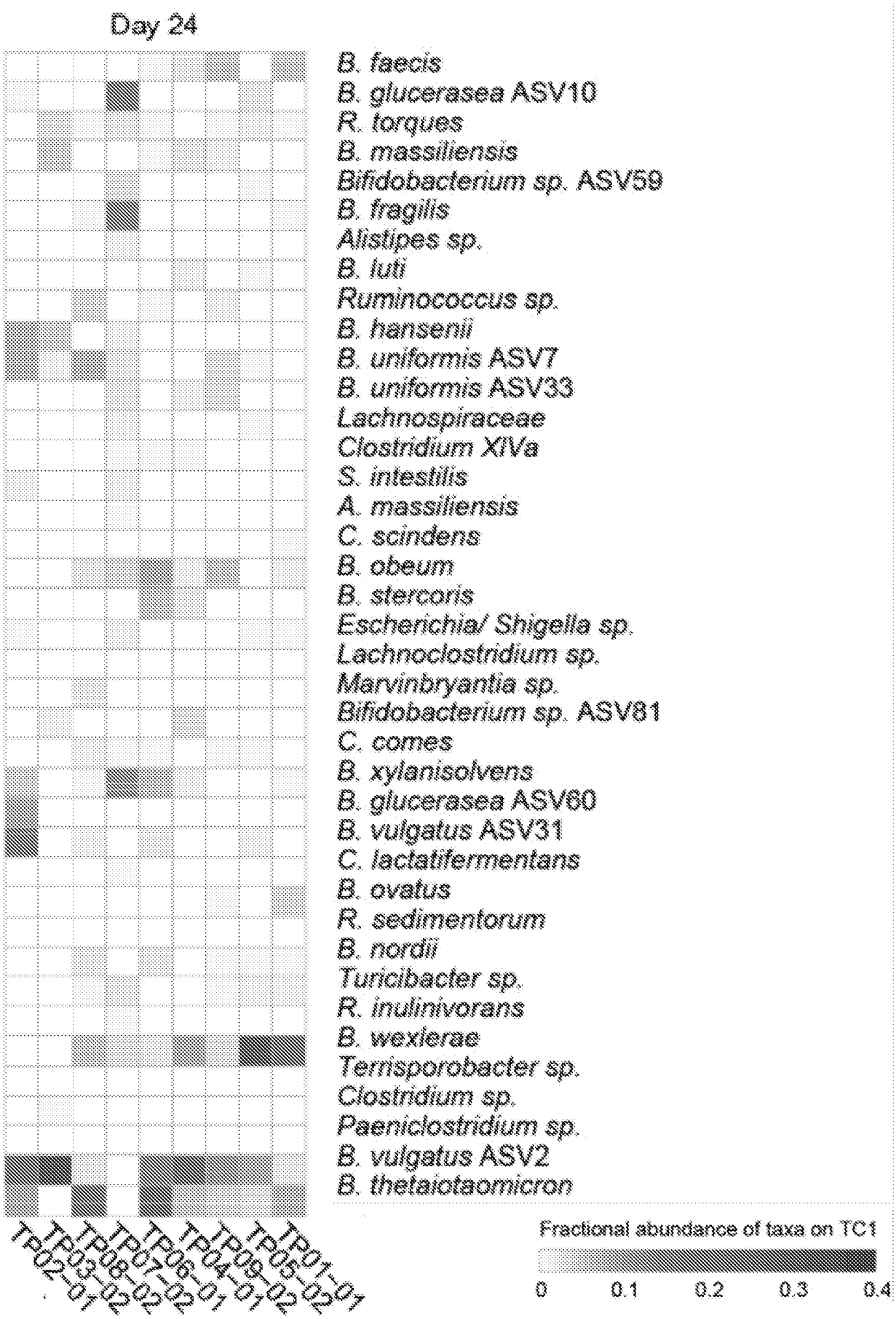

Day 24
B. faecis
B. glucerasea ASV10
R. torques
B. massiliensis
Bifidobacterium sp. ASV59
B. fragilis
Alistipes sp.
B. luti
Ruminococcus sp.
B. hansenii
B. uniformis ASV7
B. uniformis ASV33
Lachnospiraceae
Clostridium XlVa
S. intestilis
A. massiliensis
C. scindens
B. obeum
B. stercoris
Escherichia/ Shigella sp.
Lachnoclostridium sp.
Marvinbryantia sp.
Bifidobacterium sp. ASV81
C. comes
B. xylanisolvens
B. glucerasea ASV60
B. vulgatus ASV31
C. lactatifermentans
B. ovatus
R. sedimentorum
B. nordii
Turicibacter sp.
R. inulinivorans
B. wexlerae
Terrisporobacter sp.
Clostridium sp.
Paeniclostridium sp.
B. vulgatus ASV2
B. thetaiotaomicron
TP02-01
TP03-02
TP08-02
TP07-02
TP06-01
TP04-01
TP09-02
TP05-02
TP01-01
Fractional abundance of taxa on TC1
0
0.1
0.2
0.3
0.4

FIG. 35D

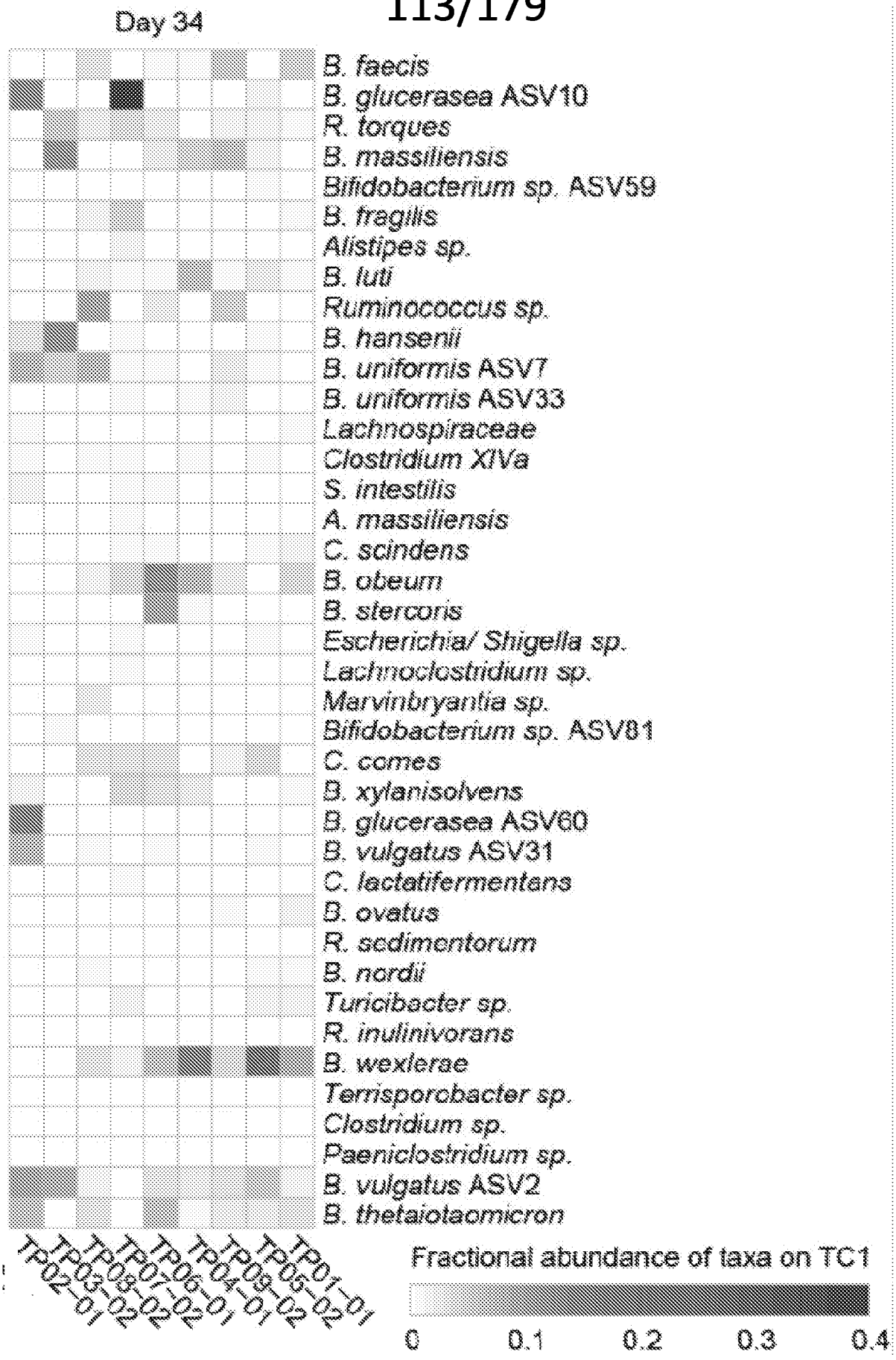

Day 34
B. faecis
B. glucerasea ASV10
R. torques
B. massiliensis
Bifidobacterium sp. ASV59
B. fragilis
Alistipes sp.
B. luti
Ruminococcus sp.
B. hansenii
B. uniformis ASV7
B. uniformis ASV33
Lachnospiraceae
Clostridium XIVa
S. intestilis
A. massiliensis
C. scindens
B. obeum
B. stercoris
Escherichia/ Shigella sp.
Lachnoclostridium sp.
Marvinbryantia sp.
Bifidobacterium sp. ASV81
C. comes
B. xylanisolvens
B. glucerasea ASV60
B. vulgatus ASV31
C. lactatifermentans
B. ovatus
R. sedimentorum
B. nordii
Turicibacter sp.
R. inulinivorans
B. wexlerae
Terrisporobacter sp.
Clostridium sp.
Paeniclostridium sp.
B. vulgatus ASV2
B. thetaiotaomicron
TP02-01
TP03-02
TP03-02
TP07-02
TP06-01
TP04-01
TP09-02
TP05-02
TP01-01
Fractional abundance of taxa on TC1
0
0.1
0.2
0.3
0.4

FIG. 35E

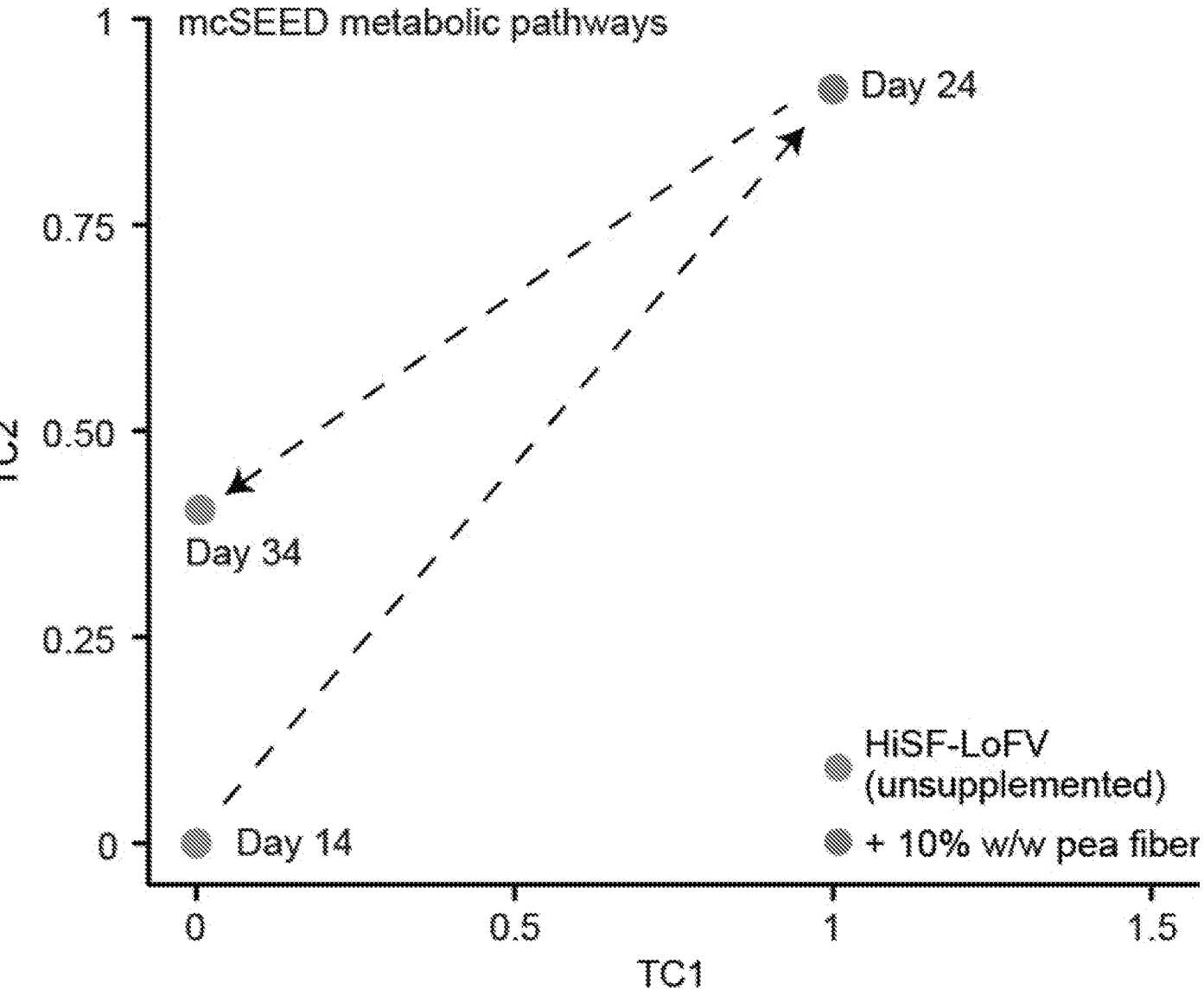

mcSEED metabolic pathways
Day 24
Day 34
Day 14
HiSF-LoFV
(unsupplemented)
+ 10% w/w pea fiber
1
0.75
0.50
0.25
0
0
0.5
1
1.5
TC1

FIG. 35F

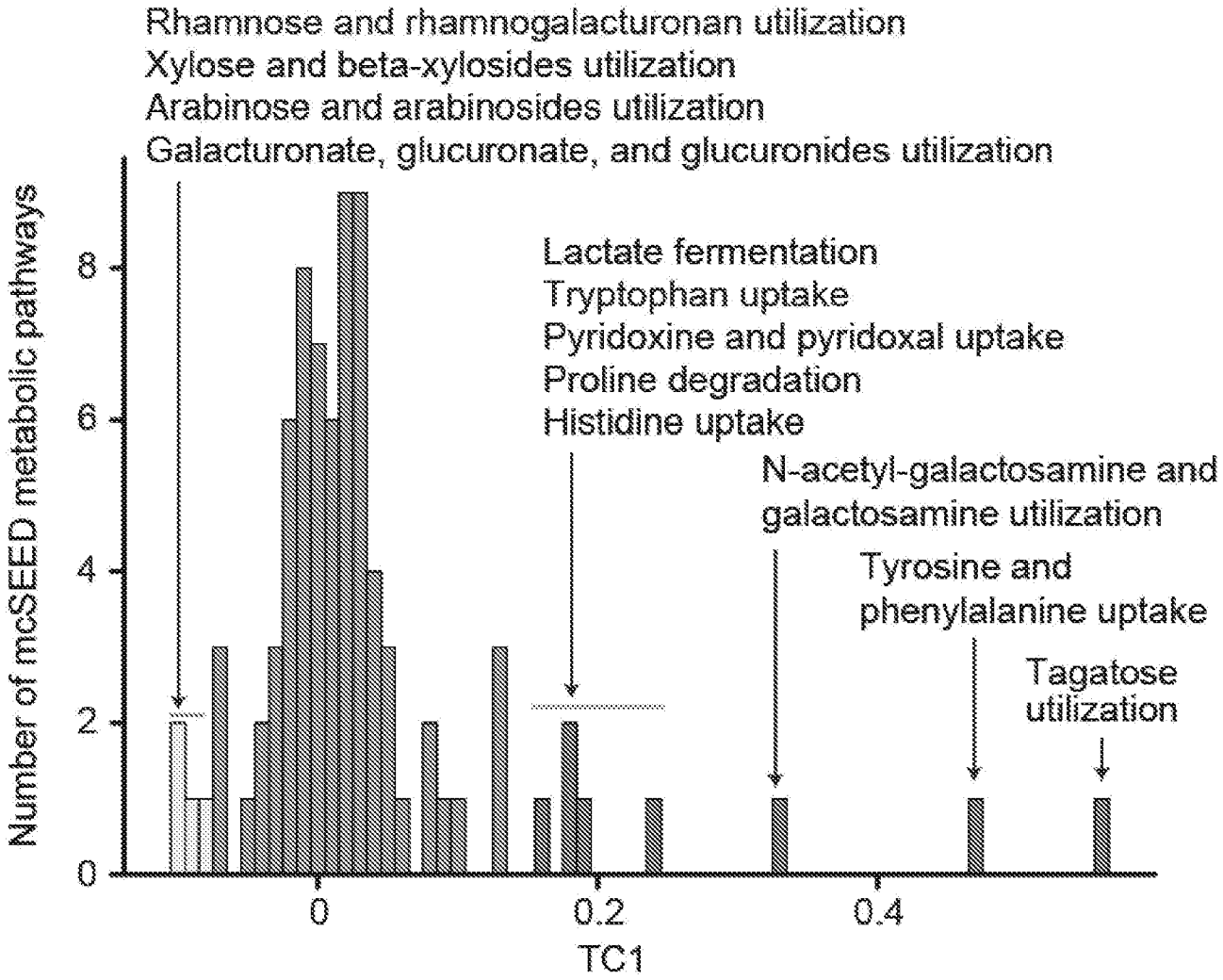

Rhamnose and rhamnogalacturonan utilization
Xylose and beta-xylosides utilization
Arabinose and arabinosides utilization
Galacturonate, glucuronate, and glucuronides utilization
Lactate fermentation
Tryptophan uptake
Pyridoxine and pyridoxal uptake
Proline degradation
Histidine uptake
N-acetyl-galactosamine and galactosamine utilization
Tyrosine and phenylalanine uptake
Tagatose utilization
Number of mcSEED metabolic pathways
0
2
4
6
8
0
0.2
0.4
TC1

FIG. 35G

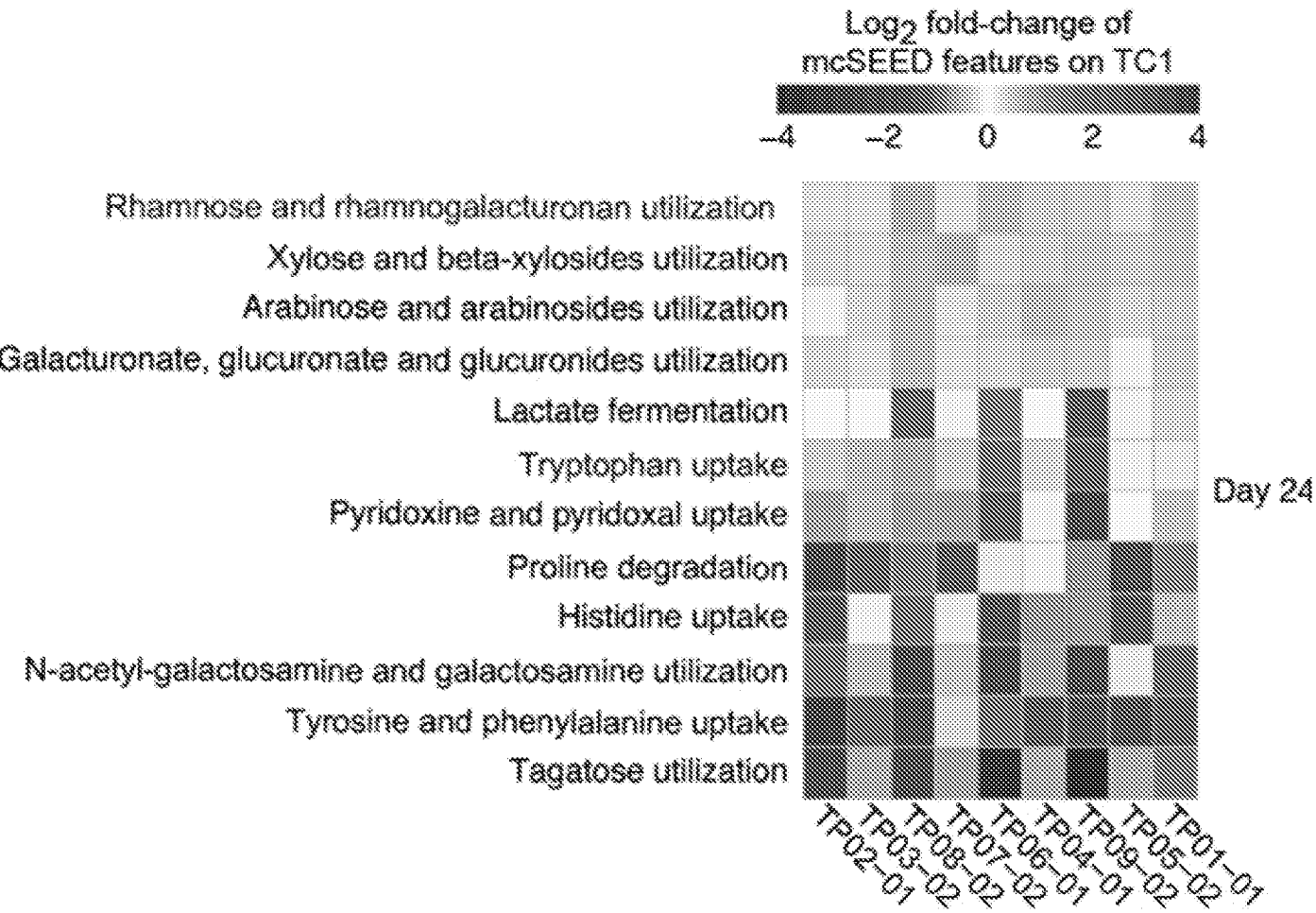

Log2 fold-change of
mcSEED features on TC1
−4
−2
0
2
4
Rhamnose and rhamnogalacturonan utilization
Xylose and beta-xylosides utilization
Arabinose and arabinosides utilization
Galacturonate, glucuronate and glucuronides utilization
Lactate fermentation
Tryptophan uptake
Pyridoxine and pyridoxal uptake
Proline degradation
Histidine uptake
N-acetyl-galactosamine and galactosamine utilization
Tyrosine and phenylalanine uptake
Tagatose utilization
Day 24
TP02_01
TP03_02
TP08_02
TP07_02
TP06_01
TP04_01
TP09_02
TP05_02
TP01_01

FIG. 35H

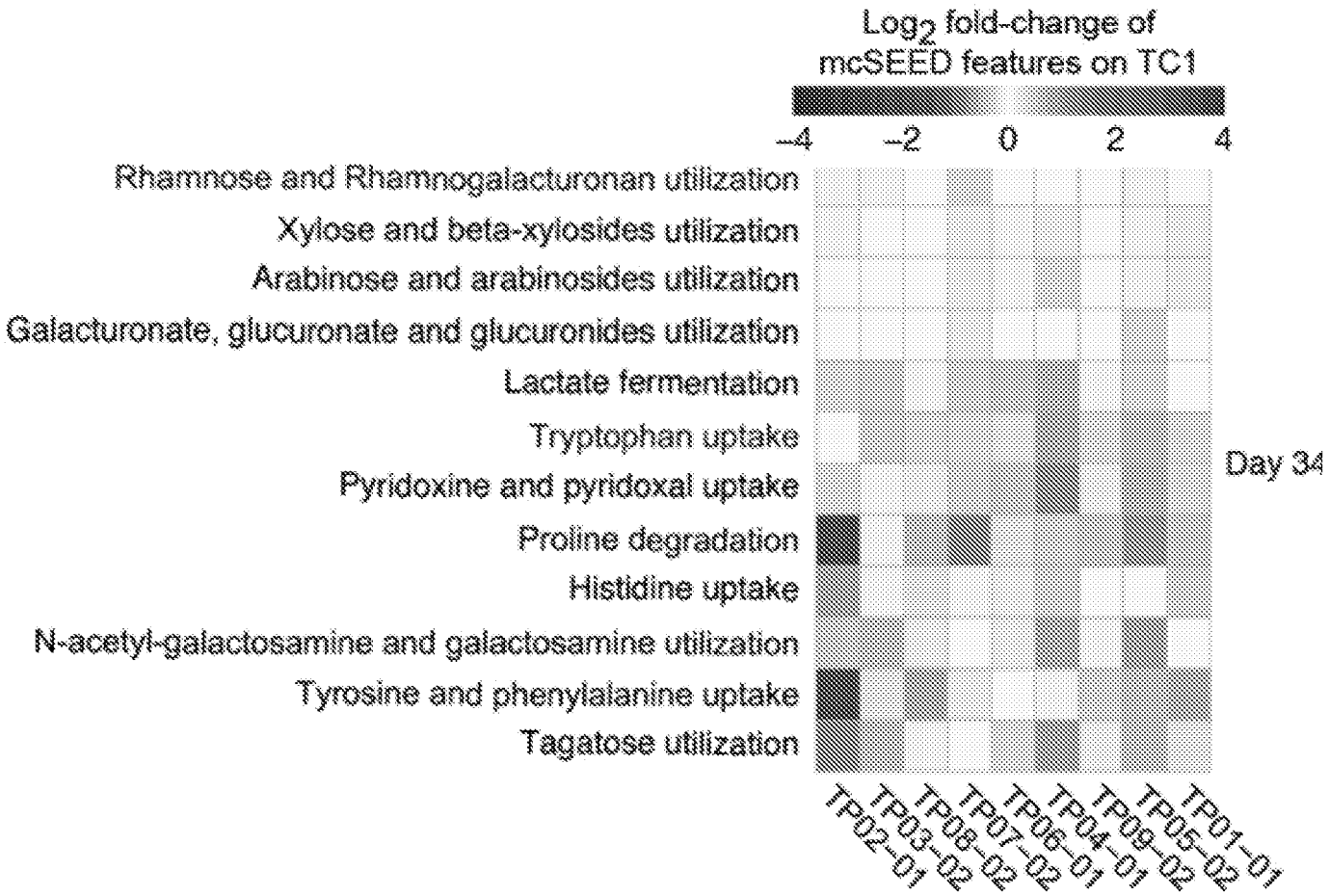

Log2 fold-change of
mcSEED features on TC1
−4
−2
0
2
4
Rhamnose and Rhamnogalacturonan utilization
Xylose and beta-xylosides utilization
Arabinose and arabinosides utilization
Galacturonate, glucuronate and glucuronides utilization
Lactate fermentation
Tryptophan uptake
Pyridoxine and pyridoxal uptake
Proline degradation
Histidine uptake
N-acetyl-galactosamine and galactosamine utilization
Tyrosine and phenylalanine uptake
Tagatose utilization
Day 34
TP02_01
TP03_02
TP08_02
TP07_02
TP06_01
TP04_01
TP09_02
TP05_02
TP01_01

FIG. 35I

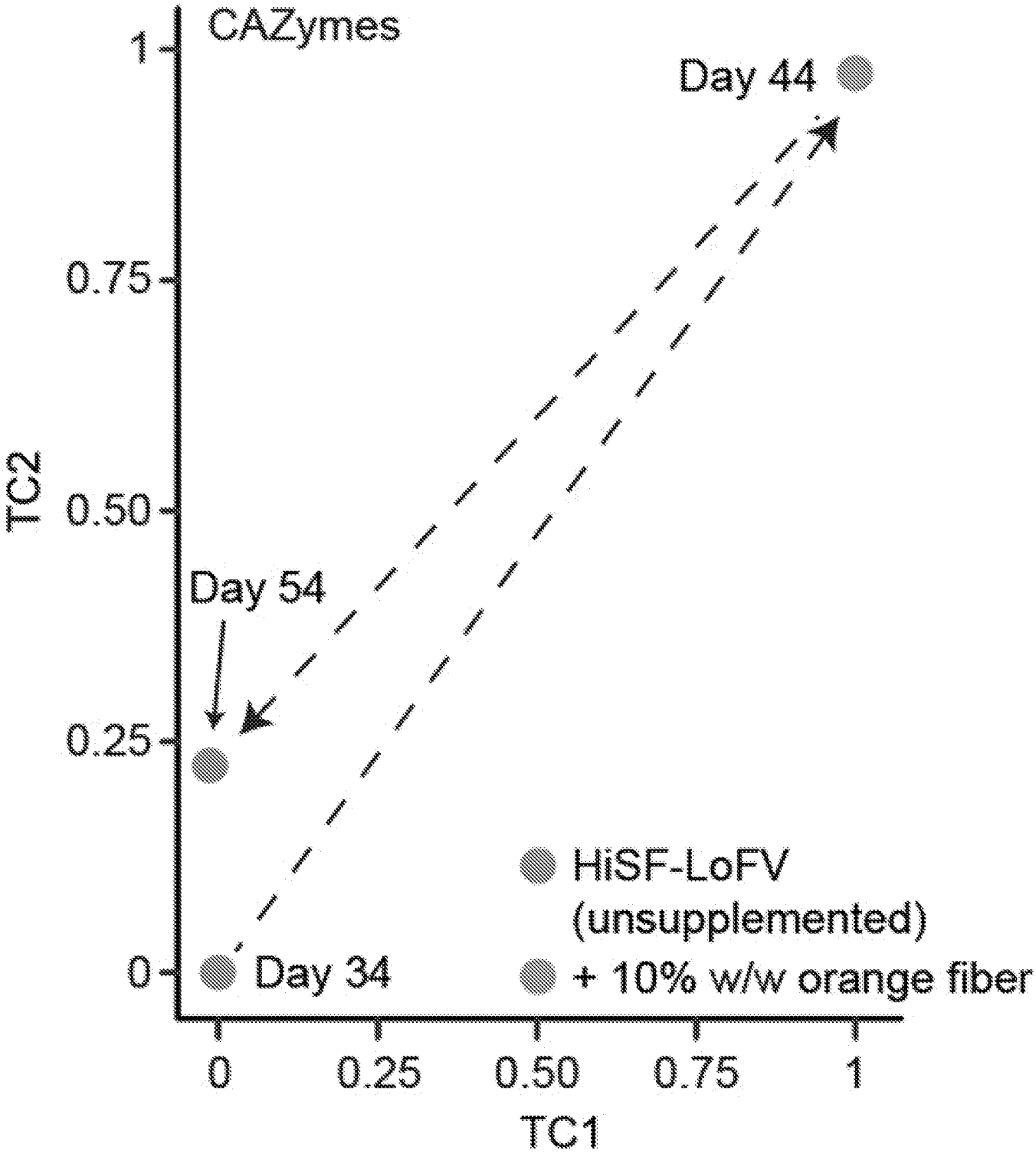

CAZymes
Day 44
Day 54
Day 34
HiSF-LoFV
(unsupplemented)
+ 10% w/w orange fiber
TC2
TC1
0
0.25
0.50
0.75
1

FIG. 36A

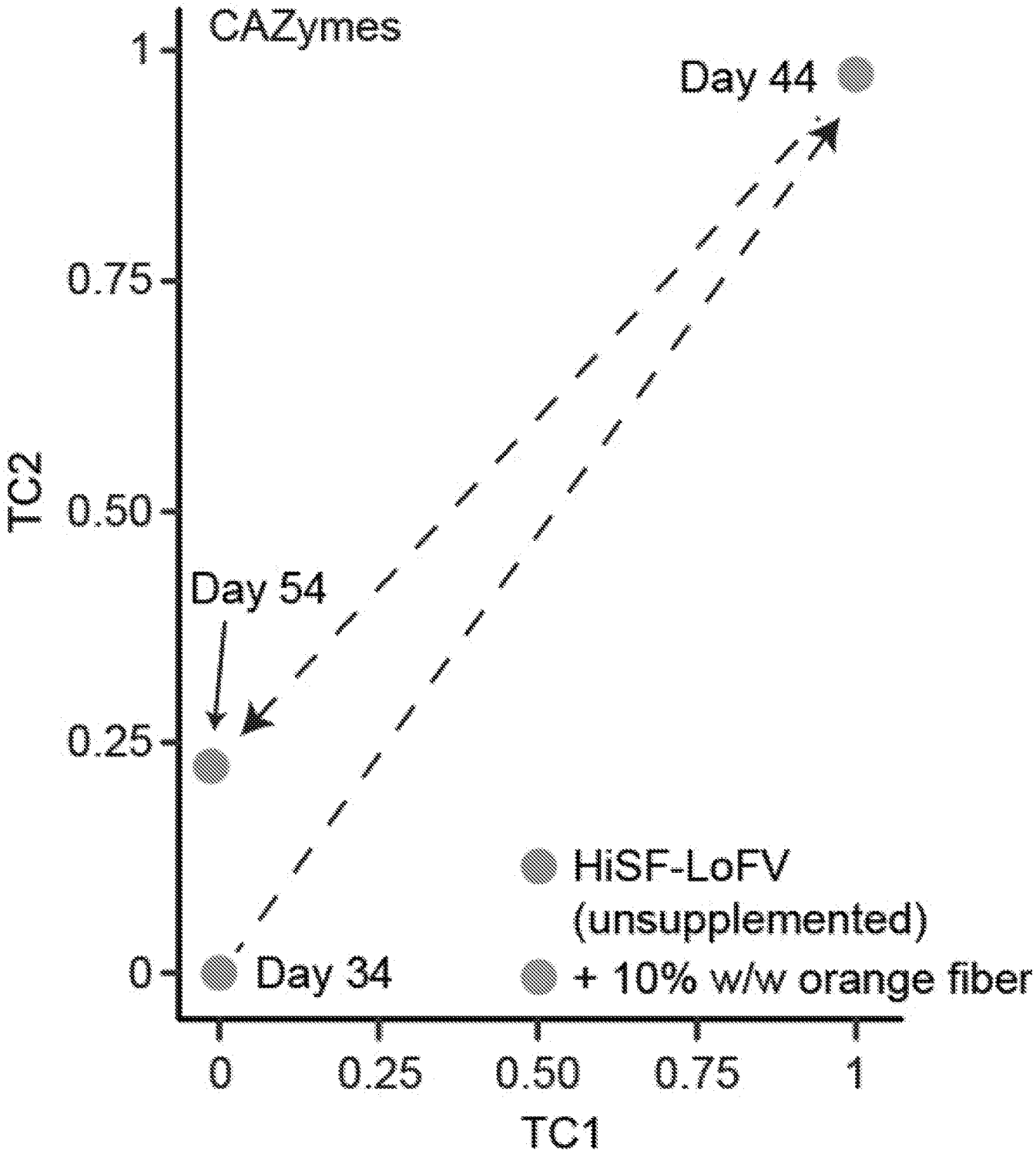

CAZymes
1
0.75
0.50
0.25
0
TC2
Day 44
Day 54
Day 34
HiSF-LoFV
(unsupplemented)
+ 10% w/w orange fiber
0
0.25
0.50
0.75
1
TC1

FIG. 36B

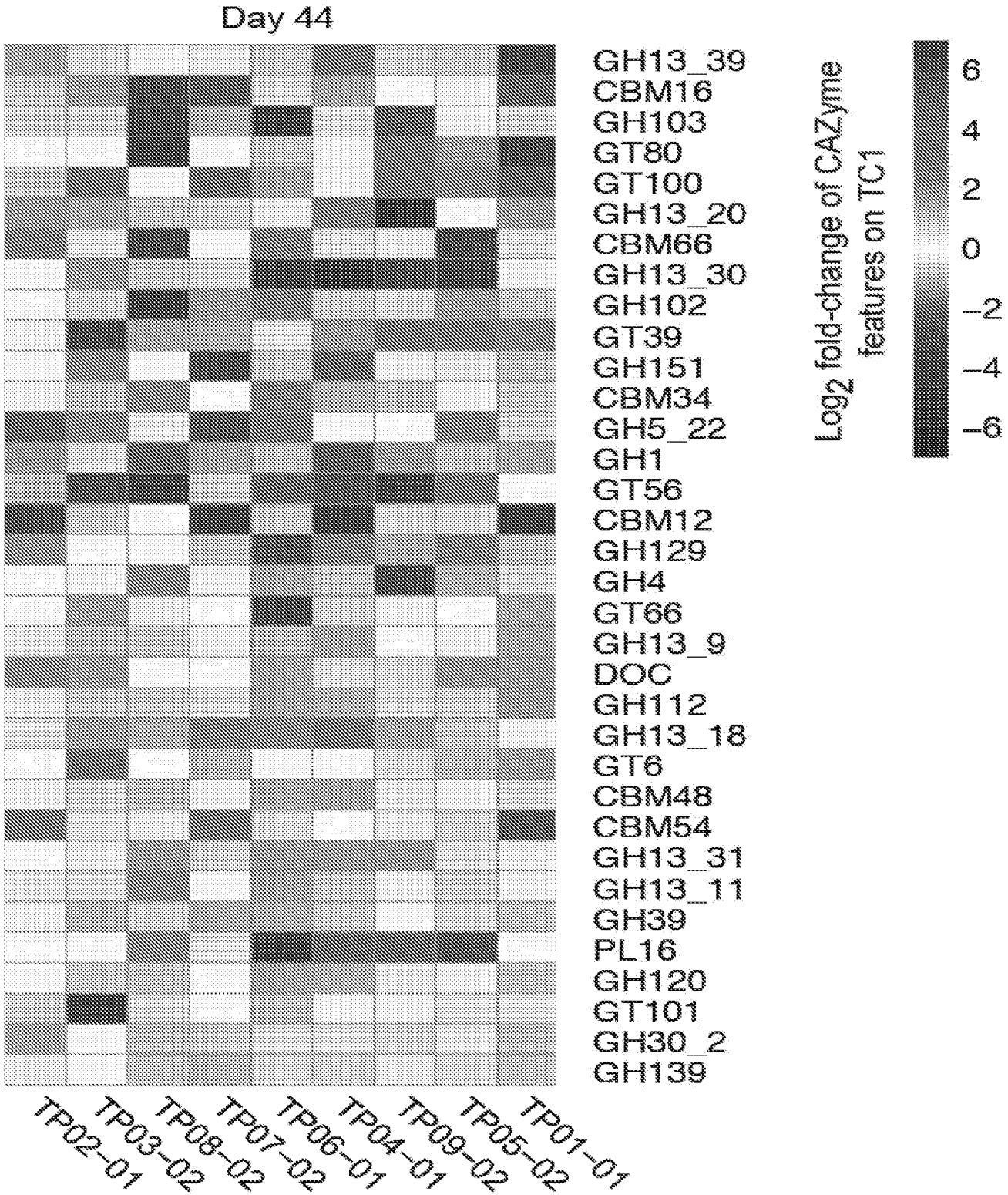

Day 44
GH13_39
CBM16
GH103
GT80
GT100
GH13_20
CBM66
GH13_30
GH102
GT39
GH151
CBM34
GH5_22
GH1
GT56
CBM12
GH129
GH4
GT66
GH13_9
DOC
GH112
GH13_18
GT6
CBM48
CBM54
GH13_31
GH13_11
GH39
PL16
GH120
GT101
GH30_2
GH139
TP02–01
TP03–02
TP08–02
TP07–02
TP06–01
TP04–01
TP09–02
TP05–02
TP01–01
Log2 fold-change of CAZyme features on TC1
6
4
2
0
–2
–4
–6

