US 20170360848A1

(54) **METHODS FOR TREATING AUTISM SPECTRUM DISORDER AND ASSOCIATED SYMPTOMS**

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Publication Classification

(57) **ABSTRACT**

The present disclosure relates to compositions and methods for treating autism spectrum disorder (ASD) by restoring an ASD patient's gut microbiota. These methods can be used with ASD patient with or without ongoing gastrointestinal symptoms. Provided here is a method for modulating the abundance of one or more gut microorganisms in a subject in need thereof, said method comprising administering to said subject a therapeutically effective amount of a pharmaceutical composition comprising a fecal microbe preparation to achieve at least a 2-fold change of the abundance of said one or more gut microorganisms, wherein said one or more gut microorganisms are selected from the group consisting of *Bifidobacterium, Prevotella, Desulfovibrio, Copprococcus, Clostridium*, and *Bacteroides*.

FIG. 1
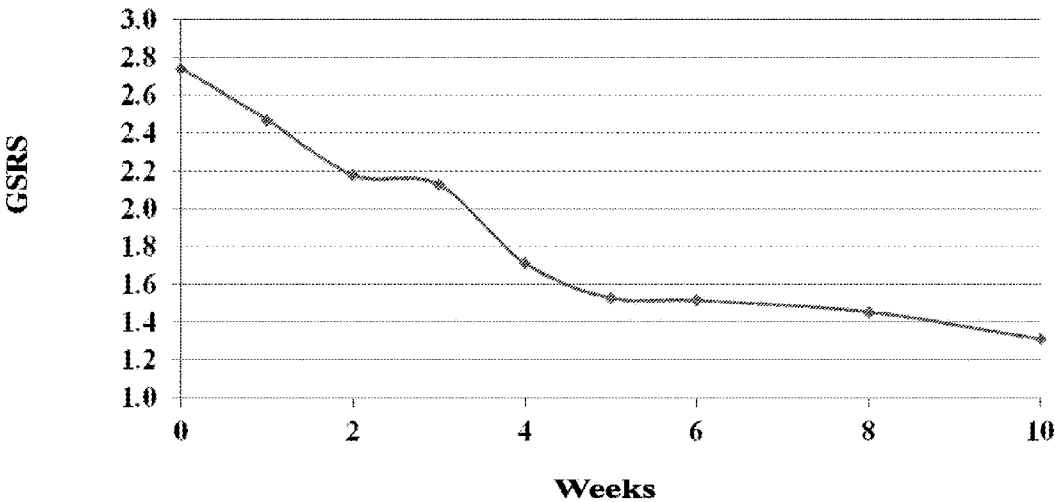
FIG. 2
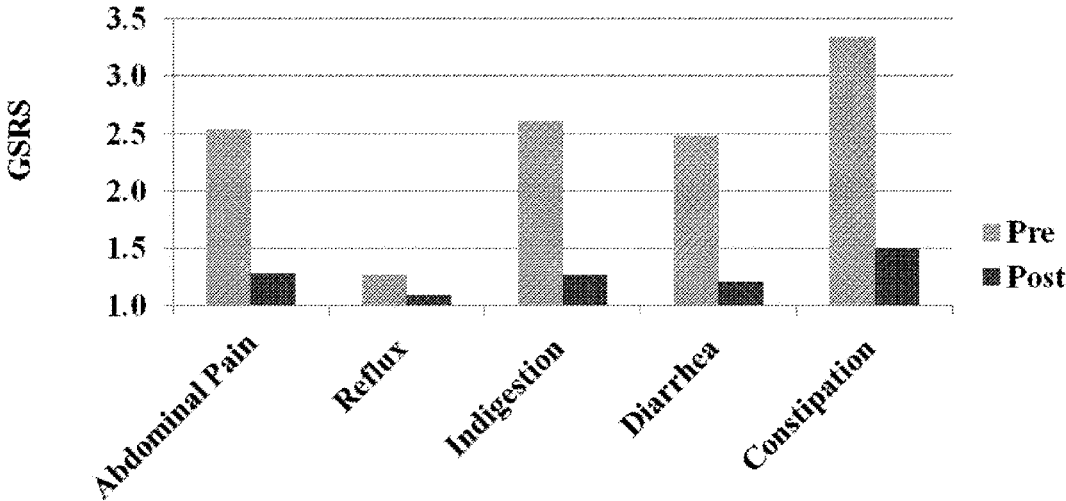
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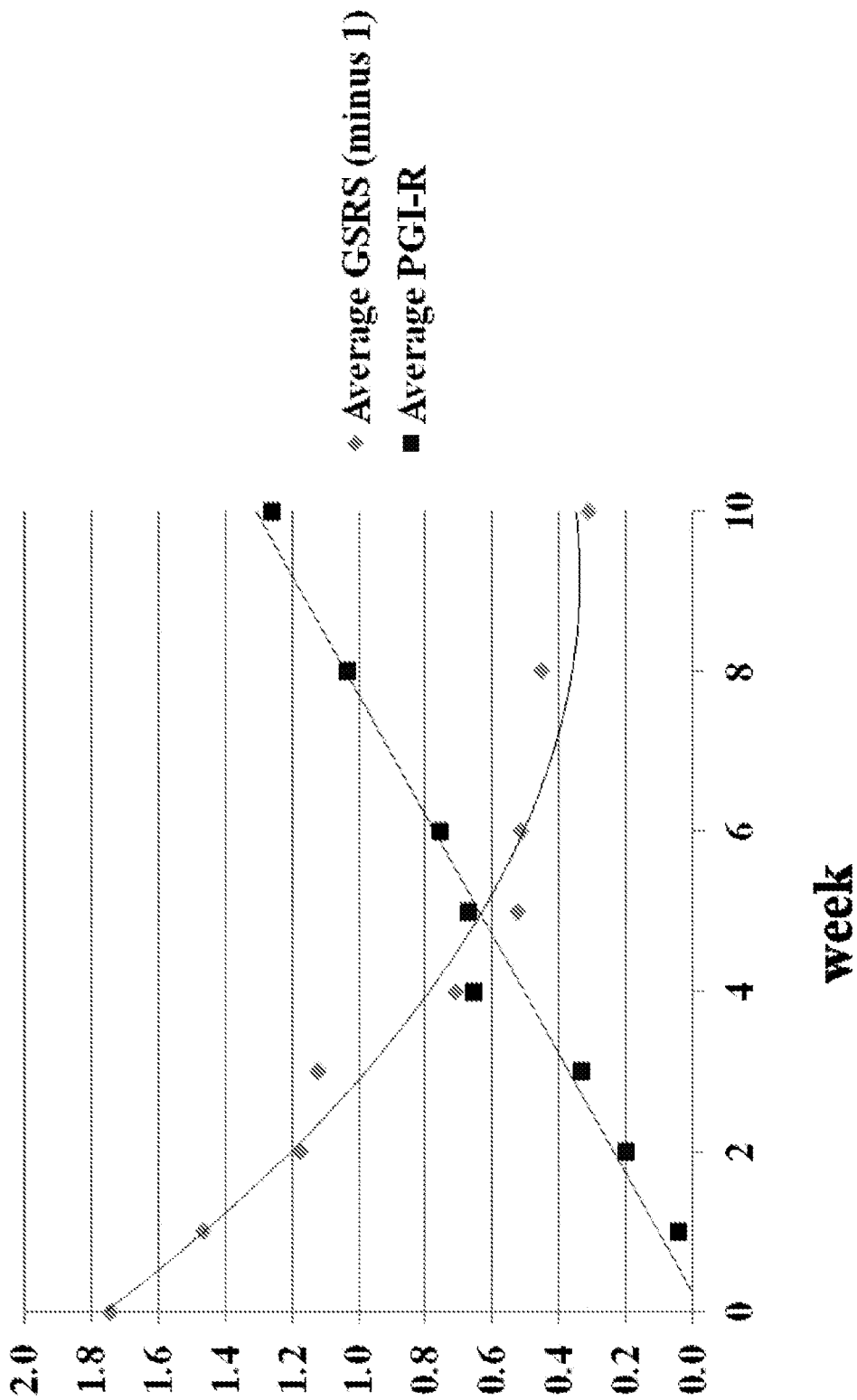
FIG. 4

	% Change	p-value
Irritability	-25%	0.03
Lethargy	-33%	0.003
Stereotopy	-21%	0.03
Hyperactivity	-28%	0.0005
Inaprorpriate Speech	-22%	0.01
Total ABC	-27%	0.001

FIG. 5

FIG. 6

Change in CARS at week 10 vs. Age

FIG. 7

PGI-R at week 10 vs. Age

FIG. 8

Change in CARS (week 10) vs. GSRS (baseline)
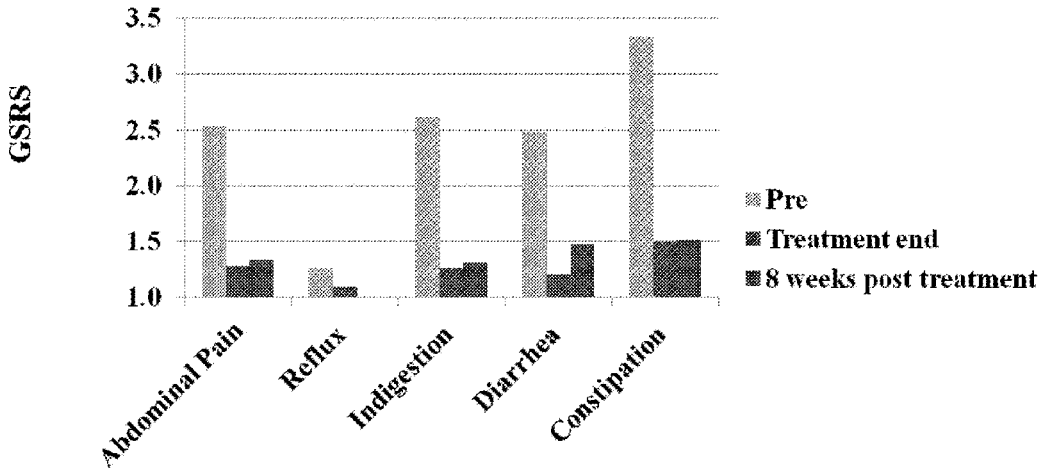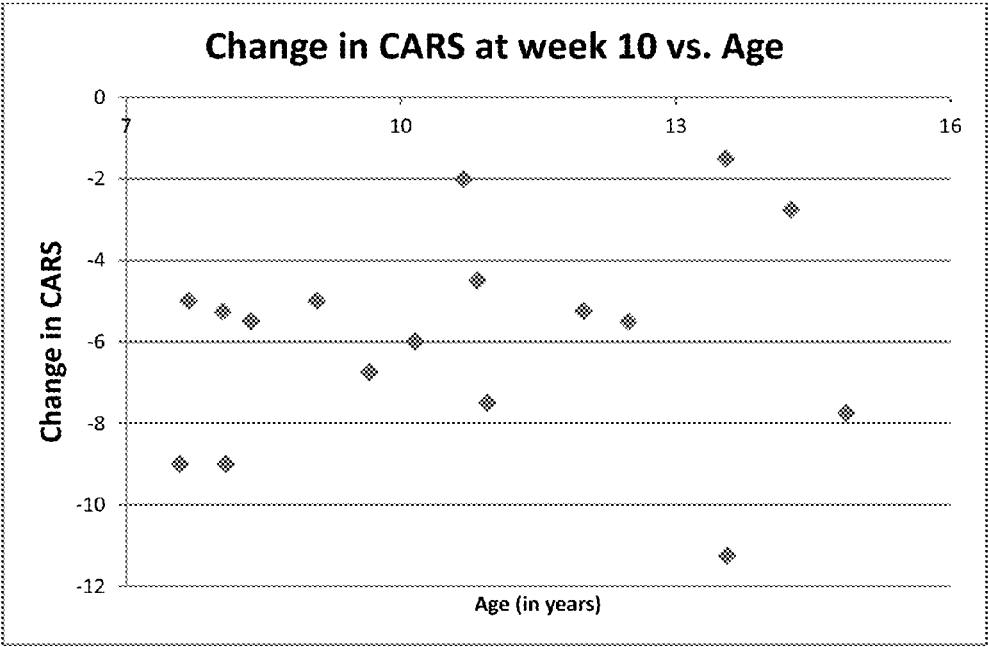
FIG. 9 (panel a)
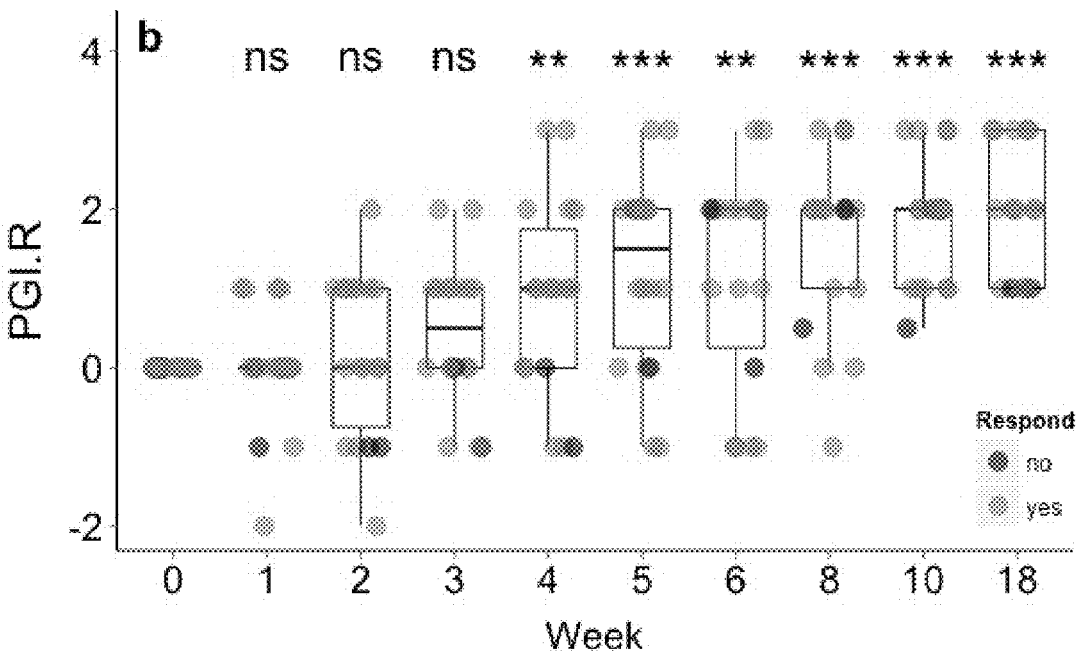
FIG. 9 (panel b)

FIG. 9 (panel c)

FIG. 9 (panel d)

FIG. 9 (panel e)

FIG. 10
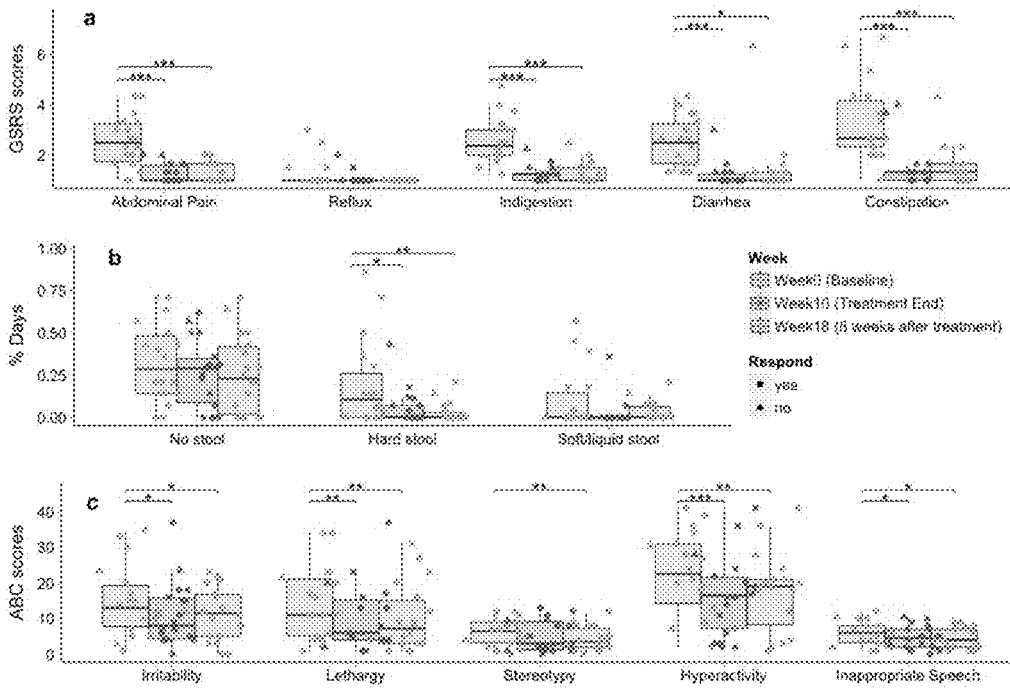
FIG. 11
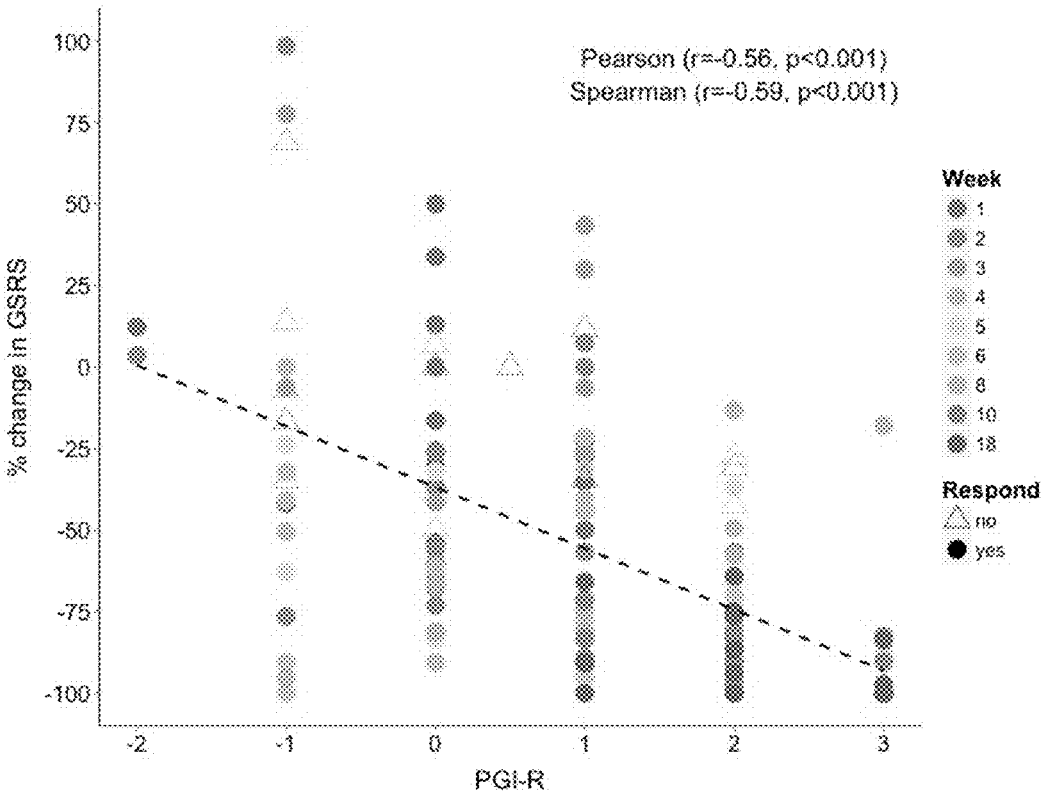
FIG. 12

FIG. 13

FIG. 14 (panel a)

FIG. 14 (panel b)

FIG. 14 (panel c)

FIG. 14 (panel d)

FIG. 14 (panel e)

FIG. 14 (panel f)

FIG. 14 (panel g)

FIG. 14 (panel h)

Bacteroides

FIG. 15

FIG. 16

FIG. 17

FIG. 18 (panel a)

a

FIG. 18 (panel b)

FIG. 18 (panel c)

FIG. 18 (panel d)

FIG. 19 (panel a)

FIG. 19 (panel b)

FIG. 19 (panel c)

FIG. 19 (panel d)

