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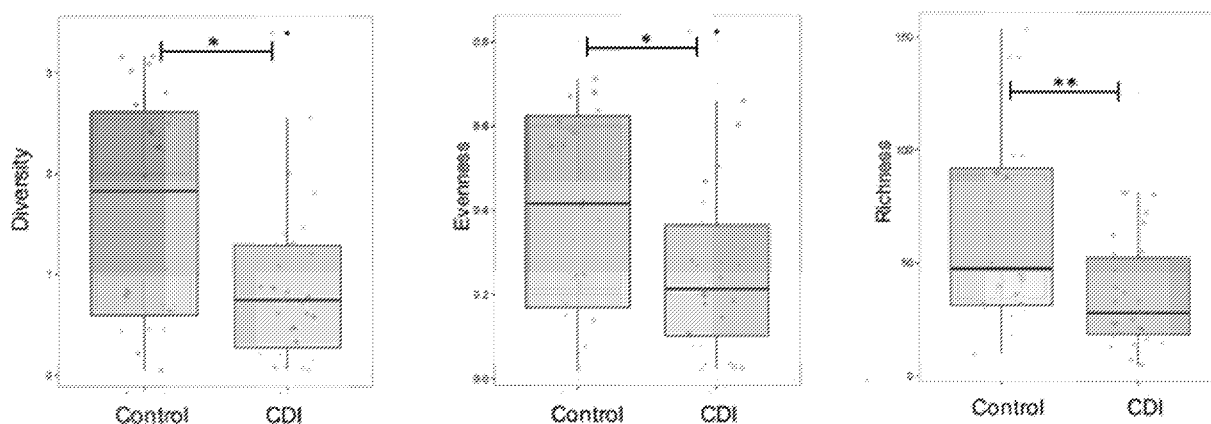


Fig. 1a

(57) Abstract: Methods are provided for identifying subjects as suitable donor or recipients for FMT, for assessing the likelihood of FMT treatment success, and for enhancing FMT treatment efficacy. Also provided are kits and compositions for FMT with enhanced efficacy.

FECAL FUNGOME AND THERAPEUTIC EFFICACY OF FECAL MICROBIOTA TRANSPLANTATION

BACKGROUND OF THE INVENTION

[0001] This application claims priority to U.S. Provisional Patent Application No. 62/625,705, filed February 2, 2018, and U.S. Provisional Patent Application No. 62/679,417, filed June 1, 2018, the contents of both are hereby incorporated by reference in the entirety for all purposes.

BACKGROUND OF THE INVENTION

[0002] *Clostridium difficile* infection (CDI) is a symptomatic infection due to the spore-forming bacterium, *Clostridium difficile*. *C. difficile* infection is spread by bacterial spores found within feces. Risk factors for infection include antibiotic or proton pump inhibitors use, hospitalization, other health problems, and older age. Its symptoms including watery diarrhea, fever, nausea, and abdominal pain, CDI makes up about 20% of cases of antibiotic-associated diarrhea. About 453,000 cases *C. difficile* infection occurred in the United States in 2011, resulting in 29,000 deaths. Each year, *C. difficile* infections accounts for health care cost of approximately \$1.5 billion. Globally, rates of *C. difficile* infection have increased between 2001 and 2016, typically with more women than men affected by the infections.

[0003] Fecal microbiota transplantation (FMT) is highly effective for the treatment of CDI, especially among patients suffering from recurrent CDI. Also known as stool transplant, FMT involves a process of transplanting fecal matter containing microorganism from a healthy individual into the gastrointestinal tract of a recipient. The goal of FMT is restoration of the gut microflora disrupted due to CDI by introducing (or re-introducing) healthy bacterial flora via various means of infusion of a healthy individual's stool, *e.g.*, by colonoscopy, enema, orogastric tube, or by mouth in the form of a capsule containing freeze-dried material obtained from a healthy donor. Aside from CDI, FMT is increasingly being used to treat other intestinal and extra-intestinal diseases, including other gastrointestinal diseases, such as inflammatory bowel disease (IBD), antibiotic-resistant bacterial infection, diarrhea, constipation, irritable bowel syndrome, autism, depression, obesity, diabetes, alopecia, and the like. In addition, FMT has been used for treating certain neurological conditions, such as multiple sclerosis and Parkinson's Disease. Considering the prevalence of CDI and other conditions treatable by FMT in the human population and their significant

economic implications, there exists an urgent need for developing new and improved methods for treating CDI and other disorders by FMT with enhanced efficacy. The present invention fulfills this and other related needs.

BRIEF SUMMARY OF THE INVENTION

[0004] The invention relates to novel methods and compositions useful for more effectively treating *Clostridium difficile* infection (CDI) and other diseases suitable by fecal microbiota transplantation (FMT) treatment. In particular, the present inventor discovered that, when elevated level of the yeast species *Candida albicans* is present in the gastrointestinal tract of an FMT recipient or in the stool of an FMT donor, therapeutic efficacy of FMT is negatively impacted. This finding allows the inventors to devise methods and compositions that can improve FMT effectiveness. Thus, in the first aspect, the present invention provides a novel method for assessing the likelihood of effective FMT. The method includes a step of determining *C. albicans* level in a stool sample obtained from a potential recipient prior to FMT is performed, *i.e.*, before the recipient is to receive transplantation of a donor fecal material.

[0005] In some embodiments, the *C. albicans* level is a percentage relative abundance, or is expressed as a percentage over the total level of all fungal species in the sample. In some embodiments, when the *C. albicans* level is determined as greater than 10% of total fungi in the sample of the recipient, FMT is assessed as unlikely to be effective for the potential recipient. Under such a determination, the recipient in some cases will not receive FMT treatment but will receive another different therapy; in other cases, the recipient is administered an effective amount of an antifungal agent that suppresses *C. albicans* growth before FMT is performed. Optionally, after the administration of the antifungal agent, the *C. albicans* level in the recipient (*e.g.*, in a recipient's stool sample) is again measured and determined to have been lowered before the recipient is transplanted with a composition containing donor fecal material. In some cases, *C. albicans* level is again determined in a stool sample obtained from the recipient after FMT.

[0006] In some embodiments, when the *C. albicans* level is no greater than 10% of total fungi in the sample, FMT is assessed as likely to be effective for the potential recipient. In some cases, the potential recipient is then immediately given FMT, without any further treatment or preparation such as administration of an antifungal agent in the effective amount. In some embodiments, the method further involves a step of determining total fungal load in

the stool sample. A potential recipient whose total fungal load in his stool sample is found to be relatively lower than that of a second potential recipient is expected to have a higher likelihood of having a successful FMT than the second recipient. In some embodiments, multiple potential recipients of FMT are tested prior to FMT using this method for assessing their relative likelihood of success upon receiving FMT treatment. For instance, *C. albicans* level is determined in a first stool sample obtained from a first potential recipient prior to FMT, and in the meantime *C. albicans* level is determined in a second stool sample obtained from a second potential recipient prior to FMT. In some embodiments, when the first potential recipient has a lower *C. albicans* level than the second potential recipient and is therefore assessed to have a higher likelihood of effective FMT than the second potential recipient. In some embodiments, the second potential recipient is then administered an effective amount of an antifungal agent that suppresses *C. albicans* growth before FMT, whereas the first potential recipient may or may need antifungal agent treatment prior to FMT. In some embodiments, *C. albicans* level is determined by quantitative polymerase chain reaction (PCR). In some embodiments, the levels of all fungal species present in the sample is determined by the Internal transcribed spacer 2 (ITS2) sequencing. In some embodiments, the recipient or recipients suffer from inflammatory bowel disease (IBD) with concurrent *Clostridium difficile* infection (CDI). The *C. albicans* level in the stool may be determined before and after their FMT process. An elevated *C. albicans* level before or after FMT is indicative of a higher likelihood of poor outcome or ineffective FMT.

[0007] In a second aspect, the present invention provides a novel, improved method for identifying a suitable donor who would provide stool or fecal material for FMT. The method includes the step of determining *C. albicans* level in a stool sample obtained from a candidate who is being considered as a potential donor for FMT.

[0008] In some embodiments, the *C. albicans* level determined in this method is a percentage relative abundance. In some cases, when the *C. albicans* level is no greater than 0.1% of all fungi present in the sample, the candidate is identified as a suitable donor for FMT. Optionally, fecal matter such as stool is immediately collected from the candidate for use in FMT. In some cases, when the *C. albicans* level is greater than 0.1%, the candidate is deemed unsuitable as a donor for FMT. As a result, either no fecal matter is collected from the candidate at all; or fecal matter is collected for processing to be used in FMT after the candidate is given an effective amount of an antifungal agent that suppresses *C. albicans* growth and again tested to find a satisfactorily reduced *C. albicans* level in the stool sample

(e.g., no greater than 0.1% of total fungi in the sample). In some embodiments, the stool sample of a candidate is tested for *Saccharomyces* level and *Aspergillus* level in addition to *C. albicans* level. In some embodiments, *C. albicans* level is determined by quantitative PCR. In some embodiments, the levels of all fungi present in the sample is determined by ITS2 sequencing. In some embodiments, the method includes the additional step of determining *Escherichia* level and *Proteus* level in the stool sample of a potential donor. Among multiple potential donors, one with a relatively high *Escherichia* level and a relatively low *Proteus* level is deemed a more suitable donor than one with a relatively low *Escherichia* level and/or a relatively high *Proteus* level. In some embodiments, the method further includes a step of determining the total fungal load in the stool sample taken from the potential donor. A potential donor whose total fungal load in his stool sample is found to be relatively lower than that of a second potential donor is expected to be a more desirable donor, i.e., provide a higher likelihood of a successful FMT, than the second donor.

[0009] In a third aspect, the present invention provides a method for improving FMT efficacy. The method includes the step of administering to an FMT recipient prior to FMT being performed an effective amount of an antifungal agent that suppresses *C. albicans* growth. In some embodiments of this method, *C. albicans* level is first determined in a stool sample from the FMT recipient prior to administration of the antifungal agent. In some embodiments, *C. albicans* level is determined in a stool sample from the FMT recipient after administration of the antifungal agent. In some embodiments, *C. albicans* level is determined by quantitative PCR. In some embodiments, the levels of all fungi present in the sample is determined by ITS2 sequencing. In some embodiments, the method further includes a step of administering to the recipient prior to FMT an effective amount of an agent (e.g., an antifungal agent, such as a broad-spectrum fungicide), which reduces total fungal load in a stool sample taken from the recipient prior to FMT. In some embodiments, the recipient is a patient suffering from inflammatory bowel disease (IBD) with concurrent *Clostridium difficile* infection (CDI).

[0010] In a fourth aspect, the present invention provides kits and compositions useful for enhanced FMT treatment with improved efficacy. In some embodiments, a kit for improving FMT efficacy includes a first composition comprising a donor stool material and a second composition comprising an effective amount of an antifungal agent capable of suppressing the growth of *C. albicans*. Typically, the first and second compositions are kept in two separate containers. In some embodiments, the first composition contains processed donor

fetal matter and is formulated for FMT by direct transfer to the GI tract (*e.g.*, via colonoscopy or via nasal intubation) or by oral ingestion. In some embodiments, the first composition comprises donor fecal matter further fortified with an additional and effective amount of one or more fungal species belonging to the genus *Saccharomyces* and/or the genus *Aspergillus*. In some embodiments, the second composition is formulated for administration of the antifungal agent (such as fluconazole) to the recipient by injection, oral ingestion, or rectal deposit. In some embodiments, the kit may further comprise, either in the second composition or in a third composition, an effective amount of an agent that reduces total fungal load. In the alternative, the kit may comprise a third composition, which comprises an effective amount of an agent that reduces total fungal load, but not the second composition. In some cases, the kit may further include printed user instructions.

[0011] Related compositions useful in FMT with improved efficacy may comprise (1) a donor stool material containing live fecal microorganisms and (2) an antifungal agent that specifically suppresses the growth or proliferation of *C. albicans* but exhibits no such suppressive or inhibitory effect against other fungal species. Instead of a broad-spectrum fungicide, such specific anti-*C. albicans* agent may be short polynucleotide in nature of (*e.g.*, a small inhibitory RNA, microRNA, miniRNA, lncRNA, or an antisense oligonucleotide that is capable of disrupting the expression of a key gene in the life cycle of *C. albicans*) that is capable of specifically targeting the species only but not other closely related fungal species.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] Fig. 1 Fungal alterations in CDI. (A) Comparison of the fecal mycobiota based on Shannon diversity, evenness, Chao1 richness in controls and CDI subjects. The bars are shown in median and interquartile range. The dots indicate individual values of the studied subjects. Statistical significance was determined by Mann-Whitney test, $*P < 0.05$, $**P < 0.01$. (B) Fungal community structure difference between controls and CDI by NMDS (Non-metric multidimensional scaling) plot based upon Bray-Curtis distance among all samples. (C) Comparison of the fecal mycobiota composition between controls and CDI subjects at the phylum level. (D) Differentially enriched fungal species between controls and CDI. Statistical significance was determined by LefSe analysis with FDR correction (only those species with q values < 0.05 and LDA effect size > 2 are shown). Heatmap of the presence of these differential fungal species is shown in relative abundance intensity. LDA effect size, q value (FDR-adjusted P value) and species annotation are shown. Green bars and dots indicate

species enriched in controls, while red bar and dot indicate species enriched in CDI. (E) Comparison of the relative abundance of fecal *C. albicans* in controls and CDI subjects. The bars are shown in median and interquartile range. Statistical significance was determined by Mann-Whitney test, $*P < 0.05$.

[0013] Fig. 2 Colonization of donor-derived fungal and bacterial taxa in FMT recipients in association with treatment response. (A) Presence of fungal operational taxonomic units (OTUs) in FMT recipients at the last follow-up. The color of the bar indicates the origin of the bacterial OTUs in the recipient. Purple indicates donor-derived OTUs colonized in the recipient, orange indicates OTUs exclusively present in recipient at baseline but not in donor at baseline, while green indicates OTUs present both in donor and in recipient before FMT. Comparison of the frequency of donor derived bacterial OTUs in FMT responders and in non-responders is shown. Statistical significance was determined by Mann-Whitney test, $**P < 0.01$. (B) Presence of bacterial OTUs in FMT recipients at the last follow-up. Comparison of the frequency of donor-derived bacterial OTUs in FMT responders and in non-responders is shown. Statistical significance was determined by Mann-Whitney test, $*P < 0.05$. (C) Heatmap of the abundance of differentially presented fungal genera in donor, pre-FMT and post-FMT last follow-up samples. Fungal genera with disparate presence between FMT responders and non-responders, as determined by LefSe analysis, are labeled with asterisk (genera with LDA effect size >2 and q value <0.01).

[0014] Fig. 3 Post-FMT alterations in the enteric mycobiota of CDI recipients in association with FMT response. Fecal fungal richness (A) and diversity (B) alterations in FMT recipients over the course of longitudinal follow-up and in their corresponding donors at baseline. Comparison of the fungal richness and diversity of pre-FMT samples and post-FMT samples collected at the last follow-up are shown in FMT responders and FMT non-responders respectively. Statistical significance was determined by paired Wilcoxon signed rank test, $*P < 0.05$. “F” indicates FMT treated subject. “W” indicates weeks post treatment. (C) Alterations in the fecal fungal composition at the genus level in CDI recipients after FMT at different time points up to the last follow-up. (D) Differentially enriched fungal taxa across post-FMT fecal samples of FMT responders versus non-responders at the genus and species level respectively. Statistical significance level was determined by LefSe analysis with FDR correction (only those taxa with q values < 0.05 and LDA effect size >2 are shown). Green bars and dots indicate taxa enriched in controls, while red bar and dot indicate taxa enriched in CDI. (E) Alterations of the relative abundance of fecal *C. albicans* after FMT at the last

follow-up in FMT recipients. Statistical significance was determined by paired Wilcoxon signed rank test, $*P < 0.05$. (F) Relative abundance of *C. albicans* in donor stool corresponding to FMT responders and non-responders. Statistical significance was determined by *Chi-square* test. (G) Relative abundance of *C. albicans* in stool of recipients before FMT in association with FMT response. Statistical significance was determined by *Chi-square* test.

[0015] Fig. 4 *C. albicans* compromises FMT efficacy in eradicating *C. difficile* infection in mice. A) Schematic diagram of *C. albicans* administration and stool infusion (FMT) in a murine *C. difficile* infection (CDI) model. Antibiotic treatment was ceased before gavage of *C. albicans* (CA) and *C. difficile*. B) Diarrhoea in mice on day 1 after stool infusion. C) Representative H&E-stained colonic sections on day 2 after stool infusion (shaded star denotes inflammatory cells infiltration, hallowed star denote ulceration, asterisk denotes oedema, arrow denotes goblet cell loss and triangle denotes herniated crypts). Scale bar, 150 μm . n=5 mice per group. D) Enumeration of *C. difficile* in feces of mice on day 0 before FMT and day 1 post FMT (n=9 mice per group). Statistical significance represents comparisons between FMT-treated mice with *C. difficile* infection versus other groups by unpaired Mann-Whitney test. $* P < 0.05$, $** P < 0.01$. E) Enumeration of *C. albicans* in feces of mice both on day 0 before FMT and day 1 post FMT (n=9 mice per group). Statistical significance represents comparison between *C. albicans* load on day 0 before FMT and day 1 post FMT, by paired Mann-Whitney test. $* P < 0.05$. Dot graphs show means $\pm$ s.e.m, performed at least two times independently.

[0016] Fig. 5 Quantification of fecal *C. albicans* levels in CDI subjects and healthy controls by qPCR. a, qPCR detection of *C. albicans* on CDI subjects and healthy controls from the discovery cohort. Comparison of the fecal *C. albicans* level between control and CDI was determined by Mann-Whitney test, $****P < 0.0001$. b, qPCR detection of *C. albicans* on an additional set of subjects (17 healthy individuals, 12 CDI subjects with and 12 without antibiotic use at inclusion). Statistical significance was tested by unpaired Mann-Whitney test. $* P < 0.05$, $** P < 0.01$. Graphs are shown in mean $\pm$ s.e.m. ND denotes no detectable *C. albicans* in the feces as determined by quantitative PCR.

[0017] Fig. 6 Longitudinal timeline of stool sample collection (expressed in weeks). “F” indicates FMT treated subject. “Donor” indicates FMT donor. “S” indicates subject treated with standard therapy (STD, vancomycin). “W” indicates weeks post treatment. Red dots indicate donor samples, green dots indicate FMT recipient samples sampled at different time points.

[0018] Fig. 7 Heatmap of the abundance of differentially presented bacterial genera in donor, pre-FMT and post-FMT last follow-up samples.

[0019] Fig. 8 Post-FMT alterations in the enteric bacterial microbiota of CDI recipients in association with FMT response. (A) Comparison of the fecal bacterial shanon diversity, evenness, chao1 richness in healthy controls and in CDI subjects. The bars are shown in median and interquartile range. The dots indicate individual values of the studied subjects. Statistical significance was determined by Mann-Whitney test, $**P < 0.01$. Fecal bacterial richness (B) and diversity (C) alterations in FMT recipients over the course of longitudinal follow-up and in their corresponding donors at baseline. Comparison of the fungal richness and diversity of pre-FMT samples and post-FMT samples collected at the last follow-up are shown in FMT responders and FMT non-responders respectively. Statistical significance was determined by paired Wilcox signed rank test, $*P < 0.05$. “F” indicates FMT treated subject. “W” indicates weeks post treatment.

[0020] Fig. 9 Post-antibiotic alterations in the enteric mycobiota of CDI subjects treated with vancomycin in association with treatment response. Fecal fungal richness (A) and diversity (B) alterations over the course of longitudinal follow-up in CDI subjects who received vancomycin treatment. “S” indicates CDI subject received vancomycin treatment (standerd therapy, STD). “W” indicates weeks post vancomycine treatment. (C) Frequencies of CDI individuals increased or decreased in fungal diversity and richness post treatment with respect to FMT and STD treatment. Statistical significance was determined by *Chi-square* test, $*P < 0.05$. (D) Comparison of post-FMT fold change (FC) of the fecal fungal diversity relative to the pre-FMT sample in FMT responders and STD responders. Statistical significance was determined by *Chi-square* test, $*P < 0.05$. (E) Comparison of post-FMT fold change (FC) of the fecal fungal richness relative to the pre-FMT sample in FMT responders and STD responders. Statistical significance was determined by Man-whitney test, $*P < 0.05$. (G) Alterations in the fecal fungal composition at the genus level in CDI subjects on vancomycin regime at different time points up to the last follow-up.

[0021] Fig. 10 Fecal bacterial microbiota richness (A) and diversity (B) alterations in STD subjects over the course of longitudinal follow-up. “S” indicates vancomycin treated subject (STD treatment). “W” indicates weeks post treatment.

[0022] Fig. 11 Differentially enriched fungal taxa across post-treatment samples of FMT responders versus STD responders at the family, genus and species levels.

Statistical significance level was determined by lefSe analysis with FDR correction (only those taxa with q values < 0.05 and LDA effect size > 2 are shown).

[0023] Fig. 12 Post-STD alterations of the relative abundance of fecal *C. albicans* at the last follow-up and at baseline in CDI subjects on vancomycin treatment. Statistical significance was determined by paired Wilcoxon signed rank test.

[0024] Fig. 13 Spearman correlation between fungal diversity, evenness, richness and bacterial diversity, evenness, richness, with respect to FMT and STD treatment and treatment response. Statistical significance was determined for all pairwise comparisons; significant correlations (P value < 0.05) are displayed with asterisk. Blue circles and positive values indicate positive correlations, red circles and negative values indicate inverse correlations. The size and shading indicate the magnitude of the correlation where darker shades are more intensively correlated than lighter ones.

[0025] Fig. 14 Trans-kingdom interactions between bacteria and fungi. Spearman correlation plots of the relative abundance of fungal genera and bacterial genera identified to be significantly associated with CDI and controls at baseline, with respect to FMT and STD treatment and treatment response. Spearman correlation coefficients were calculated for all pairwise comparisons; Blue circles and positive values indicate positive correlations, red circles and negative values indicate inverse correlations. The size and shading indicate the magnitude of the correlation where darker shades are more intensively correlated than lighter ones. Statistical significance was determined for all pairwise comparisons; only correlations tested significant (P value < 0.05) are displayed.

[0026] Fig. 15 Pre-FMT eradication of *C. albicans* in recipient mice restored FMT efficacy in clearing *C. difficile* infection. A) Schematic diagram of antifungal treatment and stool infusion (FMT) in a murine model with di-colonisation of *C. albicans* and *C. difficile*. Antifungal (fluconazole) treatment was ceased at day 4 after administration of *C. albicans* when *C. albicans* was determined negative by cultivation. “CCfF”, mouse group with di-colonisation of *C. albicans* and *C. difficile* and treatments of fluconazole and FMT; “CCF”, mouse group with di-colonisation of *C. albicans* and *C. difficile* and treatment of FMT; “CC”, mouse group with di-colonisation of *C. albicans* and *C. difficile*. B) Enumeration of *C. difficile* in feces of mice on day 0 before FMT and day 1 post FMT ($n=10$ mice per group). Statistical significance was determined by unpaired Mann-Whitney test. * $P < 0.05$, ** $P < 0.01$. C) Enumeration of *C. albicans* in feces of mice both on day 0 before FMT and day 1

post FMT (n=10 mice per group). Statistical significance represents comparison between *C. albicans* load on day 0 before FMT and day 1 post FMT, by paired Mann-Whitney test. ** $P < 0.01$. Dot graphs show means $\pm$ s.e.m, performed at least two times independently.

[0027] Fig. 16 The presence of *C. albicans* is linked to FMT outcomes in CDI. The absolute abundance of fecal *C. albicans* before and after FMT at the last follow-up in FMT recipients, assessed by quantitative PCR. Comparison of the fecal *C. albicans* levels between pre-FMT samples and post-FMT samples was performed by paired Wilcoxon signed rank test, * $P < 0.05$. Comparison of the fecal *C. albicans* levels between the post-FMT samples of FMT responders and FMT non-responders was performed by Mann-Whitney test, \$\$ $P < 0.01$. ND denotes no detectable *C. albicans* in the feces.

[0028] Fig. 17 Comparison of the total fungal load in the feces of controls and CDI subjects. Statistical significance was determined by Mann-Whitney test, *** $P < 0.001$.

[0029] Fig. 18 The total fecal fungal load and *C. albicans* in inflammatory bowel disease (IBD). a. Comparison of the total fungal load in the feces of controls and IBD subjects, including patients with CD and UC. Statistical significance was determined by Mann-Whitney test, *** $P < 0.001$, * $P < 0.05$. b. Comparison of *C. albicans* levels in the feces of controls and CDI subjects.

[0030] Fig. 19 The total fecal fungal load in irritable bowel syndrome (IBS). Comparison of the total fungal load in the feces of controls and IBS subjects. Statistical significance was determined by Mann-Whitney test, *** $P < 0.001$, ** $P < 0.05$.

[0031] Fig. 20 Post-FMT alterations in the taxonomic composition of the bacterial microbiota of CDI recipients in association with FMT response. Bacterial configurations in FMT recipients over the course of longitudinal follow-up and in their corresponding donors at baseline, at the phylum (a) and family (b) levels. c, Heatmap of the relative abundance of differentially presented bacterial genera in donor, pre-FMT and post-FMT last follow-up samples. d, Differentially presented bacteria taxa across post-FMT samples of FMT responders versus non-responders at the phylum, family, and genus levels. Statistical significance level was determined by LefSe analysis with FDR correction (only those taxa with q values < 0.05 and LDA effect size > 2 are shown).

[0032] Fig. 21 Quantification of fecal *C. albicans* levels in IBD concurrent with CDI subjects before and after FMT. The fecal *C. albicans* level (gDNA content/ fecal DNA) was determined by qPCR. PRB, peri rectal bleeding.

DEFINITIONS

[0033] The term “**fecal microbiota transplantation (FMT)**” or “stool transplant” refers to a medical procedure during which fecal matter containing live fecal microorganisms (bacteria, fungi, and he like) obtained from a healthy individual is transferred into the gastrointestinal tract of a recipient to restore healthy gut microflora that has been disrupted or destroyed by a variety of medical conditions. Typically, the fecal matter from a healthy donor is first processed into an appropriate form for the transplantation, which can be made through direct deposit into the lower gastrointestinal tract such as by colonoscopy, or by nasal intubation, or through oral ingestion of an encapsulated material containing dried and frozen fecal matter. *Clostridium difficile* infection (CDI) is the condition most commonly treated by FMT, although a number of other diseases and disorders including in the digestive system and in the nervous system have been reported to be successfully treated by FMT.

[0034] The term “**inhibiting**” or “**inhibition**,” as used herein, refers to any detectable negative effect on a target biological process, such as RNA/protein expression of a target gene, the biological activity of a target protein, cellular signal transduction, cell proliferation, and the like. Typically, an inhibition is reflected in a decrease of at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater in the target process (*e.g.*, growth or proliferation of fungal cells), or any one of the downstream parameters mentioned above, when compared to a control. “Inhibition” further includes a 100% reduction, *i.e.*, a complete elimination, prevention, or abolition of a target biological process or signal. The other relative terms such as “suppressing,” “suppression,” “reducing,” and “reduction” are used in a similar fashion in this disclosure to refer to decreases to different levels (*e.g.*, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater decrease compared to a control level) up to complete elimination of a target biological process or signal. On the other hand, terms such as “activate,” “activating,” “activation,” “increase,” “increasing,” “promote,” “promoting,” “enhance,” “enhancing,” or “enhancement” are used in this disclosure to encompass positive changes at different levels (*e.g.*, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, or greater such as 3, 5, 8, 10, 20-fold increase compared to a control level) in a target process or signal.

[0035] As used herein, “*C. albicans*” refers to a fungal species belonging to the Candida genus, Saccharomycetaceae family, Saccharomycetales order, Saccharomycetes class, and Ascomycota division. A common member of the human gut flora, *C. albicans* is a potential

yeast pathogen capable of causing opportunistic infection in humans, especially in those with compromised immune system.

[0036] The term “**antifungal agent**” refers to any substance that is capable of inhibiting, suppressing, or preventing the growth or proliferation of fungal species, especially those of the Ascomycota division, such as *C. albicans*. Known agents with fungicidal activity include amphotericin B, echinocandin, fluconazole, nystatin, and clotrimazole.

[0037] “**Percentage relative abundance**,” when used in the context of describing the presence of a particular fungal species (*e.g.*, *C. albicans*) in relation to all fungal species present in the same environment, refers to the relative amount of the fungal species out of the amount of all fungal species as expressed in a percentage form. For instance, the percentage relative abundance of *C. albicans* can be determined by comparing the quantity of *C. albicans*-specific DNA (*e.g.*, determined by quantitative polymerase chain reaction) in one given sample with the quantity of all fungal DNA (*e.g.*, determined by quantitative PCR and sequencing based on the Internal transcribed spacer 2 or ITS2 sequence) in the same sample.

[0038] “**Absolute abundance**,” when used in the context of describing the presence of a particular fungal species (*e.g.*, *C. albicans*) in the feces, refers to the amount of DNA derived from the fungal species out of the amount of all DNA in a fecal sample. For instance, the absolute abundance of *C. albicans* can be determined by comparing the quantity of *C. albicans*-specific DNA (*e.g.*, determined by quantitative polymerase chain reaction) in one given sample with the quantity of all fecal DNA in the same sample.

[0039] “**Total fungal load**” of a fecal sample, as used herein, refers to the amount of all fungal DNA out of the amount of all DNA in the fecal sample. For instance, the absolute abundance of fungi can be determined by comparing the quantity of fungal specific DNA (*e.g.*, 18S rDNA determined by quantitative polymerase chain reaction) in one given sample with the quantity of all fecal DNA in the same sample.

[0040] The term “**effective amount**,” as used herein, refers to an amount of a substance that produces a desired effect (*e.g.*, an inhibitory or suppressive effect on *C. albicans* growth) for which the substance (*e.g.*, an antifungal agent) is used or administered. The effects include the prevention, inhibition, or delaying of any pertinent biological process during *C. albicans* growth or development to any detectable extent. The exact amount will depend on the nature of the substance (the active agent), the manner of use/administration, and the

purpose of the application, and will be ascertainable by one skilled in the art using known techniques as well as those described herein.

[0041] As used herein, the term “**about**” denotes a range of value that is +/- 10% of a specified value. For instance, “about 10” denotes the value range of 10 +/- 10 x 10%, *i.e.*, 9 to 11.