FIG. 36C

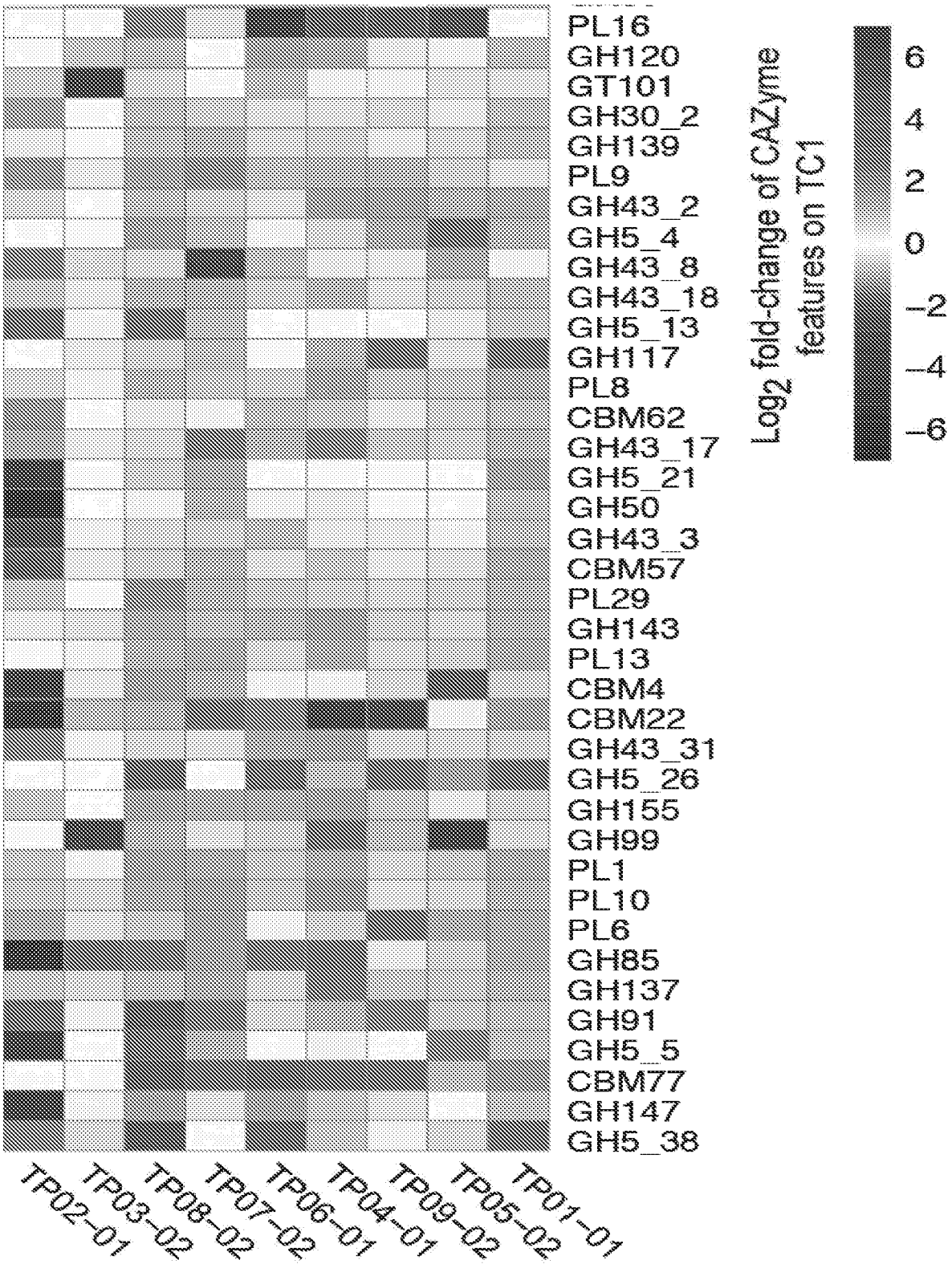

PL16
GH120
GT101
GH30_2
GH139
PL9
GH43_2
GH5_4
GH43_8
GH43_18
GH5_13
GH117
PL8
CBM62
GH43_17
GH5_21
GH50
GH43_3
CBM57
PL29
GH143
PL13
CBM4
CBM22
GH43_31
GH5_26
GH155
GH99
PL1
PL10
PL6
GH85
GH137
GH91
GH5_5
CBM77
GH147
GH5_38
TP02–01
TP03–02
TP08–02
TP07–02
TP06–01
TP04–01
TP09–02
TP05–02
TP01–01
Log2 fold-change of CAZyme features on TC1
6
4
2
0
–2
–4
–6

FIG. 36D

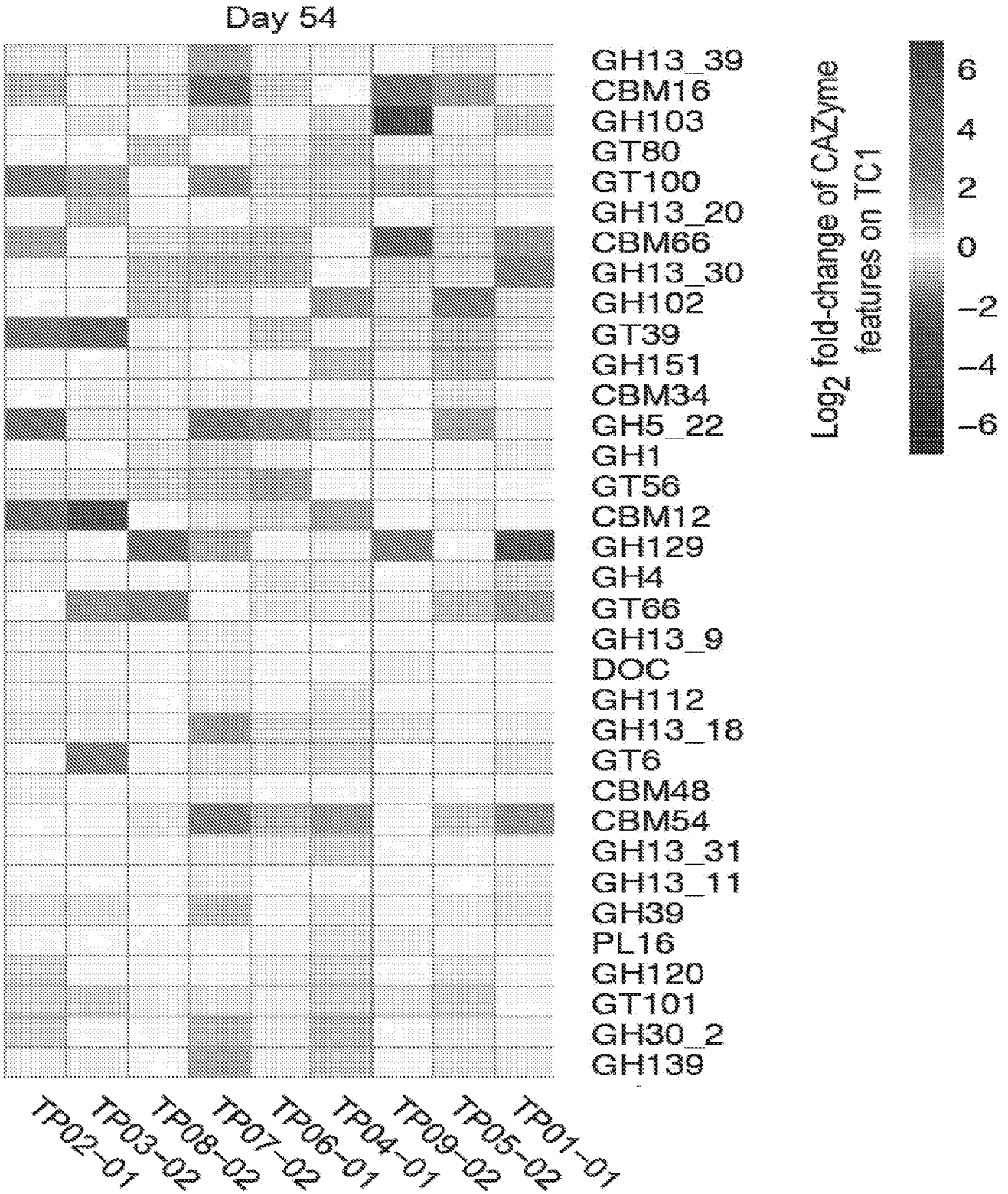

Day 54
GH13_39
CBM16
GH103
GT80
GT100
GH13_20
CBM66
GH13_30
GH102
GT39
GH151
CBM34
GH5_22
GH1
GT56
CBM12
GH129
GH4
GT66
GH13_9
DOC
GH112
GH13_18
GT6
CBM48
CBM54
GH13_31
GH13_11
GH39
PL16
GH120
GT101
GH30_2
GH139
TP02-01
TP03-02
TP08-02
TP07-02
TP06-01
TP04-01
TP09-02
TP05-02
TP01-01
Log2 fold-change of CAZyme features on TC1
6
4
2
0
−2
−4
−6

FIG. 36E

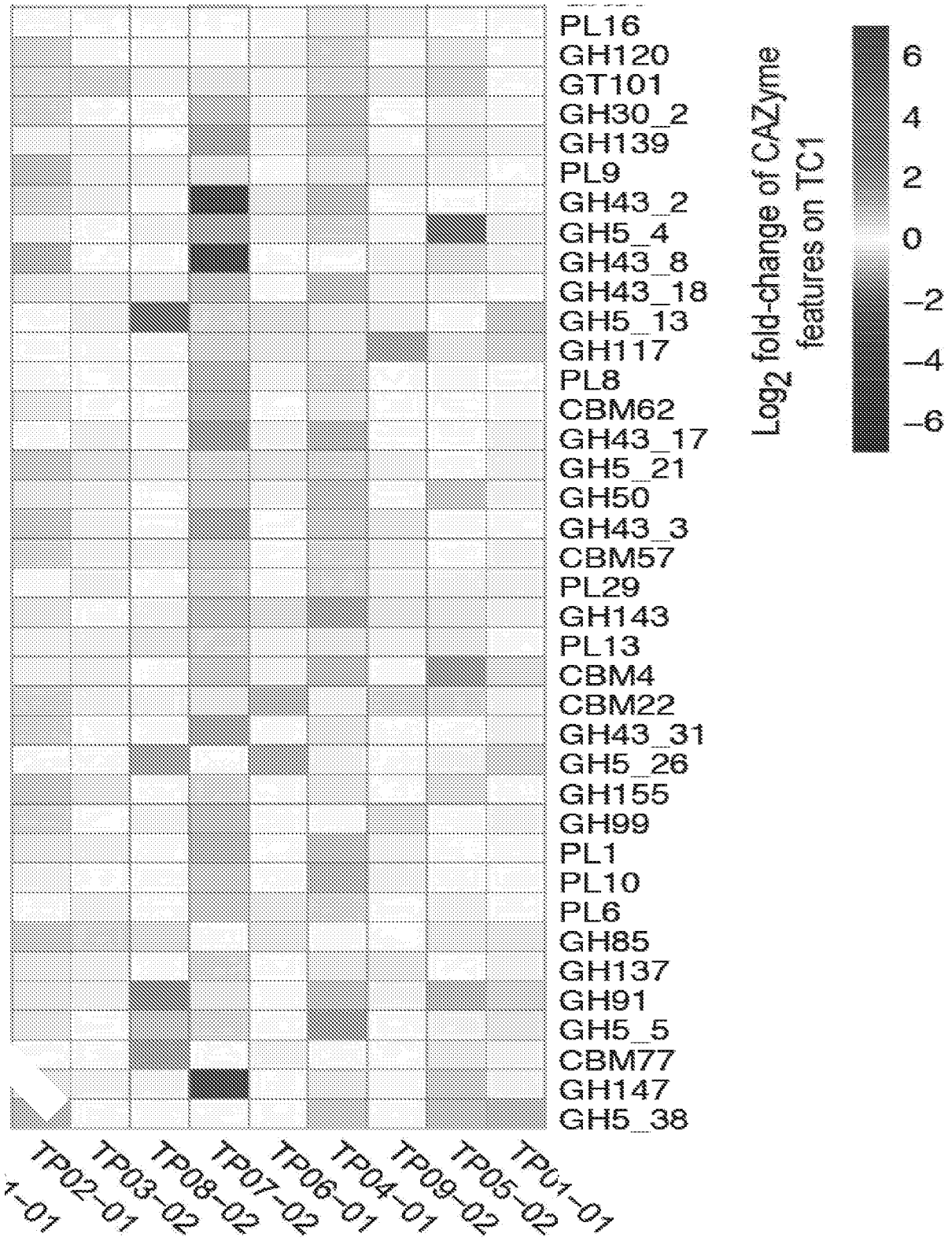

PL16
GH120
GT101
GH30_2
GH139
PL9
GH43_2
GH5_4
GH43_8
GH43_18
GH5_13
GH117
PL8
CBM62
GH43_17
GH5_21
GH50
GH43_3
CBM57
PL29
GH143
PL13
CBM4
CBM22
GH43_31
GH5_26
GH155
GH99
PL1
PL10
PL6
GH85
GH137
GH91
GH5_5
CBM77
GH147
GH5_38
TP02–01
TP03–02
TP08–02
TP07–02
TP06–01
TP04–01
TP09–02
TP05–02
TP01–01
Log2 fold-change of CAZyme features on TC1
6
4
2
0
–2
–4
–6

FIG. 36F

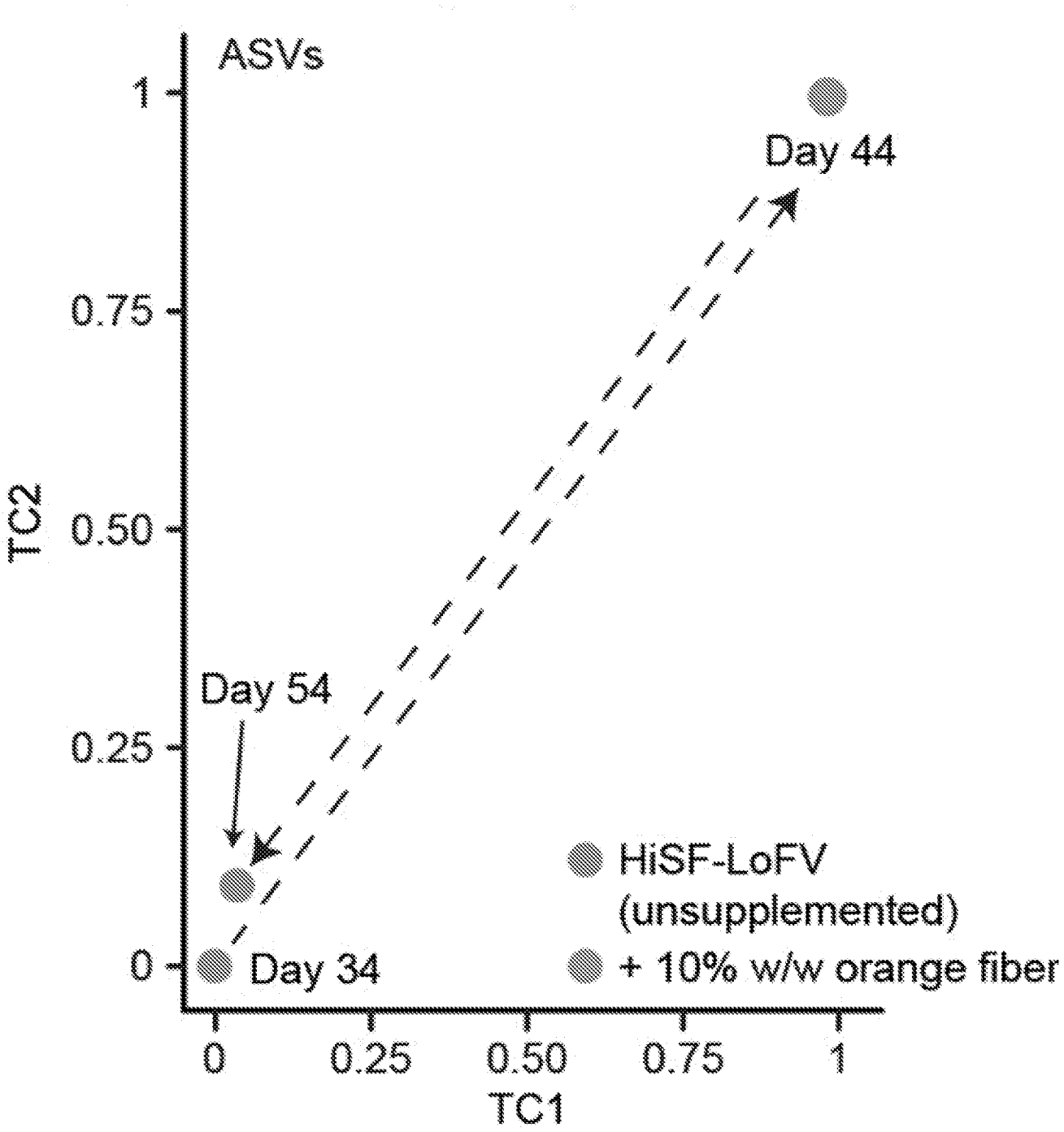

ASVs
1
0.75
0.50
0.25
0
TC2
Day 44
Day 54
Day 34
HiSF-LoFV
(unsupplemented)
+ 10% w/w orange fiber
0
0.25
0.50
0.75
1
TC1

FIG. 37A

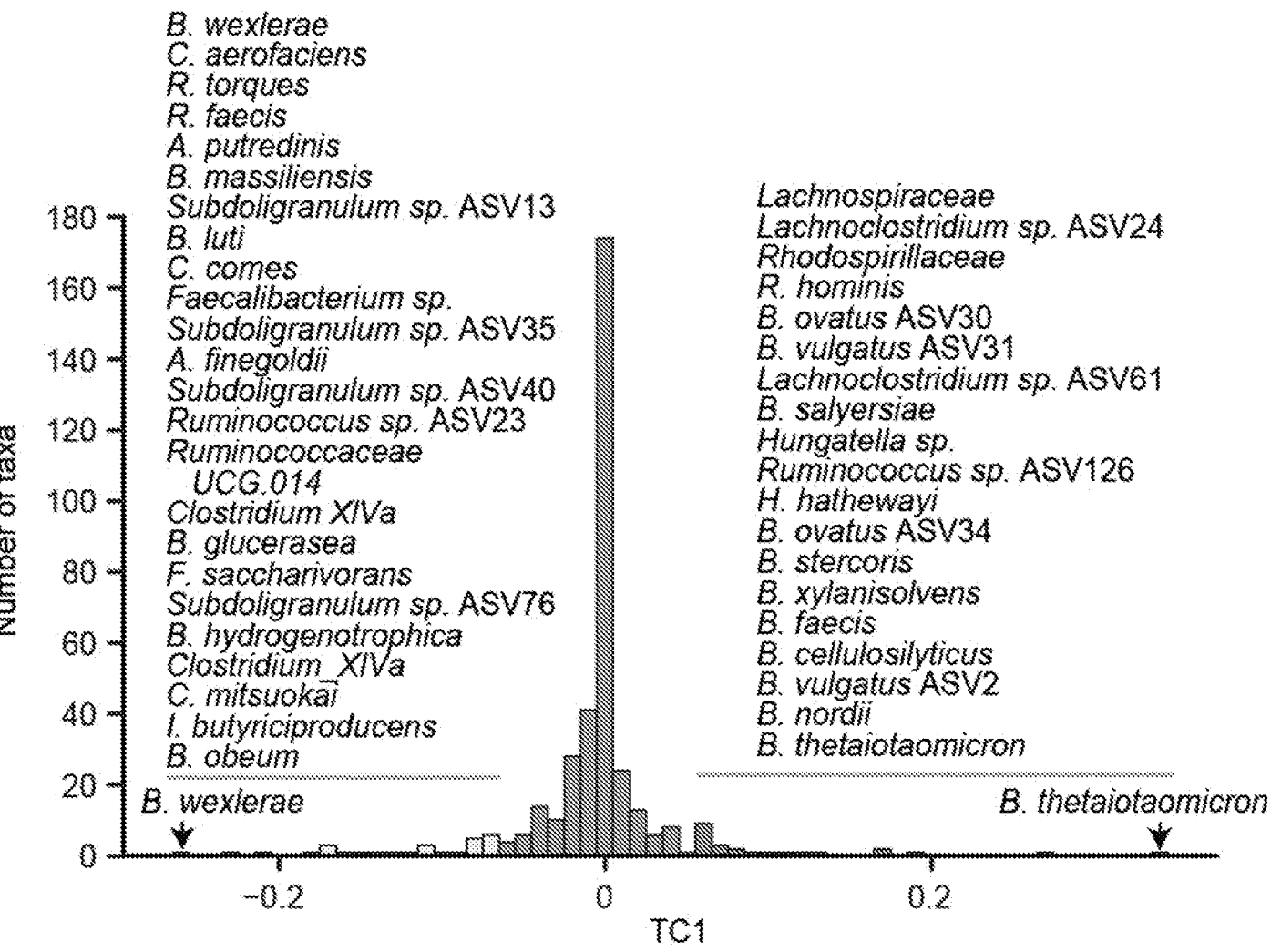

B. wexlerae
C. aerofaciens
R. torques
R. faecis
A. putredinis
B. massiliensis
Subdoligranulum sp. ASV13
B. luti
C. comes
Faecalibacterium sp.
Subdoligranulum sp. ASV35
A. finegoldii
Subdoligranulum sp. ASV40
Ruminococcus sp. ASV23
Ruminococcaceae UCG.014
Clostridium XlVa
B. glucerasea
F. saccharivorans
Subdoligranulum sp. ASV76
B. hydrogenotrophica
Clostridium_XlVa
C. mitsuokai
I. butyriciproducens
B. obeum
Lachnospiraceae
Lachnoclostridium sp. ASV24
Rhodospirillaceae
R. hominis
B. ovatus ASV30
B. vulgatus ASV31
Lachnoclostridium sp. ASV61
B. salyersiae
Hungatella sp.
Ruminococcus sp. ASV126
H. hathewayi
B. ovatus ASV34
B. stercoris
B. xylanisolvens
B. faecis
B. cellulosilyticus
B. vulgatus ASV2
B. nordii
B. thetaiotaomicron
B. wexlerae
B. thetaiotaomicron
Number of taxa
180
160
140
120
100
80
60
40
20
0
−0.2
0
0.2
TC1

FIG. 37B

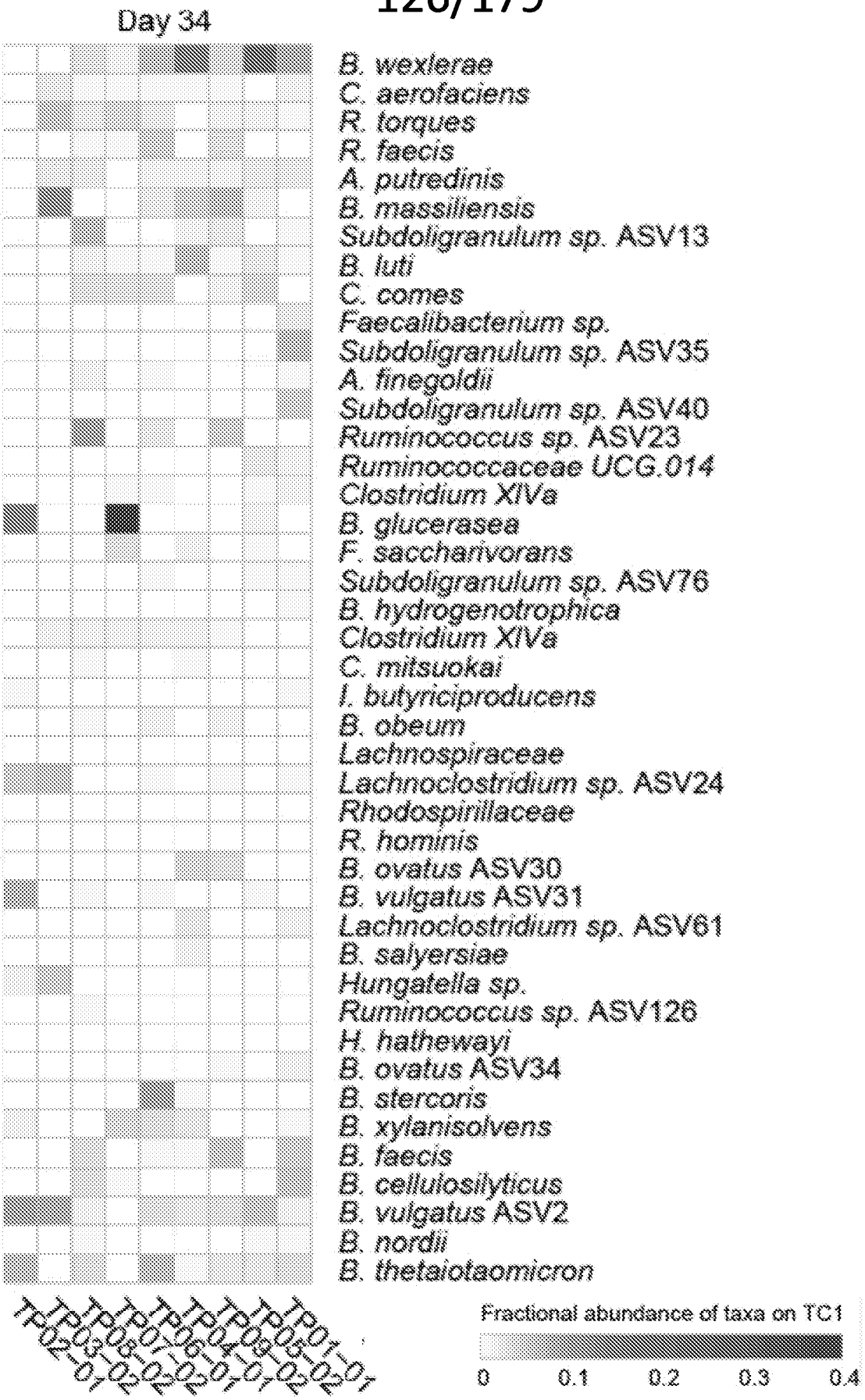

Day 34
B. wexlerae
C. aerofaciens
R. torques
R. faecis
A. putredinis
B. massiliensis
Subdoligranulum sp. ASV13
B. luti
C. comes
Faecalibacterium sp.
Subdoligranulum sp. ASV35
A. finegoldii
Subdoligranulum sp. ASV40
Ruminococcus sp. ASV23
Ruminococcaceae UCG.014
Clostridium XlVa
B. glucerasea
F. saccharivorans
Subdoligranulum sp. ASV76
B. hydrogenotrophica
Clostridium XlVa
C. mitsuokai
I. butyriciproducens
B. obeum
Lachnospiraceae
Lachnoclostridium sp. ASV24
Rhodospirillaceae
R. hominis
B. ovatus ASV30
B. vulgatus ASV31
Lachnoclostridium sp. ASV61
B. salyersiae
Hungatella sp.
Ruminococcus sp. ASV126
H. hathewayi
B. ovatus ASV34
B. stercoris
B. xylanisolvens
B. faecis
B. cellulosilyticus
B. vulgatus ASV2
B. nordii
B. thetaiotaomicron
TP-02-01
TP-03-01
TP-08-01
TP-07-01
TP-06-01
TP-04-01
TP-09-01
TP-05-01
TP-01-01
Fractional abundance of taxa on TC1
0
0.1
0.2
0.3
0.4

FIG. 37C

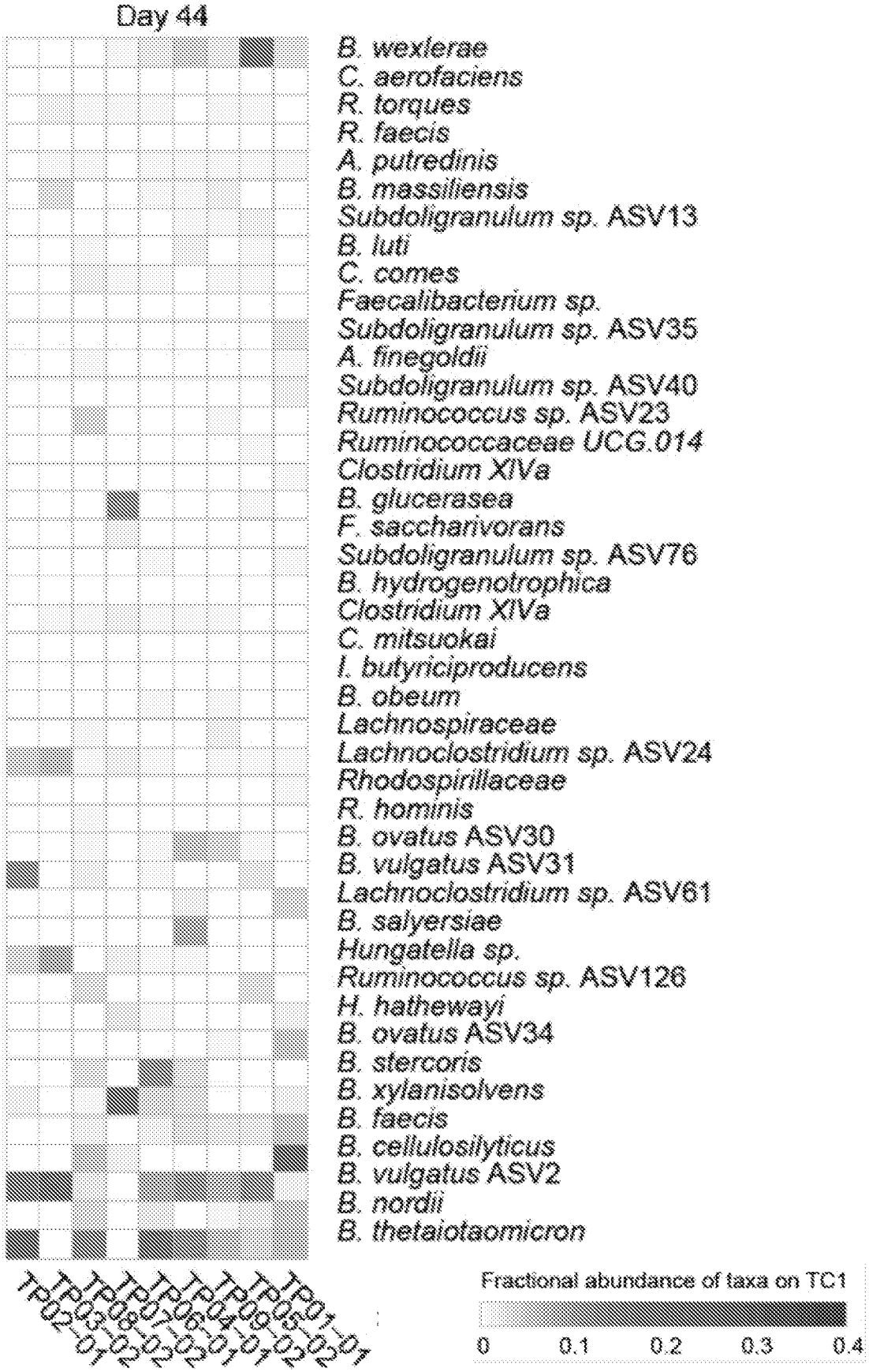

Day 44
B. wexlerae
C. aerofaciens
R. torques
R. faecis
A. putredinis
B. massiliensis
Subdoligranulum sp. ASV13
B. luti
C. comes
Faecalibacterium sp.
Subdoligranulum sp. ASV35
A. finegoldii
Subdoligranulum sp. ASV40
Ruminococcus sp. ASV23
Ruminococcaceae UCG.014
Clostridium XlVa
B. gluceraseа
F. saccharivorans
Subdoligranulum sp. ASV76
B. hydrogenotrophica
Clostridium XlVa
C. mitsuokai
I. butyriciproducens
B. obeum
Lachnospiraceae
Lachnoclostridium sp. ASV24
Rhodospirillaceae
R. hominis
B. ovatus ASV30
B. vulgatus ASV31
Lachnoclostridium sp. ASV61
B. salyersiae
Hungatella sp.
Ruminococcus sp. ASV126
H. hathewayi
B. ovatus ASV34
B. stercoris
B. xylanisolvens
B. faecis
B. cellulosilyticus
B. vulgatus ASV2
B. nordii
B. thetaiotaomicron
TP01
TP02
TP03
TP04
TP05
TP06
TP07
TP08
TP09
TP10
Fractional abundance of taxa on TC1
0
0.1
0.2
0.3
0.4

FIG. 37D

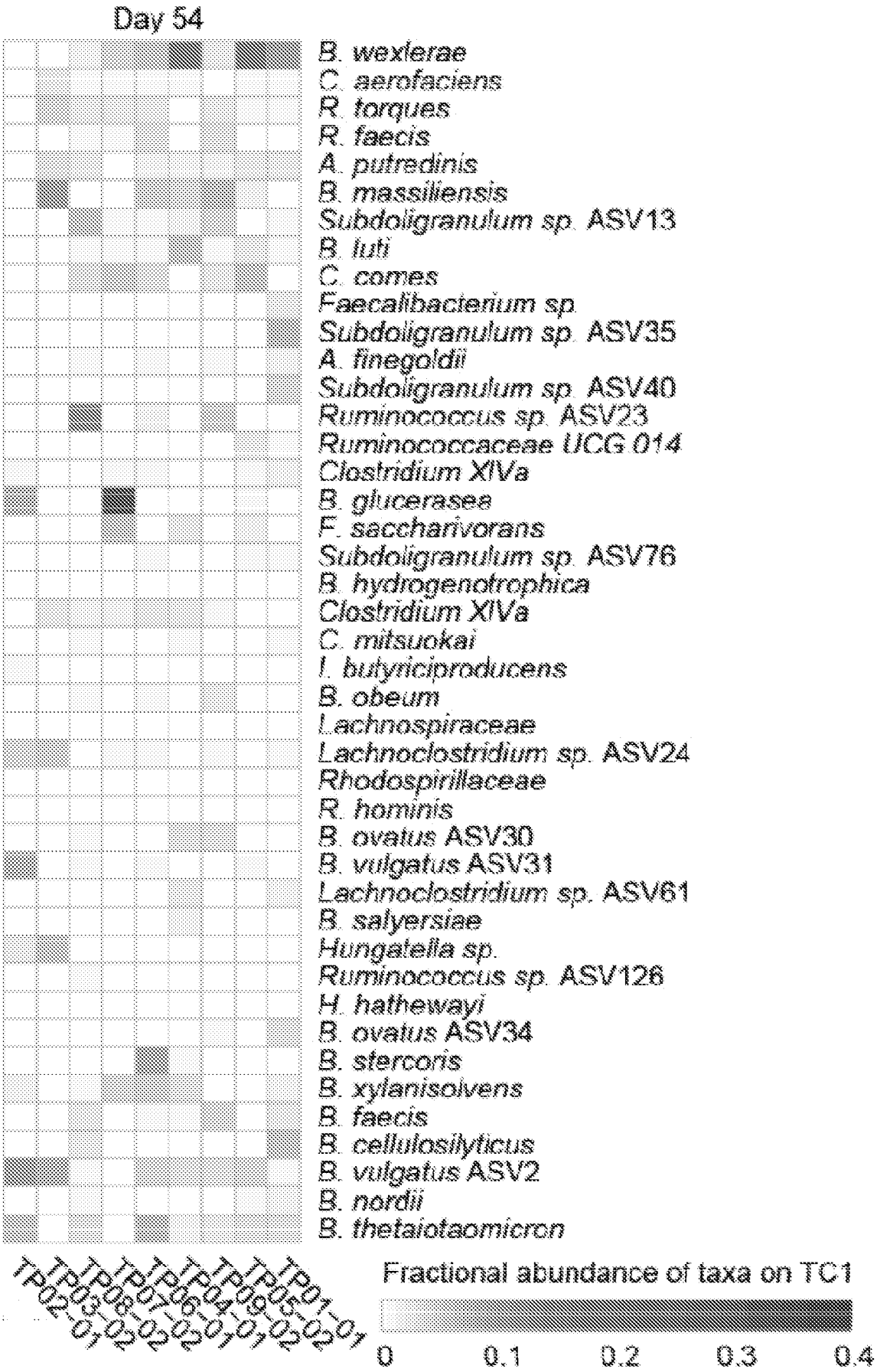

Day 54
B. wexlerae
C. aerofaciens
R. torques
R. faecis
A. putredinis
B. massiliensis
Subdoligranulum sp. ASV13
B. luti
C. comes
Faecalibacterium sp.
Subdoligranulum sp. ASV35
A. finegoldii
Subdoligranulum sp. ASV40
Ruminococcus sp. ASV23
Ruminococcaceae UCG 014
Clostridium XlVa
B. glucerasea
F. saccharivorans
Subdoligranulum sp. ASV76
B. hydrogenotrophica
Clostridium XlVa
C. mitsuokai
I. butyriciproducens
B. obeum
Lachnospiraceae
Lachnoclostridium sp. ASV24
Rhodospirillaceae
R. hominis
B. ovatus ASV30
B. vulgatus ASV31
Lachnoclostridium sp. ASV61
B. salyersiae
Hungatella sp.
Ruminococcus sp. ASV126
H. hathewayi
B. ovatus ASV34
B. stercoris
B. xylanisolvens
B. faecis
B. cellulosilyticus
B. vulgatus ASV2
B. nordii
B. thetaiotaomicron
TP01-01
TP01-02
TP02-01
TP02-02
TP03-01
TP03-02
TP04-01
TP04-02
TP05-01
TP05-02
TP06-01
TP06-02
TP07-01
TP07-02
TP08-01
TP08-02
TP09-01
TP09-02
Fractional abundance of taxa on TC1
0
0.1
0.2
0.3
0.4