FIG. 20
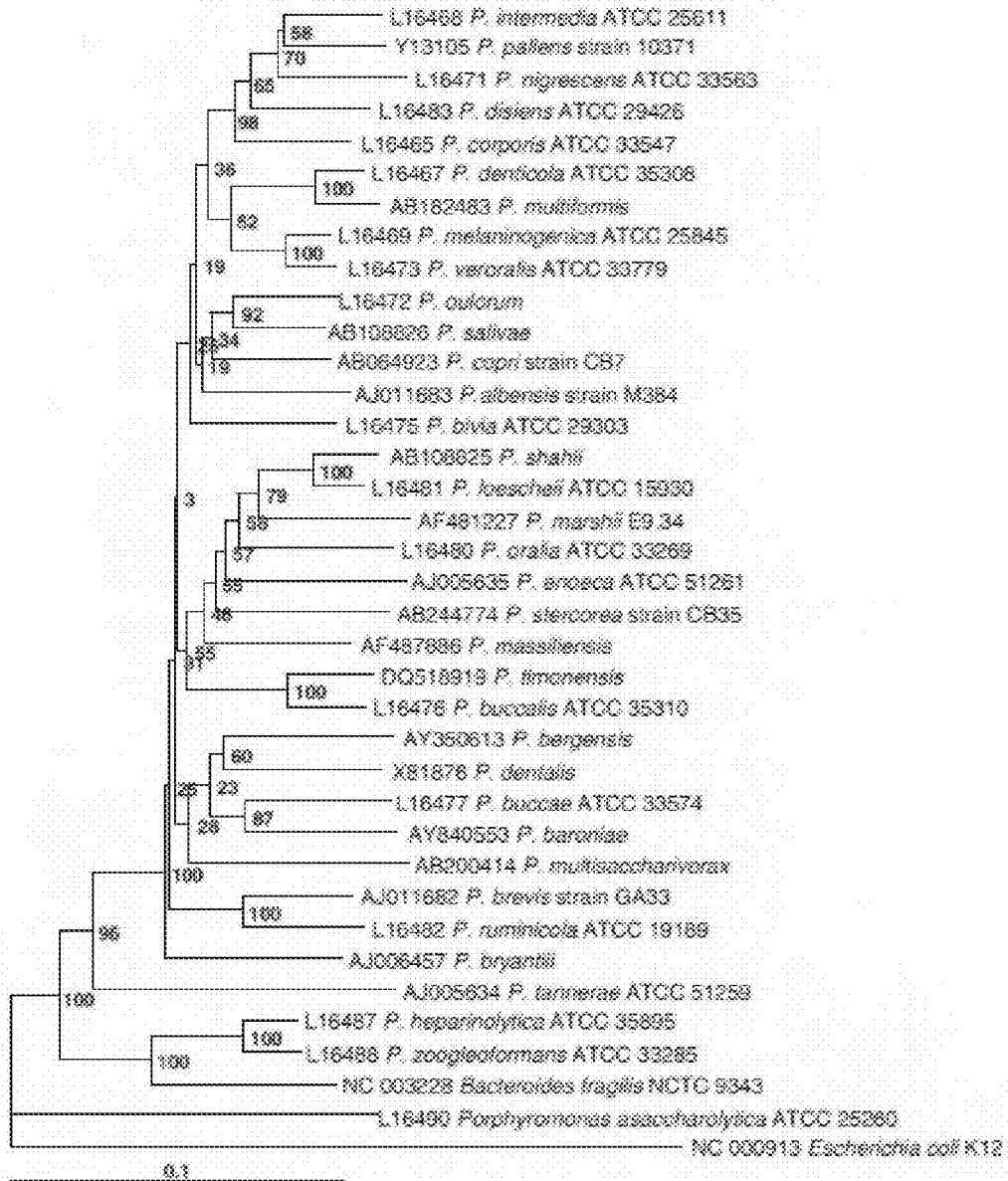
FIG. 21

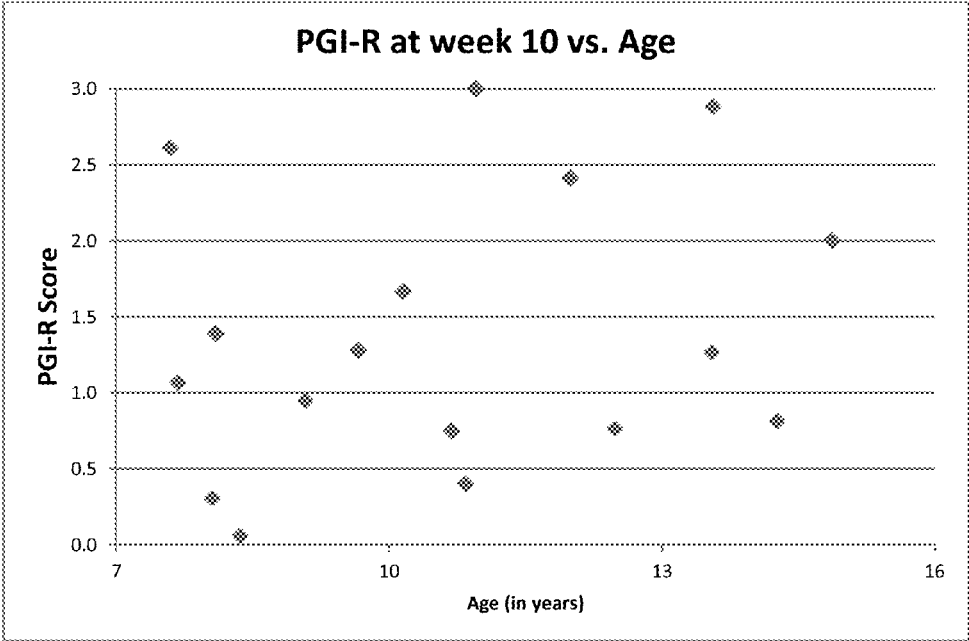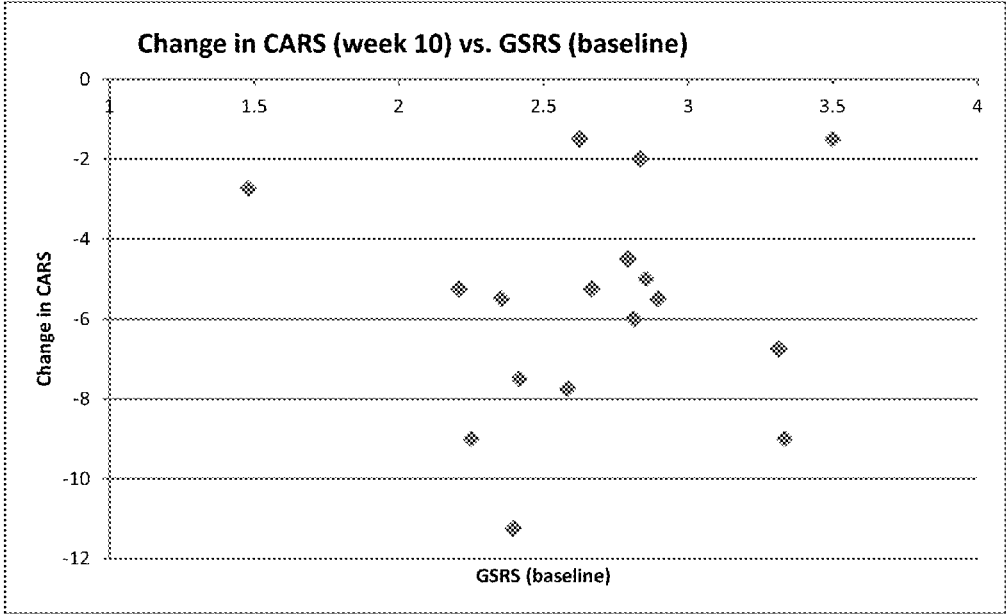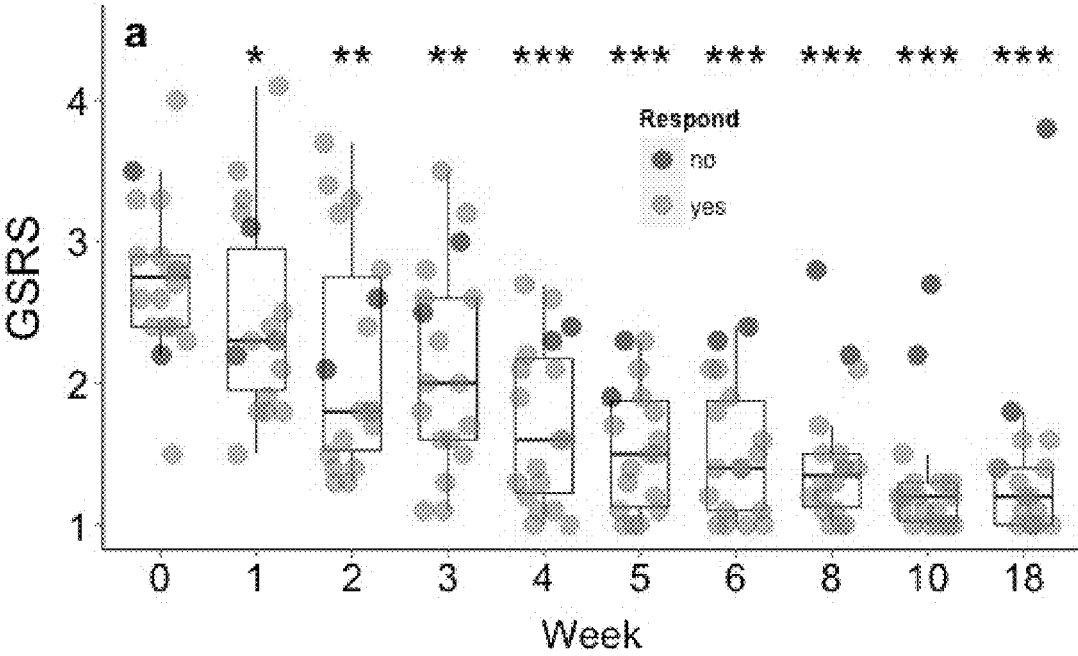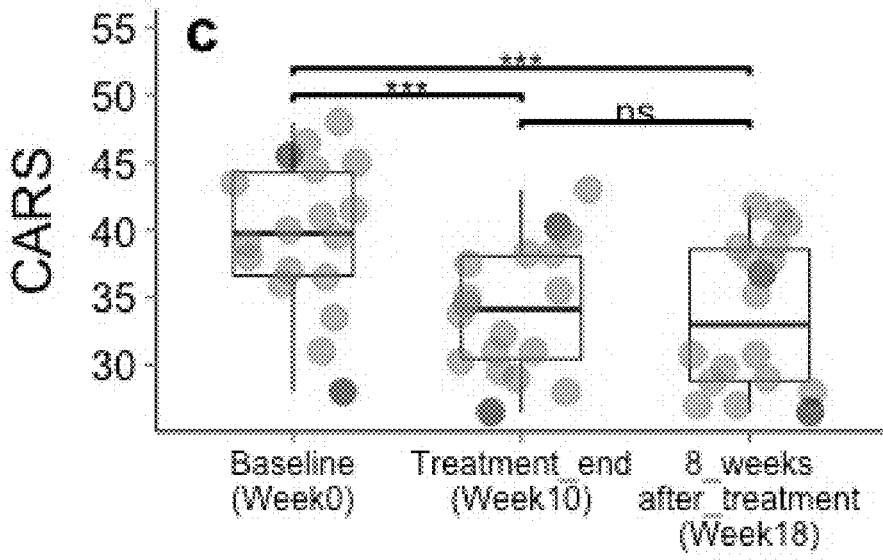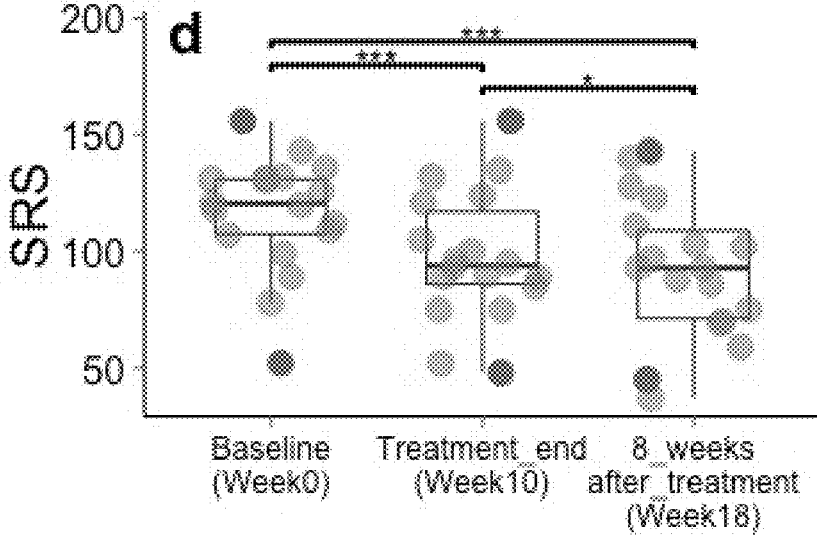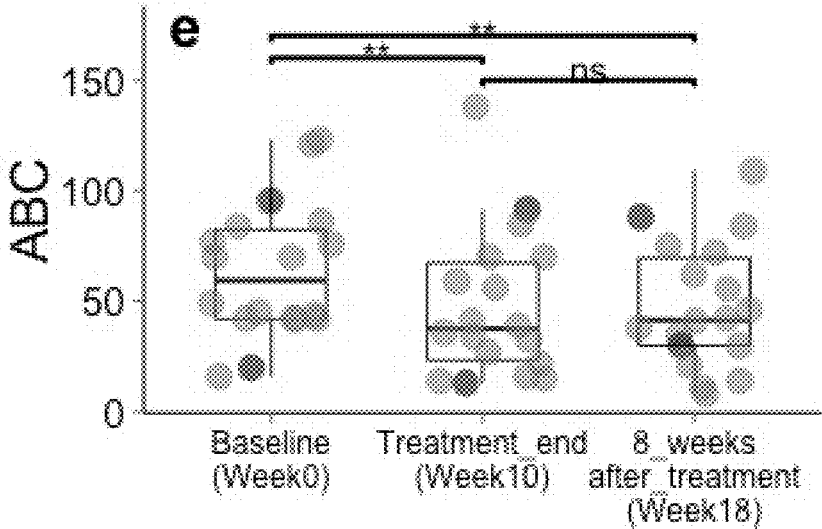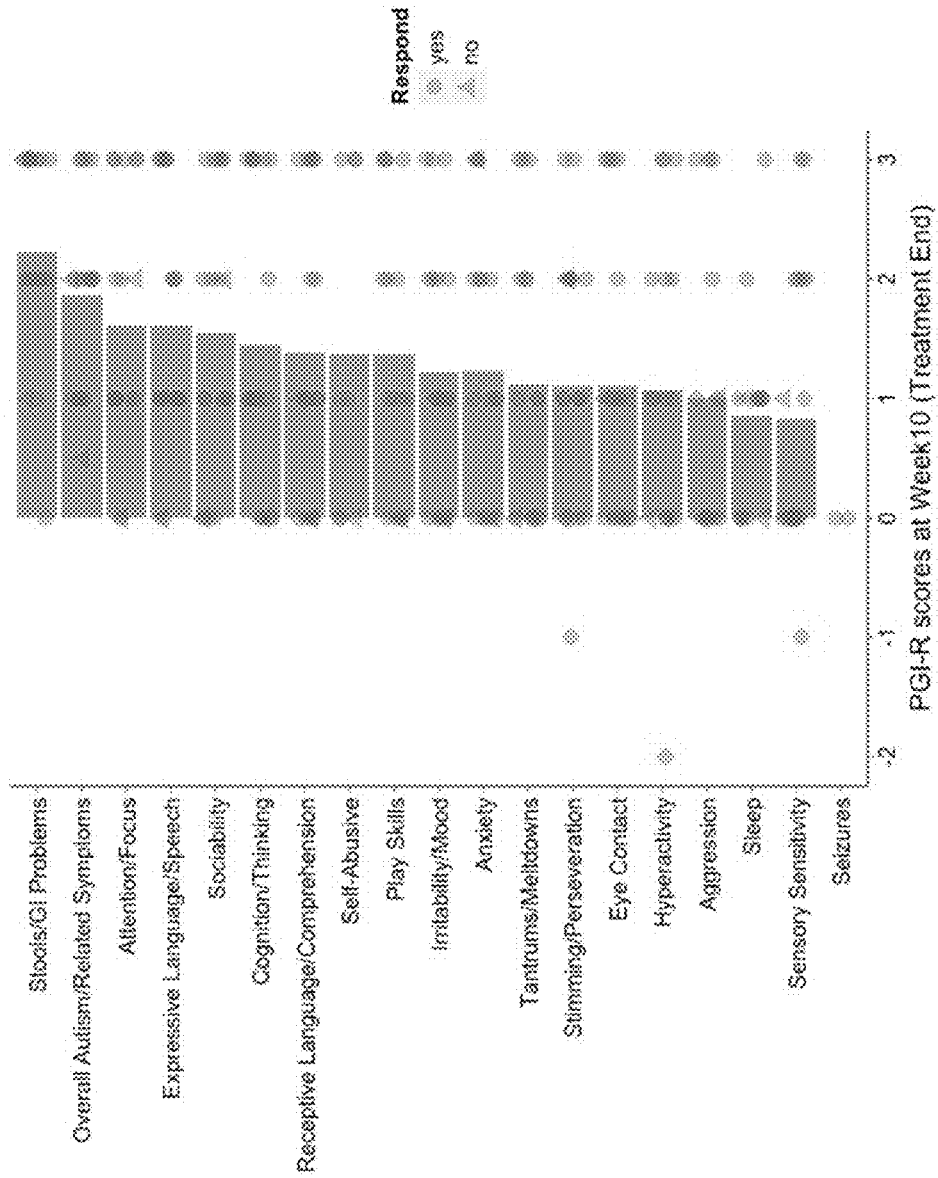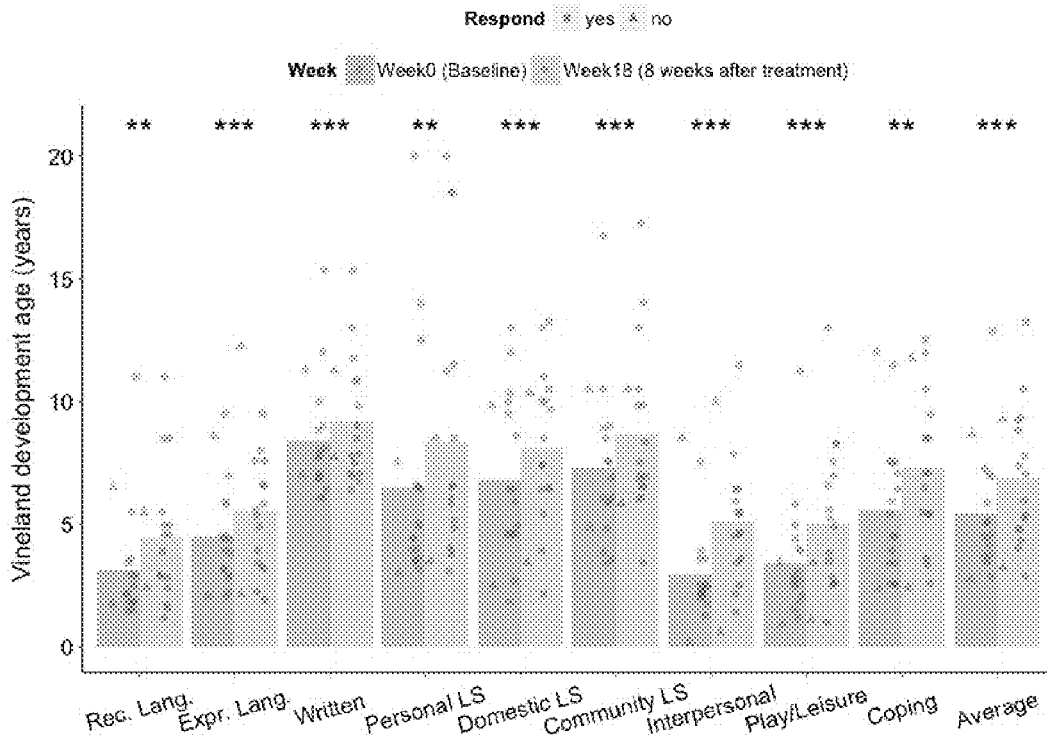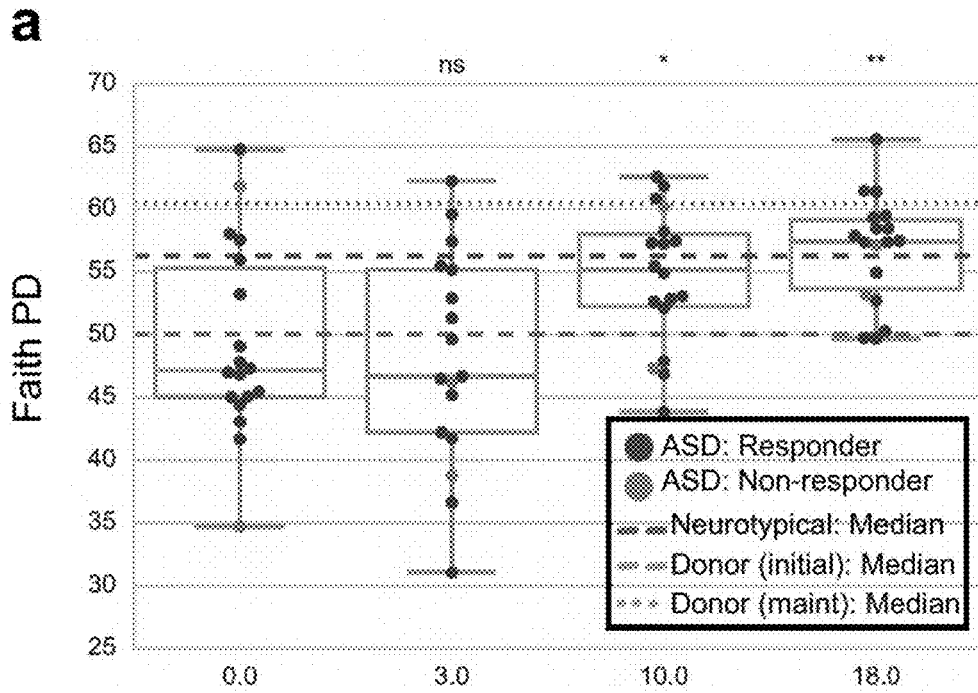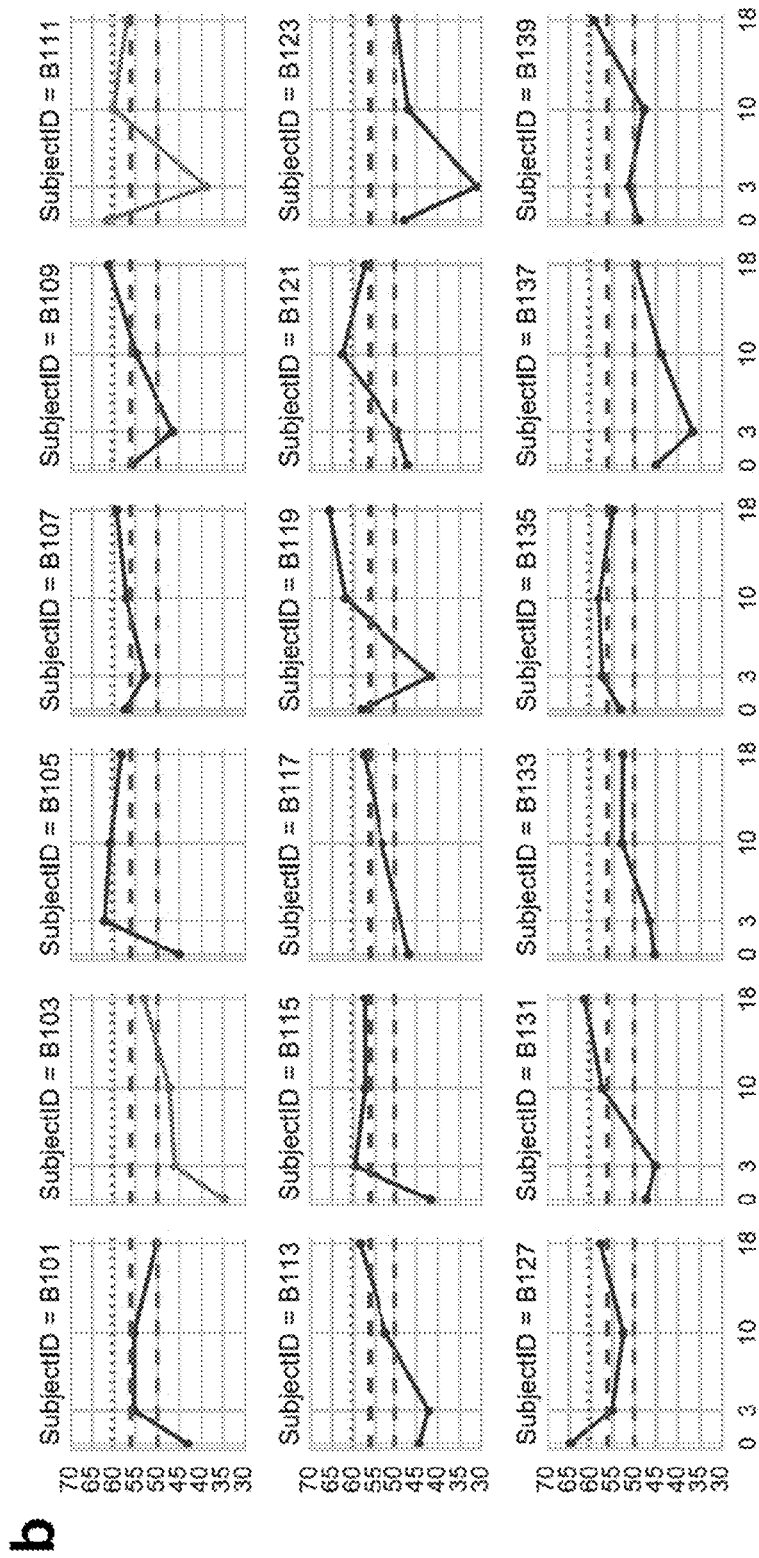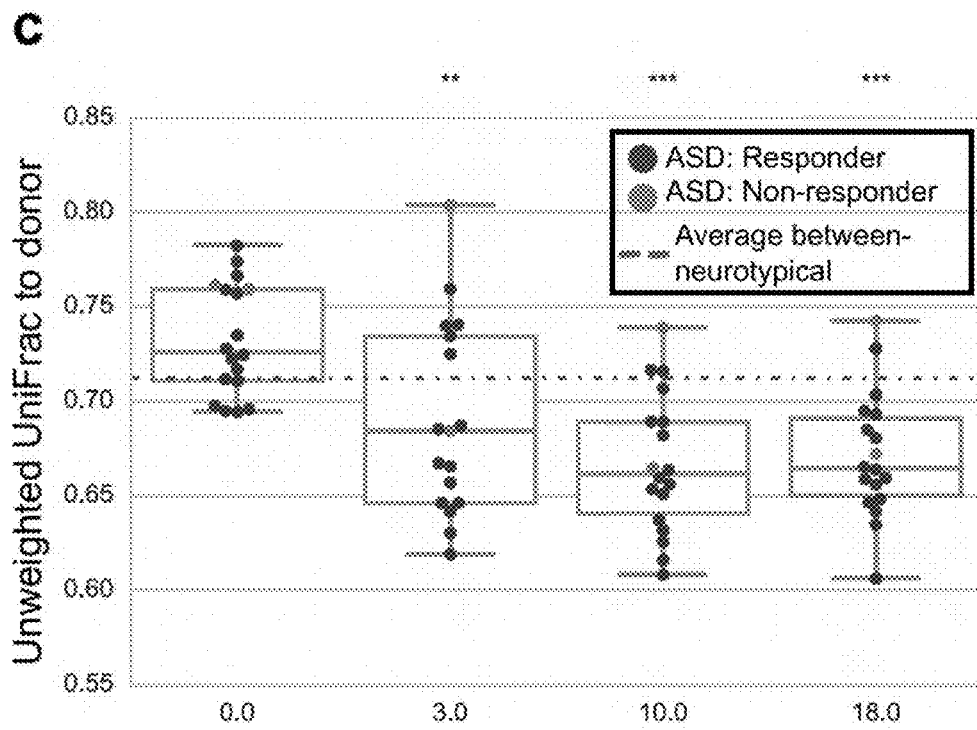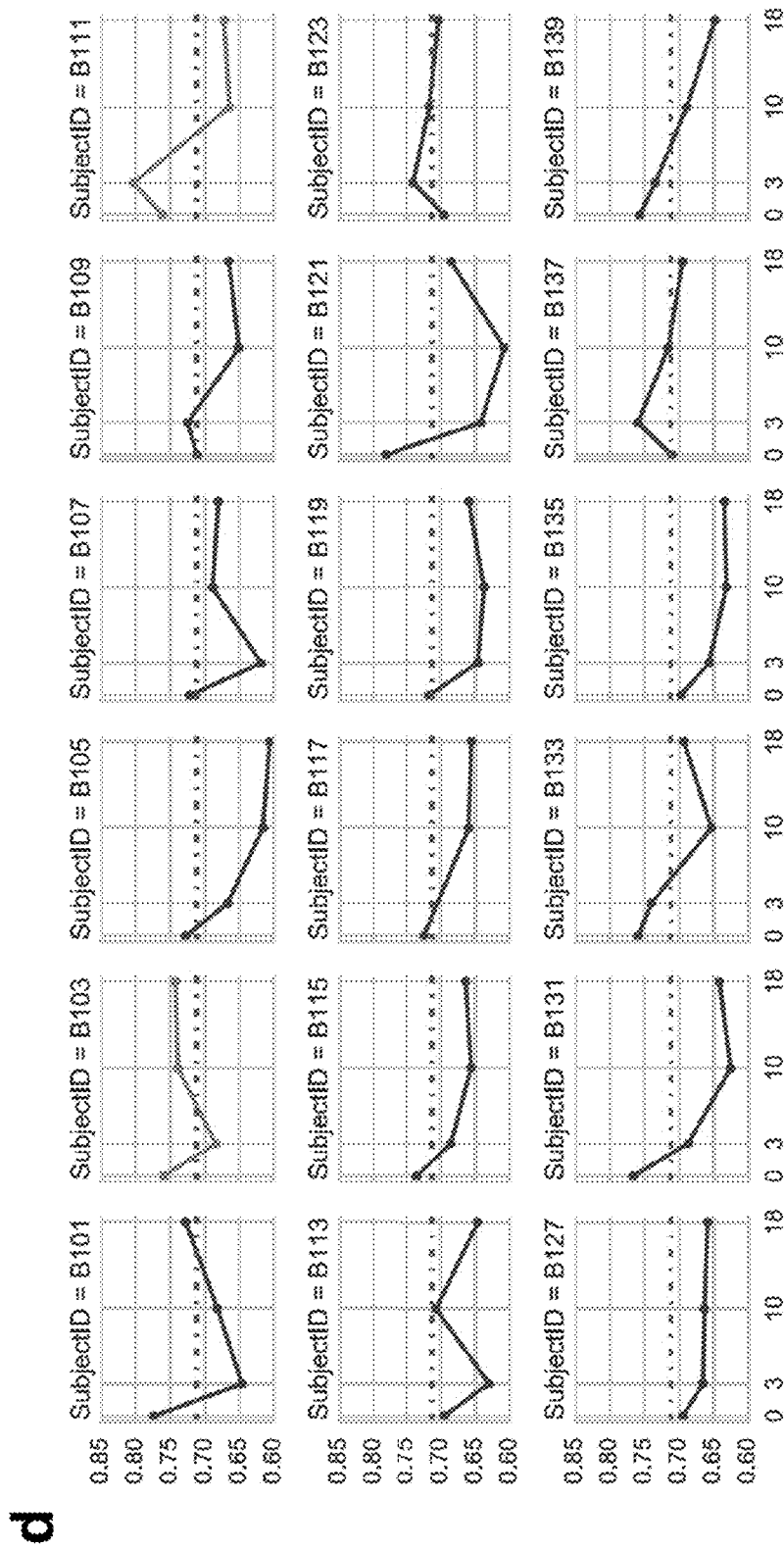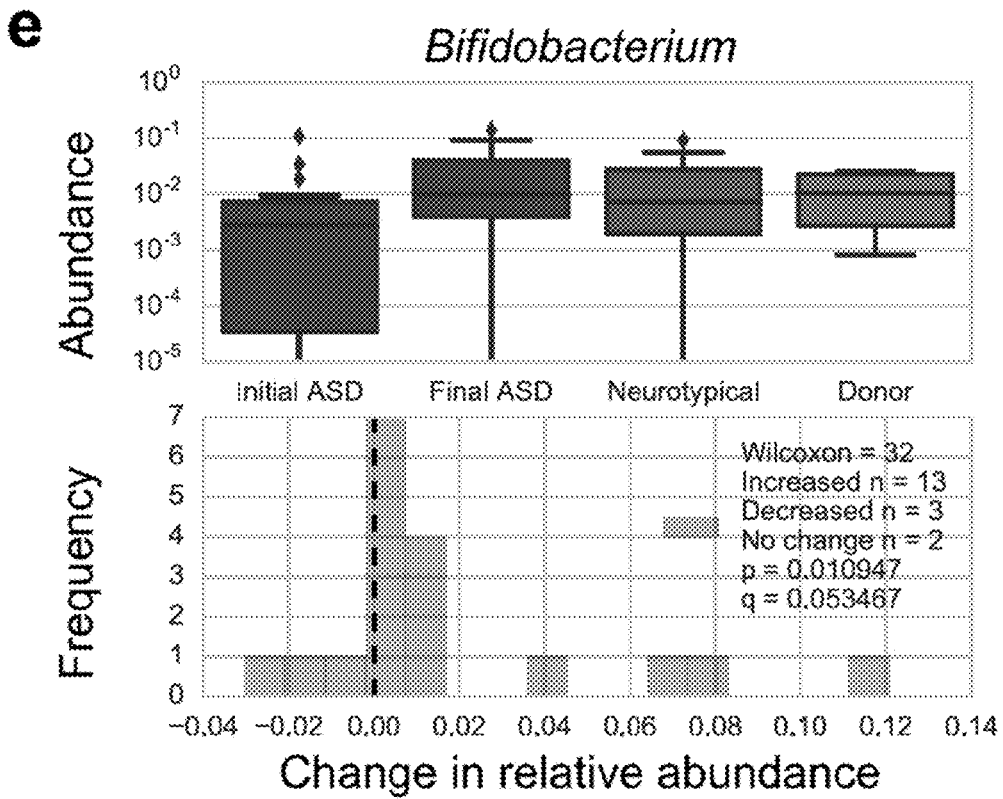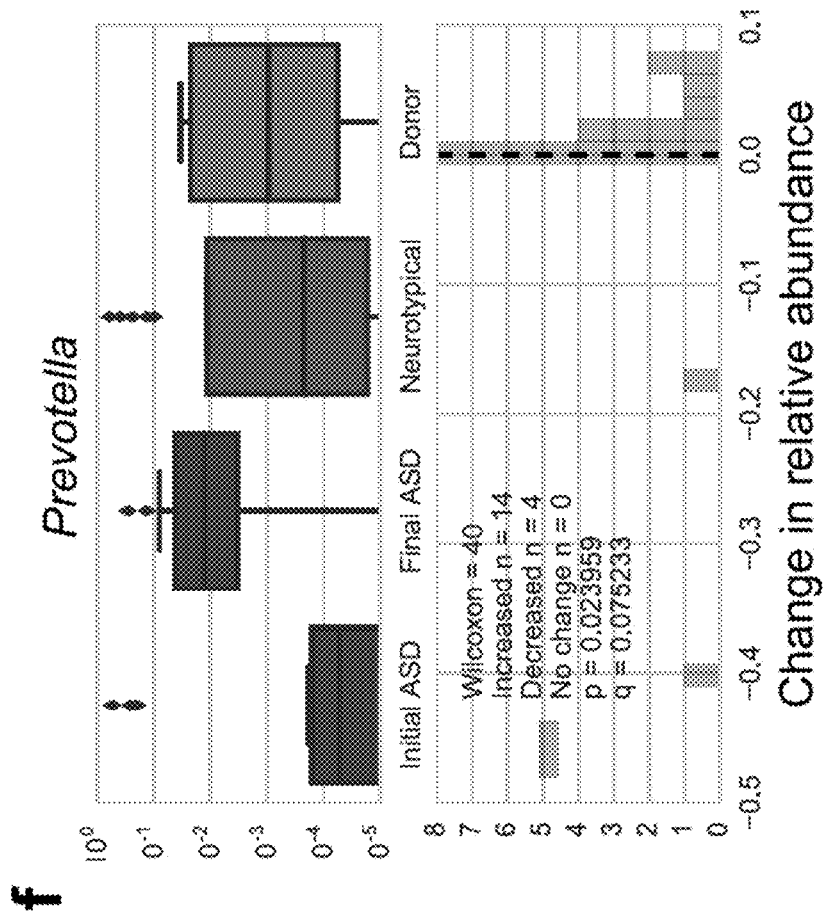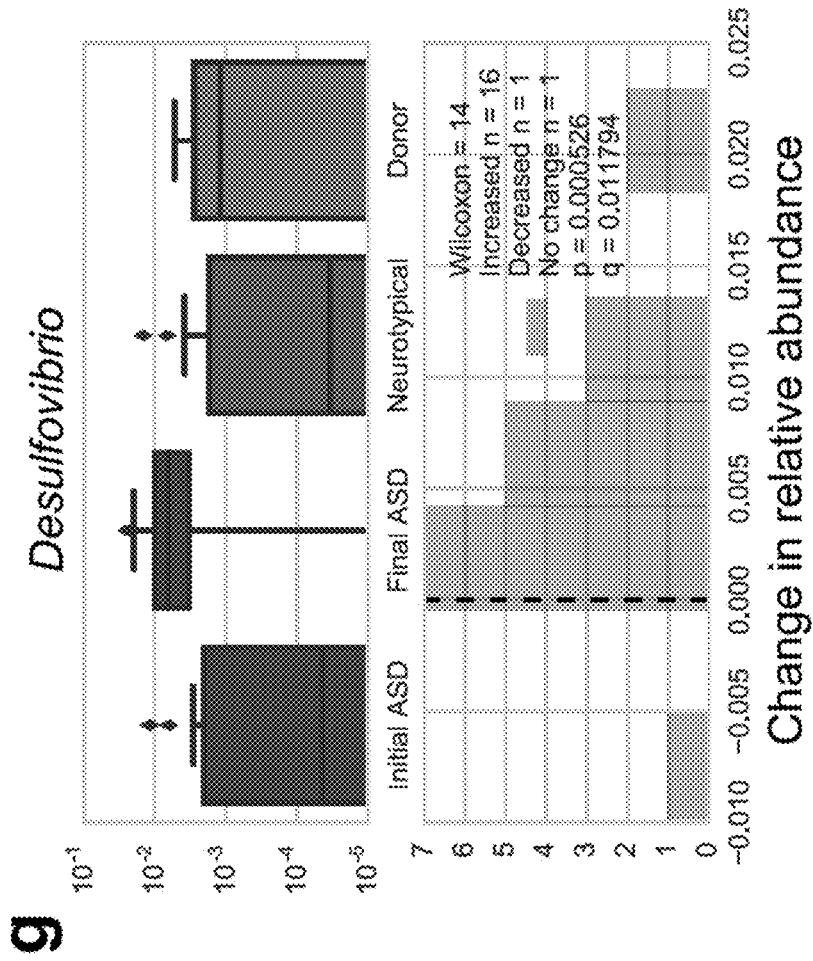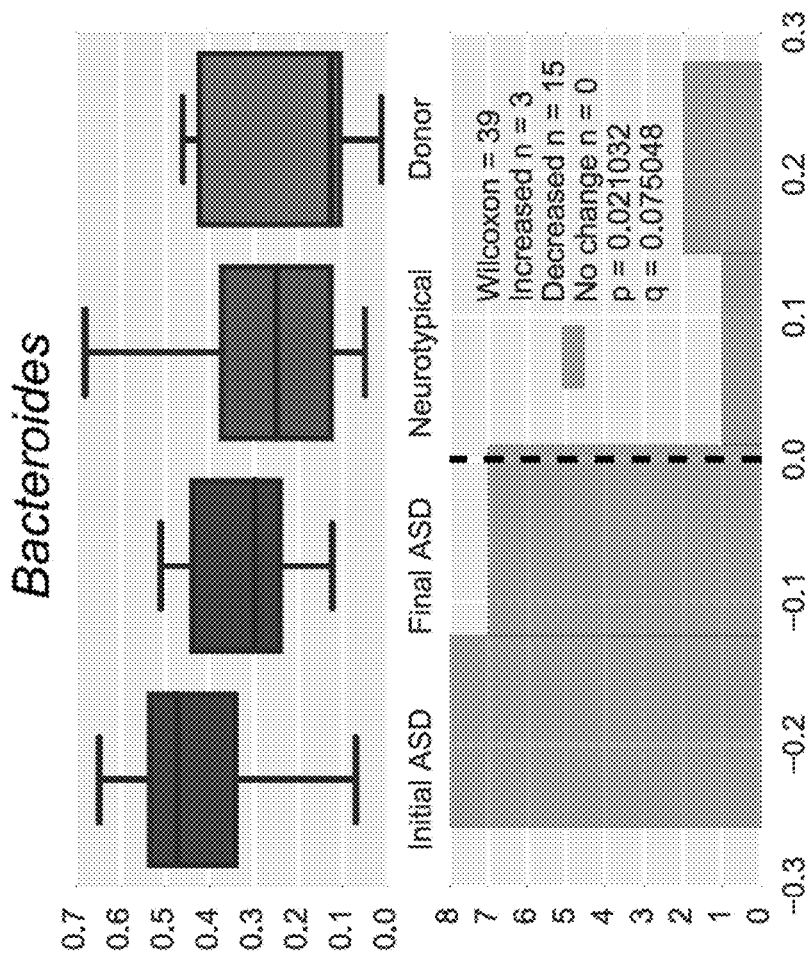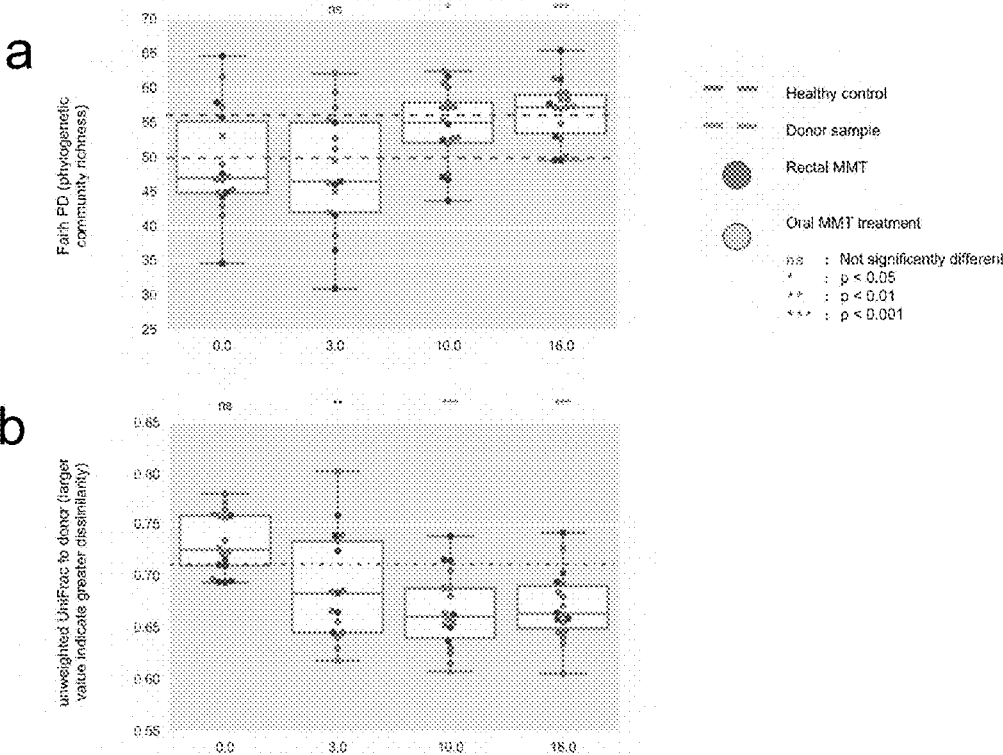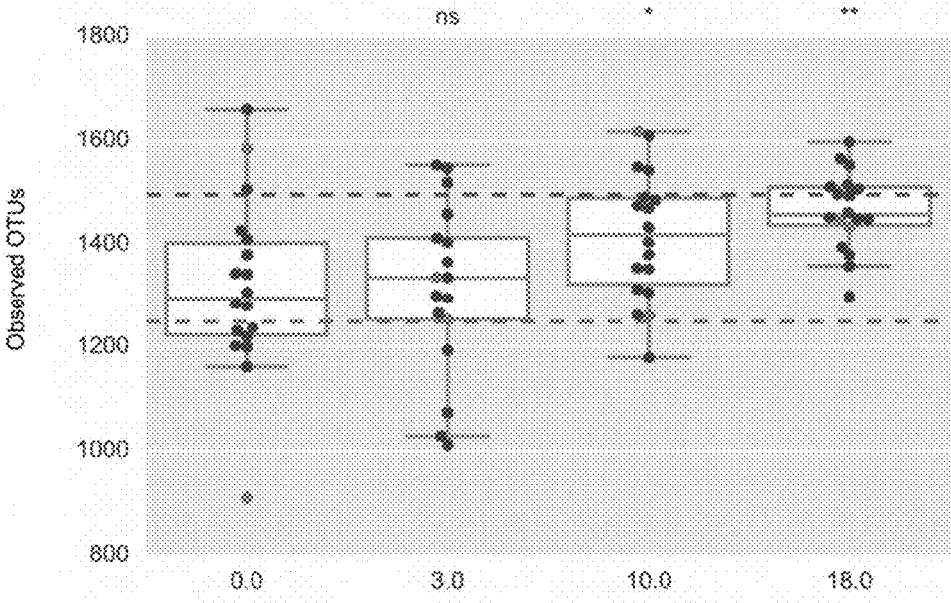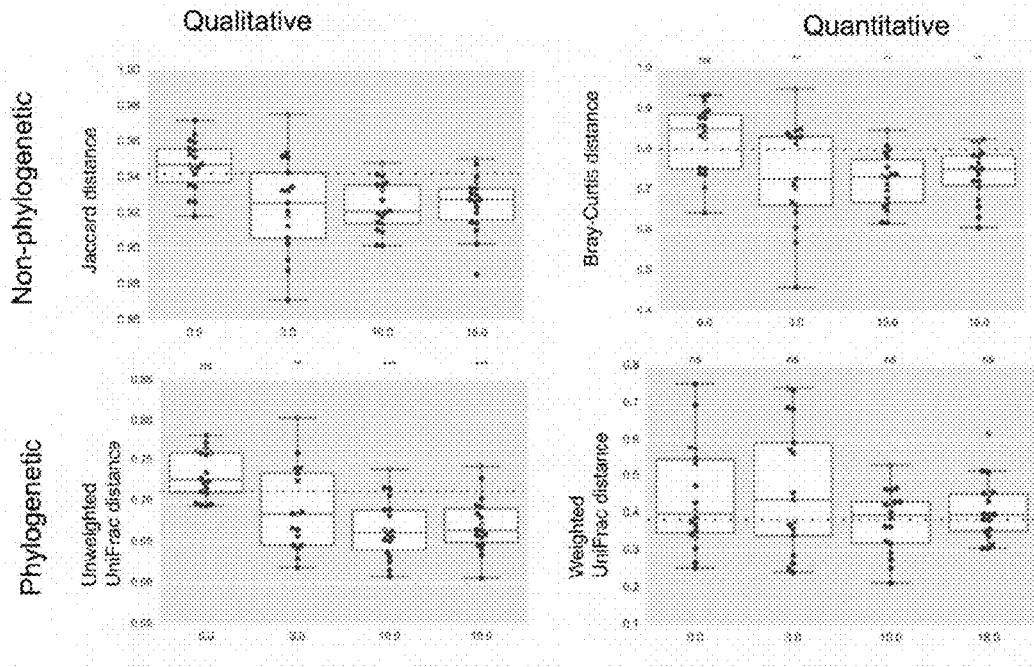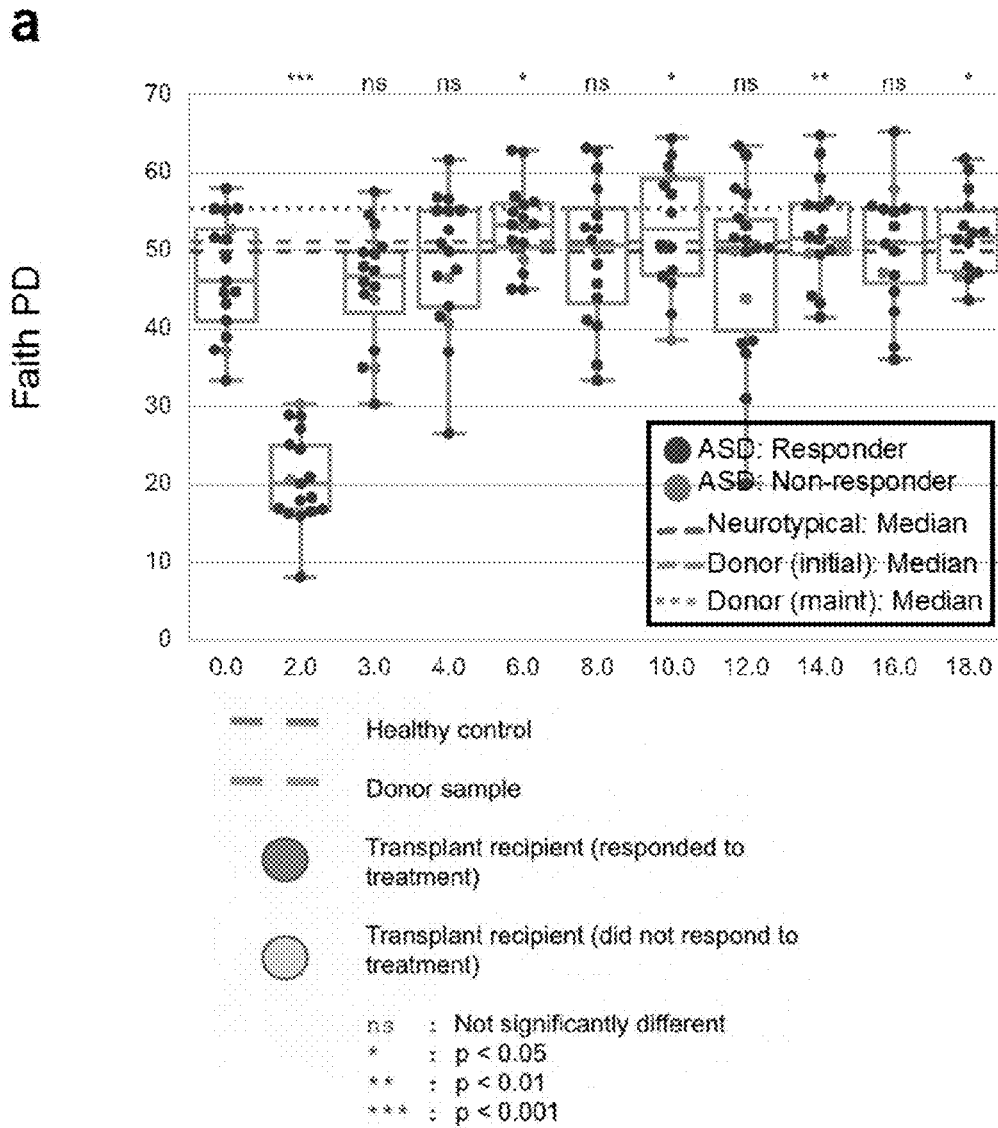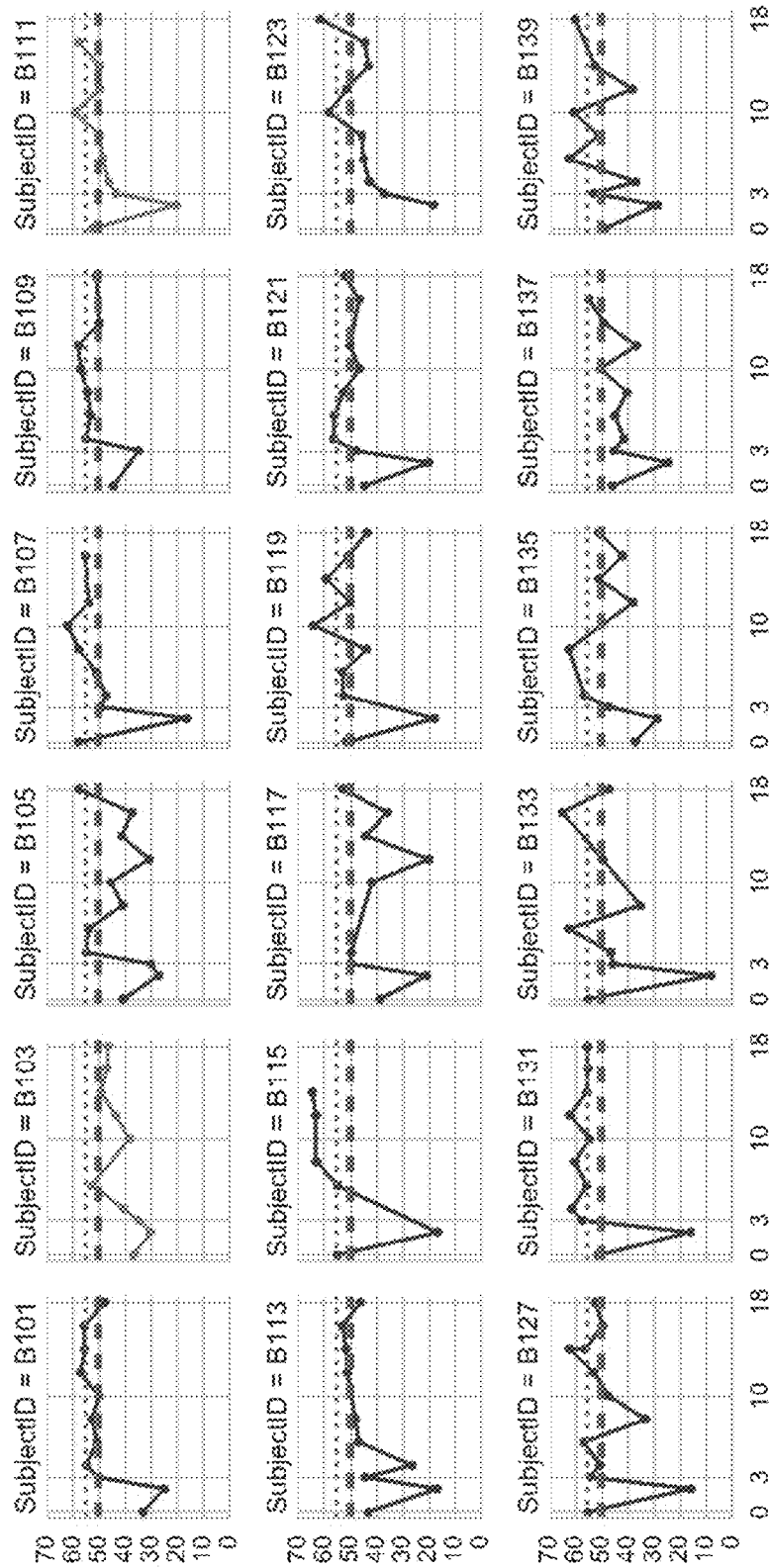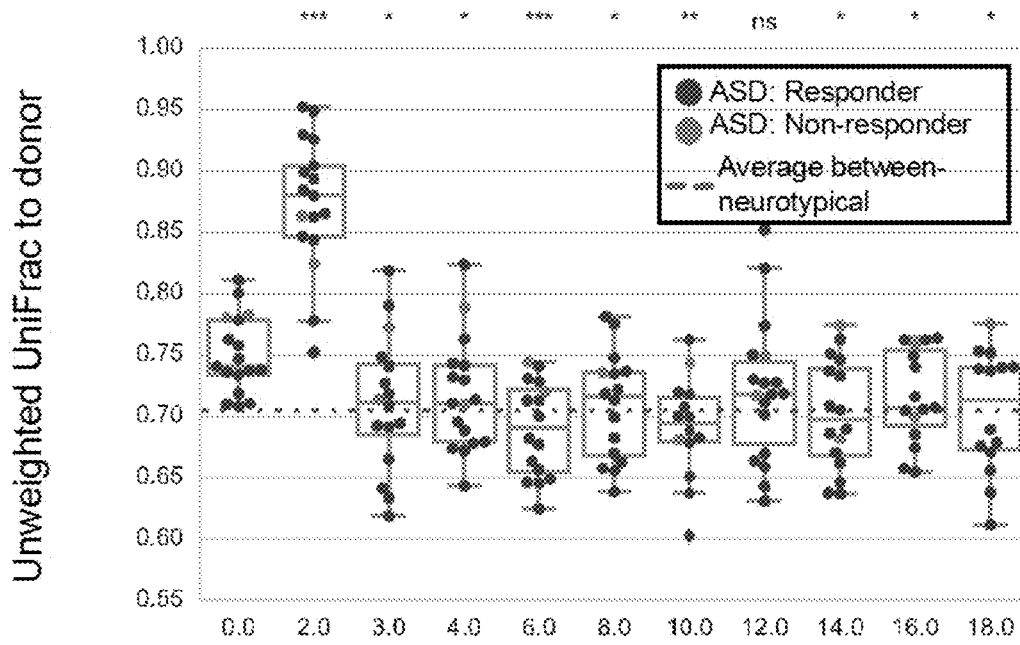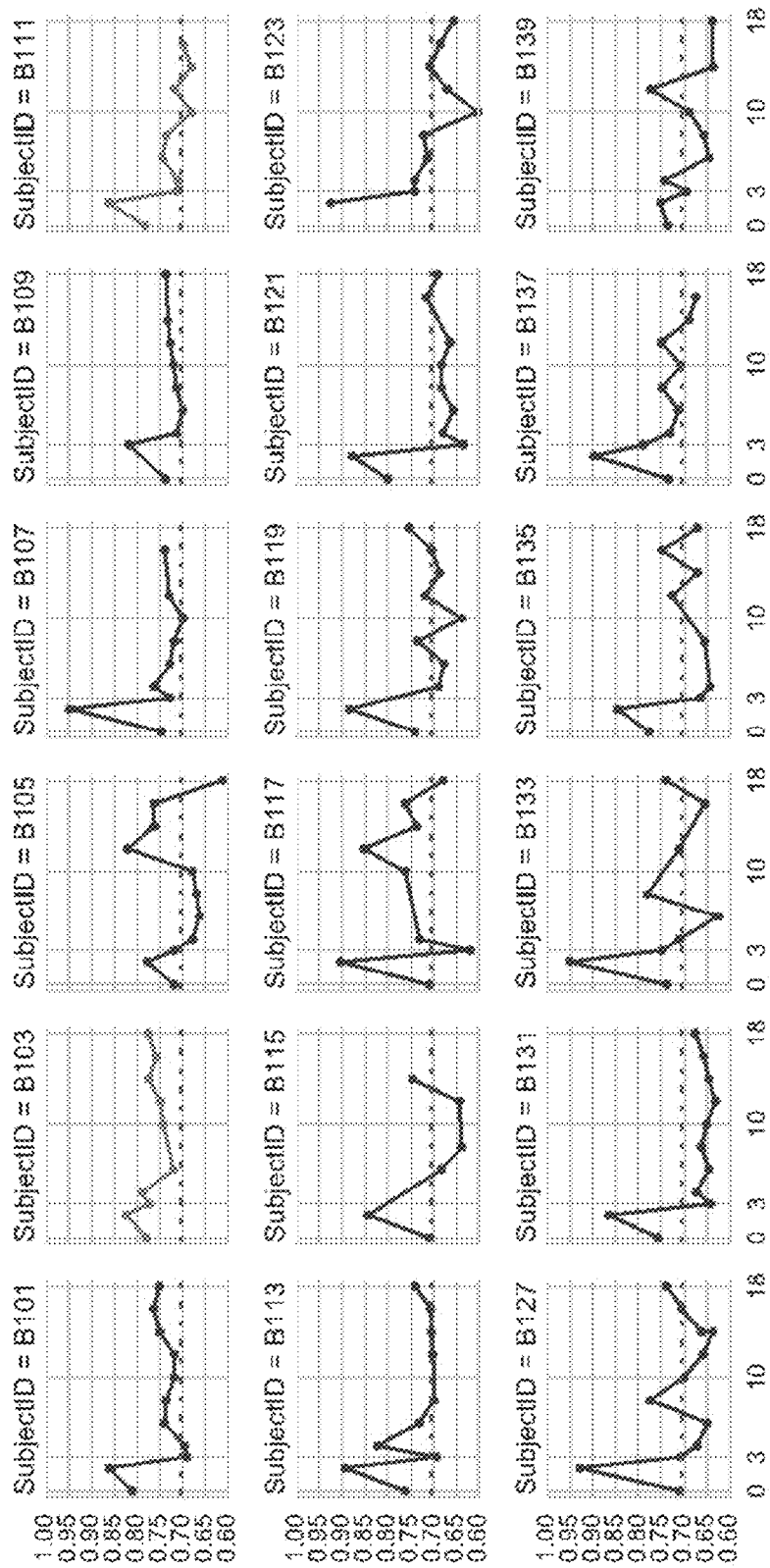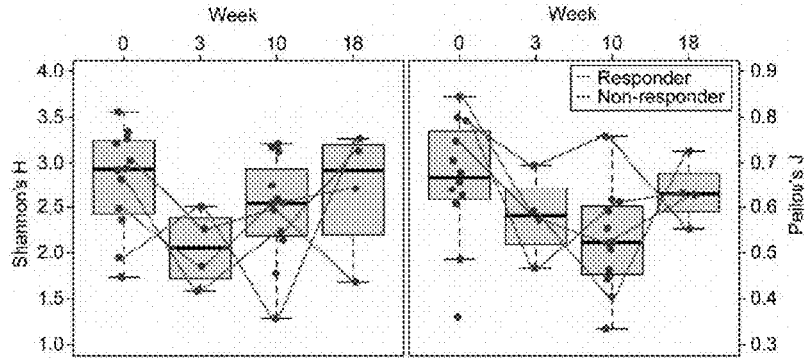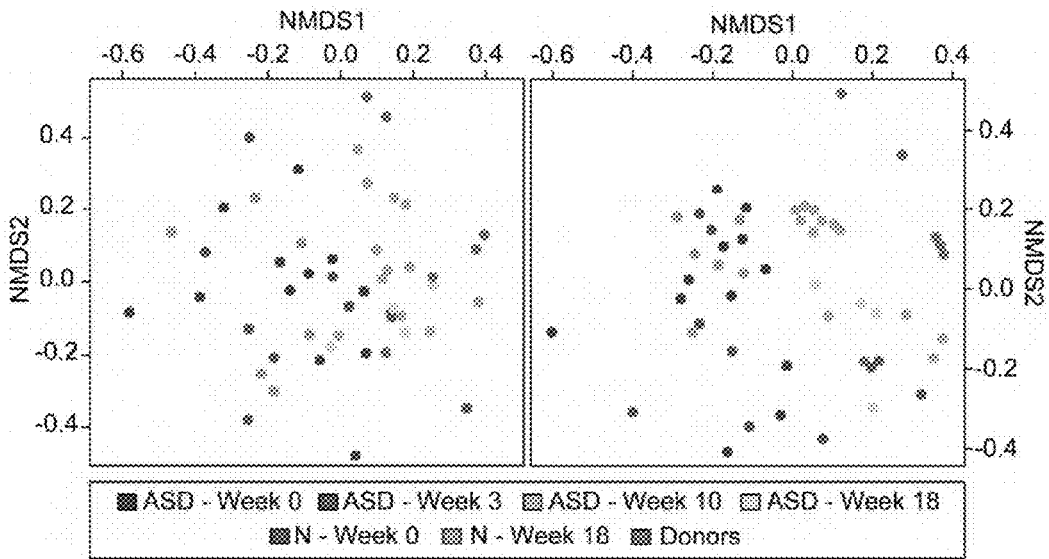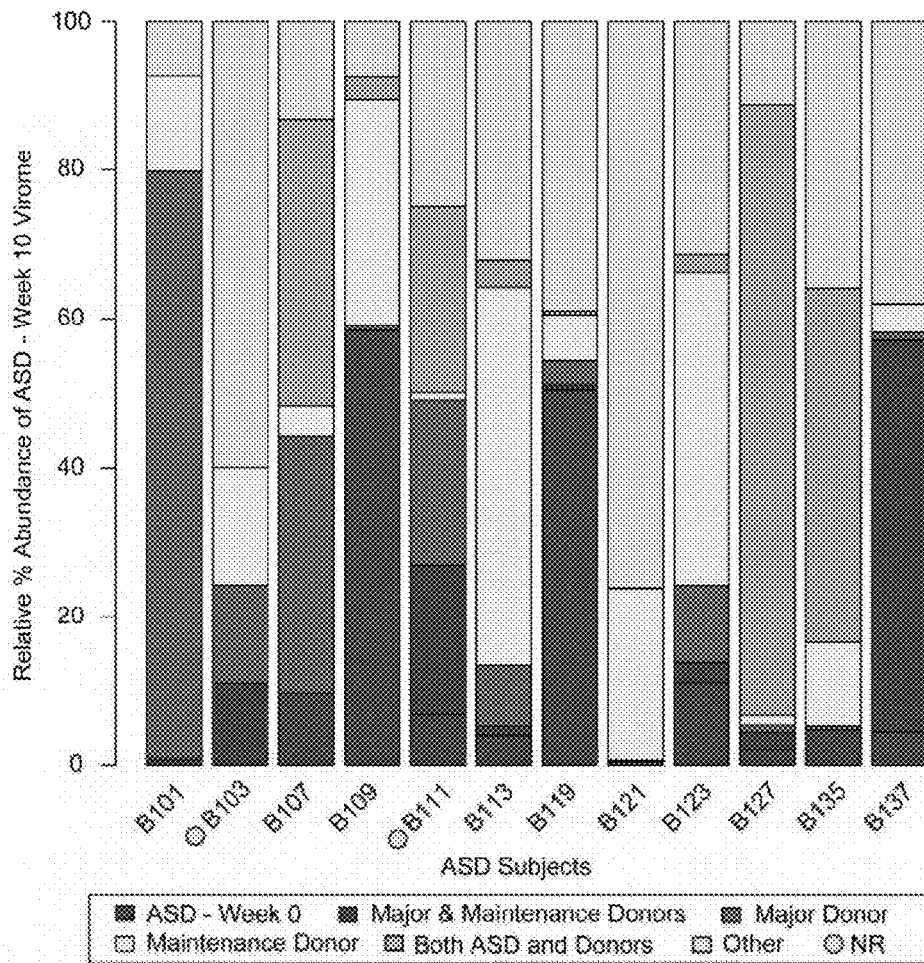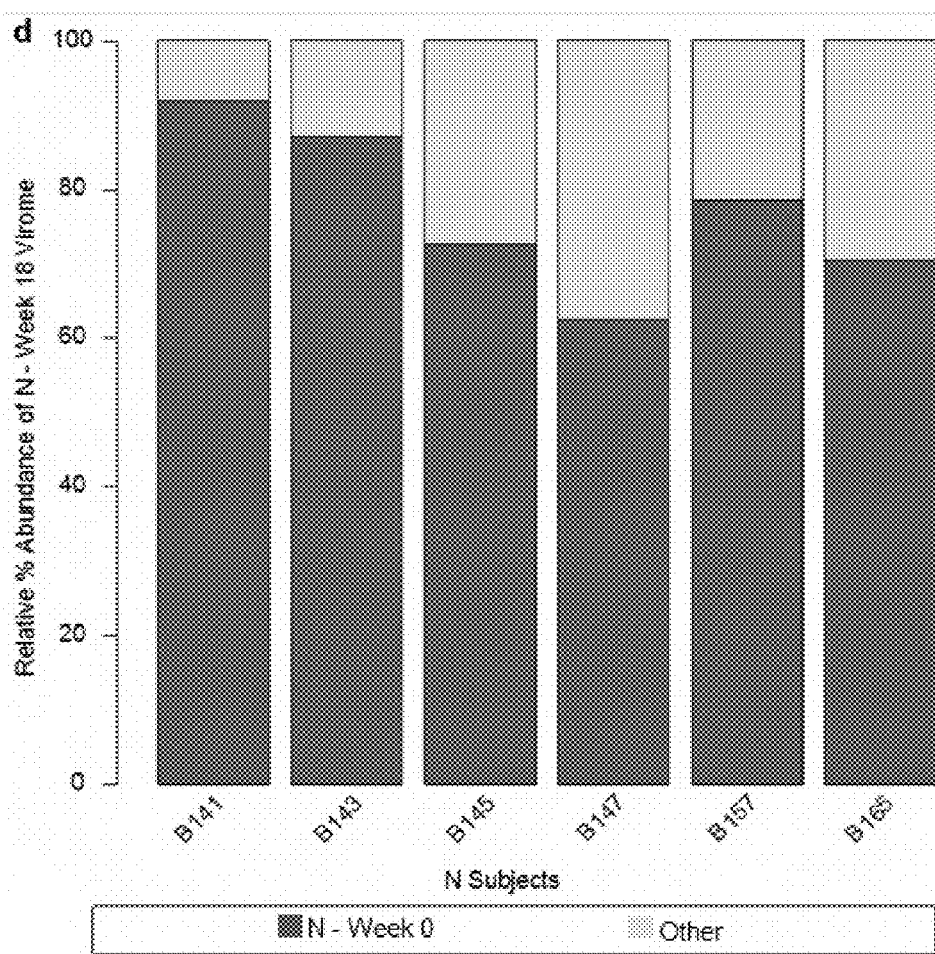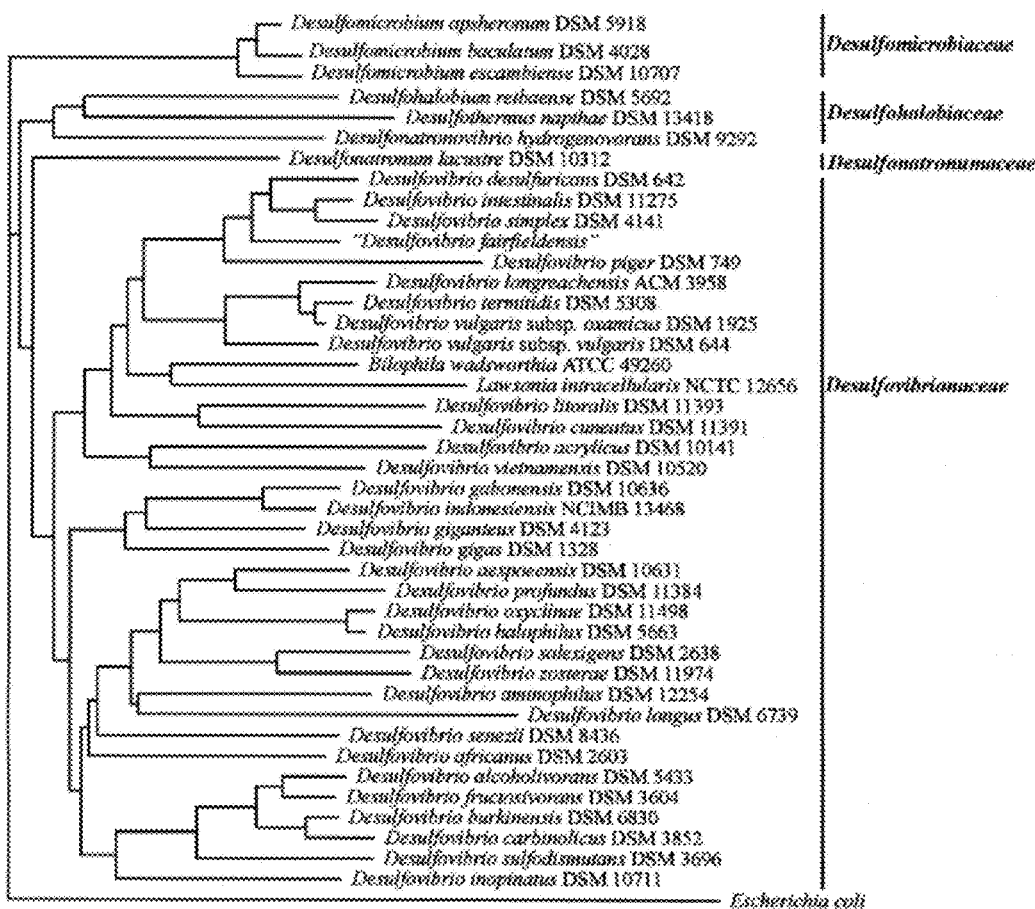# FIG. 22
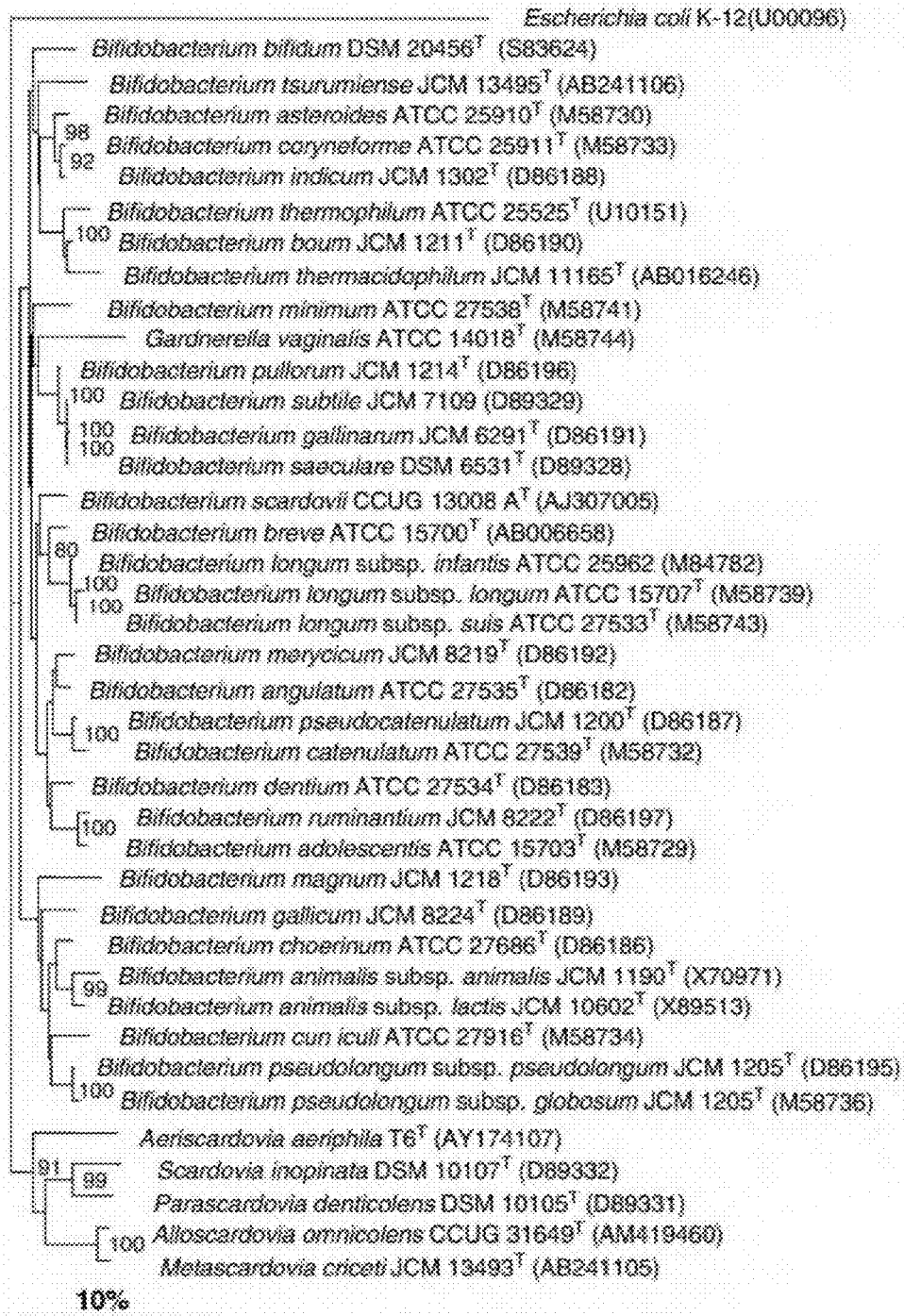
METHODS FOR TREATING AUTISM SPECTRUM DISORDER AND ASSOCIATED SYMPTOMS