DETAILED DESCRIPTION OF THE INVENTION

I. Introduction

[0042] The invention provides a novel approach for assessing the likelihood of effective FMT prior to the procedure being performed as well as for improving the effectiveness of the FMT procedure. During their studies, the present inventors discovered that the presence and relative abundance of certain fungal species both in a recipient's gastrointestinal tract and in a donor's stool directly correlate with the outcome of FMT. In particular, the fungal species *Candida albicans* of the Saccharomycetaceae family is found to negatively impact the effectiveness of FMT. The detection of *C. albicans* in a potential donor's stool thus can be used to guide donor selection, whereas analysis of *C. albicans* level in an FMT recipient can determine whether the subject is immediately ready for FMT or should be treated with an antifungal agent that suppresses *C. albicans* growth prior to FMT in order to optimize the therapeutic outcome.

II. FMT Donors and Recipients

[0043] Patients suffering from CDI, especially recurring CDI, are often considered as recipients for FMT treatment. Aside from CDI, other diseases and conditions, including those of digestive system or nervous system such as colitis, irritable bowel syndrome, multiple sclerosis, Parkinson's Disease, diabetes mellitus, and obesity are also beginning to be considered for FMT treatment.

[0044] Fecal matter used in FMT is obtained from a healthy donor and then processed into appropriate forms for the intended means of delivery in the upcoming FMT procedure. Up until now, the general criterion for an FMT donor is simply that the donor is a healthy individual without any known diseases or disorders especially in the digestive tract, although some preference is often given to the members of the same household as the recipient.

[0045] The present inventors have discovered in their studies that elevated presence of one particular fungal species, *C. albicans*, in a recipient's gastrointestinal tract or in a donor stool (which is used in the transplantation after being processed) can significantly reduce efficacy

of FMT treatment in a patient. In contrast, successful FMT has been observed as correlating with elevated presence of other fungal species, such as those belonging to the genus of *Saccharomyces* or *Aspergillus*, in a recipient or in a donor stool. This revelation enables the initial screening of individuals as appropriate FMT donors as well as the initial screening of patients as likely candidates for successful FMT treatment: if a candidate donor's stool contains an elevated level of *C. albicans* (e.g., greater than 0.1% of total fungi), the candidate is deemed as unsuitable as an FMT donor, and his stool should not be taken or used in FMT; if a candidate's stool sample shows no or only low level of *C. albicans* (e.g., no greater than 0.1% of total fungi), then the candidate is deemed an appropriate FMT donor and his fecal material can be immediately retrieved for processing and later used in FMT. On the other hand, if a patient who has been proposed to receive FMT treatment, and his stool sample shows an elevated level of *C. albicans* (e.g., greater than 10% of total fungi), then the patient is deemed to be unsuitable to receive FMT and is therefore not to be given FMT, as the therapy is likely to be unsuccessful; if a patient's stool sample shows no or only a low level of *C. albicans* (e.g., no greater than 10% of total fungi), the patient is deemed an appropriate recipient for FMT who is likely to enjoy therapeutic success from FMT, and thus can start FMT treatment immediately without other steps of preparation or pre-treatment.

[0046] Various methods have been reported in the literature for determining the levels of all fungal species in a sample, for example, amplification (e.g., by PCR) and sequencing of fungal polynucleotide sequence by using the Internal transcribed spacer 2 (ITS2) sequence. On the other hand, the level of any given fungal species may be determined by amplification and sequencing of its signature 18S rRNA sequence. A percentage abundance is often used as a parameter to indicate the relative level of a fungal species in a given environment.

III. Methods for Improving FMT Efficacy

[0047] The discovery by the present inventors revealing the direct correlation between an elevated level of *C. albicans* in FMT donor or recipient and reduced efficacy of FMT treatment not only allows one to devise an initial screening process to identify appropriate donors and recipients for the FMT procedure, it also enables different methods for improving FMT efficacy by reducing the level of *C. albicans* in a donor and in a recipient prior to the FMT treatment.

[0048] As discussed in the above section, when a candidate donor's stool is tested and found to contain an elevated level of *C. albicans* (e.g., greater than 0.1% of total fungi), the

candidate is deemed as unsuitable as an FMT donor, and his stool should not be taken for use in FMT as it is unlikely to result in a successful FMT treatment if used. Similarly, when a patient or proposed FMT recipient whose stool is tested and found to contain an elevated level of *C. albicans* (e.g., greater than 10% of total fungi), the patient is deemed as an unsuitable recipient for FMT, and he should not immediately undergo FMT due to the high probability of an ineffective outcome. Yet these cases of expected unsuccessful treatment outcome can be readily improved in view of the inventors' discovery.

[0049] First, for a patient who has been considered for receiving FMT but who has also been deemed an unsuitable recipient of FMT due to an elevated level of *C. albicans* (e.g., above 10% of total fungi) found in his/her stool sample, which indicates a diminished chance of a successful FMT, measures can be taken to lower his/her level of *C. albicans* before FMT is commenced so that a much greater efficacy can be achieved for the FMT procedure. For instance, an antifungal agent capable of suppressing the growth or proliferation of *C. albicans* can be administered to the patient in an effective amount such that the level of *C. albicans* in the patient's digestive track and in the feces is significantly reduced (e.g., no more than 10% of total fungi) prior to the start of the FMT procedure. In this case, the patient's *C. albicans* level is to be determined twice: once at the initial screening stage, a second time after the initial level is deemed too high for an effective FMT and after an antifungal agent has been given to the patient. Once the *C. albicans* level is confirmed as lowered to a percentage that would allow satisfactory FMT outcome, the patient is then ready to undergo FMT treatment.

[0050] Second, for a candidate who has been deemed improper to serve as an FMT donor due to a higher level of *C. albicans* in his stool, the expected undesirable FMT outcome can be remedied by treating the candidate donor with an effective amount of an antifungal agent capable of suppressing the growth or proliferation of *C. albicans* can be administered. Since the donor's body, especially the gastrointestinal track, contains a vast collection of microorganisms many of which are important for the health of gut microflora and for the success of FMT, a useful antifungal agent for this purpose cannot be a broad-spectrum fungicide. Rather, it should be an agent that narrowly and precisely targets the species of *C. albicans* without significantly affecting other fungal species, including those closely related to *C. albicans*. Although the agent may be of any chemical compound in nature, small polynucleotides (e.g., siRNAs, miRNAs, miniRNAs, lncRNAs, or antisense DNAs/RNAs) may be the most effective in achieving the specific task of disrupting the expression of one or

more key genes in the life cycle of *C. albicans* so as to specifically inhibit the proliferation of the target species only without significant impact on other closely related fungal species.

[0051] Immediately upon completion of FMT procedure, the recipient may be further monitored by continuous testing of the level of *C. albicans* in the stool samples on a daily basis for up to 5 days post-FMT while the clinical symptoms of the condition being treated are also being monitored in order to assess FMT outcome and the corresponding *C. albicans* level in the recipient.

IV. Kits and Compositions for Improved FMT

[0052] The present invention also provides novel kits and compositions that can be used for improving FMT efficacy. For example, in a kit for treating a patient in need of FMT, a first composition intended for transplantation into a patient or FMT recipient and a second composition intended to be administered to the recipient for reducing the level of *C. albicans* in the recipient. The first composition comprises a fecal material from a donor, which has been processed, formulated, and packaged to be in an appropriate form in accordance with the delivery means in the FMT procedure, which may be by direct deposit in the recipient's lower gastrointestinal track (*e.g.*, wet or semi-wet form) or by oral ingestion (*e.g.*, frozen dried encapsulated). The second composition comprises an antifungal agent capable of suppressing the growth/proliferation of *C. albicans*, which may be a broad-spectrum fungicide or a specific inhibitor of the *C. albicans* species, and one or more pharmaceutically acceptable excipient. The composition is formulated for the intended delivery method of the antifungal agent, for example, by injection (intravenous, intraperitoneal, intramuscular, or subcutaneous injection) or by oral ingestion or by local deposit (*e.g.*, suppositories). The first and second compositions are often kept separately in two different containers in the kit. Typically, the kit will further include printed material providing detailed instructions for users of the kit, such as providing information of the schedule and dosing arrangement for administering the first and second compositions to a recipient.

[0053] In another aspect of this invention, alternative compositions useful in FMT with improved efficacy may be devised to contain at least these two components: (1) a donor stool material containing live fecal microorganisms, and (2) an antifungal agent that specifically suppresses the growth or proliferation of *C. albicans* but exhibits no such suppressive or inhibitory effect against other fungal species. Component (2) preferably is not a broad-spectrum fungicide; rather, it should be a specific anti-*C. albicans* agent. For example, it

may be short polynucleotide in nature of, *e.g.*, a small inhibitory RNA, microRNA, miniRNA, lncRNA, or an antisense oligonucleotide, that is capable of disrupting the expression of at least one key gene in the life cycle of *C. albicans*, such that the agent is capable of specifically targeting the species only without significantly affecting other closely related fungal species. Component (2) is particularly useful in the case of a donor's stool containing a level of *C. albicans* too high to permit a satisfactory FMT outcome, as it is capable of locally and specifically suppressing the proliferation of *C. albicans* so as to ensure the success of FMT despite the less than desirable quality of the donor fecal material.

EXAMPLES

[0054] The following examples are provided by way of illustration only and not by way of limitation. Those of skill in the art will readily recognize a variety of non-critical parameters that could be changed or modified to yield essentially the same or similar results.

EXAMPLE 1

INTRODUCTION

[0055] Fecal microbiota transplantation (FMT) is effective in treating recurrent *Clostridium difficile* infection (CDI) and is increasingly being utilized in other human diseases. Whilst bacteria colonization in recipients after FMT has been established, little is known of the role of the gut mycobiota. In this study the present inventors show gut fungal dysbiosis in CDI and identify that donor-derived fungi colonization in recipient is associated with FMT response. Mycobiota profiling in CDI reveals over-presentation of *C. albicans* and decreased fungal diversity, richness and evenness compared with healthy controls. Cure after FMT was observed when donor-derived fungal taxa predominated in recipients' mycobiota. FMT responders display a high prevalence of *Saccharomyces* and *Aspergillus* whilst non-responders and individuals treated with antibiotics display a dominant presence of *Candida*. High abundance of *C. albicans* in recipient before FMT and in donor stool nullifies FMT efficacy in eradicating CDI. Furthermore, *C. albicans* compromises FMT efficacy in a mouse model of CDI, while anti-fungal treatment reestablishes its efficacy. This study furthers the knowledge of human gut mycobiota dynamics and their contribution to FMT, and it enables an understanding of personalized donor-recipient selection in future FMT studies for various human diseases.

[0056] The past decade has witnessed an increasing use of fecal microbiota transplantation (FMT) as a promising treatment option for several diseases¹⁻³, yet success rates are variable

with a cure rate of 85-90% in recurrent *Clostridium difficile* infections (CDI) ³⁻⁵ and a response rate of 30-40% in inflammatory bowel disease ⁶⁻⁸. Such variations may be related to disease traits, recipient factors or donor characteristics. The mechanisms underlying a successful FMT and its relationship with gut microbial profiles in donor-recipient pairs remain elusive. To date, the efficacy of FMT has been mostly ascribed to the restoration of the bacterial microbiota and a sustained co-existence of donor and recipient bacterial strains ⁹⁻¹². Recently, bacteriophages have been shown to be altered in CDI after FMT and these changes were associated with treatment outcome ¹³⁻¹⁵. The human gastrointestinal tract is also colonized by a large population of fungi, collectively referred to as the mycobiota, which play an important role in human health ^{16,17}. Gut mycobiota contribute to normal human physiology and in some cases can recapitulate the benefit of intestinal bacteria via regulating host immunity and maintaining intestinal homeostasis ^{16,17,18}. Whether donor-derived mycobiota can colonize a recipient host, the fate of donor and recipient mycobiota after FMT and their relationship with treatment outcomes are unknown. The inventors performed internal transcribed spacer 2 (ITS2) and 16S rDNA sequencing in FMT-treated subjects with CDI to explore the effects of FMT on the gut mycobiome in association with treatment outcome. A proof-of-causality study was conducted in *C. difficile*-infected mice to confirm the role of gut mycobiota in FMT response.

RESULTS

Gut fungal dysbiosis in CDI

[0057] The fecal mycobiomes were compared between 31 CDI subjects and 23 healthy controls. There was a significant decrease in fungal diversity, evenness and richness in CDI compared with controls (Mann-Whitney test, $p=0.0120$, 0.0309 , and 0.0043 , respectively, **Fig. 1a**). The fungal communities of CDI subjects were significantly separated from those of healthy controls at the OTU level (based on Bray-Curtis distance, adonis test $p=0.003$, **Fig. 1b**). At the phylum level, *Ascomycota* was expanded in CDI compared with controls (Mann-Whitney test, $p=0.0083$, **Fig. 1c**). At the species level, 17 fungal species were found to be differentially present between CDI and controls (LefSe analysis with FDR adjusted $q<0.05$, **Fig. 1d**). Amongst these species, only *C. albicans* was significantly enriched in CDI (**Fig. 1d, e**, Mann-Whitney test, $p=0.0080$), whereas 16 other species were enriched in controls. In line with the observation at the species level, more taxa were enriched in controls than in CDI, as determined by LefSe analysis (9 versus 1 at the order level, 15 versus 2 at the family level, 16

versus 1 at the genus level, **Table 2**). Altogether, these data indicate dysbiosis of the enteric mycobiota in patients with CDI.

[0058] Over-presentation of *C. albicans* in absolute abundance in CDI was confirmed through quantitative PCR (**Fig. 5a**). Antibiotic use has been shown to be a major contributor to the development of CDI by decreasing bacterial colonization resistance. The effect of antibiotics on *C. albicans* levels in CDI was further assessed. Stool samples were collected from new consecutive CDI patients, including 12 CDI patients with antibiotics exposure, 12 CDI patients with no antibiotics exposure at inclusion, and 17 healthy controls. Significantly higher levels of fecal *C. albicans* were found in CDI subjects exposed to antibiotics at inclusion, compared with controls (**Fig. 5b**, Mann-Whitney test, $p=0.0131$). *C. albicans* levels were also significantly higher in CDI subjects not exposed to antibiotics at inclusion when compared with controls (**Fig. 5b**, Mann-Whitney test, $p=0.0469$). These data indicate that both CDI and antibiotics are contributors to increased levels of *C. albicans*.

Donor fungi colonization in recipient is associated with FMT response

[0059] It was then explored whether FMT leads to colonization of donor-derived fungi and its association with treatment efficacy. Changes in the gut mycobiomes of recipients after FMT were monitored at multiple time points in 16 CDI subjects, using pre-FMT samples of each donor-recipient pair as a baseline for FMT (**Fig. 6**). Amongst 16 CDI subjects treated with FMT, nine remained symptom-free with a negative stool *C. difficile* toxin at the last follow-up (termed responders, FMT1-FMT9), whilst seven developed recurrence of CDI (termed non-responders, FMT10-FMT16) (**Table 1**). It was next investigated whether donor-derived fungi and bacteria in recipients may influence FMT outcomes. Subjects who responded to FMT demonstrated a larger proportion of fungal and bacterial OTUs that were transferred and predominated in the feces of recipients after FMT, compared to those who did not respond (Mann-Whitney test, $p=0.0068$ and 0.0164 respectively for comparison of donor-derived fungal and bacterial OTU ratios in recipients, **Fig. 2a, b**). The community structure at the genus level showed a higher abundance of the genera *Aspergillus* and *Penicillium* in FMT responders than in non-responders (**Fig. 2c**). In contrast, the genera *Candida* and *Simplicillium* were significantly enriched in FMT non-responders. Analogously, a similar pattern was observed at the bacterial community structure. FMT responders displayed bacterial abundance resembling that of the donor, whereas FMT non-responders showed inadequate abundance of donor-enriched bacteria at the last follow-up post FMT (**Fig. 7**). Of note, in recipients FMT12 and FMT16, bacterial configurations at the last follow-up after

FMT were similar to that of healthy controls, but their gut mycobiota configurations differed significantly from that of healthy controls. These data indicate that restoration of the gut mycobiota is at least as important as, if not more than, restoration of the bacterial microbiota in CDI recipients. Taken together, these data indicate that the final proportion of donor-derived fungal and bacterial taxa and alterations of the fecal fungal composition in the recipient post FMT were associated with treatment outcome of FMT.

FMT alters the gut mycobiota distinct from antibiotic treatment

[0060] CDI subjects who responded to FMT showed a significant increase in fungal richness and diversity (Wilcoxon matched-pairs signed rank test, $p=0.0273$ and $p=0.0474$ respectively, **Fig. 3a, b**). Although baseline bacterial diversity, evenness and richness were significantly lower in CDI subjects compared to controls (Mann-Whitney test, all $p<0.0001$, **Fig. 6a**), after FMT there was a significant increase in bacterial richness (Wilcoxon matched-pairs signed rank test, $p=0.019$) and a marginally significant increase (Wilcoxon matched-pairs signed rank test, $p=0.098$) in bacterial diversity in FMT responders. During post-FMT follow-up, there were profound differences in the gut mycobiota configurations across different donor-recipient pairs, however a significantly higher prevalence of the genus *Candida* was observed across the serial post-FMT fecal samples of FMT non-responders relative to that of responders (**Fig. 3c, d**). In contrast, the genera *Saccharomyces* and *Aspergillus* were present in higher abundances in FMT responders than in non-responders (**Fig. 3c, d**). Discriminative analysis identified disparately presented taxa between post-FMT samples of FMT responders and non-responders, at the genus and species levels (**Fig. 3d**). *C. albicans* was the most prominent species enriched after FMT in non-responders.

[0061] *C. albicans* markedly decreased after FMT (Wilcoxon matched-pairs signed rank test, $p=0.0458$, **Fig. 3d, e**). Interestingly, both the abundance of *C. albicans* in donor feces and in post-FMT recipient fecal samples were associated with FMT treatment outcome. FMT recipients transplanted with a donor feces with *C. albicans* $<0.1\%$ in the fungal community achieved a response to FMT treatment, compared to those transplanted with a donor feces with *C. albicans* $>0.1\%$ (*Chi-square* test $p=0.049$, **Fig. 3f**). Recipients with an initial high abundance of *C. albicans* before FMT and continuing to have a relative abundance of *C. albicans* $>10\%$ after FMT all experienced a disease recurrence after FMT (*Chi-square* test $p=0.029$, **Fig. 3g**). These data indicate that the presence of *C. albicans* compromises FMT efficacy. The absolute abundance of *C. albicans* (in fecal input DNA) was markedly decreased after FMT in FMT responder group (Wilcoxon matched-pairs signed rank test,

$p=0.0391$, **Fig. 16**). Interestingly, FMT non-responders exhibited significantly higher post-FMT fecal *C. albicans* levels in absolute abundance than FMT responders [Mann-Whitney test, $p=0.0018$, $\log_{10}$ transformed effect size 3.05 (95% CI: 1.48–4.29), **Fig. 16**], indicating *C. albicans* can be a marker for disease recurrence and/or pathogenesis.

[0062] The effect of antibiotics on the gut mycobiota was also assessed across longitudinal time-points in 8 CDI subjects treated with vancomycin (STD treatment, **Fig. 6, Table 1**). Five of the eight subjects remained symptom-free with a negative stool *C. difficile* toxin at the last follow-up (termed responders, STD1-STD5), while three developed recurrence of CDI (termed non-responders, STD6-STD8). Unlike FMT, vancomycin induced inconsistent alterations in the fungal richness and diversity during longitudinal follow-up (**Fig. 9a, b**). There was no significant difference in the fungal richness or diversity between STD responders and non-responders after FMT, although vancomycin resulted in a significant increase in bacterial diversity in responders after FMT (**Fig. 10**, Wilcoxon matched-pairs signed rank test, $p=0.0198$).

[0063] FMT and vancomycin led to an increase in the gut fungal diversity in 81.3% (13 out of 16) and 37.5% (3 out of 8) of CDI subjects, respectively (Chi-square test $p=0.032$, **Fig. 8c**), and an increase in the gut fungal richness in 68.8% (11 out of 16) and 37.5% (3 out of 8) of CDI subjects, respectively (**Fig. 8c**). FMT responders showed a significantly higher fold-change post FMT in both fungal richness and fungal diversity compared to STD responders (Mann-Whitney test $p=0.019$ and Chi-square test $p=0.05$ respectively, **Fig. 8d, e**). Collectively, these data indicate that FMT may be more influential in orchestrating the gut mycobiota than antibiotics.

[0064] Taxonomical analysis was performed to further elaborate the effect of antibiotics on the fungal community and to discern differences between FMT and antibiotics in modulating the gut mycobiota. After vancomycin treatment, fungal compositions exhibited similar configurations during follow-up across STD subjects, with a marked expansion of the genus *Candida* (**Fig. 8f**). To define differentially enriched fungal taxa between subjects who responded to FMT and vancomycin, we implemented LefSe analysis across all follow-up samples of treatment responders. FMT treatment enriched the genera *Saccharomyces* and *Cryptococcus* in those who responded, whereas vancomycin disparately enriched a panel of fungal genera in STD responders after treatment, which included *Candida*, *Talaromyces*, *Erythrobasidium*, *Periconia*, *Stemphylium*, *Ganoderma* (**Fig. 11**). At the family level, FMT caused an enrichment of *Saccharomycetaceae* and *Herpotrichiellaceae*, while vancomycin

caused an enrichment of Intertae sedis (**Fig. 11**). There was no statistically significant difference in the relative abundance of *C. albicans* between STD responders and non-responders, however a decrease in *C. albicans* was seen in STD responders after vancomycin treatment (**Fig. 12**). Subject STD7 who had a post-STD relative abundance of *C. albicans* >10% developed CDI recurrence after vancomycin treatment, further substantiating the importance of alleviation of *C. albicans* for eradicating CDI.

Trans-kingdom interactions between gut mycobiota and bacterial microbiota are associated with treatment outcome

[0065] To characterize the ecological network of the gut mycobiota and bacterial microbiota, the correlation of the α -diversity (diversity, evenness and richness) of the fungal community with that of the bacterial community was evaluated. Among the post-treatment samples of FMT responders, significant positive correlations were found between fungal diversity and bacterial diversity, and between fungal richness and bacterial diversity, evenness, and richness (Spearman's correlation, permutation test, $P < 0.05$, **Fig. 13**). In the post-treatment samples of FMT non-responders and STD responders, the correlation between bacterial and fungal communities showed a depletion of correlations between fungal richness and other bacterial and fungal α diversity indexes. The correlations were completely abolished across the post-treatment samples of STD non-responders. The correlations of fungal genera with bacterial genera were further assessed in controls and CDI subjects in association with treatment response. Significant inverse correlations between control-enriched bacteria, including butyrate-producing *Roseburia*, and CDI-enriched *Candida* were observed in FMT responders and STD responders after treatment, paralleling a prevalence of positive correlations between control-enriched bacteria and control-enriched fungi among which correlation of *Roseburia* and *Aspergillus* was present in both FMT responders and STD responders (**Fig. 14**). However, those who did not respond to either FMT or STD displayed an apparent contraction in the number of fungal-bacterial correlations after treatment, compared to FMT responders and STD responders. These data suggest the importance of restoration of an intricate and homeostatic fungal-bacterial ecosystem in maintaining treatment response.

C. albicans compromises FMT efficacy in a murine model of CDI

[0066] *C. albicans* was the most prominent species associated with treatment failure of FMT in CDI, suggesting a possible causal relationship. This assumption is further supported

by reports whereby CDI recurrence was observed after antibiotics treatment^{3,19}, as antibiotics contribute to the expansion of *Candida*. To determine the causal relationship between *C. albicans* and response to FMT, the efficacy of FMT in eliminating *C. difficile* was assessed using a *C. difficile* induced-diarrhea murine model in three groups of mice: (i) mice infused with human stool preparation, (ii) mice colonized with *C. albicans* then infused with human stool preparation, and (iii) mice infused with human stool preparation supplemented with *C. albicans* during fecal transplantation (**Fig. 4a**). FMT was effective in ameliorating diarrhea, intestinal inflammation, and decreasing *C. difficile* burden, compared to CDI group, while no difference in *C. difficile* load was observed among all groups before FMT (**Fig. 4b-d**). However, mice that was colonized with *C. albicans* prior to FMT or those infused with donor stool supplemented with *C. albicans* suffered significant diarrhea, intestinal inflammation, and augmented *C. difficile* burden post FMT, when compared with mice administered with a single infusion of human stool (**Fig. 4b-d**). There were high levels of *C. albicans* in these recipient mice on day 1 post FMT, though a decrease in *C. albicans* load was observed after FMT in mice colonized with *C. albicans* prior to FMT (**Fig. 4e**). An anti-fungal agent, fluconazole, to eradicate *C. albicans* in a group of recipient mice prior to human stool infusion (FMT) (**Fig. 15a**). *C. difficile* load was then compared after human stool infusion between mice with and without anti-fungal treatment. Anti-fungal treatment in recipient mice colonized with *C. albicans* before human stool infusion restored the efficacy of FMT in clearing *C. difficile* infection (**Fig. 15b**). These data demonstrate that the existence of *C. albicans*, either in the recipient or in the donor, negates the efficacy of FMT in clearing *C. difficile*, while antifungal treatment reestablishes its efficacy. These results highlight that persistent fungal dysbiosis with aberrant presence of *C. albicans* can confer an unfavourable FMT outcome in CDI.

Total fungal load is increased in CDI

[0067] The total fecal fungal load was significantly higher in CDI than in controls [Mann-Whitney test, $p=0.0004$, $\log_{10}$ transformed effect size 1.32 (95% CI: 0.62-1.97), **Fig.17**].

Total fungal load and C.albicans in inflammatory bowel disease (IBD)

[0068] The total fecal fungal load was significantly higher in patients with IBD, including patients with Crohn's disease (CD) and Ulcerative colitis (UC) – two subtypes of IBD, than in controls (Mann-Whitney test, $p=0.0003$, $p=0.0225$, respectively, **Fig.18 a**). The fecal

presence ratio of *C. albicans* is higher in CD than in controls, and 3 CD patients exhibiting the highest *C. albicans* levels had a history of recent exposure to antibiotics (**Fig.18 b**).

Total fungal load is increased in irritable bowel syndrome (IBS)

[0069] The total fecal fungal load was significantly higher in IBS than in controls [Mann-Whitney test, $p=0.0237$, **Fig.19**].

Bacterial alterations in CDI after FMT in association with FMT outcome

[0070] The present inventors explored the composition of the bacterial microbiota after FMT in relation to FMT outcomes, at various taxonomic levels (**Fig.20**). Actinobacteria, Bacteroidetes (phylum-level taxa), *Lachnospiraceae*, *Clostridiaceae*, and *Ruminococcaceae* (family-level taxa), *Clostridium*, *Blautia*, and *Faecalibacterium* (genus-level taxa) were significantly more enriched in FMT responders than in non-responders after FMT. However, bacteria from the phylum Proteobacteria were more abundant in FMT non-responders relative to FMT responders. FMT responders displayed bacterial abundances resembling that of the donor, whereas FMT non-responders showed inadequate relative abundances of donor-enriched bacteria at the last follow-up after FMT (**Fig.20c**). LefSe analysis on the fecal bacteriomes of donors at the genus level identified *Escherichia* and *Proteus* as the differentially enriched genera in FMT responders' donor stool and in FMT non-responders' donor stool respectively (LDA effect size 2.58 and 2.35, FDR adjusted $q=0.017$ and 0.006, respectively).

DISCUSSION

[0071] This is the first study to characterize the gut mycobiota in CDI and to elucidate mycobiota alterations after FMT in relation to treatment outcome. Patients with CDI showed enteric fungal dysbiosis. Importantly, disease recurrence after FMT was associated with several important findings including persistent fungal dysbiosis, low levels of donor-derived fungal colonization, high abundance of *C. albicans* in the recipient stool before FMT and the presence of *C. albicans* in the donor stool. The observations that disease cure requires both fungal and bacterial colonization from the donor provides new and important insights into the potential therapeutic importance of the gut mycobiota in treatment outcome in FMT, beyond the bacterial microbiota. These data also highlight a new concept in FMT that the abundance of fecal *C. albicans* both in recipient before treatment and in donor are critical components when considering implementation of FMT. Integration of more in-depth mycobiota analysis

in donor-recipient pair may lead to personalized and targeted gut microbial therapy in the future.

[0072] Although studies of the gut mycobiota have lagged behind that of the gut bacterial microbiota, fungi are increasingly being considered as important players of the gut and interactions between pathogenic and commensal fungal and bacterial communities are crucial in the maintenance of human health and disease pathogenesis¹⁶. Furthermore, disruption of the gut mycobiota has deleterious effect on host immunity¹⁷. Despite high interpersonal variability of the gut mycobiota in patients with CDI, there was a significant expansion of the genus *Candida* and the species *C. albicans*. Interestingly, FMT induced an increase in the genus *Saccharomyces* along with a marked contraction of *Candida* and *C. albicans* after FMT in treatment responders, while recipients who demonstrated high abundance of *C. albicans* in the stool after FMT (10%) or those whose donor had a high abundance of (0.1%) of *C. albicans* were more likely to have disease recurrence after FMT. Over-presentation of *C. albicans* in the recipient stool after FMT may largely contribute to treatment failure.

[0073] The role of fungal commensals in educating the human immune system has gained new appreciation in intestinal disease. In the steady state, bacterial communities keep fungi in check in the gut. Fungi are major causes of infections among immunocompromised or hospitalized patients with serious underlying diseases and comorbidities. *Candida* species remain the most important cause of opportunistic infections worldwide, affecting predominantly elderly patients²⁰. Candidalysin was recently unveiled as a fungal toxin from *C. albicans* critical for mucosal infection²¹. Commensal bacteria inhibit *C. albicans* colonization through activation of HIF-1 α and LL-37²². Antibiotic treatment selectively and effectively eradicates the bacterial community but consequently leads to fungal outgrowth, particularly the *Candida* species^{23,24}. Antibiotics or immunosuppressants are effective in the short term but they likely compromise the immune system in the longer term. A compromised immune system creates a more favourable environment to expansion of *Candida* and overgrowth of *Candida* can alter the recovery of the gut bacterial microbiota after cessation of antibiotic treatment^{25,26}. In this study, the over-presence of *Candida* species in recipients might account for the high failure rate of FMT in CDI. In DSS-induced colitis mouse model as well as patients with inflammatory bowel disease (IBD), *C. albicans* and *Candida* were significantly enriched²⁷⁻²⁹. Antifungal treatment decreased *Candida* prevalence and ameliorated inflammatory responses in DSS colitis mice²⁹. However,

disruption of fungal communities by long-term use of antifungals aggravated severity of DSS colitis and allergic airway³⁰. Collectively, these data implicate the importance of the gut fungal-bacterial homeostasis in host health. These data suggest that the establishment of a balanced gut fungal and bacterial community via FMT is important to eradicate CDI, as FMT non-responders showed abrogated fungi-bacteria correlations in α -diversity and taxa when compared with responders.