FIG. 37E

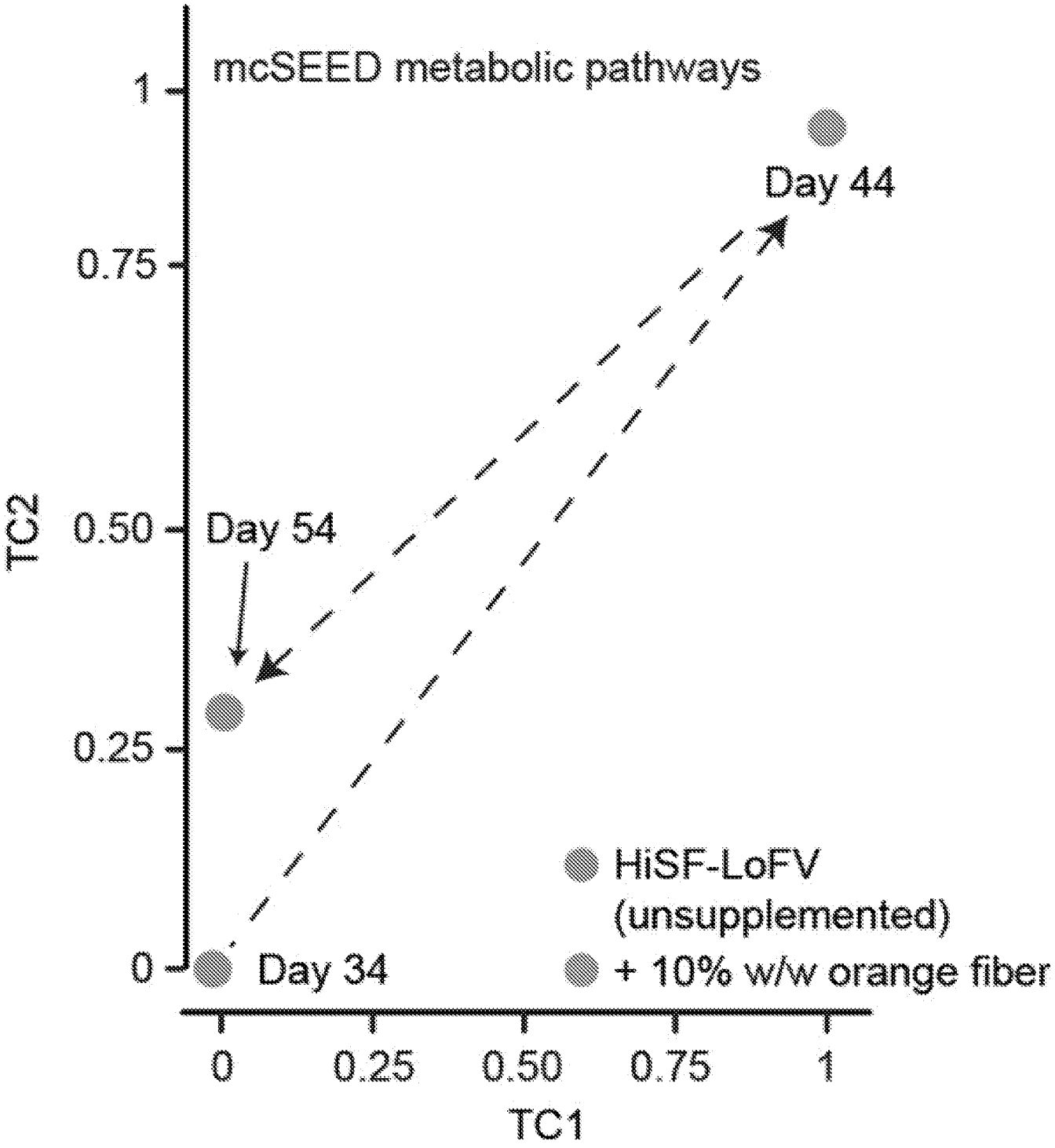

mcSEED metabolic pathways
1
0.75
0.50
0.25
0
TC2
Day 44
Day 54
Day 34
HiSF-LoFV
(unsupplemented)
+ 10% w/w orange fiber
0
0.25
0.50
0.75
1
TC1

FIG. 37F

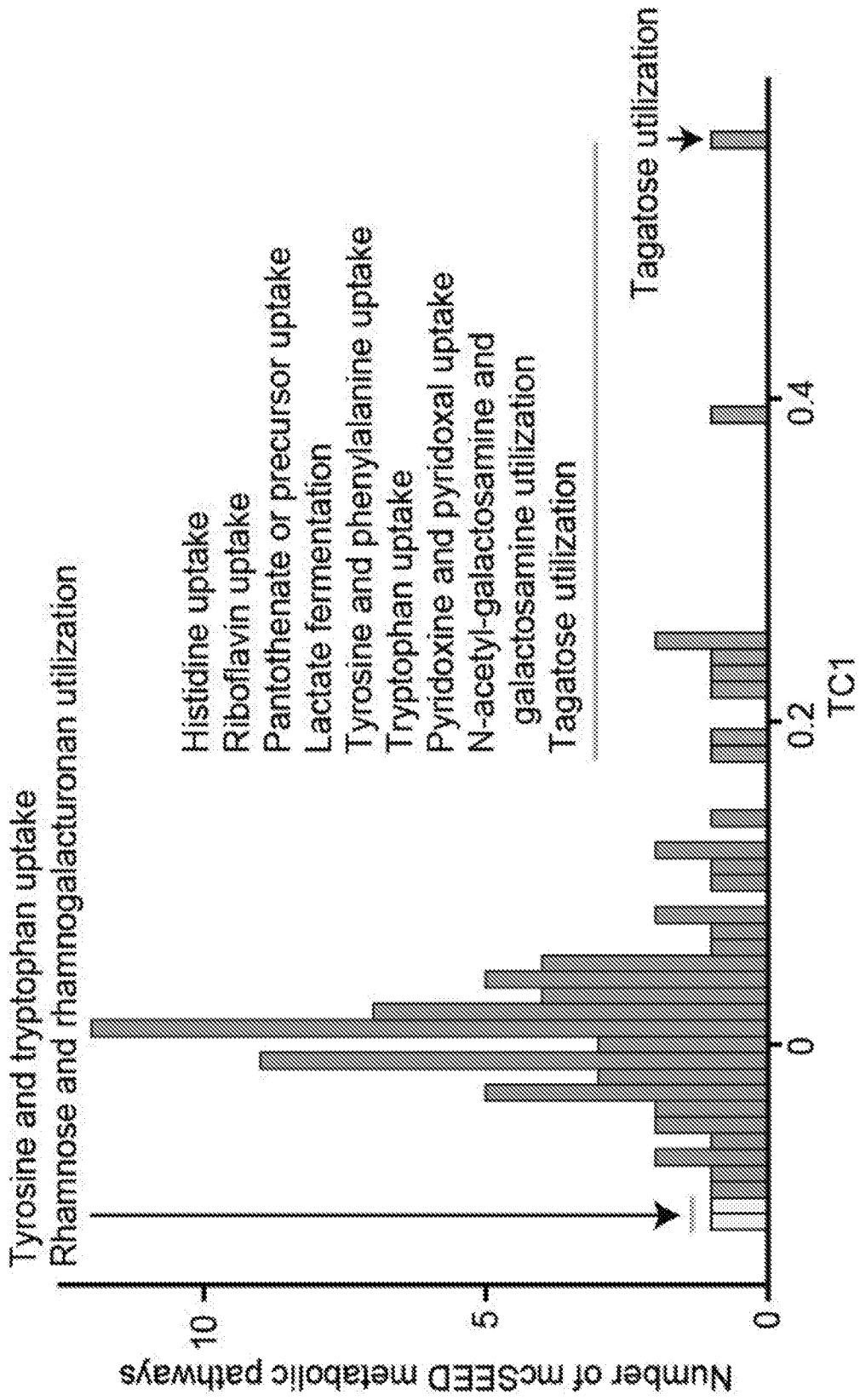

Number of mcSEED metabolic pathways
10
5
0
Tyrosine and tryptophan uptake
Rhamnose and rhamnogalacturonan utilization
Histidine uptake
Riboflavin uptake
Pantothenate or precursor uptake
Lactate fermentation
Tyrosine and phenylalanine uptake
Tryptophan uptake
Pyridoxine and pyridoxal uptake
N-acetyl-galactosamine and galactosamine utilization
Tagatose utilization
Tagatose utilization
0
0.2
0.4
TC1

FIG. 37G

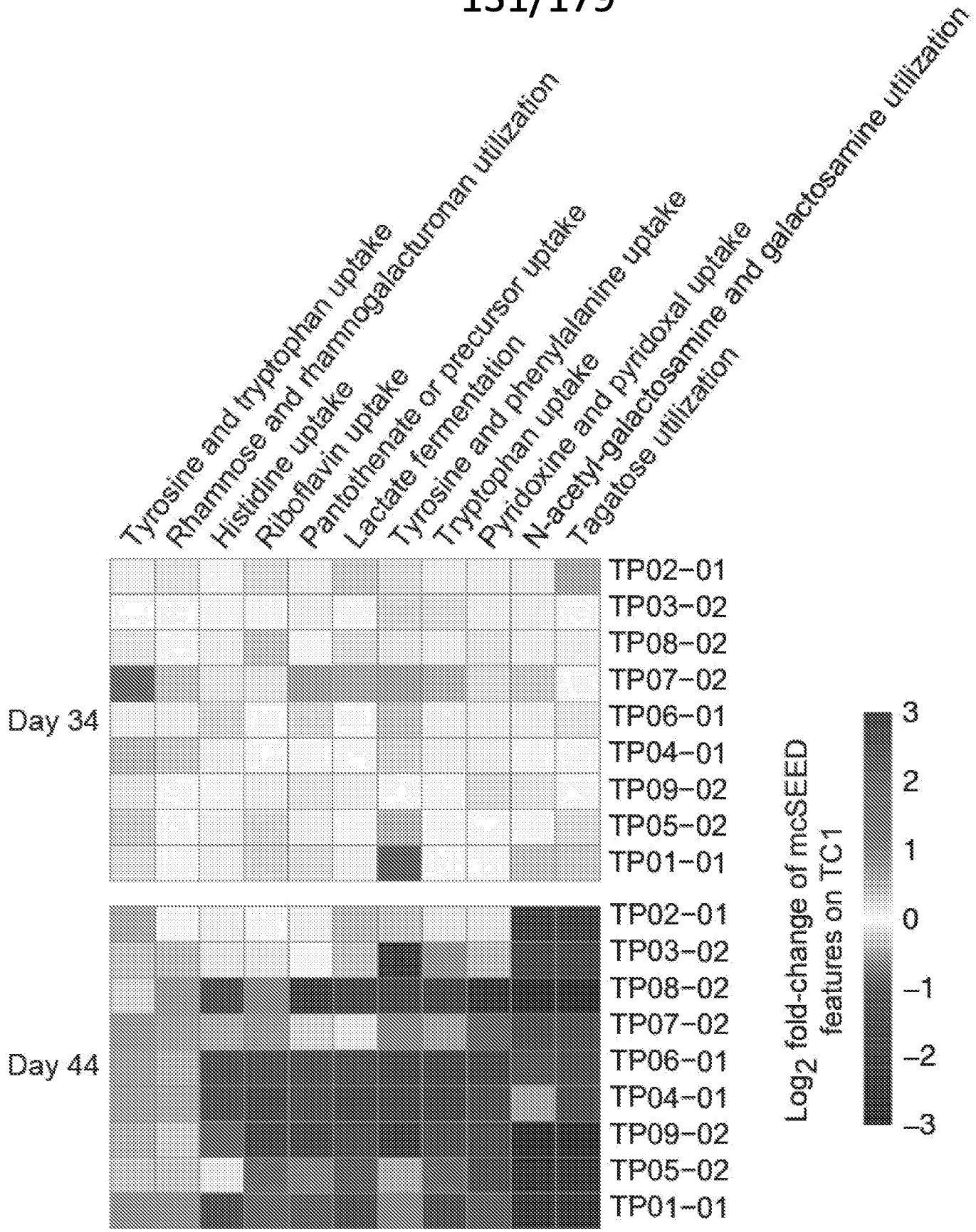

Tyrosine and tryptophan uptake
Rhamnose and rhamnogalacturonan utilization
Histidine uptake
Riboflavin uptake
Pantothenate or precursor uptake
Lactate fermentation
Tyrosine and phenylalanine uptake
Tryptophan uptake
Pyridoxine and pyridoxal uptake
N-acetyl-galactosamine and galactosamine utilization
Tagatose utilization
Day 34
TP02-01
TP03-02
TP08-02
TP07-02
TP06-01
TP04-01
TP09-02
TP05-02
TP01-01
Day 44
TP02-01
TP03-02
TP08-02
TP07-02
TP06-01
TP04-01
TP09-02
TP05-02
TP01-01
Log2 fold-change of mcSEED features on TC1
3
2
1
0
−1
−2
−3

FIG. 37H

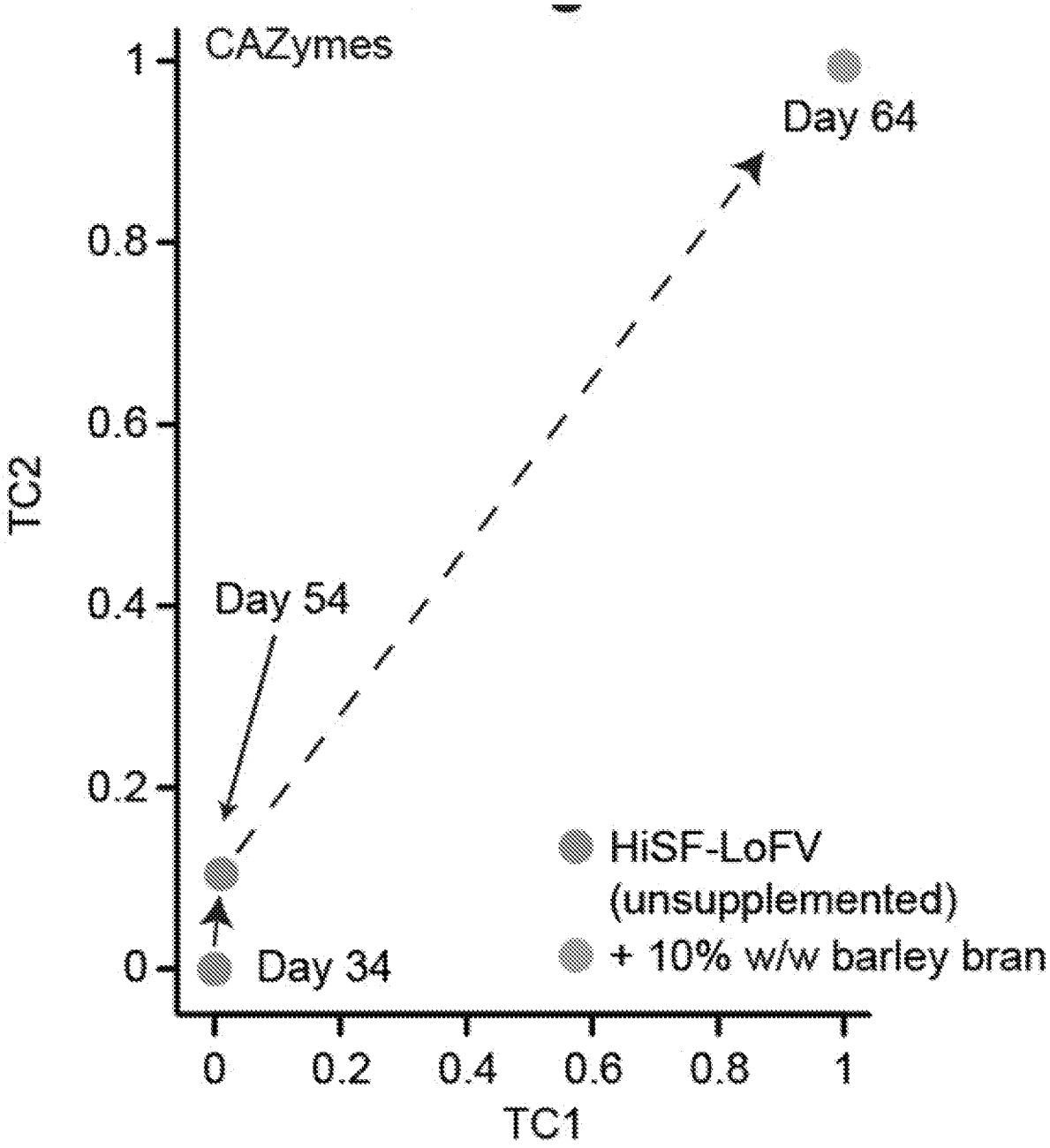

CAZymes
Day 64
Day 54
Day 34
HiSF-LoFV
(unsupplemented)
+ 10% w/w barley bran
TC2
TC1
0
0.2
0.4
0.6
0.8
1

FIG. 38A

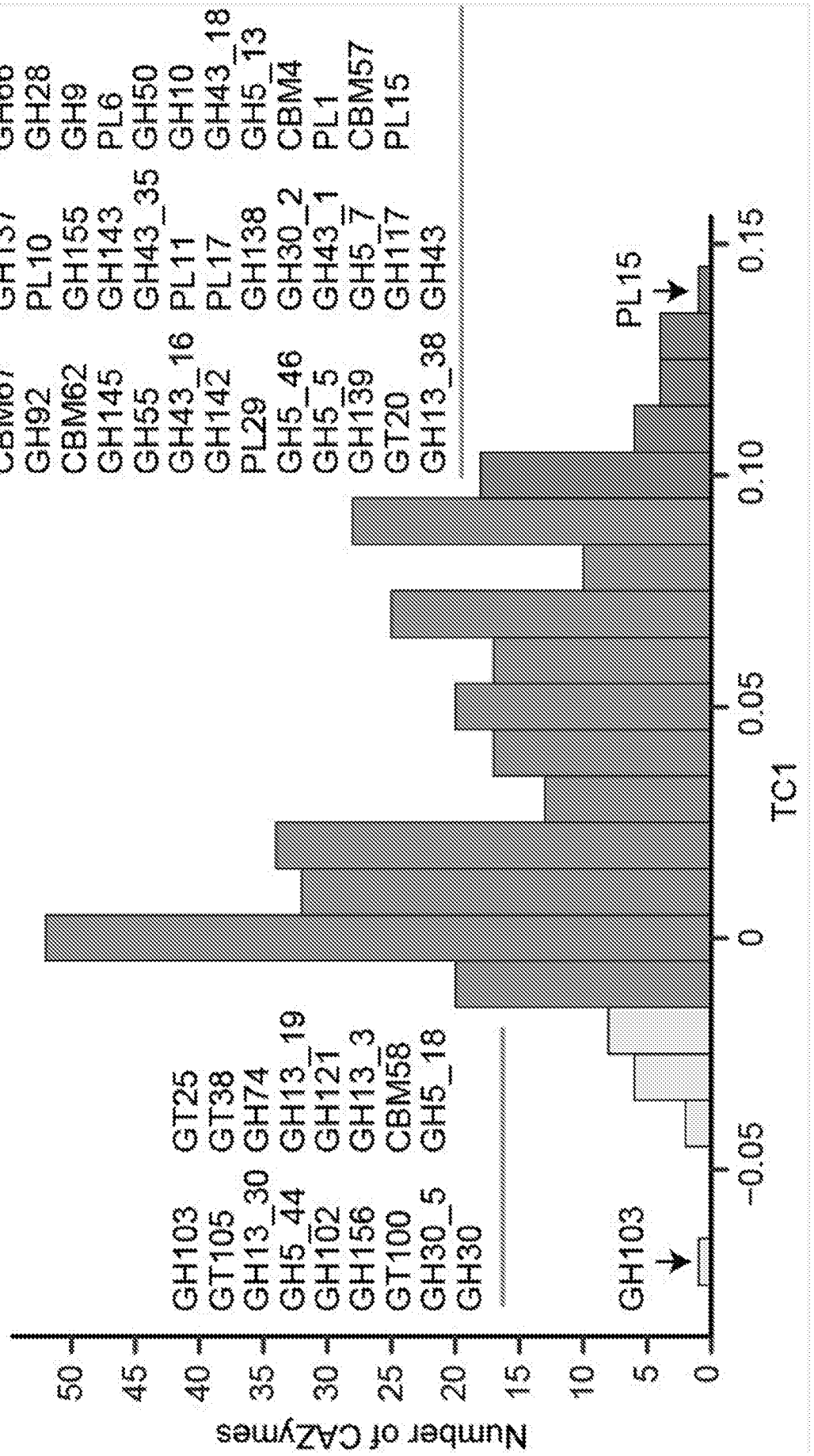

Number of CAZymes
0
5
10
15
20
25
30
35
40
45
50
GH103
GT105
GH13_30
GH5_44
GH102
GH156
GT100
GH30_5
GH30_
GT25
GT38
GH74
GH13_19
GH121_
GH13_3
CBM58
GH5_18
GH43_12
CBM67
GH92
CBM62
GH145
GH55
GH43_16
GH142
PL29
GH5_46
GH5_5
GH139
GT20
GH13_38
PL13
GH137
PL10
GH155
GH143
GH43_35
PL11
PL17
GH138
GH30_2
GH43_1
GH5_7
GH117
GH43
GH30_3
GH66
GH28
GH9
PL6
GH50
GH10
GH43_18
GH5_13
CBM4
PL1
CBM57
PL15
GH103
PL15
−0.05
0
0.05
0.10
0.15
TC1

FIG. 38B

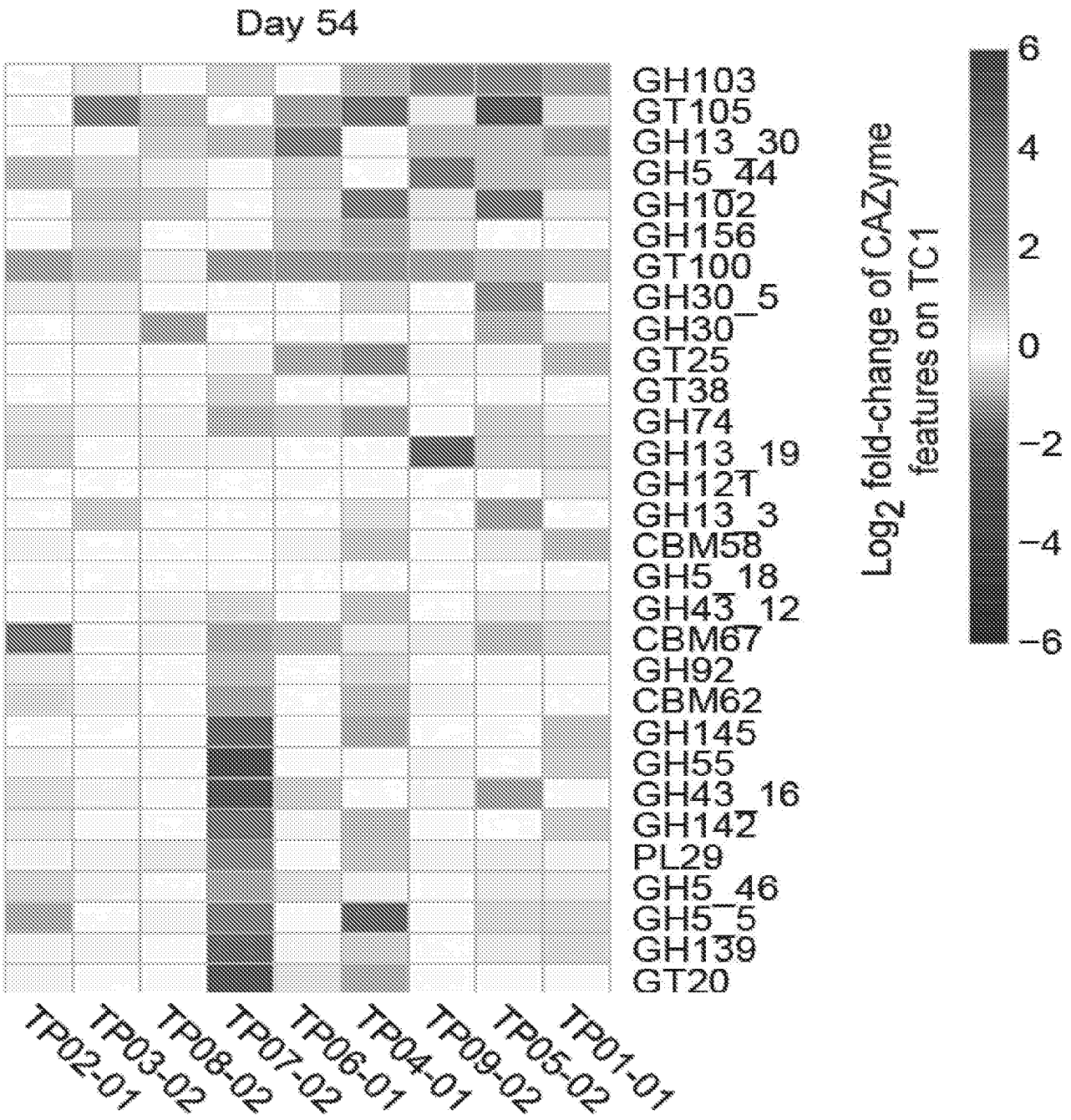

Day 54
GH103
GT105
GH13_30
GH5_44
GH102
GH156
GT100
GH30_5
GH30
GT25
GT38
GH74
GH13_19
GH121
GH13_3
CBM58
GH5_18
GH43_12
CBM67
GH92
CBM62
GH145
GH55
GH43_16
GH142
PL29
GH5_46
GH5_5
GH139
GT20
TP02-01
TP03-02
TP08-02
TP07-02
TP06-01
TP04-01
TP09-02
TP05-02
TP01-01
Log2 fold-change of CAZyme features on TC1
6
4
2
0
−2
−4
−6

FIG. 38C

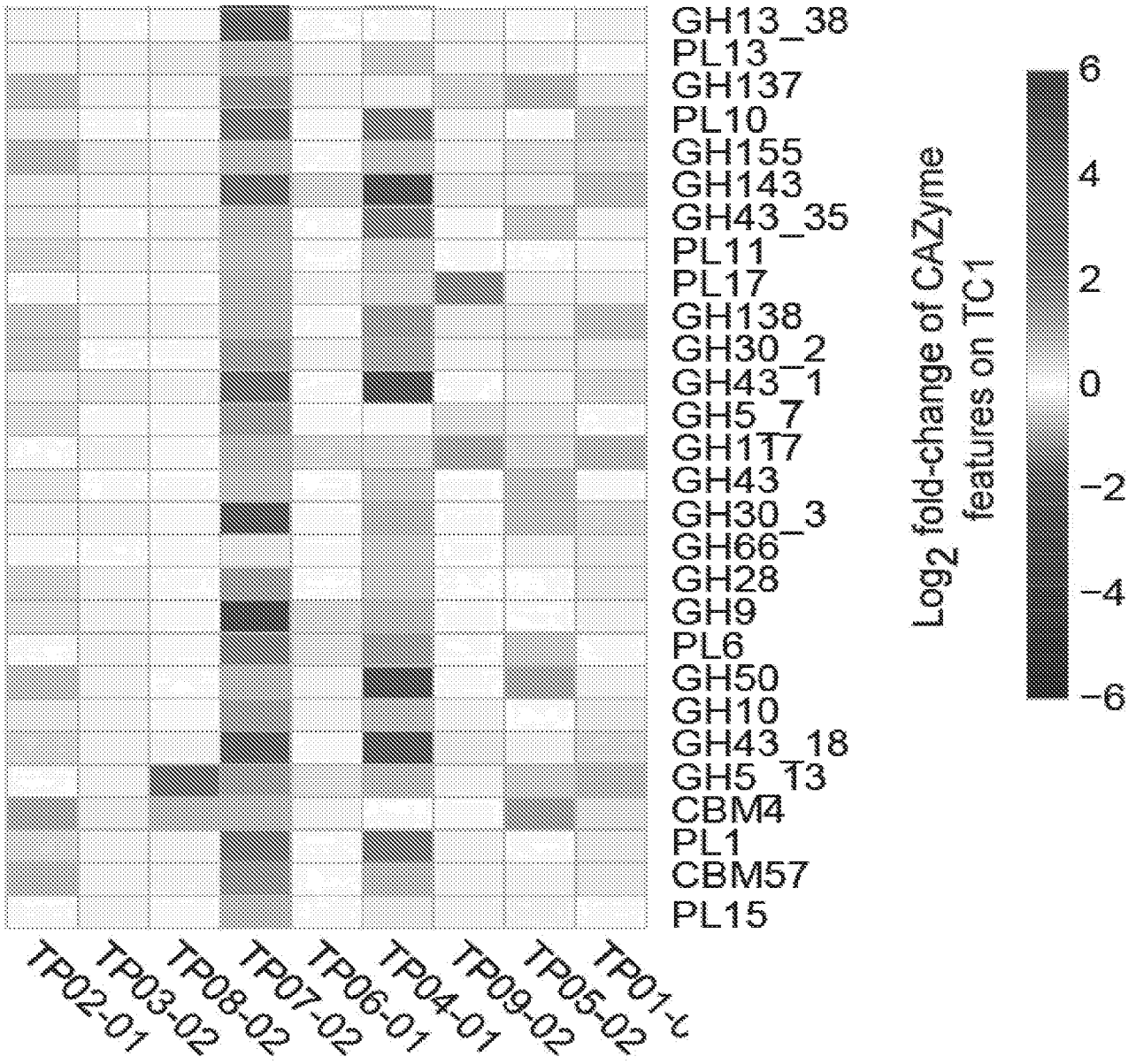

GH13_38
PL13
GH137
PL10
GH155
GH143
GH43_35
PL11
PL17
GH138
GH30_2
GH43_1
GH5_7
GH117
GH43
GH30_3
GH66
GH28
GH9
PL6
GH50
GH10
GH43_18
GH5_73
CBM4
PL1
CBM57
PL15
TP02-01
TP03-02
TP08-02
TP07-02
TP06-01
TP04-01
TP09-02
TP05-02
TP01-01
Log2 fold-change of CAZyme features on TC1
6
4
2
0
−2
−4
−6

FIG. 38D

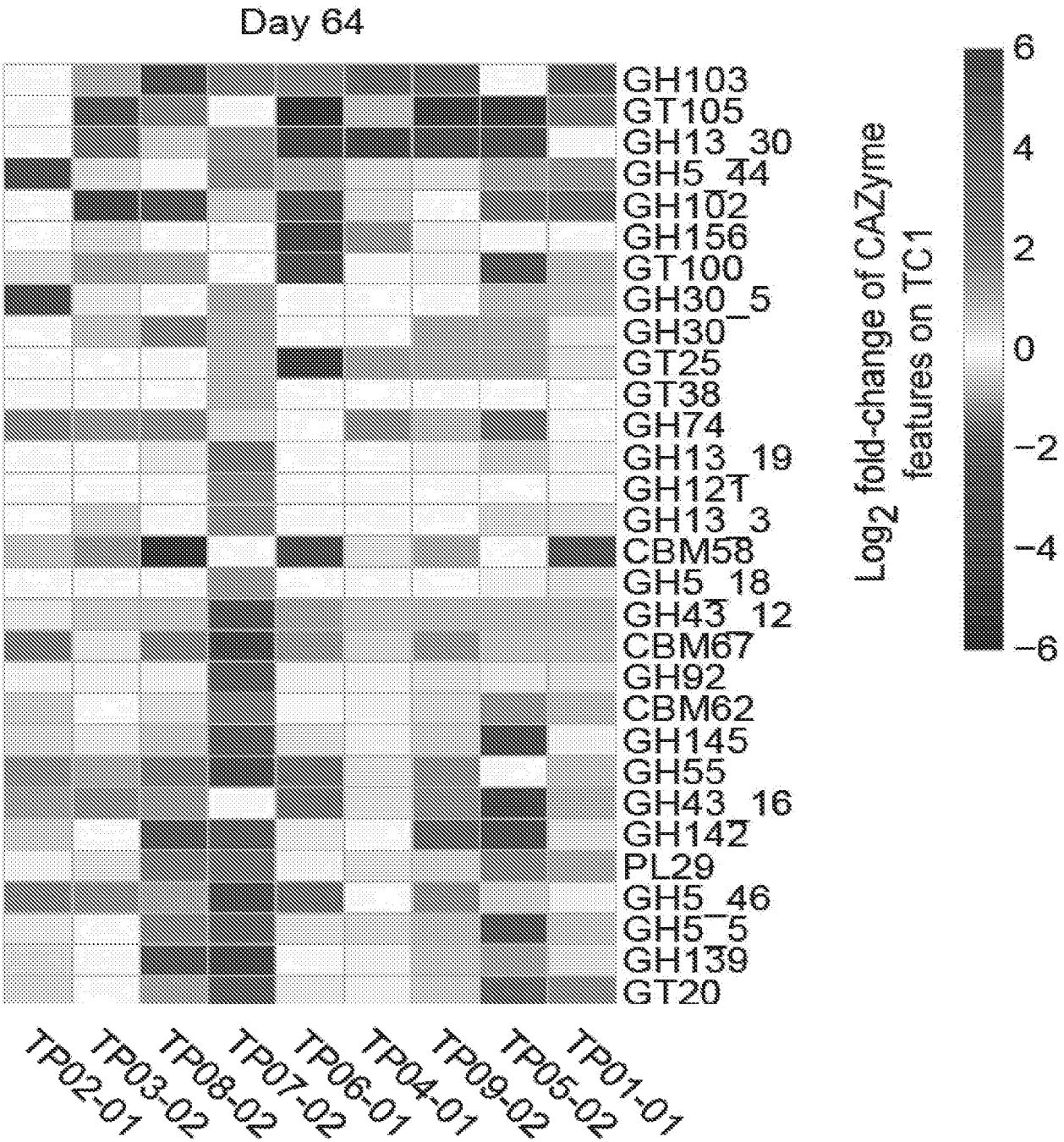

Day 64
GH103
GT105
GH13_30
GH5_44
GH102
GH156
GT100
GH30_5
GH30
GT25
GT38
GH74
GH13_19
GH121
GH13_3
CBM58
GH5_18
GH43_12
CBM67
GH92
CBM62
GH145
GH55
GH43_16
GH142
PL29
GH5_46
GH5_5
GH139
GT20
TP02-01
TP03-02
TP08-02
TP07-02
TP06-01
TP04-01
TP09-02
TP05-02
TP01-01
Log2 fold-change of CAZyme features on TC1
6
4
2
0
−2
−4
−6

FIG. 38E

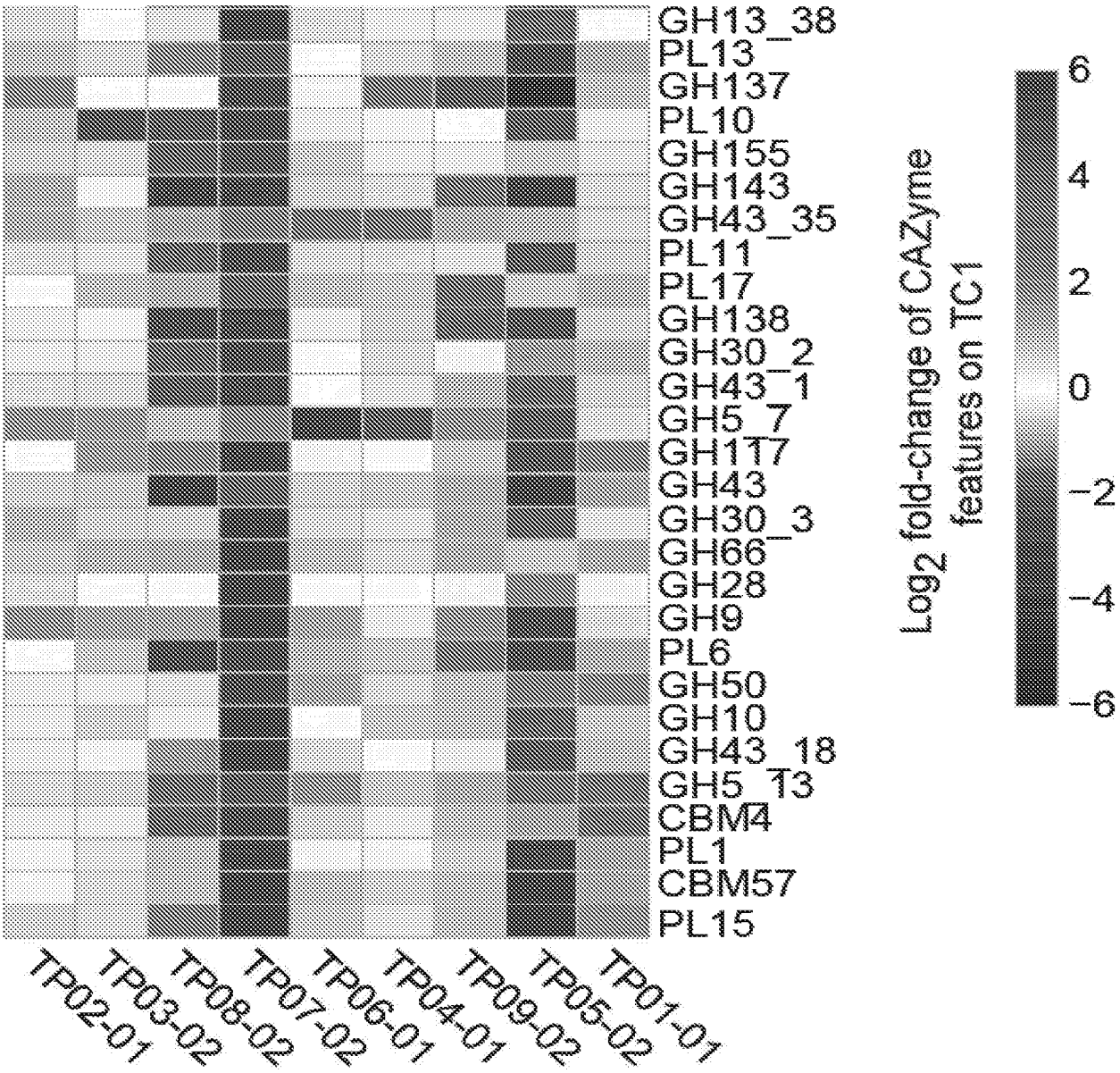

GH13_38
PL13
GH137
PL10
GH155
GH143
GH43_35
PL11
PL17
GH138
GH30_2
GH43_1
GH5_7
GH117
GH43
GH30_3
GH66
GH28
GH9
PL6
GH50
GH10
GH43_18
GH5_13
CBM4
PL1
CBM57
PL15
TP02-01
TP03-02
TP08-02
TP07-02
TP06-01
TP04-01
TP09-02
TP05-02
TP01-01
Log2 fold-change of CAZyme features on TC1
6
4
2
0
−2
−4
−6

FIG. 38F

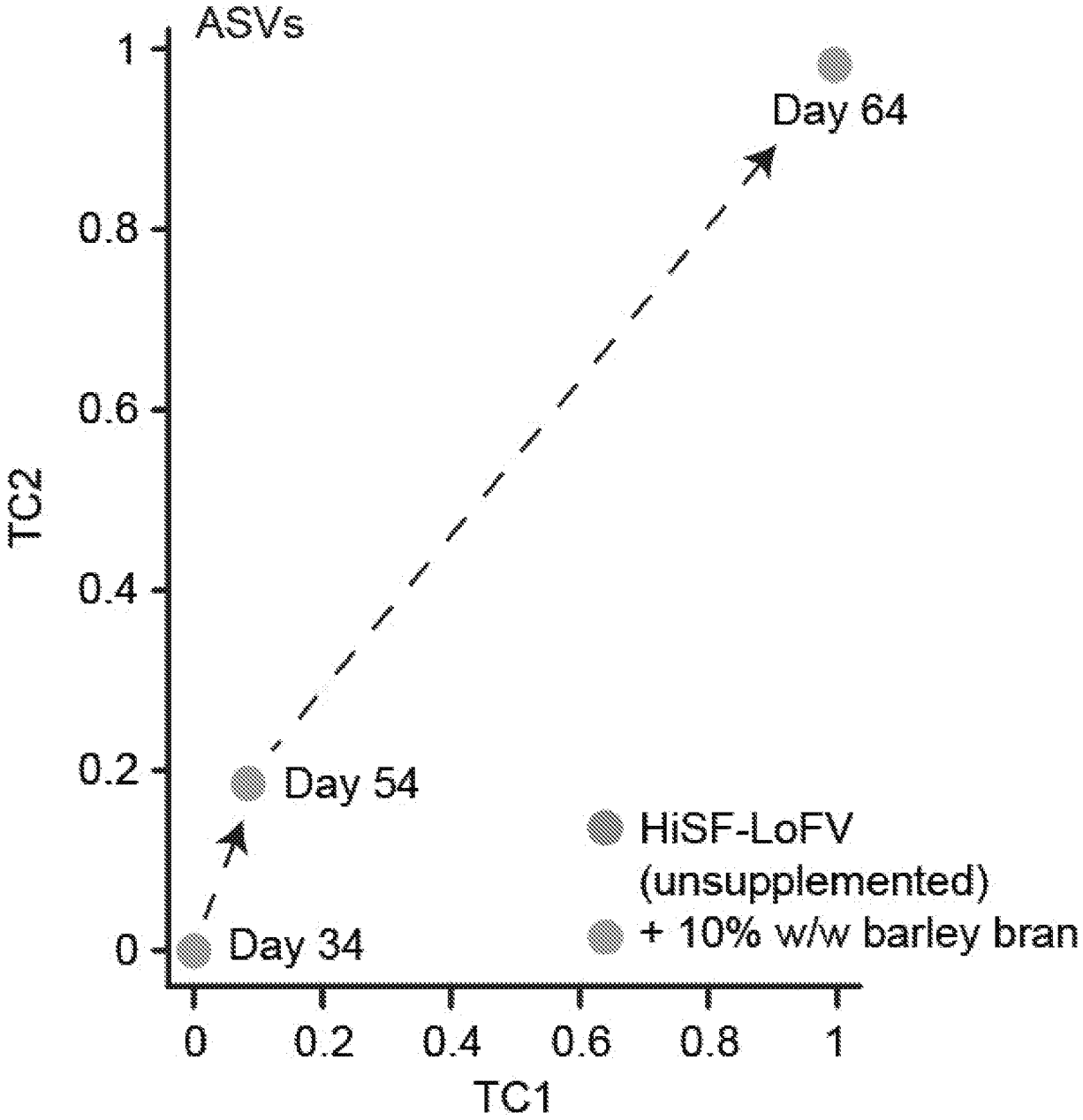

ASVs
1
0.8
0.6
0.4
0.2
0
TC2
Day 64
Day 54
Day 34
HiSF-LoFV
(unsupplemented)
+ 10% w/w barley bran
0
0.2
0.4
0.6
0.8
1
TC1