BACKGROUND

[0001] The present disclosure relates to methods of modulating gut microbiome and methods for treating autism spectrum disorder (ASD). Autism spectrum disorder (ASD) is a complex neurodevelopmental condition characterized by widespread abnormalities of social interactions and communication, as well as restricted interests and repetitive behaviors. ASD typically appears during the first three years of life and manifests in characteristic symptoms or behavioral traits. A diagnosis of ASD now includes several conditions that used to be diagnosed separately: autistic disorder, pervasive developmental disorder not otherwise specified (PDD-NOS), and Asperger syndrome. All of these conditions are now encompassed by the diagnostic criteria for autism spectrum disorder as set forth in the American Psychiatric Association's Diagnostic & Statistical Manual of Mental Disorders, Fifth Edition (DSM-V).

[0002] In addition to the spectrum of symptoms seen within these principal diagnostic criteria, ASD individuals display a wide range of neurological comorbidities, including intellectual disability, epilepsy, and anxiety and mood disorders, as well as non-neurological comorbidities, including blood hyperserotonemia, immune dysregulation, and GI dysfunction (e.g., chronic constipation, diarrhea, abdominal pain, and gastroesophageal reflux).

[0003] To date, there are no FDA-approved treatments for reducing or eliminating the core symptoms of autism spectrum disorder. The only two medications approved by the FDA for treating autism, risperidone (sold under Risperdal®) and aripiprazole (sold under Abilify®), are specifically indicated for reducing irritability in subjects having ASD. Accordingly, there remains a need in the art for improved methods for treating and reducing the severity and incidence of symptoms associated with autism spectrum disorder. This application provides a method for treating an ASD patient (with or without a GI symptom) by transferring beneficial fecal bacteria to replace, restore, or rebalance the ASD patient's gut microbiota, a treatment referred to here as Microbiota Transfer Therapy (MTT). This application further provides a method for treating an ASD in a patient by selectively modulating the abundance of certain fecal microbes (e.g., bacteria) or bacteriophages.

BRIEF DESCRIPTION OF THE DRAWINGS

[0004] FIG. 1 presents Gastrointestinal Symptom Rating Scale (GSRS) data for trial participants.

[0005] FIG. 2 presents GSRS subscale data collected prior to ("pre") and following ("post") MTT treatment.

[0006] FIG. 3 presents continuous improvements of both average GSRS and average PGI-R scores of the participants.

[0007] FIG. 4 presents Aberrant Behavior Checklist (ABC) data for MTT trial participants.

[0008] FIG. 5 presents GSRS scores collected 8 weeks post-treatment.

[0009] FIG. 6 is a graph demonstrating the lack of correlation between age and the degree of CARS score improvement.

[0010] FIG. 7 is a graph demonstrating that end-of-treatment PGI-R scores had little correlation with age.

[0011] FIG. 8 is a graph demonstrating the lack of correlation between the degree of improvement on CARS and initial GSRS score.

[0012] FIG. 9 (including panels a to e) describes the improvement of GI- and core ASD-related symptoms of 18 ASD-afflicted children treated with MTT. Children were treated with vancomycin for two weeks followed by the administering of a fecal bacteria composition for 8 weeks, with a single follow-up evaluation 8 weeks after treatment ended. Panel a, Changes in GSRS scores. GSRS is scored on a Likert scale from 1 (no symptoms) to 7 (very severe discomfort). Panel b, Changes in PGI-R scores (Overall autism/related symptoms). PGI-R is scored from −3 (much worse), −2 (worse), −1 (slightly worse), 0 (no change), 1 (slightly better), 2 (better) to 3 (much better) compared to baseline. Panel c, CARS assessment pre-treatment, post treatment and 8 weeks post treatment. Panel d, Total SRS score pre-treatment, post treatment and 8 weeks post treatment. Panel e, Total ABC score pre-treatment, post treatment and 8 weeks post treatment. The data points represent 18 individual participants, and some data points overlap in the box plot. Asterisks (at the top of the box-plot) indicate whether individuals (at each time points) have significantly decreased since pre-treatment (Week 0). ns: not-significant, *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$ (two-tailed paired t-test). Two participants who had less than 50% improvement in GSRS scores are defined as non-responders and color-coded in grey.

[0013] FIG. 10 (including panels a to c) provides a breakdown of GSRS components and improvements in patients. a, GSRS subscores at baseline, MTT treatment end, and 8 weeks after treatment. b, Results of daily stool records, averaged over 2 weeks. c, Subscales of the ABC vs. time. *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$ (two-tailed paired t-test).

[0014] FIG. 11 demonstrates a correlation between GSRS and PGI-R (based on the data shown in FIG. 10, panels a and b). The Pearson correlation test showed $r=-0.56$ and $p<0.001$.

[0015] FIG. 12 shows Vineland Developmental Age (in years) for different subscales and for the average of all the subscales, measured at baseline and at the end of observation 4 months later. Note that the average chronological age was 10.9 years at the start of treatment, so at baseline there were delays in all areas, especially in the core autism areas of language and social (interpersonal) ability. *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$ (two-tailed paired t-test).

[0016] FIG. 13 shows subscores of the PGI-R at end of treatment (week 10). The scale goes from 3 (much better) to 2 (better) to 1 (slightly better) to 0 (no change) to minus 3 (much worse). Scores were similar after 8 weeks of no treatment (week 18). The data points represent 18 individual participants, and some data points overlap in the box plot.

[0017] FIG. 14 (including panels a to h) describes stool microbiota changes in ASD patients received MTT. Panel a, Changes in Faith's Phylogenetic Diversity (PD) in the ASD microbiota as measured from stool samples. Orange line indicates median PD of the donor samples, and green line indicates median PD of neurotypical controls at week 0. Panel b, Change in Faith PD tracked on a per individual basis for all MTT recipients. Most individuals experienced an increase in gut microbiota PD. Panel c, Unweighted UniFrac distances between ASD gut microbiota and most relevant donor sample (initial donor sample at weeks 0 and 3, most recent maintenance dose sample at weeks 10 and

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18). Green line indicates the median interpersonal variation between neurotypical controls and illustrates that prior to treatment the difference in gut microbiota composition between MTT recipients and donors was on the order of normal interpersonal variation. Following treatment, the MTT recipients were more similar to donors than normal interpersonal variation. Panel d, Distances between ASD gut microbiota and donor sample on a per individual basis. Most individuals became more similar to the donor over the study period. Panels e to h, Boxplots illustrating abundance of four genera, *Bifidobacterium, Prevotella, Desulfovibrio*, and *Bacteroides*, in the gut microbiota by group (top), and change in abundance 8 weeks after MTT in the ASD group (bottom).

[0018] FIG. **15** (including panels a and b) describes stool microbiota changes in ASD patients received MTT and further classified according to the oral or rectal administration of MMT. Panel a, Changes in Faith's Phylogenetic Diversity (PD) in the ASD microbiota as measured from stool samples. Orange line indicates median PD of the donor samples, and green line indicates median PD of neurotypical controls at week 0. Panel b, Unweighted UniFrac distances between ASD gut microbiota and most relevant donor sample (initial donor sample at weeks 0 and 3, most recent maintenance dose sample at weeks 10 and 18). Green line indicates the median interpersonal variation between neurotypical controls and illustrates that prior to treatment the difference in gut microbiota composition between MTT recipients and donors was on the order of normal interpersonal variation. Following treatment, the MTT recipients were more similar to donors than normal interpersonal variation.

[0019] FIG. **16** presents Change in community richness, as measure with a non-phylogenetic diversity metric.

[0020] FIG. **17** describes engraftment plots with four diversity metrics.

[0021] FIG. **18** (including panels a to d) describes microbiota analysis of fecal swab samples from ASD patients received MTT. Each of panels a to d correspond to panels a to d of FIG. **14**, respectively. Panel a, Changes in Faith's Phylogenetic Diversity (PD) in the ASD microbiota (n=20). Orange lines indicates median PD of the donor samples (dashed line represents initial donor samples (n=4), and dashed line represents maintenance dose samples (n=2)), and green line indicates median PD of neurotypical controls at week 0 (n=18). Panel b, Change in Faith PD tracked on a per individual basis for all MTT recipients. Panel c, Unweighted UniFrac distances between ASD gut microbiota and most relevant donor sample (initial donor sample at weeks 0 and 3, most recent maintenance dose sample at weeks 10 and 18). Green line indicates the median interpersonal variation between neurotypical controls. Panel d, Distances between ASD gut microbiota and donor sample on a per individual basis.

[0022] FIG. **19** (including panels a to d) describes stool virome changes in ASD patients received MTT. Panel a, Diversity indices, Shannon's H' (a measure of biodiversity and richness; right) and Peilou's J (a measure of evenness; left), of ASD participants. ASD diversity indices decreased at week 3 following vancomycin and the initial high dose MMT treatment and then rebounded over time and stabilized following treatment with maintenance doses of MMT. Fecal samples were collected at all four time points for four out of the 12 ASD subjects where the bacteriophage communities

were assessed. The responders (indicated by a grey line) rebounded in biodiversity, richness and evenness following FMT. In contrast, the non-responder (indicated by a red line) did not recover. Panel b, Nonmetric multidimensional scaling of Bray-Curtis dissimilarity (right; 2D-stress=0.2467) and Jaccard (left; 2D-stress=0.2212) distances reveal that ASD gut bacteriophage communities are more similar to donor gut bacteriophage communities following both the high and low MTT doses. Panel c, Analyses of ASD virome composition at week 10 shows engraftment of donor bacteriophage populations across all ASD subjects. In >80% of the subjects, the starting (week 0) bacteriophage populations make up <20% of the virome at week 10. Panel d, In contrast, analyses of virome composition of the untreated neurotypical children at week 18 reveals a high percentage (>60%) of starting (week 0) bacteriophage populations.

[0023] FIG. **20** provides a neighbor-joining phylogenetic tree of 16S rRNA gene sequences of the type strains of *Prevotella* species deposited in GenBank to show the relationships of strains within the genus. Bootstrap values (expressed as percentages of 1000 replicates) are shown at the nodes. The numbers that precede each strain are the GenBank accession numbers, while those that follow the species names are the Culture Collection numbers. The type species of *Bacteroides* (*Bacteroides fragilis*, NCTC 9343) and *Porphyromonas* (*Porphyromonas asaccharolytica*, ATCC 25260), both former members of the same genus, and *Escherichia coli* (K12) are included for comparison.

[0024] FIG. **21** describes phylogenetic relationships based on 16S rRNA gene sequence analyses of the taxa within the order Desulfovibrionales for which 16S rRNA gene sequences are available. The scale bar represents 10 inferred nucleotide substitutions per 100 nucleotides. The dendrogram was reconstructed from distance matrices using the neighbor-joining method.

[0025] FIG. **22** describes a phylogenetic tree constructed from partial 16S rRNA gene sequences of members of the family Bifidobacteriaceae. The tree was rooted with *Escherichia coli*, and constructed by using the neighbor-joining method with bootstrap values calculated from 1000 trees (represented as percentages and given at each branch). For each species, the GenBank accession number for the respective 16S rRNA gene sequence is indicated. The scale bar shows the number of nucleotide substitutions per site.

SUMMARY

[0026] In one aspect, this application provides a method for increasing the abundance of one or more gut microorganisms in a subject in need thereof, the method comprising treating the subject by administering a therapeutically effective amount of a pharmaceutical composition comprising a fecal microbe preparation, wherein the subject exhibits at least a 2-fold increase of the abundance of the one or more gut microorganisms after the treatment as compared to before initiating the treatment, wherein the one or more gut microorganisms are from a genus selected from the group consisting of *Bifidobacterium, Prevotella*, and *Desulfovibrio*.

[0027] In another aspect, this application provides a method for increasing the phylogenetic diversity of fecal microbiota, fecal phage virome, or both, of a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising a fecal microbe preparation, wherein

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the subject exhibits at least a two-fold increase of fecal microbiota or fecal phage virome diversity after the treatment as compared to before initiating the treatment.

[0028] In one aspect, this application provides a method comprising: determining the relative abundance of one or more gut microorganisms in a subject having an ASD or having an ASD sibling, and administering a therapeutically effective amount of a pharmaceutical composition comprising a fecal microbe preparation to achieve at least a 2-fold change of the abundance of the one or more gut microorganisms, wherein the one or more gut microorganisms are from a genus selected from the group consisting of *Clostridium, Bacteroides, Eggerthella, Bifidobacterium, Prevotella*, and *Desulfovibrio*.

[0029] In another aspect, this application provides a method for modulating the abundance of one or more gut microorganisms in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising a fecal microbe preparation to achieve at least a 2-fold change of the abundance of the one or more gut microorganisms, wherein the one or more gut microorganisms are selected from the group consisting of *Bifidobacterium, Prevotella, Desulfovibrio, Copprococcus, Clostridium, Eggerthella*, and *Bacteroides*.

DETAILED DESCRIPTION

[0030] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs.

[0031] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

[0032] As used herein and in the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. As used herein, the term "substantially" as in, for example, the phrase "substantially all peptides of an array," refers to at least 90%, preferably at least 95%, more preferably at least 99%, and most preferably at least 99.9%, of the peptides of an array. Other uses of the term "substantially" involve an analogous definition.

[0033] Where a range of values is provided, it is understood that each intervening value, between the upper and lower limit of that range and any other stated or intervening value in that stated range is encompassed within the disclosure. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the disclosure, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either both of those included limits are also included in the disclosure.

[0034] As used herein, the term "treating" refers to (i) completely or partially inhibiting a disease, disorder or condition, for example, arresting its development; (ii) completely or partially relieving a disease, disorder or condition, for example, causing regression of the disease, disorder and/or condition; or (iii) completely or partially preventing a disease, disorder or condition from occurring in a patient that may be predisposed to the disease, disorder and/or

condition, but has not yet been diagnosed as having it. Similarly, "treatment" refers to both therapeutic treatment and prophylactic or preventative measures. In the context of autism spectrum disorder, "treat" and "treating" encompass alleviating, ameliorating, delaying the onset of, inhibiting the progression of, or reducing the severity of one or more symptoms associated with an autism spectrum disorder. In one aspect, treating can also mean modulating the abundance of selected bacterial genera.

[0035] As used herein, a "subject" can be a human or animal including, but not limited to, a dog, cat, horse, cow, pig, sheep, goat, chicken, rodent, e.g., rats and mice, and primate, e.g., monkey. Preferred subjects are human subjects. The human subject may be a pediatric, adult or a geriatric subject.

[0036] As used herein, a "microbiota" and "flora" refer to a community of microbes that live in or on a subject's body, both sustainably and transiently, including eukaryotes, archaea, bacteria, and viruses (including bacterial viruses (i.e., phage)). A "fecal microbiota" or "fecal microbiota preparation" refers to a community of microbes present in or prepared from a subject's feces. A non-selected fecal microbiota refers to a community or mixture of fecal microbes derived from a donor's fecal sample without selection and substantially resembling microbial constituents and population structure found in such fecal sample.

[0037] As used herein, "therapeutically effective amount" or "pharmaceutically active dose" refers to an amount of a composition which is effective in treating the named disease, disorder or condition.

[0038] As used herein, "isolated" or "purified" refers to a bacterium or other entity or substance that has been (1) separated from at least some of the components with which it was associated when initially produced (whether in nature or in an experimental setting), and/or (2) produced, prepared, purified, and/or manufactured by the hand of man. Isolated or purified bacteria can be separated from at least about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or more of the other components with which they were initially associated.

[0039] As used herein, the terms "non-pathogenic" in reference to a bacterium or any other organism or entity includes any such organism or entity that is not capable of causing or affecting a disease, disorder or condition of a host organism containing the organism or entity.

[0040] As used herein, "spore" or a population of "spores" includes bacteria (or other single-celled organisms) that are generally viable, more resistant to environmental influences such as heat and bacteriocidal agents than vegetative forms of the same bacteria, and typically capable of germination and out-growth. "Spore-formers" or bacteria "capable of forming spores" are those bacteria containing the genes and other necessary abilities to produce spores under suitable environmental conditions.

[0041] As used herein, "colony forming units" (cfu) refers to an estimate of the number of viable microorganism cells in a given sample. The number of cfu can be assessed by counting the number of colonies on an agar plate as in standard methods for determining the number of viable bacterial cells in a sample.

[0042] As used herein, "viable" means possessing the ability to multiply. The viability of bacterial populations can be monitored as a function of the membrane integrity of the

cell. Cells with a compromised membrane are considered to be dead or dying, whereas cells with an intact membrane are considered live. For example, SYTO 9 and propidium iodide are used to stain and differentiate live and dead bacteria. See Stocks, *Cytometry A.* 2004 October; 61(2):189-95. Cell viability can also be evaluated via molecular viability analyses, e.g., a PCR-based approach, which can differentiate nucleic acids associated with viable cells from those associated with inactivated cells. See Cangelosi and Mescheke, *Appl Environ Microbiol.* 2014 October; 80(19): 5884-5891.

[0043] As used herein, "Shannon Diversity Index" refers to a diversity index that accounts for abundance and evenness of species present in a given community using the formula $H=-\Sigma_{i=1}^{R}p_i \ln p_i$, where H is Shannon Diversity Index, R is the total number of species in the community, and p_i is the proportion of R made up of the ith species. Higher values indicate diverse and equally distributed communities, and a value of 0 indicates only one species is present in a given community. For further reference, see Shannon and Weaver, (1949) *The mathematical theory of communication.* The University of Illinois Press, Urbana. 117 pp.

[0044] As used herein, "antibiotic" refers to a substance that is used to treat and/or prevent bacterial infection by killing bacteria, inhibiting the growth of bacteria, or reducing the viability of bacteria.

[0045] Autism spectrum disorder (ASD) is a neurodevelopmental disorder that is characterized by impairments in social interaction and communication, restricted interests, and repetitive behavior. Individuals on the autism spectrum experience widely varying degrees and types of impairments, from mild to severe. Although early detection and interventions are encouraged to maximize the benefits and reduce the severity of the symptoms, individuals of any age can benefit from interventions and therapies that can reduce symptoms and increase skills and abilities. Appropriate subjects for the methods described herein include, without limitation, humans diagnosed as having or suspected of having autism spectrum disorder. In some cases, appropriate subjects for the methods provided herein are considered to be at increased risk (e.g., moderate or high risk) of developing ASD. In some cases, the subject has been diagnosed as having a condition meeting diagnostic criteria for ASD as set forth in the DSM-V. In other cases, the subject has a well-established DSM-IV diagnosis of autistic disorder, Asperger's disorder, or pervasive developmental disorder not otherwise specified (PDD-NOS).