[0074] In conclusion, gut mycobiota alterations may determine treatment outcome in FMT. The persistence of fungal dysbiosis, particularly the presence of *C. albicans*, can incur CDI recurrence. The findings disclosed herein highlight the importance of both “optimal” donor selection and pre-FMT eradication of *C. albicans* in recipient during FMT practice, where future FMT therapy should incorporate detailed characterization and stratification of both donor and recipient fecal mycobiomes. These results provide a framework for future investigations into the contribution of donor/recipient mycobiota profiles and gut fungi-bacteria interactions in FMT treatment for various human diseases.

METHODS

Study subjects and treatment outcome

[0075] The current study was a sub-study from a randomised controlled trial (RCT) of FMT versus vancomycin (standard therapy, STD) for patients with CDI. Consecutive CDI subjects enrolled into this randomised controlled trial were invited to participate in a substudy of assessment of fecal microbiota. Patients were included if they had three or more loose or watery stools per day for at least two consecutive days or eight or more soft or loose stools in 48 hours and a positive stool test for *C. difficile* based on a two-step testing algorithm in our hospital, a positive GDH (Glutamate dehydrogenase) screening test followed by a positive polymerase chain reaction (PCR) test of *C. difficile*. A total of 31 subjects with CDI and 24 healthy household controls were recruited and stool samples at baseline were obtained for analyses of fungal and bacterial microbiomes. Among them, 24 CDI subjects consented to have stool samples collected serially after treatment for microbiome analysis. 16 CDI subjects were treated with FMT and 8 were treated with vancomycin, and they were followed up at baseline and at weeks 2, 4, 10 and 16 after treatment (**Fig. 6**). Subjects in the FMT group received 5 days of vancomycin followed by donor infused stool via nasojejunal route and those who had STD received oral vancomycin 500 mg orally four times per day for 10 days. A computer-generated randomization schedule was used to assign patients to the

treatment sequences. All patients kept a stool diary and were questioned about stool frequency and consistency and medication use.

[0076] Treatment response was defined as an absence of diarrhea or persistent diarrhea that could be explained by other causes with a negative stool test for *C. difficile* toxin, while relapse was defined as diarrhea with a positive stool test for *C. difficile* toxin. Treatment cure is defined as symptom resolution and a negative *Clostridium difficile* toxin in stool until the last follow-up (last follow-up is referred to as the last stool collection time point, as shown in **Fig. 6**). 9 of the 16 subjects who had FMT (FMT1-FMT9), and 5 of the 8 patients (STD1-STD5), who had vancomycin were cured of CDI (termed responders, **Table 1**) at a median follow-up of 16 weeks. CDI recipients FMT11 and FMT12 shared the same donor, and this donor was termed “Donor11”. Clinical data of the subjects and collected stool samples are shown in **Table 3**. None of the patients had received antibiotics or proton pump inhibitors after FMT.

Study design

Patient inclusion criteria:

[0077] 1. *C. difficile* infection was defined as diarrhea (≥ 3 soft, loose or watery stools per day for at least 2 consecutive days or ≥ 8 soft or loose stools in 48 hours) and a positive stool test for *C. difficile* toxin; and

[0078] 2. Age ≥ 18 ; and

[0079] 3. Written informed consent obtained

Patient exclusion criteria:

[0080] 1. The presence of human immunodeficiency virus (HIV) infection with a CD4 count of less than 240

[0081] 2. Pregnancy

[0082] 3. GI Bleeding

[0083] 4. Acute coronary syndrome

Donor screening:

[0084] Donors included individuals who are spouses or partners, first-degree relatives, other relatives, friends, and individuals unknown to the patient. They were screened with a questionnaire and excluded if they had taken antibiotics within the preceding 3 months; were

on major immunosuppressive agents, including chemotherapeutic agents; had known or recent exposure to HIV, hepatitis B or C; had a current communicable disease; participated in high-risk sexual behaviors; used illicit drugs; traveled within 6 months to areas with endemic diarrheal illnesses; or had history of inflammatory bowel disease, irritable bowel syndrome or chronic diarrhea, gastrointestinal malignancy or polyposis. In addition, donor was screened for HBsurface Ag, Anti-HBc, Anti-HCV, Anti-HIV, Syphilis EIA, stool microscopy, culture and sensitivity, stool cyst, ova, parasite, norovirus and *C. difficile* (cytotoxin and PCR assay). All subjects and collected stool samples are listed in **Table 1**.

[0085] The donors for the FMT group were healthy household controls and the donor stool samples analyzed were the same samples used for FMT. All subjects provided written informed consent.

[0086] Family members provided donor stool for subjects randomised to FMT arm. Cure after FMT or vancomycin therapy was defined as symptom resolution and negative *Clostridium difficile* toxin in stool at last follow-up by PCR assay. Relapse was defined as diarrhea with a positive stool test for *C. difficile* toxin.

[0087] This was a randomised but not blinded study. However for mycobiome and bacterial microbiome analyses on stool samples, assessments were initially performed by analysts who were blinded to the clinical outcome of the studied subjects. When the profiled mycobiome and bacterial microbiome data were available for each individual subject, correlation was then made to associate microbiome profiles with treatment outcomes of subjects.

Infusion of donor stool

[0088] In subjects who received FMT, a nasoduodenal tube was inserted with radiology guidance. Donor feces was diluted with 500 ml of sterile saline (0.9%), blended and the supernatant was strained with filter paper and poured in a sterile bottle. Within 6 hours after collection of feces by the donor, the solution was infused through a nasoduodenal tube (2 to 3 minutes per 50 ml). The tube was removed 30 minutes after the infusion, and patients were monitored for 2 hours. In subjects with received FMT, a minimum of 50g of donor stool was collected on the same day of infusion and used within 6 hours of collection.

Fecal DNA Extraction

[0089] Fecal DNA was isolated as described below. 100mg fecal sample was pre-washed with 1 ml ddH₂O and pelleted by centrifugation at 10, 000 x g for 1 minute. The fecal pellet

was re-suspended in 800 µl TE buffer (pH 7.5), supplemented with 1.6 µl 2-Mercaptoethanol and 500 U lyticase (Sigma), and incubated at 37°C for 60 min. The sample was then centrifuged at 10,000 x g for 2 minutes and fecal DNA was subsequently extracted from the pellet using ZR Fecal DNA miniPrep kit (Zymo Research, Orange, CA) according to the protocol. Briefly, fecal pellet was added to the BashingBeadLysis Tube with 750 µl Lysis solution, and then processed at maximum speed for 10 minutes. The lysates were centrifuged at $\geq 10,000 \times g$ for 1 minute. The supernatant was transferred to a Zymo-SpinTM IV Spin Filter in a collection tube and centrifuged at 7,000 x g for 1 minute. About 1,200 µl of fecal DNA binding buffer was added to the filtrate in the collection tube, followed by concentration and purification in a new filter tube. Finally, a total of 50 µl eluted DNA with a concentration at 20-100 ng/µl was prepared for each sample.

Fungal ITS2 sequencing and quality control

[0090] The final fecal DNA for fungal sequencing was amplified based upon ITS2 (Internal transcribed spacer 2) region using primers as below and PrimeSTAR HS DNA Polymerase kit (TaKaRa, Japan). The primer pairs are ITS2-F: 5'-GCATCGATGAAGAACGCAGC-3', ITS2-R: 5'-TCCTCCGCTTATTGATATGC-3'. ITS2 amplicons were generated with 38 cycles of 3-step PCR: 98°C 10s, 59°C 10s, and 72°C 30s. PCR samples were then sequenced on the Illumina MiSeq PE300 platform (2 X 300 bp, BGI, China), 151,524 $\pm$ 97,694 (number $\pm$ SD) clean sequences obtained on average (sequence statistics in **Table 4**).

[0091] Raw reads were filtered by SOAPnuke (v 1.5.3) (web site: soap.genomics.org.cn/) developed by BGI as follows: (i) adaptors removed, (ii) read removed if N base is more than 3% of the read, (iii) read removed if bases with quality low than 20 were more than 40% of read, (iv) all duplicates removed. Quality control and data analysis were further implemented in PIPITS (v 1.4.5)³¹. Briefly, PIPITS_PREP prepares raw reads from Illumina MiSeq sequencers for ITS extraction; PIPITS_FUNITS extracts ITS2 from the reads; and PIPITS_PROCESS analyses the reads to produce operational taxonomic unit (OTU) abundance tables and the RDP taxonomic assignment table for downstream analysis. The quality trimmed and ITS2 extracted reads were aligned to fungi UNITE database exploiting RDP classifier 2.10 for taxonomic assignment to produce operational taxonomic unit (OTU) abundance table (based on sequence identity $\geq 97\%$ identity) and phylotype abundance tables at different taxonomic levels, for downstream analysis.

[0092] The fungal OTU and phylotype abundance data were imported into R 3.2.3. Richness, diversity, and evenness calculation were performed using the estimated richness function of the phyloseq package. Spearman correlation and their significance were calculated using the *cor* and *cor.test* functions in R, respectively. For the fungal-bacterial taxa comparisons, Spearman correlations were calculated for the relative abundance of the differentially presented fungal taxa and the bacterial taxa determined to be significantly associated with disease by Lefse analysis. Correlation plots were generated using the *corrplot* package in R. Heat maps were generated using the *pheatmap* package in R.

Quantitative PCR for detection of C. albicans in human fecal DNA samples

[0093] *C. albicans* loads in human stools were quantified by qPCR analysis (SsoAdvanced SYBR Green Supermix, Bio-Rad) of extracted human fecal DNA using *C. albicans* specific primers: *C. albicans*-F 5'- CCTGTTTGAGCGTCGTTTCTC -3'; *C. albicans*-R 5'- TTTGGTTAGACCTAAGCCATTGTCA -3'. *C. albicans* abundance was determined using standard curves constructed with reference genomic DNA (gDNA) of *C. albicans*.

Quantitative PCR for detection of total fungal load in human fecal DNA samples

[0094] Total fungal loads in human stools were quantified by TaqMan qPCR analysis (Premix Ex TaqTM, TaKaRa) of extracted human fecal DNA using primers³⁶: *Fungi-quant*-F 5'- GGRAAACTCACCAGGTCCAG -3'; *Fungi-quant*-R 5'- GSWCTATCCCCAKCACGA -3', and probe: 5'-TGGTGCATGGCCGTT-3'.

LefSe linear discriminant analysis

[0095] To compare differences in the configurations of fungal and bacterial microbiomes between CDI patients and healthy controls, between FMT responders and non-responders, between FMT treatment and vancomycin (STD) treatment, Lefse analyses were performed on the Huttenhower lab Galaxy server (web site: huttenhower.sph.harvard.edu/galaxy/) by importing the viral and bacterial relative abundance values and associated sample metadata, with FDR adjusted p value <0.05 considered significant and effect size calculated.

Calculation of donor transferred OTUs in recipients

[0096] In samples after FMT, if a fungal or bacterial OTU was not present in the recipient baseline sample but present both in the corresponding donor baseline sample and in the recipient post-FMT sample, the OTU was defined as “donor derived”; if an OTU was not present in the corresponding donor baseline sample but detected both in the recipient baseline

sample and in the recipient post-FMT sample, the OTU was defined as “recipient exclusive”; if an OTU was present across the recipient baseline sample, the recipient post-FMT sample and the corresponding donor baseline sample, the OTU was defined as “donor-recipient co-existed.”

Bacterial 16S rRNA sequencing and data analysis

[0097] The final fecal DNA samples were subject to bacterial 16S rRNA V4 region amplification and sequenced on the Illumina MiSeq PE250 platform (2 X 250 bp, BGI, China), $132,081 \pm 65,429$ (number $\pm$ SD) sequences obtained on average (sequence statistics in **Table 5**). Quality control and data analysis were implemented in mothur (v 1.38.0) as previously described³². Any sequences with ambiguous bases and anything longer than 275 bp were removed, and aligned against the non-redundant Greengenes database (v 13.8)³³ using the NAST algorithm. Any sequences that failed to align with the V3-4 region were discarded. The remaining sequences were trimmed to the same alignment coordinates over which they fully overlapped, followed by removal of homopolymers and detection for the presence of chimeras by UChime.

[0098] The resulting sequences were classified against the Greengenes database and annotated with deepest level taxa represented by pseudo-bootstrap confidence scores of at least 80% averaged over 1,000 iterations of the naive Bayesian classifier. Any sequences that were classified as either being originated from archaea, eukarya, chloroplasts, mitochondria, or unknown kingdoms, were removed. The annotated sequences were assigned to phylotypes according to their consensus taxonomy with which at least 80% of the sequences agreed. Closed reference operational taxonomic units (OTUs) sharing 97% identity were clustered as well and assigned taxonomy according to the Greengenes database. Lefse analysis was performed to define bacterial taxa associated with CDI and healthy controls. The relative abundance of these abundance-differential taxa identified by LefSe in pre-FMT baseline samples and post-FMT last follow-up samples were plotted using *pheatmap* R package.

*Mouse husbandry and model of *C. difficile* infection*

[0099] Studies were conducted on 4- to 6-week old female C57BL/6 that were reared in groups of 9. Individual mice were randomized after arrival. Mice were subjected to a previously described model of CDI³⁴. Briefly, mice were given an antibiotic cocktail of kanamycin (0.4 mg/mL), gentamicin (0.035 mg/mL), colistin (850 U/mL), metronidazole (0.215 mg/mL), and vancomycin (0.045 mg/mL) (all antibiotics were purchased from Sigma-

Aldrich, St. Louis, MO) in their drinking water for 3 days. Mice were then given 2 days of recovery before administration of 10^7 spores of *C. difficile* in PBS via oral gavage. Animal grouping and research scheme were designed as shown in **Fig. 4a**. On day 1 post stool infusion, diarrhea was evaluated by stool water content, calculated as stool weight loss after air drying at 70°C for 4 hours. Colons were harvested, fixed in 4% formalin solution and embedded in paraffin. Sections were stained with hematoxylin and eosin for histological assessment.

[0100] For antifungal experiment, animal grouping and research scheme were designed as shown in **Fig. 15a**. Mice was initially colonized with *C. albicans* (2×10^8 cfu per mouse) after 3 days of antibiotic cocktail treatment in the drinking water, followed by 4 days of fluconazole treatment supplemented in the drinking water (0.5mg/mL, Sigma). Then the mice were subjected to *C. difficile* administration (10^7 spores per mice) through gavage after a consecutive 1.5-day antibiotic cocktail- and 1.5-day free water- drinking. Human stool infusion was performed 2 days later after *C. difficile* gavage. Both *C. difficile* load and *C. albicans* load were enumerated by cultivation on Day 0 before FMT and Day 1 after FMT.

C. albicans administration and donor stool infusion in mice

[0101] *C. albicans* (10231, purchased from ATCC, USA) was administered to mice (2×10^8 cfu per mouse) via gavage after 3-day antibiotic treatment or supplemented in donor stool slurry at the time of donor stool infusion. Human stool from a healthy volunteer (Chinese, male, age 28 years), without presence of *C. albicans* as measured by qPCR, was obtained with informed consent. For stool microbiota infusions, approximately 500 mg of stool samples were cut in an anaerobic chamber and suspended in 5 ml of phosphate-buffered saline. Mice were colonized by oral gavage of 150 μl of fecal slurry with or without supplementation of *C. albicans* on day 2 after *C. difficile* challenge.

Quantification of C. difficile and C. albicans burdens in mouse feces

[0102] Mouse stool were collected both before and after stool infusion. Fecal *C. difficile* and *C. albicans* burdens on day 0 before and day 1 after stool infusion were measured by cultivation. Samples were diluted in PBS and respectively plated on taurocholate cycloserine cefoxitin fructose agar (TCCFA) for quantification of *C. difficile* burden, on Sabouraud dextrose agar (SDA) for quantification of *C. albicans* load. Stool samples prior to *C. albicans* colonization from antibiotic-treated mice were plated on SDA to ensure that mice were *C. albicans* culture negative.

Data availability

[0103] Sequence data and accompanying metadata have been deposited to the NCBI Sequence Read Archive under BioProject accession numbers PRJNA419097 and PRJNA419104.

EXAMPLE 2: *C. albicans* level associated with unfavorable FMT outcome in IBD

[0104] The fecal *C. albicans* level was investigated in three IBD patients with concurrent CDI, and subsequently followed them up after FMT (Fig. 21). Disease symptoms were ameliorated soon after FMT. However, all three patients manifested unfavorable FMT outcomes at different time-points post FMT. In accordance with the finding for CDI patients, these IBD patients all showed increased fecal *C. albicans* levels after FMT. Taken into consideration the previous observation that *C. albicans* levels were also higher in IBD than in Controls (Fig. 18), it indicates that *C. albicans* may play an unfavorable role in IBD and IBD-FMT.

[0105] All patents, patent applications, and other publications, including GenBank Accession Numbers, cited in this application are incorporated by reference in the entirety for all purposes.

Table 1

Subject	Sex	Age	Smoking	Severe/ Moderate	Duration of follow up (wks)	Outcome
						(till last follow up)
FMT1	M	80	Ex-smoker	Moderate	16	Cured
FMT2	M	52	No	Severe	27	Cured
FMT3	M	38	No	Moderate	17	Cured
FMT4	F	76	No	Moderate	18	Cured
FMT5	M	63	No	Severe	18	Cured
FMT6	M	88	No	Severe	23	Cured
FMT7	M	45				Cured
FMT8	M	90				Cured
FMT9	F	52				Cured
FMT10	M	45	Ex-smoker	Severe	20	Recurrence at week 19
FMT11	F	83	No	Moderate	11	Recurrence at week 5
FMT12	F	38	No	Severe	28	Recurrence at week 28
FMT13	M	81				Recurrence at week 2
FMT14	M	65				Recurrence at week 2
FMT15	F	90				Recurrence at week 4
FMT16	M	83				Recurrence at week 4
STD1	F	78	smoker	Severe	14	Cured
STD2	F	83	No	Severe	17	Cured
STD3	F	99	No	Moderate	26	Cured
STD4	F	85				Cured
STD5	F	92				Cured
STD6	M	88	Ex-smoker	Severe	20	Recurrence at week 12
STD7	M	93	No	Moderate	7	Recurrence at week 7
STD8	M	63				Recurrence at week 5

Table 2**Order level lefSe analysis**

order	enriched in group	LDA effect size	q value
o Saccharomycetales	CDI	5.26234241	0.00141993
o Incertae sedis	Control	4.22542515	0.01743251
o Ustilaginales	Control	4.64245919	0.01113039
o Wallemiales	Control	3.86277202	0.02933565
o Eurotiales	Control	5.02946522	3.45E-05
o Trechisporales	Control	4.62024264	0.00835961
o Agaricostilbales	Control	4.77590173	0.04495598
o Mucorales	Control	3.94917134	0.00275496
o Chaetothyriales	Control	3.96218634	0.03957291
o unidentified	Control	4.43843789	0.0011891

Family level lefSe analysis

family	enriched in group	LDA effect size	q value
f Incertae sedis	CDI	5.361603211	0.00032083
f Diatrypaceae	CDI	3.647432029	0.02396599
f Lichtheimiaceae	Control	3.776192836	0.03565053
f Marasmiaceae	Control	4.460718116	0.04495598
f Cordycipitaceae	Control	3.018060628	0.01080346
f Trichocomaceae	Control	4.998286724	3.71E-05
f Monascaceae	Control	4.442880786	0.00806352
f Ustilaginaceae	Control	3.308596392	0.01113039
f Agaricostilbaceae	Control	3.637118408	0.04495598
f unidentified	Control	4.382578512	0.00989125
f Pichiaceae	Control	4.234924293	0.00153938
f Rhizopodaceae	Control	3.759990223	0.03298649
f Pleosporaceae	Control	4.266635541	0.04792052
f Mucoraceae	Control	3.546579211	0.00818474
f Eremotheciaceae	Control	3.514966042	0.01499872
f Wallemiaceae	Control	3.255471141	0.02933565
f Hydnodontaceae	Control	3.172134768	0.01944714

Genus level lefSe analysis

genus	enriched in group	LDA effect size	q value
g Candida	CDI	5.382628239	0.00018873
g Wallemia	Control	3.198526789	0.02933565
g Trechispora	Control	3.55075498	0.01944714

genus	enriched in group	LDA effect size	q value
g Lentinula	Control	3.687563833	0.04495598
g Alternaria	Control	4.061524753	0.00543258
g Talaromyces	Control	3.815612518	0.04495598
g Aspergillus	Control	4.856125262	8.75E-05
g Pichia	Control	3.729764446	0.00177011
g Thermomyces	Control	3.268072407	0.0138274
g Rhizopus	Control	3.612096939	0.03298649
g unidentified	Control	4.780192159	0.00077864
g Simplicillium	Control	2.905283827	0.01080346
g Monascus	Control	4.416347845	0.00806352
g Mucor	Control	3.444062001	0.00818474
g Penicillium	Control	4.397256252	7.64E-05
g Sterigmatomyces	Control	3.736578962	0.04495598
g Eremothecium	Control	3.41388614	0.01499872

Species level lfe analysis

species	enriched in group	LDA effect size	q value
s Candida albicans	CDI	4.883094137	0.0128582
s Hanseniaspora sp	CDI	2.476489287	0.02396599
s Penicillium sp	CDI	3.159815075	0.02226132
s Aspergillus austroafricanus	Control	2.820485891	0.02586708
s Penicillium dierckxii	Control	3.112142685	0.04495598
s Eurotiomycetes sp	Control	4.236581004	0.00632072
s Pseudozyma churashimaensis	Control	2.766834362	0.04495598
s Thermomyces lanuginosus	Control	3.158801272	0.0138274
s Ustilaginaceae sp	Control	2.551290082	0.04335394
s Monascus purpureus	Control	4.436483036	0.00806352
s Penicillium brocae	Control	3.202903826	0.00668459
s Wallemia mellicola	Control	3.029805635	0.02933565
s Sterigmatomyces halophilus	Control	3.158900806	0.04495598
s Eremothecium sinicaudum	Control	3.158887769	0.04495598
s Pseudozyma sp	Control	2.865506029	0.04495598
s Rhodotorula dairenensis	Control	3.652021075	0.04495598
s Aspergillus penicillioides	Control	3.45521592	0.03459752
s Ophiostomataceae sp	Control	3.730559216	0.04495598
s Mucor racemosus	Control	2.732298798	0.00354366
s Lichtheimiaceae sp	Control	3.462009204	0.04495598
s Penicillium steckii	Control	2.538239261	0.03894247

TABLE 3

sample_name	FMT /STD number	baseline_comparasion	Sample_collection	sample_number#	Randomiz ation arm	Collect Date	time_point_ post treat- ment (FMT/STD, week)	Age	Sex	donor relation- ship to patient	House hold ID (family ID)
F1W0	FMT1	CDI	longitudinal	3	FMT	27-Oct-15	0	80	M		A
F1W2	FMT1	NA	longitudinal	4	FMT	16-Nov-15	2				A
F1W6	FMT1	NA	longitudinal	5	FMT	14-Dec-15	6				A
Control1	Donor1	Control	cross-sectional	6	Donor	27-Oct-15		35	F	Daughter	A
F2W0	FMT2	CDI	longitudinal	9	FMT	13-Feb-15	0	52	M		B
F2W2	FMT2	NA	longitudinal	10	FMT	06-Mar-15	2				B
F2W4	FMT2	NA	longitudinal	11	FMT	20-Mar-15	4				B
Control2	Donor2	Control	cross-sectional	13	Donor	12-Feb-15		51	F	Wife	B
F3W0	FMT3	CDI	longitudinal	18	FMT	20-Mar-15	0	38	M		C
F3W2	FMT3	NA	longitudinal	19	FMT	14-Apr-15	2				C
F3W4	FMT3	NA	longitudinal	20	FMT	28-Apr-15	4				C
F3W10	FMT3	NA	longitudinal	21	FMT	02-Jun-15	10				C
F3W17	FMT3	NA	longitudinal	22	FMT	28-Jul-15	17				C
Control3	Donor3	Control	cross-sectional	23	Donor	20-Mar-15		73	M	Father	C
F4W0	FMT4	CDI	longitudinal	26	FMT	03-Jun-15	0	76	F		D
F4W2	FMT4	NA	longitudinal	27	FMT	20-Jun-15	2				D
F4W4	FMT4	NA	longitudinal	28	FMT	07-Jul-15	4				D
F4W5	FMT4	NA	longitudinal	29	FMT	13-Jul-15	5				D
F4W18	FMT4	NA	longitudinal	31	FMT	16-Oct-15	18				D
Control4	Donor4	Control	cross-sectional	32	Donor	01-Jun-15		53	F	Daughter	D
F5W0	FMT5	CDI	longitudinal	38	FMT	30-Jul-15	0	63	M		E
F5W2	FMT5	NA	longitudinal	39	FMT	18-Aug-15	2				E
F5W10	FMT5	NA	longitudinal	40	FMT	19-Oct-15	10				E
F5W18	FMT5	NA	longitudinal	41	FMT	14-Dec-15	18				E
Control5	Donor5	Control	cross-sectional	42	Donor	31-Jul-15		36	F		E
F6W0	FMT6	CDI	longitudinal	43	FMT	21-Aug-15	0	88	M		F
F6W2	FMT6	NA	longitudinal	44	FMT	17-Sep-15	2				F
F6W11	FMT6	NA	longitudinal	46	FMT	20-Nov-15	11				F
Control6	Donor6	Control	cross-sectional	47	Donor	24-Aug-15		41	M	Son	F
F7W0	FMT7	CDI	longitudinal	89	FMT	01-Feb-16	0	45	M		G
F7W4	FMT7	NA	longitudinal	90	FMT	07-Mar-16	4				G
F7W12	FMT7	NA	longitudinal	91	FMT	09-May-16	12				G
Control7	Donor7	Control	cross-sectional	78	Donor	08-Jan-16		35	M	No relationship	G
F8W0	FMT8	CDI	longitudinal	83	FMT	21-Jan-16	0	90	M		H

sample_name	FMT/STD number	baseline_comparison	Sample_collection	sample_number#	Randomization arm	Collect Date	time_point_post_treatment (FMT/STD, week)	Age	Sex	donor relationship to patient	House hold ID (family ID)
F8W20	FMT8	NA	longitudinal	106	FMT	20-Jun-16	20				H
Control8	Donor8	Control	cross-sectional	79	Donor	22-Jan-16		36	F	grand daughter	H
F9W0	FMT9	CDI	longitudinal	127	FMT	15-Sep-16	0	52	F		I
F9W2	FMT9	NA	longitudinal	128	FMT	30-Sep-16	2				I
F9W4	FMT9	NA	longitudinal	129	FMT	14-Oct-16	4				I
Control9	Donor9	Control	cross-sectional	130	Donor	09-Sep-16		28	F	No relationship	I
F10W0	FMT10	CDI	longitudinal	50	FMT	26-Aug-15	0	45	M		J
F10W2	FMT10	NA	longitudinal	51	FMT	22-Sep-15	2				J
F10W6	FMT10	NA	longitudinal	52	FMT	22-Oct-15	6				J
F10W10	FMT10	NA	longitudinal	53	FMT	18-Nov-15	10				J
Control10	Donor10	Control	cross-sectional	54	Donor	02-Sep-15		21	M	Son	J
F11W0	FMT11	CDI	longitudinal	62	FMT	30-Sep-15	0	83	F		K
F11W2	FMT11	NA	longitudinal	63	FMT	18-Oct-15	2				K
F11W4	FMT11	NA	longitudinal	64	FMT	04-Nov-15	4				K
Control11	Donor11	Control	cross-sectional	65	Donor	25-Sep-15		57	M	Son	K
F12W0	FMT12	CDI	longitudinal	66	FMT	24-Sep-15	0	38	F		L
F12W4	FMT12	NA	longitudinal	68	FMT	05-Nov-15	4				L
F12W10	FMT12	NA	longitudinal	69	FMT	28-Dec-15	10				L
F13W0	FMT13	CDI	longitudinal	82	FMT	15-Jan-16	0	81	M		M
F13W2	FMT13	NA	longitudinal	102	FMT	16-Feb-16	2				M
F13W13	FMT13	NA	longitudinal	103	FMT	28-Apr-16	13				M
Control13	Donor13	NA	longitudinal	80	Donor	27-Jan-16		43	F	Daughter	M
F14W0	FMT14	CDI	longitudinal	113	FMT	21-Mar-16	0	65	M		N
F14W4	FMT14	NA	longitudinal	115	FMT	29-Apr-16	4				N
Control14	Donor14	Control	cross-sectional	119	FMT	22-Mar-16		33	M	Son	N
F15W0	FMT15	CDI	longitudinal	131	FMT	14-Sep-16	0	90	F		O
F15W2	FMT15	NA	longitudinal	132	FMT	05-Oct-16	2				O
F15W4	FMT15	NA	longitudinal	133	FMT	18-Oct-16	4				O
Control15	Donor15	Control	cross-sectional	134	Donor	13-Sep-16		52	M	Son	O
F16W0	FMT16	CDI	longitudinal	7	FMT	26-Nov-15	0	83	M		P
F16W4	FMT16	NA	longitudinal	86	FMT	08-Jan-16	4				P
Control16	Donor16	Control	cross-sectional	8	Donor	11-Dec-15		27	F	Maid	P
S1W0	STD1	CDI	longitudinal	33	Std therapy	14-Jul-15	0	78	F		

sample_name	FMT/STD number	baseline_comparison	Sample_collection	sample_number#	Randomization arm	Collect Date	time_point_post_treatment (FMT/STD, week)	Age	Sex	donor_relation-to patient	House hold ID (family ID)
S1W2	STD1	NA	longitudinal	34	Std therapy	24-Jul-15	2				
S1W4	STD1	NA	longitudinal	35	Std therapy	10-Aug-15	4				
S2W0	STD2	CDI	longitudinal	58	Std therapy	24-Sep-15	0	83	F		
S2W2	STD2	NA	longitudinal	59	Std therapy	05-Oct-15	2				
S2W4	STD2	NA	longitudinal	60	Std therapy	19-Oct-15	4				
S2W10	STD2	NA	longitudinal	61	Std therapy	30-Nov-15	10				
S3W0	STD3	CDI	longitudinal	71	Std therapy	20-Oct-15	0	99	F		
S3W2	STD3	NA	longitudinal	72	Std therapy	02-Nov-15	2				
S3W4	STD3	NA	longitudinal	73	Std therapy	16-Nov-15	4				
S4W0	STD4	CDI	longitudinal	81	Std therapy	07-Jan-16	0	85	F		
S4W2	STD4	NA	longitudinal	98	Std therapy	25-Jan-16	2				
S4W10	STD4	NA	longitudinal	100	Std therapy	21-Mar-16	10				
S5W0	STD5	CDI	longitudinal	107	Std therapy	16-Mar-16	0	92	F		
S5W2	STD5	NA	longitudinal	108	Std therapy	31-Mar-16	2				
S5W4	STD5	NA	longitudinal	109	Std therapy	19-Apr-16	4				
S5W10	STD5	NA	longitudinal	110	Std therapy	03-Jun-16	10				
S5W13	STD5	NA	longitudinal	111	Std therapy	17-Jun-16	13				
S5W23	STD5	NA	longitudinal	112	Std therapy	26-Aug-16	23				
S6W0	STD6	CDI	longitudinal	14	Std therapy	06-Mar-15	0	88	M		
S6W2	STD6	NA	longitudinal	15	Std therapy	18-Mar-15	2				
S6W10	STD6	NA	longitudinal	17	Std therapy	05-May-15	10				
S7W0	STD7	CDI	longitudinal	24	Std therapy	07-May-15	0	93	M		
S7W2	STD7	NA	longitudinal	25	Std therapy	22-May-15	2				
S8W0	STD8	CDI	longitudinal	120	Std therapy	19-Jul-16	0	63	M		
S8W4	STD8	NA	longitudinal	121	Std therapy	19-Aug-16	4				
CDI25	NA	CDI	cross-sectional	1	NA		0	86	F		Q
Control12	NA	NA	cross-sectional	2	NA	04-Mar-15					Q
CDI26	NA	CDI	cross-sectional	88	NA	30-Dec-15	0	88	M		
Control17	NA	Control	cross-sectional	37	NA	21-Jul-15		55	F		

sample_name	FMT/STD number	baseline_comparison	Sample_collection	sample_number#	Randomization arm	Collect Date	time_point_post_treatment (FMT/STD, week)	Age	Sex	donor relationship to patient	House hold ID (family ID)
CDI27	NA	CDI	cross-sectional	48	NA	31-Aug-15	0	66	F		R
Control18	NA	Control	cross-sectional	49	NA	26-Aug-15		41	M		R
CDI28	NA	CDI	cross-sectional	55	NA	07-Sep-15	0	84	M		S
Control19	NA	Control	cross-sectional	57	NA	08-Sep-15		42	M	Son	S
CDI29	NA	CDI	cross-sectional	70	NA	08-Oct-15	0	76	M		
CDI30	NA	CDI	cross-sectional	74	NA	11-Dec-15	0	25	F		T
Control20	NA	Control	cross-sectional	75	NA	24-Dec-15		33	M	Brother	T
DI31	NA	CDI	cross-sectional	122	NA	28-Jul-16		80	F		
CDI32	NA	CDI	cross-sectional	124	NA	29-Aug-16	0	52	M		
Control21	NA	Control	cross-sectional	ANS2357	NA						
Control22	NA	Control	cross-sectional	ANS2331	NA						
Control23	NA	Control	cross-sectional	ANS2237	NA						
Control24	NA	Control	cross-sectional	ANS2467	NA						