FIG. 39A

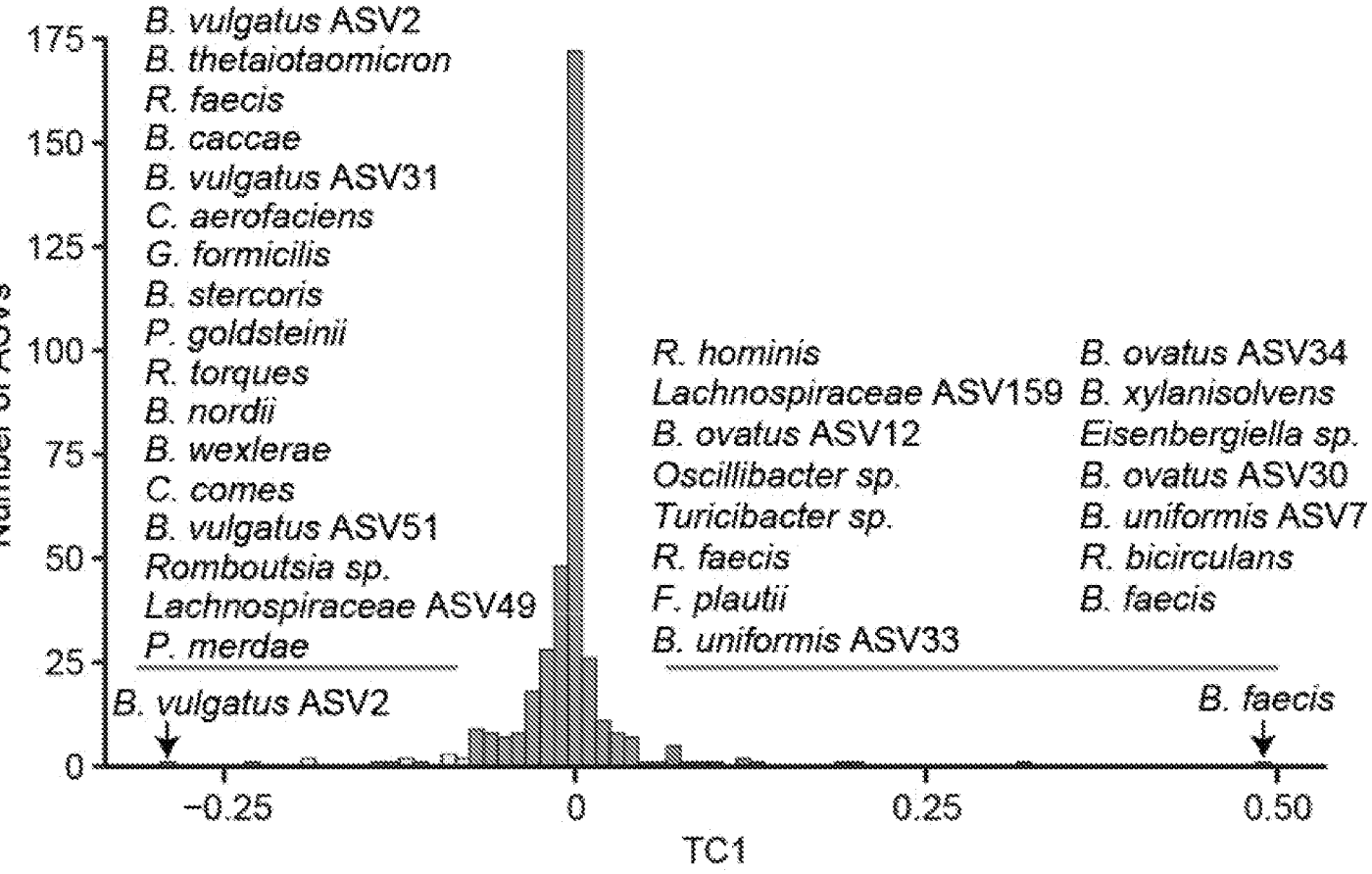

B. vulgatus ASV2
B. thetaiotaomicron
R. faecis
B. caccae
B. vulgatus ASV31
C. aerofaciens
G. formicilis
B. stercoris
P. goldsteinii
R. torques
B. nordii
B. wexlerae
C. comes
B. vulgatus ASV51
Romboutsia sp.
Lachnospiraceae ASV49
P. merdae
B. vulgatus ASV2
R. hominis
Lachnospiraceae ASV159
B. ovatus ASV12
Oscillibacter sp.
Turicibacter sp.
R. faecis
F. plautii
B. uniformis ASV33
B. ovatus ASV34
B. xylanisolvens
Eisenbergiella sp.
B. ovatus ASV30
B. uniformis ASV7
R. bicirculans
B. faecis
B. faecis
175
150
125
100
75
50
25
0
−0.25
0
0.25
0.50
TC1

FIG. 39B

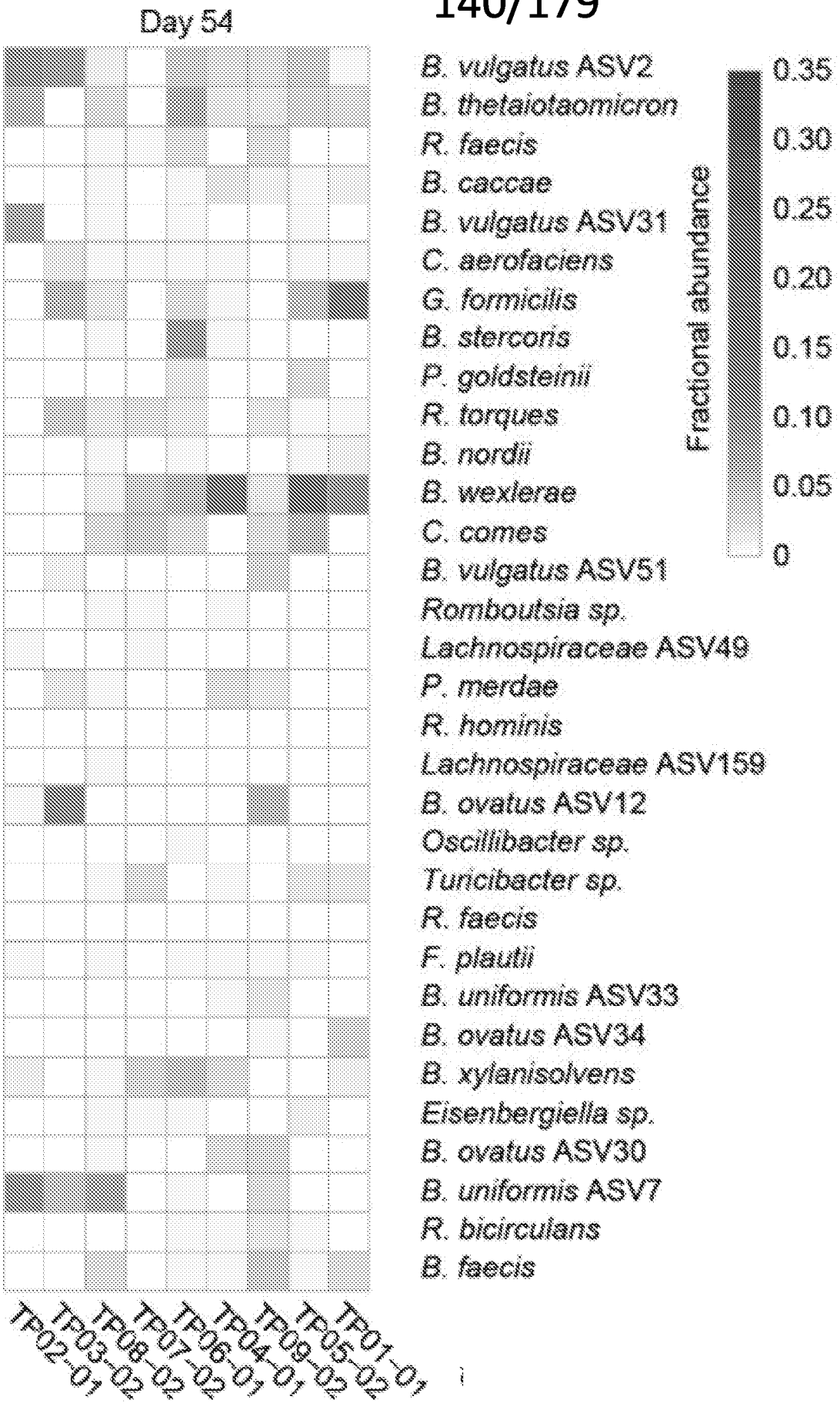

Day 54
B. vulgatus ASV2
B. thetaiotaomicron
R. faecis
B. caccae
B. vulgatus ASV31
C. aerofaciens
G. formicilis
B. stercoris
P. goldsteinii
R. torques
B. nordii
B. wexlerae
C. comes
B. vulgatus ASV51
Romboutsia sp.
Lachnospiraceae ASV49
P. merdae
R. hominis
Lachnospiraceae ASV159
B. ovatus ASV12
Oscillibacter sp.
Tuncibacter sp.
R. faecis
F. plautii
B. uniformis ASV33
B. ovatus ASV34
B. xylanisolvens
Eisenbergiella sp.
B. ovatus ASV30
B. uniformis ASV7
R. bicirculans
B. faecis
Fractional abundance
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
TP02-01
TP03-02
TP08-02
TP07-02
TP06-01
TP04-01
TP09-02
TP05-02
TP01-01

FIG. 39C

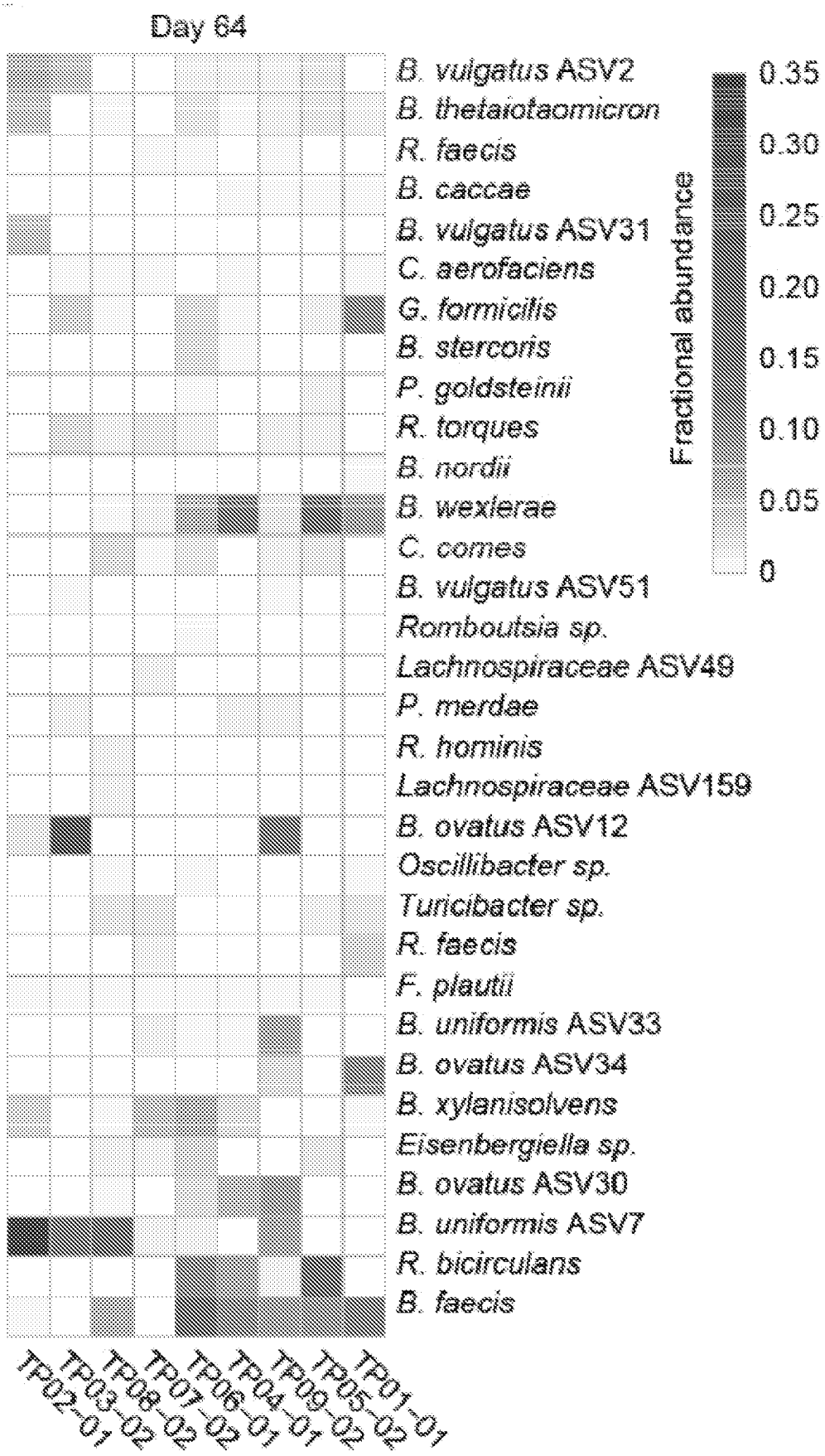

Day 64
B. vulgatus ASV2
B. thetaiotaomicron
R. faecis
B. caccae
B. vulgatus ASV31
C. aerofaciens
G. formicilis
B. stercoris
P. goldsteinii
R. torques
B. nordii
B. wexlerae
C. comes
B. vulgatus ASV51
Romboutsia sp.
Lachnospiraceae ASV49
P. merdae
R. hominis
Lachnospiraceae ASV159
B. ovatus ASV12
Oscillibacter sp.
Turicibacter sp.
R. faecis
F. plautii
B. uniformis ASV33
B. ovatus ASV34
B. xylanisolvens
Eisenbergiella sp.
B. ovatus ASV30
B. uniformis ASV7
R. bicirculans
B. faecis
Fractional abundance
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
TP02_01
TP03_02
TP08_02
TP07_02
TP06_01
TP04_01
TP09_02
TP05_02
TP01_01

FIG. 39D

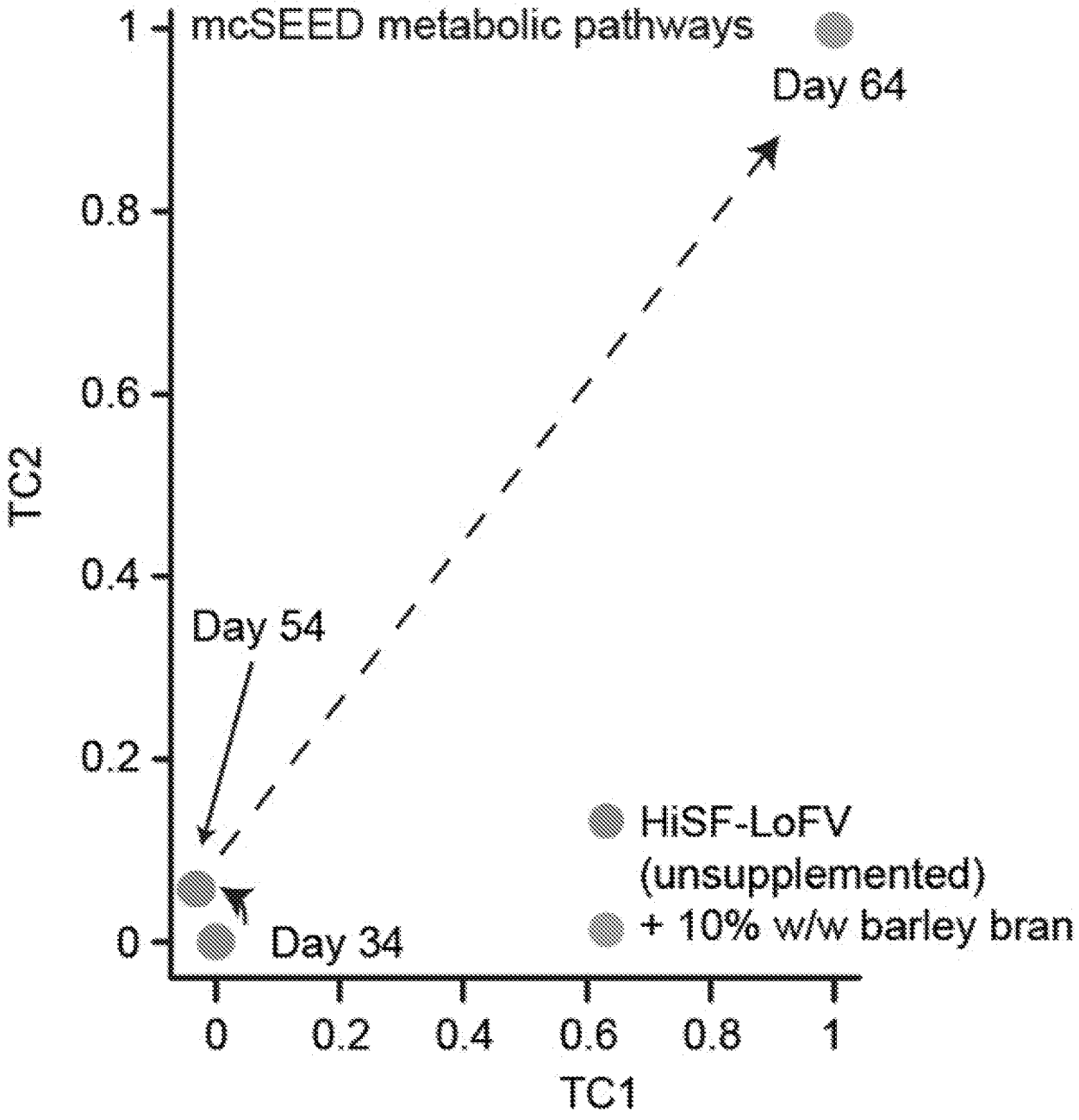

mcSEED metabolic pathways
Day 64
Day 54
Day 34
HiSF-LoFV
(unsupplemented)
+ 10% w/w barley bran
TC2
TC1
0
0.2
0.4
0.6
0.8
1

FIG. 39E

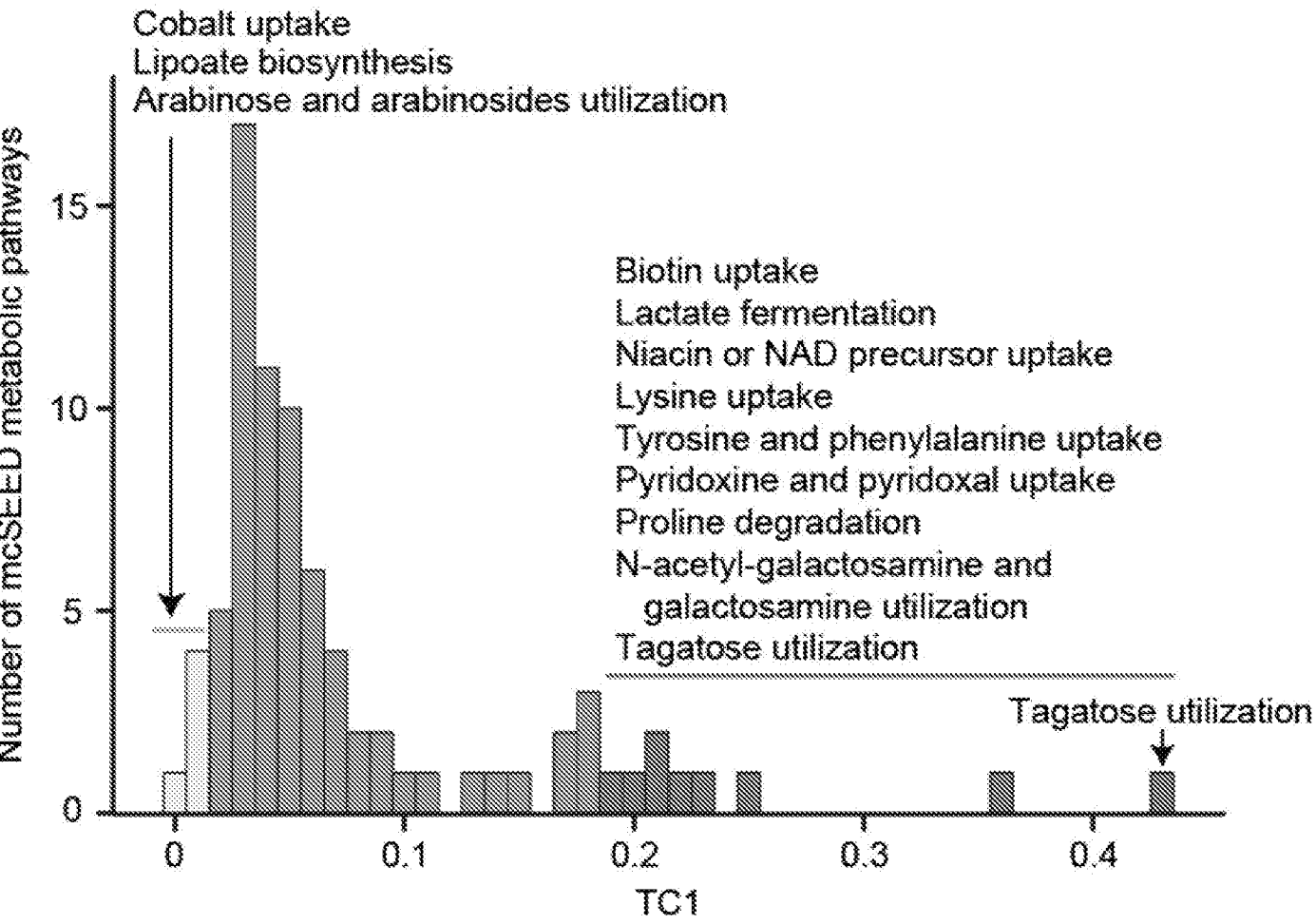

Cobalt uptake
Lipoate biosynthesis
Arabinose and arabinosides utilization
Biotin uptake
Lactate fermentation
Niacin or NAD precursor uptake
Lysine uptake
Tyrosine and phenylalanine uptake
Pyridoxine and pyridoxal uptake
Proline degradation
N-acetyl-galactosamine and
galactosamine utilization
Tagatose utilization
Tagatose utilization
Number of mcSEED metabolic pathways
15
10
5
0
0
0.1
0.2
0.3
0.4
TC1

FIG. 39F

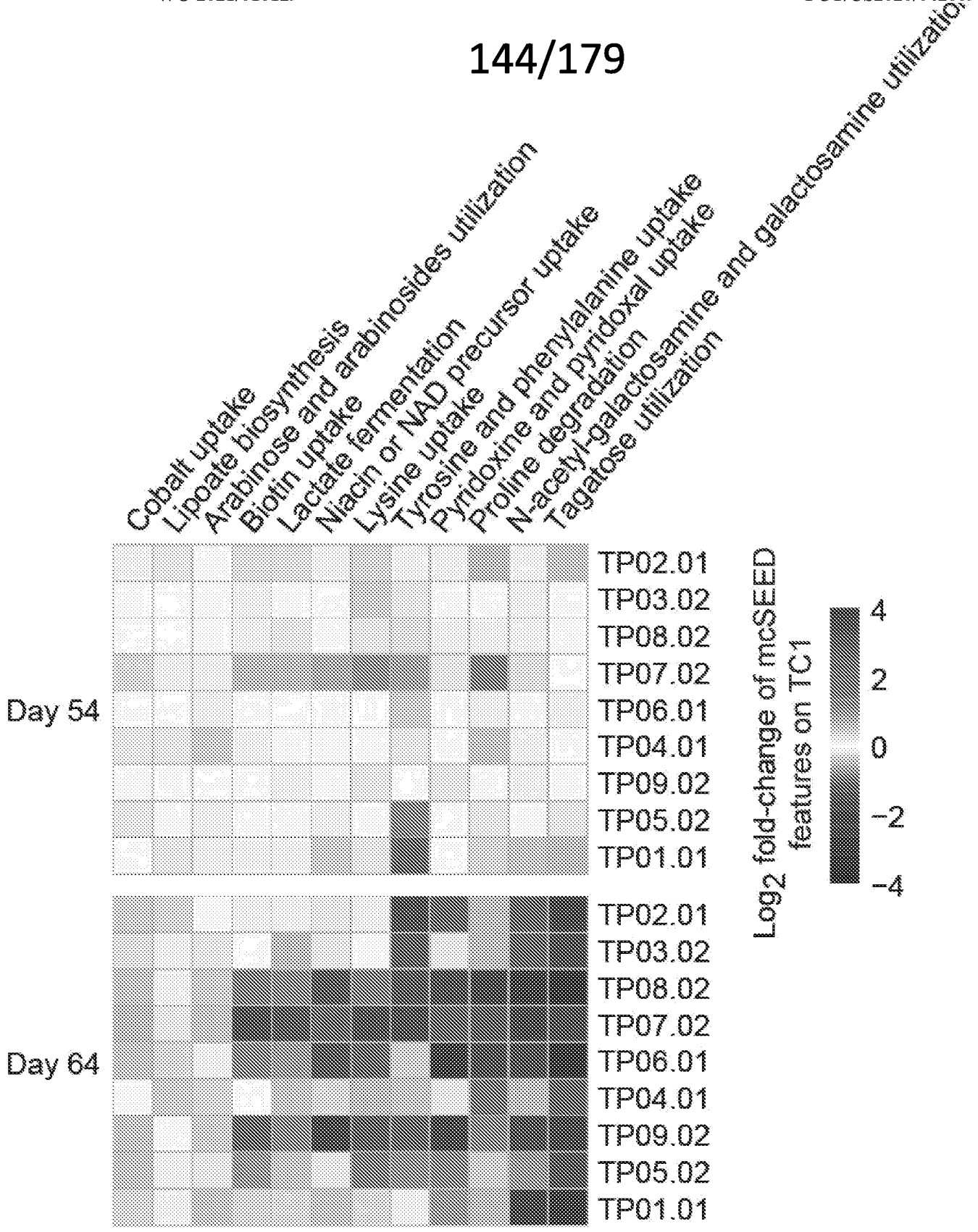

Cobalt uptake
Lipoate biosynthesis
Arabinose and arabinosides utilization
Biotin uptake
Lactate fermentation
Niacin or NAD precursor uptake
Lysine uptake
Tyrosine and phenylalanine uptake
Pyridoxine and pyridoxal uptake
Proline degradation
N-acetyl-galactosamine and galactosamine utilization
Tagatose utilization
Day 54
Day 64
TP02.01
TP03.02
TP08.02
TP07.02
TP06.01
TP04.01
TP09.02
TP05.02
TP01.01
Log2 fold-change of mcSEED features on TC1
4
2
0
−2
−4

FIG. 39G

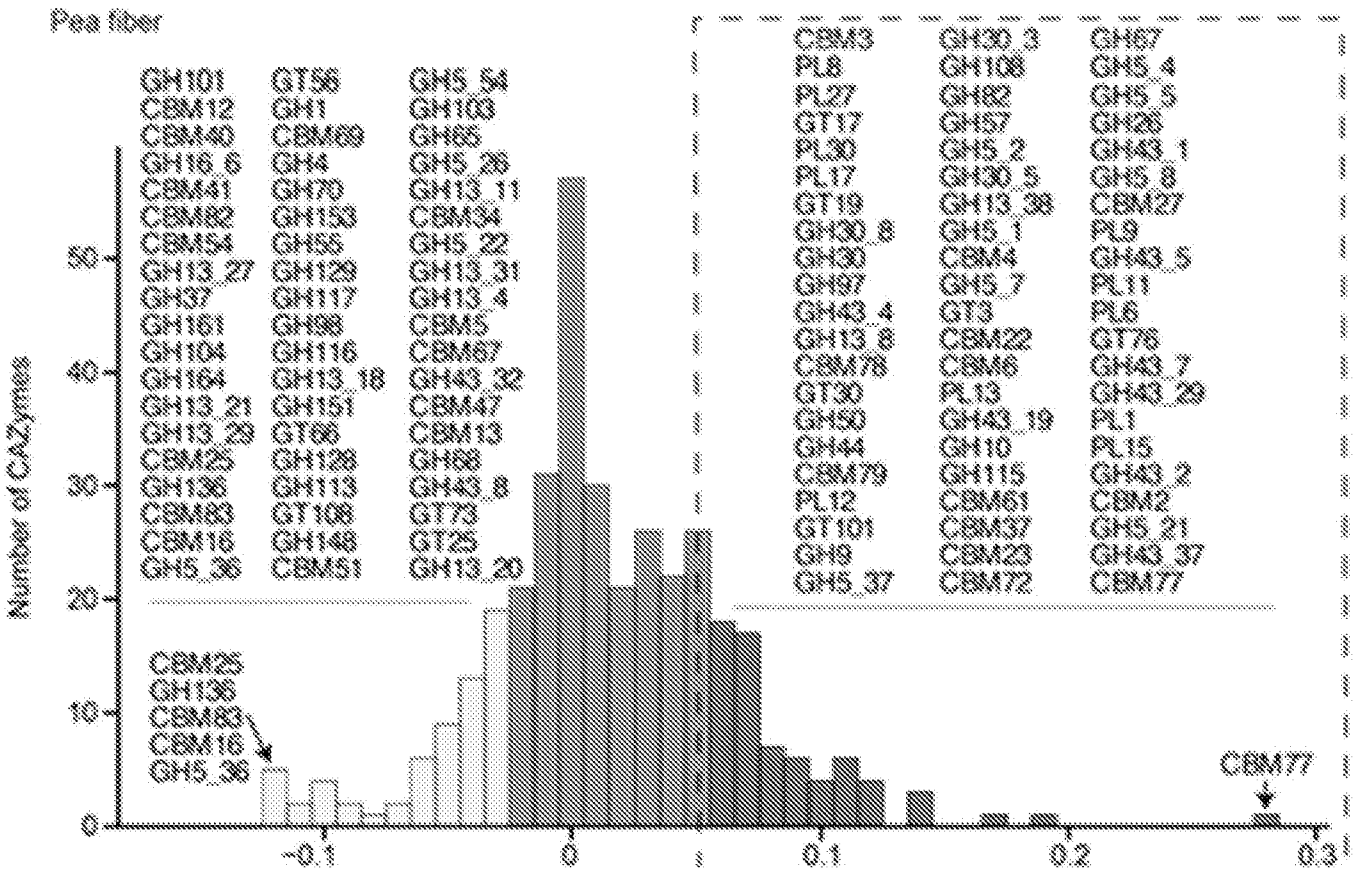

Pea fiber
Number of CAZymes
GH101
CBM12
CBM40
GH16_6
CBM41
CBM82
CBM54
GH13_27
GH37
GH161
GH104
GH164
GH13_21
GH13_29
CBM25
GH136
CBM83
CBM16
GH5_36
GT56
GH1
CBM69
GH4
GH70
GH153
GH55
GH129
GH117
GH98
GH116
GH13_18
GH151
GT66
GH128
GH113
GT108
GH148
CBM51
GH5_54
GH103
GH65
GH5_26
GH13_11
CBM34
GH5_22
GH13_31
GH13_4
CBM5
CBM67
GH43_32
CBM47
CBM13
GH68
GH43_8
GT73
GT25
GH13_20
CBM3
PL8
PL27
GT17
PL30
PL17
GT19
GH30_8
GH30
GH97
GH43_4
GH13_8
CBM78
GT30
GH50
GH44
CBM79
PL12
GT101
GH9
GH5_37
GH30_3
GH108
GH62
GH57
GH5_2
GH30_5
GH13_38
GH5_1
CBM4
GH5_7
GT3
CBM22
CBM6
PL13
GH43_19
GH10
GH115
CBM61
CBM37
CBM23
CBM72
GH67
GH5_4
GH5_5
GH26
GH43_1
GH5_8
CBM27
PL9
GH43_5
PL11
PL6
GT76
GH43_7
GH43_29
PL1
PL15
GH43_2
CBM2
GH5_21
GH43_37
CBM77
CBM25
GH136
CBM83
CBM16
GH5_36
CBM77
0
10
20
30
40
50
-0.1
0
0.1
0.2
0.3

FIG. 40A

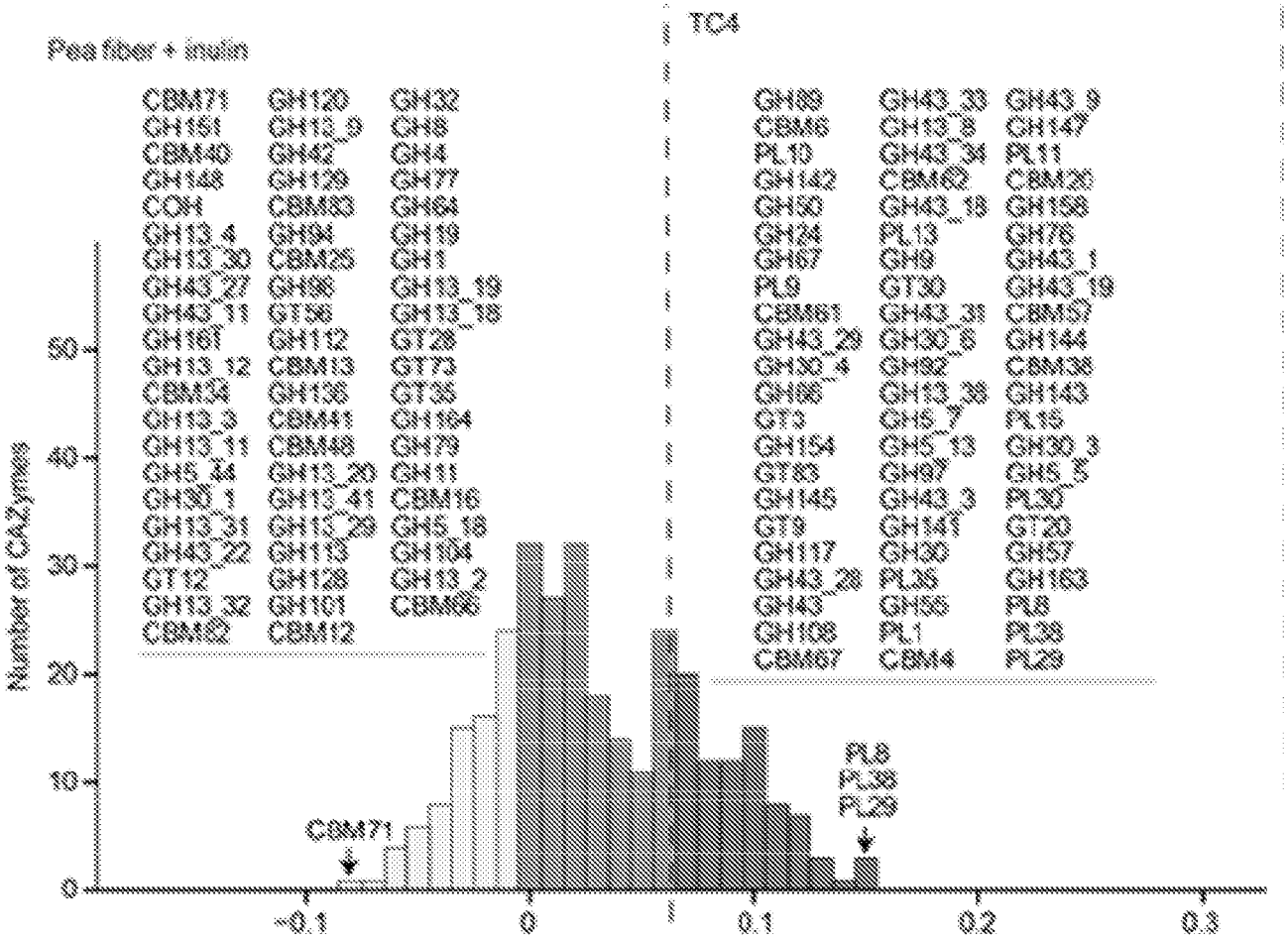

Pea fiber + inulin
TC4
Number of CAZymes
0
10
20
30
40
50
-0.1
0
0.1
0.2
0.3
CBM71
PL9
PL38
PL29
CBM71 GH120 GH32
GH151 GH13_9 GH8
CBM40 GH42 GH4
GH148 GH129 GH77
COH CBM83 GH64
GH13_4 GH94 GH19
GH13_30 CBM25 GH1
GH43_27 GH96 GH13_19
GH43_11 GT56 GH13_18
GH16T GH112 GT28
GH13_12 CBM13 GT73
CBM34 GH136 GT35
GH13_3 CBM41 GH164
GH13_11 CBM48 GH79
GH5_44 GH13_20 GH11
GH30_1 GH13_41 CBM16
GH13_31 GH13_29 GH5_18
GH43_22 GH113 GH104
GT12 GH128 GH13_2
GH13_32 GH101 CBM66
CBM62 CBM12
GH88 GH43_33 GH43_9
CBM6 GH13_8 GH147
PL10 GH43_34 PL11
GH142 CBM62 CBM26
GH50 GH43_19 GH158
GH24 PL13 GH78
GH67 GH9 GH43_1
PL9 GT30 GH43_19
CBM61 GH43_31 CBM57
GH43_29 GH30_6 GH144
GH30_4 GH92 CBM36
GH66 GH13_38 GH143
GT3 GH5_7 PL15
GH154 GH5_13 GH30_3
GT83 GH97 GH5_5
GH145 GH43_3 PL30
GT9 GH141 GT20
GH117 GH30 GH57
GH43_28 PL35 GH163
GH43 GH56 PL8
GH106 PL1 PL39
CBM67 CBM14 PL29