[0046] The methods provided herein result in, or are aimed at achieving a detectable improvement in one or more indicators or symptoms of ASD including, without limitation, including, but not limited to, changes in eye tracking, skin conductance and/or EEG measurements in response to visual stimuli, difficulties engaging in and responding to social interaction, verbal and nonverbal communication problems, repetitive behaviors, intellectual disability, difficulties in motor coordination, attention issues, sleep disturbances, and physical health issues such as gastrointestinal disturbances.

[0047] Several screening instruments are known in the art for evaluating a subject's social and communicative development and thus can be used as aids in screening for and detecting changes in the severity of impairment in communication skills, social interactions, and restricted, repetitive and stereotyped patterns of behavior characteristic of autism

spectrum disorder. Evaluation can include neurologic and genetic assessment, along with in-depth cognitive and language testing. Additional measures developed specifically for diagnosing and assessing autism include the Autism Diagnosis Interview-Revised (ADI-R), the Autism Diagnostic Observation Schedule (ADOS-G) and the Childhood Autism Rating Scale (CARS).

[0048] According to CARS, evaluators rate the subject on a scale from 1 to 4 in each of 15 areas: Relating to People; Imitation; Emotional Response; Body Use; Object Use; Adaptation to Change; Visual Response; Listening Response; Taste, Smell, and Touch Response and Use; Fear; Verbal Communication; Nonverbal Communication; Activity; Level and Consistency of Intellectual Response; and General Impressions.

[0049] A second edition of CARS, known as the Childhood Autism Rating Scale—2 or CARS-2, was developed by Schopler et al. (Childhood Autism Rating Scale—Second edition (CARS2): Manual. Los Angeles: Western Psychological Services, 2010). The original CARS was developed primarily with individuals with co-morbid intellectual functioning and was criticized for not accurately identifying higher functioning individuals with ASD. CARS-2 retained the original CARS form for use with younger or lower functioning individuals (now renamed the CARS2-ST for "Standard Form"), but also includes a separate rating scale for use with higher functioning individuals (named the CARS2-HF for "High Functioning") and an unscored information-gathering scale ("Questionnaire for Parents or Caregivers" or CARS2-QPC) that has utility for making CARS2ST and CARS2-HF ratings.

[0050] Another symptom rating instrument useful for assessing changes in symptom severity before, during, or following treatment according to a method provided herein is the Aberrant Behavior Checklist (ABC). See Aman et al., Psychometric characteristics of the aberrant behavior checklist. *Am J Ment Defic.* 1985 March; 89(5):492-502. The ABC is a symptom rating checklist used to assess and classify problem behaviors of children and adults in a variety of settings. The ABC includes 58 items that resolve onto five subscales: (1) irritability/agitation, (2) lethargy/social withdrawal, (3) stereotypic behavior, (4) hyperactivity/noncompliance, and (5) inappropriate speech.

[0051] The present inventors observed that autistic individuals, regardless of the presence or absence of comorbid gastrointestinal distress, have fewer species of gut bacteria as compared to neurotypical individuals. The present inventors also found that restoring the species diversity of gut bacteria helps to treat autistic symptoms in patients in need thereof.

[0052] In one aspect, this application provides a method for increasing the abundance of one or more gut microorganisms in a subject in need thereof, the method comprising treating the subject by administering a therapeutically effective amount of a pharmaceutical composition comprising a fecal microbe preparation, wherein the subject exhibits at least a 50-fold increase of the abundance of the one or more gut microorganisms after the treatment as compared to before initiating the treatment, wherein the one or more gut microorganisms are from a genus selected from the group consisting of *Bifidobacterium, Prevotella,* and *Desulfovibrio.* In one aspect, a subject being treated exhibits at least 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, 60-fold, 70-fold, 80-fold, 90-fold, 100-

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fold, 110-fold, 120-fold, 130-fold, 140-fold, 150-fold, 160-fold, 170-fold, 180-fold, 190-fold, or 200-fold increase of the abundance of one or more *Bifidobacterium, Prevotella,* or *Desulfovibrio* species after a treatment as compared to before initiating the treatment. In another aspect, this application provides a method for modulating the abundance of one or more gut microorganisms in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising a fecal microbe preparation to achieve at least a 2-fold, 5-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, 60-fold, 70-fold, 80-fold, 90-fold, 100-fold, 110-fold, 120-fold, 130-fold, 140-fold, 150-fold, 160-fold, 170-fold, 180-fold, 190-fold, or 200-fold change of the abundance of the one or more gut microorganisms, wherein the one or more gut microorganisms are selected from the group consisting of *Bifidobacterium, Prevotella, Desulfovibrio, Copprococcus, Clostridium, Eggerthella,* and *Bacteroides.* In a further aspect, a treated subject exhibit a bacterial abundance fold increase (e.g., *Bifidobacterium, Prevotella,* or *Desulfovibrio*) of between 2 and 10, between 3 and 10, between 4 and 10, between 5 and 10, between 6 and 10, between 10 and 20, between 10 and 30, between 10 and 40, between 10 and 50, between 10 and 60, between 10 and 70, between 10 and 80, between 10 and 90, between 20 and 30, between 20 and 40, between 20 and 50, between 20 and 60, between 20 and 70, between 20 and 80, between 20 and 90, between 30 and 40, between 30 and 50, between 30 and 60, between 30 and 70, between 30 and 80, between 30 and 90, between 40 and 50, between 40 and 60, between 40 and 70, between 40 and 80, between 40 and 90, between 50 and 60, between 50 and 70, between 50 and 80, between 50 and 90, between 50 and 100, between 50 and 150, between 50 and 200, between 50 and 250, between 50 and 300 fold after 8 or more weeks of treatment as compared to before initiating the treatment.

[0053] *Prevotella* is an anaerobic, non-spore forming, non-motile, rod that stains Gram-negative. *Desulfovibrio* is a gram negative, rod shaped, sulfate reducing bacterium, which is an anaerobe and which may compete with the host for sulfur. Its metal corroding ability has led to numerous health and safety concerns; and its production of H_2S and endotoxin can lead to genotoxic and other toxic-related problems. *Bifidobacterium* is a Gram-positive, non-acid-fast, non-sporeforming, and nonmotile anaerobe. *Eggerthella* are Gram-positive obligately anaerobic bacilli, which are nonmotile and do not produce endospores. *Prevotella* and *Bifidobacterium* are generally believed to be beneficial intestinal bacteria, but the role of *Desulfovibrio* is less understood. This application illustrates that increases in all three are beneficial in children with ASD.

[0054] In one aspect, an increase in *Prevotella* abundance is an increase of one or more, two or more, three or more, four or more, five or more, six or more, or seven or more species selected from the group consisting of *P. intermedia, P. pallens, P. nigrescens, P. disiens, P. corporis, P. denticola, P. multiformis, P. melaninogenica, P. veroralis, P. oulorum, P. salivae, P. copri, P. albensis, P. bivia, P. shahii, P. loescheii, P. marshii, P. oralia, P. enoeca, P. stercorea, P. massiliensis, P. timonensis, P. buccalis, P. bergensis, P. dentalis, P. buccae, P. baroniae, P. multisaccharivorax, P. brevis, P. ruminicola, P. bryantiii, P. tannerae, P. heparinolytica,* and *P. zoogleoformans.* In one aspect, an increase in *Prevotella* abundance is an increase of one or more, two or more, three or more, four or more, five or more, six or

more, or seven or more species listed in FIG. **20**. In one aspect, an increase in *Desulfovibrio* abundance is an increase of one or more, two or more, three or more, four or more, five or more, six or more, or seven or more *Desulfovibrio* species listed in FIG. **21**. In one aspect, an increase in *Bifidobacterium* abundance is an increase of one or more, two or more, three or more, four or more, five or more, six or more, or seven or more *Bifidobacterium* species selected from the group consisting of *B. adolescentis, B. angulatum, B. animalis, B. asteroides, B. bifidum, B. boum, B. breve, B. catenulatum, B. choerinum, B. coryneforme, B. cuniculi, B. denticolens, B. dentium, B. gallicum, B. gallinarum, B. indicum, B. inopinatum, B. infantis, B. lactis, B. longum, B. magnum, B. merycicum, B. minimum, B. pseudocatenulatum, B. pseudolongum, B. pullorum, B. ruminantium, B. saeculare, B. subtile, B. thermacidophilum, B. thermophilum,* and *B. tsurumiense.* In one aspect, an increase in *Bifidobacterium* abundance is an increase of one or more, two or more, three or more, four or more, five or more, six or more, or seven or more *Bifidobacterium* species listed in FIG. **22**. In one aspect, a change in *Eggerthella* abundance is a decrease of one or more or two or more *Eggerthella* species selected from the group consisting of *Eggerthella lenta, Eggerthella sinensis,* and *Eggerthella* hongkongensis.

[0055] In one aspect, a bacterial abundance change (e.g., increase of *Bifidobacterium, Prevotella,* or *Desulfovibrio,* or decrease of *Bacteroides* or *Eggerthella*) is ongoing during a treatment or sustained after finishing or discontinuing a treatment. In one aspect, a bacterial abundance change (e.g., increase of *Bifidobacterium, Prevotella,* or *Desulfovibrio,* or decrease of *Bacteroides* or *Eggerthella*) is assessed at a specific time point during or post treatment, e.g., about 2, 4, 6, 8, 12, 18, 24, 32, 40, 48 weeks after initiating a treatment, or about 2, 4, 6, 8, 12, 18, 24, 32, 40, 48 weeks after finishing or discontinuing a treatment.

[0056] In one aspect, a method further comprises determining in a subject a relative abundance of one or more gut microorganisms selected from the group consisting of *Bifidobacterium, Prevotella, Desulfovibrio, Copprococcus, Clostridium, Eggerthella,* and *Bacteroides.* In one aspect, a relative abundance is determined via an assay selected from the group consisting of qPCR, RT-qPCR, clone libraries, DGGE, T-RFLP, ARISA, microarrays, FISH, dot-blot hybridization, and a DNA hybridization method. In another aspect, a relative abundance is determined via 16S rDNA-targeted pyrosequencing. In a further aspect, a relative abundance is determined via a DNA hybridization assay based on a 16S rDNA sequence. In one aspect, a relative abundance is determined via detecting one or more proteins or metabolites specific to one or more gut microorganisms. In another aspect, a relative abundance is determined via an assay selected from the group consisting of 2-Dimensional Gel Electrophoresis, 2-Diminsional Difference Gel Electrophoresis (2D-DIGE), MALDI TOF-MS, (2D-) LC-ESI-MS/MS, AQUA and 1TRAQ.

[0057] In one aspect, this application provides a method for modulating the abundance of *Eggerthella* or *Bacteroides* in a subject in need thereof, the method comprising treating the subject by administering a therapeutically effective amount of a pharmaceutical composition comprising a fecal microbe preparation, wherein the subject exhibits at least a 20% change in the abundance of *Eggerthella* or *Bacteroides* after the treatment as compared to before initiating the treatment. In one aspect, a subject being treated exhibits at

least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% change in *Eggerthella* or *Bacteroides* abundance after a treatment as compared to before initiating the treatment. In one aspect, a subject being treated exhibits between 20% and 30%, between 20% and 40%, between 20% and 50%, between 20% and 60%, between 20% and 70%, between 20% and 80%, between 20% and 90%, between 30% and 40%, between 30% and 50%, between 30% and 60%, between 30% and 70%, between 30% and 80%, between 30% and 90%, between 40% and 50%, between 40% and 60%, between 40% and 70%, between 40% and 80%, between 40% and 90%, between 50% and 60%, between 50% and 70%, between 50% and 80%, or between 50% and 90% change in *Eggerthella* or *Bacteroides* abundance after a treatment as compared to before initiating the treatment. In one aspect, a change in *Eggerthella* or *Bacteroides* abundance is an abundance increase. In one aspect, a change in *Eggerthella* or *Bacteroides* abundance is an abundance decrease.

[0058] In another aspect, this application provides a method for increasing the phylogenetic diversity of fecal microbiota, fecal phage virome, or both, of a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising a fecal microbe preparation, wherein the subject exhibits at least a two-fold increase of fecal microbiota or fecal phage virome diversity after the treatment as compared to before initiating the treatment.

[0059] In one aspect, a subject being treated exhibits at least 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, 60-fold, 70-fold, 80-fold, 90-fold, 100-fold, 110-fold, 120-fold, 130-fold, 140-fold, 150-fold, 160-fold, 170-fold, 180-fold, 190-fold, or 200-fold increase of fecal microbiota or fecal phage virome diversity after a treatment as compared to before initiating the treatment. In another aspect, a treated subject exhibit an increase in fecal microbiota or fecal phage virome diversity of between 2 and 10, between 3 and 10, between 4 and 10, between 5 and 10, between 6 and 10, between 10 and 20, between 10 and 30, between 10 and 40, between 10 and 50, between 10 and 60, between 10 and 70, between 10 and 80, between 10 and 90, between 20 and 30, between 20 and 40, between 20 and 50, between 20 and 60, between 20 and 70, between 20 and 80, between 20 and 90, between 30 and 40, between 30 and 50, between 30 and 60, between 30 and 70, between 30 and 80, between 30 and 90, between 40 and 50, between 40 and 60, between 40 and 70, between 40 and 80, between 40 and 90, between 50 and 60, between 50 and 70, between 50 and 80, between 50 and 90, between 50 and 100, between 50 and 150, between 50 and 200, between 50 and 250, between 50 and 300 fold after 8 or more weeks of treatment as compared to before initiating the treatment. In one aspect, an increase in fecal microbiota or fecal phage virome diversity is ongoing during a treatment or sustained after finishing or discontinuing a treatment. In one aspect, a subject being treated exhibits at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% increase of fecal microbiota or fecal phage virome diversity after a treatment as compared to before initiating the treatment. In another aspect, a treated subject exhibit an increase in fecal microbiota or fecal phage virome diversity of between 20% and 30%, between 20% and 40%, between 20% and 50%, between 20% and 60%, between 20% and 70%, between 20% and 80%, between 20% and 90%, between 30% and 40%, between 30% and

50%, between 30% and 60%, between 30% and 70%, between 30% and 80%, between 30% and 90%, between 40% and 50%, between 40% and 60%, between 40% and 70%, between 40% and 80%, between 40% and 90%, between 50% and 60%, between 50% and 70%, between 50% and 80%, or between 50% and 90% after 8 or more weeks of treatment as compared to before initiating the treatment. In one aspect, an increase in fecal microbiota or fecal phage virome diversity is ongoing during a treatment or sustained after finishing or discontinuing a treatment. In another aspect, an increase in fecal microbiota or fecal phage virome diversity is assessed at a specific time point during or post treatment, e.g., about 2, 4, 6, 8, 12, 18, 24, 32, 40, 48 weeks after initiating a treatment, or about 2, 4, 6, 8, 12, 18, 24, 32, 40, 48 weeks after finishing or discontinuing a treatment.

[0060] In one aspect, fecal microbiota or fecal phage virome diversity is assessed using Shannon's Index. In another aspect, fecal microbiota or fecal phage virome diversity is assessed using Faith phylogenetic diversity. In another aspect, fecal microbiota or fecal phage virome diversity is assessed using an observed OTUs metric. In one aspect, fecal microbiota or fecal phage virome diversity is assessed using a qualitative non-phylogenetic metric (Jaccard distance), a quantitative non-phylogenetic diversity metric (Bray-Curtis distance), a qualitative phylogenetic diversity metric (unweighted UniFrac), or a quantitative phylogenetic diversity metric (weighted UniFrac).

[0061] In one aspect, this application provides a method comprising: determining the relative abundance of one or more gut microorganisms in a subject having an ASD or having an ASD sibling, and administering a therapeutically effective amount of a pharmaceutical composition comprising a fecal microbe preparation to achieve at least a 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, 60-fold, 70-fold, 80-fold, 90-fold, 100-fold, 110-fold, 120-fold, 130-fold, 140-fold, 150-fold, 160-fold, 170-fold, 180-fold, 190-fold, or 200-fold change of the abundance of the one or more gut microorganisms, wherein the one or more gut microorganisms are from a genus selected from the group consisting of *Clostridium, Bacteroides, Eggerthella, Bifidobacterium, Prevotella*, and *Desulfovibrio*. In another aspect, a subject exhibits an abundance change of one or more gut microorganisms selected from the group consisting of *Clostridium, Bacteroides, Eggerthella, Bifidobacterium, Prevotella*, and *Desulfovibrio*, of between 3 and 10, between 4 and 10, between 5 and 10, between 6 and 10, between 10 and 20, between 10 and 30, between 10 and 40, between 10 and 50, between 10 and 60, between 10 and 70, between 10 and 80, between 10 and 90, between 20 and 30, between 20 and 40, between 20 and 50, between 20 and 60, between 20 and 70, between 20 and 80, between 20 and 90, between 30 and 40, between 30 and 50, between 30 and 60, between 30 and 70, between 30 and 80, between 30 and 90, between 40 and 50, between 40 and 60, between 40 and 70, between 40 and 80, between 40 and 90, between 50 and 60, between 50 and 70, between 50 and 80, between 50 and 90, between 50 and 100, between 50 and 150, between 50 and 200, between 50 and 250, between 50 and 300 fold after 8 or more weeks. In one aspect, a subject having an ASD exhibits no gastrointestinal symptom. In another aspect, a subject having an ASD does not exhibit one or more, two or more, three or more, four or more gastrointestinal symptoms selected from the group consisting of abdominal pain, reflux,

indigestion, irritable bowel syndrome, chronic persistent diarrhoea, diarrhoea, flatulence, constipation, and alternating constipation/diarrhoea. In a further aspect, a subject having an ASD exhibits no gastrointestinal symptom selected from the group consisting of abdominal pain, reflux, indigestion, irritable bowel syndrome, chronic persistent diarrhoea, diarrhoea, flatulence, constipation, and alternating constipation/diarrhoea. In one aspect, one or more gut microorganisms with abundance change are from *Clostridium*, and wherein the *Clostridium* microorganisms increase their abundance by at least 2 fold. In another aspect, one or more gut microorganisms with abundance change are from *Bacteroides*, and wherein the *Bacteroides* microorganisms decrease their abundance by at least 2 fold. In another aspect, one or more gut microorganisms with abundance change are from *Eggerthella*, and wherein the *Eggerthella* microorganisms decrease their abundance by at least 2 fold. In another aspect, one or more gut microorganisms with abundance change are from *Bifidobacterium*, and wherein the *Bifidobacterium* microorganisms increase their abundance by at least 2 fold. In another aspect, one or more gut microorganisms with abundance change are from *Prevotella*, and wherein the *Prevotella* microorganisms increase their abundance by at least 2 fold. In another aspect, one or more gut microorganisms with abundance change are from *Desulfovibrio*, and wherein the *Desulfovibrio* microorganisms increase their abundance by at least 2 fold.

[0062] In one aspect, a subject being treated has an ASD and the treatment improves one or more ASD symptoms. In one aspect, a subject exhibits at least a 10% reduction in ASD symptom severity after the treatment as compared to before initiating the treatment, and based on an assessment system selected from the group consisting of Childhood Autism Rating Scale (CARS), Childhood Autism Rating Scale 2—Standard Form (CARS2-ST), Childhood Autism Rating Scale 2—High Functioning (CARS2-HF), Aberrant Behavior Checklist (ABC), Social Responsiveness Scale (SRS), and Vineland Adaptive Behavior Scale II (VABS-II).

[0063] In one aspect, a treatment results in an improvement of at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% based on the Leiter International Performance Scale (see Roid, G. H., & Miller, L. J. (1997). Leiter International Performance Scale—Revised. Wood Dale, Ill.: Stoelting) in an ASD patient. In another aspect, a Leiter score improvement is measured after at least 8, 16, 24, 32, 40, 50, 60, or 80 weeks of treatment and compared to a Leiter score prior to the treatment.

[0064] One of ordinary skill in the art understands that the foregoing assessment systems are only exemplary tools for evaluating ASD-related social and cognitive symptoms. Other similar tools can be used or designed to evaluate core ASD-related symptoms. For example, in one aspect, a

treatment results in an improvement of at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% based on Autism Treatment Evaluation Checklist (ATEC). See Rimland and Edelson: Autism Treatment Evaluation Checklist: Statistical Analyses. Autism Research Institute 2000. In another aspect, a treatment results in an improvement of at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% based on Pervasive Developmental Disorders Behavior Inventory (PDD-BI). See Cohen et al., The PDD Behavior Inventory: a rating scale for assessing response to intervention in children with pervasive developmental disorder. *J Autism Dev Disord.* 2003 33(1):31-45. In yet another aspect, a treatment results in an improvement of at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% based on Severity of Autism Scale (SAS). See Adams et al., The severity of autism is associated with toxic metal body burden and red blood cell glutathione levels. *J Toxicol.* 2009, 2009:532640. In a further aspect, an improvement of autism-related symptoms or an symptom severity reduction is assessed based on any one of the system or scale mentioned in Aman et al., Outcome Measures for Clinical Drug Trials in Autism, *CNS Spectr.* 9(1): 36-47 (2004). In a further aspect, an improvement of autism-related symptoms or an symptom severity reduction is assessed based on any one of the symptom characterization systems listed in Table 1. In another aspect, a method described here achieve an improvement of autism-related symptoms or an symptom severity reduction of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 graduations in a scale described here (e.g., in Table 1). In another aspect, a method described here achieve an improvement of autism-related symptoms or an symptom severity reduction of between 1 and 10, between 2 and 10, between 3 and 10, between 4 and 10, between 5 and 10, between 6 and 10, between 1 and 9, between 2 and 9, between 3 and 9, between 4 and 9, between 5 and 9, between 6 and 9, between 1 and 8, between 2 and 8, between 3 and 8, between 4 and 8, between 5 and 8, between 6 and 8, between 1 and 7, between 2 and 7, between 3 and 7, between 4 and 7, between 5 and 7, between 6 and 7, between 1 and 6, between 2 and 6, between 3 and 6, between 4 and 6, between 5 and 6, between 1 and 5, between 2 and 5, between 3 and 5, between 4 and 5, between 1 and 4, between 2 and 4, between 3 and 4, between 1 and 3, between 2 and 3, or between 1 and 2 graduations in a scale described here (e.g., CARS, CARS2-ST, CARS2-HF, ABC, SRS, VABS-II, PGI-R2, or a scale in Table 1). In one aspect, an symptom improvement over any one of the foregoing systems is measured after at least 8, 16, 24, 32, 40, 50, 60, or 80 weeks of treatment and compared to a Leiter score prior to the treatment. In one aspect, an symptom improvement over any one of the foregoing systems is measured after discontinuing treatment for at least 2, 4, 6, 8, 10 or more weeks and compared to a measurement prior to the treatment.

TABLE 1

Selected outcome measures that can be used to monitor core ASD-related social and cognitive symptoms.
Validated Outcome Measures

Tool	Description	
	Autism Symptoms	Rater
ADOS	The Autism Diagnostic Observation Schedule (ADOS) is a gold standards instrument for diagnosing ASD with the largest evidence base and highest sensitivity and specificity	Trained Examiner

TABLE 1-continued

Selected outcome measures that can be used to monitor core ASD-related social and
cognitive symptoms.
Validated Outcome Measures

Tool	Description	
OACIS	The Ohio Autism Clinical Impression Scale was developed to be sensitive to subtle, but clinically-meaningful changes in core and associated ASD symptoms using a focused scaling system that assesses severity and improvement in ASD behaviors similar to the widely used Clinical Global	Clinician
SRS	The Social Responsiveness Scale is a standardized and validated quantitative scale that measures the severity and type of social impairments that are characteristic of ASD	Parent or Teacher
SCQ	Social Communication Questionnaire is brief instrument that evaluates communication skills and social functioning. Both the current and lifetime editions will be used as appropriate	Parent or Teacher
AIM	The Autism Impact Measure is a recently developed parent-report measure that assesses both frequency and impact of current core ASD symptoms during the past 2-weeks. Initial studies have demonstrated excellent psychometric properties and construct validity Behavior	Parent
ABC	The Aberrant Behavior Checklist is a validated questionnaire that rates symptoms of hyperactivity, irritability, lethargy, and stereotypic behavior in individuals with developmental disabilities. It has been used in multiple clinical trials in ASD and has convergent and divergent validity	Parent or Teacher
CBCL	Child Behavior Checklist is an easy to complete standardized questionnaire that assesses a wide range of behaviors associated with ASD symptoms, including anxiety, depression, withdraw, sleep problems, somatic problems, and aggressive and destructive behavior	Parent or Teacher
BASC	The Behavioral Assessment System for Children provides scales of cognition function, behavior, social function, and academic problems. This scale measures a wide range of behaviors including hyperactivity, attention, depression, anxiety, and executive function. Language	Parent or Teacher
CELF	The Clinical Evaluations of Language Fundamentals is one of the only standardized, well-validated language assessment instruments that spans the age range of most participants (using both CELF-preschool-2 and CELF-4). It assesses a wide range of language skills that are only partially measured by other language tests, including high-level language skills that are abnormal in individuals with ASD, such as language pragmatics and has been used in several recent studies focusing on core language deficits in ASD	Trained Examiner
PLS	The Preschool Language Scale-4 is used in conjunction with the CELF since it is also a standardized, well-validated language assessment instrument and can measure subtle changes in language in children with poor language abilities Adaptive Behavior	Trained Examiner
VABS	The Vineland Adaptive Behavior Scale is a widely used standardized, well-validated assessment tool for children with developmental delays that measures functional abilities within several domains. It is particularly useful for children with intellectual disability which commonly co-occurs with ASD and has valid measures of social impairments in children with ASD Intellect	Trained Interviewer
Leiter-R	The Leiter-R, due to its non-verbal nature, is an excellent unbiased measure of intellect when language impairment exists. It assesses a wide range of ages (2-21 years) and contains attention and memory batteries which are skills often disrupted in ASD. The Leiter-R is designed to measure growth in all domains it assesses, making it sensitive to change due to treatment. Studies have shown good psychometric properties and verified that it is generally recommended for use in children with ASD	Trained Examiner
WISC/ WPPSI	The Wechsler Intelligence Scale for Children is one of the oldest and most widely used tests of intelligence for children. For children younger than 6 years the Wechsler Preschool and Primary Scale of Intelligence test is used. One disadvantage when using this with children with ASD is its reliance on language.	Trained Examiner

[0065] In one aspect, a treatment described here achieves at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% reduction in ASD symptom severity after 2 or more weeks of treatment as compared to before initiating the treatment, where the ASD symptom severity is assessed by a method selected from the group consisting of CARS, CARS2-ST, CARS2-HF, ABC, SRS, and VABS-II. In one aspect, a treatment achieves at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% reduction in ASD symptom severity after 4 or more weeks of treatment as compared to before initiating the treatment, where the ASD symptom severity is assessed by a method selected from the group consisting of CARS, CARS2-ST, CARS2-HF, ABC, SRS, and VABS-II. In one aspect, a treatment achieves at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% reduction in ASD symptom severity after 6 or more weeks of treatment as compared to before initiating the treatment, where the ASD symptom severity is assessed by a method selected from the group consisting of CARS, CARS2-ST, CARS2-HF, ABC, SRS, and VABS-II. In one aspect, a treatment achieves at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% reduction in ASD symptom severity after 8 or more weeks of treatment as compared to before initiating the treatment, where the ASD symptom severity is assessed by a method selected from the group consisting of CARS, CARS2-ST, CARS2-HF, ABC, SRS, and VAB S-II.

[0066] In another aspect, a treatment achieves between 10% and 20%, between 10% and 30%, between 10% and 40%, between 10% and 50%, between 10% and 60%, between 10% and 70%, between 10% and 80%, between 10% and 90%, between 20% and 30%, between 20% and 40%, between 20% and 50%, between 20% and 60%, between 20% and 70%, between 20% and 80%, between 20% and 90%, between 30% and 40%, between 30% and 50%, between 30% and 60%, between 30% and 70%, between 30% and 80%, between 30% and 90%, between 40% and 50%, between 40% and 60%, between 40% and 70%, between 40% and 80%, between 40% and 90%, between 50% and 60%, between 50% and 70%, between 50% and 80%, or between 50% and 90% reduction in ASD symptom severity after 8 or more weeks of treatment as compared to before initiating the treatment, where the ASD symptom severity is assessed by a method selected from the group consisting of CARS, CARS2-ST, CARS2-HF, ABC, SRS, and VABS-II. In another aspect, a treatment achieves between 10% and 90%, between 20% and 80%, between 30% and 70%, or between 40% and 60% reduction in ASD symptom severity after 8 or more weeks of treatment as compared to before initiating the treatment, where the ASD symptom severity is assessed by a method selected from the group consisting of CARS, CARS2-ST, CARS2-HF, ABC, SRS, and VABS-II. In another aspect, a treatment achieves between 10% and 90%, between 20% and 80%, between 30% and 70%, or between 40% and 60% reduction in ASD symptom severity after 12 or more weeks of treatment as compared to before initiating the treatment, where the ASD symptom severity is assessed by a method selected from the group consisting of CARS, CARS2-ST, CARS2-HF, ABC, SRS, and VABS-II. In another aspect, a treatment achieves between 10% and 90%, between 20% and 80%, between 30% and 70%, or between 40% and 60% reduction in ASD symptom severity after 18 or more weeks of treatment as compared to before initiating the treatment, where the ASD symptom severity is assessed by a method selected from the

group consisting of CARS, CARS2-ST, CARS2-HF, ABC, SRS, and VABS-II. In another aspect, a treatment achieves between 10% and 90%, between 20% and 80%, between 30% and 70%, or between 40% and 60% reduction in ASD symptom severity after 24 or more weeks of treatment as compared to before initiating the treatment, where the ASD symptom severity is assessed by a method selected from the group consisting of CARS, CARS2-ST, CARS2-HF, ABC, SRS, and VABS-II.

[0067] In one aspect, a treatment achieves at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% reduction in ASD symptom severity and substantially maintains the symptom severity reduction for at least 8, 12, 16, 20, 24, or 28 weeks after discontinuing the treatment, where the ASD symptom severity is assessed by CARS. In one aspect, a treatment achieves at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% reduction in ASD symptom severity and substantially maintains the symptom severity reduction for at least 8, 12, 16, 20, 24, or 28 weeks after discontinuing the treatment, where the ASD symptom severity is assessed by CARS2-ST. In one aspect, a treatment achieves at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% reduction in ASD symptom severity and substantially maintains the symptom severity reduction for at least 8, 12, 16, 20, 24, or 28 weeks after discontinuing the treatment, where the ASD symptom severity is assessed by CARS2-HF. In one aspect, a treatment achieves at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% reduction in ASD symptom severity and substantially maintains the symptom severity reduction for at least 8, 12, 16, 20, 24, or 28 weeks after discontinuing the treatment, where the ASD symptom severity is assessed by ABC. In one aspect, a treatment achieves at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% reduction in ASD symptom severity and substantially maintains the symptom severity reduction for at least 8, 12, 16, 20, 24, or 28 weeks after discontinuing the treatment, where the ASD symptom severity is assessed by SRS. In one aspect, a treatment achieves at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% reduction in ASD symptom severity and substantially maintains the symptom severity reduction for at least 8, 12, 16, 20, 24, or 28 weeks after discontinuing the treatment, where the ASD symptom severity is assessed by VABS-II.

[0068] In one aspect, an ASD subject being treated exhibits no gastrointestinal (GI) symptom prior to initiating a treatment. In another aspect, an ASD subject being treated exhibits one or more GI symptoms prior to initiating a treatment. In one aspect, an ASD subject being treated exhibits at least a 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% reduction in GI symptom severity after a treatment as compared to before initiating the treatment. In one aspect, GI symptom severity is assessed by the Gastrointestinal Symptom Rating Scale (GSRS). In another aspect, a treatment achieves between 20% and 30%, between 20% and 40%, between 20% and 50%, between 20% and 60%, between 20% and 70%, between 20% and 80%, between 20% and 90%, between 30% and 40%, between 30% and 50%, between 30% and 60%, between 30% and 70%, between 30% and 80%, between 30% and 90%, between 40% and 50%, between 40% and 60%, between 40% and 70%, between 40% and 80%, between 40% and 90%, between 50% and 60%, between 50% and 70%, between 50% and 80%, or between 50% and 90% reduction in GI symptom severity in an ASD patient after 8 or more weeks

of treatment as compared to before initiating the treatment, where the GI symptom severity is assessed by one or more symptom assessment systems selected from the group consisting of Gastrointestinal Severity Index (GSI) (Schneider et al., *J Autism Dev Disord* 2006; 36: 1053-64); 6-item Gastrointestinal Severity Index (6-GSI) (Adams et al., *BMC Gastroenterology* 2011, 11:22); Gastrointestinal Symptom Questionnaire—GISQ (Bovenschen et al., *Dig Dis Sci* (2006) 51:1509-15); GSRS (original) (Svedlund et al., Dig Dis Sci 1988; 33: 129-134); GSRS (Likert version) (Revicki et al., Quality of Life Research Vol 7 1998 75-83); GSRS-IBS (Wiklund et al., Scand J Gastroenterol 2003; 38(9): 947-954); PROMIS (Spiegel et al., *Am J Gastroenterol* 2014; 109(11): 1804-1814); PedsQL GI Distress Domain (Varni et al., JPGN 2014; 59: 347-355); Pediatric Functional Gastrointestinal Disorders (PFGD) (Caplan et al., J Pediatric Gastroenterol Nutrition 41, 305-316); Autism Network GI Symptom Inventory (Mazefsky, Autism Treatment Network. Autism Treatment Network GI Symptom Inventory Questionnaire, version 3.0. New York, N.Y.: Autism Speaks; 2005); Questionnaire on Pediatric GI Symptoms (QPGS) (ROME III criteria; Caplan et al., J Pediatric Gastroenterol Nutrition 2005 4, 296-304); GI Symptom Questionnaire (Chandler et al., J Autism Dev Disord 2013; 43: 2737-2747); Birmingham IBS (Roalfe et al., BMD Gastroenterology 2008; 8:30); Gastrointestinal Symptom Score in Kids (GISSK) (Brunner et al., *J Clin Rheumatol* 2005; 11: 194-204); Gastro-Q (Liebbrand et al., *Int J Behav Med* 2002; 9: 155-72); IBS-SSS (Francis et al., *Aliment Pharmacol Ther* 1997; 11: 395-402); and any other similar, corresponding, or modified systems.

[0069] The GSRS is a disease-specific instrument of 15 items combined into five symptom clusters depicting Reflux, Abdominal pain, Indigestion, Diarrhoea and Constipation. See Svedlund et al., *Dig. Dis. Sci.,* 33(2): 129-34(1988). The GSRS has a seven-point graded Likert-type scale where 0 represents absence of troublesome symptoms and 3 represents an extreme degree of the symptoms with half-steps to increase the sensitivity of the scales. In one aspect, a treatment method provided here reduces, alleviates, or eliminates one or more, two or more, three or more, four or more, five or more, six or more, or seven or more GI symptoms selected from the group consisting of epigastric pain, colicky abdominal pain, dull abdominal pain, undefined abdominal pain, heartburn, acid regurgitation, sucking sensations in the epigastrium, nausea and vomiting, borborygmus, abdominal distension, eructation, increased flatus, decreased passage of stools, increased passage of stools, loose stool, hard stools, urgent need for defecation, feeling of incomplete evacuation.

[0070] In one aspect, a treated subject's abdominal pain decreases from a more severe level to a less severe level, where the pain levels are selected from the group consisting of severe or crippling pains with impact on all social activities, prolonged and troublesome aches and pains causing requests for relief and interfering with many social activities, occasional aches and pains interfering with some social activities, and no or transient pain.

[0071] In another aspect, a treated subject's heartburn decreases from a more severe level to a less severe level, where the pain levels are selected from the group consisting of continuous discomfort with only transient relief by ant-

acids, frequent episodes of prolonged discomfort; requests for relief, occasional discomfort of short duration, and no or transient heartburn.

[0072] In another aspect, a treated subject's acid regurgitation condition improves from a more severe level to a less severe level, where the condition levels are selected from the group consisting of regurgitation several times a day; only transient and insignificant relief by antacids, regurgitation once or twice a day; requests for relief, occasional troublesome regurgitation, and no or transient regurgitation.

[0073] In another aspect, a treated subject's sucking sensations in the epigastrium improves from a more severe level to a less severe level, where the condition levels are selected from the group consisting of continuous discomfort; frequent requests for food or antacids between meals, frequent episodes of prolonged discomfort, requests for food and antacids between meals, occasional discomfort of short duration; no requests for food or antacids between meals, and no or transient sucking sensation. As used herein, sucking sensation in the epigastrium represents a sucking sensation in the epigastrium with relief by food or antacids. If food or antacids are not available, the sucking sensations progress to ache, and pains.

[0074] In another aspect, a treated subject's nausea or vomiting condition improves from a more severe level to a less severe level, where the condition levels are selected from the group consisting of continuous nausea coupled with frequent vomiting, frequent and prolonged nausea with no vomiting, occasional nausea episodes of short duration, and no nausea.

[0075] In another aspect, a treated subject's borborygmus condition improves from a more severe level to a less severe level, where the condition levels are selected from the group consisting of continuous borborygmus severely interfering with social performance, frequent and prolonged episodes which can be mastered by moving without impairing social performance, occasional troublesome borborygmus of short duration, and no or transient borborygmus.

[0076] In another aspect, a treated subject's abdominal distension condition improves from a more severe level to a less severe level, where the condition levels are selected from the group consisting of continuous discomfort seriously interfering with social performance, frequent and prolonged episodes which can be mastered by adjusting the clothing, occasional discomfort of short duration, and no or transient distension.

[0077] In another aspect, a treated subject's eructation condition improves from a more severe level to a less severe level, where the condition levels are selected from the group consisting of frequent episodes seriously interfering with social performance, frequent episodes interfering with some social activities, occasional troublesome eructation, and no or transient eructation.

[0078] In another aspect, a treated subject's increased flatus condition improves from a more severe level to a less severe level, where the condition levels are selected from the group consisting of frequent episodes seriously interfering with social performance, frequent and prolonged episodes interfering with some social activities, occasional discomfort of short duration, and no increase flatus.

[0079] In another aspect, a treated subject's decreased stool frequency improves from a more severe level to a less severe level, where the levels are selected from the group

consisting of every seventh day or less freluently, every sixth day, every fifth day, every fourth day, every third day, every second day, and once a day.

[0080] In another aspect, a treated subject's increased stool frequency improves from a more severe level to a less severe level, where the levels are selected from the group consisting of seven times a day or more frequently, six times a day, five times a day, four times a day, three times a day, twice a day, and once a day.