Table 4

sample_name	sample_num-Aer	Sequencing_platform	strategy	length	Nreads	raw reads	clean_reads	clean_data_raw data	Duplication	Clean.data. Mbp.
F1W0	A3	Illumina Miseq	PE300	294	0.16	179802	81730	45.46	0	24.27
F1W2	A4	Illumina Miseq	PE300	300	0	616376	139656	22.66	0	41.9
F1W6	A5	Illumina Miseq	PE300	300	0.228	1031864	316410	30.66	0	94.92
Control1	A6	Illumina Miseq	PE300	300	0.139	433810	181802	41.91	0	54.54
F2W0	A9	Illumina Miseq	PE300	299	0.14	202246	158372	78.31	0	47.43
F2W2	A10	Illumina Miseq	PE300	300	0.13	238288	185840	77.99	0	55.75
F2W4	A11	Illumina Miseq	PE300	298	0.14	274630	230666	83.99	0	68.97
Control2	A13	Illumina Miseq	PE300	300	0.165	339154	46712	13.77	0	14.01
F3W0	A18	Illumina Miseq	PE300	300	0	231822	93316	40.25	0	27.99
F3W2	A19	Illumina Miseq	PE300	300	0.105	618984	262320	42.38	0	78.7
F3W4	A20	Illumina Miseq	PE300	300	0.13	801066	294282	36.74	0	88.28
F3W10	A21	Illumina Miseq	PE300	300	0.131	762700	277712	36.41	0	83.31
F3W17	A22	Illumina Miseq	PE300	300	0.139	696480	71662	10.29	0	21.5
Control3	A23	Illumina Miseq	PE300	300	0	207068	84600	40.86	0	25.38
F4W0	A26	Illumina Miseq	PE300	300	0	209102	67856	32.45	0	20.36
F4W2	A27	Illumina Miseq	PE300	300	0	216998	88906	40.97	0	26.67
F4W4	A28	Illumina Miseq	PE300	300	0.112	240322	93194	38.78	0	27.96
F4W5	A29	Illumina Miseq	PE300	300	0.001	132580	50098	37.79	0	15.03
F4W18	A31	Illumina Miseq	PE300	300	0	173430	49710	28.66	0	14.91
Control4	A32	Illumina Miseq	PE300	300	0.001	1708684	752020	44.01	0	225.61

sample_name	sample_num-Aer	Sequencing_platform	strategy	length	Nreads	raw_reads	clean_reads	clean_data.raw_data	Duplication	Clean_data.Mbp.
F5W0	A38	Illumina_Miseq	PE300	293	0.14	248084	109264	44.04	0	32.34
F5W2	A39	Illumina_Miseq	PE300	297	0.14	181924	81388	44.74	0	24.25
F5W10	A40	Illumina_Miseq	PE300	300	0.075	140624	54390	38.68	0	16.32
F5W18	A41	Illumina_Miseq	PE300	296	0.13	192510	94050	48.85	0	27.89
Control5	A42	Illumina_Miseq	PE300	300	0.208	215010	91448	42.53	0	27.43
F6W0	A43	Illumina_Miseq	PE300	298	0.15	282544	219516	77.69	0	65.64
F6W2	A44	Illumina_Miseq	PE300	300	0.035	298672	118338	39.62	0	35.5
F6W11	A46	Illumina_Miseq	PE300	300	0.045	275570	122034	44.28	0	36.61
Control6	A47	Illumina_Miseq	PE300	297	0.12	344408	244256	70.92	0	72.54
F7W0	A89	Illumina_Miseq	PE300	300	0.083	257738	78176	30.33	0	23.45
F7W4	A90	Illumina_Miseq	PE300	293	0.18	202056	105384	52.16	0	31.25
F7W12	A91	Illumina_Miseq	PE300	300	0.068	211482	17624	8.33	0	5.29
Control7	A78	Illumina_Miseq	PE300	300	0.074	321992	36754	11.41	0	11.03
F8W0	A83	Illumina_Miseq	PE300	300	0	400454	105068	26.24	0	31.52
F8W20	A106	Illumina_Miseq	PE300	300	0.018	329840	55682	16.88	0	16.7
Control8	A79	Illumina_Miseq	PE300	300	0.037	271614	136442	50.23	0	40.93
F9W0	A127	Illumina_Miseq	PE300	297	0.15	222020	181952	81.95	0	54.22
F9W2	A128	Illumina_Miseq	PE300	296	0.15	303888	236462	77.81	0	69.64
F9W4	A129	Illumina_Miseq	PE300	294	0.14	378688	327536	86.49	0	96.13
Control9	A130	Illumina_Miseq	PE300	300	0.055	308178	107542	34.9	0	32.26
F10W0	A50	Illumina_Miseq	PE300	299	0.15	365022	166948	45.74	0	49.5
F10W2	A51	Illumina_Miseq	PE300	298	0.13	342394	166724	48.69	0	49.6
F10W6	A52	Illumina_Miseq	PE300	294	0.14	444320	194454	43.76	0	57.07
F10W10	A53	Illumina_Miseq	PE300	300	0.019	652970	251992	38.59	0	75.6
Control10	A54	Illumina_Miseq	PE300	300	0.017	888528	237676	26.75	0	71.3
F11W0	A62	Illumina_Miseq	PE300	293	0.15	220522	108276	49.1	0	31.89
F11W2	A63	Illumina_Miseq	PE300	300	0.117	347680	112438	32.34	0	33.73
F11W4	A64	Illumina_Miseq	PE300	300	0.14	265458	123800	46.64	0	36.89
Control11	A65	Illumina_Miseq	PE300	300	0.076	364878	147312	40.37	0	44.19
F12W0	A66	Illumina_Miseq	PE300	299	0.13	385064	168008	43.63	0	49.98
F12W4	A68	Illumina_Miseq	PE300	293	0.13	239996	94998	39.58	0	28.12
F12W10	A69	Illumina_Miseq	PE300	294	0.13	367404	180104	49.02	0	52.86
F13W0	A82	Illumina_Miseq	PE300	300	0.015	505218	166652	32.99	0	50
F13W2	A102	Illumina_Miseq	PE300	300	0.073	233864	84538	36.15	0	25.36
F13W13	A103	Illumina_Miseq	PE300	297	0.14	196280	83390	42.49	0	24.64
Control13	A80	Illumina_Miseq	PE300	300	0.058	306382	124604	40.67	0	37.38
F14W0	A113	Illumina_Miseq	PE300	298	0.15	183402	137498	74.97	0	40.84
F14W4	A115	Illumina_Miseq	PE300	297	0.12	298982	138884	46.45	0	41.11
Control14	A119	Illumina_Miseq	PE300	300	0.13	205292	159234	77.56	0	47.37
F15W0	A131	Illumina_Miseq	PE300	300	0.14	629728	298766	47.44	0	88.58
F15W2	A132	Illumina_Miseq	PE300	299	0.15	357030	171648	48.08	0	50.81

sample_name	sample_num-Aer	Sequencing_platform	strategy	length	Nreads	raw reads	clean_reads	clean_data_raw_data	Duplica-tion	Clean.data.Mbp.
F15W4	A133	Illumina_Miseq	PE300	300	0.15	309054	151058	48.88	0	45.09
Control15	A134	Illumina_Miseq	PE300	300	0	471798	185850	39.39	0	55.76
F16W0	A7	Illumina_Miseq	PE300	300	0.185	346110	73132	21.13	0	21.94
F16W4	A86	Illumina_Miseq	PE300	299	0.12	327154	132150	40.39	0	39.58
Control16	A8	Illumina_Miseq	PE300	300	0.147	292252	39628	13.56	0	11.89
S1W0	A33	Illumina_Miseq	PE300	300	0.001	770414	328156	42.59	0	98.45
S1W2	A34	Illumina_Miseq	PE300	299	0.14	175118	75890	43.34	0	22.65
S1W4	A35	Illumina_Miseq	PE300	298	0.15	234894	110164	46.9	0	32.83
S2W0	A58	Illumina_Miseq	PE300	297	0.14	335058	249376	74.43	0	73.94
S2W2	A59	Illumina_Miseq	PE300	294	0.14	405606	276022	68.05	0	81.98
S2W4	A60	Illumina_Miseq	PE300	296	0.12	176070	132478	75.24	0	39.21
S2W10	A61	Illumina_Miseq	PE300	300	0.068	346262	38884	11.23	0	11.67
S3W0	A71	Illumina_Miseq	PE300	300	0.108	199368	44866	22.5	0	13.46
S3W2	A72	Illumina_Miseq	PE300	296	0.15	269004	119998	44.61	0	35.52
S3W4	A73	Illumina_Miseq	PE300	293	0.07	291644	58196	19.95	0	17.05
S4W0	A81	Illumina_Miseq	PE300	300	0.015	712372	170362	23.91	0	51.11
S4W2	A98	Illumina_Miseq	PE300	299	0.15	410054	186270	45.43	0	55.32
S4W10	A100	Illumina_Miseq	PE300	300	0.066	223792	94782	42.35	0	28.43
S5W0	A107	Illumina_Miseq	PE300	300	0	431392	221564	51.36	0	66.47
S5W2	A108	Illumina_Miseq	PE300	296	0.13	209076	72748	34.8	0	21.53
S5W4	A109	Illumina_Miseq	PE300	294	0.14	245218	101786	41.51	0	30.03
S5W10	A110	Illumina_Miseq	PE300	293	0.14	267352	112302	42.01	0	33.07
S5W13	A111	Illumina_Miseq	PE300	293	0.14	309046	123178	39.86	0	36.21
S5W23	A112	Illumina_Miseq	PE300	300	0.086	407234	89072	21.87	0	26.72
S6W0	A14	Illumina_Miseq	PE300	296	0.13	300734	121426	40.38	0	36.12
S6W2	A15	Illumina_Miseq	PE300	294	0.14	381812	175862	46.06	0	52.14
S6W10	A17	Illumina_Miseq	PE300	293	0.09	373944	109182	29.2	0	32.26
S7W0	A24	Illumina_Miseq	PE300	300	0	385622	128024	33.2	0	38.41
S7W2	A25	Illumina_Miseq	PE300	297	0.12	280482	115920	41.33	0	34.54
S8W0	A120	Illumina_Miseq	PE300	298	0.13	211678	100168	47.32	0	29.95
S8W4	A121	Illumina_Miseq	PE300	299	0.14	219782	94604	43.04	0	28.33
CDI25	A1	Illumina_Miseq	PE300	297	0.13	266926	117804	44.13	0	35.16
Control12	A2	Illumina_Miseq	PE300	296	0.13	380378	163882	43.08	0	48.84
CDI26	A88	Illumina_Miseq	PE300	300	0.029	539830	199604	36.98	0	59.88
Control17	A37	Illumina_Miseq	PE300	300	0.256	343310	112180	32.68	0	33.65
CDI27	A48	Illumina_Miseq	PE300	300	0.12	386578	128450	33.23	0	38.53
Control18	A49	Illumina_Miseq	PE300	297	0.13	353998	247752	69.99	0	73.09
CDI28	A55	Illumina_Miseq	PE300	296	0.14	288796	124028	42.95	0	36.96
Control19	A57	Illumina_Miseq	PE300	300	0.071	866640	91022	10.5	0	27.31
CDI29	A70	Illumina_Miseq	PE300	298	0.12	331604	159998	48.25	0	47.52
CDI30	A74	Illumina_Miseq	PE300	294	0.15	222838	163664	73.45	0	48.2

sample_name	sample_num-Aer	Sequencing_platform	strategy	length	Nreads	raw_reads	clean_reads	clean_data_raw_data	Duplication	Clean_data.Mbp.
CDI31	A122	Illumina_Miseq	PE300	300	0.071	218720	46710	21.36	0	14.01
CDI32	A124	Illumina_Miseq	PE300	298	0.15	541002	285192	52.72	0	84.42
Control20	A136	Illumina_Miseq	PE300	300	0.056	425346	154348	36.29	0	46.3
Control21	A137	Illumina_Miseq	PE300	300	0.125	817664	347726	42.53	0	104.32
Control22	A138	Illumina_Miseq	PE300	300	0.147	1584946	635954	40.12	0	190.79
Control23	A139	Illumina_Miseq	PE300	300	0.155	540728	250244	46.28	0	75.07

Table 5

sample_name	sample_NUMBER	Sequencing_platform	Sequencing_Strategy.PE.SE.	Raw-Reads	Read_length.bp.	Clean_Data.Raw_Data...	Clean_Reads	Read_GC...	Adapter_Rate...	Duplication_Rate...	N_rate...
F1W0	B3	Illumina_Miseq	PE250	139020	250	36.41	50614	54.77	0	-	0
F1W2	B4	Illumina_Miseq	PE250	453216	250	49.63	224930	55.19	0	-	0
F1W6	B5	Illumina_Miseq	PE250	210766	250	54.7	115298	54.43	0	-	0
Control1	B6	Illumina_Miseq	PE250	197162	250	56.09	110582	53.81	0	-	0
F2W0	B9	Illumina_Miseq	PE250	145174	250	36.12	52432	56.19	0	-	0
F2W2	B10	Illumina_Miseq	PE250	165058	250	39.87	65806	53.31	0	-	0
F2W4	B11	Illumina_Miseq	PE250	157286	250	51.06	80306	55.57	0	-	0
Control2	B13	Illumina_Miseq	PE250	178348	250	53.95	96226	53.59	0	-	0
F3W0	B18	Illumina_Miseq	PE250	392454	250	37.82	148440	51.66	0	-	0
F3W2	B19	Illumina_Miseq	PE250	230982	250	44.39	102532	54.01	0	-	0
F3W4	B20	Illumina_Miseq	PE250	112378	250	47.17	53006	53.36	0	-	0
F3W10	B21	Illumina_Miseq	PE250	230772	250	44.67	103090	52.81	0	-	0
F3W17	B22	Illumina_Miseq	PE250	311224	250	44.88	139674	54.17	0	-	0
Control3	B23	Illumina_Miseq	PE250	523854	250	48.24	252724	53.82	0	-	0
F4W0	B26	Illumina_Miseq	PE250	174036	250	41.06	71452	54.33	0	-	0
F4W2	B27	Illumina_Miseq	PE250	220990	250	43.23	95524	55.18	0	-	0
F4W4	B28	Illumina_Miseq	PE250	241624	250	36.16	87376	55.01	0	-	0
F4W5	B29	Illumina_Miseq	PE250	180292	250	47.99	86526	58.15	0	-	0
F4W18	B31	Illumina_Miseq	PE250	201408	250	43.77	88154	54.42	0	-	0
Control4	B32	Illumina_Miseq	PE250	421468	250	49.82	209980	53.83	0	-	0
F5W0	B38	Illumina_Miseq	PE250	141266	250	37.14	52462	53.82	0	-	0
F5W2	B39	Illumina_Miseq	PE250	144132	250	32.13	46310	53.24	0	-	0
F5W10	B40	Illumina_Miseq	PE250	218376	250	38.59	84272	54.1	0	-	0
F5W18	B41	Illumina_Miseq	PE250	238492	250	36.26	86488	53.76	0	-	0
Control5	B42	Illumina_Miseq	PE250	350844	250	46.31	162472	54.36	0	-	0
F6W0	B43	Illumina_Miseq	PE250	266526	250	37.13	98972	54.74	0	-	0

sample_name	sample_NUMBER	Sequencing_platform	Sequencing_Strategy_PE.SE.	Raw_Reads	Read_length_bp.	Clean_Data_Raw_Data...	Clean_Reads	Read_GC...	Adapter_Rate...	Duplication_Rate...	N_rate...
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F6W11	B46	Illumina_Miseq	PE250	233562	250	45.15	105462	54.06	0	-	0
Control6	B47	Illumina_Miseq	PE250	305446	250	55.31	168944	54.78	0	-	0
F7W0	B89	Illumina_Miseq	PE250	237086	250	79.42	188300	54.91	0	-	0
F7W4	B90	Illumina_Miseq	PE250	235976	250	83.49	197021	53.01	0	-	0
F7W12	B91	Illumina_Miseq	PE250	250973	250	79.33	199092	53.2	0	-	0
Control7	B78	Illumina_Miseq	PE250	203468	250	50.32	102382	54.61	0	-	0
F8W0	B83	Illumina_Miseq	PE250	131878	250	26.67	35168	54.94	0	-	0
F8W20	B106	Illumina_Miseq	PE250	167805	250	83.11	139457	53.09	0	-	0
Control8	B79	Illumina_Miseq	PE250	242486	250	58.72	142396	55.31	0	-	0
F9W0	B127	Illumina_Miseq	PE250	196849	250	80.26	157992	52.39	0	-	0
F9W2	B128	Illumina_Miseq	PE250	204701	250	77.69	159032	51.85	0	-	0
F9W4	B129	Illumina_Miseq	PE250	174369	250	79.82	139188	53.08	0	-	0
Control9	B130	Illumina_Miseq	PE250	158498	250	81.99	129950	52.62	0	-	0
F10W0	B50	Illumina_Miseq	PE250	283910	250	33.77	95884	55.08	0	-	0
F10W2	B51	Illumina_Miseq	PE250	200238	250	30.26	60588	55.17	0	-	0
F10W6	B52	Illumina_Miseq	PE250	170482	250	38.49	65614	54.36	0	-	0
F10W10	B53	Illumina_Miseq	PE250	178994	250	36.65	65606	54.59	0	-	0
Control10	B54	Illumina_Miseq	PE250	824186	250	40.69	335336	53.36	0	-	0
F11W0	B62	Illumina_Miseq	PE250	215272	250	42.74	92012	53.28	0	-	0
F11W2	B63	Illumina_Miseq	PE250	195352	250	44.86	87634	53.31	0	-	0
F11W4	B64	Illumina_Miseq	PE250	193674	250	29.48	57096	55.18	0	-	0
Control11	B65	Illumina_Miseq	PE250	183434	250	47.41	86968	55.82	0	-	0
F12W0	B66	Illumina_Miseq	PE250	130132	250	28.16	36646	51.56	0	-	0
F12W4	B68	Illumina_Miseq	PE250	442748	250	38.11	168732	52.41	0	-	0
F12W10	B69	Illumina_Miseq	PE250	376794	250	44.61	168084	54.27	0	-	0
F13W0	B82	Illumina_Miseq	PE250	295522	250	35.97	106314	54.32	0	-	0
F13W2	B102	Illumina_Miseq	PE250	163065	250	82.16	133971	54.28	0	-	0
F13W13	B103	Illumina_Miseq	PE250	207191	250	80.73	167256	53.56	0	-	0
Control13	B80	Illumina_Miseq	PE250	462154	250	48.56	224408	53.21	0	-	0
F14W0	B113	Illumina_Miseq	PE250	223935	250	81.37	182212	52.37	0	-	0
F14W4	B115	Illumina_Miseq	PE250	259086	250	76.58	198409	53.36	0	-	0
Control14	B119	Illumina_Miseq	PE250	247736	250	78.41	194254	51.97	0	-	0
F15W0	B131	Illumina_Miseq	PE250	154491	250	79.7	123134	56.75	0	-	0
F15W2	B132	Illumina_Miseq	PE250	148992	250	77.43	115366	51.66	0	-	0

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		Miseq									
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Control15	B134	Illumina_Miseq	PE250	253469	250	74.17	187995	52.93	0	-	0
F16W0	B7	Illumina_Miseq	PE250	115544	250	47.42	54786	55.67	0	-	0
F16W4	B86	Illumina_Miseq	PE250	281377	250	73.93	208021	51.76	0	-	0
Control16	B8	Illumina_Miseq	PE250	192726	250	49.58	95562	55.47	0	-	0
S1W0	B33	Illumina_Miseq	PE250	127724	250	48.49	61934	53.3	0	-	0
S1W2	B34	Illumina_Miseq	PE250	156738	250	40.52	63506	53.78	0	-	0
S1W4	B35	Illumina_Miseq	PE250	173534	250	38.11	66142	54.08	0	-	0
S2W0	B58	Illumina_Miseq	PE250	223636	250	31.94	71434	55.8	0	-	0
S2W2	B59	Illumina_Miseq	PE250	185150	250	32.01	59258	55.44	0	-	0
S2W4	B60	Illumina_Miseq	PE250	159388	250	28.23	44998	55.05	0	-	0
S2W10	B61	Illumina_Miseq	PE250	344816	250	39.19	135144	52.08	0	-	0
S3W0	B71	Illumina_Miseq	PE250	241924	250	32.86	79506	55.38	0	-	0
S3W2	B72	Illumina_Miseq	PE250	273290	250	38.59	105472	55.84	0	-	0
S3W4	B73	Illumina_Miseq	PE250	332982	250	37.47	124756	53.04	0	-	0
S4W0	B81	Illumina_Miseq	PE250	192778	250	45.56	87836	53.87	0	-	0
S4W2	B98	Illumina_Miseq	PE250	198910	250	81.35	161811	53.93	0	-	0
S4W10	B100	Illumina_Miseq	PE250	157771	250	76.87	121286	51.88	0	-	0
S5W0	B107	Illumina_Miseq	PE250	239494	250	84.11	201431	55.84	0	-	0
S5W2	B108	Illumina_Miseq	PE250	230334	250	82.31	189592	55.29	0	-	0
S5W4	B109	Illumina_Miseq	PE250	264477	250	78.33	207172	52.18	0	-	0
S5W10	B110	Illumina_Miseq	PE250	283660	250	81.24	230451	52.84	0	-	0
S5W13	B111	Illumina_Miseq	PE250	281066	250	78.64	221027	55.94	0	-	0
S5W23	B112	Illumina_Miseq	PE250	269234	250	76.53	206040	52.41	0	-	0
S6W0	B14	Illumina_Miseq	PE250	144110	250	50.6	72918	54.66	0	-	0
S6W2	B15	Illumina_Miseq	PE250	122768	250	35.01	42986	55.06	0	-	0
S6W10	B17	Illumina_Miseq	PE250	177230	250	52.6	93230	53.52	0	-	0
S7W0	B24	Illumina_Miseq	PE250	141758	250	41.43	58734	54.25	0	-	0
S7W2	B25	Illumina_Miseq	PE250	135760	250	33.35	45270	55.72	0	-	0
S8W0	B120	Illumina_Miseq	PE250	225001	250	77.52	174431	52.82	0	-	0
S8W4	B121	Illumina_Miseq	PE250	231234	250	78.69	181960	52.87	0	-	0
CDI25	B1	Illumina_Miseq	PE250	93298	250	29.23	27272	54.54	0	-	0
Control12	B2	Illumina_Miseq	PE250	127534	250	52.3	66694	55.41	0	-	0
CDI26	B88	Illumina_Miseq	PE250	205071	250	61.56	126248	53.34	0	-	0

sample_name	sample_NUMBER	Sequencing_platform	Sequencing_Strategy. PE,SE.	Raw-Reads	Read_length. bp.	Clean_Data. Raw Data...	Clean_Reads	Read_GC...	Adapter_Rate...	Duplication_Rate...	N_rate...
Control17	B37	Illumina_Miseq	PE250	253674	250	46.68	118412	51.69	0	-	0
CDI27	B48	Illumina_Miseq	PE250	168938	250	32.75	55330	54.64	0	-	0
Control18	B49	Illumina_Miseq	PE250	356614	250	48.47	172844	54.23	0	-	0
CDI28	B55	Illumina_Miseq	PE250	144894	250	27.61	39998	54.42	0	-	0
Control19	B57	Illumina_Miseq	PE250	474850	250	50.34	239062	55.47	0	-	0
CDI29	B70	Illumina_Miseq	PE250	225218	250	28.02	63114	55.92	0	-	0
CDI30	B74	Illumina_Miseq	PE250	232140	250	36.89	85644	56.9	0	-	0
Control20	B75	Illumina_Miseq	PE250	279832	250	57.13	159864	54.04	0	-	0
DI31	B122	Illumina_Miseq	PE250	241976	250	82.29	199112	52.56	0	-	0
CDI32	B124	Illumina_Miseq	PE250	166333	250	81.76	135989	54.71	0	-	0

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WHAT IS CLAIMED IS:

1. A method for assessing likelihood of effective fecal microbiota transplantation (FMT), comprising determining *C. albicans* level in a stool sample obtained from a potential recipient prior to FMT.
2. The method of claim 1, wherein the *C. albicans* level is a percentage relative abundance.
3. The method of claim 2, wherein the *C. albicans* level is greater than 10% and FMT is assessed as unlikely to be effective for the potential recipient.
4. The method of claim 3, wherein the recipient is administered an effective amount of an antifungal agent that suppresses *C. albicans* growth before FMT.
5. The method of claim 4, further comprising determining *C. albicans* level in a stool sample obtained from the recipient after FMT.
6. The method of claim 2, wherein the *C. albicans* level is no greater than 10% and FMT is assessed as likely to be effective for the potential recipient.
7. The method of claim 1, further comprising determining total fungal load in the stool sample.
8. The method of claim 7, further comprising performing FMT on the potential recipient.
9. The method of claim 1, wherein *C. albicans* level is determined in a first stool sample obtained from a first potential recipient prior to FMT and in a second stool sample obtained from a second potential recipient prior to FMT.
10. The method of claim 9, wherein the first potential recipient has a lower *C. albicans* level than the second potential recipient and is assessed to have a higher likelihood of effective FMT than the potential recipient.
11. The method of claim 9, wherein the second potential recipient is administered an effective amount of an antifungal agent that suppresses *C. albicans* growth before FMT.

- 12.** The method of claim **1**, wherein the potential recipient has inflammatory bowel disease (IBD) with concurrent *Clostridium difficile* infection (CDI).
- 13.** The method of claim **12**, wherein the *C. albicans* level is determined in stool samples taken from the recipient before and after FMT.
- 14.** A method for identifying a suitable donor for FMT, comprising the step of determining *C. albicans* level in a stool sample obtained from a candidate.
- 15.** The method of claim **14**, wherein the *C. albicans* level is a percentage relative abundance.
- 16.** The method of claim **15**, wherein the *C. albicans* level is no greater than 0.1% and the candidate is identified as a suitable donor for FMT.
- 17.** The method of claim **15**, wherein the *C. albicans* level is greater than 10% and the candidate is identified as an unsuitable donor for FMT.
- 18.** The method of claim **14**, further comprising determining *Saccharomyces* level and *Aspergillus* level in the stool sample.
- 19.** The method of claim **14**, further comprising determining *Escherichia* level and *Proteus* level in the stool sample.
- 20.** The method of claim **14**, further comprising determining total fungal load in the stool sample.
- 21.** A method for improving FMT efficacy, comprising administering to an FMT recipient prior to FMT an effective amount of an antifungal agent that suppresses *C. albicans* growth.
- 22.** The method of claim **21**, wherein *C. albicans* level is determined in a stool sample from the FMT recipient prior to administration of the antifungal agent.
- 23.** The method of claim **21**, wherein *C. albicans* level is determined in a stool sample from the FMT recipient after administration of the antifungal agent.