FIG. 40B

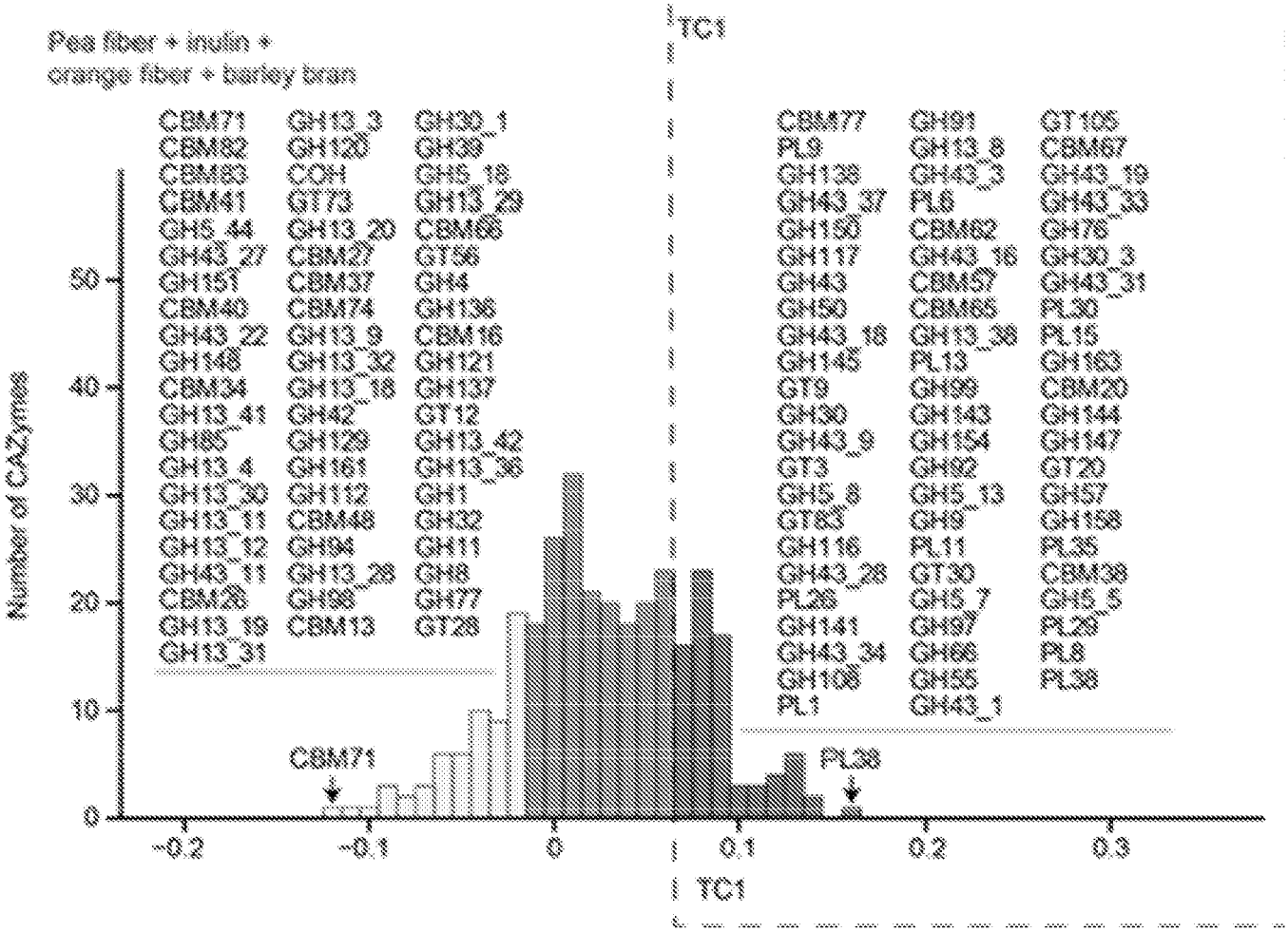

Pea fiber + inulin +
orange fiber + barley bran
TC1
CBM71
CBM82
CBM83
CBM41
GH5_44
GH43_27
GH151
CBM40
GH43_22
GH148
CBM34
GH13_41
GH85
GH13_4
GH13_30
GH13_11
GH13_12
GH43_11
CBM28
GH13_19
GH13_31
GH13_3
GH120
COH
GT73
GH13_20
CBM27
CBM37
CBM74
GH13_9
GH13_32
GH13_18
GH42
GH129
GH161
GH112
CBM48
GH94
GH13_28
GH98
CBM13
GH30_1
GH39
GH5_18
GH13_29
CBM66
GT56
GH4
GH136
CBM16
GH121
GH137
GT12
GH13_42
GH13_36
GH1
GH32
GH11
GH8
GH77
GT28
CBM77
PL9
GH138
GH43_37
GH150
GH117
GH43
GH50
GH43_18
GH145
GT9
GH30
GH43_9
GT3
GH5_8
GT83
GH116
GH43_28
PL26
GH141
GH43_34
GH108
PL1
GH91
GH13_8
GH43_3
PL6
CBM62
GH43_16
CBM57
CBM65
GH13_38
PL13
GH99
GH143
GH154
GH92
GH5_13
GH9
PL11
GT30
GH5_7
GH97
GH68
GH55
GH43_1
GT105
CBM67
GH43_19
GH43_33
GH76
GH30_3
GH43_31
PL30
PL15
GH163
CBM20
GH144
GH147
GT20
GH57
GH158
PL35
CBM39
GH5_5
PL29
PL8
PL38
Number of CAZymes
0
10
20
30
40
50
-0.2
-0.1
0
0.1
0.2
0.3
CBM71
PL38
TC1

FIG. 40C

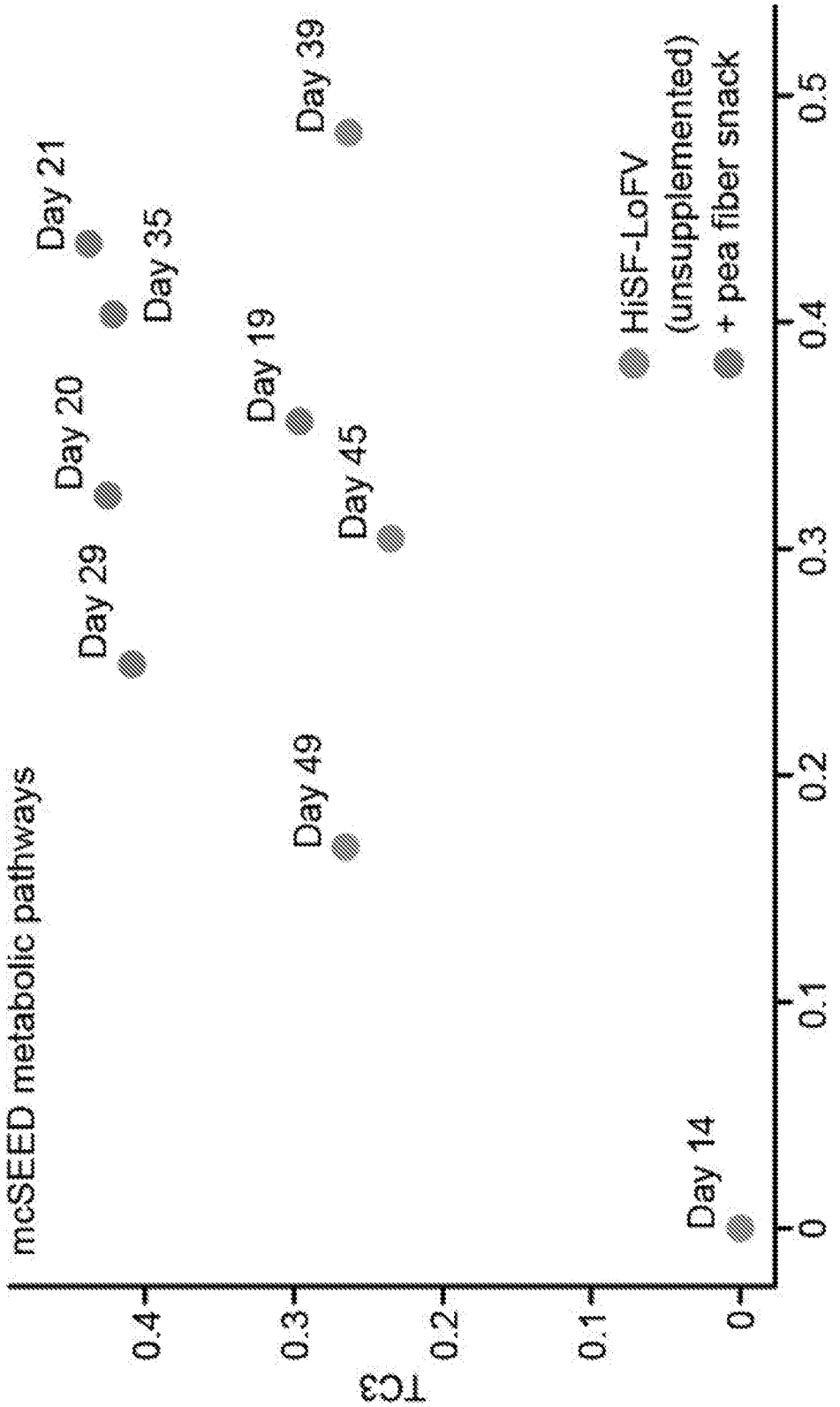

mcSEED metabolic pathways
PC3
PC1
0
0.1
0.2
0.3
0.4
0.5
Day 14
Day 21
Day 35
Day 39
Day 20
Day 19
Day 45
Day 29
Day 49
HiSF-LoFV (unsupplemented)
+ pea fiber snack

FIG. 41A

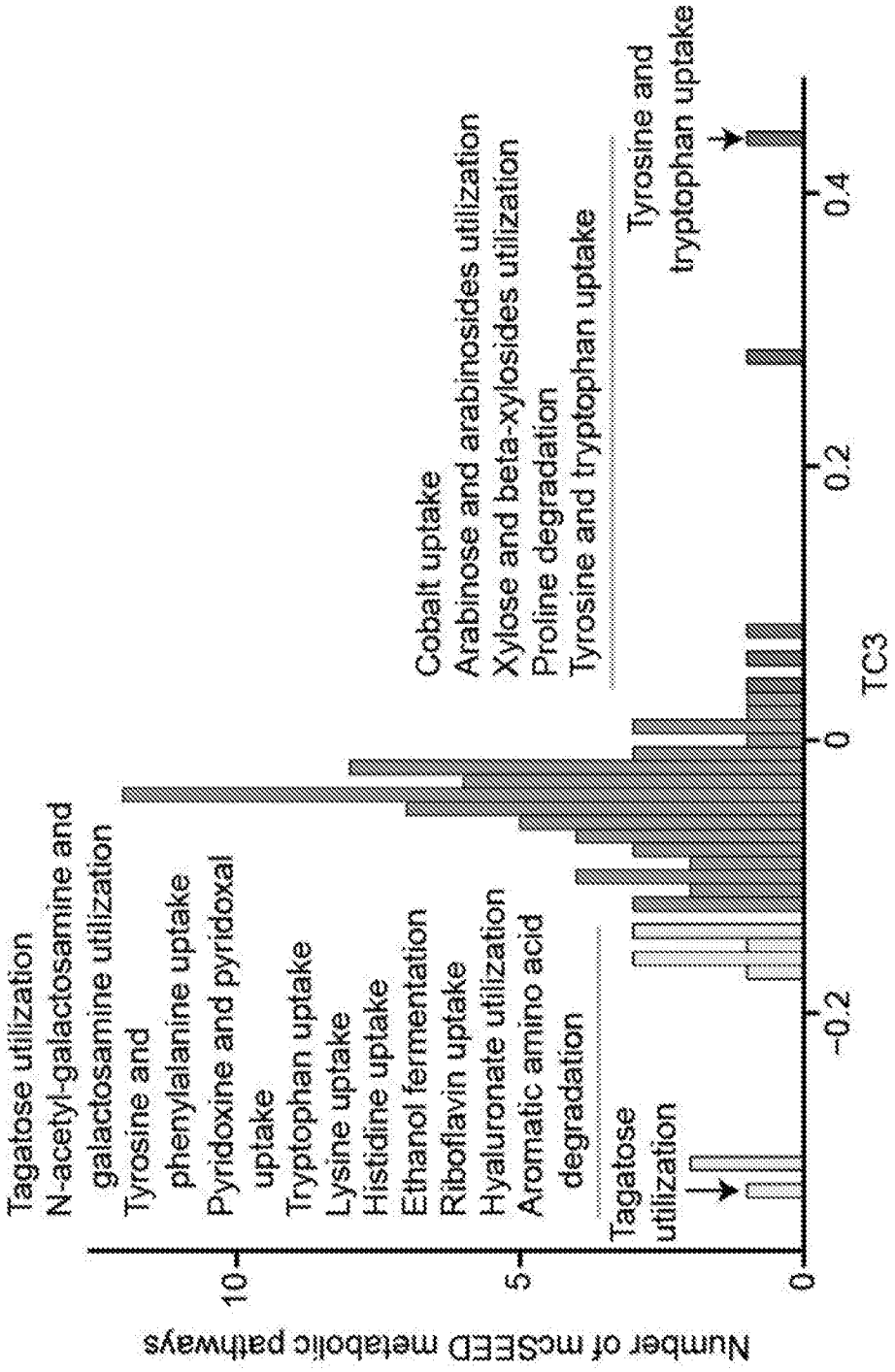

Number of mCSEED metabolic pathways
0
5
10
−0.2
0
0.2
0.4
TC3
Tagatose utilization
N-acetyl-galactosamine and galactosamine utilization
Tyrosine and phenylalanine uptake
Pyridoxine and pyridoxal uptake
Tryptophan uptake
Lysine uptake
Histidine uptake
Ethanol fermentation
Riboflavin uptake
Hyaluronate utilization
Aromatic amino acid degradation
Tagatose utilization
Cobalt uptake
Arabinose and arabinosides utilization
Xylose and beta-xylosides utilization
Proline degradation
Tyrosine and tryptophan uptake
Tyrosine and tryptophan uptake

FIG. 41B

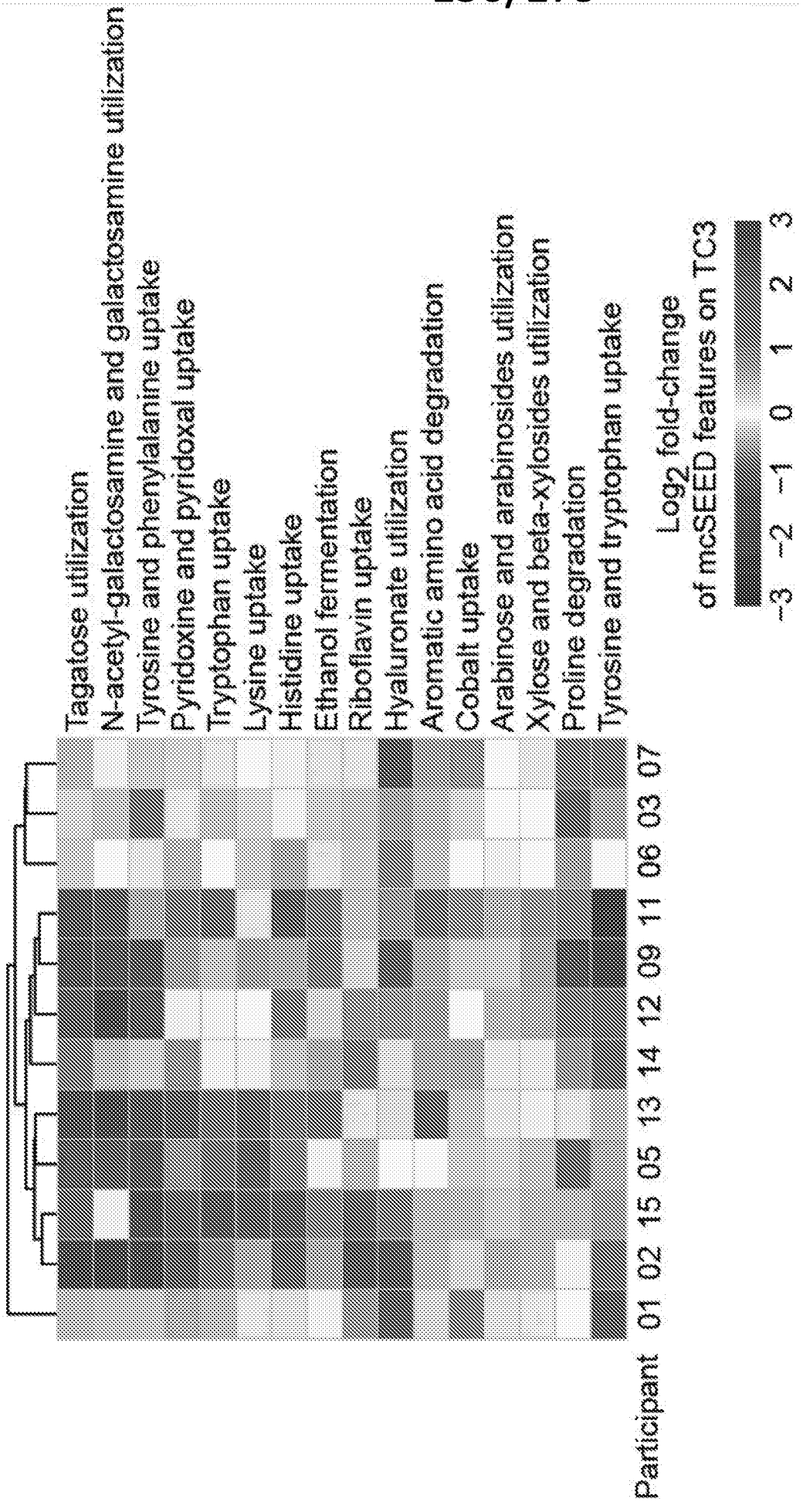

Tagatose utilization
N-acetyl-galactosamine and galactosamine utilization
Tyrosine and phenylalanine uptake
Pyridoxine and pyridoxal uptake
Tryptophan uptake
Lysine uptake
Histidine uptake
Ethanol fermentation
Riboflavin uptake
Hyaluronate utilization
Aromatic amino acid degradation
Cobalt uptake
Arabinose and arabinosides utilization
Xylose and beta-xylosides utilization
Proline degradation
Tyrosine and tryptophan uptake
Participant 01 02 15 05 13 14 12 09 11 06 03 07
Log2 fold-change
of mcSEED features on TC3
−3 −2 −1 0 1 2 3

FIG. 41C

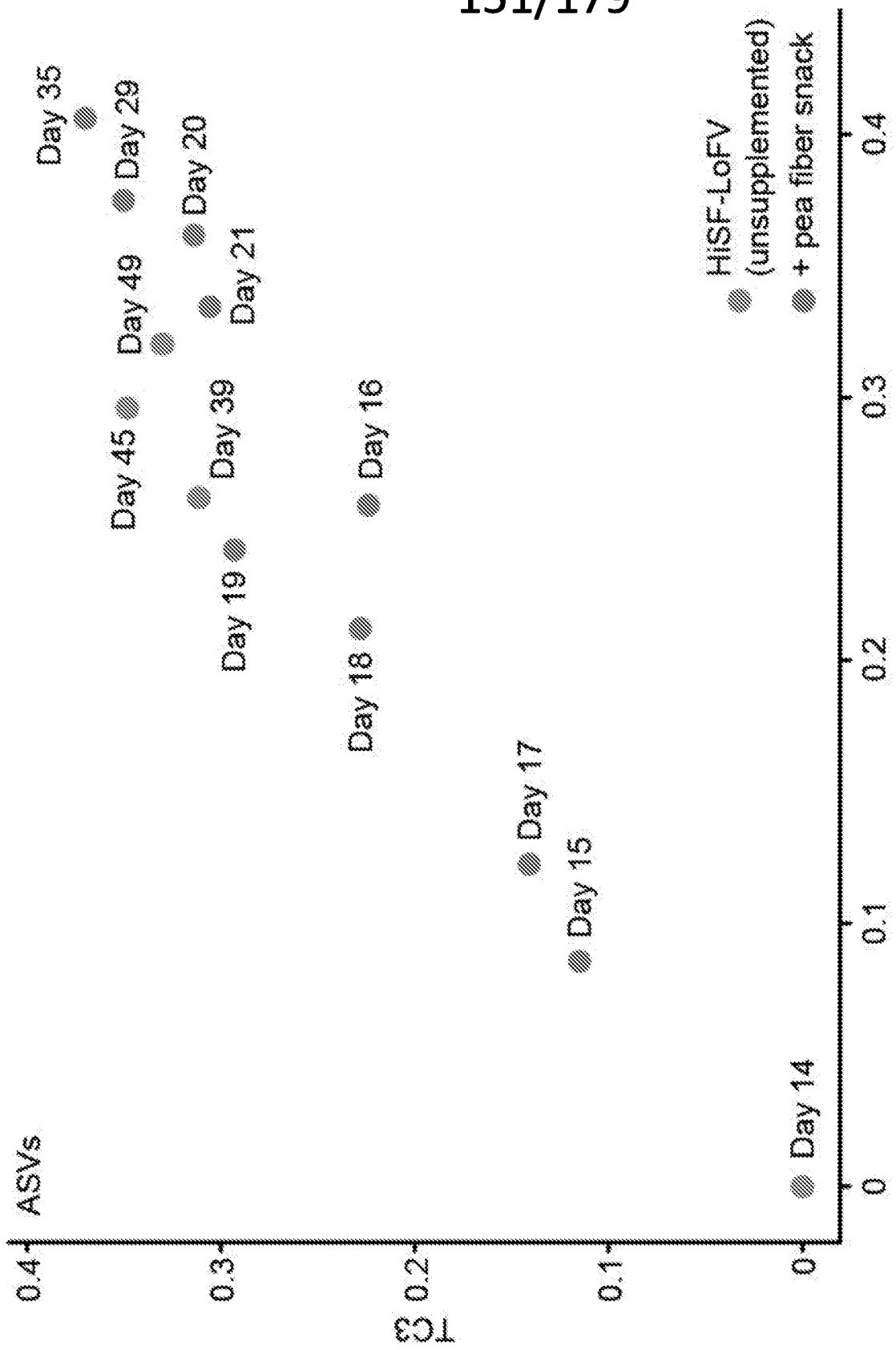

ASVs
0.4
0.3
0.2
0.1
0
TC3
0
0.1
0.2
0.3
0.4
TC2
Day 35
Day 29
Day 20
Day 21
Day 49
Day 45
Day 39
Day 19
Day 18
Day 16
Day 17
Day 15
Day 14
HiSF-LoFV (unsupplemented)
+ pea fiber snack

FIG. 41D

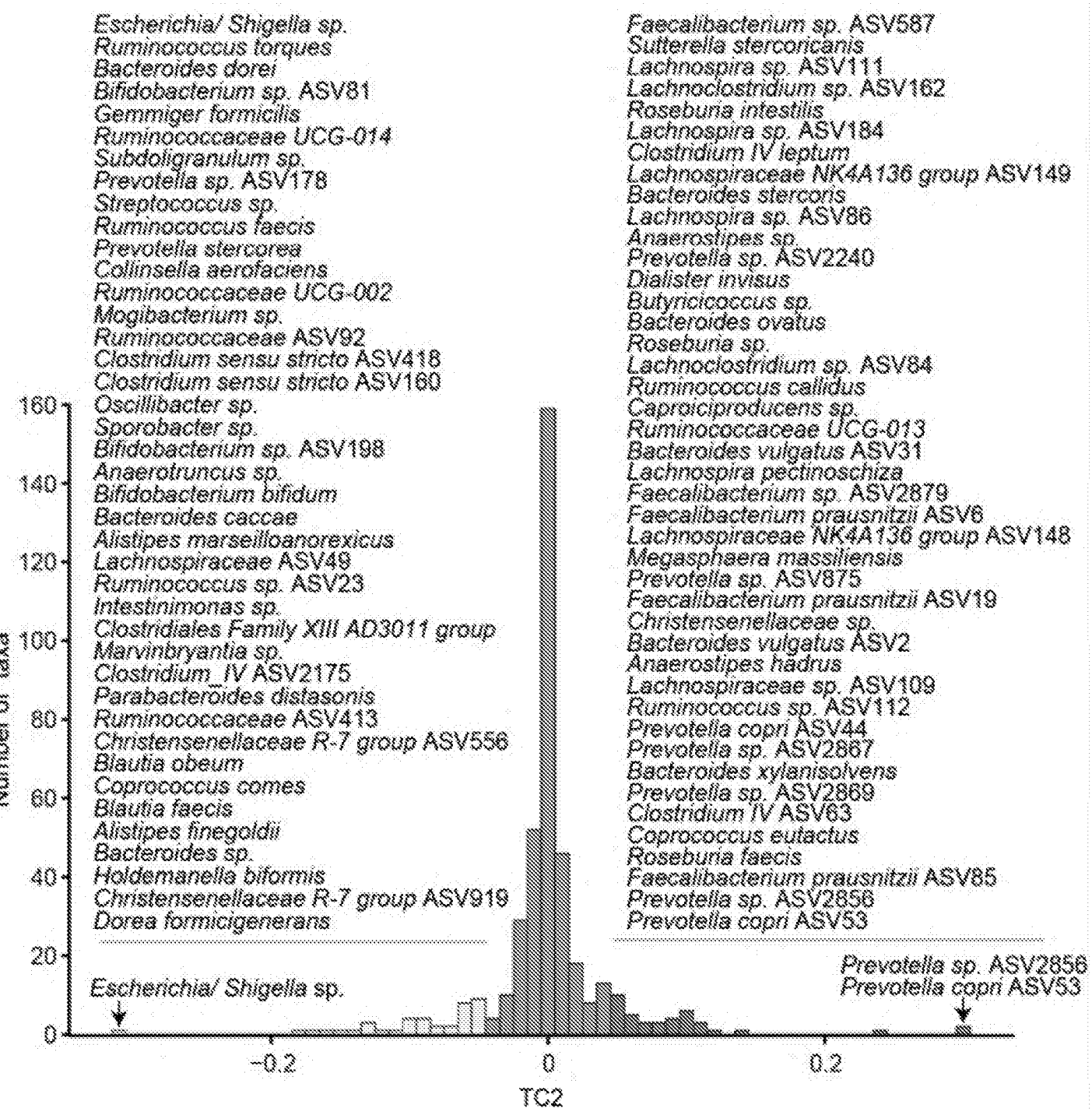

Escherichia/ Shigella sp.
Ruminococcus torques
Bacteroides dorei
Bifidobacterium sp. ASV81
Gemmiger formicilis
Ruminococcaceae UCG-014
Subdoligranulum sp.
Prevotella sp. ASV178
Streptococcus sp.
Ruminococcus faecis
Prevotella stercorea
Collinsella aerofaciens
Ruminococcaceae UCG-002
Mogibacterium sp.
Ruminococcaceae ASV92
Clostridium sensu stricto ASV418
Clostridium sensu stricto ASV160
Oscillibacter sp.
Sporobacter sp.
Bifidobacterium sp. ASV198
Anaerotruncus sp.
Bifidobacterium bifidum
Bacteroides caccae
Alistipes marseilloanorexicus
Lachnospiraceae ASV49
Ruminococcus sp. ASV23
Intestinimonas sp.
Clostridiales Family XIII AD3011 group
Marvinbryantia sp.
Clostridium_IV ASV2175
Parabacteroides distasonis
Ruminococcaceae ASV413
Christensenellaceae R-7 group ASV556
Blautia obeum
Coprococcus comes
Blautia faecis
Alistipes finegoldii
Bacteroides sp.
Holdemanella biformis
Christensenellaceae R-7 group ASV919
Dorea formicigenerans
Faecalibacterium sp. ASV587
Sutterella stercoricanis
Lachnospira sp. ASV111
Lachnoclostridium sp. ASV162
Roseburia intestilis
Lachnospira sp. ASV184
Clostridium IV leptum
Lachnospiraceae NK4A136 group ASV149
Bacteroides stercoris
Lachnospira sp. ASV86
Anaerostipes sp.
Prevotella sp. ASV2240
Dialister invisus
Butyricicoccus sp.
Bacteroides ovatus
Roseburia sp.
Lachnoclostridium sp. ASV84
Ruminococcus callidus
Caproiciproducens sp.
Ruminococcaceae UCG-013
Bacteroides vulgatus ASV31
Lachnospira pectinoschiza
Faecalibacterium sp. ASV2879
Faecalibacterium prausnitzii ASV6
Lachnospiraceae NK4A136 group ASV148
Megasphaera massiliensis
Prevotella sp. ASV875
Faecalibacterium prausnitzii ASV19
Christensenellaceae sp.
Bacteroides vulgatus ASV2
Anaerostipes hadrus
Lachnospiraceae sp. ASV109
Ruminococcus sp. ASV112
Prevotella copri ASV44
Prevotella sp. ASV2867
Bacteroides xylanisolvens
Prevotella sp. ASV2869
Clostridium IV ASV63
Coprococcus eutactus
Roseburia faecis
Faecalibacterium prausnitzii ASV85
Prevotella sp. ASV2856
Prevotella copri ASV53
Number of taxa
160
140
120
100
80
60
40
20
0
Escherichia/ Shigella sp.
Prevotella sp. ASV2856
Prevotella copri ASV53
−0.2
0
0.2
TC2

FIG. 41E

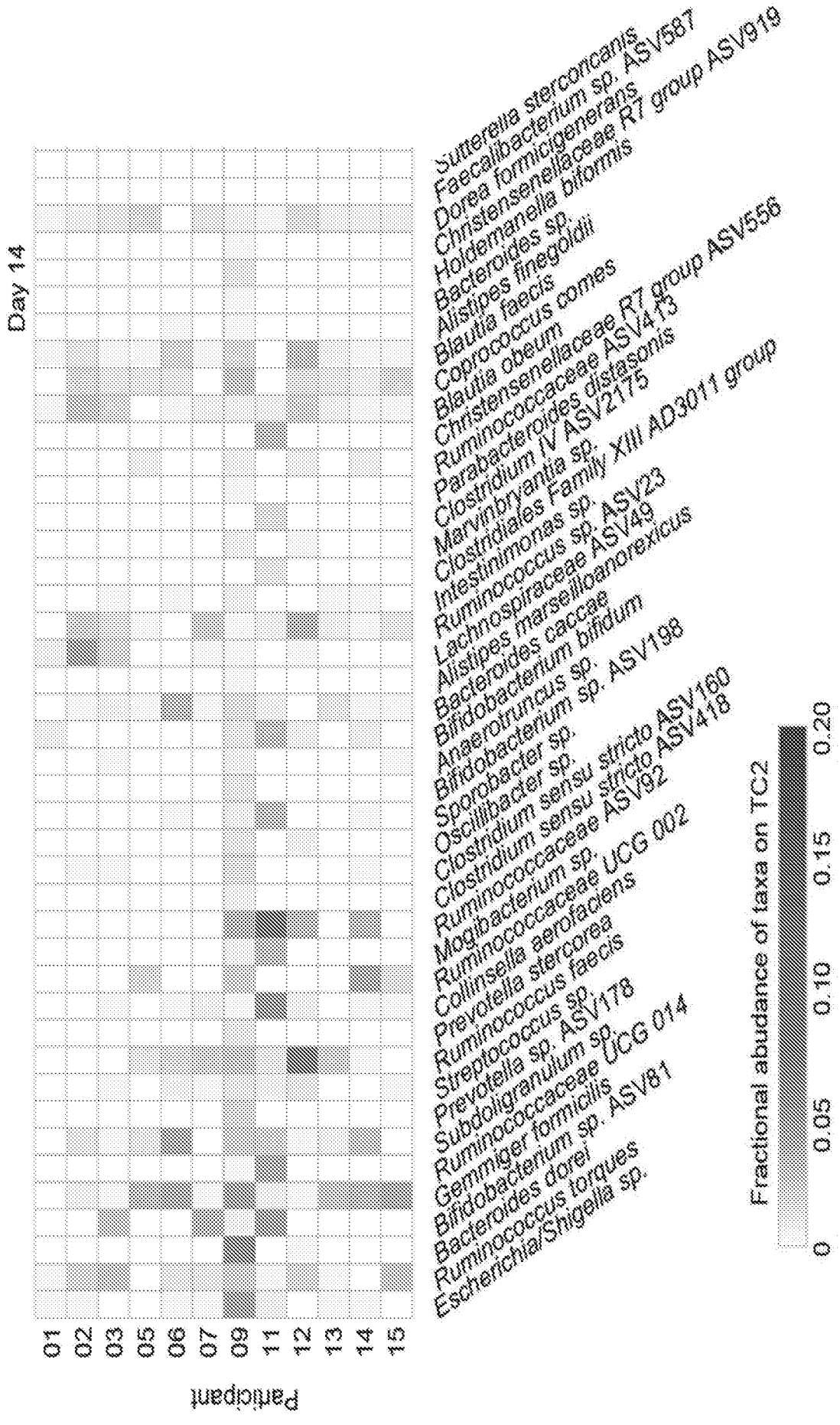

Day 14
Participant
01
02
03
05
06
07
09
11
12
13
14
15
Sutterella stercoricanis
Faecalibacterium sp. ASV587
Dorea formicigenerans
Christensenellaceae R7 group ASV919
Holdemanella biformis
Bacteroides sp.
Alistipes finegoldii
Blautia faecis
Coprococcus comes
Blautia obeum
Christensenellaceae R7 group ASV556
Ruminococcaceae ASV413
Parabacteroides distasonis
Clostridium IV ASV2175
Marvinbryantia sp.
Clostridiales Family XIII AD3011 group
Clostridium sp. ASV23
Intestinimonas sp.
Ruminococcus sp. ASV49
Lachnospiraceae ASV49
Alistipes marseilloanorexicus
Bacteroides caccae
Bifidobacterium bifidum
Anaerotruncus sp.
Bifidobacterium sp. ASV198
Sporobacter sp.
Oscillibacter sp.
Clostridium sensu stricto ASV160
Clostridium sensu stricto ASV418
Ruminococcaceae ASV92
Mogibacterium sp.
Ruminococcaceae UCG 002
Collinsella aerofaciens
Prevotella stercorea
Ruminococcus faecis
Ruminococcus sp.
Streptococcus sp. ASV178
Prevotella sp.
Subdoligranulum sp.
Ruminococcaceae UCG 014
Gemmiger formicilis
Bifidobacterium sp. ASV81
Bacteroides dorei
Ruminococcus torques
Escherichia/Shigella sp.
Fractional abudance of taxa on TC2
0
0.05
0.10
0.15
0.20

FIG. 41F

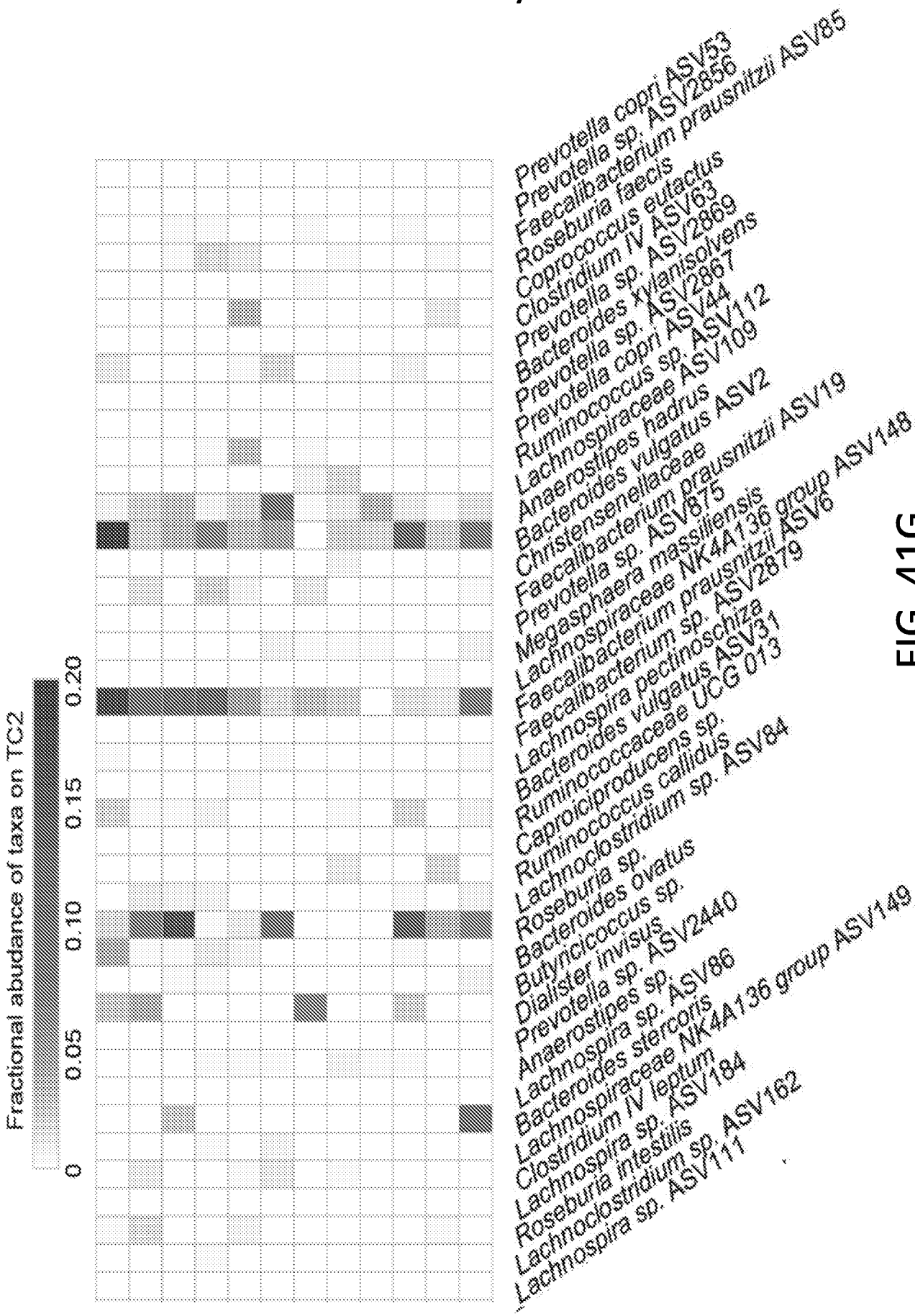

Fractional abudance of taxa on TC2
0
0.05
0.10
0.15
0.20
Prevotella copri ASV53
Prevotella sp. ASV2856
Faecalibacterium prausnitzii ASV85
Roseburia faecis
Coprococcus eutactus
Clostridium IV ASV63
Prevotella sp. ASV2869
Bacteroides xylanisolvens
Prevotella sp. ASV2867
Prevotella copri ASV44
Ruminococcus sp. ASV112
Lachnospiraceae ASV109
Anaerostipes hadrus
Christensenellaceae
Bacteroides vulgatus ASV2
Faecalibacterium prausnitzii ASV19
Megasphaera massiliensis
Prevotella sp. ASV875
Lachnospiraceae NK4A136 group ASV148
Faecalibacterium sp. ASV2879
Faecalibacterium prausnitzii ASV6
Lachnospira pectinoschiza
Bacteroides vulgatus ASV31
Ruminococcaceae UCG 013
Caproiciproducens sp.
Ruminococcus callidus
Ruminoclostridium sp. ASV84
Lachnoclostridium sp.
Roseburia sp.
Bacteroides ovatus
Butyricicoccus sp.
Dialister invisus
Prevotella sp. ASV2440
Anaerostipes sp.
Lachnospira sp. ASV86
Bacteroides stercoris
Lachnospiraceae NK4A136 group ASV149
Clostridium IV leptum
Lachnospira sp. ASV184
Roseburia intestinis
Lachnoclostridium sp. ASV162
Lachnospira sp. ASV111