[0081] In another aspect, a treated subject's loose-stool condition improves from a more severe level to a less severe level, where the levels are selected from the group consisting of watery, runny, somewhat loose, and normal consistency.

[0082] In another aspect, a treated subject's hard-stool condition improves from a more severe level to a less severe level, where the levels are selected from the group consisting of hard and fragmented with occasional diarrhea, hard, somewhat hard, and normal consistency.

[0083] In another aspect, a treated subject's urgency for defecation improves from a more severe level to a less severe level, where the levels are selected from the group consisting of inability to control defecation, frequent feelings of urgent need for defecation with sudden need for a toilet interfering with social performance, occasional feelings of urgent need for defecation, and normal control of defecation.

[0084] In another aspect, a treated subject's feeling of incomplete evacuation improves from a more severe level to a less severe level, where the levels are selected from the group consisting of defecation extremely difficult with regular feelings of incomplete evacuation, defecation definitely difficult with often feelings of incomplete evacuation, defecation somewhat difficult; occasional feelings of incomplete evacuation, and feeling of complete evacuation without straining.

[0085] In another aspect, a treated subject's one or more additional GI symptoms improve from a more severe level to a less severe level, where these one or more additional GI symptoms are selected from the group consisting of unusually large amount and/or large diameter of stools, unusually foul-smelling stools, unusual color of stool (medium brown is normal). In another aspect, a treated subject's stool also improves to a form corresponding to Type 3 or 4 of the Bristol stool scale or improves from a more irregular type to a type closer of the normal stool form (e.g., from Types 1-2 or Types 5-7 to Type 3 or 4. Bristol stool scale is a medical aid designed to classify the form of human feces into seven types. See Lewis and Heaton, *Scand J Gastroenterol.* 32(9): 920-24 (1997). The seven types of stool are: Type 1: Separate hard lumps, like nuts (hard to pass); Type 2: Sausage-shaped, but lumpy; Type 3: Like a sausage but with cracks on its surface; Type 4: Like a sausage or snake, smooth and soft; Type 5: Soft blobs with clear cut edges (passed easily); Type 6: Fluffy pieces with ragged edges, a mushy stool; and Type 7: Watery, no solid pieces, entirely liquid.

[0086] In one aspect, a symptom severity reduction (e.g., for ASD symptoms, GI symptoms, or both) is ongoing during a treatment or sustained after finishing or discontinuing a treatment. In one aspect, a symptom severity reduction (e.g., for ASD symptoms, GI symptoms, or both) is assessed at a specific time point during or post treatment, e.g., about 2, 4, 6, 8, 12, 18, 24, 32, 40, 48 weeks after initiating a

treatment, or about 2, 4, 6, 8, 12, 18, 24, 32, 40, 48 weeks after finishing or discontinuing a treatment.

[0087] In one aspect, a method further comprises administering an antibiotic to a subject prior to administering a pharmaceutical composition comprising a fecal microbe preparation. In another aspect, a method further comprises subjecting a subject to a bowel cleanse.

[0088] In another aspect, a pharmaceutical composition used herein comprises a non-selected and substantially complete fecal microbiota supplemented with one or more viable, non-pathogenic microorganisms selected from the group consisting of *Bifidobacterium, Prevotella, Desulfovibrio, Copprococcus*, and *Clostridium*. In another aspect, a pharmaceutical composition used herein comprises a synthetic fecal composition of predetermined flora. In another aspect, a pharmaceutical composition used herein comprises a predetermined flora comprises a preparation of viable flora in proportional content that resembles a normal healthy human fecal flora and comprises no antibiotic resistant populations. In another aspect, a pharmaceutical composition used herein is administered as a solid dosage form selected from the group consisting of capsule, tablet, powder, and granule. In another aspect, a pharmaceutical composition used herein is formulated as an acid resistant capsule.

[0089] In another aspect, provided herein is a method of treating an autism spectrum disorder in a human subject. In exemplary aspects, the method comprises or consists essentially of the following steps: administering an antibiotic to a human subject; subjecting the human subject to a bowel cleanse; and administering purified fecal microbiota to the human subject, wherein an autism spectrum disorder is treated in the human subject.

[0090] In exemplary aspects, treating ASD comprises alleviating, ameliorating, delaying the onset of, inhibiting the progression of, or reducing the severity of one or more, two or more, three or more, four or more, five or more, six or more, seven or more, eight or more symptoms characteristic of ASD. In one aspect, a treatment alleviates, ameliorates, delays the onset of, inhibits the progression of, or reduces the severity of one or more social and cognitive core ASD-related symptoms. In some aspects, the symptom(s) is selected from the group consisting of: (i) insistence on sameness or resistance to change; (ii) difficulty in expressing needs; (iii) repeating words or phrases in place of normal, responsive language; (iv) laughing, crying, showing distress for reasons not apparent to others; (v) prefers to be alone or aloof manner; (vi) tantrums; (vii) difficulty in mixing with others; (viii) may not want to cuddle or be cuddled; (ix) little or no eye contact; (x) unresponsive to normal teaching methods; (xi) sustained odd play; (xii) apparent over-sensitivity or under-sensitivity to pain; (xiii) little or no real fears of danger; (xiv) noticeable physical over-activity or extreme under-activity; (xv) uneven gross/fine motor skills; and/or (xvi) non-responsiveness to verbal cues. In some aspects, the symptom(s) is selected from the group consisting of compulsive behavior, ritualistic behavior, restricted behavior, stereotypy, sameness, or self-injury. The methods described here can lead to improvement of any combination of the foregoing symptoms.

[0091] In exemplary aspects, the human subject exhibits a significant reduction in autism symptom severity as assessed according to a ASD rating scale. In some cases, for example, the human subject exhibits at least a 10% or 20% reduction

in autism symptom severity as assessed by the Childhood Autism Rating Scale (CARS) relative to severity as assessed prior to initiating the method.

[0092] Subjects appropriate for treatment according to a method provided herein may not present with or report gastrointestinal distress symptoms prior to initiating a method as provided herein. In some cases, for example, a human subject appropriate for treatment according to a method provided herein manifests no gastrointestinal symptoms prior to or at the time at which treatment is begun. In one aspect, an ASD subject treated herein exhibit one or more or two or more GI symptoms selected from the group consisting of abdominal pain, reflux, indigestion, irritable bowel syndrome, chronic persistent diarrhoea, diarrhoea, flatulence, constipation, and alternating constipation/diarrhoea.

[0093] Regardless of the presence or absence of gastrointestinal distress symptoms, human subjects appropriate for the methods provided herein typically have significantly fewer species of gut bacteria before the method of treatment as compared to a neurotypical human. In some cases, the human subject to be treated by the method exhibits at least about 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% fewer species of gut bacterial prior to administration of the purified fecal microbiota dosage as compared to a neurotypical human.

[0094] Also provided herein are methods for reducing autism severity in an autistic human subject. In exemplary aspects, the method comprises or consists essentially of the following steps: orally-administering a non-absorbable antibiotic to an autistic human subject; subjecting the autistic human subject to a bowel cleanse; and administering purified fecal microbiota from a neurotypical human donor to the human subject, wherein the human subject exhibits a significant reduction in autism symptom severity as assessed by the Childhood Autism Rating Scale (CARS) after the method as compared to before initiating the method. In some cases, the human subject exhibits at least a 10% or 20% reduction in autism symptom severity as assessed by the Childhood Autism Rating Scale (CARS) relative to severity as assessed prior to initiating the method.

[0095] In one aspect, a fecal microbiota preparation used in a method described here comprises a donor's entire or substantially complete microbiota. In one aspect, a fecal microbiota preparation comprises a non-selected fecal microbiota. In another aspect, a fecal microbiota preparation comprises an isolated or purified population of live non-pathogenic fecal bacteria. In a further aspect, a fecal microbiota preparation comprises a non-selected and substantially complete fecal microbiota preparation from a single donor. In another aspect, a therapeutic composition used herein comprises a mixture of live, non-pathogenic, synthetic bacteria or live, non-pathogenic, purified or extracted, fecal microbiota.

[0096] In one aspect, the preparation of a fecal microbiota preparation involves a treatment selected from the group consisting of ethanol treatment, detergent treatment, heat treatment, irradiation, and sonication, or a combination thereof. In one aspect, the preparation of a fecal microbiota preparation involves no treatment selected from the group consisting of ethanol treatment, detergent treatment, heat treatment, irradiation, and sonication. In one aspect, the preparation of a fecal microbiota preparation involves a separation step selected from the group consisting of filter-

ing, sieving, density gradients, filtration, chromatography, and a combination thereof. In one aspect, the preparation of a fecal microbiota preparation does not require one or more separation steps selected from the group consisting of filtering, sieving, density gradients, filtration, and chromatography. In one aspect, a fecal microbiota preparation is substantially free of non-living matter. In one aspect, a fecal microbiota preparation is substantially free of acellular material selected from the group consisting of residual fiber, DNA, viral coat material, and non-viable material. In one aspect, a fecal microbiota preparation is substantially free of eukaryotic cells from the fecal microbiota's donor.

[0097] In one aspect, the present disclosure provides a method for treating ASD in a subject in need thereof, where the method comprises administering to the subject a pharmaceutically active dose of a therapeutic composition described herein. In one aspect, the present disclosure provides a method for treating ASD in a subject in need thereof, where the method comprises administering daily to the subject a pharmaceutically active dose of a therapeutic composition described herein. In one aspect, a therapeutic composition is administered to a patient in need thereof at least once daily for at least two consecutive days. In one aspect, a therapeutic composition is administered at least once daily for at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 consecutive days. In another aspect, a therapeutic composition is administered at least once daily for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 consecutive weeks. In one aspect, a therapeutic composition is administered at least once daily for at most 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 consecutive days or weeks. In another aspect, a therapeutic composition is administered at least once daily for at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 consecutive weeks or months. In a further aspect, a therapeutic composition is administered at least once for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 consecutive months or years, chronically for a subject's entire life span, or an indefinite period of time.

[0098] In one aspect, a therapeutic composition is administered to a patient in need thereof at least twice daily for at least two consecutive days. In one aspect, a therapeutic composition is administered at least twice daily for at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 consecutive days. In another aspect, a therapeutic composition is administered at least twice daily for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 consecutive weeks. In one aspect, a therapeutic composition is administered at least twice daily for at most 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 consecutive days or week. In another aspect, a therapeutic composition is administered at least twice daily for at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 consecutive weeks or months. In a further aspect, a therapeutic composition is administered at least twice for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 consecutive months or years, chronically for a subject's entire life span, or an indefinite period of time.

[0099] In one aspect, a therapeutic composition is administered to a patient in need thereof at least three times daily for at least two consecutive days. In one aspect, a therapeutic composition is administered at least three times daily for at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 consecutive days. In another aspect, a therapeutic composition is administered at least three times daily for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 consecutive weeks. In one aspect, a therapeutic composition is administered at least three times

daily for at most 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 consecutive days or weeks. In another aspect, a therapeutic composition is administered at least three times daily for at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 consecutive weeks or months. In a further aspect, a therapeutic composition is administered at least three times for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 consecutive months or years, chronically for a subject's entire life span, or an indefinite period of time.

[0100] In one aspect, the present disclosure provides a method for treating ASD in a subject in need thereof, where the method comprises administering orally to the subject a pharmaceutically active dose of a therapeutic composition comprising live, non-pathogenic, synthetic bacterial mixture or live, non-pathogenic, purified or extracted, fecal microbiota, where the dose is administered at a dosing schedule of at least once or twice daily for at least three consecutive days or weeks. In another aspect, a dose is administered at least once, twice, or three times daily for a period between 1 and 12 weeks, between 2 and 12 weeks, between 3 and 12 weeks, between 4 and 12 weeks, between 5 and 12 weeks, between 6 and 12 weeks, between 7 and 12 weeks, between 8 and 12 weeks, between 9 and 12 weeks, between 10 and 12 weeks, between 1 and 2 weeks, between 2 and 3 weeks, between 3 and 4 weeks, between 4 and 5 weeks, between 5 and 6 weeks, between 6 and 7 weeks, between 7 and 8 weeks, between 8 and 9 weeks, between 9 and 10 weeks, or between 10 and 11 weeks.

[0101] In one aspect, the present disclosure provides a method for treating ASD in a subject in need thereof by administering a pharmaceutical composition described herein, where the method comprises a first dosing schedule followed by a second dosing schedule. In one aspect, a first dosing schedule comprises a treatment or induction dose. In one aspect, a first dosing schedule comprises a continuous dosing schedule. In another aspect, a second dosing schedule comprises a maintenance dose lower than or equal to a pharmaceutically active dose of a first dosing schedule. In another aspect, a second dosing schedule lasts for at least about 2, 4, 6, 8, 10, 12, 18, 24, 36, 48, 72, or 96 months. In one aspect, a second dosing schedule lasts permanently, for a treated subject's entire life span, or an indefinite period of time. In one aspect, a second dosing schedule is a continuous dosing schedule. In another aspect, a second dosing schedule is an intermittent dosing schedule. In a further aspect, a second dosing schedule is an intermittent dosing schedule comprising a treatment period of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 days followed by a resting period of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 days. In another aspect, a second dosing schedule comprises administering a second dose (e.g., a maintenance dose) every other day, every two days, or every 3, 4, 5, 6, 7, 8 days. In another aspect, a maintenance dose is administered for an extended period of time with or without titration (or otherwise changing the dosage or dosing schedule). In one aspect, the interval between a first and a second dosing schedule is at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 weeks. In another aspect, a second dosing schedule (e.g., a maintenance dose) comprises a dosage about 2, 5, 10, 50, 100, 200, 400, 800, 1000, 5000 or more folds lower than the dosage used in a first dosing schedule (e.g., an initial treatment dose). In another aspect, a second dosing schedule (e.g., a maintenance dosing schedule) has an equal or lower dosing frequency than a first dosing schedule (e.g., an initial treat-

ment dosing schedule). In another aspect, a second dosing schedule (e.g., a maintenance dosing schedule) has a higher dosing interval than a first dosing schedule (e.g., an initial treatment dosing schedule).

[0102] In one aspect, a first or second dosing schedule used in a method can be once-a-week, twice-a-week, or thrice-a-week. The term "once-a-week" means that a dose is administered once in a week, preferably on the same day of each week. "Twice-a-week" means that a dose is administered two times in a week, preferably on the same two days of each weekly period. "Thrice-a-week" means that a dose is administered three times in a week, preferably on the same three days of each weekly period. In one aspect, a first or second dosing schedule can use fecal microbiota prepared from two or more different donors. In another aspect, a first dose schedule (e.g., a treatment, induction, or initial loading dose) comprises a fecal microbiota preparation from a donor different from the donor providing the fecal microbiota preparation used in a second dose schedule (e.g., a maintenance dose).

[0103] In one aspect, a subject being treated is a subject already with a disorder (e.g., ASD). Administration of a disclosed therapeutic composition to clinically, asymptomatic human subject who is genetically predisposed or prone to a disorder (e.g., ASD) is also useful in preventing the onset of clinical symptoms. A human subject genetically predisposed or prone to ASD can be a human subject having a close family member or relative exhibiting or having suffered a disorder (e.g., ASD). In another aspect, a subject being treated is a subject in which ASD is to be prevented. In another aspect, a subject being treated is predisposed or susceptible to a disorder (e.g., ASD). In another aspect, a subject being treated is a subject diagnosed as having a disorder (e.g., ASD). In one aspect, a subject being treated is a patient in need thereof.

[0104] In one aspect, a subject being treated is a human patient. In one aspect, a patient is a male patient. In one aspect, a patient is a female patient. In one aspect, a patient is a premature newborn. In one aspect, a patient is a term newborn. In one aspect, a patient is a neonate. In one aspect, a patient is an infant. In one aspect, a patient is a toddler. In one aspect, a patient is a young child. In one aspect, a patient is a child. In one aspect, a patient is an adolescent. In one aspect, a patient is a pediatric patient. In one aspect, a patient is a geriatric patient. In one aspect, a human patient is a child patient below about 18, 15, 12, 10, 8, 6, 4, 3, 2, or 1 year old. In another aspect, a human patient is an adult patient. In another aspect, a human patient is an elderly patient. In a further aspect, a human patient is a patient above about 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, or 95 years old. In another aspect, a patient is about between 1 and 5, between 2 and 10, between 3 and 18, between 21 and 50, between 21 and 40, between 21 and 30, between 50 and 90, between 60 and 90, between 70 and 90, between 60 and 80, or between 65 and 75 years old. In one aspect, a patient is a young old patient (65-74 years). In one aspect, a patient is a middle old patient (75-84 years). In one aspect, a patient is an old patient (>85 years).

[0105] In one aspect, a method comprises administering a therapeutic composition orally, by enema, or via rectal suppository. In one aspect, a pharmaceutical composition is formulated as a geltab, pill, microcapsule, capsule, or tablet. In one aspect, a therapeutic composition is formulated as an enteric coated capsule or microcapsule, acid-resistant cap-

sule or microcapsule, or formulated as part of or administered together with a food, a food additive, a dairy-based product, a soy-based product or a derivative thereof, a jelly, or a yogurt. In another aspect, a therapeutic composition is formulated as an acid-resistant enteric coated capsule. A therapeutic composition can be provided as a powder for sale in combination with a food or drink. A food or drink can be a dairy-based product or a soy-based product. In another aspect, a food or food supplement contains enteric-coated and/or acid-resistant microcapsules containing a therapeutic composition.

[0106] In an aspect, a therapeutic composition comprises a liquid culture. In another aspect, a therapeutic composition is lyophilized, pulverized and powdered. It may then be infused, dissolved such as in saline, as an enema. Alternatively the powder may be encapsulated as enteric-coated and/or acid-resistant capsules for oral administration. These capsules may take the form of enteric-coated and/or acid-resistant microcapsules. A powder can preferably be provided in a palatable form for reconstitution for drinking or for reconstitution as a food additive. In a further aspect, a food is yogurt. In one aspect, a powder may be reconstituted to be infused via naso-duodenal infusion.

[0107] In another aspect, a therapeutic composition is in a liquid, frozen, freeze-dried, spray-dried, lyophilized, or powder formulation. In a further aspect, a therapeutic composition is formulated as a delayed or gradual enteric release form. In another aspect, a therapeutic composition comprises an excipient, a saline, a buffer, a buffering agent, or a fluid-glucose-cellobiose agar (RGCA) media.

[0108] In one aspect, a therapeutic composition further comprises an acid suppressant, an antacid, an H2 antagonist, a proton pump inhibitor or a combination thereof. In one aspect, a therapeutic composition substantially free of non-living matter. In another aspect, a therapeutic composition substantially free of acellular material selected from the group consisting of residual fiber, DNA, viral coat material, and non-viable material.

[0109] In one aspect, a therapeutic composition comprises a cryoprotectant. In another aspect, a cryoprotectant comprises, consisting essentially or, or consisting of polyethylene glycol, skim milk, erythritol, arabitol, sorbitol, glucose, fructose, alanine, glycine, proline, sucrose, lactose, ribose, trehalose, dimethyl sulfoxide (DMSO), glycerol, or a combination thereof.

[0110] In another aspect, a therapeutic composition comprises a lyoprotectant. In one aspect, the same substance or the same substance combination is used as both a cryoprotectant and a lyoprotectant. Exemplary lyoprotectants include sugars such as sucrose or trehalose; an amino acid such as monosodium glutamate or histidine; a methylamine such as betaine; a lyotropic salt such as magnesium sulfate; a polyol such as trihydric or higher sugar alcohols, e.g. glycerin, erythritol, glycerol, arabitol, xylitol, sorbitol, and mannitol; propylene glycol; polyethylene glycol; Pluronics; and combinations thereof. In one aspect, a lyoprotectant is a non-reducing sugar, such as trehalose or sucrose. In one aspect, a cryoprotectant or a lyoprotectant consisting essentially of, or consisting of, one or more substances mentioned in this paragraph and the paragraph above.

[0111] In one aspect, a lyophilized formulation comprises trehalose. In one aspect, a lyophilized formulation comprises 2% to 30%, 3% to 25%, 4% to 20%, 5% to 15%, 6% to 10%, 2% to 30%, 2% to 25%, 2% to 20%, 2% to 15%, or 2% to 10% trehalose. In one aspect, a lyophilized formulation comprises at least 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, or 15% trehalose. In one aspect, a lyophilized formulation comprises at most 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, or 15% trehalose. In one aspect, a lyophilized formulation comprises about 5% trehalose. In one aspect, a lyophilized formulation comprises trehalose and sucrose. In one aspect, a lyophilized formulation comprises between about 8% to 12% trehalose with between about 1.5% to 3.5% sucrose and between about 0.5% to 1.5% NaCl.

[0112] In one aspect, a therapeutic composition also comprises or is supplemented with a prebiotic nutrient selected from the group consisting of polyols, fructooligosaccharides (FOSs), oligofructoses, inulins, galactooligosaccharides (GOSs), xylooligosaccharides (XOSs), polydextroses, monosaccharides, tagatose, and/or mannooligosaccharides.

[0113] In one aspect, a method further comprises pretreating a subject with an antibiotic composition prior to administering a therapeutic bacterial or microbiota composition. In one aspect, an antibiotic composition comprises an antibiotic selected from the group consisting of rifabutin, clarithromycin, clofazimine, vancomycin, rifampicin, nitroimidazole, chloramphenicol, and a combination thereof. In another aspect, an antibiotic composition comprises an antibiotic selected from the group consisting of rifaximin, rifamycin derivative, rifampicin, rifabutin, rifapentine, rifalazil, bicozamycin, aminoglycoside, gentamycin, neomycin, streptomycin, paromomycin, verdamicin, mutamicin, sisomicin, netilmicin, retymicin, kanamycin, aztreonam, aztreonam macrolide, clarithromycin, dirithromycin, roxithromycin, telithromycin, azithromycin, bismuth subsalicylate, vancomycin, streptomycin, fidaxomicin, amikacin, arbekacin, neomycin, netilmicin, paromomycin, rhodostreptomycin, tobramycin, apramycin, and a combination thereof. In a further aspect, a method further comprises pretreating a subject with an anti-inflammatory drug prior to administration of a therapeutic bacterial or microbiota composition.

[0114] In one aspect, every about 200 mg of a pharmaceutical composition comprises a pharmacologically active dose. In one aspect, every about 75, 100, 125, 150, 175, 200, 250, 300, 350, 400, 450, 500, 750, 1000, 1500, or 2000 mg of a pharmaceutical composition comprises a pharmacologically active dose.

[0115] In one aspect, a pharmaceutically active or therapeutic effective dose comprises at least about 10^5, 10^6, 10^7, 10^8, 10^9, 10^{10}, 10^{11}, 10^{12}, or 10^{13} cfu. In another aspect, a pharmaceutically active therapeutic effective dose comprises at most about 10^5, 10^6, 10^7, 10^8, 10^9, 10^{10}, 10^{11}, 10^{12}, or 10^{13} cfu. In a further aspect, a pharmacologically active therapeutic effective dose is selected, from the group consisting of from 10^8 cfu to 10^{14} cu from 10^9 cfu to 10^{13} cfu, from 10^{10} cfu to 10^{12} cfu, from 10^9 cfu to 10^{14} cfu, from 10^9 cfu to 10^{12} cfu, from 10^9 cfu to 10^{11} cfu, from 10^9 cfu to 10^{10} cfu, from 10^{10} cfu to 10^{14} cfu, from 10^{10} cfu to 10^{13} cfu, from 10^{11} cfu to 10^{14} cfu, from 10^{11} cfu to 10^{13} cfu, from 10^{12} cfu to 10^{14} cfu, and from 10^{13} cfu to 10^{14} cfu. In one aspect, a pharmaceutical composition comprises the foregoing pharmaceutically active or therapeutic effective dose in a unit weight of about 0.2, 0.4, 0.6, 0.8 or 1.0 gram, or a unit volume of about 0.2, 0.4, 0.6, 0.8 or 1.0 milliliter.

[0116] In one aspect, a pharmaceutically active or therapeutic effective dose comprises at least 12, about 10^5, 10^6, 10^7, 10^8, 10^9, 10^{10}, 10^{11}, 10^{12}, or 10^{13} cells or spores. In

another aspect, a pharmaceutically active or therapeutic effective dose comprises at most about 10^5, 10^6, 10^7, 10^8, 10^9, 10^{10}, 10^{11}, 10^{12}, or 10^{13} total cells or spores. In a further aspect, a pharmacologically active or therapeutic effective dose is selected from the group consisting of from 10^8 to 10^{14}, from 10^9 to 10^{13}, from 10^{10} to 10^{12}, from 10^9 to 10^{14}, from 10^9 to 10^{12}, from 10^9 to 10^{11}, from 10^9 to 10^{10}, from 10^{10} to 10^{14}, from 10^{10} to 10^{13}, from 10^{11} to 10^{14}, from 10^{11} to 10^{13}, from 10^{12} to 10^{14}, and from 10^{13} to 10^{14} cells or spores. In an aspect, the pharmaceutically active or therapeutic effective dose cell count is directed to live cells. In one aspect, a pharmaceutical composition comprises the foregoing pharmaceutically active or therapeutic effective dose in a unit weight of about 0.2, 0.4, 0.6, 0.8 or 1.0 gram, or a unit volume of about 0.2, 0.4, 0.6, 0.8 or 1.0 milliliter.

[0117] In one aspect, a therapeutic composition described and used here comprises one or more, two or more, three or more, four or more, or five or more isolated, purified, or cultured microorganisms selected from the group consisting of *Clostridium, Bacillus, Collinsella, Bacteroides, Eggerthella, Eubacterium, Fusobacterium, Propionibacterium, Lactobacillus, Ruminococcus, Escherichia coli, Gemmiger, Desulfomonas, Peptostreptococcus, Bifidobacterium, Coprococcus, Dorea*, and *Monilia*.

[0118] In one aspect, a fecal microbiota preparation described herein comprises a purified or reconstituted fecal bacterial mixture. In one aspect, a fecal microbiota preparation described and used here comprises one or more, one or more, two or more, three or more, four or more, or five or more live fecal microorganisms are selected from the group consisting of *Acidaminococcus, Akkermansia, Alistipes, Anaerotruncus, Bacteroides, Eggerthella, Bifidobacterium, Blautia, Butyrivibrio, Clostridium, Collinsella, Coprococcus, Corynebacterium, Dorea, Enterococcus, Escherichia, Eubacterium, Faecalibacterium, Haemophilus, Holdemania, Lactobacillus, Moraxella, Parabacteroides, Prevotella, Propionibacterium, Raoultella, Roseburia, Ruminococcus, Staphylococcus, Streptococcus, Subdoligranulum*, and *Veillonella*. In one aspect, a fecal microbiota preparation comprises one or more, one or more, two or more, three or more, four or more, or five or more live fecal microorganisms are selected from the group consisting of *Bacteroides fragilis* ssp. *vulgatus, Collinsella aerofaciens, Bacteroides fragilis* ssp. *thetaiotaomicron*, Parabacteroides *distasonis, Faecalibacterium prausnitzii, Coprococcus eutactus, Peptostreptococcus productus* I, *Ruminococcus bromii, Bifidobacterium adolescentis, Gemmiger formicilis, Bifidobacterium longum, Eubacterium siraeum, Ruminococcus torques, Eubacterium rectale, Eubacterium eligens, Bacteroides eggerthii, Clostridium leptum, Bacteroides fragilis* ssp. A, *Eubacterium biforme, Bifidobacterium infantis, Eubacterium rectale, Coprococcus comes, Pseudoflavonifractor capillosus, Ruminococcus albus, Dorea formicigenerans, Eubacterium hallii, Eubacterium ventriosum* I, *Fusobacterium russi, Ruminococcus obeum, Eubacterium rectale, Clostridium ramosum, Lactobacillus leichmannii, Ruminococcus callidus, Butyrivibrio crossotus, Acidaminococcus fermentans, Eubacterium ventriosum, Bacteroides fragilis* ssp. *fragilis, Coprococcus catus, Aerostipes hadrus, Eubacterium cylindroides, Eubacterium ruminantium, Staphylococcus epidermidis, Eubacterium limosum, Tissirella praeacuta, Fusobacterium mortiferum* I, *Fusobacterium naviforme, Clostridium innocuum, Clostridium ramosum, Propionibacterium acnes, Ruminococcus flavefaciens, Bacteroides fragilis* ssp. *ovatus, Fusobacterium nucleatum, Fusobacterium mortiferum, Escherichia coli, Gemella morbillorum, Finegoldia magnus, Streptococcus intermedius, Ruminococcus lactaris, Eubacterium tenue, Eubacterium ramulus, Bacteroides clostridiiformis* ssp. *clostridliformis, Bacteroides coagulans, Prevotella oxalis, Prevotella ruminicola, Odoribacter splanchnicus*, and *Desuifomonas pigra*.

[0119] In one aspect, a fecal microbiota preparation described and used here lacks or is substantially devoid of one or more, one or more, two or more, three or more, four or more, or five or more live fecal microorganisms are selected from the group consisting of *Acidaminococcus, Akkermansia, Alistipes, Anaerotruncus, Bacteroides, Eggerthella, Bifidobacterium, Blautia, Butyrivibrio, Clostridium, Collinsella, Coprococcus, Corynebacterium, Dorea, Enterococcus, Escherichia, Eubacterium, Faecalibacterium, Haemophilus, Holdemania, Lactobacillus, Moraxella, Parabacteroides, Prevotella, Propionibacterium, Raoultella, Roseburia, Ruminococcus, Staphylococcus, Streptococcus, Subdoligranulum*, and *Veillonella*. In one aspect, a fecal microbiota preparation lacks or is substantially devoid of one or more, one or more, two or more, three or more, four or more, or five or live more fecal microorganisms are selected from the group consisting of *Bacteroides fragilis* ssp. *vulgatus, Collinsella aerofaciens, Bacteroides fragilis* ssp. *thetaiotaomicron, Peptostreptococcus productus* II, *Parabacteroides distasonis, Faecalibacterium prausnitzii, Coprococcus eutactus, Peptostreptococcus productus* I, *Ruminococcus bromii, Bifidobacterium adolescentis, Gemmiger formicilis, Bifidobacterium longum, Eubacterium siraeum, Ruminococcus torques, Eubacterium rectale, Eubacterium eligens, Bacteroides eggerthii, Clostridium leptum, Bacteroides fragilis* ssp. A, *Eubacterium biforme, Bifidobacterium infantis, Eubacterium rectale, Coprococcus comes, Pseudoflavonifractor capillosus, Ruminococcus albus, Dorea formicigenerans, Eubacterium hallii, Eubacterium ventriosum* I, *Fusobacterium russi, Ruminococcus obeum, Eubacterium rectale, Clostridium ramosum, Lactobacillus leichmannii, Ruminococcus callidus, Butyrivibrio crossotus, Acidaminococcus fermentans, Eubacterium ventriosum, Bacteroides fragilis* ssp. *fragilis, Coprococcus catus, Aerostipes hadrus, Eubacterium cylindroides, Eubacterium ruminantium, Staphylococcus epidermidis, Eubacterium limosum, Tissirella praeacuta, Fusobacterium mortiferum* I, *Fusobacterium naviforme, Clostridium innocuum, Clostridium ramosum, Propionibacterium acnes, Ruminococcus flavefaciens, Bacteroides fragilis* ssp. *ovatus, Fusobacterium nucleatum, Fusobacterium mortiferum, Escherichia coli, Gemella morbillorum, Finegoldia magnus, Streptococcus intermedius, Ruminococcus lactaris, Eubacterium tenue, Eubacterium ramulus, Bacteroides clostridiiformis* ssp. *clostridliformis, Bacteroides coagulans, Prevotella oxalis, Prevotella ruminicola, Odoribacter splanchnicus*, and *Desuifomonas pigra*.

[0120] In another aspect, a therapeutic composition comprises a fecal microbiota further supplemented, spiked, or enhanced with a fecal microorganism. In one aspect, a fecal microbiota is supplemented with a non-pathogenic (or with attenuated pathogenicity) bacterium of *Clostridium, Collinsella, Dorea, Ruminococcus, Coprococcus, Prevotella, Veillonella, Bacteroides, Eggerthella, Bacillus*, or a combination thereof. In another aspect, a therapeutic composition com-

prises a fecal microbiota further supplemented, spiked, or enhanced with a species of *Veillonellaceae, Firmicutes, Gammaproteobacteria, Bacteroidetes*, or a combination thereof. In another aspect, a therapeutic composition comprises a fecal microbiota further supplemented with fecal bacterial spores. In one aspect, fecal bacterial spores are *Clostridium* spores, *Bacillus* spores, or both. In another aspect, a therapeutic composition comprises a fecal microbiota further supplemented, spiked, or enhanced with a *Bacteroides* species selected from the group consisting of *Bacteroides coprocola, Bacteroides plebeius, Bacteroides massiliensis, Bacteroides vulgatus, Bacteroides helcogenes, Bacteroides pyogenes, Bacteroides tectus, Bacteroides uniformis, Bacteroides stercoris, Bacteroides eggerthii, Bacteroides finegoldii, Bacteroides thetaiotaomicron, Bacteroides ovatus, Bacteroides acidifaciens, Bacteroides caccae, Bacteroides nordii, Bacteroides salyersiae, Bacteroides fragilis, Bacteroides intestinalis, Bacteroides coprosuis, Bacteroides distasonis, Bacteroides goldsteinii, Bacteroides merdae, Bacteroides forsythus, Bacteroides splanchnicus, Bacteroides capillosus, Bacteroides cellulosolvens*, and *Bacteroides ureolyticus*.

[0121] In an aspect, a therapeutic composition comprises a fecal microbiota from a subject selected from the group consisting of a human, a bovine, a dairy calf, a ruminant, an ovine, a caprine, or a *cervine*. In another aspect, a therapeutic composition can be administered to a subject selected from the group consisting of a human, a bovine, a dairy calf, a ruminant, an ovine, a caprine, or a *cervine*. In an aspect, a therapeutic composition is substantially or nearly odourless.

[0122] In an aspect, a therapeutic composition provided here comprises a fecal microbiota preparation comprising a Shannon Diversity Index of greater than or equal to 0.3, greater than or equal to 0.4, greater than or equal to 0.5, greater than or equal to 0.6, greater than or equal to 0.7, greater than or equal to 0.8, greater than or equal to 0.9, greater than or equal to 1.0, greater than or equal to 1.1, greater than or equal to 1.2, greater than or equal to 1.3, greater than or equal to 1.4, greater than or equal to 1.5, greater than or equal to 1.6, greater than or equal to 1.7, greater than or equal to 1.8, greater than or equal to 1.9, greater than or equal to 2.0, greater than or equal to 2.1, greater than or equal to 2.2, greater than or equal to 2.3, greater than or equal to 2.4, greater than or equal to 2.5, greater than or equal to 3.0, greater than or equal to 3.1, greater than or equal to 3.2, greater than or equal to 3.3, greater than or equal to 3.4, greater than or equal to 3.5, greater than or equal to 3.6, greater than or equal to 3.7, greater than or equal to 3.8, greater than or equal to 3.9, greater than or equal to 4.0, greater than or equal to 4.1, greater than or equal to 4.2, greater than or equal to 4.3, greater than or equal to 4.4, greater than or equal to 4.5, or greater than or equal to 5.0. In another aspect, a therapeutic composition comprises fecal microbiota comprising a Shannon Diversity Index of between 0.1 and 3.0, between 0.1 and 2.5, between 0.1 and 2.4, between 0.1 and 2.3, between 0.1 and 2.2, between 0.1 and 2.1, between 0.1 and 2.0, between 0.4 and 2.5, between 0.4 and 3.0, between 0.5 and 5.0, between 0.7 and 5.0, between 0.9 and 5.0, between 1.1 and 5.0, between 1.3 and 5.0, between 1.5 and 5.0, between 1.7 and 5.0, between 1.9 and 5.0, between 2.1 and 5.0, between 2.3 and 5.0, between 2.5 and 5.0, between 2.7 and 5.0, between 2.9 and 5.0, between 3.1 and 5.0, between 3.3 and

5.0, between 3.5 and 5.0, between 3.7 and 5.0, between 31.9 and 5.0, or between 4.1 and 5.0. In one aspect, a Shannon Diversity Index is calculated at the phylum level. In another aspect, a Shannon Diversity Index is calculated at the family level. In one aspect, a Shannon Diversity Index is calculated at the genus level. In another aspect, a Shannon Diversity Index is calculated at the species level. In a further aspect, a therapeutic composition comprises a preparation of flora in proportional content that resembles a normal healthy human fecal flora.

[0123] In a further aspect, a therapeutic composition comprises fecal bacteria from at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 different families. In an aspect, a therapeutic composition provided here comprises a fecal microbiota comprising a weight ratio between fecal-derived non-living material and fecal-derived biological material of no greater than 0.05%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, or 10%. In another aspect, a therapeutic composition provided here comprises a fecal microbiota comprising a weight ratio between fecal-derived non-living material and fecal-derived biological material of no greater than 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%. In another aspect, a therapeutic composition provided here comprises, consists of, or consists essentially of, particles of non-living material and/or particles of biological material of a fecal sample that passes through a sieve, a column, or a similar filtering device having a sieve, exclusion, or particle filter size of 2.0 mm, 1.0 mm, 0.5 mm, 0.25 mm, 0.212 mm, 0.180 mm, 0.150 mm, 0.125 mm, 0.106 mm, 0.090 mm, 0.075 mm, 0.063 mm, 0.053 mm, 0.045 mm, 0.038 mm, 0.032 mm, 0.025 mm, 0.020 mm, 0.01 mm, or 0.2 mm. "Non-living material" does not include an excipient, e.g., a pharmaceutically inactive substance, such as a cryoprotectant, added to a processed fecal material. "Biological material" refers to the living material in fecal material, and includes microbes including prokaryotic cells, such as bacteria and archaea (e.g., living prokaryotic cells and spores that can sporulate to become living prokaryotic cells), eukaryotic cells such as protozoa and fungi, and viruses. In one aspect, "biological material" refers to the living material, e.g., the microbes, eukaryotic cells, and viruses, which are present in the colon of a normal healthy human. In an aspect, a therapeutic composition provided or comprises an extract of human feces where the composition is substantially odorless. In an aspect, a therapeutic composition provided or comprises fecal material or a fecal floral preparation in a lyophilized, crude, semi-purified or purified formulation.

[0124] In an aspect, a fecal microbiota in a therapeutic composition comprises highly refined or purified fecal flora, e.g., substantially free of non-floral fecal material. In an aspect, a fecal microbiota can be further processed, e.g., to undergo microfiltration before, after, or before and after sieving. In another aspect, a highly purified fecal microbiota product is ultra-filtrated to remove large molecules but retain the therapeutic microflora, e.g., bacteria.