24. The method of claim **21**, further comprising administering to the recipient prior to FMT an effective amount of an agent that reduces total fungal load in a stool sample taken from the recipient prior to FMT.

25. The method of claim **21**, wherein the recipient has inflammatory bowel disease (IBD) with concurrent *Clostridium difficile* infection (CDI).

26. A kit comprising (1) a first composition comprising donor stool; and (2) a second composition comprising an effective amount of an antifungal agent that suppresses *C. albicans* growth.

27. The kit of claim **22**, wherein the first composition comprises donor stool that has been dried, frozen, and placed in a capsule for oral ingestion.

28. The kit of claim **22**, further comprising in the second composition or in a third composition an effective amount of an agent that reduces total fungal load.

29. The method of claim **1, 9, 12, 20, or 21**, wherein *C. albicans* level is determined by quantitative polymerase chain reaction (PCR).

Fig. 1a

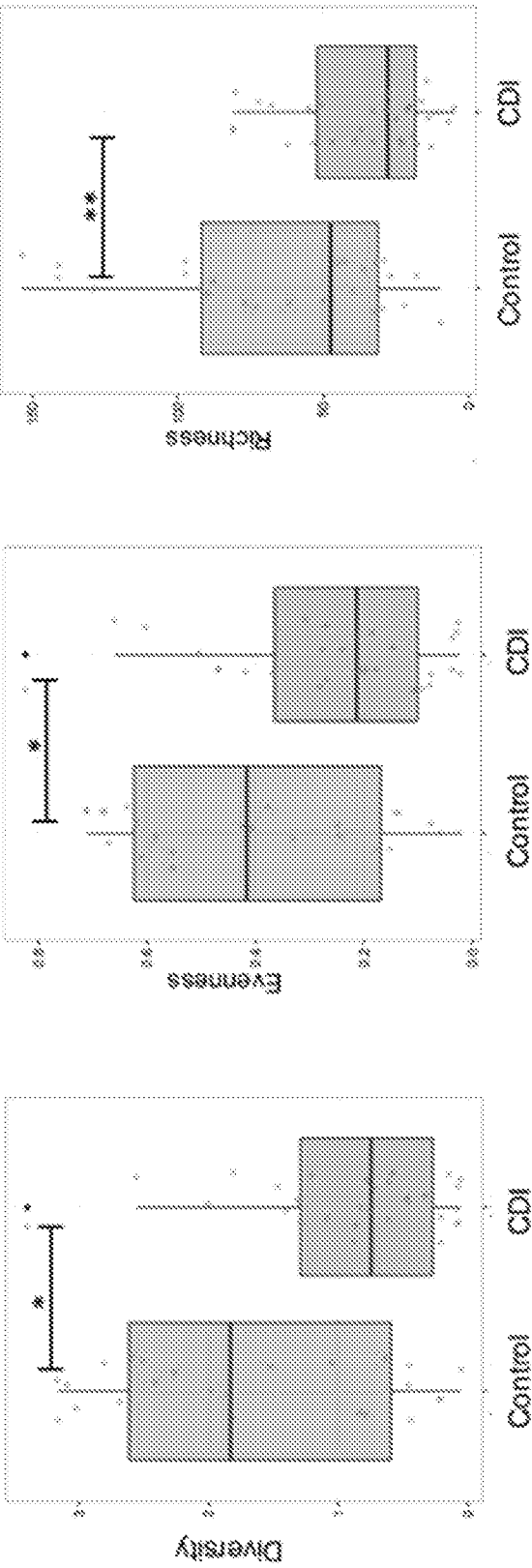


Fig. 1b

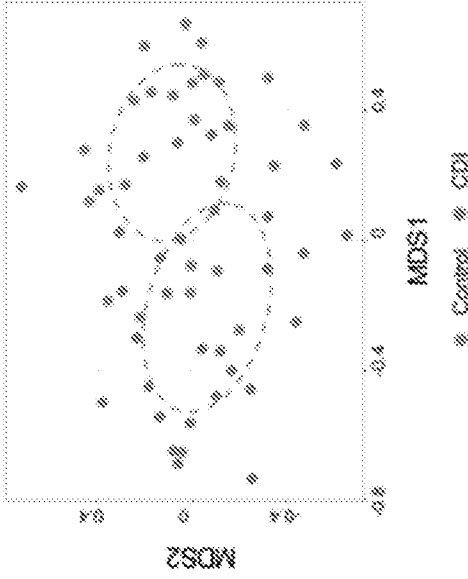


Fig. 1c

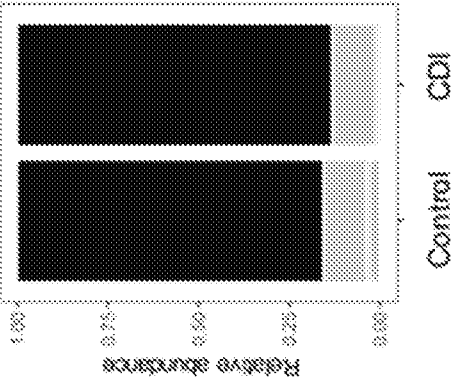


Fig. 1e

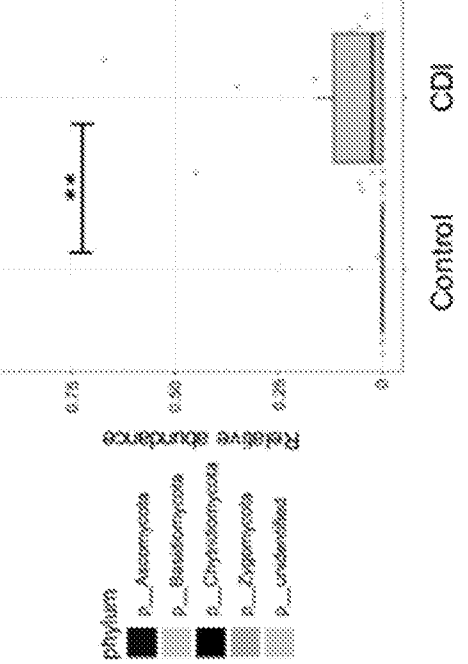
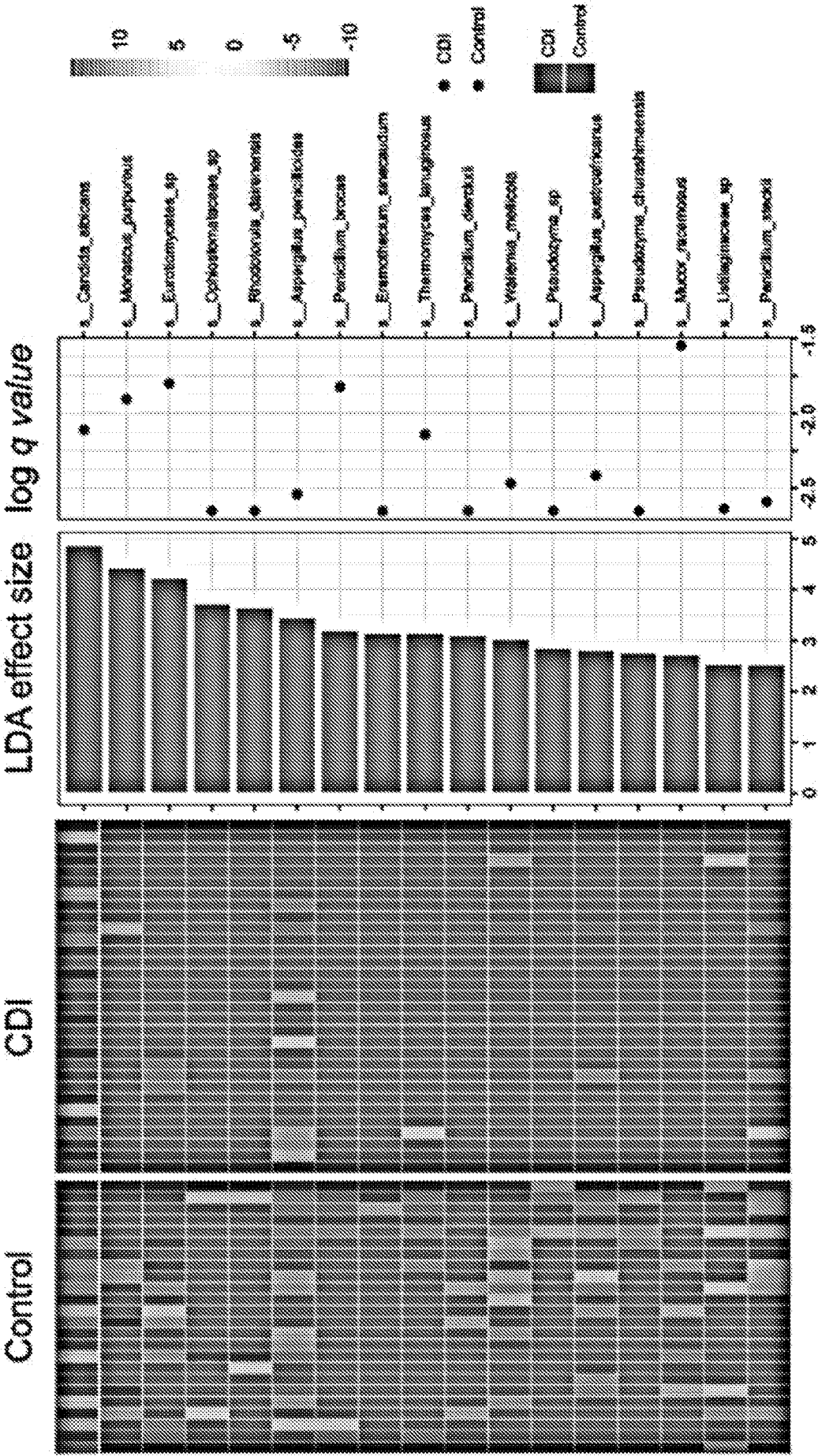


Fig. 1d



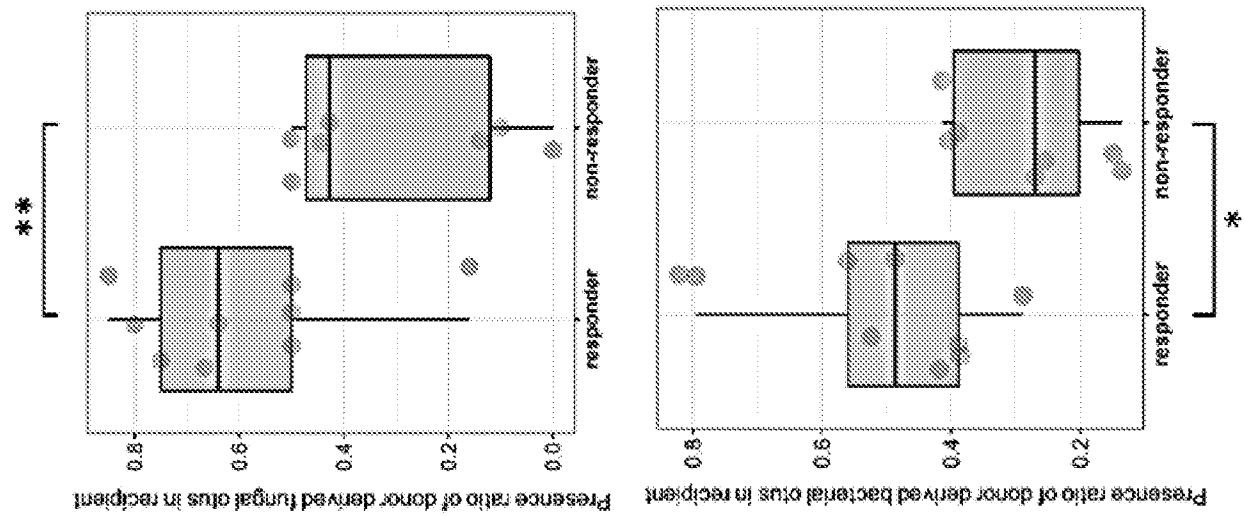
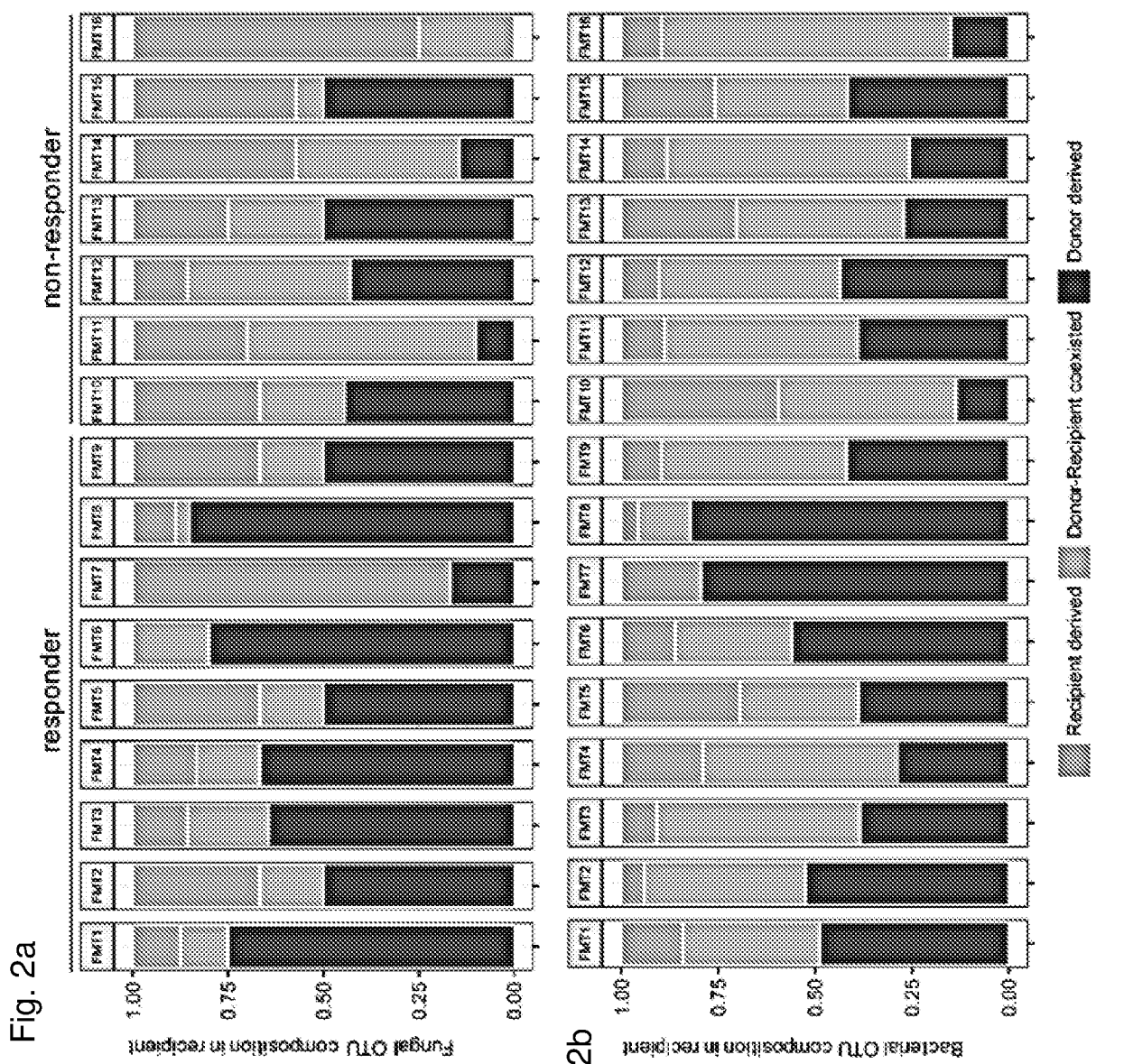
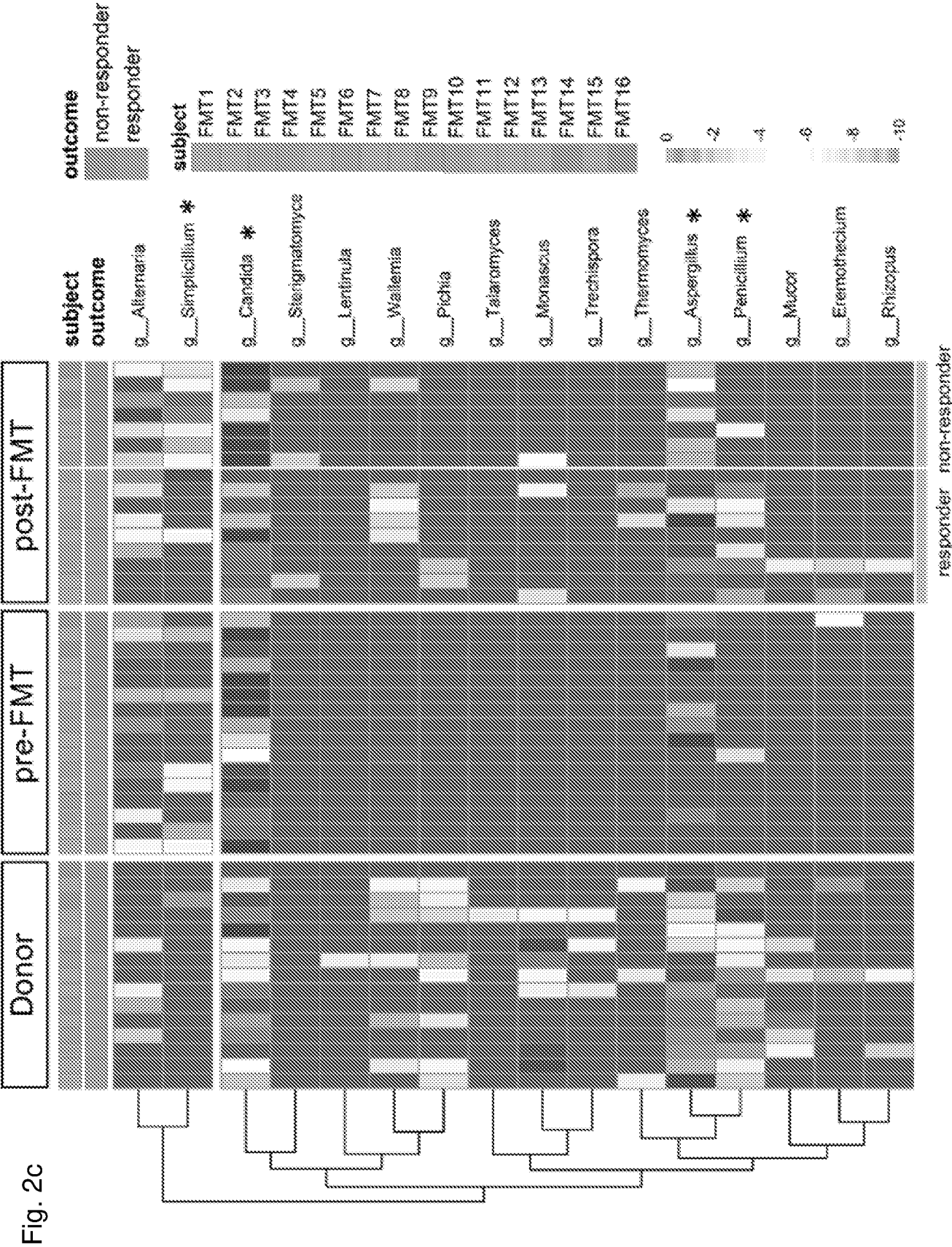


Fig. 2a

Fig. 2b



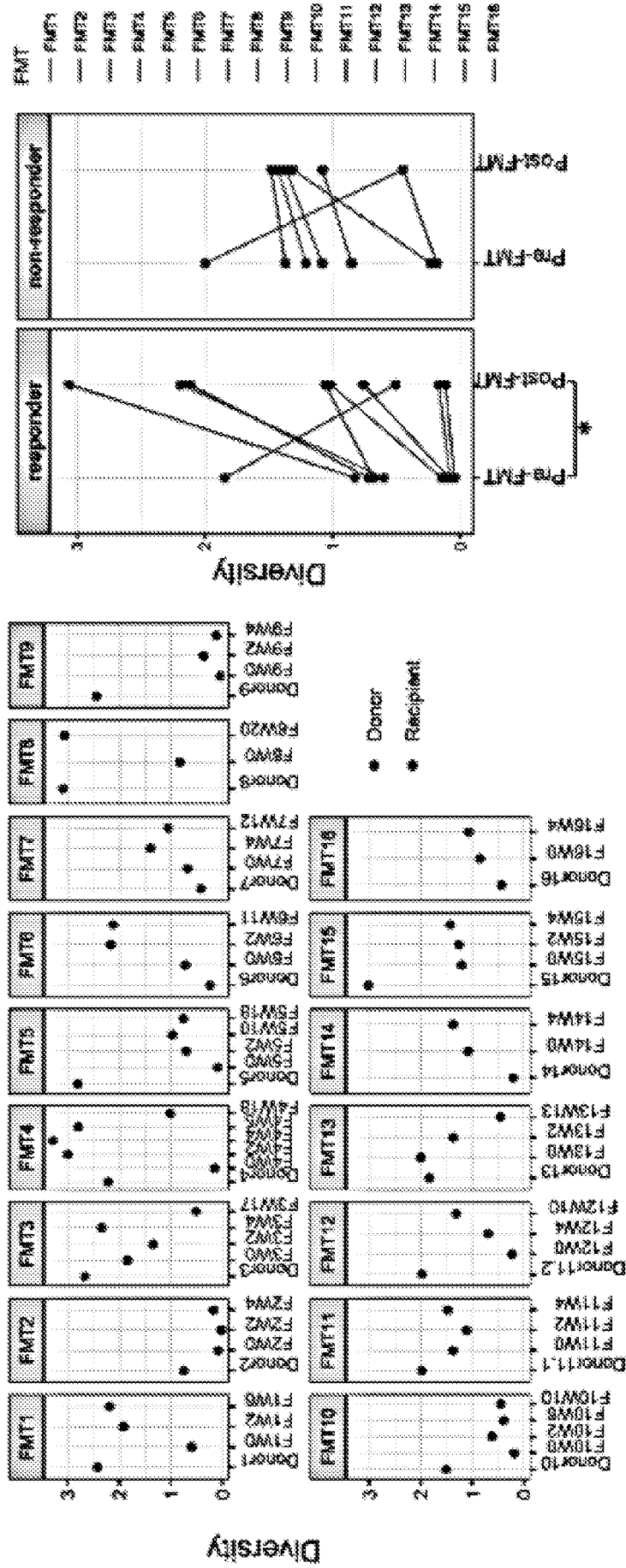
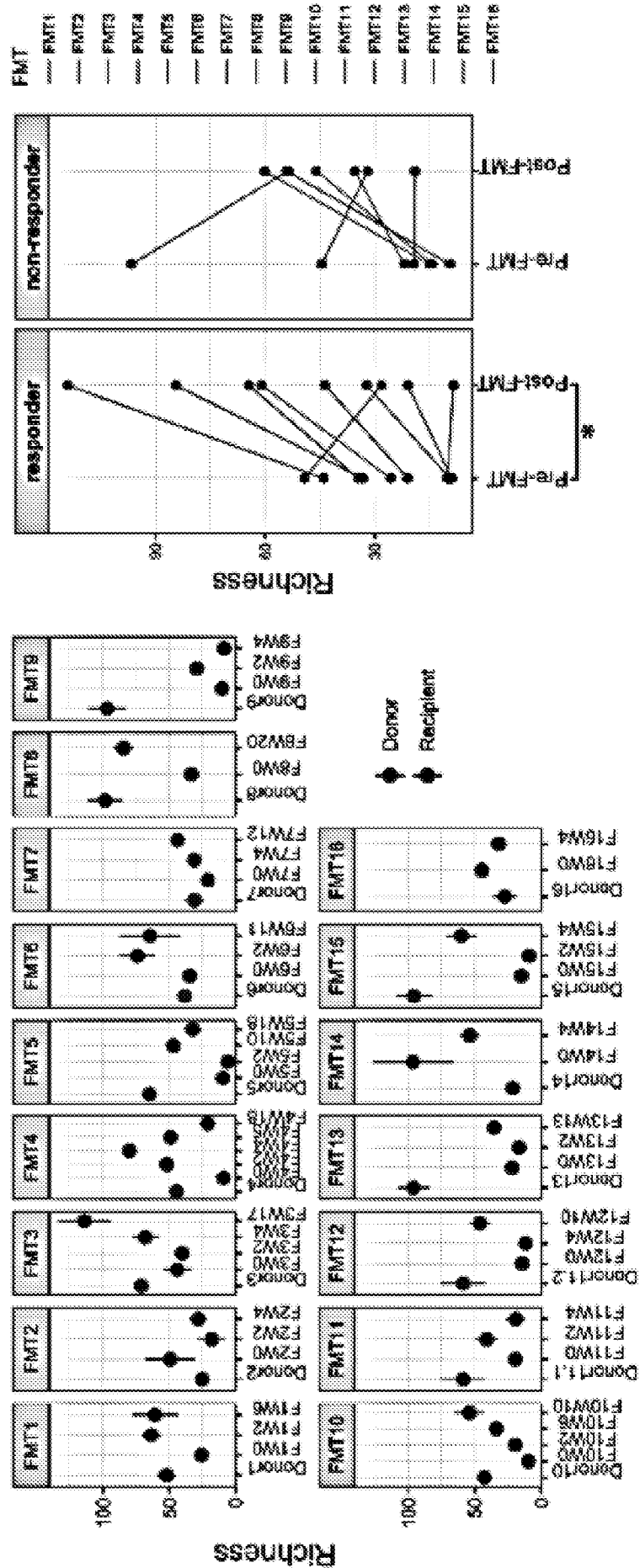


Fig. 3b



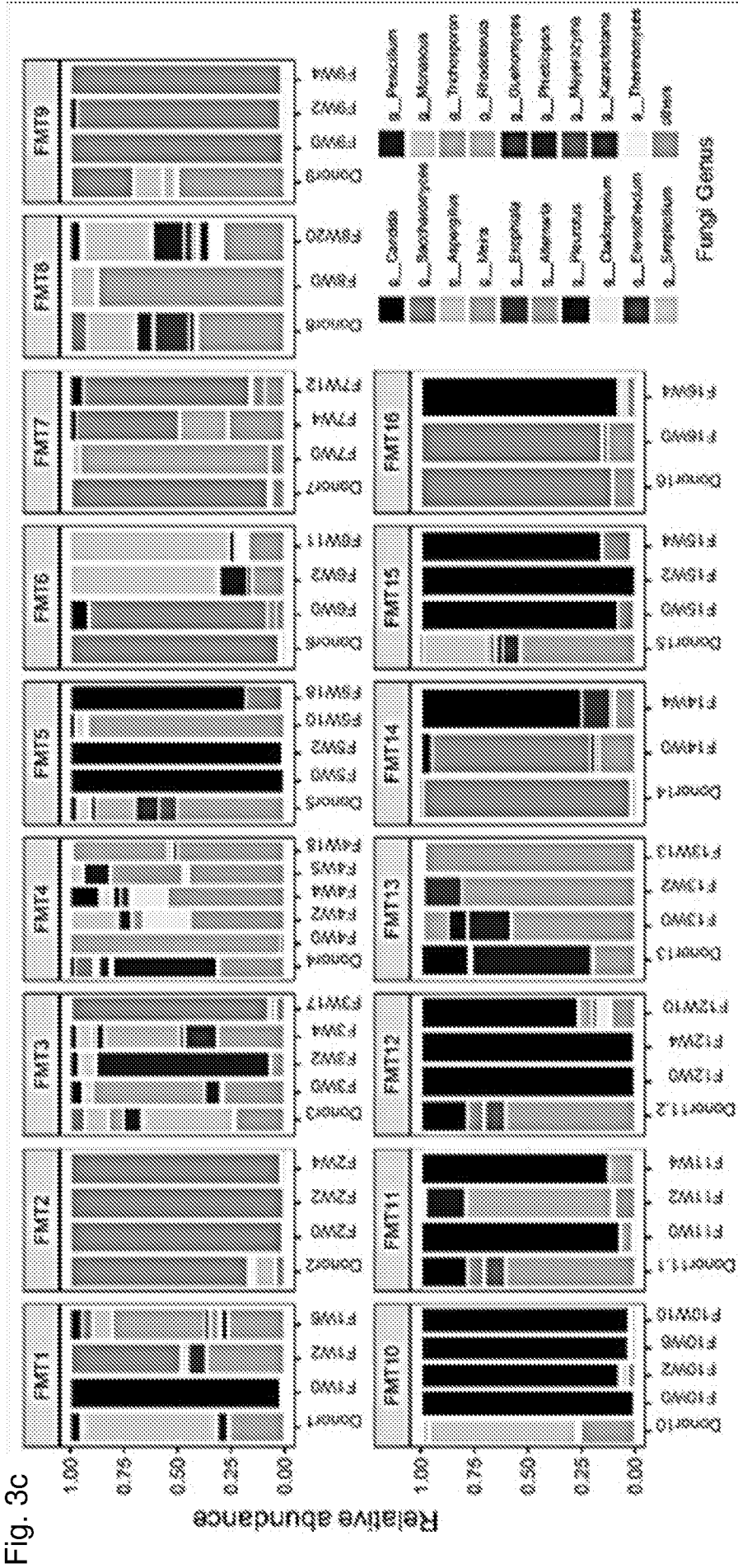


Fig. 3d

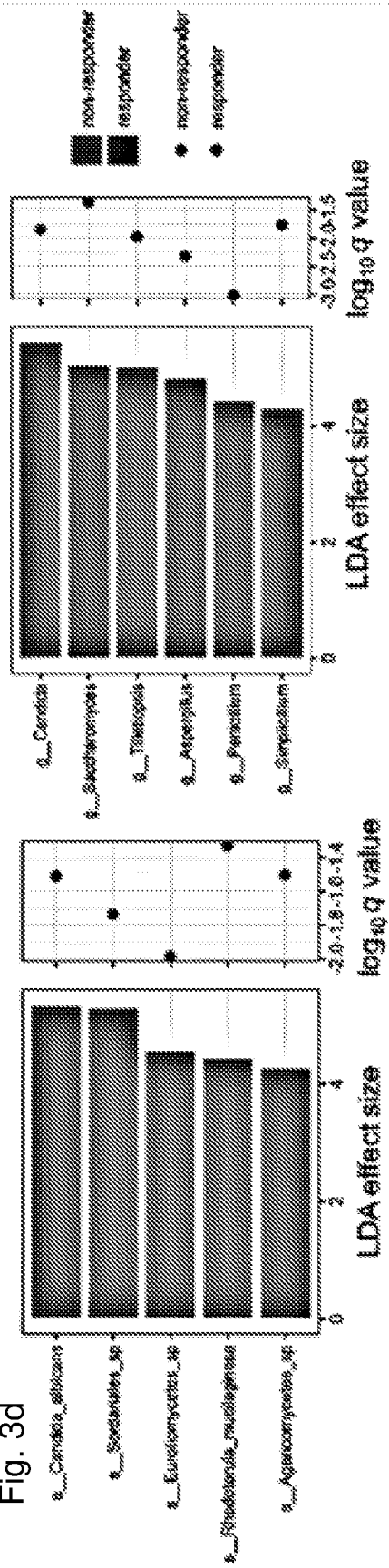


Fig. 3e

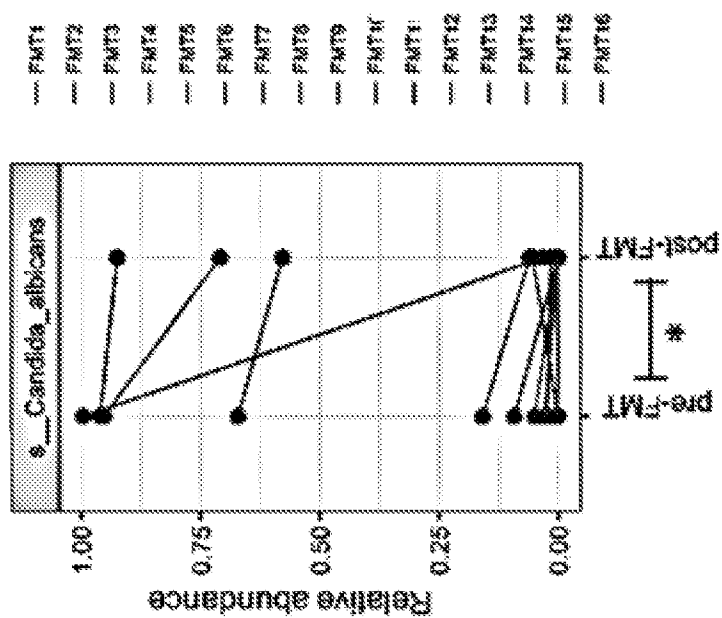


Fig. 3f

Relative abundance of *C. albicans* in donor and FMT response

	Responder	Non-responder
Donors with > 0.1% <i>C. albicans</i>	2	5
Donors with < 0.1% <i>C. albicans</i>	7	2