FIG. 41G

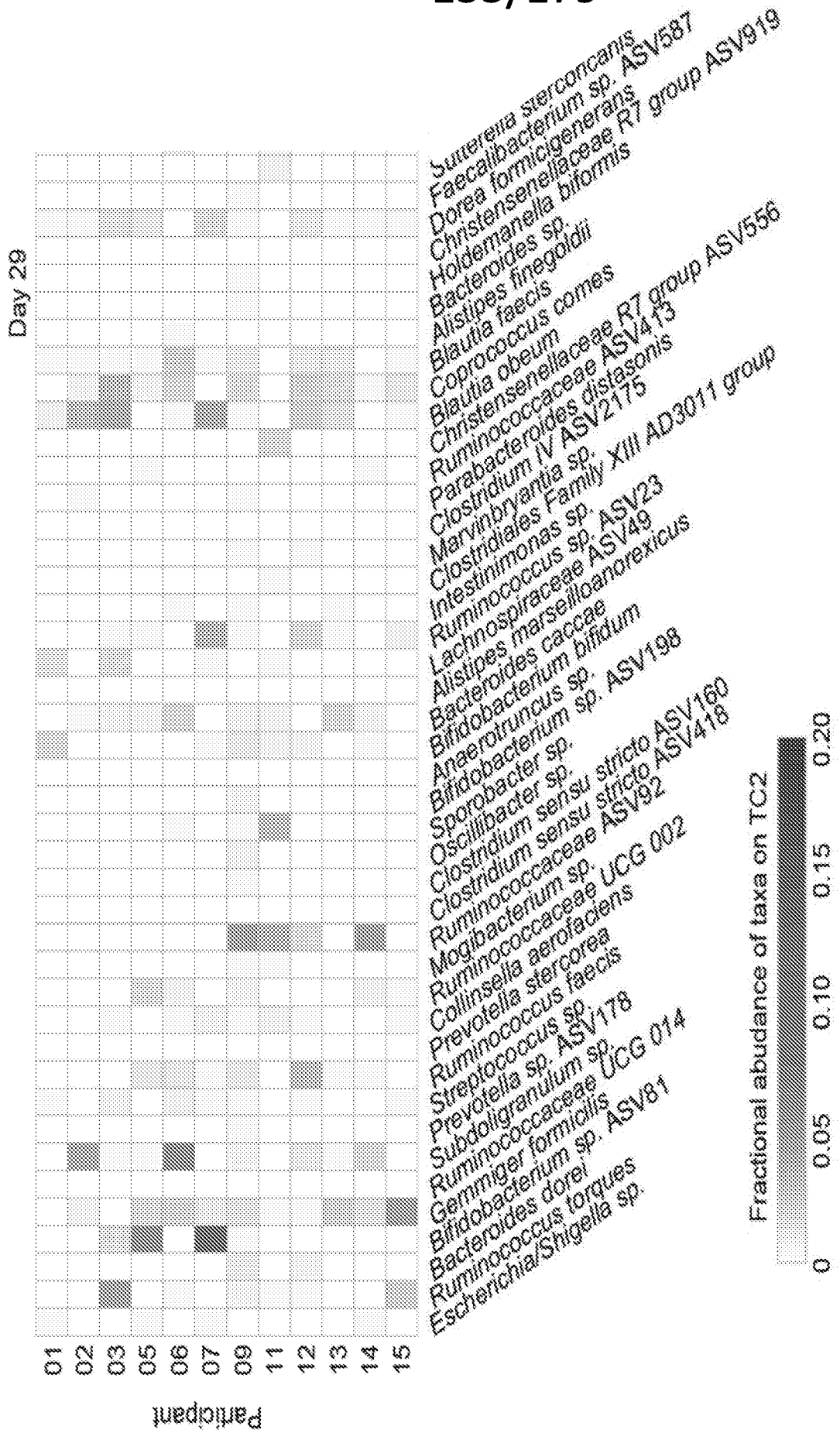

Day 29
Participant
01
02
03
05
06
07
09
11
12
13
14
15
Sutterella stercoricanis
Faecalibacterium sp. ASV587
Dorea formicigenerans
Christensenellaceae R7 group ASV919
Holdemanella biformis
Bacteroides sp.
Alistipes finegoldii
Blautia faecis
Coprococcus comes
Blautia obeum
Christensenellaceae R7 group ASV556
Ruminococcaceae ASV413
Parabacteroides distasonis
Clostridium IV ASV2175
Marvinbryantia sp.
Clostridiales Family XIII AD3011 group
Intestinimonas sp.
Ruminococcus sp. ASV23
Lachnospiraceae ASV49
Alistipes marseilloanorexicus
Bacteroides caccae
Bifidobacterium bifidum
Anaerotruncus sp.
Bifidobacterium sp. ASV198
Sporobacter sp.
Oscillibacter sp.
Clostridium sensu stricto ASV160
Clostridium sensu stricto ASV418
Ruminococcaceae ASV92
Mogibacterium sp.
Ruminococcaceae UCG 002
Collinsella aerofaciens
Prevotella stercorea
Ruminococcus faecis
Streptococcus sp.
Prevotella sp. ASV178
Subdoligranulum sp.
Ruminococcaceae UCG 014
Gemmiger formicilis
Bifidobacterium sp. ASV81
Bacteroides dorei
Ruminococcus torques
Escherichia/Shigella sp.
Fractional abudance of taxa on TC2
0
0.05
0.10
0.15
0.20

FIG. 41H

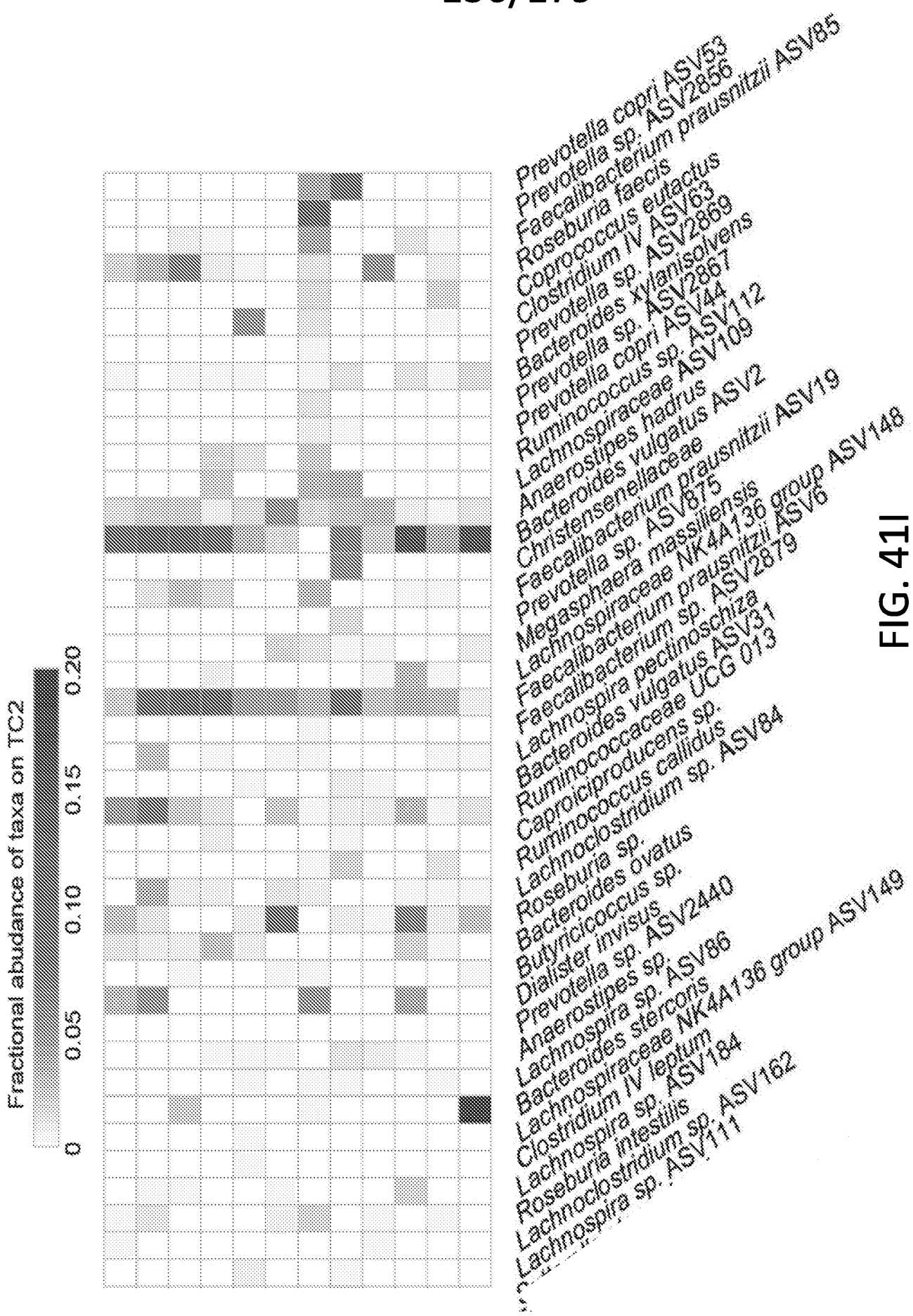

Fractional abundance of taxa on TC2
0
0.05
0.10
0.15
0.20
Prevotella copri ASV53
Prevotella sp. ASV2856
Faecalibacterium prausnitzii ASV85
Roseburia faecis
Coprococcus eutactus
Clostridium IV ASV63
Prevotella sp. ASV2869
Bacteroides xylanisolvens
Prevotella sp. ASV2867
Prevotella copri ASV44
Ruminococcus sp. ASV112
Lachnospiraceae ASV109
Anaerostipes hadrus
Bacteroides vulgatus ASV2
Christensenellaceae
Faecalibacterium prausnitzii ASV19
Prevotella sp. ASV875
Megasphaera massiliensis
Lachnospiraceae NK4A136 group ASV148
Faecalibacterium sp. ASV2879
Faecalibacterium prausnitzii ASV6
Lachnospira pectinoschiza
Bacteroides vulgatus ASV31
Ruminococcaceae UCG 013
Caproiciproducens sp.
Ruminococcus callidus
Lachnoclostridium sp. ASV84
Roseburia sp.
Bacteroides ovatus
Butyricicoccus sp.
Dialister invisus
Prevotella sp. ASV2440
Anaerostipes sp.
Lachnospira sp. ASV86
Bacteroides stercoris
Lachnospiraceae NK4A136 group ASV149
Clostridium IV leptum
Lachnospira sp. ASV184
Roseburia intestinalis
Lachnoclostridium sp. ASV162
Lachnospira sp. ASV111

FIG. 41I

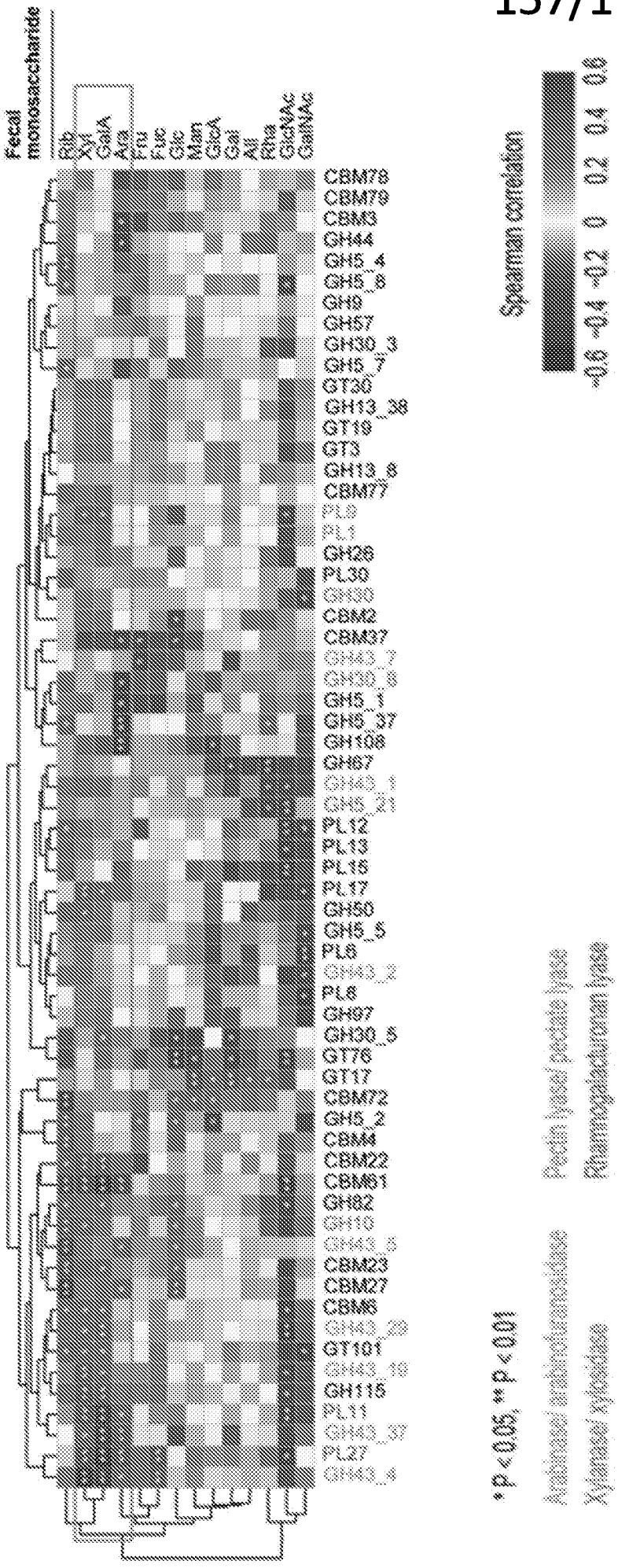

Fecal monosaccharide
Rib
Xyl
GalA
Ara
Fru
Fuc
Glc
Man
GlcA
Gal
All
Rha
GlcNAc
GalNAc
CBM78
CBM79
CBM3
GH44
GH5_4
GH5_8
GH9
GH57
GH30_3
GH5_7
GT30
GH13_38
GT19
GT3
GH13_6
CBM77
PL9
PL1
GH28
PL30
GH30
CBM2
CBM37
GH43_7
GH30_8
GH5_1
GH5_37
GH106
GH67
GH43_1
GH5_21
PL12
PL13
PL15
PL17
GH50
GH5_5
PL6
GH43_2
PL8
GH97
GH30_5
GT76
GT17
CBM72
GH5_2
CBM4
CBM22
CBM61
GH82
GH10
GH43_6
CBM23
CBM27
CBM6
GH43_29
GT101
GH43_16
GH115
PL11
GH43_37
PL27
GH43_4
Spearman correlation
-0.6 -0.4 -0.2 0 0.2 0.4 0.6
* P < 0.05, ** P < 0.01
Arabinanase/rhamnosidase
Pectin lyase/pectate lyase
Xylanase/xylosidase
Rhamnogalacturonan lyase

FIG. 42A

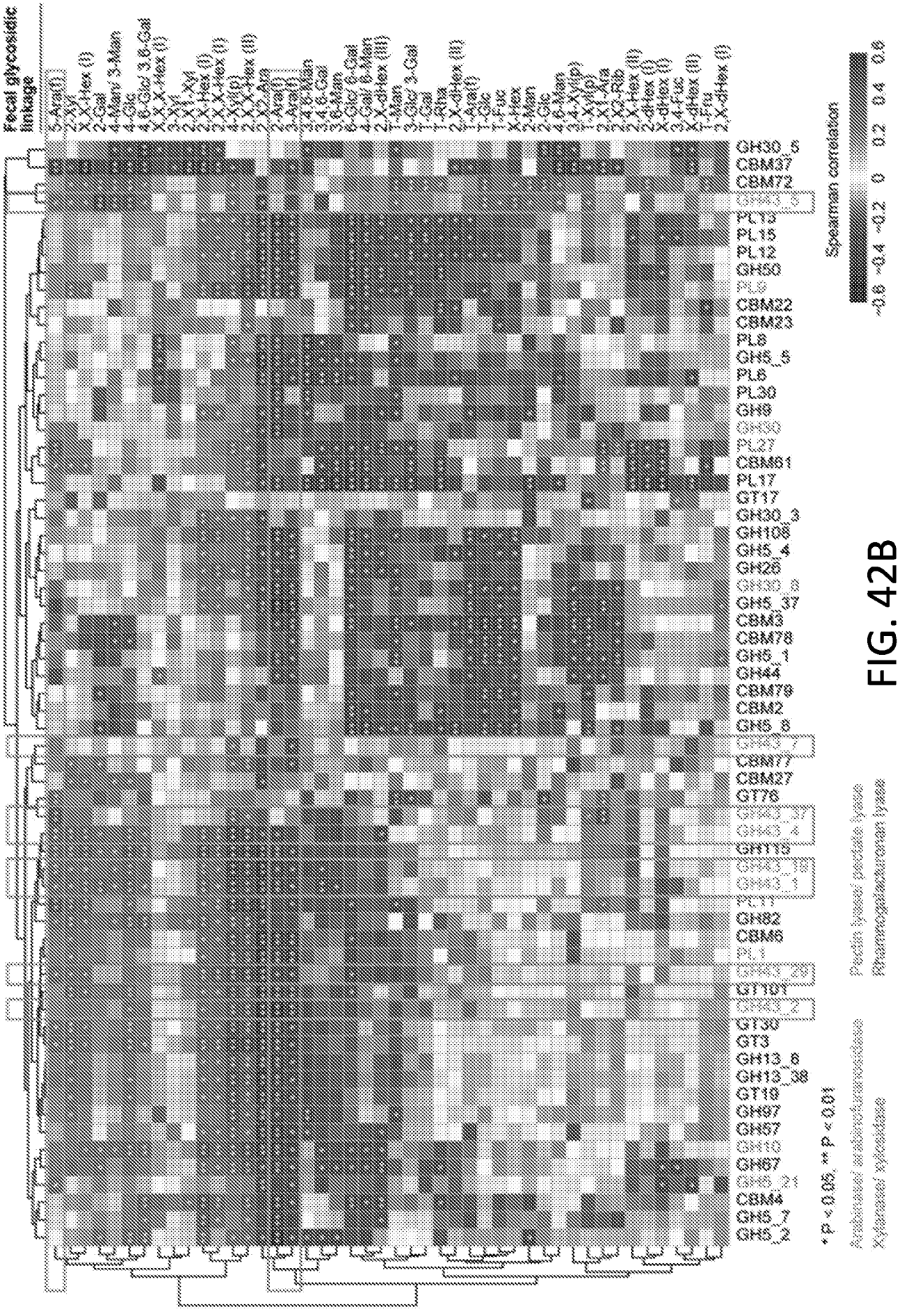

Focal glycosidic linkage
5-Ara(f)
2-Xyl
2-X,X-Hex (t)
2-Gal
4-Man/ 3-Man
4-Glc
4,6-Glc/ 3,6-Gal
X-X,X-Hex (t)
3-Xyl
2-X,t-Xyl
2-X,t-Hex (t)
2-X,X-Hex (t)
4-X,t(p)
4-X,X-Hex (t)
2-X,2-Ara
2-Ara(f)
3-Ara(f)
3,4,6-Man
3,4,6-Gal
3,6-Man
3-Glc/ 6-Gal
4-Gal/ 6-Man
2-X,dHex (t)
t-Man
3-Glc/ 3-Gal
t-Gal
t-Ara
2-X,dHex (t)
2-Ara(f)
t-Glc
t-Fuc
X-Hex
X-Man
2-Glc
4,6-Man
3,4-Xyl(p)
3-Xyl(p)
2-X,t-Ara
2-X,Rib
2-X-Hex (t)
2-dHex (t)
X-dHex (t)
3,4-Fuc
X-dHex (t)
t-Fru
2,X-dHex (t)
GH30_5
CBM37
CBM72
GH43_6
PL13
PL15
PL12
GH50
PL9
CBM22
CBM23
PL8
GH5_5
PL6
PL30
GH9
GH30
PL27
CBM61
PL17
GT17
GH30_3
GH105
GH5_4
GH28
GH30_8
GH5_37
CBM3
CBM78
GH5_1
GH44
CBM79
CBM2
GH5_8
GH43_7
CBM77
CBM27
GT76
GH43_17
GH43_4
GH115
GH43_18
GH43_1
PL11
GH82
CBM6
PL1
GH43_29
GT101
GH43_2
GT30
GT3
GH13_6
GH13_38
GT19
GH97
GH57
GH10
GH67
GH8_21
CBM4
GH5_7
GH5_2
Spearman correlation
-0.6 -0.4 -0.2 0 0.2 0.4 0.6
* P < 0.05, ** P < 0.01
Arabinanase/ arabinofuranosidase
Xylanase/ xylosidase
Pectin lyase/ pectate lyase
Rhamnogalacturonan lyase

FIG. 42B

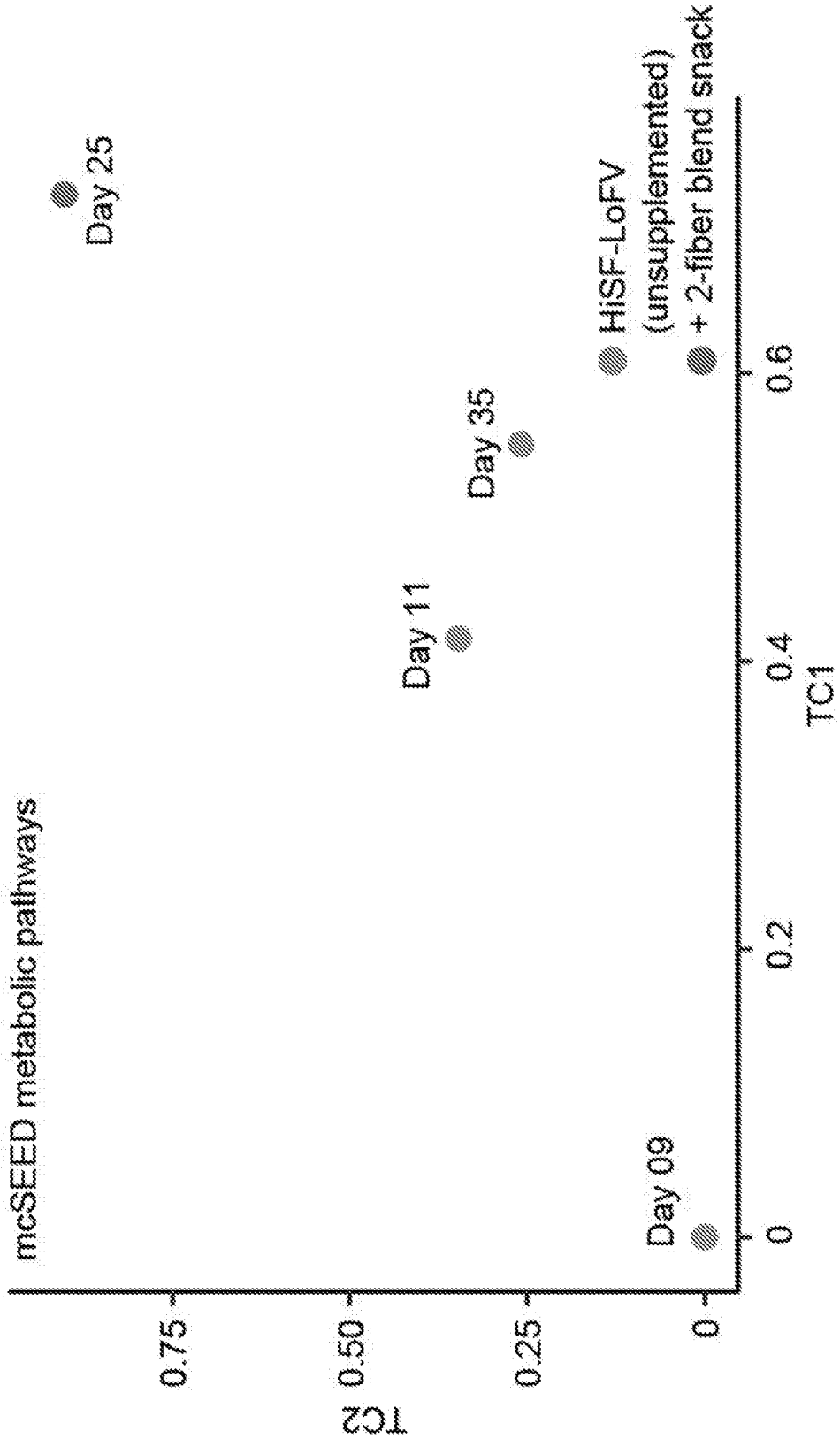

mcSEED metabolic pathways
Day 25
Day 35
Day 11
Day 09
HiSF-LoFV
(unsupplemented)
+ 2-fiber blend snack
0.75
0.50
0.25
0
TC2
0
0.2
0.4
0.6
TC1

FIG. 43A

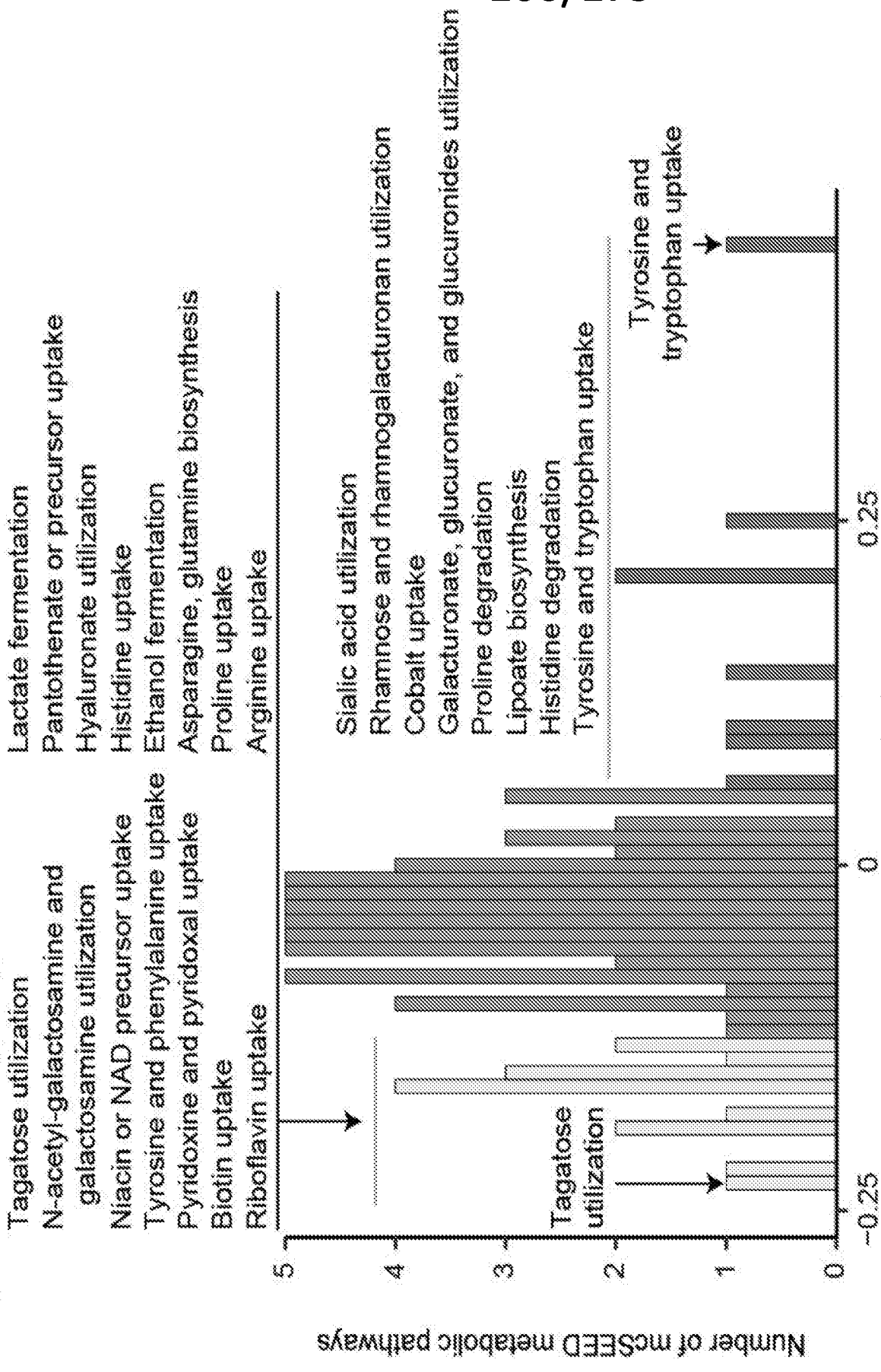

Tagatose utilization
N-acetyl-galactosamine and galactosamine utilization
Niacin or NAD precursor uptake
Tyrosine and phenylalanine uptake
Pyridoxine and pyridoxal uptake
Biotin uptake
Riboflavin uptake
Lactate fermentation
Pantothenate or precursor uptake
Hyaluronate utilization
Histidine uptake
Ethanol fermentation
Asparagine, glutamine biosynthesis
Proline uptake
Arginine uptake
Sialic acid utilization
Rhamnose and rhamnogalacturonan utilization
Cobalt uptake
Galacturonate, glucuronate, and glucuronides utilization
Proline degradation
Lipoate biosynthesis
Histidine degradation
Tyrosine and tryptophan uptake
Tagatose utilization
Tyrosine and tryptophan uptake
Number of mcSEED metabolic pathways
0
1
2
3
4
5
−0.25
0
0.25
TC2

FIG. 43B

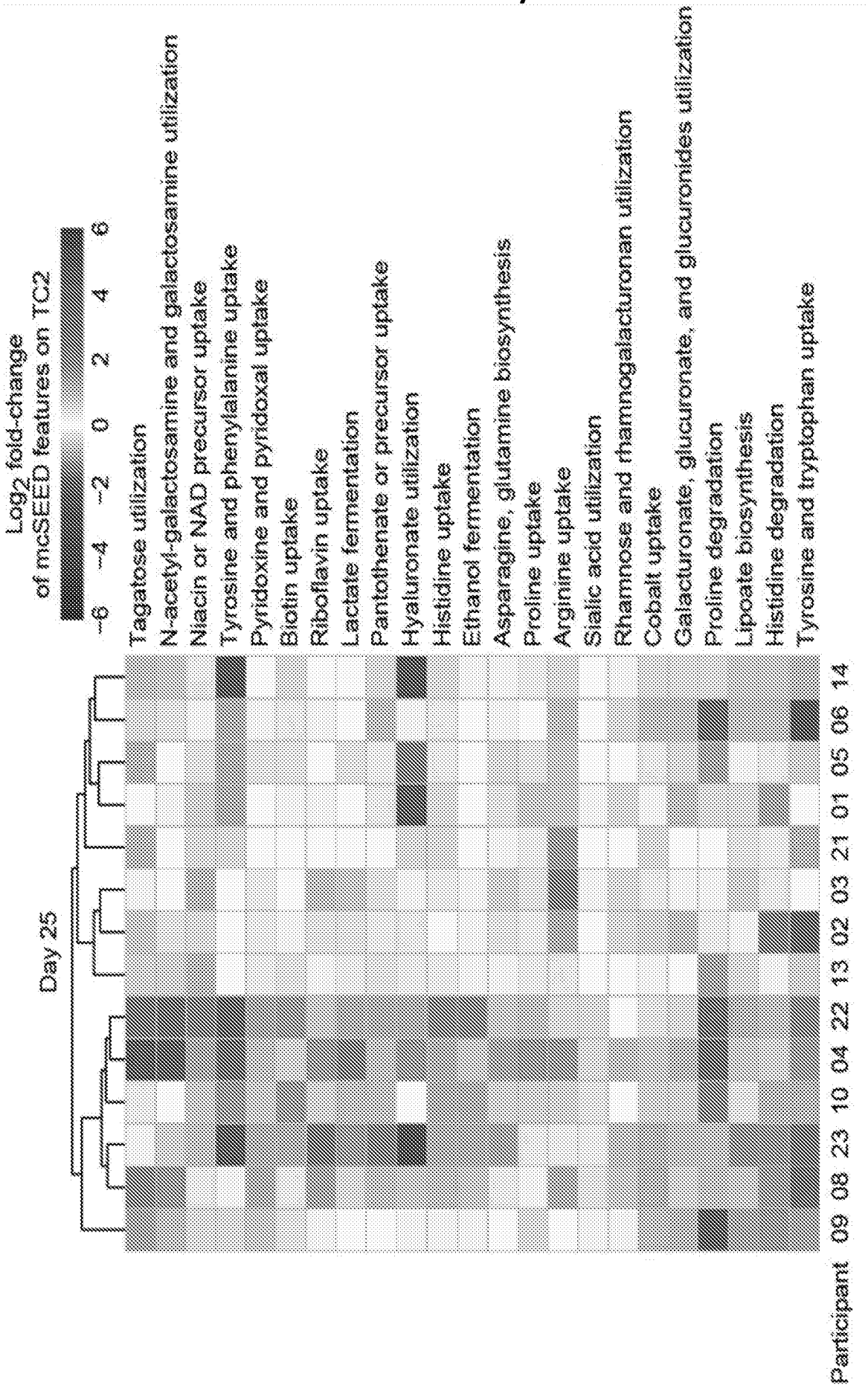

Log2 fold-change
of mcSEED features on TC2
−6 −4 −2 0 2 4 6
Tagatose utilization
N-acetyl-galactosamine and galactosamine utilization
Niacin or NAD precursor uptake
Tyrosine and phenylalanine uptake
Pyridoxine and pyridoxal uptake
Biotin uptake
Riboflavin uptake
Lactate fermentation
Pantothenate or precursor uptake
Hyaluronate utilization
Histidine uptake
Ethanol fermentation
Asparagine, glutamine biosynthesis
Proline uptake
Arginine uptake
Sialic acid utilization
Rhamnose and rhamnogalacturonan utilization
Cobalt uptake
Galacturonate, glucuronate, and glucuronides utilization
Proline degradation
Lipoate biosynthesis
Histidine degradation
Tyrosine and tryptophan uptake
Day 25
Participant 09 08 23 10 04 22 13 02 03 21 01 05 06 14

FIG. 43C

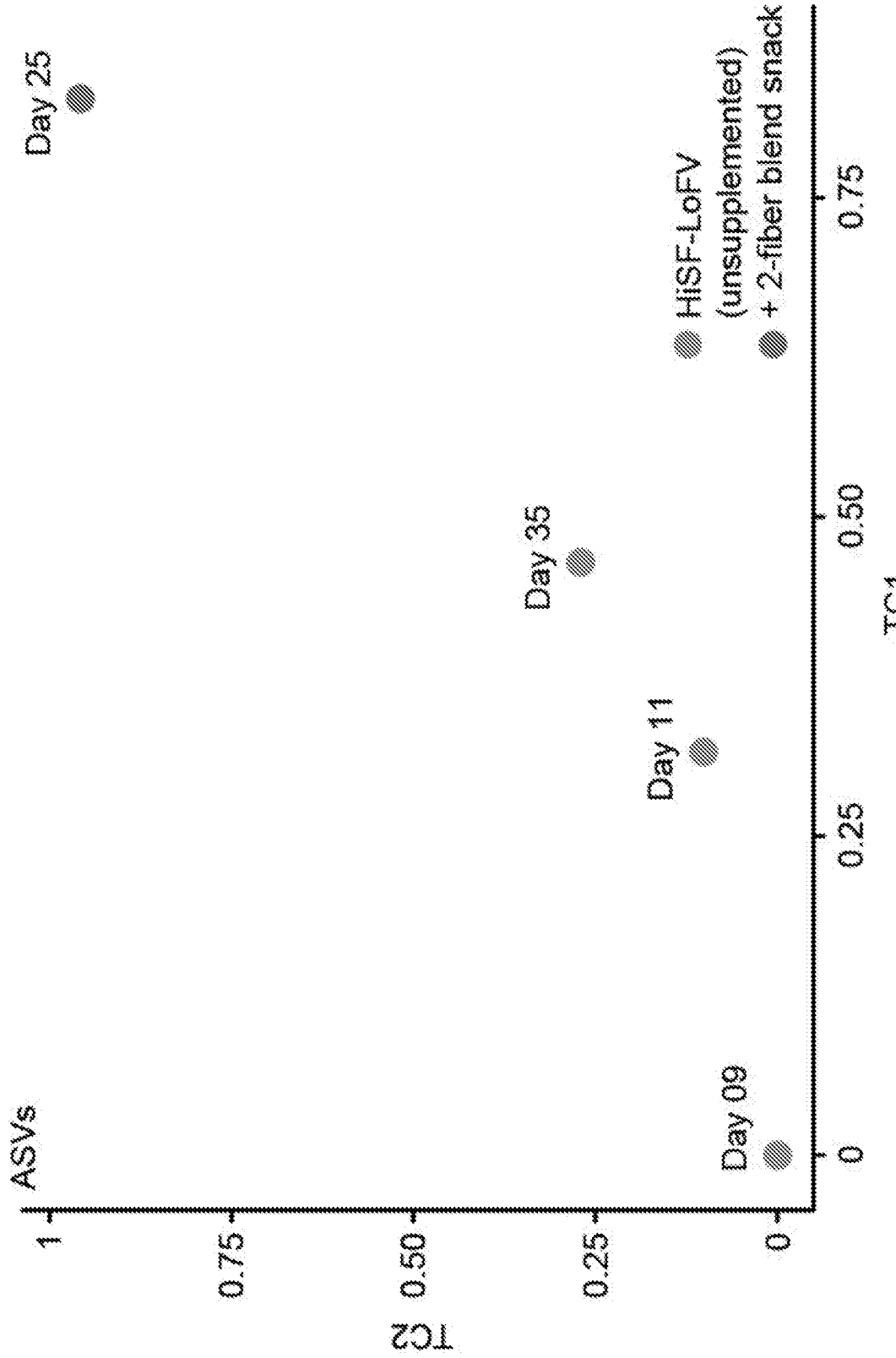

ASVs
1
0.75
0.50
0.25
0
TC2
0
0.25
0.50
0.75
TC1
Day 25
Day 35
Day 11
Day 09
HiSF-LoFV (unsupplemented)
+ 2-fiber blend snack