[0125] In another aspect, a fecal microbiota in a therapeutic composition used herein comprises or consists essentially of a substantially isolated or a purified fecal flora or entire (or substantially entire) microbiota that is (or comprises) an isolate of fecal flora that is at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.6%, 99.7%, 99.8% or 99.9% isolated or pure, or having no more

than about 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9% or 1.0% or more non-fecal floral material; or, a substantially isolated, purified, or substantially entire microbiota as described in Sadowsky et al., WO 2012/122478 A1, or as described in Borody et al., WO 2012/016287 A2. In one aspect, a fecal microbiota preparation comprises a weight ratio between fecal-derived non-living material and fecal-derived biological material of no greater than about 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1%, 2%, 5%, 8%, 10%, 15%, 20%, 30%, 40$, or 50%.

[0126] In an aspect, a fecal microbiota in a therapeutic composition comprises a donor's substantially entire or non-selected fecal microbiota, reconstituted fecal material, or synthetic fecal material. In another aspect, the fecal microbiota in a therapeutic composition comprises no antibiotic resistant population. In another aspect, a therapeutic composition comprises a fecal microbiota and is largely free of extraneous matter (e.g., non-living matter including acellular matter such as residual fiber, DNA, RNA, viral coat material, non-viable material; and living matter such as eukaryotic cells from the fecal matter's donor).

[0127] In an aspect, a fecal microbiota in a therapeutic composition used herein is derived from disease-screened fresh homologous feces or equivalent freeze-dried and reconstituted feces. In an aspect, a fresh homologous feces does not include an antibiotic resistant population. In another aspect, a fecal microbiota in a therapeutic composition is derived from a synthetic fecal composition. In an aspect, a synthetic fecal composition comprises a preparation of viable flora which preferably in proportional content, resembles normal healthy human fecal flora which does not include antibiotic resistant populations. Suitable microorganisms may be selected from the following: *Bacteroides, Eggerthella, Eubacterium, Fusobacterium, Propionibacterium, Lactobacillus, Ruminococcus, Escherichia coli, Gemmiger, Clostridium, Desulfomonas, Peptostreptococcus, Bifidobacterium, Collinsella, Coprococcus, Dorea,* and *Ruminococcus.*

[0128] In an aspect, a therapeutic composition is combined with other adjuvants such as antacids to dampen bacterial inactivation in the stomach. (e.g., Mylanta, Mucaine, Gastrogel). In another aspect, acid secretion in the stomach could also be pharmacologically suppressed using H2-antagonists or proton pump inhibitors. An example H2-antagonist is ranitidine. An example proton pump inhibitor is omeprazole. In one aspect, an acid suppressant is administered prior to administering, or in co-administration with, a therapeutic composition.

[0129] In an aspect, a therapeutic composition is administered in the form of: an enema composition which can be reconstituted with an appropriate diluent; enteric-coated capsules; enteric-coated microcapsules; acid-resistant tablet; acid-resistant capsules; acid-resistant microcapsules; powder for reconstitution with an appropriate diluent for nasoenteric infusion or colonoscopic infusion; powder for reconstitution with appropriate diluent, flavoring and gastric acid suppression agent for oral ingestion; powder for reconstitution with food or drink; or food or food supplement comprising enteric-coated and/or acid-resistant microcapsules of the composition, powder, jelly, or liquid.

[0130] In an aspect, a treatment method effects a cure, reduction of the symptoms, or a percentage reduction of symptoms of a disorder (e.g., ASD). The change of flora is preferably as "near-complete" as possible and the flora is replaced by viable organisms which will crowd out any remaining, original flora. Typically the change in enteric flora comprises introduction of an array of predetermined flora into the gastro-intestinal system, and thus in a preferred form the method of treatment comprises substantially or completely displacing pathogenic enteric flora in patients requiring such treatment.

[0131] In another aspect, a therapeutic composition can be provided together with a pharmaceutically acceptable carrier. As used herein, a "pharmaceutically acceptable carrier" refers to a non-toxic solvent, dispersant, excipient, adjuvant, or other material which is mixed with a live bacterium in order to permit the formation of a pharmaceutical composition, e.g., a dosage form capable of administration to the patient. A pharmaceutically acceptable carrier can be liquid (e.g., saline), gel or solid form of diluents, adjuvant, excipients or an acid resistant encapsulated ingredient. Suitable diluents and excipients include pharmaceutical grades of physiological saline, dextrose, glycerol, mannitol, lactose, starch, magnesium stearate, sodium saccharin, cellulose, magnesium carbonate, and the like, and combinations thereof. In another aspect, a therapeutic composition may contain auxiliary substances such as wetting or emulsifying agents, stabilizing or pH buffering agents. In an aspect, a therapeutic composition contains about 1%-5%, 5%-10%, 10%-15%, 15-20%, 20%-25%, 25-30%, 30-35%, 40-45%, 50%-55%, 1%-95%, 2%-95%, 5%-95%, 10%-95%, 15%-95%, 20%-95%, 25%-95%, 30%-95%, 35%-95%, 40%-95%, 45%-95%, 50%-95%, 55%-95%, 60%-95%, 65%-95%, 70%-95%, 45%-95%, 80%-95%, or 85%-95% of active ingredient. In an aspect, a therapeutic composition contains about 2%-70%, 5%-60%, 10%-50%, 15%-40%, 20%-30%, 25%-60%, 30%-60%, or 35%-60% of active ingredient.

[0132] In an aspect, a therapeutic composition can be incorporated into tablets, drenches, boluses, capsules or premixes. Formulation of these active ingredients into such dosage forms can be accomplished by means of methods well known in the pharmaceutical formulation arts. See, e.g., U.S. Pat. No. 4,394,377. Filling gelatin capsules with any desired form of the active ingredients readily produces capsules. If desired, these materials can be diluted with an inert powdered diluent, such as sugar, starch, powdered milk, purified crystalline cellulose, or the like to increase the volume for convenience of filling capsules.

[0133] In an aspect, conventional formulation processes can be used to prepare tablets containing a therapeutic composition. In addition to the active ingredients, tablets may contain a base, a disintegrator, an absorbent, a binder, and a lubricant. Typical bases include lactose, sugar, sodium chloride, starch and mannitol. Starch is also a good disintegrator as is alginic acid. Surface-active agents such as sodium lauryl sulfate and dioctyl sodium sulphosuccinate are also sometimes used. Commonly used absorbents include starch and lactose. Magnesium carbonate is also useful for oily substances. As a binder there can be used, for example, gelatin, gums, starch, dextrin, polyvinyl pyrrolidone and various cellulose derivatives. Among the commonly used lubricants are magnesium stearate, talc, paraffin wax, various metallic soaps, and polyethylene glycol.

[0134] In an aspect, for preparing solid compositions such as tablets, an active ingredient is mixed with a pharmaceutical carrier, e.g., conventional tableting ingredients such as corn starch, lactose, sucrose, sorbitol, talc, stearic acid,

magnesium stearate, dicalcium phosphate or gums, or other pharmaceutical diluents, e.g. water, to form a solid preformulation composition containing a homogeneous mixture of a composition of the present disclosure. When referring to these preformulation compositions as homogeneous, it is meant that the active ingredient is dispersed evenly throughout the composition so that the composition may be readily subdivided into equally effective unit dosage forms such as tablets, pills and capsules. This solid preformulation composition is then subdivided into unit dosage forms of the type described above containing a desired amount of an active ingredient (e.g., at least about 10^5, 10^6, 10^7, 10^8, 10^9, 10^{10}, 10^{11}, 10^{12}, or 10^{13} cfu). A therapeutic composition used herein can be flavored.

[0135] In an aspect, a therapeutic composition can be a tablet or a pill. In one aspect, a tablet or a pill can be coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action. For example, a tablet or pill can comprise an inner dosage and an outer dosage component, the latter being in the form of an envelope over the former. The two components can be separated by an enteric layer which serves to resist disintegration in the stomach and permits the inner component to pass intact into the duodenum or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, such materials including a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol and cellulose acetate.

[0136] In an aspect, a therapeutic composition is formulated as a delayed or gradual enteric release form. In an aspect, a delayed or gradual enteric release formulation comprises the use of cellulose acetate, polyethylene glycerol, or both. In an aspect, a delayed or gradual enteric release formulation comprises the use of a hydroxypropylmethylcellulose (HPMC), a microcrystalline cellulose (MCC), magnesium stearate, or a combination thereof. In an aspect, a delayed or gradual enteric release formulation comprises the use of a poly(meth)acrylate, a methacrylic acid copolymer B, a methyl methacrylate, a methacrylic acid ester, a polyvinylpyrrolidone (PVP), a PVP-K90, or a combination thereof. In an aspect, a delayed or gradual enteric release formulation comprises the use of a solid inner layer sandwiched between two outer layers; wherein the solid inner layer comprises the pharmaceutical composition and another component selected from the group consisting of a disintegrant, an exploding agent, an effervescent or any combination thereof wherein the outer layer comprises a substantially water soluble, a crystalline polymer, or both. In an aspect, a delayed or gradual enteric release formulation comprises the use of a non-swellable diffusion matrix.

[0137] In another aspect, a delayed or gradual enteric release formulation comprises the use of a bilayer tablet or capsule which comprises a first layer comprising a polyalkylene oxide, a polyvinylpyrrolidone, a lubricant, or a mixture thereof, and a second osmotic push layer comprising polyethylene oxide, carboxy-methylcellulose, or both. In an aspect, a delayed or gradual enteric release formulation comprises the use of a release-retarding matrix material selected from the group consisting of an acrylic polymer, a cellulose, a wax, a fatty acid, shellac, zein, hydrogenated vegetable oil, hydrogenated castor oil, polyvinylpyrrolidine, a vinyl acetate copolymer, a vinyl alcohol copolymer, polyethylene oxide, an acrylic acid and methacrylic acid copolymer, a methyl methacrylate copolymer, an ethoxyethyl methacrylate polymer, a cyanoethyl methacrylate polymer, an aminoalkyl methacrylate copolymer, a poly(acrylic acid), a poly(methacrylic acid), a methacrylic acid alkylamide copolymer, a poly(methyl methacrylate), a poly(methacrylic acid anhydride), a methyl methacrylate polymer, a polymethacrylate, a poly(methyl methacrylate) copolymer, a polyacrylamide, an aminoalkyl methacrylate copolymer, a glycidyl methacrylate copolymer, a methyl cellulose, an ethylcellulose, a carboxymethylcellulose, a hydroxypropylmethylcellulose, a hydroxymethyl cellulose, a hydroxyethyl cellulose, a hydroxypropyl cellulose, a crosslinked sodium carboxymethylcellulose, a crosslinked hydroxypropylcellulose, a natural wax, a synthetic wax, a fatty alcohol, a fatty acid, a fatty acid ester, a fatty acid glyceride, a hydrogenated fat, a hydrocarbon wax, stearic acid, stearyl alcohol, beeswax, glycowax, castor wax, carnauba wax, a polylactic acid, polyglycolic acid, a co-polymer of lactic and glycolic acid, carboxymethyl starch, potassium methacrylate/divinylbenzene copolymer, crosslinked polyvinylpyrrolidone, poly inylalcohols, polyvinylalcohol copolymers, polyethylene glycols, non-crosslinked polyvinylpyrrolidone, polyvinylacetates, polyvinylacetate copolymers, or any combination thereof. In an aspect, a delayed or gradual enteric release formulation comprises the use of a microenvironment pH modifier.

[0138] In an aspect, a therapeutic composition can be a drench. In one aspect, a drench is prepared by choosing a saline-suspended form of a therapeutic composition. A water-soluble form of one ingredient can be used in conjunction with a water-insoluble form of the other by preparing a suspension of one with an aqueous solution of the other. Water-insoluble forms of either active ingredient may be prepared as a suspension or in some physiologically acceptable solvent such as polyethylene glycol. Suspensions of water-insoluble forms of either active ingredient can be prepared in oils such as peanut, corn, sesame oil or the like; in a glycol such as propylene glycol or a polyethylene glycol; or in water depending on the solubility of a particular active ingredient. Suitable physiologically acceptable adjuvants may be necessary in order to keep the active ingredients suspended. Adjuvants can include and be chosen from among the thickeners, such as carboxymethylcellulose, polyvinyl pyrrolidone, gelatin and the alginates. Surfactants generally will serve to suspend the active ingredients, particularly the fat-soluble propionate-enhancing compounds. Most useful for making suspensions in liquid nonsolvents are alkylphenol polyethylene oxide adducts, naphthalenesulfonates, alkylbenzene-sulfonates, and the polyoxyethylene sorbitan esters. In addition many substances, which affect the hydrophilicity, density and surface tension of the liquid, can assist in making suspensions in individual cases. For example, silicone anti-foams, glycols, sorbitol, and sugars can be useful suspending agents.

[0139] In an aspect, a therapeutic composition comprises non-pathogenic spores of one or more, two or more, three or more, or four or more *Clostridium* species selected from the group consisting of *Clostridium absonum, Clostridium argentinense, Clostridium baratii, Clostridium botulinum, Clostridium cadaveris, Clostridium carnis, Clostridium celatum, Clostridium chauvoei, Clostridium clostridioforme, Clostridium cochlearium, Clostridium fallax, Clostridium felsineum, Clostridium ghonii, Clostridium glycolicum, Clostridium haemolyticum, Clostridium hastiforme, Clostridium histolyticum, Clostridium indolis,*

Clostridium irregulare, Clostridium limosum, Clostridium malenominatum, Clostridium novyi, Clostridium oroticum, Clostridium paraputrificum, Clostridium perfringens, Clostridium piliforme, Clostridium putrefaciens, Clostridium putrificum, Clostridium sardiniense, Clostridium sartagoforme, Clostridium scindens, Clostridium septicum, Clostridium sordellii, Clostridium sphenoides, Clostridium spiroforme, Clostridium sporogenes, Clostridium subterminale, Clostridium symbiosum, Clostridium tertium, Clostridium tetani, Clostridium welchii, and *Clostridium villosum*. In an aspect, a therapeutic composition comprises one or more, two or more, three or more, or four or more non-pathogenic *Bacteroides* species selected from the group of *Bacteroides coprocola, Bacteroides plebeius, Bacteroides massiliensis, Bacteroides vulgatus, Bacteroides helcogenes, Bacteroides pyogenes, Bacteroides tectus, Bacteroides uniformis, Bacteroides stercoris, Bacteroides eggerthii, Bacteroides finegoldii, Bacteroides thetaiotaomicron, Bacteroides ovatus, Bacteroides acidifaciens, Bacteroides caccae, Bacteroides nordii, Bacteroides salyersiae, Bacteroides fragilis, Bacteroides intestinalis, Bacteroides coprosuis, Bacteroides distasonis, Bacteroides goldsteinii, Bacteroides merdae, Bacteroides forsythus, Bacteroides splanchnicus, Bacteroides capillosus, Bacteroides cellulosolvens*, and *Bacteroides ureolyticus*. The foregoing *Clostridium* and *Bacteroides* can be either cultured or purified and can be used in combination in a single combination for a synergistic effect.

[0140] In an aspect, a therapeutic composition comprises purified, isolated, or cultured viable non-pathogenic *Clostridium* and a plurality of purified, isolated, or cultured viable non-pathogenic microorganisms from one or more genera selected from the group consisting of *Collinsella, Coprococcus, Dorea, Eubacterium*, and *Ruminococcus*. In another aspect, a therapeutic composition comprises a plurality of purified, isolated, or cultured viable non-pathogenic microorganisms from one or more genera selected from the group consisting of *Clostridium, Collinsella, Coprococcus, Dorea, Eubacterium*, and *Ruminococcus*.

[0141] In an aspect, a therapeutic composition comprises two or more genera selected from the group consisting of *Collinsella, Coprococcus, Dorea, Eubacterium*, and *Ruminococcus*. In another aspect, a therapeutic composition comprises two or more genera selected from the group consisting of *Coprococcus, Dorea, Eubacterium*, and *Ruminococcus*. In a further aspect, a therapeutic composition comprises one or more, two or more, three or more, four or more, or five or more species selected from the group consisting of *Coprococcus catus, Coprococcus comes, Dorea longicatena, Eubacterium eligens, Eubacterium hadrum, Eubacterium hallii, Eubacterium rectale*, and *Ruminococcus torques*.

[0142] In one aspect, a pharmaceutical composition is in an anaerobic package or container. In another aspect, a pharmaceutical composition further comprises an oxygen scavenger. In one aspect, a container can be made oxygen free by e.g., incorporating into the container a built in or clipped-on oxygen-scavenging mechanism, e.g., oxygen scavenging pellets as described e.g., in U.S. Pat. No. 7,541,091. In another aspect, the container itself is made of an oxygen scavenging material, e.g., oxygen scavenging iron, e.g., as described by O2BLOCK™, or equivalents, which uses a purified and modified layered clay as a performance-enhancing carrier of oxygen-scavenging iron; the active iron

is dispersed directly in the polymer. In one aspect, oxygen-scavenging polymers are used to make the container itself or to coat the container, or as pellets to be added; e.g., as described in U.S. Pat. App. Pub. 20110045222, describing polymer blends having one or more unsaturated olefinic homopolymers or copolymers; one or more polyamide homopolymers or copolymers; one or more polyethylene terephthalate homopolymers or copolymers; that exhibit oxygen-scavenging activity. In one aspect, oxygen-scavenging polymers are used to make the container itself or to coat the container, or as pellets to be added; e.g., as described in U.S. Pat. App. Pub. 20110008554, describing compositions comprising a polyester, a copolyester ether and an oxidation catalyst, wherein the copolyester ether comprises a polyether segment comprising poly(tetramethylene-co-alkylene ether). In one aspect, oxygen-scavenging polymers are used to make the container itself or to coat the container, or as pellets to be added; e.g., as described in U.S. Pat. App. Pub. 201000255231, describing a dispersed iron/salt particle in a polymer matrix, and an oxygen scavenging film with oxygen scavenging particulates.

[0143] In preferred aspects, purified fecal microbiota is obtained from a carefully screened, healthy, neurotypical human donor. Microbiota is separated from fecal material collected from healthy donors, mixed with a cryopreservative, stored as a frozen liquid suspension with the cryopreservative, and thawed prior to administration in liquid form. Based on the route of administration, the purified fecal microbiota can be provided as fresh, frozen-thawed, or lyophilized live microbiota. In some cases, purified fecal microbiota is administered to a human subject in the form of an oral dose. In other cases, purified fecal microbiota is administered in the form of a rectal dose.

[0144] In some cases, the dosage form comprises any suitable form of live microbiota (fresh, frozen, lyophilized, etc.) and is formulated for administration to a human subject orally, by nasogastric tube, by colonoscopy, or anally. In some cases, the dosage is administered as a solution. In other cases, the dosage is administered as solid dosage forms such as, for example, capsules, tablets, powders, and granules. In such solid dosage forms, purified fecal microbiota is admixed with at least one inert excipient (or carrier), a filler or extender (e.g., starches, lactose, sucrose, mannitol, or silicic acid), a binder (e.g., carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidone, sucrose, or acacia), a humectant (e.g., glycerol), a disintegrating agent (e.g., agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, a silicate, sodium carbonate), an absorption accelerators, a wetting agent (e.g., cetyl alcohol or glycerol monostearate), an adsorbent (e.g., kaolin or bentonite), and/or a lubricant (e.g., talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, or mixtures thereof). In the case of capsules and tablets, the dosage forms may also comprise buffering agents.

[0145] A tablet comprising purified fecal microbiota can, for example, be made by compressing or molding the active ingredient, optionally with one or more additional ingredients. Compressed tablets can be prepared by compressing, in a suitable device, the active ingredient in a free-flowing form such as a powder or granular preparation, optionally mixed with one or more of a binder, a lubricant, an excipient, a surface active agent, and a dispersing agent. Molded tablets can be made by molding, in a suitable device, a mixture of the active ingredient, a pharmaceutically acceptable carrier,

and at least sufficient liquid to moisten the mixture. In exemplary aspects, the dosage form comprises a powder prepared by lyophilization ("freeze drying"), whereby the process involves removing water from purified, frozen fecal microbiota at extremely low pressures.

[0146] The specific dosage and dosage range that can be used depends on a number of factors, and the determination of dosage ranges and optimal dosages for a particular patient is well within the ordinary skill of one in the art in view of this disclosure. It is further understood, however, that the specific dose level for any particular human will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, gender, and diet of the human, the time of administration, the route of administration, the rate of excretion, any drug combination, and the severity of any disorder being treated.

[0147] In exemplary aspect, purified fecal microbiota is administered to a subject in multiple doses. For example, purified fecal microbiota can be administered to a subject according to a method provided herein in multiple doses over a time period of about two days to about eight weeks.

[0148] Prior to administration of purified fecal microbiota, any suitable antibiotic can be administered to the subject. In exemplary aspects, the antibiotic is a non-absorbed or minimally-absorbed antibiotic such as, for example, vancomycin or rifaximin. Antibiotics are administered to the subject via any appropriate delivery route. One of skill in the art can develop appropriate dose delivery methods. Preferably, the antibiotic is administered to the subject orally. In another aspect, an ASD treatment method requires no antibiotic pretreatment. In a further aspect, an ASD treatment method requires no bowel preparation or bowel cleansing. In another aspect, an ASD treatment method requires neither antibiotic pretreatment nor bowel cleansing prior to administering a pharmaceutical composition comprising a fecal microbiota preparation.

[0149] In some cases, the antibiotic is administered in multiple doses before a bowel cleanse is performed. In some cases, administration of the antibiotic is initiated at least seven days (e.g., at least 7, 9, 10, 12, 14, 18, or 21 days) before the bowel cleanse. In preferred aspects, the bowel cleanse is preceded by fasting of the human subject.

[0150] Following administration of an antibiotic, the subject undergoes a bowel cleanse. In exemplary aspects, the bowel cleanse comprises administering to the subject a product such as MoviPrep®, a commercial bowel prep for colonoscopy. Preferably, the bowel cleanse removes residual vancomycin and cleanses the lower gastrointestinal tract.

[0151] In exemplary aspects, the method further comprises administering to the subject a stomach acid suppressant. Stomach acid suppressants, also known as gastric acid suppressants, suitable for use according to a method provided herein include, without limitation, proton pump inhibitors (PPIs) and histamine blockers. In some cases, the stomach acid suppressant is Prilosec and is administered to the subject one or more days in advance of oral administration of purified fecal microbiota. In some cases, the stomach acid suppressant is administered one week prior to oral administration of purified fecal microbiota.

[0152] In another aspect, provided herein are unit dosage forms comprising purified fecal microbiota. In some cases, unit dosage forms described herein are provided as part of a kit. Such a kit could include a purified fecal microbiota dosage and, optionally, a delivery device to administer the composition to the subject or instructions for administering the dosage to a subject via an appropriate delivery route. In some cases, the dosage form comprises any suitable form of live microbiota (fresh, frozen, lyophilized, etc.) and is formulated for administration to a human subject orally, by nasogastric tube, by colonoscopy, or anally. As described herein, dosage forms suitable for kits provided herein include, without limitation, liquid solutions, capsules, tablets, powders, granules, and lyophilized forms.

[0153] In a further aspect, provided herein is use of a purified composition for manufacture of a medicament for treating autism spectrum disorder or for reducing the severity of one or more symptoms of autism spectrum disorder.

[0154] It will be appreciated that compositions, dosage forms, and medicaments as described herein include combination pharmaceutical compositions in which one or more additional compounds or medications are added to or otherwise co-administered with a purified fecal microbiota composition.

Embodiment 1

[0155] A method for increasing the abundance of one or more gut microorganisms in a subject in need thereof, the method comprising treating the subject by administering a therapeutically effective amount of a pharmaceutical composition comprising a fecal microbe preparation, wherein the subject exhibits at least a 2-fold increase of the abundance of the one or more gut microorganisms after the treatment as compared to before initiating the treatment, wherein the one or more gut microorganisms are selected from the group consisting of *Bifidobacterium, Prevotella* and *Desulfovibrio.*

Embodiment 2

[0156] A method for modulating the abundance of one or more gut microorganisms in a subject in need thereof, the method comprising treating the subject by administering a therapeutically effective amount of a pharmaceutical composition comprising a fecal microbe preparation, wherein the subject exhibits at least a 2-fold change of the abundance of the one or more gut microorganisms after the treatment as compared to before initiating the treatment, wherein the one or more gut microorganisms are selected from the group consisting of *Bifidobacterium, Prevotella, Desulfovibrio, Copprococcus, Clostridium, Eggerthella*, and *Bacteroides.*

Embodiment 3

[0157] The method of Embodiment 1, wherein the method further comprises determining in the subject a relative abundance of the one or more gut microorganisms.

Embodiment 4

[0158] The method of Embodiment 3, wherein the relative abundance is determined via an assay selected from the group consisting of qPCR, RT-qPCR, clone libraries, DGGE, T-RFLP, ARISA, microarrays, FISH, dot-blot hybridization, and a DNA hybridization method.

Embodiment 5

[0159] The method of Embodiment 3, wherein the relative abundance is determined via 16S rDNA-targeted pyrosequencing.

Embodiment 6

[0160] The method of Embodiment 3, wherein the relative abundance is determined via a DNA hybridization assay based on a 16S rDNA sequence.

Embodiment 7

[0161] The method of Embodiment 3, wherein the relative abundance is determined via detecting one or more proteins or metabolites specific to the one or more gut microorganisms.

Embodiment 8

[0162] The method of Embodiment 7, wherein the relative abundance is determined via an assay selected from the group consisting of 2-Dimensional Gel Electrophoresis, 2-Diminsional Difference Gel Electrophoresis (2D-DIGE), MALDI TOF-MS, (2D-) LC-ESI-MS/MS, AQUA and 1TRAQ.

Embodiment 9

[0163] The method of Embodiment 1, wherein the subject has an ASD and the method improves one or more ASD symptoms.

Embodiment 10

[0164] The method of Embodiment 9, wherein the one or more ASD symptoms are selected from the group consisting of gastrointestinal (GI) condition, speech, sociability, receptive language, cognition, irritability, mood, anxiety, lethargy, stereotypy, hyperactivity, and play skills.

Embodiment 11

[0165] The method of Embodiment 1, wherein the subject exhibits at least a 10% reduction in ASD symptom severity after the treatment as compared to before initiating the treatment, and based on an assessment system selected from the group consisting of Childhood Autism Rating Scale (CARS), Childhood Autism Rating Scale 2—Standard Form (CARS2-ST), Childhood Autism Rating Scale 2—High Functioning (CARS2-HF), Aberrant Behavior Checklist (ABC), Social Responsiveness Scale (SRS), and Vineland Adaptive Behavior Scale II (VABS-II).

Embodiment 12

[0166] The method of Embodiment 1, where the at least 2-fold increase is achieved after 2 or more weeks of initiating the treatment.

Embodiment 13

[0167] The method of Embodiment 1, where the at least 2-fold increase is maintained for at least 8 weeks after discontinuing the treatment.

Embodiment 14

[0168] The method of Embodiment 1, where the subject exhibits no gastrointestinal (GI) symptom prior to initiating the treatment.

Embodiment 15

[0169] The method of Embodiment 1, where the subject further exhibits one or more GI symptoms prior to initiating the treatment.

Embodiment 16

[0170] The method of Embodiment 15, where the subject exhibits at least a 50% reduction in GI symptom severity based on the Gastrointestinal Symptom Rating Scale (GSRS) after the treatment as compared to before initiating the treatment.

Embodiment 17

[0171] The method of Embodiment 1, where the method further comprises administering an antibiotic to the subject prior to administering the pharmaceutical composition.

Embodiment 18

[0172] The method of Embodiment 1, where the method further comprises subjecting the subject to a bowel cleanse.

Embodiment 19

[0173] The method of Embodiment 11, where the at least 10% reduction in ASD symptom severity is achieved after 2 or more weeks of initiating the treatment.

Embodiment 20

[0174] The method of Embodiment 11, where the at least 10% reduction in ASD symptom severity is maintained for at least 8 weeks after discontinuing the treatment.

Embodiment 21

[0175] The method of Embodiment 11, where the subject exhibits no gastrointestinal (GI) symptom prior to initiating the treatment.

Embodiment 22

[0176] The method of Embodiment 11, where the subject further exhibits one or more GI symptoms prior to initiating the treatment.

Embodiment 23

[0177] The method of Embodiment 22, where the subject exhibits at least a 50% reduction in GI symptom severity based on the Gastrointestinal Symptom Rating Scale (GSRS) after the treatment as compared to before initiating the treatment.

Embodiment 24

[0178] The method of Embodiment 11, where the method further comprises administering an antibiotic to the subject prior to administering the pharmaceutical composition.

Embodiment 25

[0179] The method of Embodiment 11, where the method further comprises subjecting the subject to a bowel cleanse.

Embodiment 26

[0180] The method of Embodiment 11, wherein the ASD is selected from the group consisting of autistic disorder, pervasive developmental disorder not otherwise specified (PDD-NOS), and Asperger syndrome.

Embodiment 27

[0181] The method of Embodiment 1, wherein the pharmaceutical composition is administered orally.

Embodiment 28

[0182] The method of Embodiment 1, wherein the fecal microbe preparation is lyophilized.

Embodiment 29

[0183] The method of Embodiment 1, wherein the fecal microbe preparation comprises a non-selected and substantially complete fecal microbiota from a single donor.

Embodiment 30

[0184] The method of Embodiment 29, wherein the non-selected and substantially complete fecal microbiota is supplemented with one or more viable, non-pathogenic microorganisms selected from the group consisting of *Bifidobacterium, Prevotella, Desulfovibrio, Copprococcus, Clostridium*, and *Bacteroides*.

Embodiment 31

[0185] The method of Embodiment 1, wherein the fecal microbe preparation comprises a synthetic fecal composition of predetermined flora.

Embodiment 32

[0186] The method of Embodiment 31, wherein the predetermined flora comprises a preparation of viable flora in proportional content that resembles a normal healthy human fecal flora and comprises no antibiotic resistant populations.

Embodiment 33

[0187] The method of Embodiment 1, wherein the pharmaceutical composition is administered as a solid dosage form selected from the group consisting of capsule, tablet, powder, and granule.

Embodiment 34

[0188] The method of Embodiment 1, wherein the pharmaceutical composition is formulated as an acid resistant capsule.

Embodiment 35

[0189] A method for increasing the phylogenetic diversity of fecal microbiota, fecal phage virome, or both, of a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising a fecal microbe preparation, wherein the subject exhibits at least a two-fold increase of fecal microbiota or fecal phage virome diversity after the treatment as compared to before initiating the treatment.

Embodiment 36

[0190] The method of Embodiment 35, wherein the subject has an ASD and the method improves one or more ASD symptoms.

Embodiment 37

[0191] A method comprising

[0192] a. determining the relative abundance of one or more gut microorganisms in a subject having an ASD or having an ASD sibling, and

[0193] b. administering a therapeutically effective amount of a pharmaceutical composition comprising a fecal microbe preparation to achieve at least a 2-fold change of the abundance of the one or more gut microorganisms, wherein the one or more gut microorganisms are from a genus selected from the group consisting of *Clostridium, Bacteroides, Bifidobacterium, Prevotella, Eggerthella*, and *Desulfovibrio*.

Embodiment 38

[0194] The method of Embodiment 37, wherein the subject having an ASD exhibits no gastrointestinal symptom.

Embodiment 39

[0195] The method of Embodiment 37, wherein the subject having an ASD does not exhibit one or more, two or more, three or more, four or more gastrointestinal symptoms selected from the group consisting of abdominal pain, reflux, indigestion, irritable bowel syndrome, chronic persistent diarrhoea, diarrhoea, flatulence, constipation, and alternating constipation/diarrhoea.

Embodiment 40

[0196] The method of Embodiment 37, wherein the subject having an ASD exhibits no gastrointestinal symptom selected from the group consisting of abdominal pain, reflux, indigestion, irritable bowel syndrome, chronic persistent diarrhoea, diarrhoea, flatulence, constipation, and alternating constipation/diarrhoea.

Embodiment 41

[0197] The method of Embodiment 37, wherein the one or more gut microorganisms are from *Clostridium*, and wherein the *Clostridium* microorganisms increase their abundance by at least 2 fold.

Embodiment 42

[0198] The method of Embodiment 37, wherein the one or more gut microorganisms are from *Bacteroides*, and wherein the *Bacteroides* microorganisms decrease their abundance by at least 2 fold.

Embodiment 43

[0199] The method of Embodiment 37, wherein the one or more gut microorganisms are from *Prevotella*, and wherein the *Prevotella* microorganisms increase their abundance by at least 2 fold.

Embodiment 44

[0200] The method of Embodiment 37, wherein the one or more gut microorganisms are from *Desulfovibrio*, and wherein the *Desulfovibrio* microorganisms increase their abundance by at least 2 fold.

[0201] The disclosure may be better understood by reference to the following non-limiting Examples, which are provided as exemplary of the disclosure. The following examples are presented in order to more fully illustrate the preferred aspects of the disclosure and should in no way be construed, however, as limiting the broad scope of the disclosure. Therefore, the scope of the appended claims should not be limited to the description of the aspects contained herein.

EXAMPLES

Example 1: Treating Autistic Children Using Microbiota Transfer Therapy (MTT)

[0202] A clinical study (ClinicalTrials.gov Identifier: NCT02504554) of 20 autistic children, ages 7-17, was conducted to evaluate the safety and tolerability of a fecal microbiota-based treatment designed to reduce the symptoms of autism by improving the gastrointestinal microbiota function. This treatment included transfer of purified gut bacteria from a healthy person to children diagnosed as having autism spectrum disorder. Details of this trial are described in PCT Application No. PCT/US2016/033747 and U.S. application Ser. No. 15/161,654, both filed May 23, 2016 (both incorporated by reference in their entirety).

[0203] The general study design was an open-label clinical trial involving 18 children (ages 7-17 years) with ASD who were diagnosed by the Autism Diagnostic Interview-Revised (ADI-R) and had moderate to severe gastrointestinal problems. Each child participated in the study for 18 weeks in total, a 10 week treatment and a follow-up 8 week observation period after the treatment stopped. For the fecal material transplant (FMT) treatment, two routes of administration, oral versus rectal, were compared for the initial dose, followed by a lower maintenance dosage given orally for 7-8 weeks.

[0204] The protocol was approved by FDA (Investigational new drug number 15886) and the Institutional Review Board of Arizona State University (ASU IRB Protocol #: 00001053). The study was advertised by email to approximately 2500 ASD families in Arizona, using the contact list of the Autism Society of Greater Phoenix and the Autism/Asperger's Research Program at Arizona State University. Families with children who met the study inclusion and exclusion criteria had a 1-hour individual phone call to discuss the study. After the phone call, families who signed the parent permission form and child assent form were provided with initial questionnaires to complete. A letter was also sent to them for their personal physician to double-check their medications and for the physician to be aware of the delivery of the vancomycin, Prilosec, and the fecal transplant

[0205] Beneficial bacteria (a non-selected fecal microbiota preparation) were prepared from human donor stools. Fecal samples were collected from carefully-screened healthy donors (90% of general population rejected) and purified extensively to retain only bacteria. Specifically, the microbiota was separated from fecal material collected from

carefully screened, healthy donors, stored in a cryopreservative in a frozen liquid suspension with a cryopreservative, and thawed prior to administration in liquid form. Each purified sample of beneficial bacteria contained 1000 or more bacterial species. By comparison, standard commercially available probiotics include 1 to 10 bacterial species.

Example 2: Subject Recruitment

[0206] The study began with a verification of an autism spectrum diagnosis using the Autism Diagnostic Interview-Revised (ADI-R), which involved a phone interview of the parents with our ADI-R evaluator. The study physician assessed general physical health through an initial 30 minute meeting with participants and an extensive review of the participants' last 2 years of medical records and height/weight/growth charts in order to check for exclusion criteria. Participant exclusion criteria include antibiotics in last 6 months and probiotics in last 3 months, single-gene disorder, major brain malformation, tube feeding, severe GI problems that require immediate treatment (life-threatening), Ulcerative Colitis, Crohn's disease, diagnosed Celiac Disease, Eosinophilic Gasteoenteritis, severely underweight/malnourished, and recent/scheduled surgeries. None of the neurotypical children was diagnosed with mental disorders including ASD, ADHD, depression, and anxiety, and neurotypical children did not have first-degree relatives of individuals with ASD. From participants, initial blood, urine, and stool samples were collected and parents were asked to fill in diet diaries of their child for one week at the beginning of the study. Participants were recruited primarily from the greater Phoenix, Ariz. area, but three participants were from outside that area. Neurotypical families were recruited from friends of the ASD families and professionals who work with ASD families.

Example 3: Trial Participants

[0207] Eighteen autism participants (each from a different family) ages 7-17 years with moderate to severe GI problems and moderate to high cognitive functioning. Twenty participants were recruited into the study, but two did not enter the treatment phase of the study before the treatment started. One participant was disqualified due to a change in medication, and one decided not to participate. Characteristics of 18 study participants and their medical history are listed in Table 2. All 18 participants that entered the treatment phase completed the 19-week trial. The post-treatment data presented herein were collected for 13 of these 18 participants. In addition, 20 age- and gender-matched neurotypical children from 13 families (6 families had 1 neurotypical participant, and 7 families had 2 neurotypical participants) are also recruited. These 20 neurotypical children were monitored for 18 weeks but not treated.

TABLE 2

Characteristics of study participants and their medical history.

	Children with ASD	Neurotypical children	p-value
Total Number	18	20	
Male/Female	16/2	18/2	
Age	11.0 +/− 2.7	11.1 +/− 2.5	n.s.
BMI	19.9 +/− 5.4	18.1 +/− 3.4	n.s.

TABLE 2-continued

Characteristics of study participants and their medical history.

	Children with ASD	Neurotypical children	p-value
GSRS 4-point scale (sum of all 15 items, minimum score for no symptoms is 15)	28.1 +/− 4.3	18.8 +/− 4.0	P < 0.001
Born by C-Section	61%	16%	P < 0.01
Number of months of breastfeeding exclusively (no formula)	3.3 +/− 3.9	9.3 +/− 7.8	P < 0.01
% using non-standard formula (soy or other)	39%	8%	P < 0.05
Food allergy (moderate or severe)	56%	5%	P < 0.01
Other allergies (moderate or severe)	44%	10%	P < 0.01
Eczema	56%	5%	P < 0.01
Fiber consumption - child	8.9 +/− 4.3	11.8 +/− 4.9	P < 0.07
Fiber consumption - mother	6.7 +/− 3.9	10.5 +/− 4.5	P < 0.02
Oral antibiotic use during first 4 years of life (number of rounds)	4.6 +/− 5.2	4.1 +/− 6.0	n.s.

TABLE 3

MTT Treatment Timeline Summary.