Chi-square test: $p=0.0490$ *

Fig. 3g

Post-FMT relative abundance of *C. albicans* in recipient and FMT response

	Responder	Non-responder
Recipients with > 10% <i>C. albicans</i>	0	3
Recipients with < 10% <i>C. albicans</i>	9	4

Chi-square test: $p=0.0293$ *

Fig. 4a

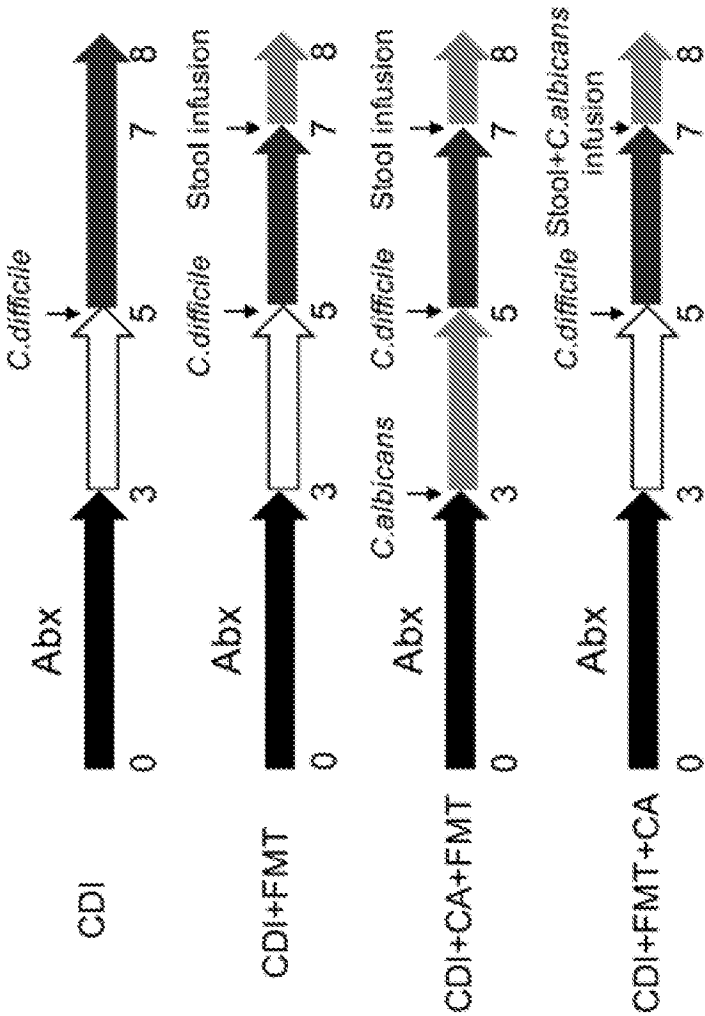


Fig. 4b

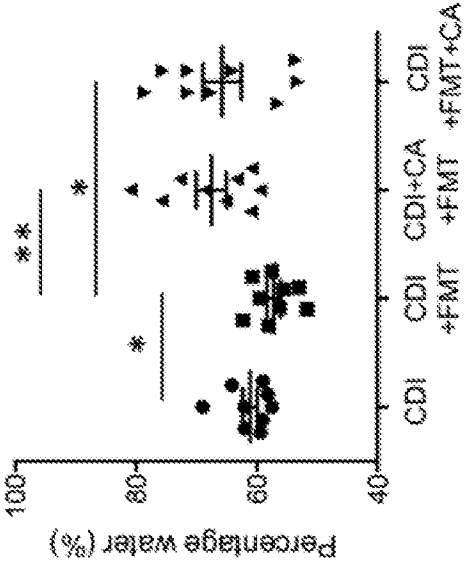


Fig. 4c

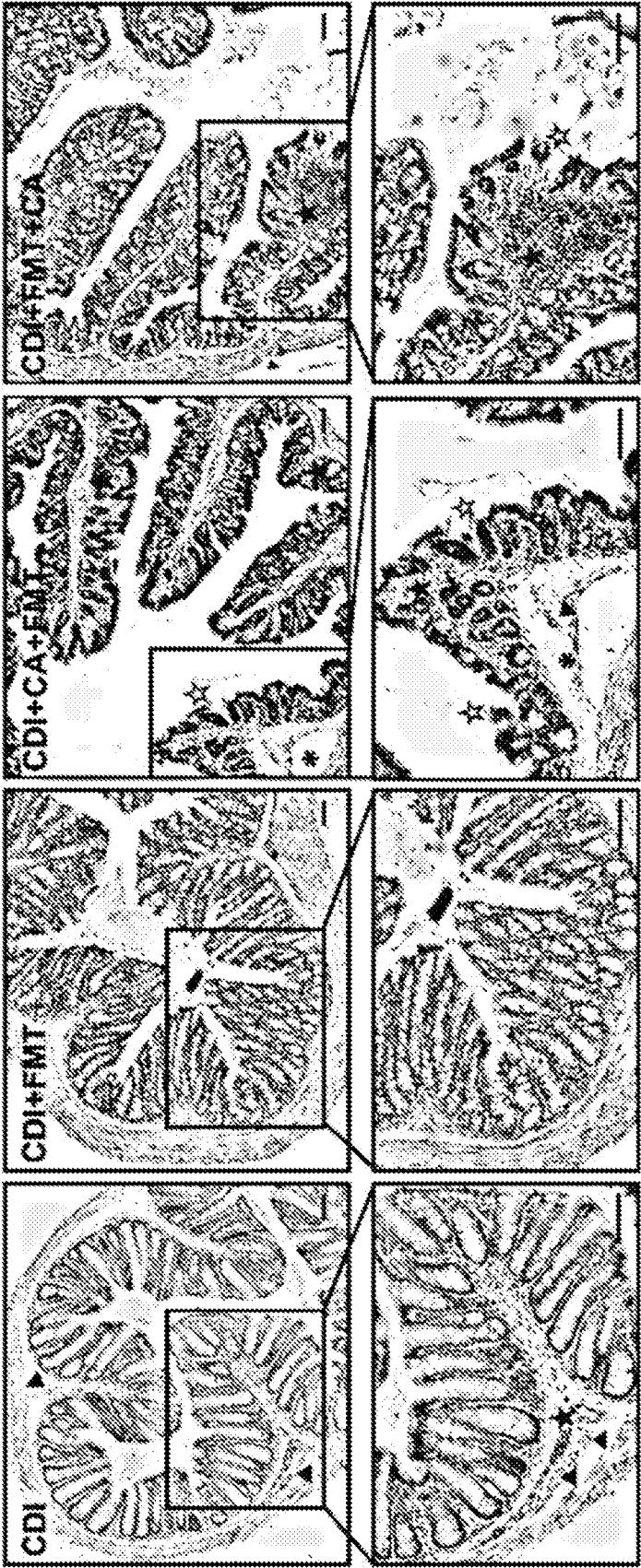


Fig. 4d

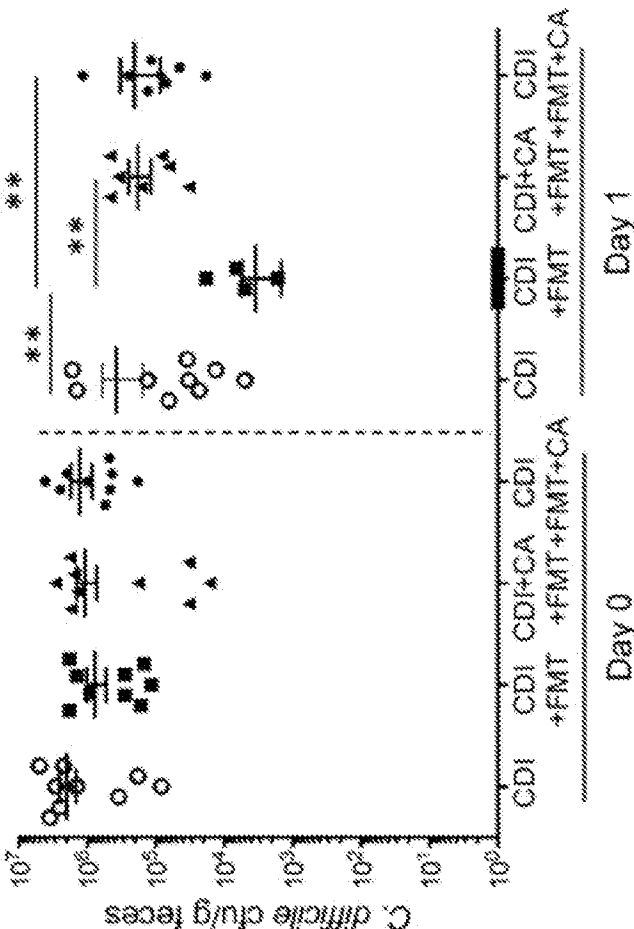


Fig. 4e

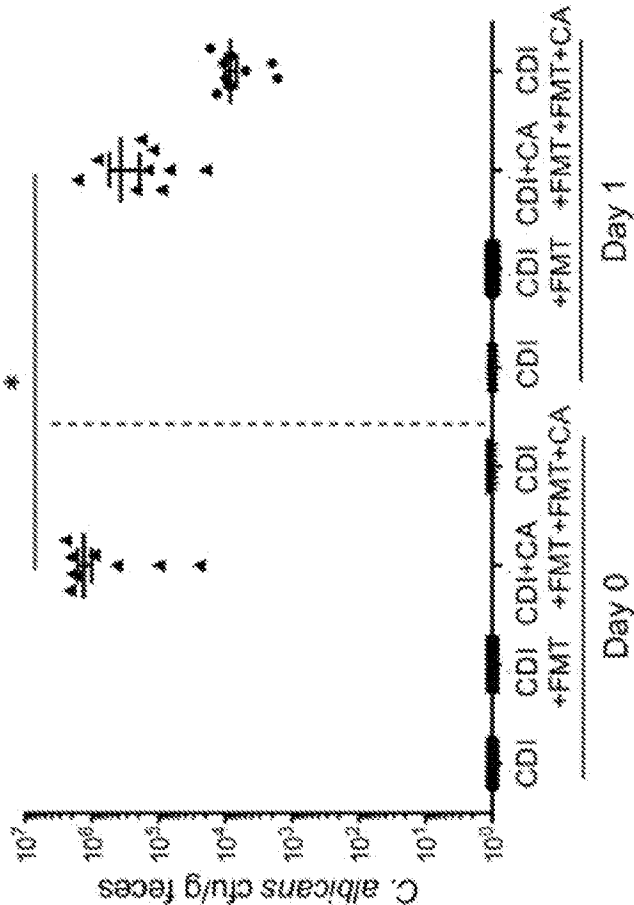


Fig. 5b

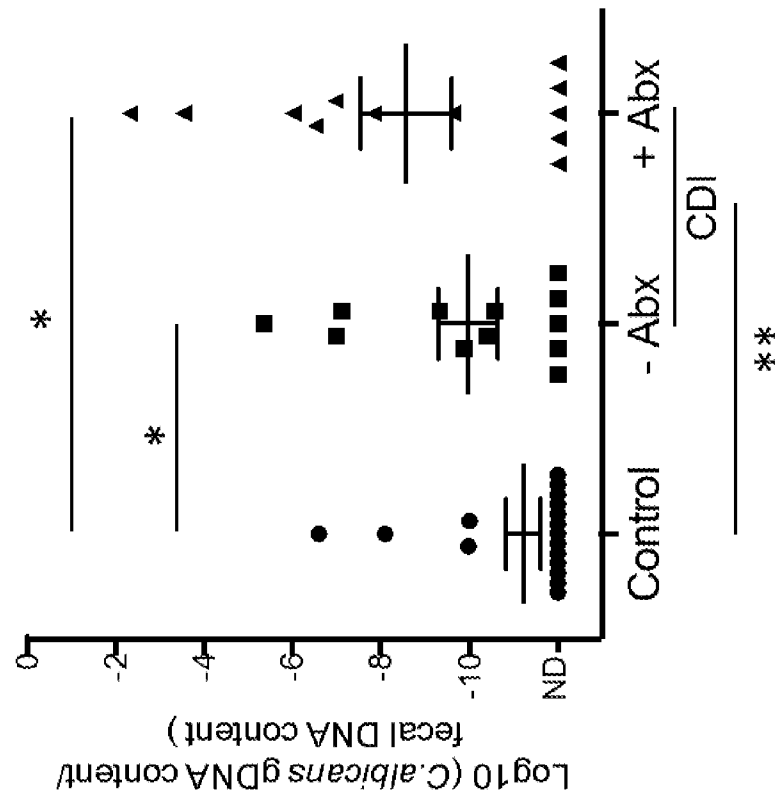


Fig. 5a

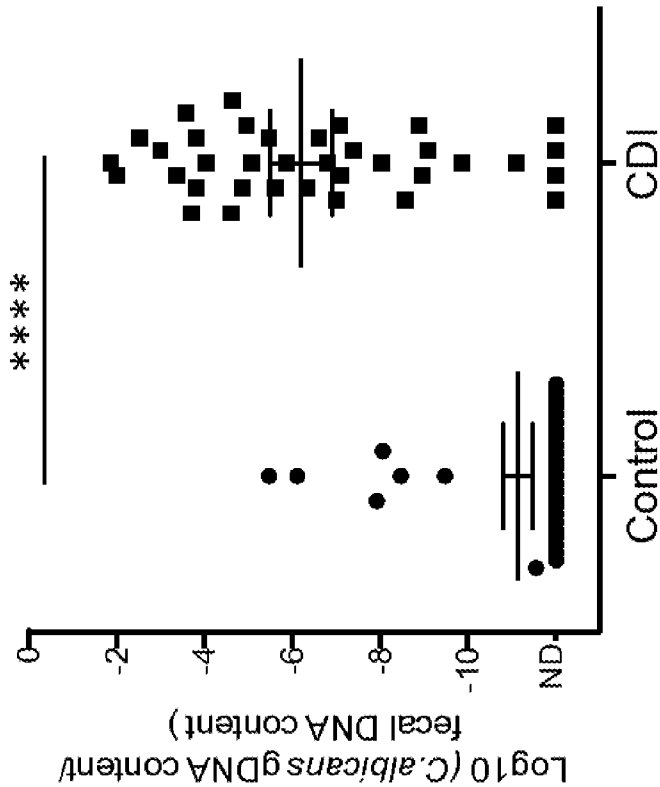


Fig. 6

FMT

FMT1	Device1	F1100	F1102	F1104
	Device2	F1200	F1202	F1204
FMT2	Device3	F2100	F2102	F2104
	Device4	F2200	F2202	F2204
FMT3	Device5	F3100	F3102	F3104
	Device6	F3200	F3202	F3204
FMT4	Device7	F4100	F4102	F4104
	Device8	F4200	F4202	F4204
FMT5	Device9	F5100	F5102	F5104
	Device10	F5200	F5202	F5204
FMT6	Device11	F6100	F6102	F6104
	Device12	F6200	F6202	F6204
FMT7	Device13	F7100	F7102	F7104
	Device14	F7200	F7202	F7204
FMT8	Device15	F8100	F8102	F8104
	Device16	F8200	F8202	F8204
FMT9	Device17	F9100	F9102	F9104
	Device18	F9200	F9202	F9204
FMT10	Device19	F10100	F10102	F10104
	Device20	F10200	F10202	F10204
FMT11	Device21	F11100	F11102	F11104
	Device22	F11200	F11202	F11204
FMT12	Device23	F12100	F12102	F12104
	Device24	F12200	F12202	F12204
FMT13	Device25	F13100	F13102	F13104
	Device26	F13200	F13202	F13204
FMT14	Device27	F14100	F14102	F14104
	Device28	F14200	F14202	F14204
FMT15	Device29	F15100	F15102	F15104
	Device30	F15200	F15202	F15204
FMT16	Device31	F16100	F16102	F16104
	Device32	F16200	F16202	F16204

responder

non-responder

STD

STD1	S1100	S1102	S1104
	S1200	S1202	S1204
STD2	S2100	S2102	S2104
	S2200	S2202	S2204
STD3	S3100	S3102	S3104
	S3200	S3202	S3204
STD4	S4100	S4102	S4104
	S4200	S4202	S4204
STD5	S5100	S5102	S5104
	S5200	S5202	S5204
STD6	S6100	S6102	S6104
	S6200	S6202	S6204
STD7	S7100	S7102	S7104
	S7200	S7202	S7204
STD8	S8100	S8102	S8104
	S8200	S8202	S8204

responder

non-responder

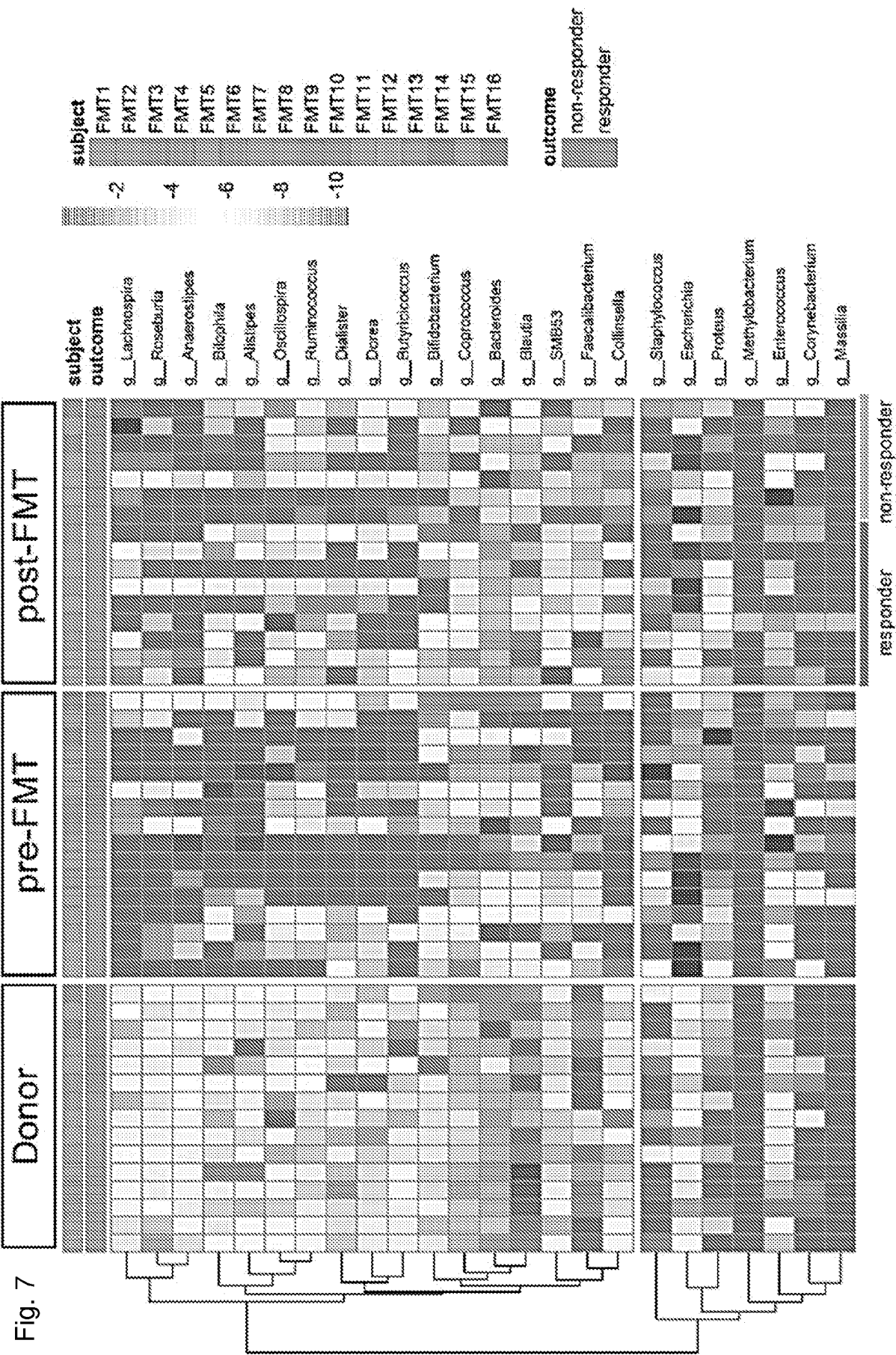
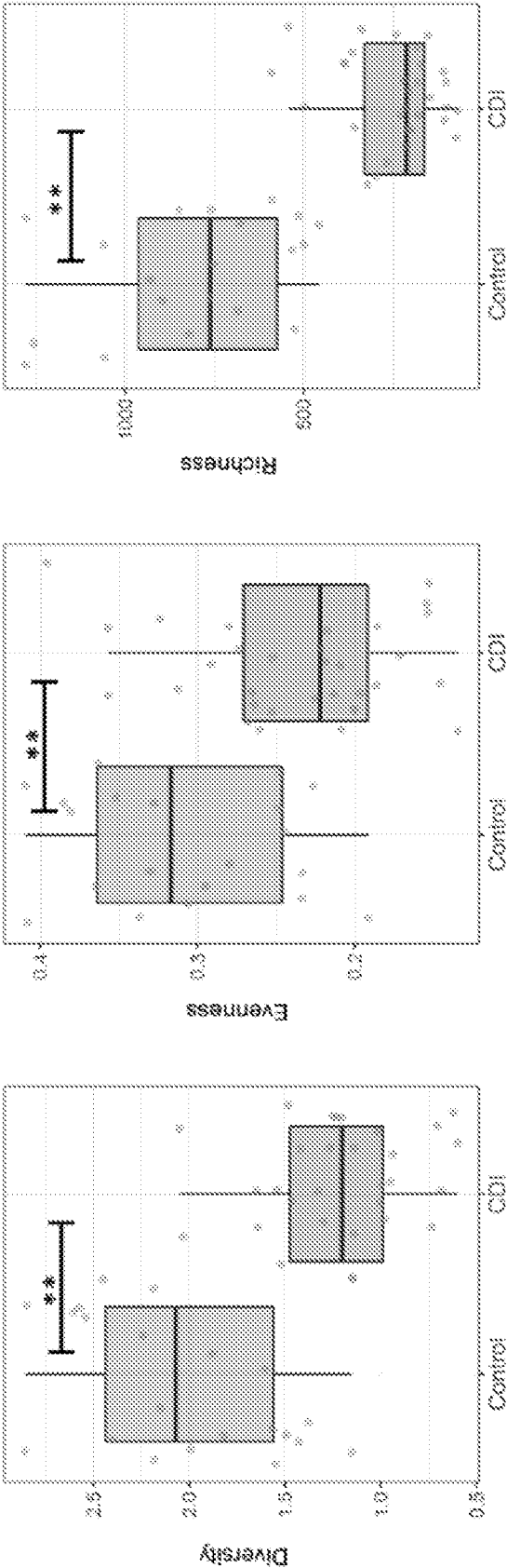
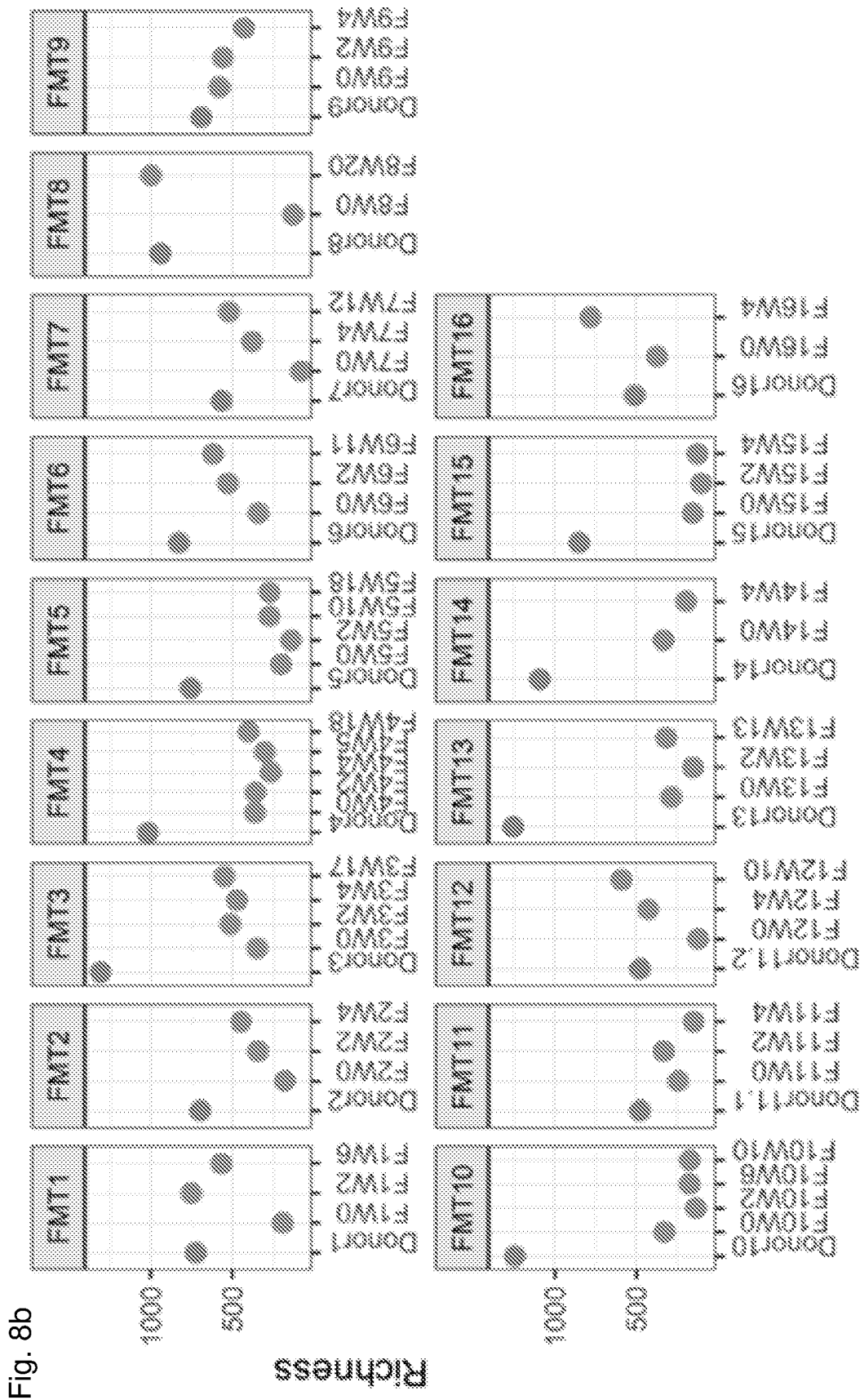