FIG. 43D

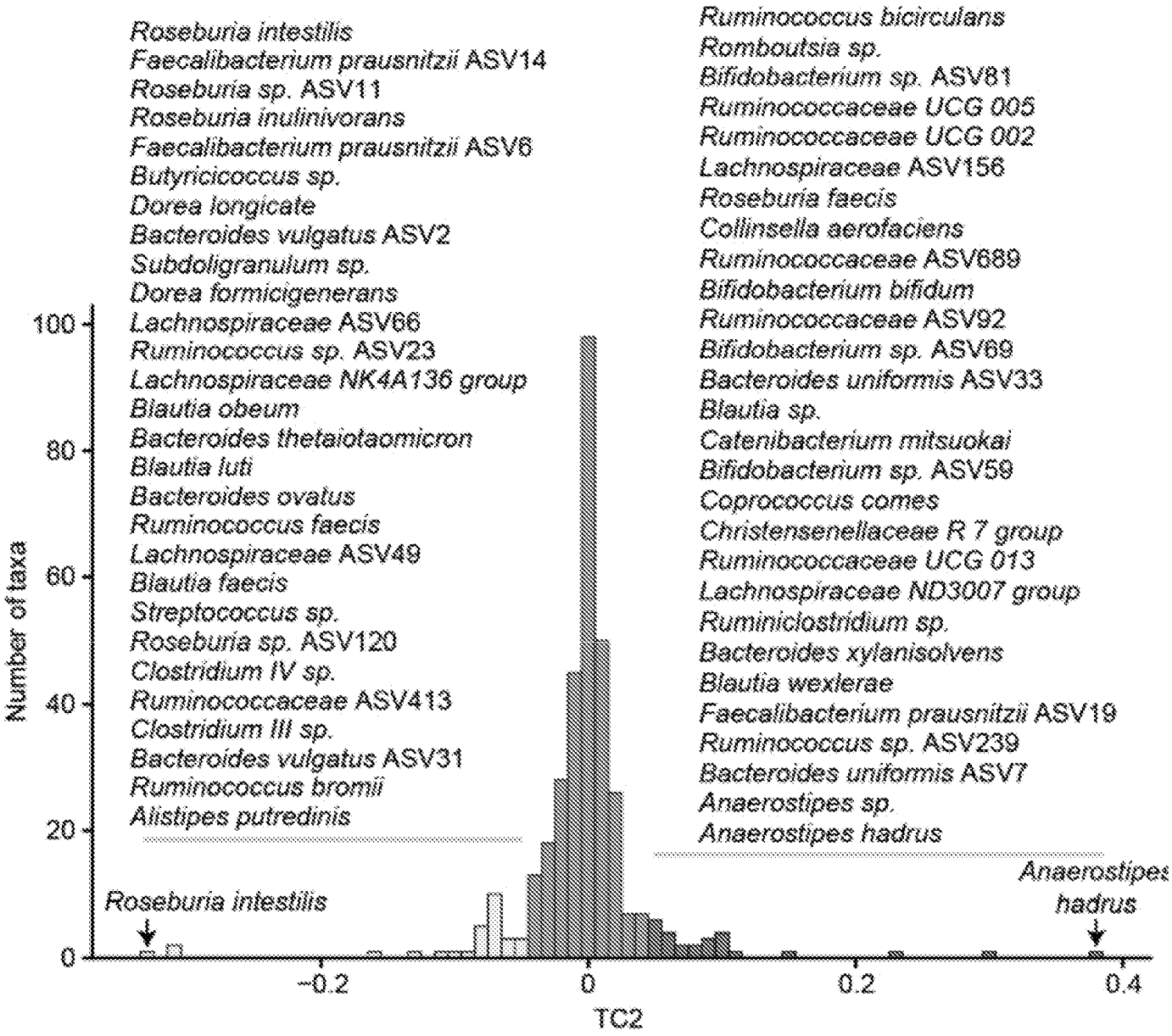

Roseburia intestilis
Faecalibacterium prausnitzii ASV14
Roseburia sp. ASV11
Roseburia inulinivorans
Faecalibacterium prausnitzii ASV6
Butyricicoccus sp.
Dorea longicate
Bacteroides vulgatus ASV2
Subdoligranulum sp.
Dorea formicigenerans
Lachnospiraceae ASV66
Ruminococcus sp. ASV23
Lachnospiraceae NK4A136 group
Blautia obeum
Bacteroides thetaiotaomicron
Blautia luti
Bacteroides ovatus
Ruminococcus faecis
Lachnospiraceae ASV49
Blautia faecis
Streptococcus sp.
Roseburia sp. ASV120
Clostridium IV sp.
Ruminococcaceae ASV413
Clostridium III sp.
Bacteroides vulgatus ASV31
Ruminococcus bromii
Alistipes putredinis
Ruminococcus bicirculans
Romboutsia sp.
Bifidobacterium sp. ASV81
Ruminococcaceae UCG 005
Ruminococcaceae UCG 002
Lachnospiraceae ASV156
Roseburia faecis
Collinsella aerofaciens
Ruminococcaceae ASV689
Bifidobacterium bifidum
Ruminococcaceae ASV92
Bifidobacterium sp. ASV69
Bacteroides uniformis ASV33
Blautia sp.
Catenibacterium mitsuokai
Bifidobacterium sp. ASV59
Coprococcus comes
Christensenellaceae R 7 group
Ruminococcaceae UCG 013
Lachnospiraceae ND3007 group
Ruminiclostridium sp.
Bacteroides xylanisolvens
Blautia wexlerae
Faecalibacterium prausnitzii ASV19
Ruminococcus sp. ASV239
Bacteroides uniformis ASV7
Anaerostipes sp.
Anaerostipes hadrus
Roseburia intestilis
Anaerostipes hadrus
Number of taxa
100
80
60
40
20
0
−0.2
0
0.2
0.4
TC2

FIG. 43E

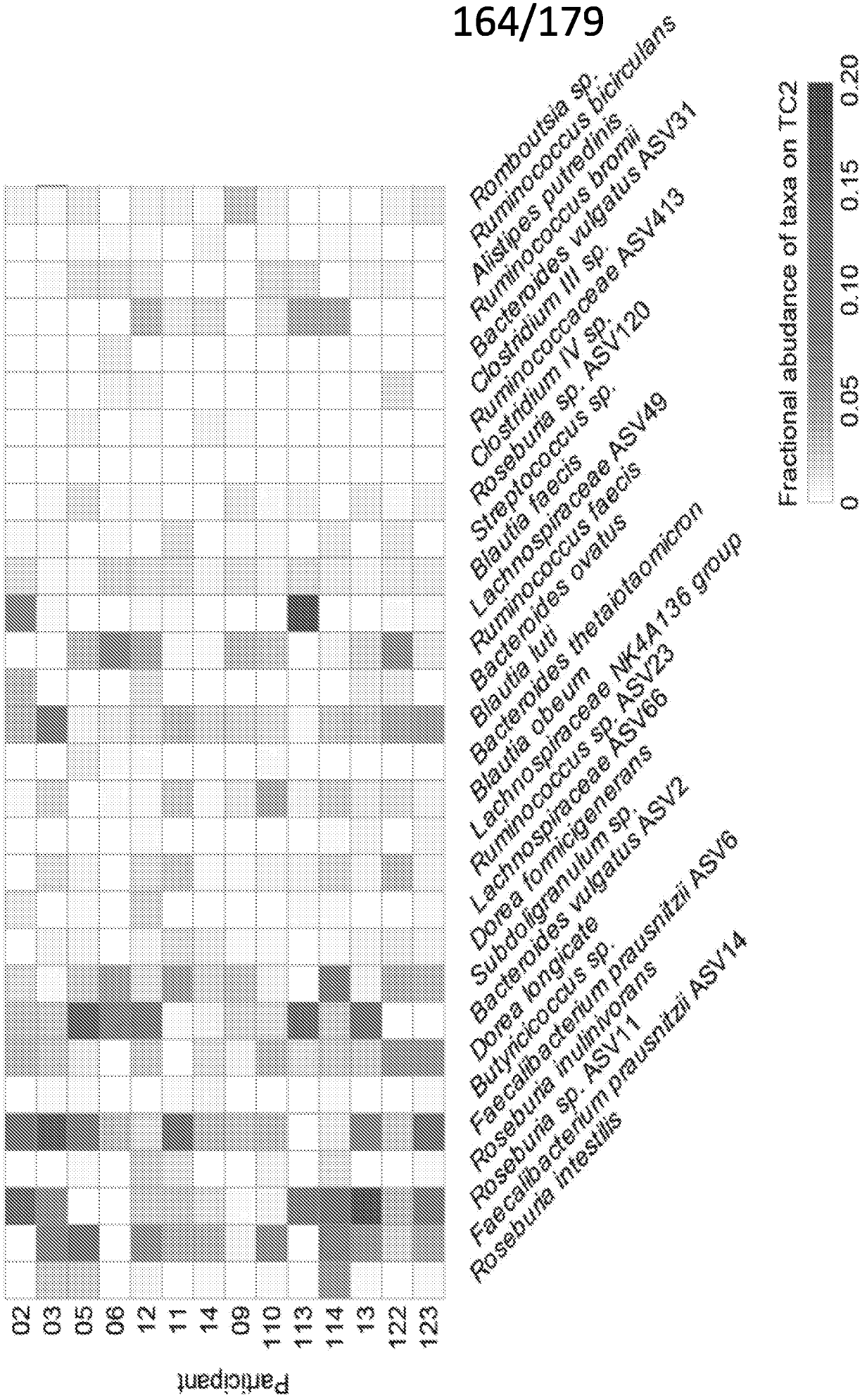

Romboutsia sp.
Ruminococcus bicirculans
Alistipes putredinis
Ruminococcus bromii
Bacteroides vulgatus ASV31
Clostridium III sp.
Ruminococcaceae ASV413
Clostridium IV sp.
Roseburia sp. ASV120
Streptococcus sp.
Blautia faecis
Lachnospiraceae ASV49
Ruminococcus faecis
Bacteroides ovatus
Blautia luti
Bacteroides thetaiotaomicron
Blautia obeum
Lachnospiraceae NK4A136 group
Ruminococcus sp. ASV23
Lachnospiraceae ASV66
Dorea formicigenerans
Subdoligranulum sp.
Bacteroides vulgatus ASV2
Dorea longicate
Butyricicoccus sp.
Faecalibacterium prausnitzii ASV6
Roseburia inulinivorans
Roseburia sp. ASV11
Faecalibacterium prausnitzii ASV14
Roseburia intestilis
Fractional abudance of taxa on TC2
0
0.05
0.10
0.15
0.20
02
03
05
06
12
11
14
08
110
113
114
13
122
123
Participant

FIG. 43F

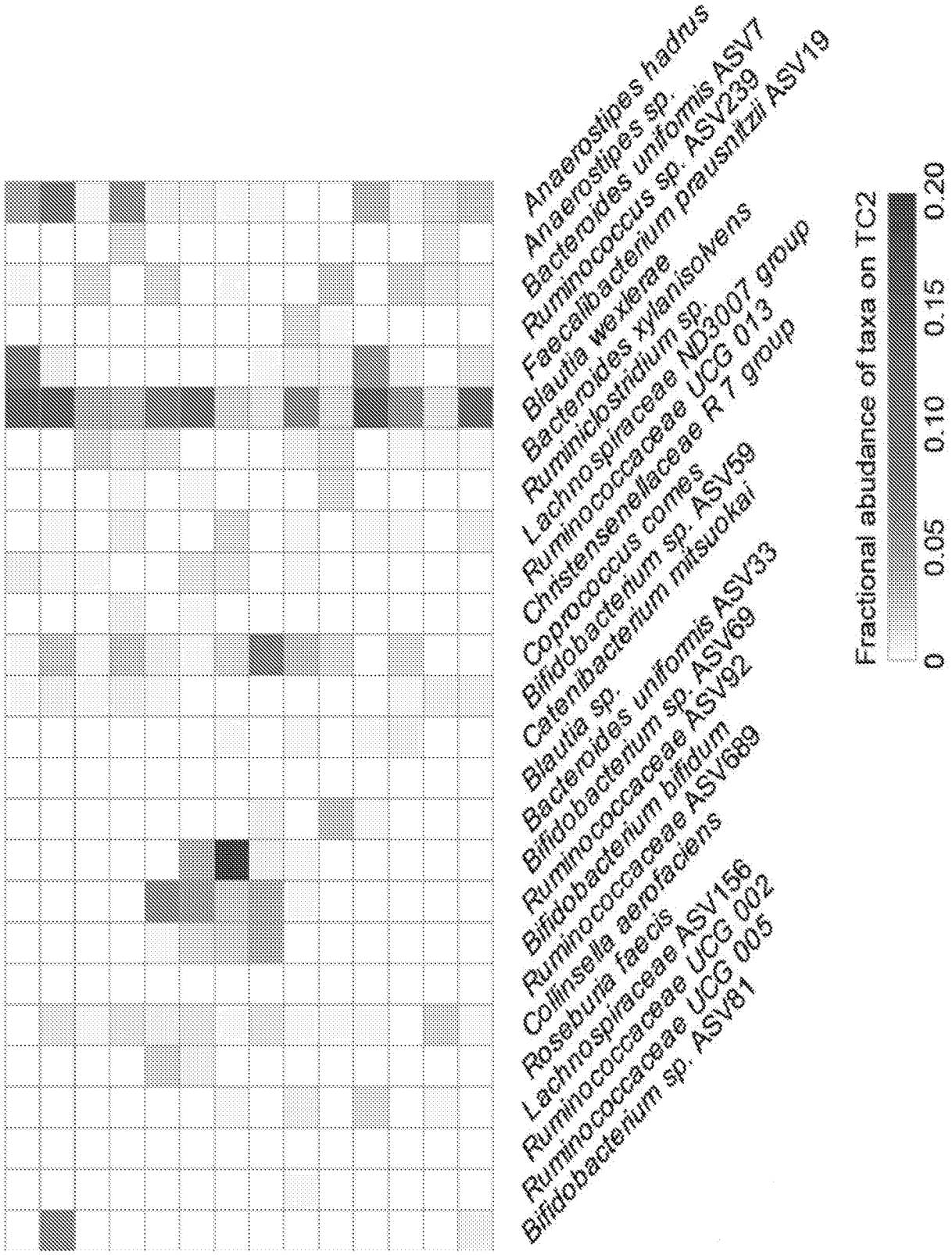

Anaerostipes hadrus
Anaerostipes sp.
Bacteroides uniformis ASV7
Ruminococcus sp. ASV239
Faecalibacterium prausnitzii ASV19
Blautia wexlerae
Bacteroides xylanisolvens
Ruminiclostridium sp.
Lachnospiraceae ND3007 group
Ruminococcaceae UCG 013
Coprococcus comes
Christensenellaceae R 7 group
Bifidobacterium sp. ASV59
Catenibacterium mitsuokai
Blautia sp.
Bacteroides uniformis ASV33
Bifidobacterium sp. ASV69
Ruminococcaceae ASV92
Bifidobacterium bifidum
Ruminococcaceae ASV689
Collinsella aerofaciens
Roseburia faecis
Lachnospiraceae ASV156
Ruminococcaceae UCG 002
Ruminococcaceae UCG 005
Bifidobacterium sp. ASV81
Fractional abudance of taxa on TC2
0
0.05
0.10
0.15
0.20

FIG. 43G

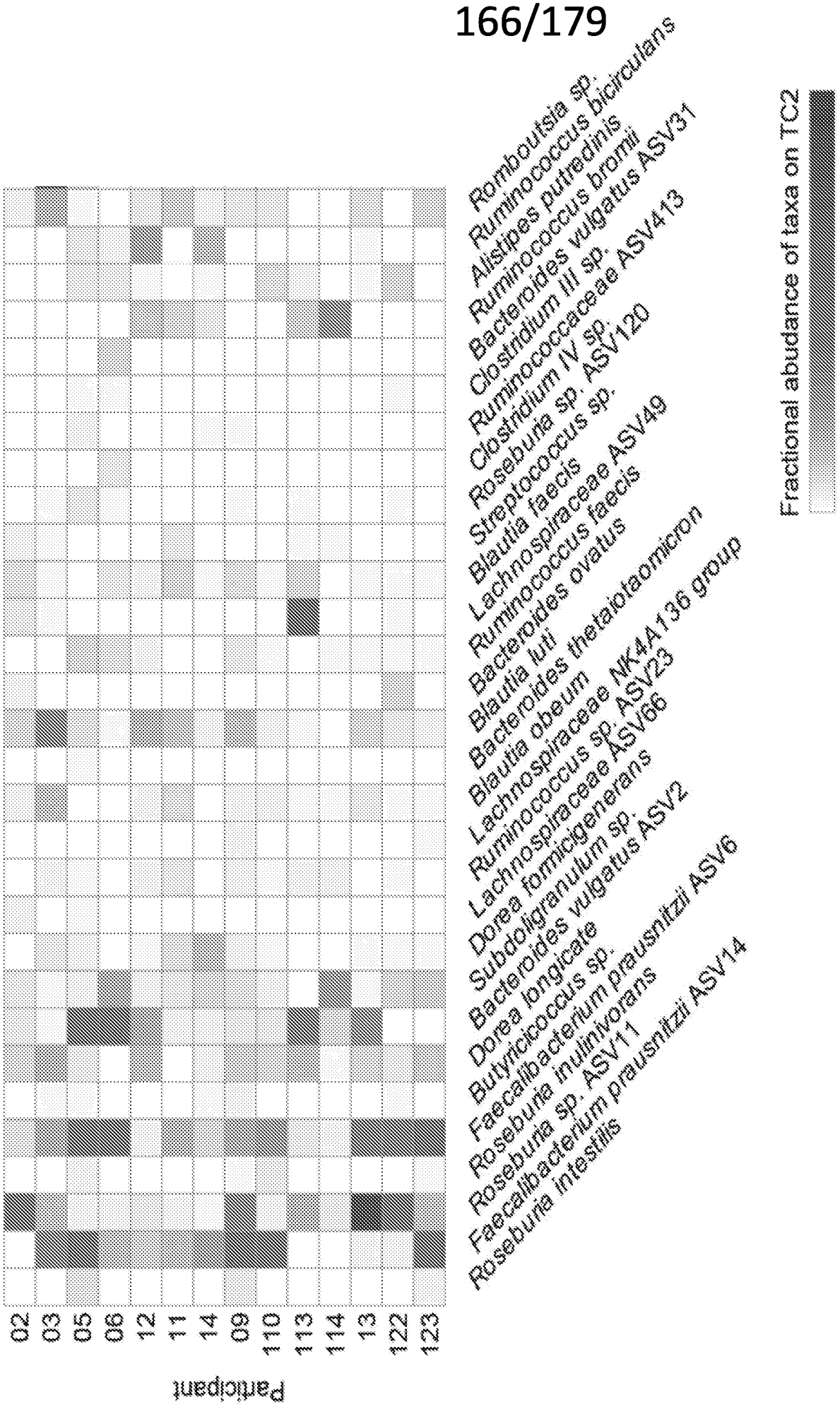

Romboutsia sp.
Ruminococcus bicirculans
Alistipes putredinis
Ruminococcus bromii
Bacteroides vulgatus ASV31
Clostridium III sp.
Ruminococcaceae ASV413
Ruminococcaceae IV sp.
Clostridium sp. ASV120
Roseburia sp.
Streptococcus sp.
Blautia faecis
Lachnospiraceae ASV49
Ruminococcus faecis
Bacteroides ovatus
Blautia luti
Bacteroides thetaiotaomicron
Blautia obeum
Lachnospiraceae NK4A136 group
Ruminococcus sp. ASV23
Lachnospiraceae ASV66
Dorea formicigenerans
Subdoligranulum sp.
Bacteroides vulgatus ASV2
Dorea longicate
Butyricicoccus sp.
Faecalibacterium prausnitzii ASV6
Roseburia inulinivorans
Roseburia sp. ASV11
Faecalibacterium prausnitzii ASV14
Roseburia intestilis
Fractional abudance of taxa on TC2
0
0.05
0.10
0.15
0.20
02
03
05
06
12
11
14
09
110
113
114
13
122
123
Participant

FIG. 43H

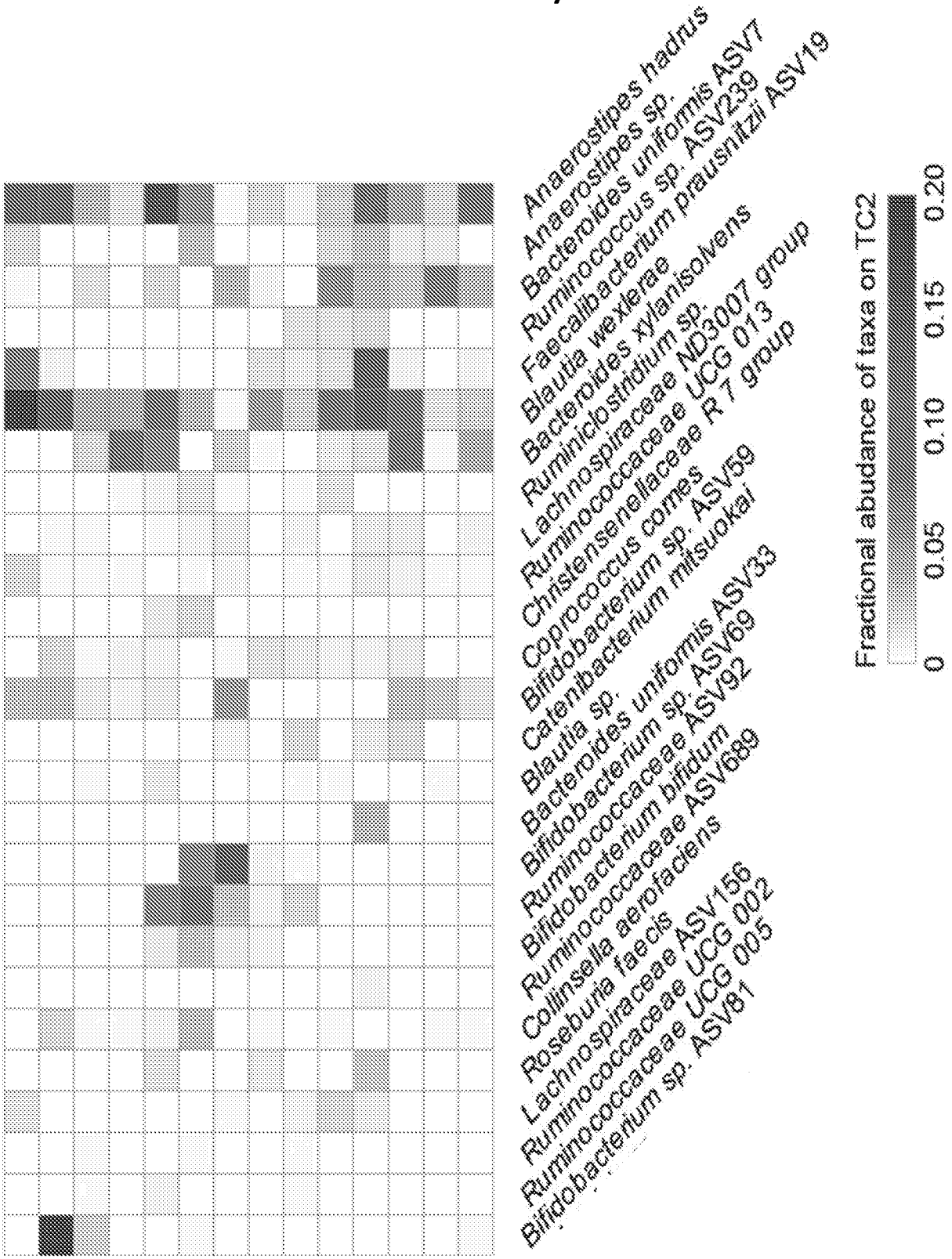

Anaerostipes hadrus
Anaerostipes sp.
Bacteroides uniformis ASV7
Ruminococcus sp. ASV239
Faecalibacterium prausnitzii ASV19
Blautia wexlerae
Bacteroides xylanisolvens
Ruminiclostridium sp.
Lachnospiraceae ND3007 group
Ruminococcaceae UCG 013
Christensenellaceae R 7 group
Coprococcus comes
Bifidobacterium sp. ASV59
Catenibacterium mitsuokai
Blautia sp.
Bacteroides uniformis ASV33
Bifidobacterium sp. ASV69
Ruminococcaceae ASV92
Bifidobacterium bifidum
Ruminococcaceae ASV689
Collinsella aerofaciens
Roseburia faecis
Lachnospiraceae ASV156
Ruminococcaceae UCG 002
Ruminococcaceae UCG 005
Bifidobacterium sp. ASV81
Fractional abudance of taxa on TC2
0
0.05
0.10
0.15
0.20

FIG. 43FI

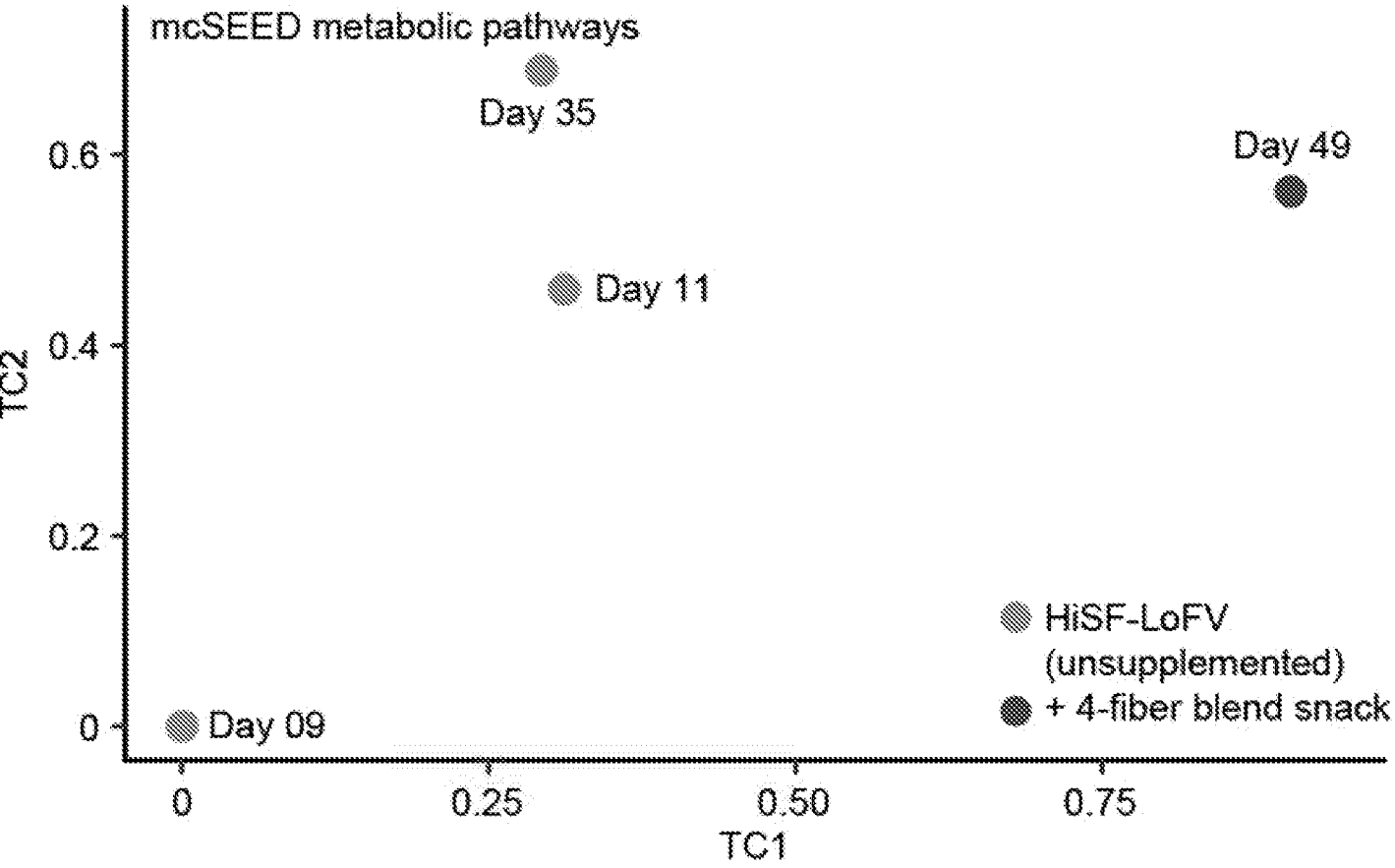

mcSEED metabolic pathways
Day 35
Day 49
Day 11
HiSF-LoFV
(unsupplemented)
+ 4-fiber blend snack
Day 09
0.6
0.4
0.2
0
TC2
0
0.25
0.50
0.75
TC1

FIG. 44A

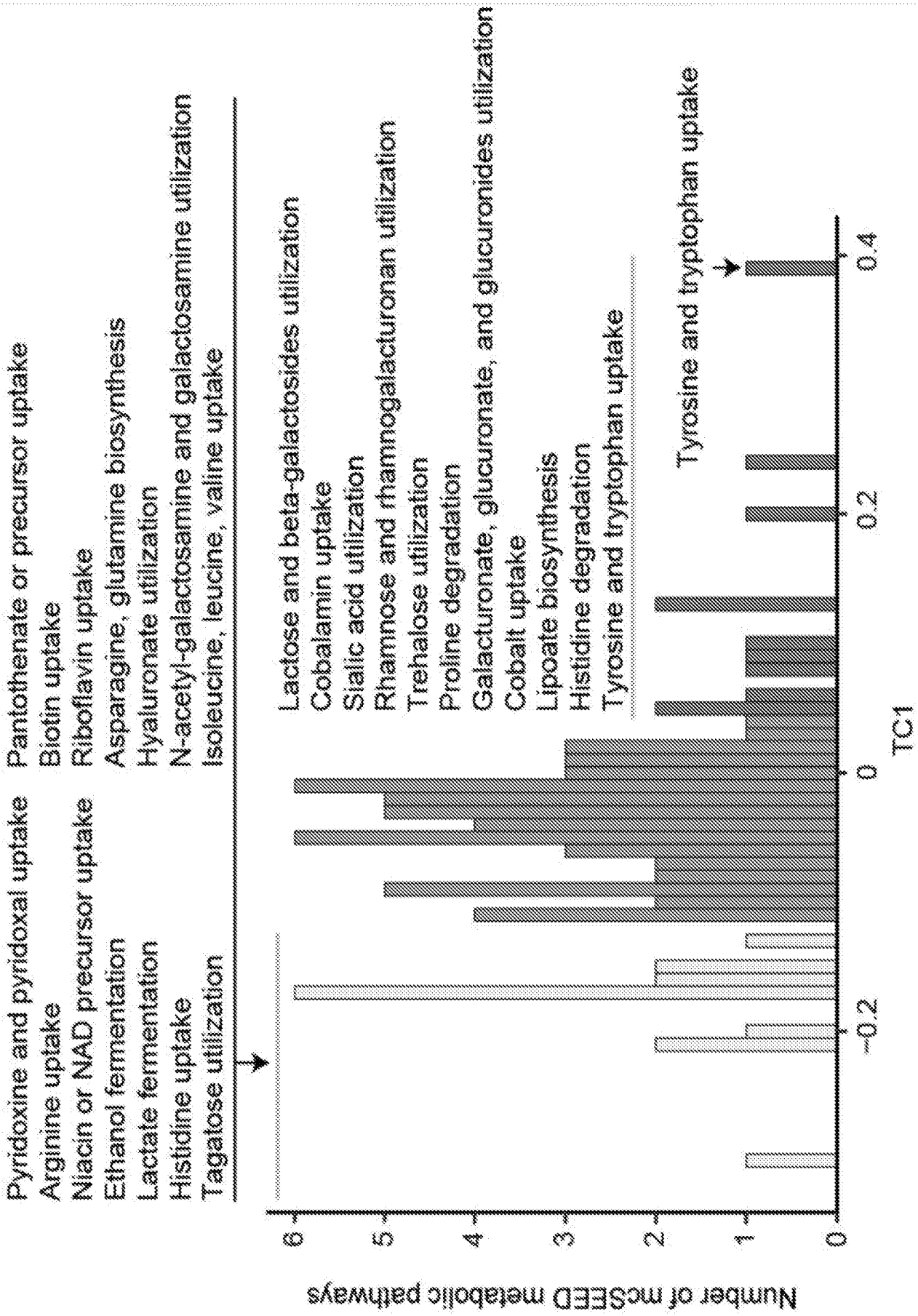

Pyridoxine and pyridoxal uptake
Arginine uptake
Niacin or NAD precursor uptake
Ethanol fermentation
Lactate fermentation
Histidine uptake
Tagatose utilization
Pantothenate or precursor uptake
Biotin uptake
Riboflavin uptake
Asparagine, glutamine biosynthesis
Hyaluronate utilization
N-acetyl-galactosamine and galactosamine utilization
Isoleucine, leucine, valine uptake
Lactose and beta-galactosides utilization
Cobalamin uptake
Sialic acid utilization
Rhamnose and rhamnogalacturonan utilization
Trehalose utilization
Proline degradation
Galacturonate, glucuronate, and glucuronides utilization
Cobalt uptake
Lipoate biosynthesis
Histidine degradation
Tyrosine and tryptophan uptake
Tyrosine and tryptophan uptake
Number of mcSEED metabolic pathways
0
1
2
3
4
5
6
−0.2
0
0.2
0.4
TC1

FIG. 44B

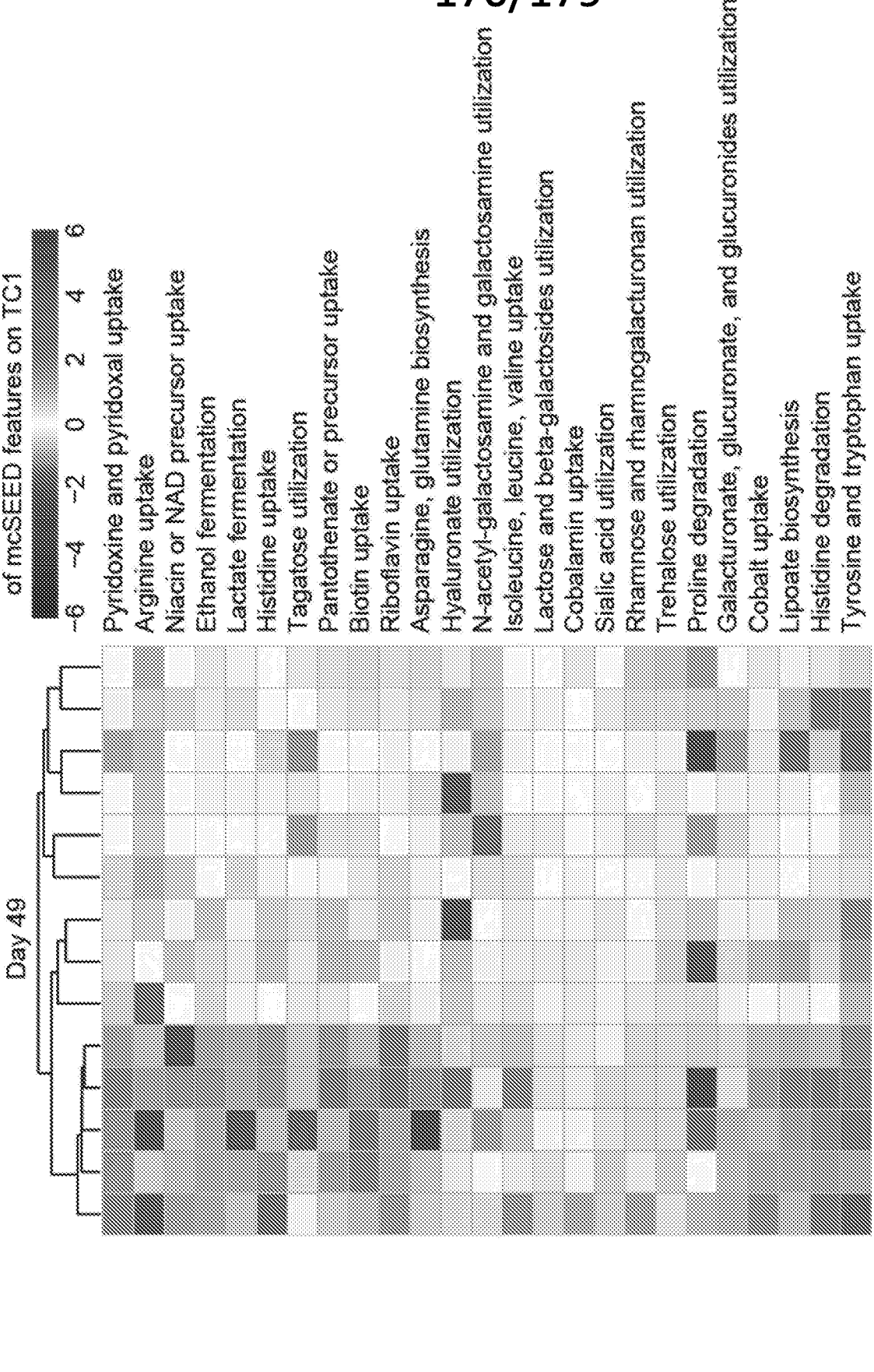

Log2 fold-change
of mcSEED features on TC1
−6 −4 −2 0 2 4 6
Day 49
Pyridoxine and pyridoxal uptake
Arginine uptake
Niacin or NAD precursor uptake
Ethanol fermentation
Lactate fermentation
Histidine uptake
Tagatose utilization
Pantothenate or precursor uptake
Biotin uptake
Riboflavin uptake
Asparagine, glutamine biosynthesis
Hyaluronate utilization
N-acetyl-galactosamine and galactosamine utilization
Isoleucine, leucine, valine uptake
Lactose and beta-galactosides utilization
Cobalamin uptake
Sialic acid utilization
Rhamnose and rhamnogalacturonan utilization
Trehalose utilization
Proline degradation
Galacturonate, glucuronate, and glucuronides utilization
Cobalt uptake
Lipoate biosynthesis
Histidine degradation
Tyrosine and tryptophan uptake
Participant 08 10 02 14 21 13 06 23 03 04 05 09 01 22