Time (Day)	Initial oral administration	Initial rectal administration
Day 1-14	Vancomycin*	
Day 12-74	Prilosec*	
Day 15	MoviPrep*	
Day 16	Major oral dose of MTT**	Major rectal dose of MTT**
Day 17	Major oral dose of MTT	—
Day 18-24	Lower maintenance oral dose of MTT	—
Day 25-74	Lower maintenance oral dose of MTT***	
Day 75-130	No treatment, observation period	

*Vancomycin: 40 mg/kg P.O. per day, divided into three doses, not to exceed 2 gm per day; Prilosec: 20 mg PO QD; MoviPrep: Standard kit was used with half the dosage being administered at approximately 10 am and the other half at 4 pm on day fifteen only, to cleanse the bowel of vancomycin and feces. The dosage varies proportionately based on the body mass.
**Initial oral route: The dosage for the first 2 days will be 8.3×10^{11} cells, t.i.d, for a total daily dose of 2.5×10^{12} cells/day, for Day 16 and 17 only; Initial rectal route: 2.5×10^{12} cells, 1x (Day 16 only)
***Maintenance dose: 2.5×10^9 cells, 1x/day P.O.

Example 4: Trial Protocol

[0208] The participants were given oral vancomycin (a non-absorbable broad spectrum antibiotic that stays in the GI tract) for 2 weeks to reduce levels of pathogenic bacteria, and then 1 day of low-volume colonoscopy prep MoviPrep® (a drink that flushes the bowels, to remove most remaining gut bacteria and vancomycin) to clear the residual vancomycin and feces. The vancomycin was intended to kill off harmful bacteria, the fasting was intended to remove any remaining bacteria and to minimize other luminal fecal material, and the colon cleanse helped remove the vancomycin and cleanse the lower GI Tract.

[0209] Following vancomycin treatment and bowel cleanse, participants received either 2 days of high dose oral Microbiota Transfer Therapy (MTT, mixed in a chocolate milk, milk substitute, or juice) (dosage of 2.5×10^{12} CFU per day) or a single dose of rectal MTT (dosage of 2.5×10^{12} CFU for one given similar to an enema). The rectal dose was administered under the direct supervision of the study physician, and the first oral dose was similarly administered in the presence of the physician. Participants were randomly assigned to either the oral or rectal route of administration. If one administration route was not tolerated, or if the family preferred the other route, then participants had the option of trying the other route. For the participants with initial oral dose, a lower oral maintenance dose (2.5×10^9 CFU) was followed for 8 weeks right after the major oral initial dose. Whereas, the major rectal initial dose was followed by waiting period of 1 week followed by a lower oral maintenance dose (2.5×10^9 CFU) for 7 weeks. The maintenance SHGM dose were self-administered orally every day up to week 10. After treatment was stopped, participants were monitored for another 8 weeks.

[0210] Prilosec (omeprazole) was administered daily to reduce stomach acid and thereby increase viability of the MTT, starting on the 12th day of oral vancomycin treatment and continuing until the end of the maintenance dose. Table 3 provides a general treatment timeline.

Example 5: Fecal Microbiota Preparation Used for MTT

[0211] A human microbiota preparation, which comprises a highly purified standardized extract from human feces (also called Standardized Human Gut Microbiota (SHGM)) was used. This is a full-spectrum product, containing all the bacteria present in the gut of very healthy donors. First, donors were carefully screened using an extensive health questionnaire and extensive medical testing to ensure optimal GI and overall health; the screening process is so rigorous that 90% of donors are eliminated, leaving only the 10% healthiest portion of the population. The donated material is then extensively filtered and standardized, following FDA Good Manufacturing Processes (GMP). The final product is liquid form which can be frozen, and was proven to be highly effective for treating *C. difficile* (Hamilton et al., *Am J Gastroenterol.* 2012 May; 107(5):761-7). The SHGM was stored in −80° C. freezers and then delivered to families on dry ice every week during the study. Families were instructed to keep the SHGM in a container with dry ice, and thaw it shortly before use.

[0212] Two different doses of SHGM were used; the high major dose and a lower maintenance dose. The high-dose SHGM was at a daily dosage of 2.5×10^{12} cells. The rationale for two days of high dose was that after the MoviPrep and 1-day fast is presumably the most critical time in which to provide new beneficial bacteria. The low-dose SHGM was at a dosage of 2.5×10^9 cells.

Example 6: Toleration of Study Medications

[0213] Vancomycin: The vancomycin was associated with two types of minor adverse events. One child developed an allergic rash upon administration of oral vancomycin, but they were switched to vancomycin without orange flavoring and the rash disappeared. Twelve of the 18 children had a behavioral reaction to the vancomycin, starting 1-4 days after the start of the vancomycin, and lasting 1-3 days in most cases, although 1 participant had symptoms lasting for 3 weeks. In 7 cases, the symptoms were mild to moderate increase in hyperactivity, and in 5 cases the symptoms were mild to moderate increase in tantrumming/aggression. After these behavioral symptoms disappeared, GI symptoms and

autism symptoms began improving. Similar results were reported in a previous study (Sandler, 2000), and parents of the study subjected had been informed to expect this. The reaction may be due to release of bacterial toxins as the vancomycin kills off harmful bacteria.

[0214]   Prilosec: This was generally well-tolerated.

[0215]   MoviPrep®: Many children had difficulty consuming this medication due to taste.

[0216]   Rectal administration of Microbiota Transfer Therapy (MTT): This was surprisingly well-tolerated by 6 of 6 recipients.

[0217]   Oral administration of high-dose MTT: This was well-tolerated by 12 of 13 recipients, but 1 participant experienced vomiting and was switched to the rectal route.

[0218]   Oral administration of maintenance dose MTT: This was well-tolerated by all participants.

[0219]   CBC/ChemPanel: There were no major concerns regarding changes in Complete Blood Count (CBC) or blood chemistry panel (CBC). The following minor changes were observed. There was a 5% decrease in potassium (p=0.01) from beginning to end of treatment, but all levels remain in the normal range. After the vancomycin (2nd week of study), there was a 8% increase in platelets (p=0.03). Four subjects had elevated levels at start, and only 2 had elevated levels after vancomycin. There was a 26% drop in blood urea nitrogen (BUN) (p=0.002), but all stayed in normal range. There was a 6% increase in albumin to globulin (A/G) ratio (p=0.03), with 1 slightly elevated. There was a 17% increase in aspartate amino transferase (AST) (p=0.01), but all remained in normal range. There was a 24% increase in alanine amino transferase (ALT) (p=0.003), where 1 remained elevated and 2 became slightly elevated. All of these values (platelets, BUN, A/G, AST, ALT) returned to similar to baseline at the 3rd and 4th tests. Slight changes (1-2%) in red blood cell indices (Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), and Red cell distribution width (RDW)) were observed.

Example 7: Adverse Effects

[0220]   Children with ASD experienced temporary adverse effects at the beginning of vancomycin treatment. As listed in Table 4, one participant among the 18 children with ASD (5%) developed an extensive rash, but the rash disappeared when vancomycin was switched from a natural orange flavor to an unflavored form. Within 1-4 days after the start of the vancomycin, 12 children with ASD had a temporary behavioral reaction to the vancomycin either involving hyperactivity (7 out of 12 cases; 39%) or Tantrums/Aggression (5 out of 12 cases; 28%). The symptoms lasted 1-3 days in most cases, except for one participant that had symptoms lasting for 3 weeks. After the symptoms disappeared, GI symptoms and behavioral symptoms began improving, which is similar to what Sandler et al., *Journal of Child Neurology* 15, 429-35, (2000) reported in their oral vancomycin therapy for children with autism. Only one participant did not tolerate the initial high-dose oral SHGM (nausea/vomiting) and was switched to initial rectal administration.

TABLE 4

Adverse effects.

Adverse effect	adverse effects
Rash	5% (due to natural orange flavor in vancomycin)
Hyperactivity	39%* (temporary: start of vancomycin only)
Tantrums/Aggression	28%* (temporary: start of vancomycin only)
Nausea/vomiting	5% (due to high-dose SHGM)

*The severity of symptoms ranged from mild to moderate.

Example 8: Assessments of Gastrointestinal Symptoms

[0221]   Gastrointestinal Symptom Rating Scale (GSRS) is an assessment of GI symptoms during the previous week, based on 15 questions, which are then scored in 5 domains: Abdominal Pain, Reflux, Indigestion, Diarrhea, and Constipation. A score is reported for each domain based on the average within the questions in that domain. The original GSRS used a 4-point scale, but a revised version was used which included 7-point Likert scale which also has simpler language. The GSRS was assessed on days 0, 7, 14, 21, 28, 35, 42, 56, 74, and 130. One of ordinary skill in the art understands that GSRS is only one way to assess GI symptoms. Other similar tools can be used or designed to evaluate GI symptoms.

[0222]   Daily Stool Records (DSR) were collected at baseline for two weeks, daily during the treatment phase, and the last two weeks of the observation period. These records included a rating of the stool using the Bristol Stool Form scale (1=very hard, 7=liquid).

Example 9: Assessments of Autism and Related Symptoms

[0223]   Autism Diagnostic Interview-Revised (ADI-R) is a 2-hour structured interview and is one of the primary tools used for clinical diagnosis of autism and autism spectrum disorders. It is not designed to be a measure of autism severity, but higher scores are generally consistent with more severe symptoms. The ADI-R was be used to verify the diagnosis of ASD for admission into the study.

[0224]   Parent Global Impressions—III is introduced here as an expanded version of the PGI-R. See Adams et al., Effect of a Vitamin/Mineral Supplement on Children with Autism, *BMC Pediatrics,* 11:111(2011). The PGI-III evaluates changes in 17 areas (see FIG. 13), and overall, using a 7-point scale ranging from "much worse" to "much better". An "Average Change" is computed by computing the average in all 18 scores of the PGI-2-Final. This tool was chosen because it was found that it is more reliable to ask parents directly about observed changes than to have them estimate symptom severity at beginning and end and then compute a difference. Also, the use of a 7-point scale to detect changes seems to yield a high sensitivity to changes.

[0225]   Childhood Autism Rating Scale (CARS) is a 15-item scale that can be used to both diagnose autism and ASD and to assess the overall severity of symptoms. The CARS assessment was done subsequent to the ADIR assessment by the same evaluator.

[0226]   Aberrant Behavior Checklist (ABC) assesses problem behaviors in five areas common in children with ASD, including irritability, lethargy, stereotypy, hyperactivity, and inappropriate speech.

[0227] Social Responsiveness Scale (SRS) is a 65-item scale that assesses social impairments, a core issue in autism, including social awareness, social information processing, capacity for reciprocal social communication, social anxiety/avoidance, and autistic preoccupations and traits. See Constantino et al., Validation of a brief quantitative measure of autistic traits: comparison of the social responsiveness scale with the autism diagnostic interview-revised. *J Autism Dev Disord.* 2003 August; 33(4):427-33.

[0228] Vineland Adaptive Behavior Scale II (VABS-II) is a measure of the functioning level in four different domains: Communication, Daily Living Skills, Socialization, and Motor Skills, and 11 sub-domains. The raw scores were converted into an age equivalent score. It complements the ABC, which assesses problem behaviors. See Sara et al., Vineland Adaptive Behavior Scales, Second Edition (Vineland™-II), Pearson Publishing, 2005.

[0229] The GSRS and PGI-R3 were assessed on days 0, 7, 14, 21, 28, 35, 42, 56, 74, and 130. The Stool Record was assessed every day during the treatment. The CARS, ABC, and SRS were assessed at baseline, at the end of treatment, and at the end of the observation period. The VABS-II was assessed at baseline and at the end of the observation period only, because it is lengthy and believed to be less sensitive to short time periods since it assesses changes in specific adaptive skills. The CARS was assessed by a professional evaluator, and the GSRS, PGI-R2, ABC, SRS, and VABS-II were assessed by parents.

Example 10: Initial Observations

[0230] GI Symptoms:

[0231] During the 2 weeks of vancomycin and then 8 weeks of beneficial bacteria, there was a rapid improvement in GI symptoms in most children. At the end of treatment there was an 82% reduction in average scores on the Gastrointestinal Symptom Rating Scale (GSRS) (FIG. **1** and FIG. **3**). As shown in FIG. **2** and FIG. **5**, roughly equal decrease in all 4 GSRS subscale areas (abdominal pain, indigestion, diarrhea, constipation). There was no change in the reflux subscale because none of the children had a significant reflux problem. Sixteen of 18 children had a 70% or greater reduction, 1 had a 30% reduction, and 1 exhibited no change. Similar results were obtained for both the rectal-administration group and the oral-administration group.

[0232] Autism Symptoms:

[0233] By the end of the treatment phase, the parents rated their children's autism symptoms on the Overall scale of the Parent Global Impressions as: Much Better—4; Better—8; Slightly Better—5; Little/No change—1. The largest improvements were in GI, speech, sociability, receptive language, cognition, irritability/mood, anxiety, and play skills (FIG. **3**). For the Childhood Autism Rating Scale (CARS) rated by our experienced evaluator, there was a 22% decrease in the CARS scores, p<0.001, which is consistent with the observations by the parents. For the Aberrant Behavior Checklist (ABC), there was a 27% reduction in the total score, p=0.001 (FIG. **4**). Similar results were obtained for both the rectal-administration group and the oral-administration group.

[0234] Post-Treatment:

[0235] Among the first 5 participants that completed the 8-week post-treatment observation period, after two months of receiving no treatment, on average no change in improvements of GI symptoms was observed (73% reduction in GSRS at end of treatment vs. start; 71% reduction after 8 weeks of no treatment vs. start). With respect to post-treatment autism symptoms, PGI-Scores continued to improve over those collected at the end of treatment, with medium to large improvements in 3 participants and no detected change in 2 participants. (FIG. **7**). With regard to post-treatment CARS scores, these 5 children had a 16% decrease in CARS scores at the end of treatment, and a 25% decrease compared to baseline at the end of the no-treatment (observation) period. So, there appeared to be a surprising continued improvement in symptoms even after treatment stopped.

[0236] These data demonstrates a 22% reduction in autism severity scores assigned using the Childhood Autism Rating Scale (CARS) after only 10 weeks of the combined therapy (FIG. **3**). The degree of improvement on the CARS did not appear to correlate with age (FIG. **6**). This suggests that the treatment is useful for both younger children and adults. Furthermore, the degree of improvement on the CARS did not correlate with initial GSRS score (FIG. **8**). This suggests that the treatment is helpful to those with mild GI symptoms as well as those without GI symptoms. In other words, the treatment appears to be effective to reduce autism symptoms regardless of the presence or absence of GI symptoms. This observation is consistent with data reported in our previous study (Kang et al., *PLOS One* 8(7):e68322 (2013)), from which it was concluded that children with ASD had a low diversity of gut bacteria that was independent of their gastrointestinal symptoms.

Example 11: Trial Outcome and Analysis

[0237] Clinically, this study was broadly successful. First, all ASD participants completed the 18-week study. Second, GI symptoms, as assessed by the Gastrointestinal Symptom Rating Scale (GSRS), significantly improved for abdominal pain, indigestion, diarrhea, and constipation, such that the average GSRS score dropped 82% from the beginning to end of treatment and remained improved (77% decrease from baseline) at 8 weeks after treatment stopped (two-tailed paired t-test t=−9.45, p<0.001, t=−7.64, p<0.001, respectively) (FIG. **9**, panel a). A steady and large degree of improvement in most areas of GSRS evaluation including abdominal pain, indigestion, diarrhea, and constipation (FIG. **10**, panel a) was observed. There was little change in reflux since no children had significant reflux at the start of the study. Notably, two seemingly opposite GI symptoms—diarrhea and constipation—responded to the MTT treatment effectively.

[0238] Similarly, the Daily Stool Record (DSR), showed significant decreases in the number of days with abnormal or no stools, and those improvements remained after 8 weeks of no treatment (Table 5, FIG. **10**, panel b). The Daily Stool Record (DSR) was collected and averaged it over two weeks in order to assess changes in stool hardness/softness during the study. Overall, a significant decrease was observed in "% days of abnormal stool" that combines % days of hard, soft/liquid, and no stool, from 62% to 34% (p=0.001) during the 10-week MTT treatment (Table 5 and FIG. **10**, panel b). The improvements remained stable for the following 8 weeks during the observation period. In detail, both "% days of hard stools" (type 1 or 2) and "% days of soft/liquid stools" (type 6 or 7) significantly decreased during the 10-week MTT treatment, but the decrease in "% days of no stool" was not significant. (Table 5).

TABLE 5

Percent days of no stool, stool hardness and softness based on the daily stool
record (DSR) and the Bristol Stool Form Scale.

	Baseline	Treatment end	p-value	8 weeks after treatment	p-value
No stool	33%	26%	0.27	26%	0.38
Hard stool (type 1 or 2)	19%	6%	0.04	3%	0.01
Soft/liquid stool (type 6 or 7)	10%	2%	0.05	3%	0.11
Abnormal stool (in total of hard, soft/liquid/, no stool)	62%	34%	0.007	32%	0.001

[0239] Third, there were only temporary adverse effects (primarily mild to moderate hyperactivity and tantrums/aggression) from vancomycin treatment (Table 4), but no major changes in blood chemistry or long-term adverse effects.

[0240] Beyond these GI improvements, ASD-related behavior also improved following MTT. First, the Parent Global Impressions (PGI-R) assessment, which evaluates 17 ASD-related symptoms, revealed significant improvement during treatment and no reversion 8 weeks after treatment ended (FIG. 9, panel b). Further, a significant negative correlation between GSRS and PGI-R (Spearman correlation test showed r=−0.59 and p<0.001, FIG. 11) suggests that GI symptoms impact ASD behaviors, and that these can be altered via MTT. By the end of the MTT treatment at week 10, the parents rated the change in their children's autism symptoms using the PGI-R, and the largest improvements were in the GI subscore among 17 subscales and "Overall autism/related symptoms" of the PGI-R (FIG. 12). Specifically, the overall scale of PGI-R was rated as Much Better: n=4 (22%); Better: n=8 (44%); Slightly Better: n=5 (28%); Little/No change: n=1 (6%). The improvement in the other subscales is shown in FIG. 12.

[0241] Second, the Childhood Autism Rating Scale (CARS), which rates core ASD symptoms, decreased by 22% from beginning to end of treatment and 24% (relative to baseline) after 8 weeks of no treatment (p<0.001, FIG. 9, panel c).

[0242] Third, ASD-afflicted children saw improvement in their scores in the Social Responsiveness Scale (SRS), which assesses social skill deficits (FIG. 9, panel d), and the Aberrant Behavior Checklist (ABC), which evaluates irritability, hyperactivity, lethargy, stereotypy, and aberrant speech (FIG. 9, panel e). FIG. 10, panel c also shows a more detailed breakdown of ABC analysis to assess treatment effects on behaviors common in children with ASD: irritability, lethargy, stereotypy, hyperactivity, and inappropriate speech. In all five subscales, a significant reduction at the end of treatment was observed.

[0243] Fourth, the Vineland Adaptive Behavior Scale II (VABS-II) scoring found that the average developmental age increased by 1.4 years (p<0.001, VABS-II) and across all sub-domain areas (FIG. 13) during MTT; though the final VABS-II score was still lower than their chronological age. VABS-II is a measure of the functioning level in four different domains: Communication, Daily Living Skills, Socialization, and Motor Skills, based on 11 sub-domains. Among 11 subscales, Fine and Gross Motor skills were excluded, since these two subscales for the Vineland are only calculated up to 6.8 years and most children with ASD improved near to the limit of the scale. The other 9 subscales

and their average were compared between the baseline and at the end of the study. The MTT treatment resulted in a significant increase in average developmental age, from 5.4 years at baseline to 6.8 years at the end of the study (p<0.001). A gain of 1.4 years within 18 weeks of the study is a substantial increase, but they still remained below their chronological age of 10.9 years. Significant improvements were also observed in all 9 subscale areas with the largest gains in Interpersonal Skills (2.2 years), Personal Living Skills (1.8 years), and Coping Skills (1.7 years) (FIG. 13). It is notable that the major impairments in ASD, namely Receptive language, Expressive language, and Interpersonal skills, were among the lowest initial scores, with initial developmental ages of 3.1 years, 4.5 years, and 2.9 years, respectively; all three areas had substantial improvements of 1.3, 1.1, and 2.2 years, respectively.

[0244] Finally, the MTT appears to be beneficial across both younger and older individuals (no significant correlations between age and GSRS or CARS improvement) and whether the initial MTT does was received orally or rectally. Under our sample size, no difference was observed in efficacy of treatment or clinical outcomes whether MTT was initially administered rectally or orally.

[0245] Together these findings show that MTT is safe and well-tolerated across an age-diverse cohort of 18 ASD-afflicted children. MTT is also effective as it led to significant improvements in both GI- and behavior-related symptoms that were sustained at least 8 weeks after treatment.

Example 12: Gut Microbiome Analysis

[0246] Changes in the bacterial diversity of gut samples from ASD patients or neurotypical participants were evaluated for correlates to the observed clinical improvements. Briefly, bacteria were surveyed by standard sequencing of 16S rRNA gene PCR-amplicons and definition of operational taxonomic units (OTUs). Abundance profiles of microbial OTUs were then statistically evaluated for changes over time.

[0247] It was observed that gut microbiota were significantly less diverse in ASD children than controls at baseline (FIG. 14, panel a; one-tailed t-test t=−2.25, p=0.015, n=18). After MTT, however, bacterial diversity increased in ASD children (FIG. 14, panel a; paired two-tailed t-test t=4.56, p=0.0003, n=18) such that median richness at week 18 was statistically indistinguishable across the ASD and control groups (FIG. 14, panel a; two-tailed t-test t=0.47, p=0.64, n=18). This increase was observed in nearly all individuals including one of the two non-responders (subjects whose GI symptoms did not improve) (FIG. 14, panel b). Higher gut microbiota richness is associated with healthy states. This is

presumably, without being bound to any scientific theory, due to resilience afforded by higher functional redundancy.

[0248] It was also observed that the donor microbiota at least partially engrafted in the recipient gut. Specifically, the unweighted UniFrac distance, a qualitative measure of microbiota phylogenetic similarity, between the recipient gut and their most recent donor sample significantly decreased over time (FIG. **14**, panel c; p<0.01 at three weeks and p<0.001 at 10 and 18 weeks), and remained more similar to the donor's microbiota 8 weeks after treatment stopped. By the end of treatment (week 10) and even 8 weeks after treatment stopped (week 18), the distance between the recipient and the donor microbiota was less than normal interpersonal microbiota variation (FIG. **14**, panel c). Since exogenous bacteria are usually washed out within a month in the human gut, these signatures of engraftment indicate that MTT overcame "colonization resistance". The degree of engraftment varied across participants, which potentially reflects variation in starting gut microbiota and diets.

[0249] It was further observed that, consistent with a lack of difference in treatment efficacy between rectal and oral administration, receiving MTT either orally or rectally did not lead to different patterns in patients' gut microbiota diversity changes. (FIG. **15**).

[0250] Procedures for microbiome 16S rDNA library preparation, sequencing, and analysis are summarized below.

[0251] Parents were asked to collect stool samples from their child on approximately days 0, 21, 70, and 126, and to collect fecal swabs bi-weekly on days 0, 14, 21, 28, 42, 56, 70, 84, 98, 112, and 126. The stool samples were analyzed to determine the types and amounts of gut microbiota present. For safety tests, blood and urine samples were also collected on approximately days 0, 19, 33, and 74. During the study, the participants met with the physician for an initial physical evaluation (including review of medical history) and following evaluations on days 16, 30, and 74. The physician had a phone consult with families on days 7, 21, 42, and 130, and more frequently if adverse symptoms occurred, or if families had any questions. Neurotypical participants did not receive any treatment. They simply provided stool samples (at weeks 0 and 19), and swab samples every 2 weeks.

[0252] Microbial DNA from stools, swabs, and donor samples was extracted with PowerSoil® DNA Isolation Kit (Mobio Carlsbard, Calif.). 16S rRNA library for MiSeq Illumina platform, was constructed according to the protocol from Earth Microbiome Project. The barcoded primer set 515f-806r for pair-ended sequencing of the 16s rRNA V4 region was used (Caporaso et al. *ISME Journal*, 6:1621-24 (2012)). Library preparation and sequencing work was performed at the Microbiome Analysis Laboratory in the Swette Center for Environmental Biotechnology. The used primers amplify both bacterial and archaeal 16S rRNA. No changes specific to archaea were observed.

[0253] Sequencing data were analyzed using QIIME 1.9.1 (Caporaso et al. *Nature Methods* 7:335-36 (2010)), biom-format version 2.1.5 (McDonald et al. *Gigascience*, 1:6 (2012)), vsearch version 1.7.0 (https://github.com/torognes/ vsearch), SSU-ALIGN 0.1 (Nawrocki, *Bioinformatics*, 25:1335-37 (2009)) and FastTree (Price et al. *Molecular Biology and Evolution*, 26:1641-50 (2009)). Sequence quality control and demultiplexing was performed using QIIME's split_libraries_fastq.py with default parameters as

described in (Bokulich et al, *Nature Methods*, 10:57-U11 (2013)) on a per run basis. The sequences were combined across runs by merging the resulting files using the cat Unix command, and sequences were clustered into OTUs at sequence similarities of 100% and 97%. All commands that were applied for these analyses are provided in the GitHub repository available at http://github.com/gregcaporaso/au- tism-fmtl.

[0254] Microbiome profiles across even sampling depths and OTU percent identity thresholds were analyzed to achieve the maximum resolution in OTUs. This can reveal important differences in microbiomes that are apparent only by observing differences in presence, absence, and abun- dance of closely related taxa that may be grouped into single OTUs at the commonly used 97% similarity threshold. Both 100% and 97% OTUs were therefore defined. The analysis here focused on the 100% OTUs, but also compared features of the microbiome to those computed based on 97% OTUs for validation.

[0255] To validate the method, it was further confirmed that the measures of community richness and composition used in our study were correlated between the 100% and 97% OTUs, such that if analyses were instead performed on 97% OTUs, similar results would be achieved. Specifically, Faith PD (Pearson r=0.97038, p<0.001, n=569), unweighted UniFrac (Mantel r: 0.92999, p<0.001, n=569), and weighted UniFrac (Mantel r: 0.76651, p<0.001, n=569) were all highly correlated across the two OTU clustering thresholds, suggesting that the same conclusions would be drawn with either approach. Small differences are expected, as there are many more 100% OTUs than 97% OTUs.

[0256] Similarly, the richness and composition metrics were computed at even sampling (rarefaction) depths of 5721 and 10,040 on our 100% OTU data. The lower depth allowed for maximizing the number of samples that could be analyzed, and comparison to the higher depth allows for confirming that results would be similar if more sequences were retained. Faith PD (Pearson r=0.99738, p<0.001, n=548), unweighted UniFrac (Mantel r: 0.97296, p<0.001), and weighted UniFrac (Mantel r: 0.99953, p<0.001) were all highly correlated across sampling depths, suggesting that the same conclusions would be drawn based from either sam- pling depth.

[0257] A customized pipeline was used to compute 100% OTUs. First, sequences were clustered into 100% OTUs with vsearch. The resulting data were loaded into a BIOM table using the biom from-uc command. OTUs that occurred in only one sample were filtered from the table for compu- tational efficiency. OTU representative sequences were aligned with ssu-align, and high entropy positions were filtered with ssu-mask. A phylogenetic tree was built using FastTreeMP for phylogenetic diversity analyses. Taxonomy was assigned to the representative sequences using QIIME's RDP Classifier wrapper against the Greengenes 13_5 refer- ence database. Alpha and beta diversity analyses were performed using QIIME's core diversity, analyses.py, at rarefaction depths of 5721 (to retain as many samples as possible), and 10,000 to confirm that results were similar with more sequences per sample. Meanwhile, 97% OTUs were computed using QIIME's pick_open_reference_otus. py with the Greengenes 13_5 reference database and default parameters. Alpha and beta diversity analyses were per- formed using QIIME's core diversity analyses.py at a rar- efaction depth of 5721.

Example 13: MTT Changes the Abundance of
Selected Bacterial Genera in ASD Patients

[0258] Several bacterial genera changed their abundance significantly following MTT. These genera include *Bifidobacterium* (FIG. **14**, panel e), *Prevotella* (FIG. **14**, panel f) and *Desulfovibrio* (FIG. **14**, panel g) which both increased in abundance, and *Bacteroides* (FIG. **14**, panel h), which decreased in abundance. Additional information for taxonomic changes accompanying MTT is provided in Table 6.

[0259] Previous reports asserted that *Bifidobacterium* were under-represented in ASD-afflicted children (Buie et al., *Pediatrics* 125, S1-S18 (2010); Bravo et al., *PNAS* 108:16050-55 (2011)). This was also observed in this study at baseline (two-tailed Mann-Whitney U-test $p<0.05$), but following MTT, *Bifidobacterium* significantly increased 4-fold to a median final relative abundance of 1.0% (FIG. **14**, panel e), this suggests strong engraftment by these microbes in particular.

[0260] *Prevotella* were observed to be under-represented in ASD-afflicted children (at baseline median neurotypical and ASD abundances were 0.022% and 0.005%, respectively), but following MTT *Prevotella* increased 250-fold to a median final abundance of approximately 1.3% (Wilcoxon statistic: 40, $p=0.024$, FDR-corrected $p=0.075$). Along with *Prevotella, Desulfovibrio* also increased ~150-fold after MTT from baseline to 8 weeks after treatment. Without being bound to any scientific theory, this suggests strong engraftment by this microbe in particular. Both *Desulfovibrio* and *Prevotella* were on average more abundant in MTT recipients following treatment than in the donor samples, illustrating that the transferred microbiota adapts in its new host, but also that MTT changes the gut environment in a way that is more hospitable to recruit new commensal bacteria. Taken together, these data suggest that MTT successfully shifts the ASD microbiota toward that of age/gender matched healthy controls and to that of their donors.

TABLE 6

Selected fecal bacterial genera change their abundance in response to MTT. This table shows the abundance of different genera of bacteria at baseline and 8 weeks after MTT for the ASD group. The p-values show which results are statistically significant without correction for multiple hypothesis testing, and the q values show which results are significant (q < 0.05) or marginally significant (q < 0.10) after correction for multiple hypothesis testing. The following genera show a significant increase: Bifidobacterium, Prevotella, and Desulfovibrio; The following genus showed a marginally significant decrease: Bacteroides and Eggerthella.

Taxonomy	Fold donor enrichment	Fold final enrichment	Donor median abundance	Neurotypical median abundance	Initial ASD median abundance	Final ASD median abundance	Wilcoxon	p-value	FDR-corrected p-value (q)
k_Bacteria; p_Proteobacteria; c_Betaproteobacteria; o_Burkholderiales; f_Oxalobacteraceae	inf	inf	2.10E-05	0	0	0.000142986	0	6.32E-05	0.00273954
k_Bacteria; p_Proteobacteria; c_Betaproteobacteria; o_Burkholderiales; f_Oxalobacteraceae; g_Oxalobacter	inf	inf	0	0	0	0.000116622	0	7.90E-05	0.00273954
k_Bacteria; p_Bacteroidetes; c_Bacteroidia; o_Bacteroidales; f_S24-7; g_	inf	inf	0.003035093	0	0	0.000957645	3	8.72E-05	0.00273954
k_Bacteria; p_Bacteroidetes; c_Bacteroidia; o_Bacteroidales; f_S24-7	inf	inf	0.003035093	0	0	0.000957645	3	8.72E-05	0.00273954
k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Ruminococcaceae; g_Gemmiger	2.001821375	6.148439423	0.005964801	0.007595364	0.002979687	0.018320426	5	6.48E-05	0.00273954
k_Bacteria; p_Proteobacteria; c_Betaproteobacteria; o_Burkholderiales; f_Comamonadaceae	inf	inf	0.000505136	0	0	5.84E-05	13	0.00032	0.00840514
k_Bacteria; p_Proteobacteria; c_Deltaproteobacteria; o_Desulfovibrionales; f_Desulfovibrionaceae; g_Desulfovibrio	26.54064356	147.603739	0.001180314	3.36E-05	4.45E-05	0.006564225	14	0.00053	0.01179367
k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Ruminococcaceae; g_Anaerofilum	inf	inf	0	0	0	4.71E-05	19	0.00238	0.02011689
k_Bacteria; p_Proteobacteria; c_Betaproteobacteria; o_Burkholderiales; Other; Other	inf	inf	0.00065189	0	0	5.62E-05	20	0.00115	0.02011689
k_Bacteria; p_Proteobacteria; c_Betaproteobacteria; o_Burkholderiales; Other	inf	inf	0.00065189	0	0	5.62E-05	20	0.00115	0.02011689
k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Ruminococcaceae; g_Subdoligranulum	inf	inf	0	4.21E-05	0	5.58E-05	22	0.00297	0.0249607
k_Bacteria; p_Proteobacteria; c_Alphaproteobacteria; o_RF32	inf	inf	0.00035831	0	0	0.001304964	23	0.00295	0.0249607
k_Bacteria; p_Proteobacteria; c_Alphaproteobacteria; o_RF32; f_; g_	inf	inf	0.00035831	0	0	0.001304964	23	0.00295	0.0249607
k_Bacteria; p_Proteobacteria; c_Alphaproteobacteria; o_RF32; f_	inf	inf	0.00035831	0	0	0.001304964	23	0.00295	0.0249607
k_Bacteria; p_Lentisphaerae; c_[Lentisphaeria]; o_Victivallales	inf	inf	0	0	0	3.00E-05	24	0.00228	0.0249607
k_Bacteria; p_Lentisphaerae; c_[Lentisphaeria]	inf	inf	0	0	0	3.00E-05	24	0.00228	0.0249607
k_Bacteria; p_Lentisphaerae; c_Lentisphaerae	inf	inf	0	0	0	3.00E-05	24	0.00228	0.0249607
k_Bacteria; p_Lentisphaerae; c_[Lentisphaeria]; o_Victivallales; f_Victivallaceae	inf	inf	0	0	0	3.00E-05	24	0.00228	0.0249607
k_Bacteria; p_Actinobacteria; c_Coriobacteriia; o_Coriobacteriales; f_Coriobacteriaceae; g_Adlercreutzia	0	4.081749272	0	5.23E-05	4.89E-05	0.000199396	26	0.00315	0.0249607
k_Bacteria; p_Proteobacteria; c_Deltaproteobacteria; o_Desulfovibrionales; f_Desulfovibrionaceae	1.313603645	3.843000937	0.002789742	0.001461841	0.002123732	0.008161503	26	0.00315	0.0249607
k_Bacteria; p_Proteobacteria; c_Deltaproteobacteria; o_Desulfovibrionales	1.313603645	3.843000937	0.002789742	0.001461841	0.002123732	0.008161503	26	0.00315	0.0249607

TABLE 6-continued

Selected fecal bacterial genera change their abundance in response to MTT. This table shows the abundance of different genera of bacteria at baseline and 8 weeks after MTT for the ASD group. The p-values show which results are statistically significant without correction for multiple hypothesis testing, and the q values show which results are significant (q < 0.05) or marginally significant (q < 0.10) after correction for multiple hypothesis testing. The following genera show a significant increase: Bifidobacterium, Prevotella, and Desulfovibrio; The following genus showed a marginally significant decrease: Bacteroides and Eggerthella.

Taxonomy	Fold donor enrichment	Fold final enrichment	Donor median abundance	Neurotypical median abundance	Initial ASD median abundance	Final ASD median abundance	Wilcoxon	p-value	FDR-corrected p-value (q)
k_Bacteria; p_Proteobacteria; c_Deltaproteobacteria	1.313603645	3.843000937	0.002789742	0.001461841	0.002123732	0.008161503	26	0.00315	0.02249607
k_Bacteria; p_Bacteroidetes; c_Bacteroidia; o_Bacteroidales; f_[Odoribacteraceae]; g_Butyricimonas	inf	inf	0.000105144	0	0	0.004541428	28	0.00433	0.02954072
k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_Ruminococcus	0	0	0	9.72E-05	0.000225701	0	28	0.00625	0.03922132
k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Peptostreptococcaceae; g_	6.827225474	2.419218284	0.007000977	0.002181422	0.00102545	0.002480787	29	0.00505	0.033057
k_Bacteria; p_Proteobacteria; c_Alphaproteobacteria	inf	inf	0.000505306	3.41E-05	0	0.001329582	30	0.0083	0.04495085
k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Veillonellaceae; g_Veillonella	0.522843938	0	4.59E-05	4.54E-05	8.79E-05	0	30	0.01124	0.0534674
k_Bacteria; p_Actinobacteria	2.88572859	3.028147004	0.010686058	0.00745319	0.003703071	0.011213443	31	0.00684	0.04132322
k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_	5.713487631	6.369176097	0.018777814	0.015906199	0.003286576	0.020932784	32	0.00794	0.04450209
k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_; g_	5.713487631	6.369176097	0.018777814	0.015906199	0.003286576	0.020932784	32	0.00794	0.04450209
k_Bacteria; p_Actinobacteria; c_Actinobacteria; o_Bifidobacteriales; f_Bifidobacteriaceae	3.807675238	3.673005325	0.010264517	0.007225959	0.002695744	0.009901481	32	0.01095	0.0534674
k_Bacteria; p_Actinobacteria; c_Actinobacteria; o_Bifidobacteriales; f_Bifidobacteriaceae;	3.807675238	3.673005325	0.010264517	0.007225959	0.002695744	0.009901481	32	0.01095	0.0534674
k_Bacteria; p_Actinobacteria; c_Actinobacteria; o_Bifidobacteriales; f_Bifidobacteriaceae; g_Bifidobacterium	3.807675238	3.664672999	0.010264517	0.007225959	0.002695744	0.009879019	32	0.01095	0.0534674
k_Bacteria; p_Actinobacteria; c_Actinobacteria	3.742668409	3.626520891	0.010264517	0.007271405	0.002742566	0.009945975	35	0.01221	0.05640206
k_Bacteria; p_Actinobacteria; c_Coriobacteria; o_Coriobacteriales; f_Coriobacteriaceae; g_Eggerthella	0	0.585844581	0	0	9.42E-05	5.52E-05	36	0.0229	0.07523254
k_Bacteria; p_Bacteroidetes	0.344495799	0.826138917	0.210193161	0.404768129	0.610147241	0.5406638	37	0.0161	0.06831558
k_Bacteria; p_Bacteroidetes; c_Bacteroidia	0.344418317	0.825842598	0.21011159	0.404637424	0.610047663	0.503803347	37	0.0161	0.06831558
k_Bacteria; p_Bacteroidetes; c_Bacteroidia; o_Bacteroidales	0.344418317	0.825842598	0.21011159	0.404637424	0.610047663	0.503803347	37	0.0161	0.06831558
k_Bacteria; p_Firmicutes; Other; Other; Other; Other	1.03418292	2.13255533	0.000147539	0.0002105	0.000142663	0.000304236	38	0.01842	0.06886366
k_Bacteria; p_Firmicutes; Other; Other; Other	1.03418292	2.13255533	0.000147539	0.0002105	0.000142663	0.000304236	38	0.01842	0.06886366
k_Bacteria; p_Firmicutes; Other; Other; Other; Other	1.03418292	2.13255533	0.000147539	0.0002105	0.000142663	0.000304236	38	0.01842	0.06886366
k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Ruminococcaceae; Other	1.747310327	2.016617709	0.007878736	0.008887959	0.004509065	0.00909306	38	0.01842	0.06886366
k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Ruminococcaceae; g_Anaerotruncus	0.247844155	0.709404861	1.63E-05	3.36E-05	6.58E-05	4.67E-05	39	0.027	0.08312852
k_Bacteria; p_Bacteroidetes; c_Bacteroidia; o_Bacteroidales; f_Bacteroidaceae	0.268505262	0.630391858	0.127006656	0.248714588	0.473013658	0.298183959	39	0.02103	0.07504768
k_Bacteria; p_Bacteroidetes; c_Bacteroidia; o_Bacteroidales; f_Bacteroidaceae; g_Bacteroides	0.268505262	0.630340467	0.127006656	0.248714588	0.473013658	0.29815965	39	0.02103	0.07504768
k_Bacteria; p_Bacteroidetes; c_Bacteroidia; o_Bacteroidales; f_Prevotellaceae	17.65719148	248.1220024	0.000948467	0.000221251	5.37E-05	0.013328022	40	0.02396	0.07523254
k_Bacteria; p_Bacteroidetes; c_Bacteroidia; o_Bacteroidales; f_Prevotellaceae; g_Prevotella	17.65719148	247.9923587	0.000948467	0.000221251	5.37E-05	0.013321059	40	0.02396	0.07523254
k_Bacteria; p_Proteobacteria	2.192234341	2.272050415	0.038251251	0.01496568	0.017448523	0.039643924	40	0.02396	0.07523254

TABLE 6-continued

Selected fecal bacterial genera change their abundance in response to MTT. This table shows the abundance of different genera of bacteria at baseline and 8 weeks after MTT for the ASD group. The p-values show which results are statistically significant without correction for multiple hypothesis testing, and the q values show which results are significant (q < 0.05) or marginally significant (q < 0.10) after correction for multiple hypothesis testing. The following genera show a significant increase: Bifidobacterium, Prevotella, and Desulfovibrio; The following gems showed a marginally significant decrease: Bacteroides and Eggerthella.