Fig. 8a





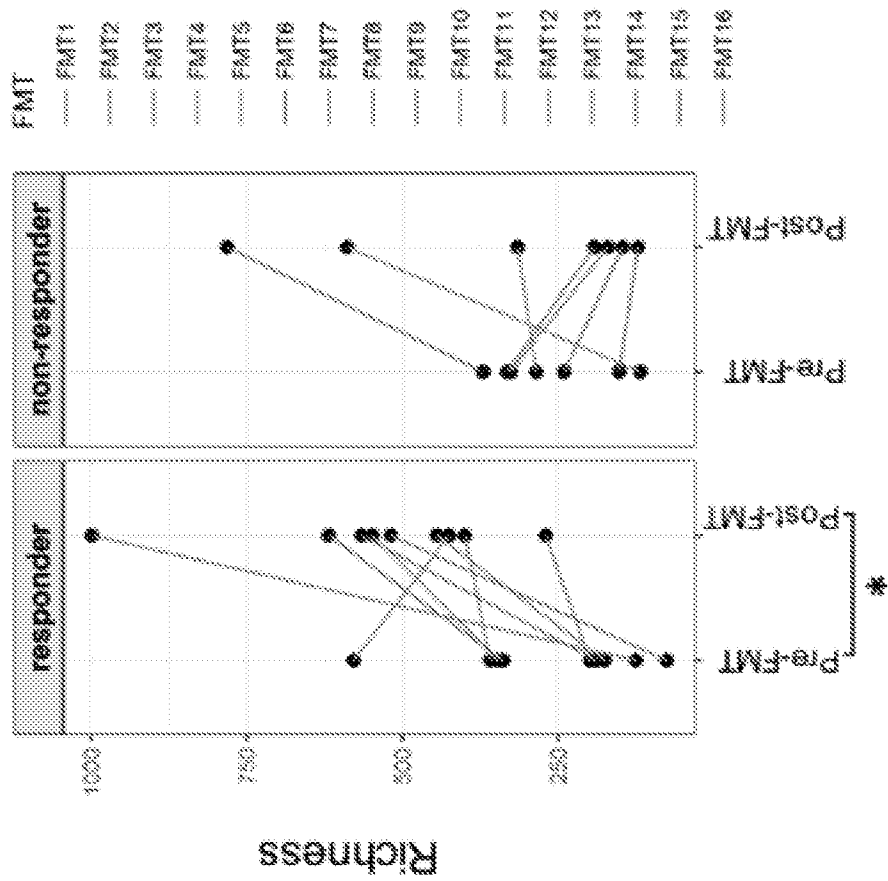


Fig. 8b (cont'd)

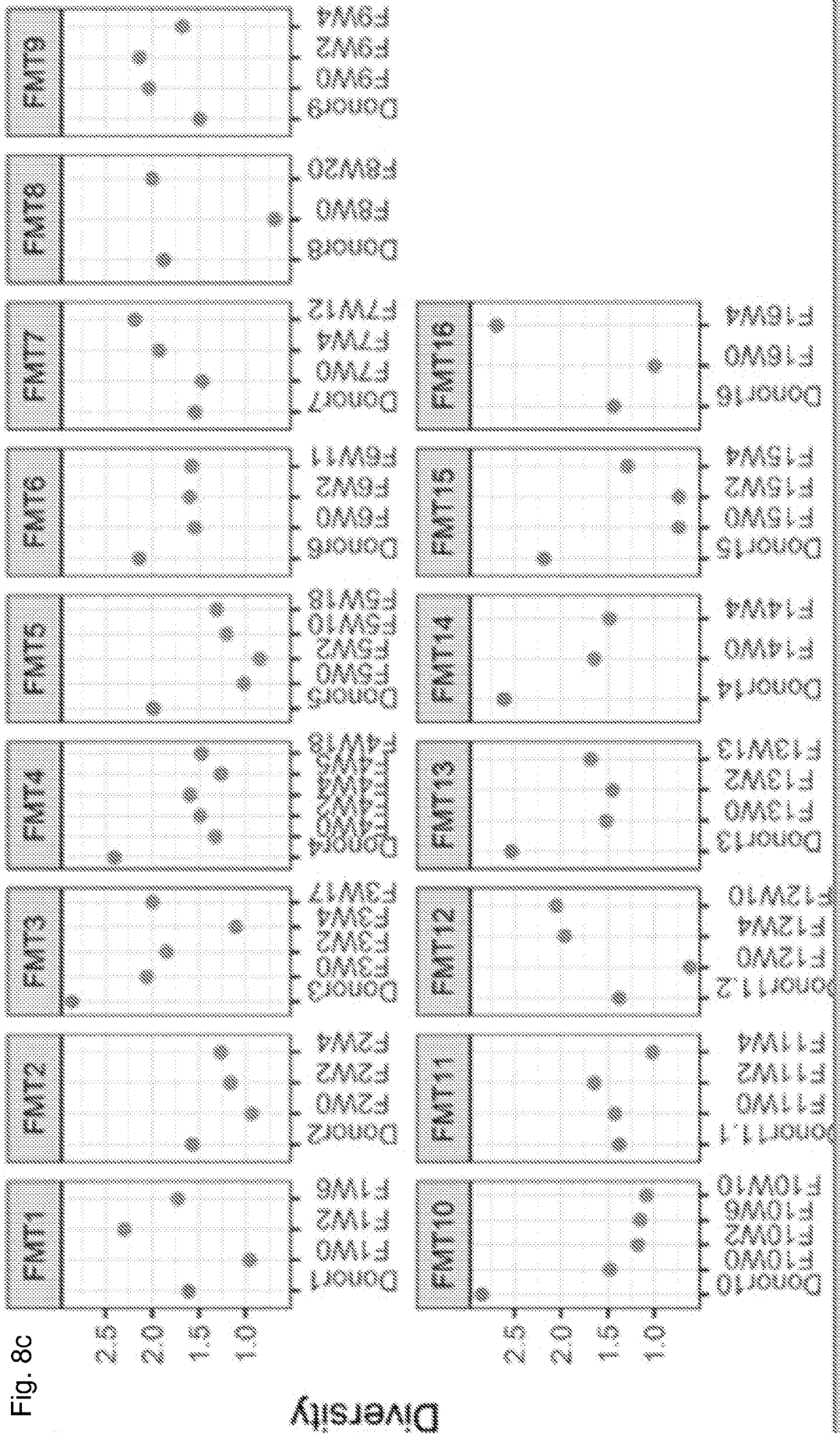
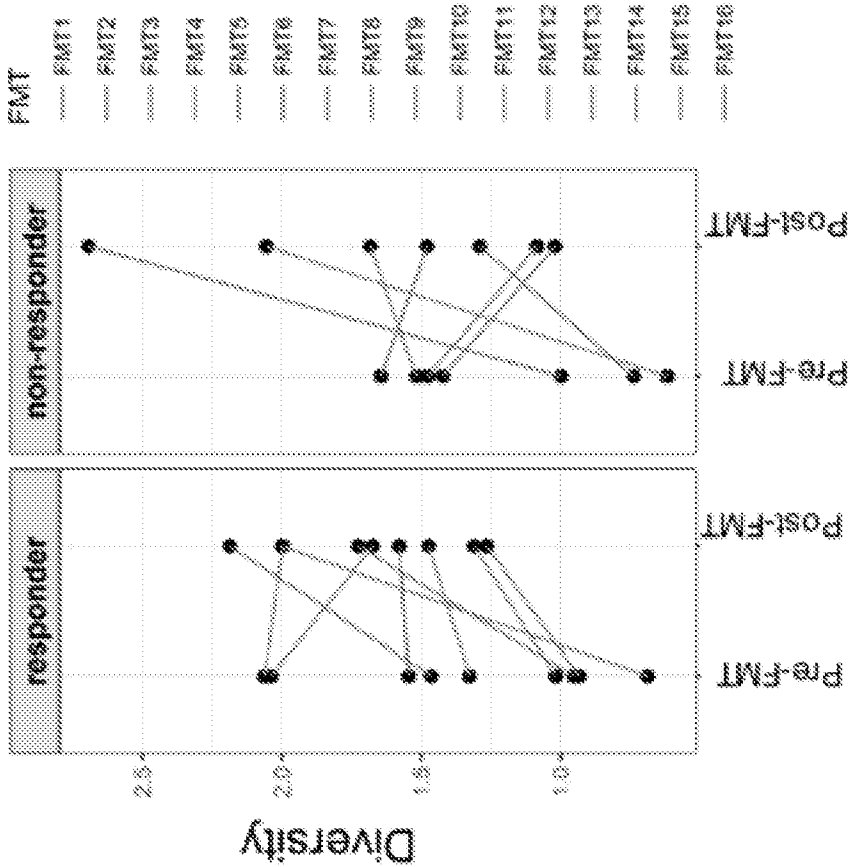
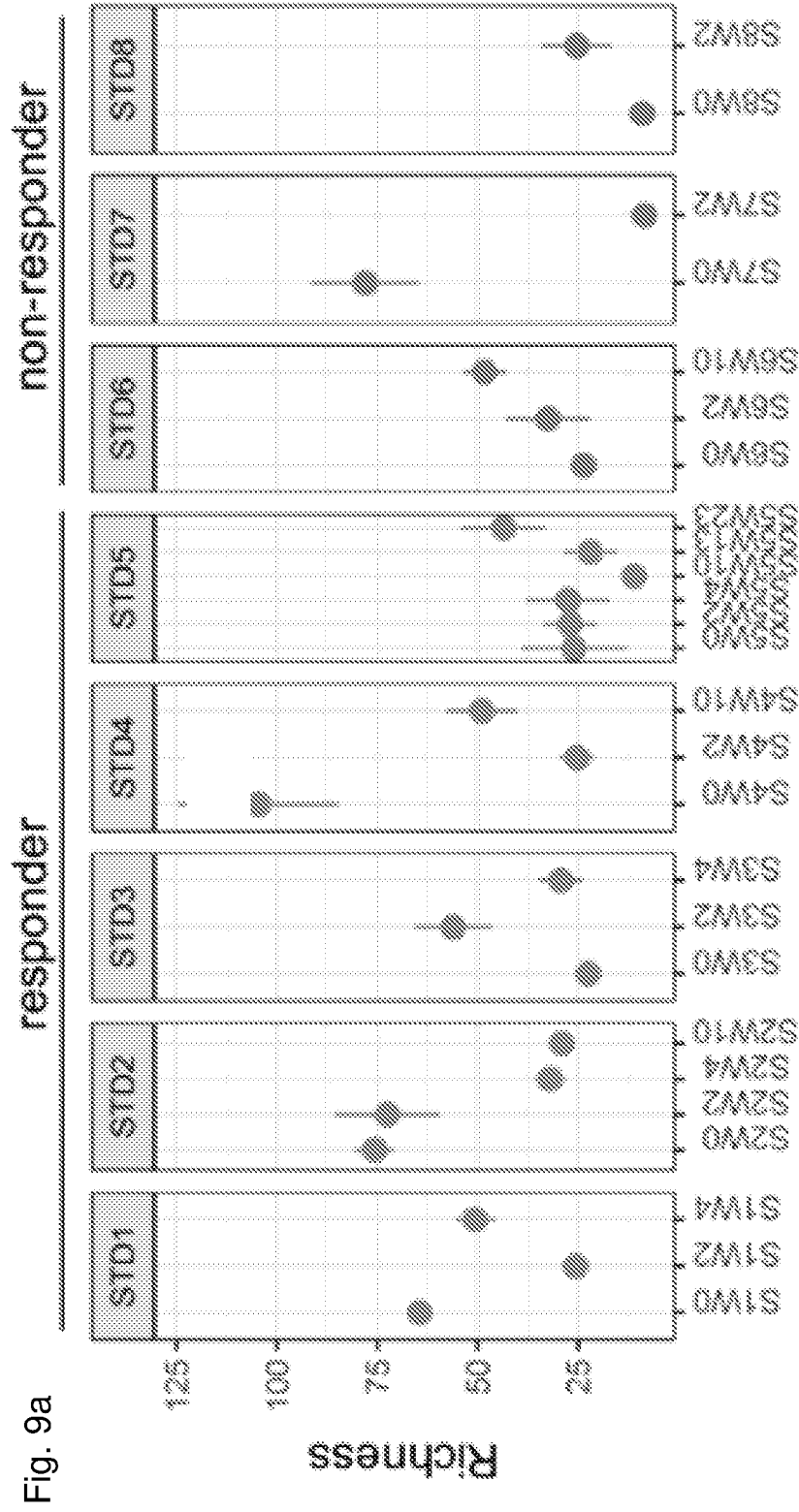


Fig. 8c (cont'd)





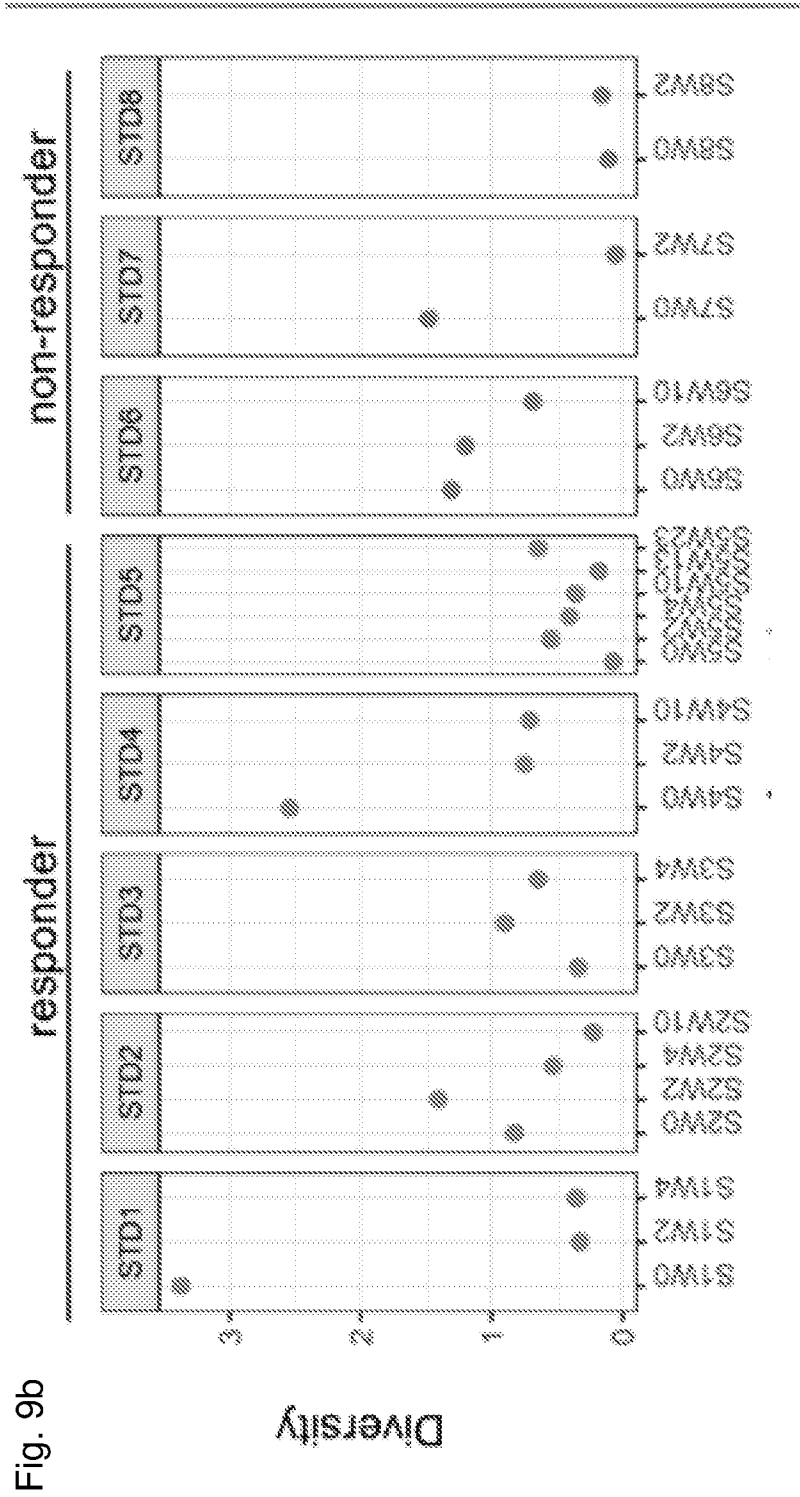


Fig. 9c Post-treatment alteration in fungal diversity

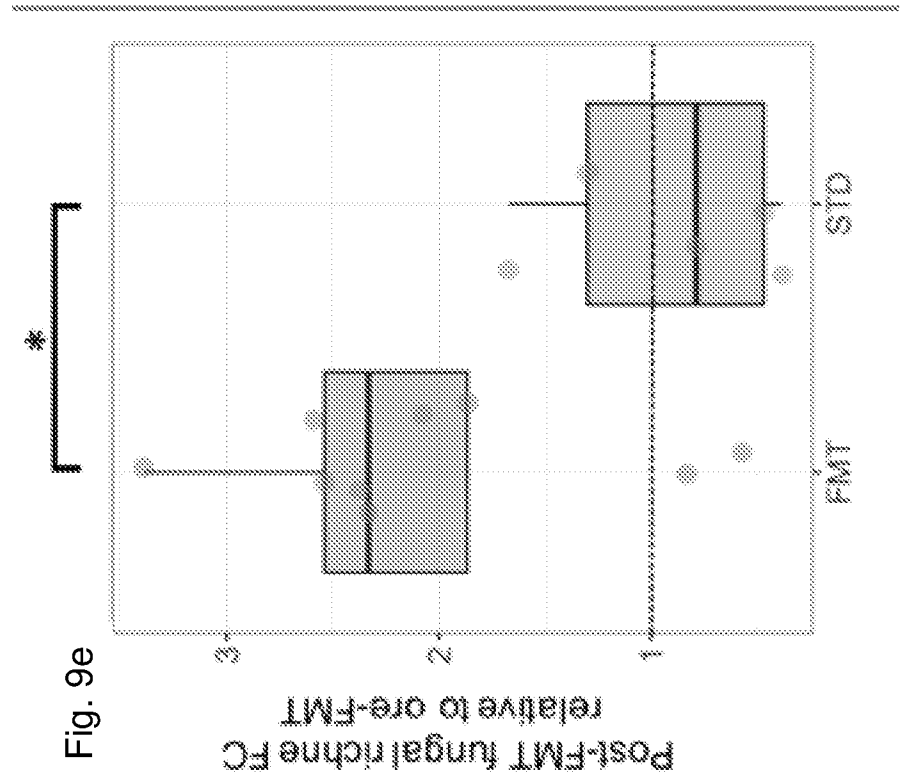
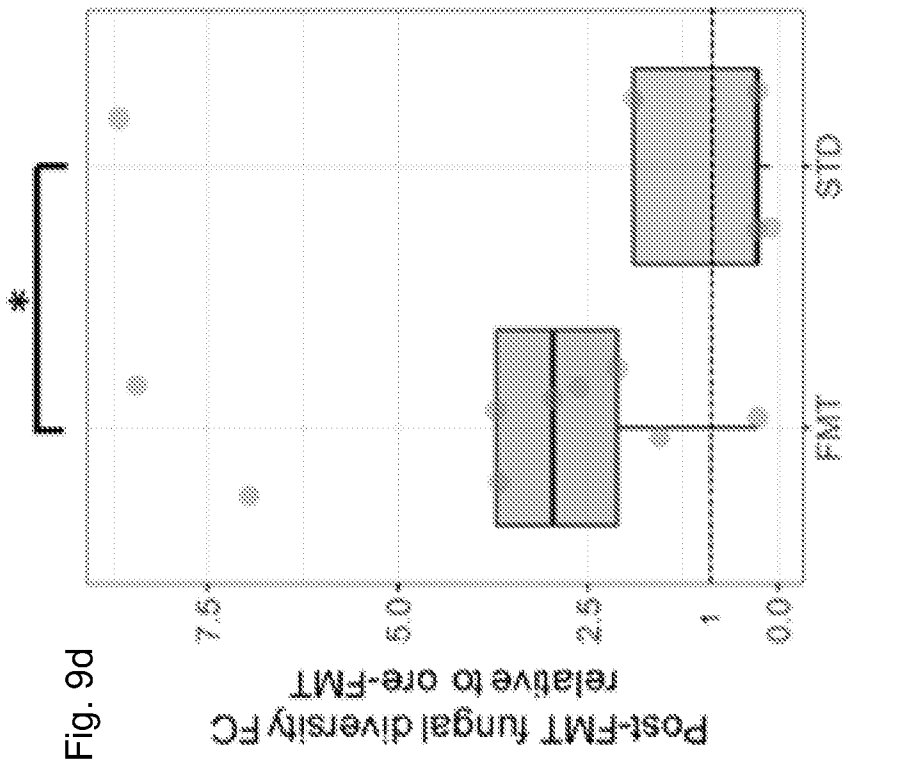
	FMT	STD
Individuals increased in diversity	13	3
Individuals decreased in diversity	3	5

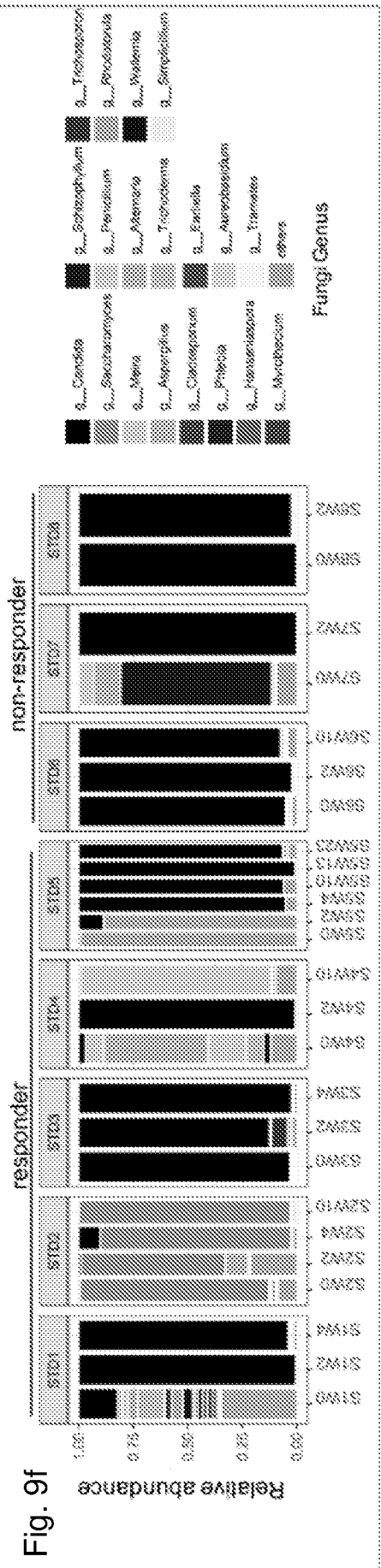
Chi-square test: $p=0.032$ *

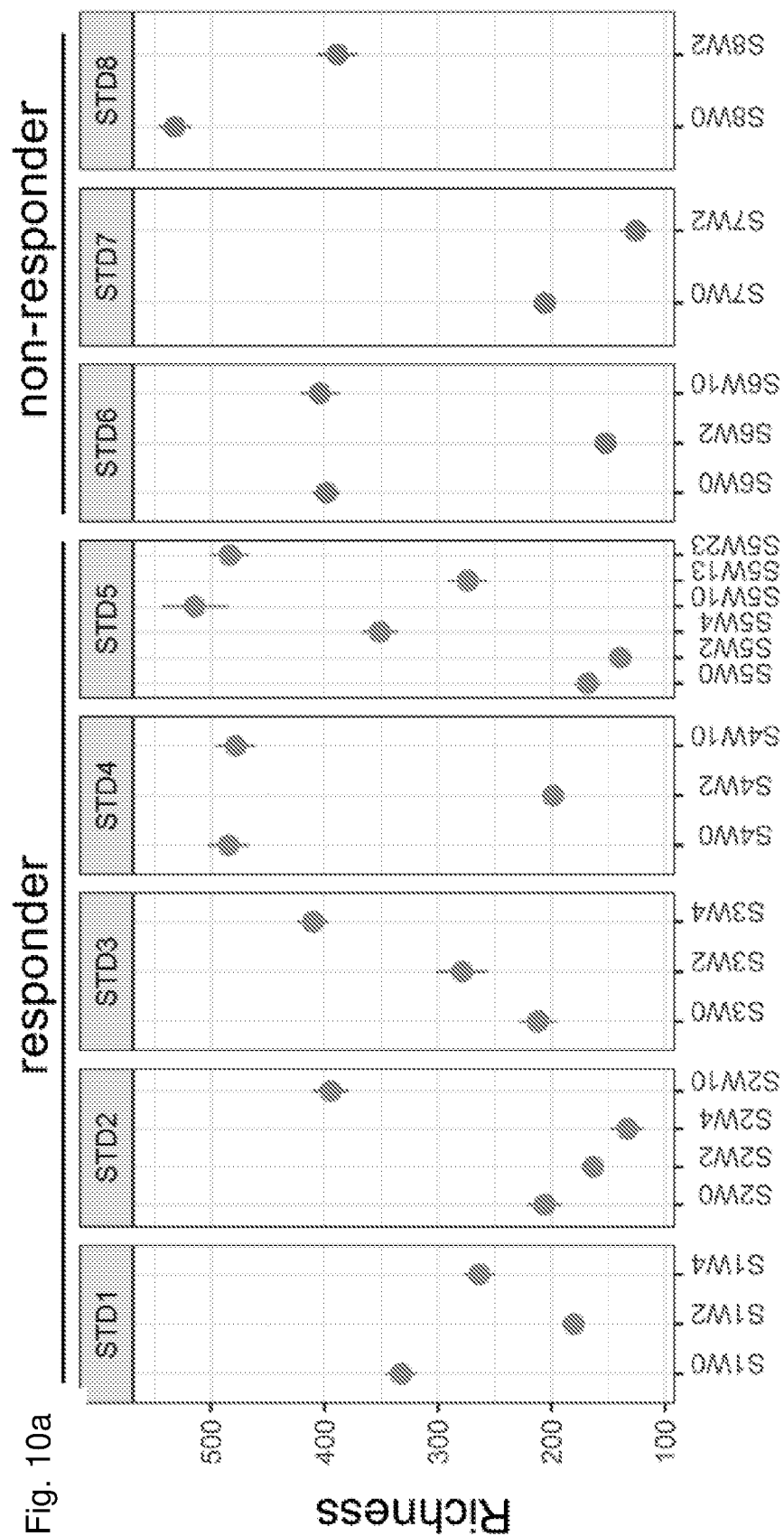
Post-treatment alteration in fungal richness

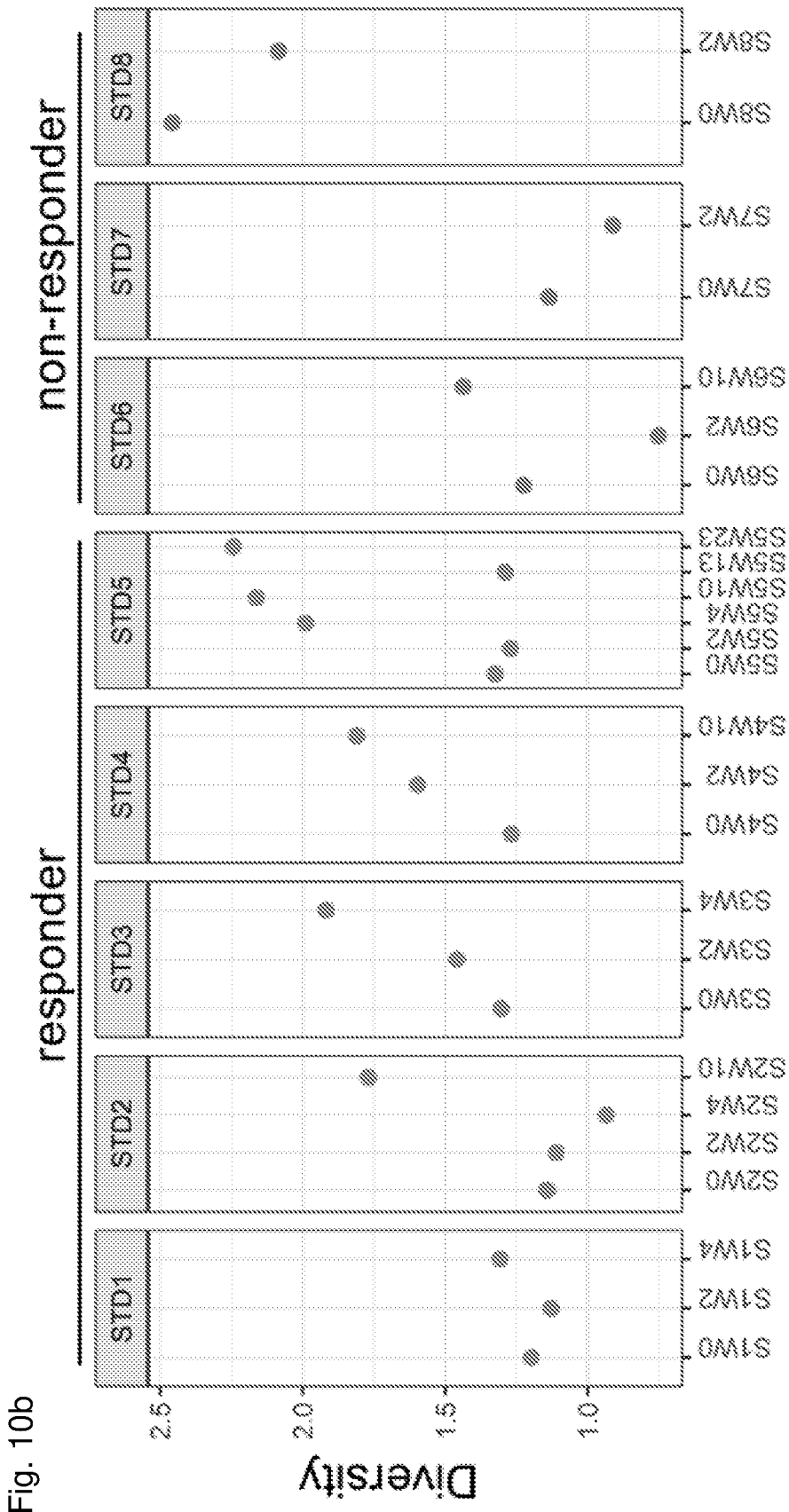
	FMT	STD
Individuals increased in richness	11	3
Individuals decreased in richness	5	5