FIG. 44C

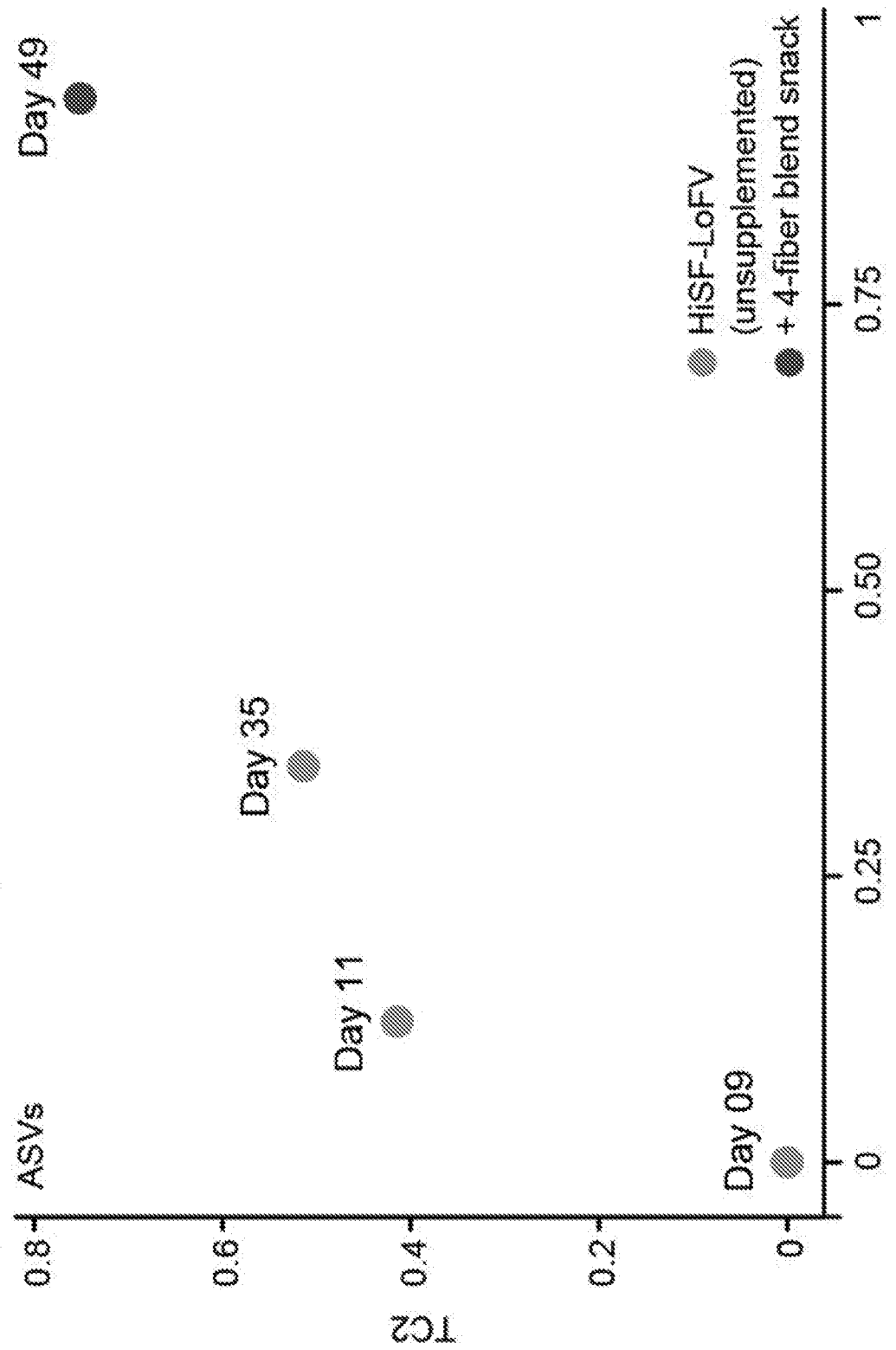

ASVs
Day 49
Day 35
Day 11
Day 09
HiSF-LoFV (unsupplemented)
+ 4-fiber blend snack
TC1
TC2
0
0.25
0.50
0.75
1
0
0.2
0.4
0.6
0.8

FIG. 44D

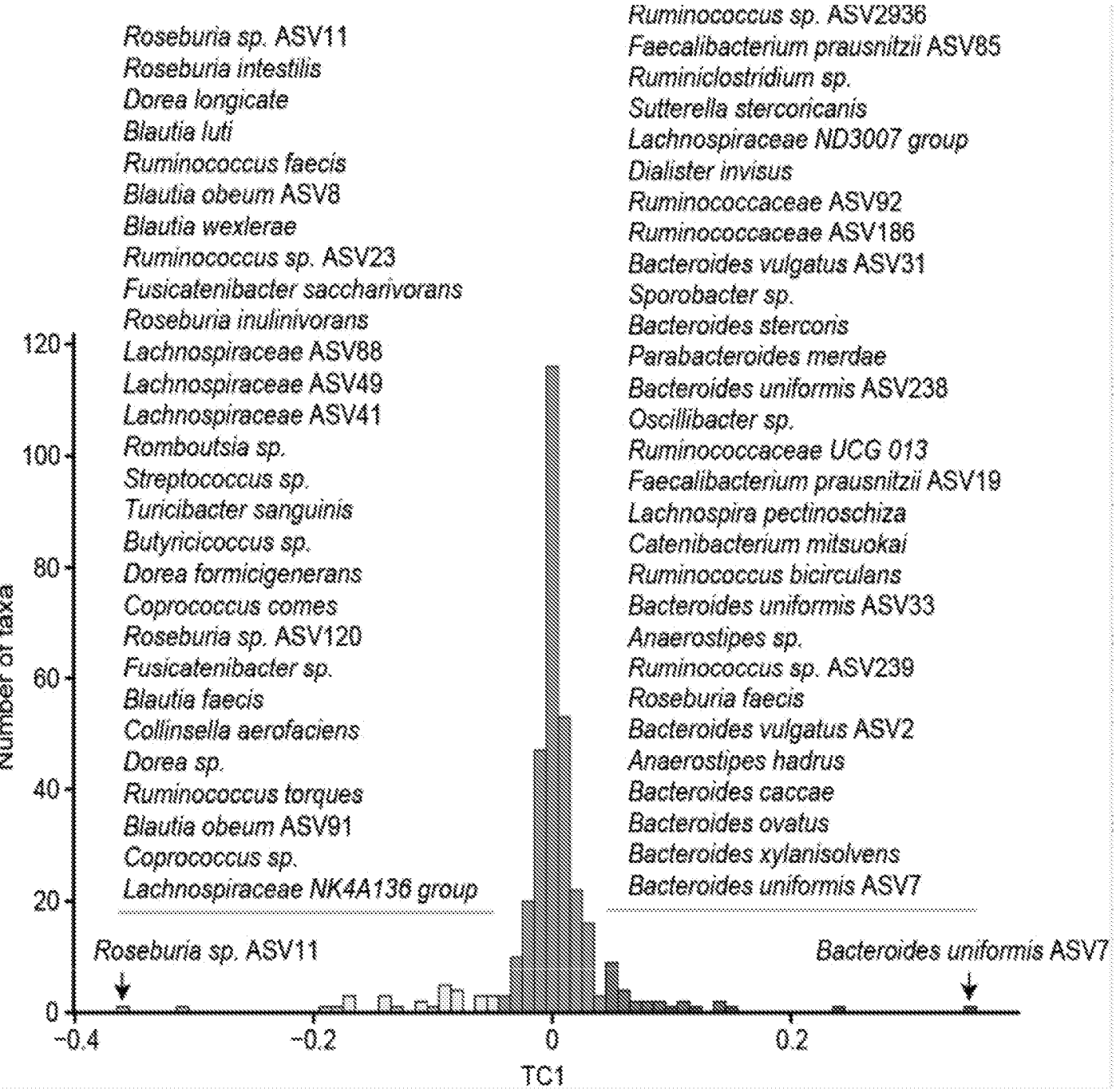

Roseburia sp. ASV11
Roseburia intestilis
Dorea longicate
Blautia luti
Ruminococcus faecis
Blautia obeum ASV8
Blautia wexlerae
Ruminococcus sp. ASV23
Fusicatenibacter saccharivorans
Roseburia inulinivorans
Lachnospiraceae ASV88
Lachnospiraceae ASV49
Lachnospiraceae ASV41
Romboutsia sp.
Streptococcus sp.
Turicibacter sanguinis
Butyricicoccus sp.
Dorea formicigenerans
Coprococcus comes
Roseburia sp. ASV120
Fusicatenibacter sp.
Blautia faecis
Collinsella aerofaciens
Dorea sp.
Ruminococcus torques
Blautia obeum ASV91
Coprococcus sp.
Lachnospiraceae NK4A136 group
Ruminococcus sp. ASV2936
Faecalibacterium prausnitzii ASV85
Ruminiclostridium sp.
Sutterella stercoricanis
Lachnospiraceae ND3007 group
Dialister invisus
Ruminococcaceae ASV92
Ruminococcaceae ASV186
Bacteroides vulgatus ASV31
Sporobacter sp.
Bacteroides stercoris
Parabacteroides merdae
Bacteroides uniformis ASV238
Oscillibacter sp.
Ruminococcaceae UCG 013
Faecalibacterium prausnitzii ASV19
Lachnospira pectinoschiza
Catenibacterium mitsuokai
Ruminococcus bicirculans
Bacteroides uniformis ASV33
Anaerostipes sp.
Ruminococcus sp. ASV239
Roseburia faecis
Bacteroides vulgatus ASV2
Anaerostipes hadrus
Bacteroides caccae
Bacteroides ovatus
Bacteroides xylanisolvens
Bacteroides uniformis ASV7
Roseburia sp. ASV11
Bacteroides uniformis ASV7
Number of taxa
120
100
80
60
40
20
0
−0.4
−0.2
0
0.2
TC1

FIG. 44E

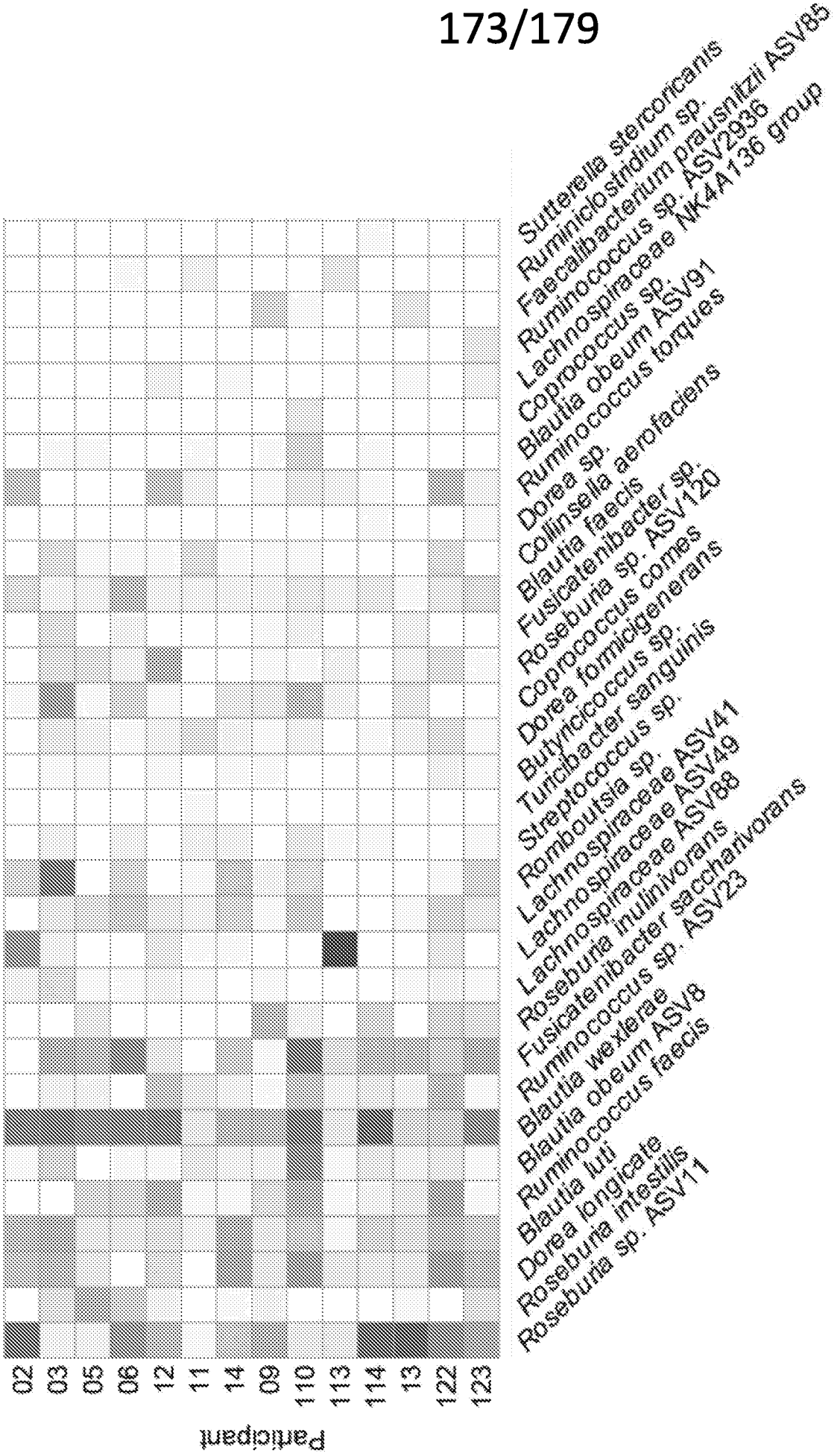

Sutterella stercoricanis
Ruminiclostridium sp.
Faecalibacterium prausnitzii ASV85
Ruminococcus sp. ASV2936
Lachnospiraceae NK4A136 group
Coprococcus sp.
Blautia obeum ASV91
Ruminococcus torques
Dorea sp.
Collinsella aerofaciens
Blautia faecis
Fusicatenibacter sp.
Roseburia sp. ASV120
Coprococcus comes
Roseburia cf. inulinivorans
Dorea formicigenerans
Butyricicoccus sp.
Turicibacter sanguinis
Streptococcus sp.
Romboutsia sp. ASV41
Lachnospiraceae ASV49
Lachnospiraceae ASV88
Roseburia inulinivorans
Fusicatenibacter saccharivorans
Ruminococcus sp. ASV23
Blautia wexlerae
Blautia obeum ASV8
Ruminococcus faecis
Blautia luti
Dorea longicate
Roseburia intestilis
Roseburia sp. ASV11
02
03
05
06
12
11
14
09
110
113
114
13
122
123
Participant

FIG. 44F

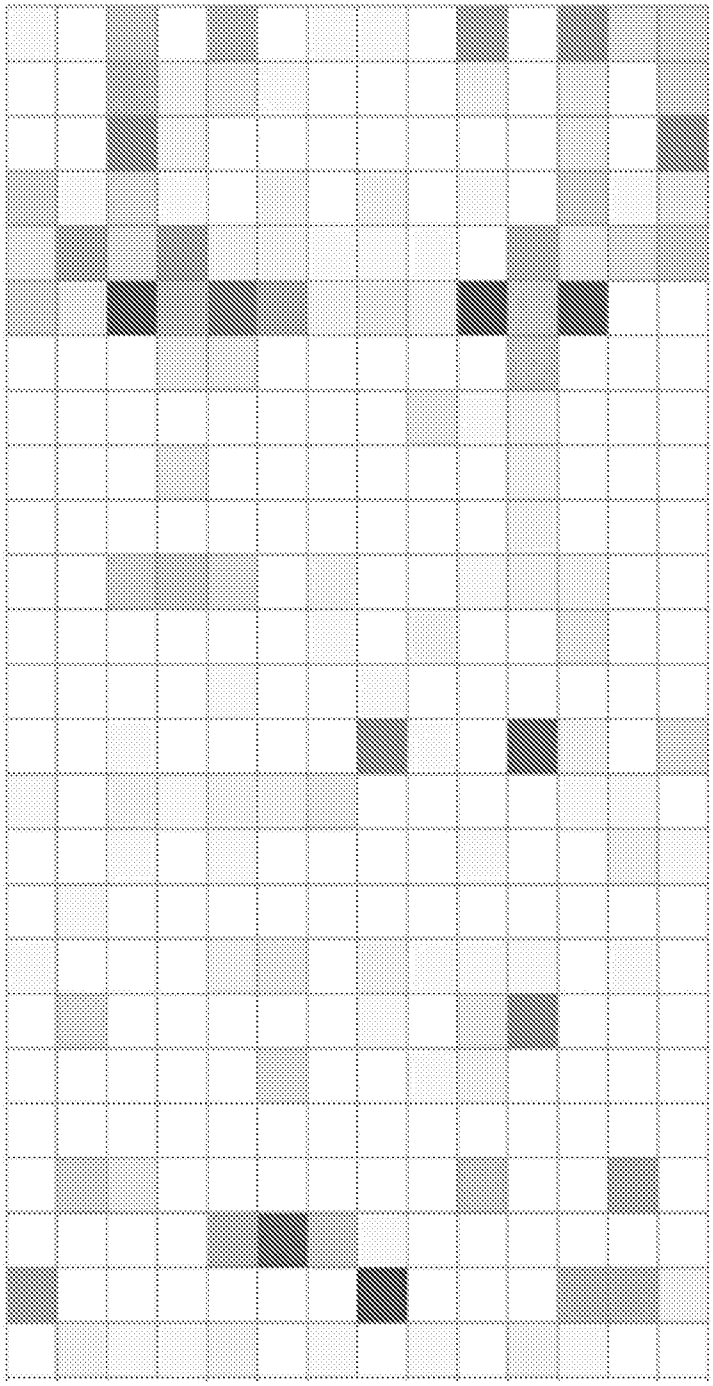

Bacteroides uniformis ASV7
Bacteroides xylanisolvens
Bacteroides ovatus
Bacteroides caccae
Anaerostipes hadrus
Bacteroides vulgatus ASV2
Roseburia faecis
Ruminococcus sp. ASV239
Anaerostipes sp.
Bacteroides uniformis ASV33
Ruminococcus bicirculans
Catenibacterium mitsuokai
Lachnospira pectinoschiza
Faecalibacterium prausnitzii ASV19
Ruminococcaceae UCG 013
Oscillibacter sp.
Bacteroides uniformis ASV238
Parabacteroides merdae
Bacteroides stercoris
Sporobacter sp.
Bacteroides vulgatus ASV31
Ruminococcaceae ASV186
Dialister invisus
Ruminococcaceae ASV92
Lachnospiraceae ND3007 group

FIG. 44G

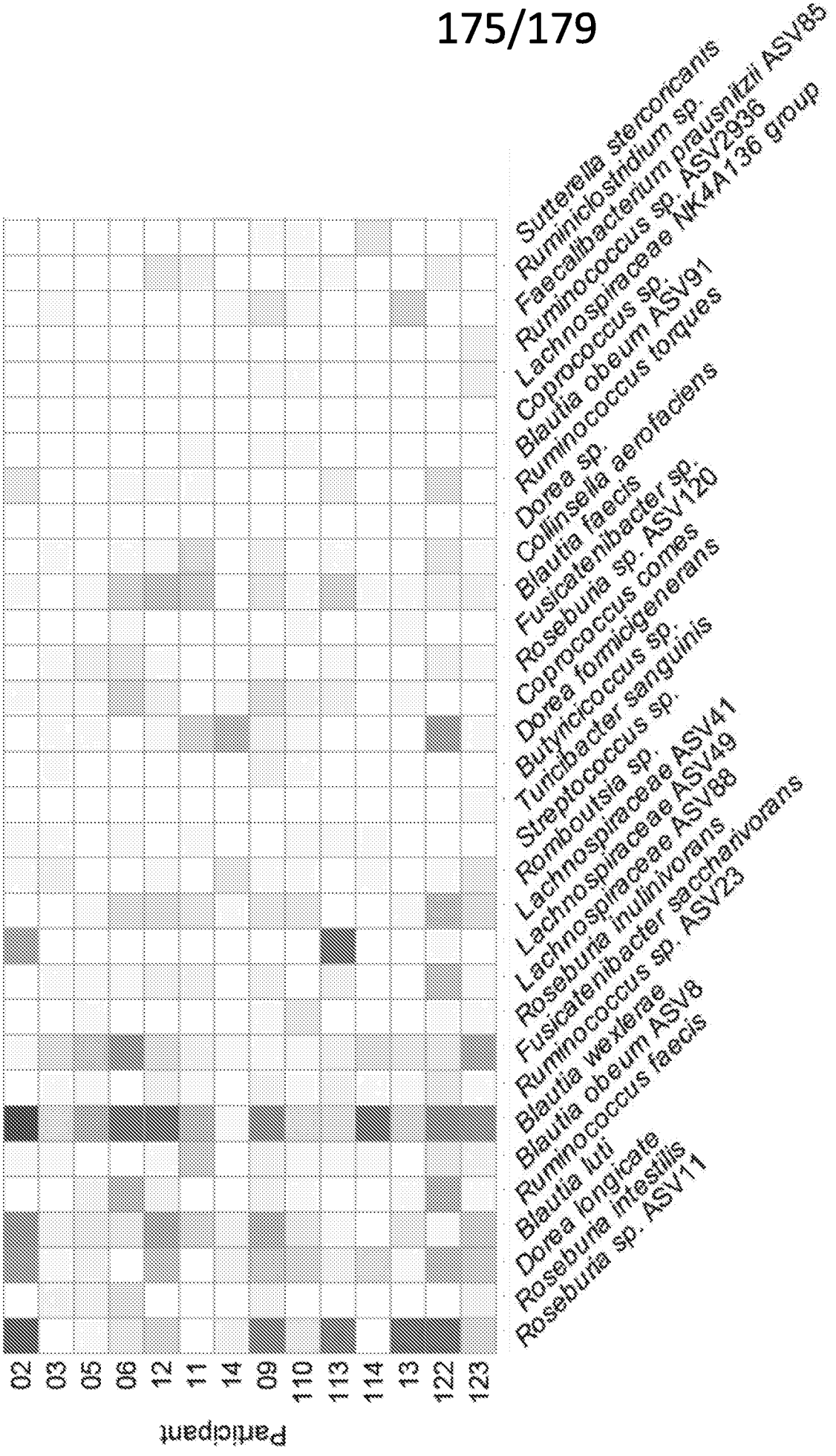

Sutterella stercoricanis
Ruminiclostridium sp.
Faecalibacterium prausnitzii ASV85
Ruminococcus sp. ASV2936
Lachnospiraceae NK4A136 group
Coprococcus sp.
Blautia obeum ASV91
Ruminococcus torques
Dorea sp.
Collinsella aerofaciens
Blautia faecis
Fusicatenibacter sp.
Roseburia sp. ASV120
Coprococcus comes
Roseburia cornes
Dorea formicigenerans
Butyricicoccus sp.
Turicibacter sanguinis
Streptococcus sp.
Romboutsia sp. ASV41
Lachnospiraceae ASV49
Lachnospiraceae ASV88
Roseburia inulinivorans
Fusicatenibacter saccharivorans
Ruminococcus sp. ASV23
Blautia wexlerae
Blautia obeum ASV8
Ruminococcus faecis
Blautia luti
Dorea longicate
Roseburia intestilis
Roseburia sp. ASV11
02
03
05
06
12
11
14
09
110
113
114
13
122
123
Participant

FIG. 44H

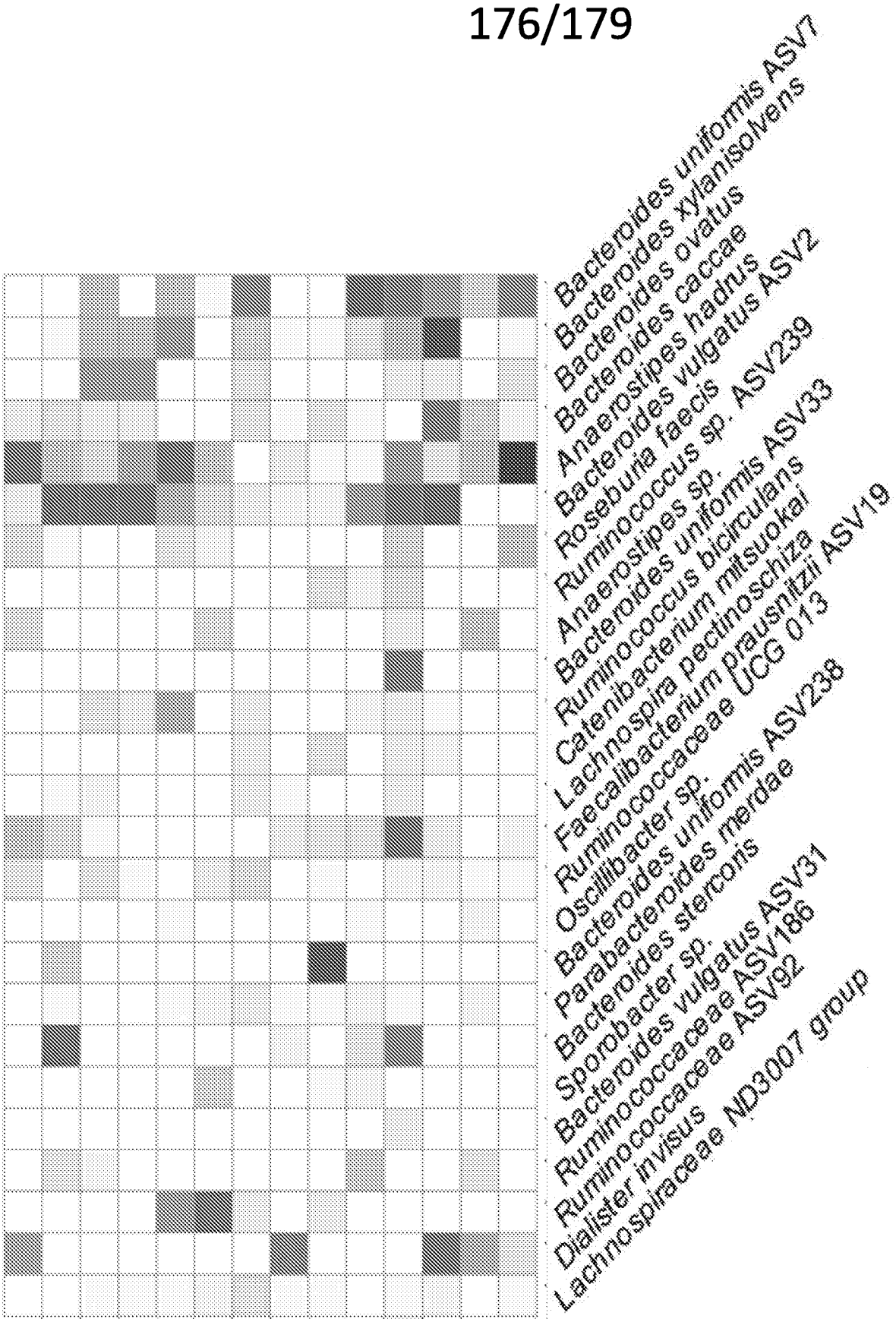

Bacteroides uniformis ASV7
Bacteroides xylanisolvens
Bacteroides ovatus
Bacteroides caccae
Anaerostipes hadrus
Bacteroides vulgatus ASV2
Roseburia faecis
Ruminococcus sp. ASV239
Anaerostipes sp.
Bacteroides uniformis ASV33
Ruminococcus bicirculans
Catenibacterium mitsuokai
Lachnospira pectinoschiza
Faecalibacterium prausnitzii ASV19
Ruminococcaceae UCG 013
Oscillibacter sp.
Ruminococcaceae ASV238
Bacteroides uniformis
Parabacteroides merdae
Bacteroides stercoris
Sporobacter sp.
Bacteroides vulgatus ASV31
Ruminococcaceae ASV186
Dialister invisus
Ruminococcaceae ASV92
Lachnospiraceae ND3007 group

FIG. 44I

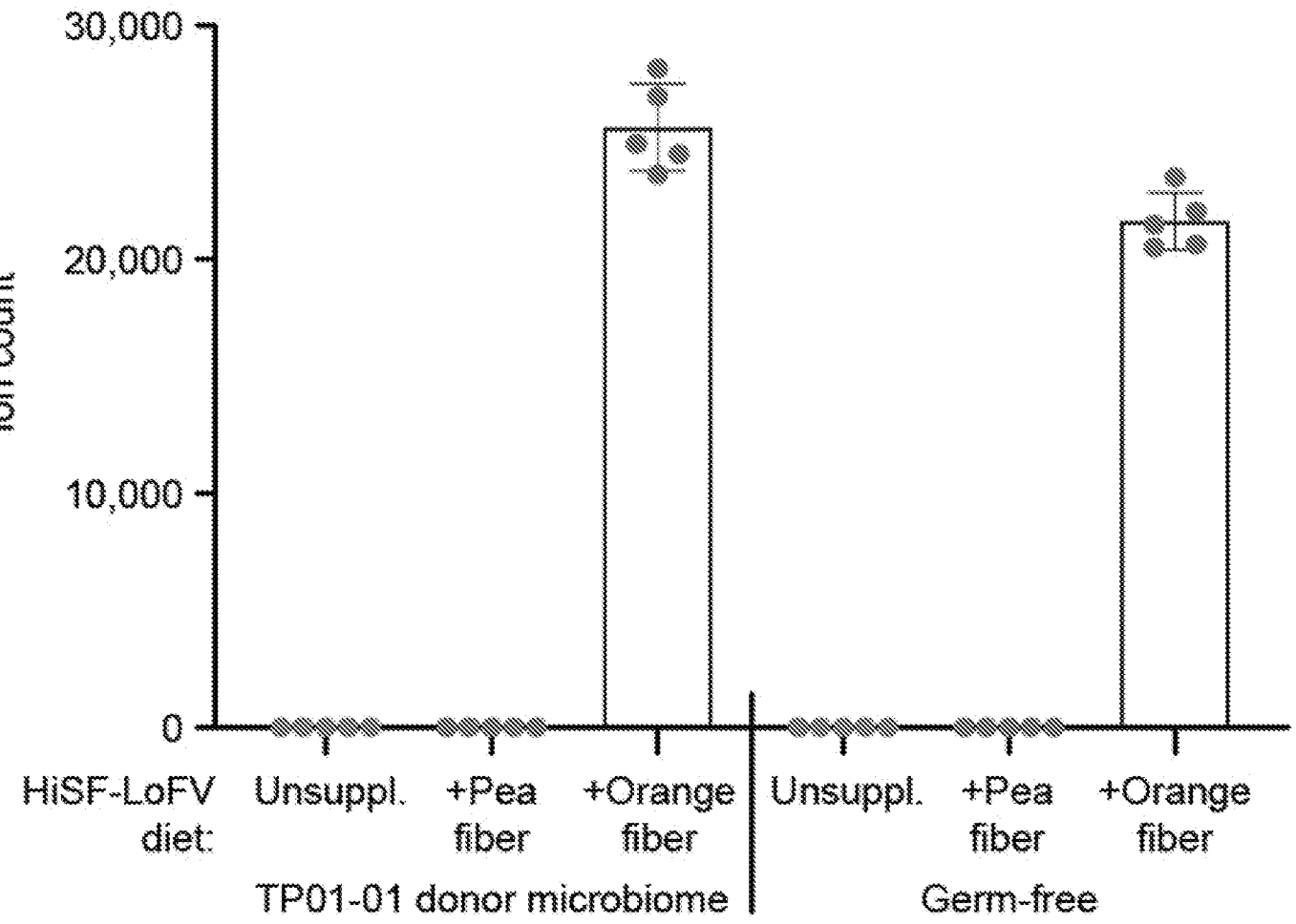

30,000
20,000
10,000
0
HiSF-LoFV diet:
Unsuppl.
+Pea fiber
+Orange fiber
Unsuppl.
+Pea fiber
+Orange fiber
TP01-01 donor microbiome
Germ-free

FIG. 45A

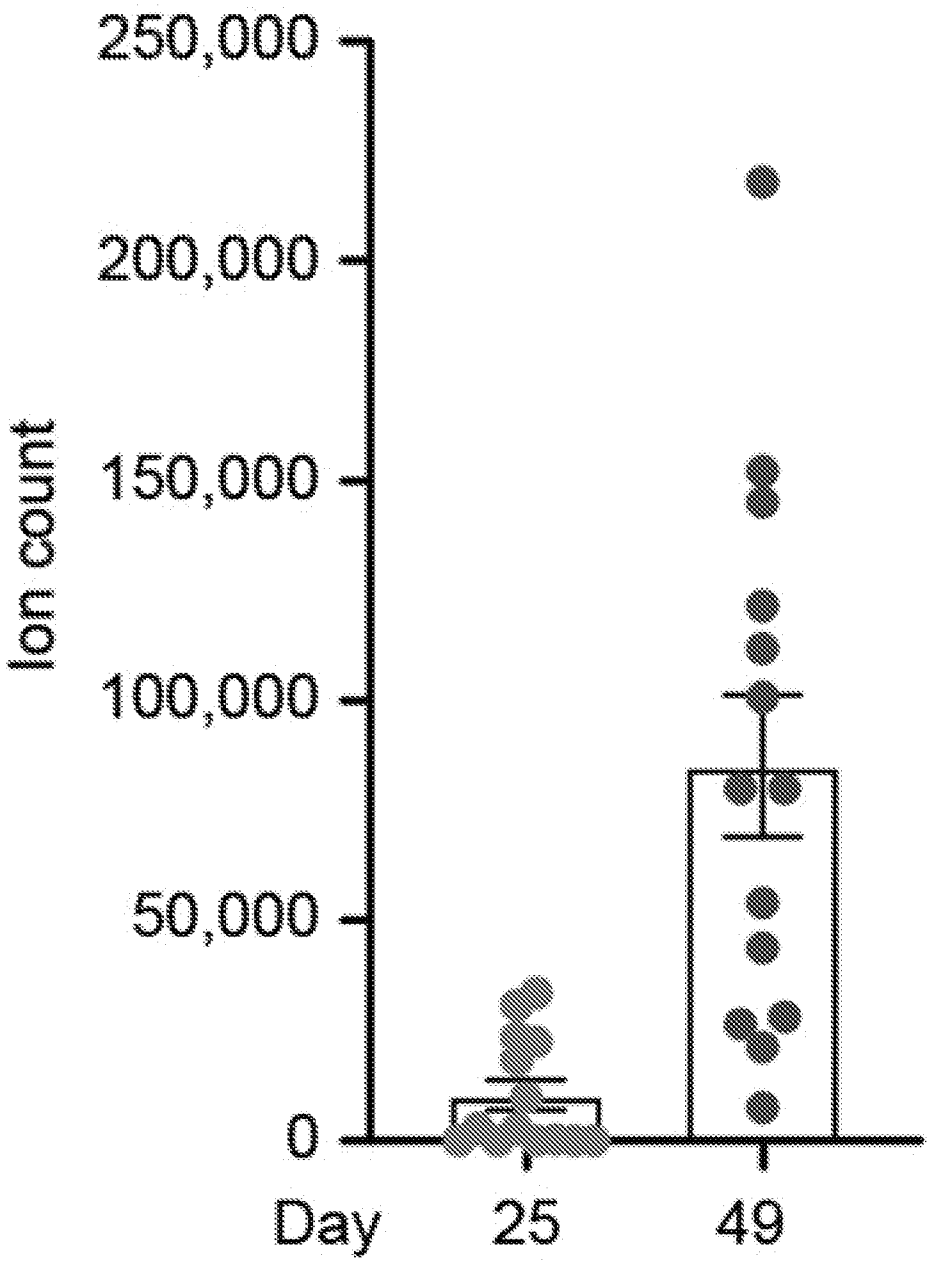


FIG. 45B

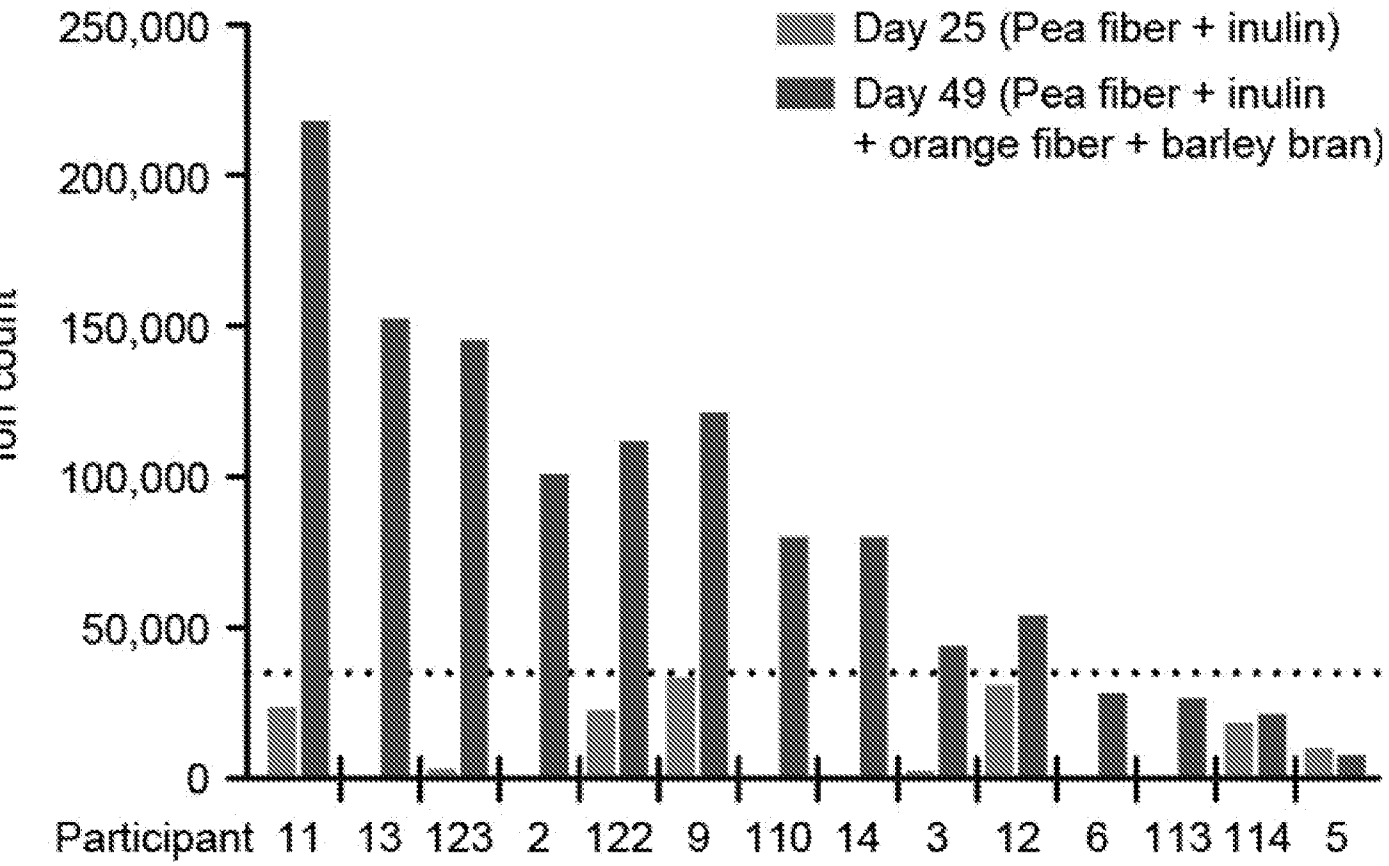

Day 25 (Pea fiber + inulin)
Day 49 (Pea fiber + inulin + orange fiber + barley bran)
250,000
200,000
150,000
100,000
50,000
0
Participant 11 13 123 2 122 9 110 14 3 12 6 113 114 5

FIG. 45C

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 20/42669

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61B 10/00; A61B 5/00; A61B 5/07 (2020.01)

CPC - A61B 10/0038; A61B 10/0045; A61B 10/04; A61B 5/073

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 5,972,415 A (Brassart et al.) 26 October 1999 (26.10.1999) entire document especially Col 5, Line 16-17	1-3 and 10 --- 4, 11 and 12
Y	US 2005/0118326 A1 (Anfinsen et al.) 02 June 2005 (02.06.2005) entire document especially Para [0199]	4 and 11
Y	US 2015/0366256 A1 (CARGILL, INCORPORATED) 24 December 2015 (24.12.2015) entire document especially Para [0019]	12
A	US 2013/0344010 A1 (Pompejus) 26 December 2013 (26.12.2013) entire document	1-4 and 10-12
A	US 6,254,913 B1 (Wadsworth et al.) 03 July 2001 (03.07.2001) entire document	1-4 and 10-12
A	US 2015/0086686 A1 (Access Business Group International LLC) 26 March 2015 (26.03.2015) entire document	1-4 and 10-12
A	US 5,085,883 A (Garleb et al.) 04 February 1992 (04.02.1992) entire document	1-4 and 10-12
A,P -	"Inulin" Wikipedia, Wikimedia Foundation, 12 Sepetember 2020 (12.09.2020) entire document especially Page 2 Para 2 Retrieved from en.wikipedia.org/wiki/Inulin.	1, 3, and 10

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 September 2020	14 OCT 2020
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Lee Young Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 20/42669

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 5-9 and 13-46
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.