Taxonomy	Fold donor enrichment	Fold final enrichment	Donor median abundance	Neurotypical median abundance	Initial ASD median abundance	Final ASD median abundance	Wilcoxon	p-value	FDR-corrected p-value (q)
k_Bacteria; p_Bacteroidetes; c_Bacteroidia; o_Bacteroidales; f_Porphyromonadaceae; g_Parabacteroides	0.433529741	2.062863986	0.009768805	0.026376327	0.022533183	0.046482891	40	0.02396	0.07523254
k_Bacteria; p_Bacteroidetes; c_Bacteroidia; o_Bacteroidales; f_Porphyromonadaceae	0.411630428	1.959236616	0.009768805	0.026376327	0.02373198	0.046496563	40	0.02396	0.07523254
k_Bacteria; p_Firmicutes; c_Clostridia; Other; Other; Other	inf	inf	0	0	0	3.28E-05	41	0.045	0.10868376
k_Bacteria; p_Firmicutes; c_Clostridia; Other; Other	inf	inf	0	0	0	3.28E-05	41	0.045	0.10868376
k_Bacteria; p_Actinobacteria; c_Coriobacteria; o_Coriobacteriales; f_Coriobacteriaceae; g_Collinsella	0.426757048	7.362725286	3.26E-05	0	7.65E-05	0.000562932	41	0.03992	0.10275249
k_Bacteria; p_Actinobacteria; c_Coriobacteria; o_Coriobacteriales; f_Coriobacteriaceae	0.517161809	1.879491712	0.0003426	0.000522821	0.000662462	0.001245091	42	0.03089	0.08979861
k_Bacteria; p_Actinobacteria; c_Coriobacteria	0.517161809	1.879491712	0.0003426	0.000522821	0.000662462	0.001245091	42	0.03089	0.08979861
k_Bacteria; p_Actinobacteria; c_Coriobacteria; o_Coriobacteriales	0.517161809	1.879491712	0.0003426	0.000522821	0.000662462	0.001245091	42	0.03089	0.08979861
k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; Other	3.017549229	0.696152119	0.036168755	0.013559835	0.011986136	0.008344174	43	0.03495	0.09977185
k_Bacteria; Other; Other; Other	1.301001458	1.570025829	0.000513831	0.000716455	0.00039495	0.000620082	44	0.03947	0.10275249
k_Bacteria; Other; Other; Other; Other	1.301001458	1.570025829	0.000513831	0.000716455	0.00039495	0.000620082	44	0.03947	0.10275249
k_Bacteria; Other; Other; Other	1.301001458	1.570025829	0.000513831	0.000716455	0.00039495	0.000620082	44	0.03947	0.10275249
k_Bacteria; Other	1.301001458	1.570025829	0.000513831	0.000716455	0.00039495	0.000620082	44	0.03947	0.10275249
k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_Pseudobutyrivibrio	2.683981412	0	0.000126462	0	4.71E-05	0	44	0.06007	0.13667366
k_Bacteria; p_Bacteroidetes; c_Bacteroidia; o_Bacteroidales; f_Rikenellaceae; g_Alistipes	0.058011525	0.514869524	0.002008032	0.015744349	0.034614365	0.017821882	45	0.04447	0.10868376
k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_Anaerostipes	1.276736899	1.974407509	0.000542711	0.000836392	0.000425077	0.000839275	46	0.04999	0.11714052
k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Ruminococcaceae; g_Oscillospira	0.524902192	1.417823714	0.01100073	0.020311601	0.020957675	0.029714289	46	0.04999	0.11714052
k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Ruminococcaceae; g_Butyricicoccus	5.455129435	2.094743099	0.002339551	0.000637078	0.000428872	0.000898376	47	0.05608	0.12947425
k_Bacteria; p_Actinobacteria; c_Actinobacteria; o_Actinomycetales; f_Actinomycetaceae; g_Actinomyces	0	0	0	0	3.15E-05	0	47	0.07548	0.16459142
k_Bacteria; p_Bacteroidetes; c_Bacteroidia; o_Bacteroidales; f_Rikenellaceae; g_	0.120110717	2.207642958	0.000967321	0.009714929	0.008053381	0.017779431	48	0.06277	0.13880434
k_Bacteria; p_Firmicutes; c_Bacilli; o_Lactobacillales	0.769966567	0.649684935	0.00048844	0.000793279	0.000634366	0.000412138	48	0.06277	0.13880434
k_Bacteria; p_Proteobacteria; c_Gammaproteobacteria; o_Enterobacteriales; f_Enterobacteriaceae; g_Klebsiella	0	0	0	0	9.56E-06	0	48	0.08672	0.18192579
k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_[Tissierellaceae]	0.994459647	1.599142849	3.26E-05	1.88E-05	3.28E-05	5.25E-05	49	0.07673	0.16501965
k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Ruminococcaceae	1.453704892	1.110880946	0.230557214	0.230537983	0.158599738	0.176185427	51	0.08691	0.18192579

TABLE 6-continued

Selected fecal bacterial genera change their abundance in response to MTT. This table shows the abundance of different genera of bacteria at baseline and 8 weeks after MTT for the ASD group. The p-values show which results are statistically significant without correction for multiple hypothesis testing, and the q values show which results are significant (q < 0.05) or marginally significant (q < 0.10) after correction for multiple hypothesis testing. The following genera show a significant increase: Bifidobacterium, Prevotella, and Desulfovibrio; The following gems showed a marginally significant decrease: Bacteroides and Eggerthella.

Taxonomy	Fold donor enrichment	Fold final enrichment	Donor median abundance	Neurotypical median abundance	Initial ASD median abundance	Final ASD median abundance	Wilcoxon	p-value	FDR-corrected p-value (q)
k_Bacteria; p_Bacteroidetes; c_Bacteroidia; o_Bacteroidales; f_[Odoribacteraceae]	0.831155585	4.796755878	0.001177609	0.001908744	0.001416833	0.006796202	52	0.09645	0.19924207
k_Bacteria; p_Firmicutes; c_Bacilli; o_Lactobacillales; f_Streptococcaceae	0.699563524	0.706905977	0.000407857	0.000242931	0.000583016	0.000412138	53	0.10681	0.21778096
k_Bacteria; p_Proteobacteria; c_Gammaproteobacteria; o_Enterobacteriales; f_Enterobacteriaceae; g_Shigella	1.193837765	0	4.59E-05	0	3.85E-05	0	53	0.14088	0.2764745
k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_Lachnospira	5.11663948	1.440152127	0.021681676	0.005215694	0.004237484	0.006102621	54	0.11803	0.23457433
k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_Clostridium	2.201981808	0.70751989	0.01978803	0.012243708	0.008986464	0.006358102	54	0.11803	0.23457433
k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Veillonellaceae; g_Phascolarctobacterium	3.523547829	3.422413343	0.003709558	0.001636066	0.001052791	0.003603085	57	0.1573	0.29754187
k_Bacteria; p_Firmicutes; c_Bacilli; o_Lactobacillales; f_Streptococcaceae; g_Streptococcus	0.590616304	0.722103841	0.000336757	0.000242931	0.000570179	0.000411728	57	0.1573	0.29754187
k_Bacteria; p_Proteobacteria; c_Gammaproteobacteria; o_Pasteurellales; f_Pasteurellaceae; g_Haemophilus	6.817633863	0.337394452	0.000954145	6.29E-05	0.000139953	4.72E-05	57	0.1573	0.29754187
k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Peptostreptococcaceae	3.357260558	1.594334641	0.012807989	0.00340103	0.003815012	0.006082406	58	0.17238	0.31469611
k_Bacteria; p_Proteobacteria; c_Gammaproteobacteria; o_Pasteurellales	7.00622958	0.430120039	0.00107048	0.00010618	0.00015279	6.57E-05	58	0.17238	0.31469611
k_Bacteria; p_Proteobacteria; c_Gammaproteobacteria; o_Pasteurellales; f_Pasteurellaceae	7.00622958	0.430120039	0.00107048	0.00010618	0.00015279	6.57E-05	58	0.17238	0.31469611
k_Bacteria; p_Firmicutes; c_Bacilli	1.467041864	0.940659447	0.00162713	0.001510384	0.001109123	0.001043307	59	0.18852	0.34020243
k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae	1.813106928	0.790901395	0.282307099	0.178019629	0.155703503	0.123146118	60	0.20575	0.3670003
k_Bacteria; p_Bacteroidetes; Other; Other; Other; Other	0.516176178	1.201854876	8.16E-05	7.56E-05	0.00015803	0.000189929	61	0.2348	0.39217229
k_Bacteria; p_Bacteroidetes; Other; Other	0.516176178	1.201854876	8.16E-05	7.56E-05	0.00015803	0.000189929	61	0.2348	0.39217229
k_Bacteria; p_Bacteroidetes; Other	0.516176178	1.201854876	8.16E-05	7.56E-05	0.00015803	0.000189929	61	0.2348	0.39217229
k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; Other; Other	0.981525478	0.90949021	0.002480592	0.003488372	0.002527282	0.002298538	61	0.2348	0.39217229
k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; Other	0.981525478	0.90949021	0.002480592	0.003488372	0.002527282	0.002298538	61	0.22409	0.3909104
k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_Coprococcus	1.850680871	0.812111071	0.035388344	0.020691033	0.019121797	0.015529023	63	0.26421	0.43211663
k_Bacteria; p_Verrucomicrobia; c_Verrucomicrobiae; o_Verrucomicrobiales; f_Verrucomicrobiaceae; g_Akkermansia	0.257961535	0.728898333	0.000551243	0.004473737	0.000136918	0.001557596	63	0.27523	0.43211663
k_Bacteria; p_Verrucomicrobia; c_Verrucomicrobia; c_Verrucomicrobiae; o_Verrucomicrobiales	0.254058759	0.717870614	0.000551243	0.004473737	0.002169744	0.001557596	63	0.27523	0.43211663
k_Bacteria; p_Verrucomicrobia; c_Verrucomicrobiae; f_Verrucomicrobiaceae	0.254058759	0.717870614	0.000551243	0.004473737	0.002169744	0.001557596	63	0.27523	0.43211663
k_Bacteria; p_Verrucomicrobia	0.254058759	0.717870614	0.000551243	0.004473737	0.002169744	0.001557596	63	0.27523	0.43211663
k_Bacteria; p_Verrucomicrobia; c_Verrucomicrobiae	0.254058759	0.717870614	0.000551243	0.004473737	0.002169744	0.001557596	63	0.27523	0.43211663
k_Bacteria; p_Tenericutes	inf	inf	0.000880971	0.000499909	0	0.000126247	64	0.32999	0.49815875

TABLE 6-continued

Selected fecal bacterial genera change their abundance in response to MTT. This table shows the abundance of different genera of bacteria at baseline and 8 weeks after MTT for the ASD group. The p-values show which results are statistically significant without correction for multiple hypothesis testing, and the q values show which results are significant (q < 0.05) or marginally significant (q < 0.10) after correction for multiple hypothesis testing. The following genera show a significant increase: Bifidobacterium, Prevotella, and Desulfovibrio; The following genus showed a marginally significant decrease: Bacteroides and Eggerthella.

Taxonomy	Fold donor enrichment	Fold final enrichment	Donor median abundance	Neurotypical median abundance	Initial ASD median abundance	Final ASD median abundance	Wilcoxon	p-value	FDR-corrected p-value (q)
k_Bacteria; p_Firmicutes; c_Clostridia	1.625088942	1.142513703	0.515044329	0.496217231	0.31693301	0.362100306	64	0.28603	0.43599322
k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales	1.625088942	1.142513703	0.515044329	0.495923993	0.31693301	0.362100306	64	0.28603	0.43599322
k_Bacteria; p_Firmicutes; c_Erysipelotrichi; o_Erysipelotrichales; f_Erysipelotrichaceae; g_	1.269226262	0.43813951	0.0056043	0.005308983	0.004415525	0.001934616	64	0.28603	0.43599322
k_Bacteria; p_Actinobacteria; c_Coriobacteria; o_Coriobacteriales; f_Coriobacteriaceae; g_	1.243790419	1.288374997	0.000105144	9.09E-05	8.45E-05	0.000108913	66	0.34435	0.51488091
k_Bacteria; p_Firmicutes	1.601955527	1.167896058	0.521797051	0.523011392	0.325725054	0.380413007	67	0.35862	0.53116324
k_Bacteria; p_Proteobacteria; c_Betaproteobacteria	3.265311462	1.561772748	0.023853623	0.008376604	0.00730516	0.011409	68	0.38519	0.54481563
k_Bacteria; p_Proteobacteria; c_Betaproteobacteria; o_Burkholderiales	3.265311462	1.557543063	0.023853623	0.008376604	0.00730516	0.011378101	68	0.38519	0.54481563
k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_Lachnobacterium	9.072677791	0.974782185	0.0007994	9.09E-05	8.81E-05	8.59E-05	68	0.41288	0.56373387
k_Bacteria; p_Firmicutes; c_Erysipelotrichi; o_Erysipelotrichales	0.908561486	0.469731563	0.006201479	0.007736019	0.006825602	0.003206201	68	0.38519	0.54481563
k_Bacteria; p_Firmicutes; c_Erysipelotrichi	0.908561486	0.469731563	0.006201479	0.007736019	0.006825602	0.003206201	68	0.38519	0.54481563
k_Bacteria; p_Firmicutes; c_Erysipelotrichi; o_Erysipelotrichales; f_Erysipelotrichaceae	0.908561486	0.469731563	0.006201479	0.007736019	0.006825602	0.003206201	68	0.38519	0.54481563
k_Bacteria; p_Proteobacteria; c_Betaproteobacteria; o_Burkholderiales; f_Alcaligenaceae	3.148210789	1.472594374	0.02228209	0.008376604	0.0070777	0.010422581	69	0.41293	0.56373387
k_Bacteria; p_Proteobacteria; c_Betaproteobacteria; o_Burkholderiales; f_Alcaligenaceae; g_Sutterella	3.140280787	1.464825274	0.022225964	0.008376604	0.0070777	0.010367593	69	0.41293	0.56373387
k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_Blautia	1.306385665	1.029190844	0.025648711	0.021260472	0.019633338	0.020206452	69	0.41293	0.56373387
k_Bacteria; p_Firmicutes; c_Erysipelotrichi; o_Erysipelotrichales; f_Erysipelotrichaceae; g_Coprobacillus	0	0	0	6.29E-05	4.53E-05	0	69	0.45471	0.58515603
k_Bacteria; p_Bacteroidetes; c_Bacteroidia; o_Bacteroidales; Other	0.536601183	1.743188226	0.0001142	0.000221251	0.000212821	0.000370987	70	0.44181	0.57326273
k_Bacteria; p_Bacteroidetes; c_Bacteroidia; o_Bacteroidales; Other; Other	0.536601183	1.743188226	0.0001142	0.000221251	0.000212821	0.000370987	70	0.44181	0.57326273
k_Bacteria; p_Actinobacteria; c_Actinobacteria; o_Actinomycetales	0	1.218333024	0	3.75E-05	4.61E-05	5.62E-05	70	0.44181	0.57326273
k_Bacteria; p_Proteobacteria; c_Deltaproteobacteria; o_Desulfovibrionales; f_Desulfovibrionaceae; g_Bilophila	0.869794269	1.174806104	0.001031152	0.000932554	0.001185512	0.001392747	70	0.44181	0.57326273
k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Peptostreptococcaceae; Other	2.084966197	1.007207434	0.004700179	0.001875469	0.002254319	0.002270567	70	0.44181	0.57326273
k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Clostridiaceae; g_Clostridium	1.518402522	0.797478568	0.005661055	0.003284265	0.003728296	0.002973236	71	0.47183	0.59261272
k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_Dorea	1.084132934	1.272777939	0.003169645	0.004715194	0.002923668	0.00372118	71	0.47183	0.59261272
k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Ruminococcaceae; g_Faecalibacterium	3.022482575	1.27215778	0.157954842	0.071420012	0.052259968	0.066482925	71	0.47183	0.59261272
k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Peptostreptococcaceae; g_Clostridium	1.048841882	1.267345199	0.000277343	0.000252042	0.000264428	0.000335121	71	0.47183	0.59261272
k_Bacteria; p_Firmicutes; c_Erysipelotrichi; o_Erysipelotrichales; f_Erysipelotrichaceae; g_Clostridium	0.621776194	0.823944028	0.000309971	0.000388689	0.000498525	0.000410757	71	0.48196	0.60054299

TABLE 6-continued

Selected fecal bacterial genera change their abundance in response to MTT. This table shows the abundance of different genera of bacteria at baseline and 8 weeks after MTT for the ASD group. The p-values show which results are statistically significant without correction for multiple hypothesis testing, and the q values show which results are significant (q < 0.05) or marginally significant (q < 0.10) after correction for multiple hypothesis testing. The following genera show a significant increase: Bifidobacterium, Prevotella, and Desulfovibrio; The following genus showed a marginally significant decrease: Bacteroides and Eggerthella.

Taxonomy	Fold donor enrichment	Fold final enrichment	Donor median abundance	Neurotypical median abundance	Initial ASD median abundance	Final ASD median abundance	Wilcoxon	p-value	FDR-corrected p-value (q)
k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Clostridiaceae; g_	2.50352946	13.14941079	4.21E-05	0.000971723	1.68E-05	0.000220828	72	0.5276	0.64211601
k_Bacteria; p_Firmicutes; c_Erysipelotrichi; o_Erysipelotrichales; f_Erysipelotrichaceae; g_Holdemania	2.309460668	1.428020468	0.000183748	9.29E-05	7.96E-05	0.000113618	72	0.51269	0.6284726
k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Clostridiaceae	1.362144975	0.610778314	0.008692301	0.006038584	0.006381333	0.00389758	72	0.50293	0.62172928
k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Ruminococcaceae; g_Clostridium	0	0.901570882	0	6.29E-05	8.79E-05	7.92E-05	73	0.55219	0.66178605
k_Bacteria; p_Firmicutes; c_Erysipelotrichi; o_Erysipelotrichales; f_Erysipelotrichaceae; g_[Eubacterium]	0	0.419550281	0	0.000212359	0.000157073	6.59E-05	73	0.53508	0.64621276
k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_Roseburia	2.583386922	0.717930175	0.074168314	0.042863063	0.02870972	0.020611574	74	0.56824	0.6758595
k_Bacteria; p_Actinobacteria; c_Actinobacteria; o_Actinomycetales; f_Actinomycetaceae	0	0.647292947	0	0	3.47E-05	2.25E-05	74	0.59051	0.69707032
k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Clostridiaceae; g_SMB53	3.296707194	0.766585538	0.000954145	0.000201633	0.000289424	0.000221868	75	0.60235	0.70573682
k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_[Ruminococcus]	1.00601522	1.062751999	0.007882812	0.007641034	0.007835678	0.008327383	76	0.63735	0.74121664
k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Christensenellaceae; g_	0.536877265	1.443733623	0.000275621	0.001406267	0.000513379	0.000741182	78	0.70977	0.81936988
k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_	2.670911283	0.683634656	0.033267443	0.014794342	0.012455465	0.008514988	79	0.74704	0.8557764
k_Bacteria; p_Proteobacteria; c_Gammaproteobacteria; o_Enterobacteriales; f_Enterobacteriaceae; g_Escherichia	1.022906515	1.930806554	0.000505136	0.000243811	0.000493824	0.000953478	80	0.78492	0.8557764
k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_[Mogibacteriaceae]; g_	1.137356676	1.741723987	0.00055665	0.001437758	0.000489424	0.000852442	80	0.78492	0.8557764

TABLE 6-continued

Selected fecal bacterial genera change their abundance in response to MTT. This table shows the abundance of different genera of bacteria at baseline and 8 weeks after MTT for the ASD group. The p-values show which results are statistically significant without correction for multiple hypothesis testing, and the q values show which results are significant (q < 0.05) or marginally significant (q < 0.10) after correction for multiple hypothesis testing. The following genera show a significant increase: Bifidobacterium, Prevotella, and Desulfovibrio; The following genus showed a marginally significant decrease: Bacteroides and Eggerthella.

Taxonomy	Fold donor enrichment	Fold final enrichment	Donor median abundance	Neurotypical median abundance	Initial ASD median abundance	Final ASD median abundance	Wilcoxon	p-value	FDR-corrected p-value (q)
k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Christensenellaceae	0.536877265	1.518256548	0.000275621	0.001406267	0.000513379	0.00077944	80	0.78492	0.8557764
k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Ruminococcaceae; g_Ruminococcus	1.472940024	1.51485443	0.028288958	0.0437382	0.019205778	0.029093957	80	0.78492	0.8557764
k_Bacteria; p_Firmicutes; c_Bacilli; o_Turicibacterales; f_Turicibacteraceae; g_Turicibacter	1.215935696	0.955712453	0.000299735	0.000145758	0.000246505	0.000235588	80	0.78492	0.8557764
k_Bacteria; p_Firmicutes; c_Bacilli; o_Turicibacterales; f_Turicibacteraceae	1.215935696	0.955712453	0.000299735	0.000145758	0.000246505	0.000235588	80	0.78492	0.8557764
k_Bacteria; p_Firmicutes; c_Bacilli; o_Turicibacterales	1.215935696	0.955712453	0.000299735	0.000145758	0.000246505	0.000235588	80	0.78492	0.8557764
k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Veillonellaceae; g_Dialister	87.44384578	1.70820069	0.023443618	0.00337408	0.000268099	0.000457967	82	0.86212	0.90840897
k_Bacteria; p_Proteobacteria; c_Gammaproteobacteria; o_Enterobacteriales; f_Enterobacteriaceae	2.302029237	1.663637994	0.001682405	0.0003368	0.000730836	0.001215846	82	0.86212	0.90840897
k_Bacteria; p_Proteobacteria; c_Gammaproteobacteria; o_Enterobacteriales	2.302029237	1.663637994	0.001682405	0.0003368	0.000730836	0.001215846	82	0.86212	0.90840897
k_Bacteria; p_Proteobacteria; c_Gammaproteobacteria; o_Enterobacteriales; f_Enterobacteriaceae; Other	0.509840777	1.391330932	5.43E-05	7.21E-05	0.000106447	0.000148103	82	0.86212	0.90840897
k_Bacteria; p_Bacteroidetes; c_Bacteroidia; o_Bacteroidales; f_Rikenellaceae; Other	0.147361573	1.109505141	0.00033646	0.001516181	0.002283225	0.00253325	82	0.86212	0.90840897
k_Bacteria; p_Bacteroidetes; c_Bacteroidia; o_Bacteroidales; f_[Barnesiellaceae]	9.667924155	25.23844637	0.000379898	0.007586842	3.93E-05	0.000991737	83	0.90127	0.92483534
k_Bacteria; p_Bacteroidetes; c_Bacteroidia; o_Bacteroidales; f_[Barnesiellaceae]; g_	9.667924155	25.23844637	0.000379898	0.007153308	3.93E-05	0.000991737	83	0.90127	0.92483534
k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_[Mogibacteriaceae]	1.137356676	1.741723987	0.00055665	0.001437758	0.000489424	0.000852442	83	0.90127	0.92483534
k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Veillonellaceae	4.806417425	0.984253727	0.030337566	0.00905059	0.006311888	0.006212499	83	0.90127	0.92483534
k_Bacteria; p_Bacteroidetes; c_Bacteroidia; o_Bacteroidales; f_[Odoribacteraceae]; g_Odoribacter	1.068251023	1.746723821	0.001072465	0.001052221	0.001003945	0.001753614	84	0.94067	0.95280405
k_Bacteria; p_Bacteroidetes; c_Bacteroidia; o_Bacteroidales; f_Rikenellaceae	0.060561698	0.789582481	0.00330151	0.03397749	0.054514817	0.043043944	84	0.94067	0.95280405
k_Bacteria; p_Proteobacteria; c_Gammaproteobacteria	1.745513701	1.302022307	0.002229045	0.000759536	0.001277014	0.0016627	85	0.98021	0.98020591
k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Ruminococcaceae; g_	0.708114468	0.856367273	0.010703294	0.031857844	0.015115203	0.012944165	85	0.98021	0.98020591

Example 14: Microbiome Analysis Across Common Diversity Metrics

[0261] Parallel analyses were performed for multiple diversity metrics to understand the effect of metric on the microbiome findings. With the Observed OTUs metric (a count of the number of OTUs observed at least one time in a sample (FIG. 16), the same patterns as in FIG. 14, panel a were observed. This confirms that both phylogenetic and non-phylogenetic metrics illustrate the same patterns of change in community richness with MTT.

[0262] Similarly, pairwise distances were computed between samples using four diversity metrics, a qualitative non-phylogenetic metric (Jaccard distance), and quantitative non-phylogenetic diversity metric (Bray-Curtis distance), a qualitative phylogenetic diversity metric (unweighted Uni-Frac), and a quantitative phylogenetic diversity metric (weighted UniFrac) (FIG. 17). While the same pattern was observed with the first three metrics, no significant engraftment was seen with weighted UniFrac (though less variation was observed across individuals at weeks 10 and 18 than in earlier timepoints). Quantitative metrics give more weight to higher abundance OTUs than qualitative metrics. Because differences were observed in composition with our quantitative non-phylogenetic metric but not our quantitative phylogenetic metric, without being bound to any scientific theory, this may suggest that when changes occur in high abundance OTUs, those OTUs are generally closely related (thus the change is down-weighted with a phylogenetic diversity metric relative to a non-phylogenetic diversity metric).

Example 15: Microbiome Analysis Based on Fecal Swab Samples

[0263] In addition to stool collection over the 18 week period, fecal swab samples were collected nearly every other week. These samples were obtained by swabbing bottoms or used toilet paper with a sterile swab, and thus are easier to collect than stool samples. The general patterns observed from the stool data are present in the swab data. However, the fecal swab samples did not achieve the same statistical significance as in the stool samples (FIG. 18). This could be due to greater variability in the handling of the swab samples, which were shipped to our collection facility at ASU, and thus spent a varied amount of time at ambient temperature, ranging from a few hours to multiple days.

Example 16: Bacteriophage Virome Analysis

[0264] Changes in the phage diversity of gut samples from ASD or neurotypical participants were also evaluated. Briefly, phages were surveyed by sequencing community DNA from purified viral fraction samples, assembling them, and defining phage populations from the assembled contigs as established in Brum et al., Patterns and ecological drivers of ocean viral communities. *Science* 348, doi:10.1126/science.1261498 (2015). Abundance profiles of phage populations were then statistically evaluated for changes over time.

[0265] In contrast to the microbiota, phage richness and evenness did not significantly change following MTT during the timeframe of this study (FIG. 19, panel a). Without being bound to any scientific theory, at the population level, phage communities are reliant on their host communities and, thus, significant changes in phage diversity can lag behind bacterial community changes (Rodriguez-Brito et al. 2010).

However, a number of metrics suggested phage communities also responded to MTT as follows.

[0266] First, in four individuals tracked across all four stool samples, phage diversity of three MTT responders decreased and then recovered, while the non-responder decreased but did not recover. Second, analyses of quantitative (Bray-Curtis; FIG. 19, panel b, right) and qualitative (Jaccard; FIG. 19, panel b, left) measures of community dissimilarity between samples revealed phage communities of ASD-afflicted children were significantly more similar to those from the donor following MTT. Permutation based fitting of subject variables to Bray-Curtis and Jaccard NMDS plots uncovered significant clustering based on subject type (e.g. autism, neutrotypic and donor; $r^2 \geq 0.2120$, p-value$\leq$0.0001, 9999 permutations) and among ASD subjects based on treatment stage ($r^2 \geq 0.4021$, p$\leq$0.0002, 9999 permutations), and high ($r^2 \geq 0.2066$, p$\leq$0.0149, 9999 permutations) and low ($r^2 \geq 0.1851$, p$\leq$0.0023, 9999 permutations) dose donor. Not surprisingly, in addition to microbiota, FMT also transfers phages. Indeed, phage communities of ASD children appear driven by the successful transfer of donor viral populations during MTT (FIG. 19, panel c). Following the high and low doses of MTT, phage populations from the donor engrafted across all ASD subjects, while the abundance of phage populations originally in their pre-MTT virome were completely eliminated or decreased (FIG. 19, panel c). In contrast, across neurotypical subjects high abundance (>60%) of phage populations of the starting virome remained indicating that the changes observed are not due to normal temporal variability (FIG. 19, panel d). The results indicate that MTT shifts phage communities of ASD children towards those of donors

[0267] Procedures for gut virome preparation, sequence, and analysis are summarized below. Viral DNA was isolated from stool samples as previously described by (Minot et al. *Genome Research* 21:1616-25 (2011)) with slight modifications. Briefly, 0.5 g of stool was resuspended into 40 mL of SM buffer and spun down at 4000 rpm for 30 min and the supernatant was filtered at 0.2 um. The filtrate was then ultra-centrifuged in a step CsCl gradient as detailed in (Thurber et al., *Environmental Microbiology* 11:2148-63 (2009)). To target dsDNA bacteriophages, the 1.35-1.5 g/mL fraction was collected from the CsCl column, treated with chloroform (Vega Thurber et al 2009) and then with DNase I (100 U/mL) followed by the addition of 0.1M EDTA and 0.1M EGTA to halt enzyme activity as described (Hurwitz et al., *Environmental Microbiology* 15:1428-40 (2013)). Viral DNA was then extracted using the DNeasy Blood and Tissue Kit.

[0268] Following DNA extraction, the NexteraXT kit was used to prepare the sequencing libraries with two minor changes. During the library preparation, input DNA was PCR amplified with 18-25 cycles. When input DNA concentrations was low, the buffer ATM was added at a 1:10 dilution. Sequencing was performed on a MiSeq v3 2×300 at one-sixth of a lane per sequencing library.

[0269] For Virome bioinformatic analysis, reads were QC'd using Trimmomatic (Bolger et al., *Bioinformatics* 30:2114-20 (2014)) to remove adaptors, trim low-quality ends of reads (reads were cut as soon as the base quality dropped below 20 on a 4 bp window), and discard short reads (<50 bp). The reads from each sample were then

assembled using Idba_ud (Peng et al., *Bioinformatics* 28(11):1420-8(2012)) with kmer size varying from 20 to 100 by increment of 10.

[0270] The assembled contigs were screened with Vir-Sorter (Roux et al., 2015) to identify and remove all micro-bial genomes sequences (i.e. all contigs >10 kb and not detected as viral by VirSorter in "virome decontamination" mode). Then, a non-redundant dataset of viral contigs was generated by clustering all viral contigs with Cd-hit (Li et al., *Bioinformatics* 22:1658-59 (2006), threshold of 95% ANI on 80% of the shortest sequence). This resulted in 4,759 non-redundant viral sequences longer than 10 kb.

[0271] To determine the viral population relative abun-dances in the initial samples, the QC reads were mapped back to this non-redundant contigs database with bowtie2 (Langmead et al., *Nature Methods* 9:357-U354 (2012), option-non-deterministic and -sensitive, default otherwise). A contig was considered as detected in a sample if covered by reads on more than 75% of its length, and its abundance was computed as the contig average coverage (number of bp mapped to the contig divided by contig length) normalized by the total number of bp sequenced in the metagenome (as in Brum et al., *Science* 348, doi:10.1126/science.1261498 (2015)).

[0272] The diversity indices, Shannon's H' and Peilou's J, and Bray-Curtis distances analyses were calculated using the vegan package (Oksanen et al., Community ecology pack-age, version 2 (2013)) in R version 3.2.3 (R Core Team, 2015). Bray-Curtis distances were statistically ordinated using the nonmetric multidimensional scaling (NMDS) and then the influence of the metadata on sample ordination was evaluated using the "envfit" function with a total of 9999 permutations in the vegan package.

1. A method for increasing the abundance of one or more gut microorganisms in a subject in need thereof, said method comprising treating said subject by administering a thera-peutically effective amount of a pharmaceutical composition comprising a fecal microbe preparation comprising a sub-stantially complete fecal microbiota from a single donor, wherein said subject exhibits at least a 2-fold increase of the abundance of said one or more gut microorganisms after said treatment as compared to before initiating said treatment, wherein said one or more gut microorganisms are selected from the group consisting of *Bifidobacterium, Prevotella* and *Desulfovibrio.*

2. A method for modulating the abundance of one or more gut microorganisms in a subject in need thereof, said method comprising treating said subject by administering a thera-peutically effective amount of a pharmaceutical composition comprising a fecal microbe preparation comprising a sub-stantially complete fecal microbiota from a single donor, wherein said subject exhibits at least a 2-fold change of the abundance of said one or more gut microorganisms after said treatment as compared to before initiating said treatment, wherein said one or more gut microorganisms are selected from the group consisting of *Bifidobacterium, Prevotella, Desulfovibrio, Copprococcus, Clostridium, Eggerthella*, and *Bacteroides.*

3. The method of claim 1, wherein said method further comprises determining in said subject a relative abundance of said one or more gut microorganisms.

4. The method of claim 1, wherein said subject has an autism spectrum disorder (ASD) and said method improves one or more ASD symptoms.

5. The method of claim 4, wherein said one or more ASD symptoms are selected from the group consisting of gastro-intestinal (GI) condition, speech, sociability, receptive lan-guage, cognition, irritability, mood, anxiety, lethargy, ste-reotypy, hyperactivity, and play skills.

6. The method of claim 1, wherein said subject exhibits at least a 10% reduction in assessment score after said treat-ment as compared to before initiating said treatment based on an assessment system selected from the group consisting of Childhood Autism Rating Scale (CARS), Childhood Autism Rating Scale 2—Standard Form (CARS2-ST), Childhood Autism Rating Scale 2—High Functioning (CARS2-HF), Aberrant Behavior Checklist (ABC), Social Responsiveness Scale (SRS), and Vineland Adaptive Behav-ior Scale II (VABS-II).

7. The method of claim 1, where said at least 2-fold increase is achieved after 2 or more weeks of initiating said treatment.

8. The method of claim 1, where said at least 2-fold increase is maintained for at least 8 weeks after discontinu-ing said treatment.

9. The method of claim 1, where said subject exhibits no gastrointestinal (GI) symptom prior to initiating said treat-ment.

10. The method of claim 1, where said subject further exhibits one or more GI symptoms prior to initiating said treatment.

11. The method of claim 10, where said subject exhibits at least a 50% reduction in assessment score based on the Gastrointestinal Symptom Rating Scale (GSRS) assessment system after said treatment as compared to before initiating said treatment.

12. The method of claim 1, where said method further comprises administering an antibiotic to said subject prior to administering said pharmaceutical composition.

13. The method of claim 1, wherein said pharmaceutical composition is administered orally.

14. The method of claim 1, wherein said fecal microbe preparation is lyophilized.

15. The method of claim 1, wherein said fecal microbe preparation comprises a non-selected and substantially com-plete fecal microbiota from a single donor.

16. The method of claim 1, wherein said fecal microbe preparation comprises a preparation of viable flora in pro-portional content that resembles a normal healthy human fecal flora and comprises no antibiotic resistant populations.

17. The method of claim 1, wherein said pharmaceutical composition is formulated as an acid-resistant capsule.

18. A method comprising
  a. determining a relative abundance of one or more gut microorganisms in a subject having an ASD or having an ASD sibling, and
  b. administering to said subject a therapeutically effective amount of a pharmaceutical composition comprising a fecal microbe preparation comprising a substantially complete fecal microbiota from a single donor to achieve at least a 2-fold change of the abundance of said one or more gut microorganisms, wherein said one or more gut microorganisms are from a genus selected from the group consisting of *Clostridium, Bacteroides, Bifidobacterium, Prevotella, Eggerthella*, and *Desulfo-vibrio.*

19. The method of claim 18, wherein said subject having an ASD exhibits no gastrointestinal symptom.

20. The method of claim **18**, wherein said subject having an ASD does not exhibit one or more, two or more, three or more, four or more gastrointestinal symptoms selected from the group consisting of abdominal pain, reflux, indigestion, irritable bowel syndrome, chronic persistent diarrhoea, diarrhoea, flatulence, constipation, and alternating constipation/diarrhoea.

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