Chi-square test: $p=0.371$









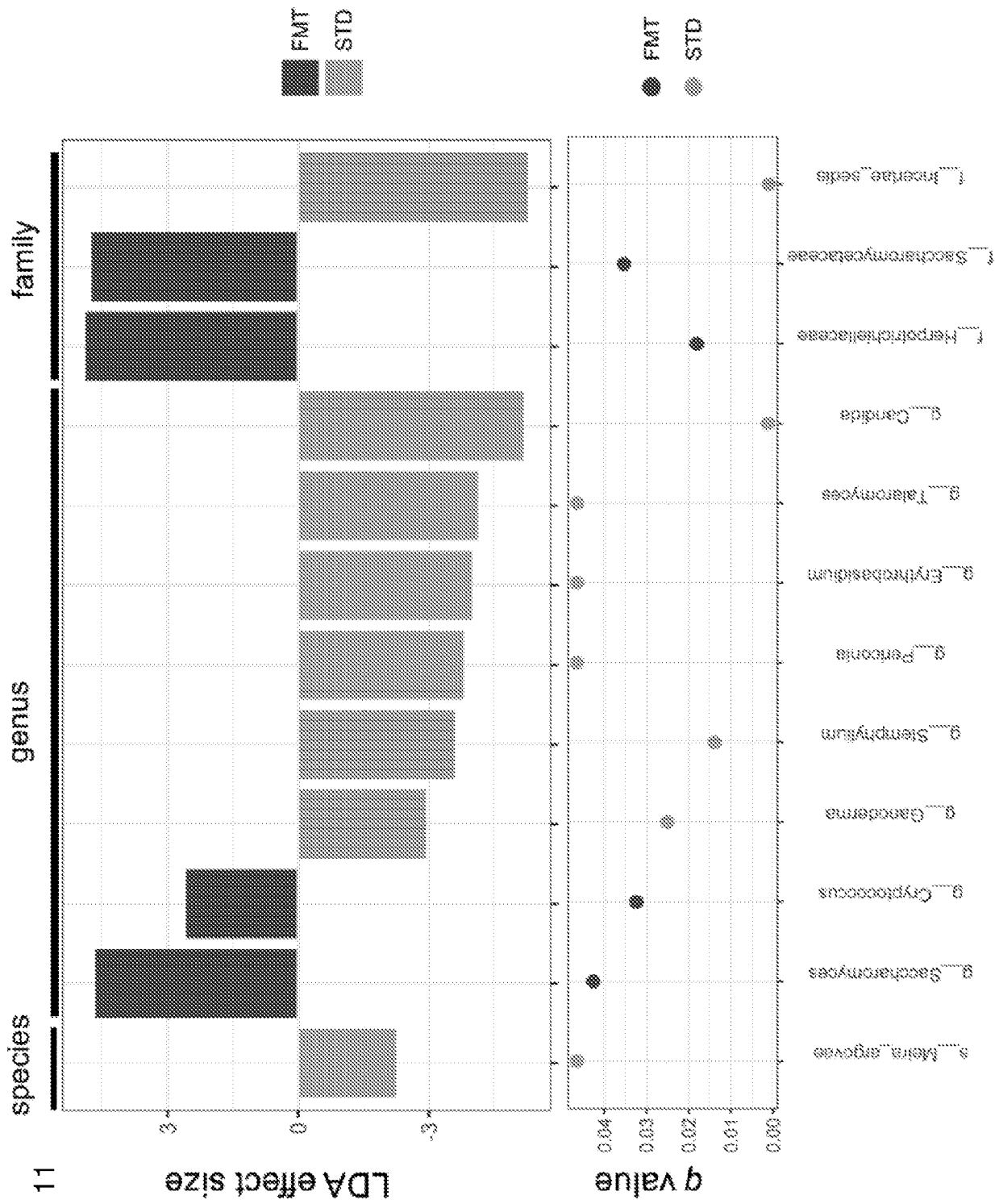


Fig. 11

Fig. 12

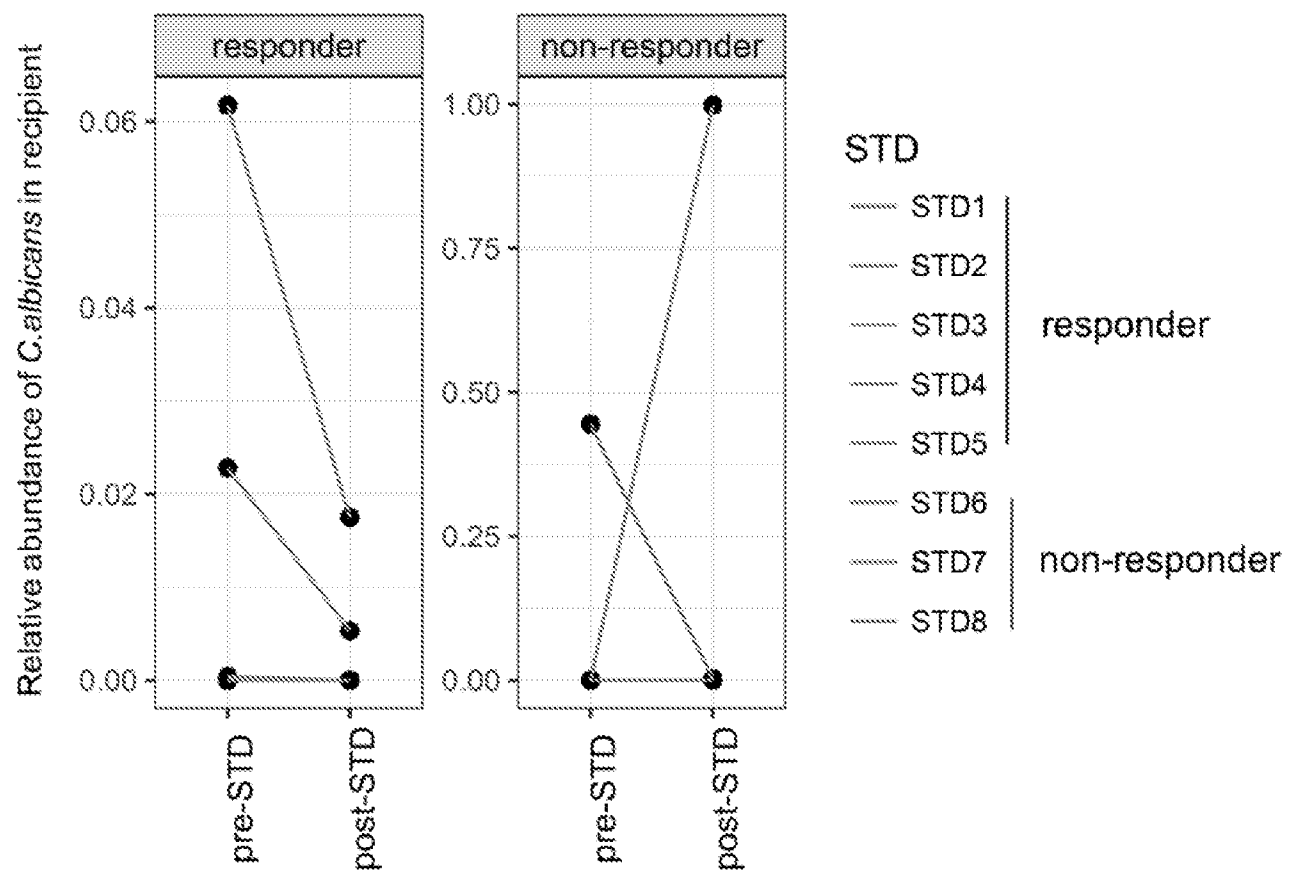
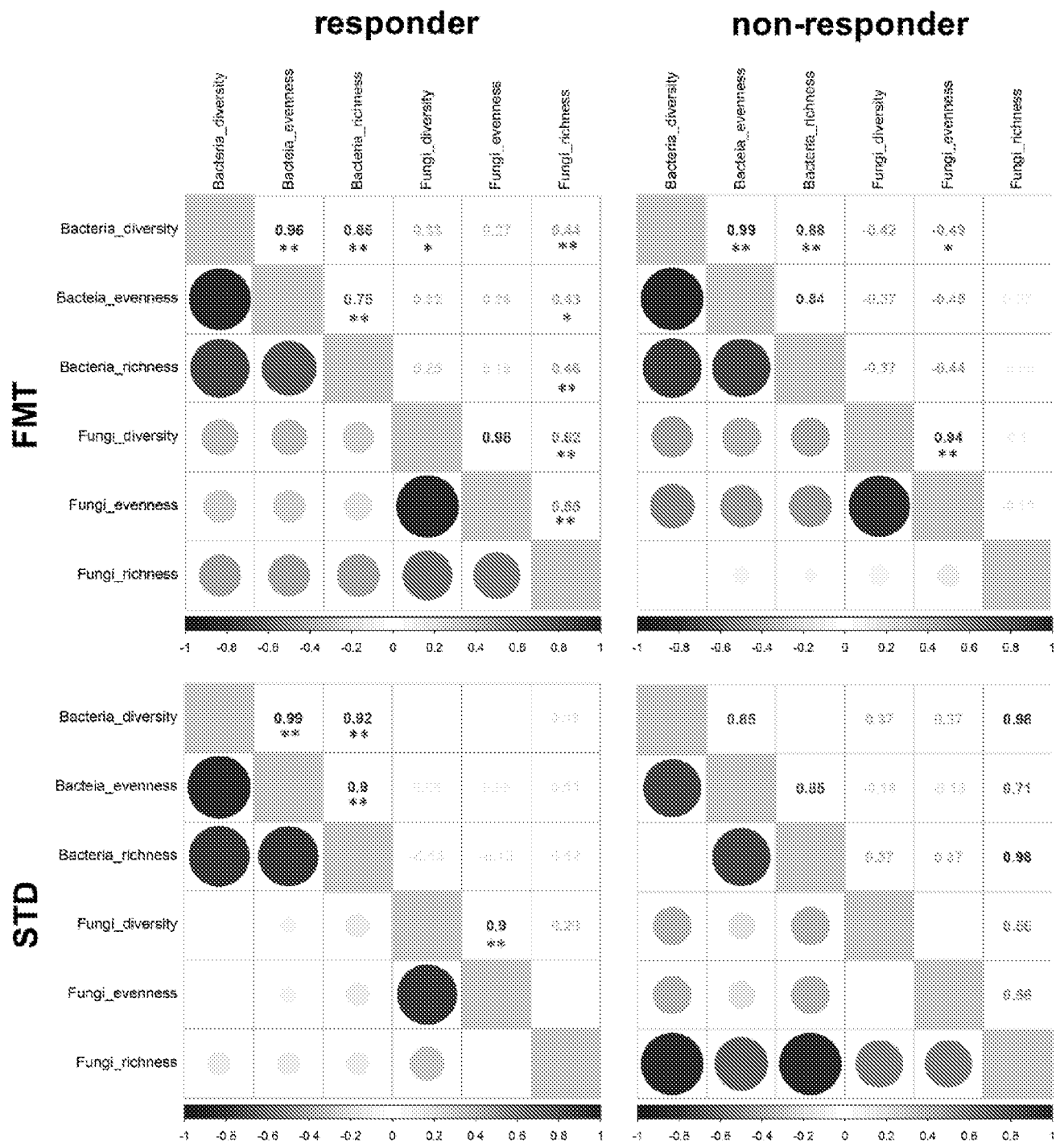


Fig. 13



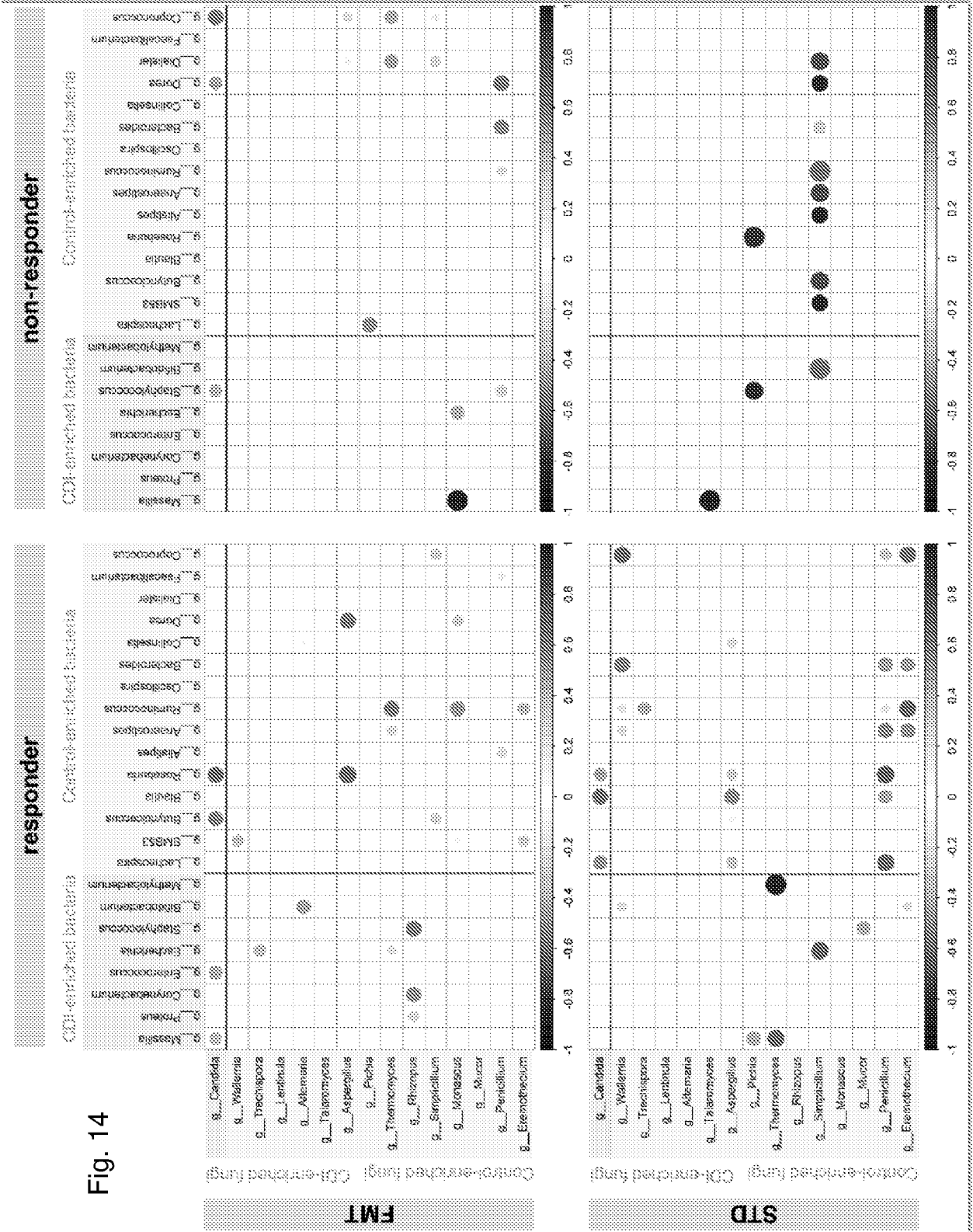


Fig. 15a

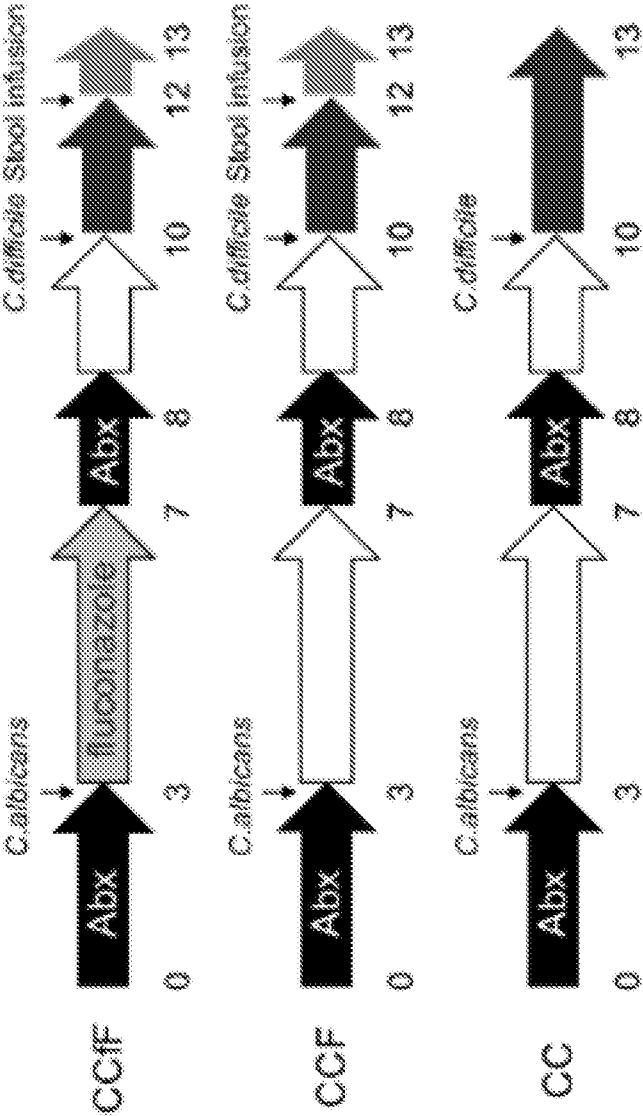


Fig. 15b

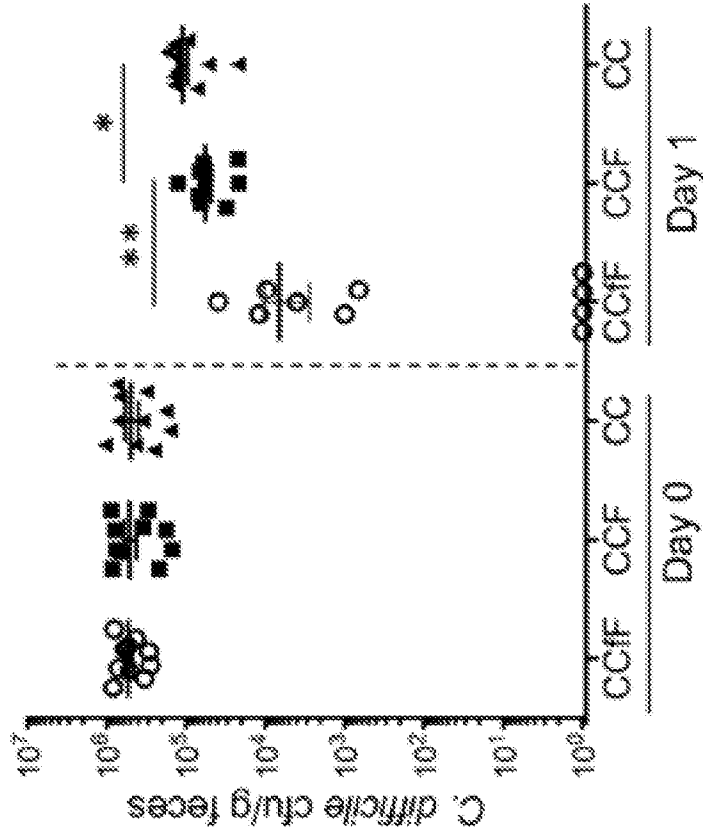
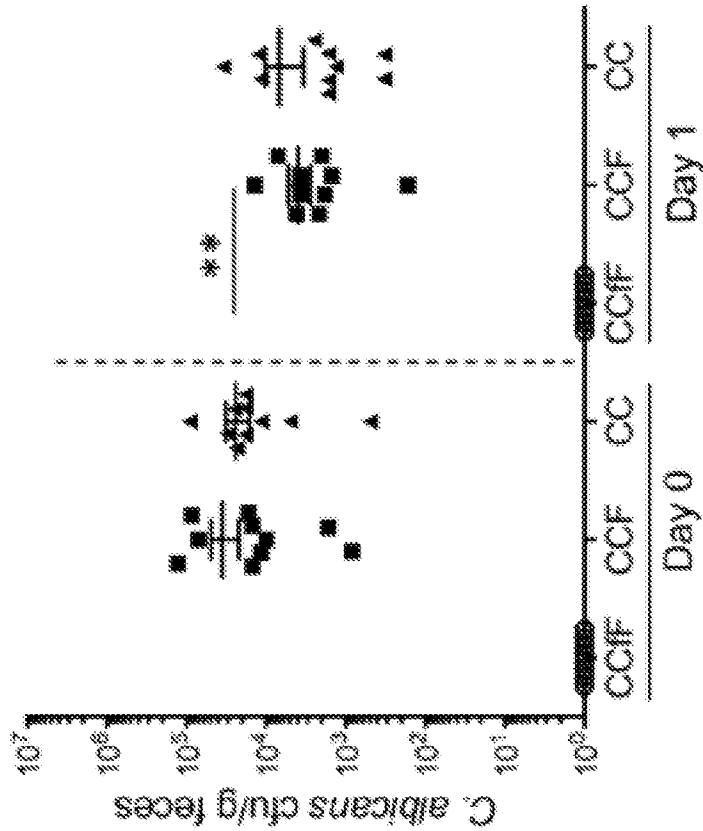


Fig. 15c



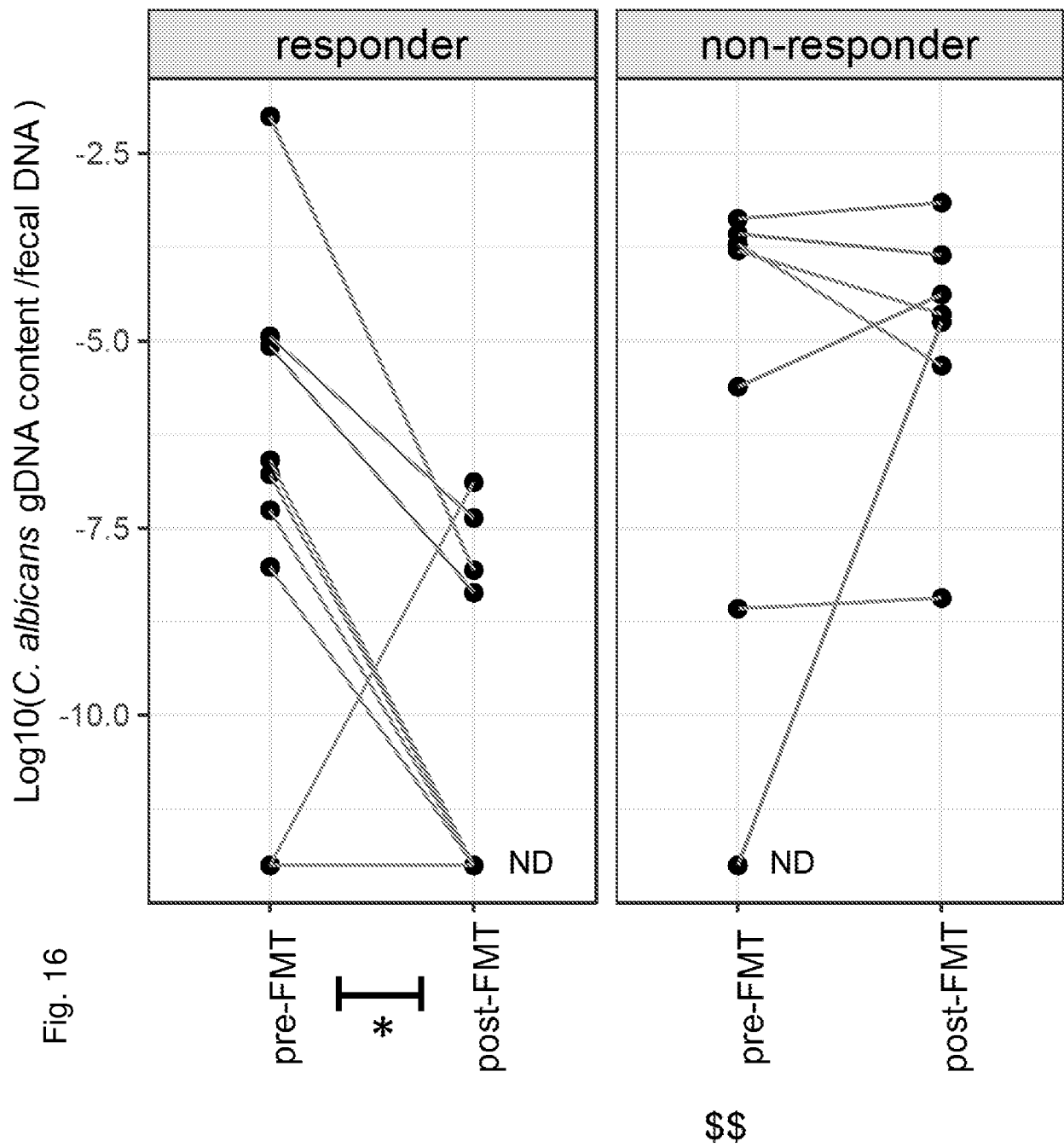


Fig. 17

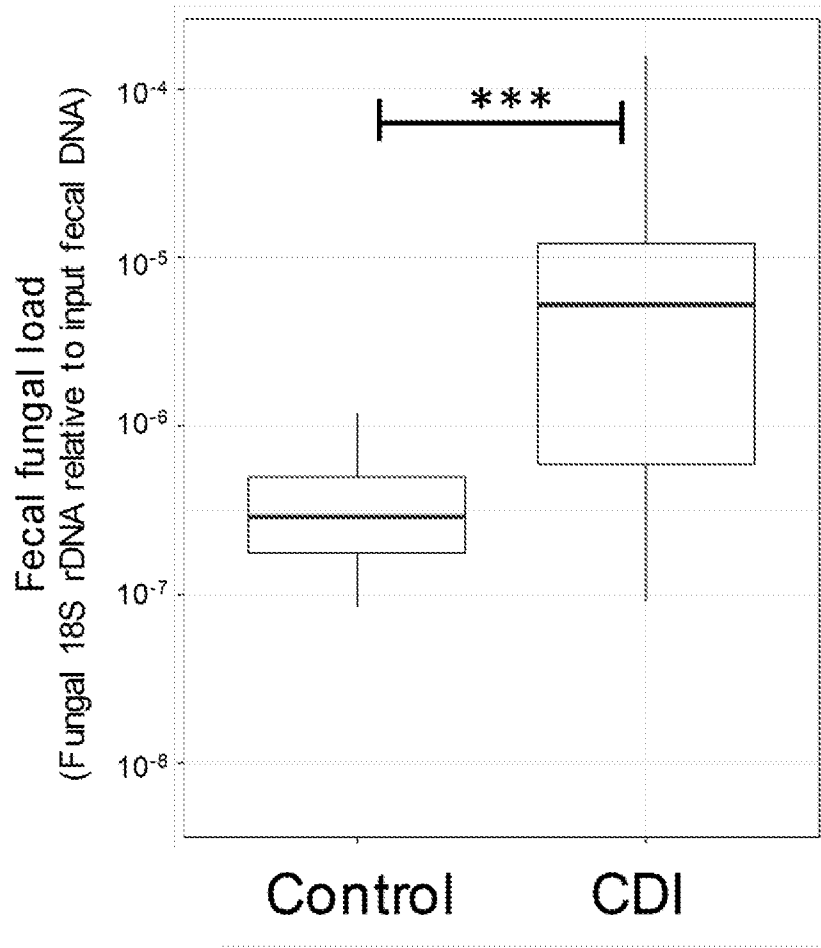


Fig. 18a

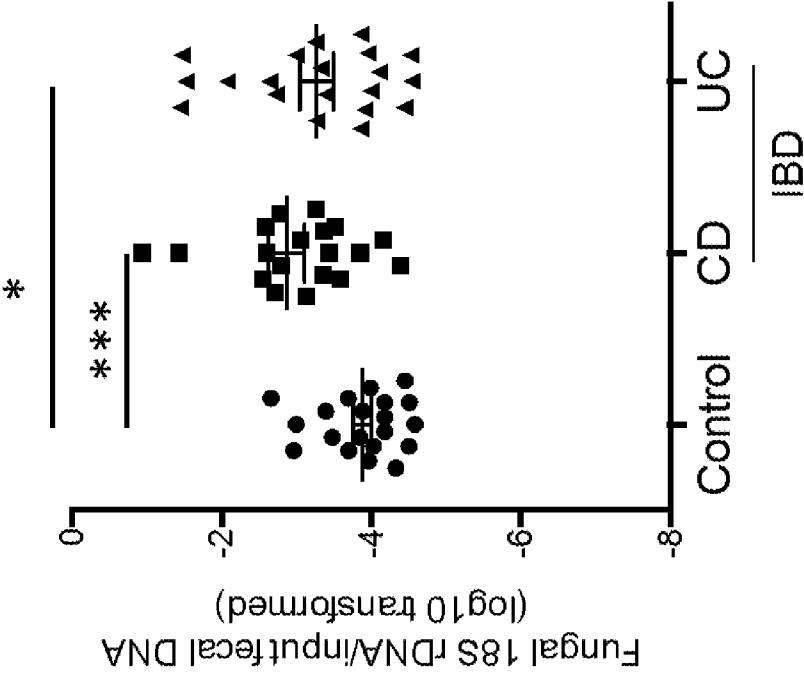


Fig. 18b

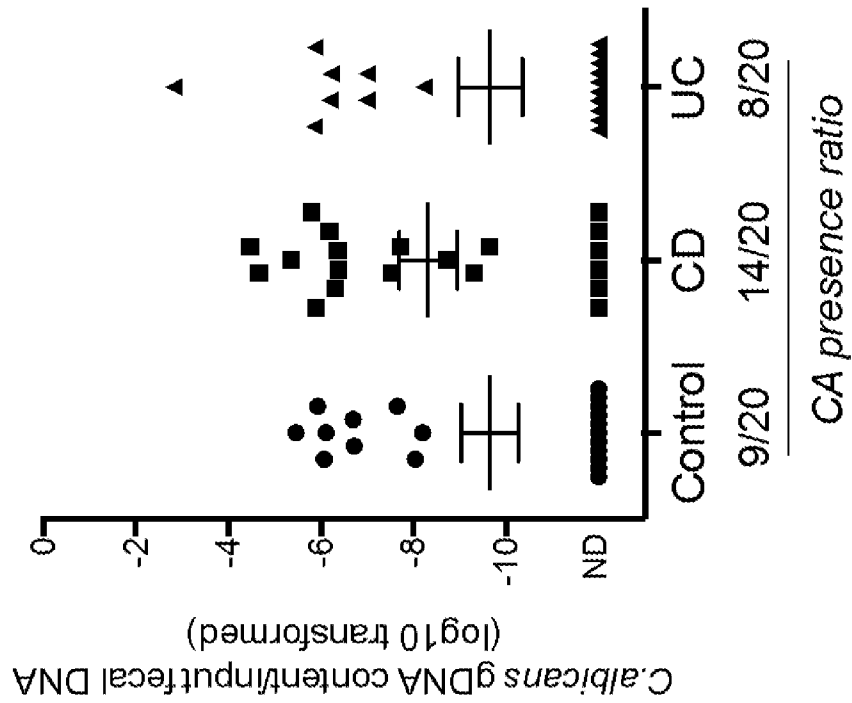


Fig. 19

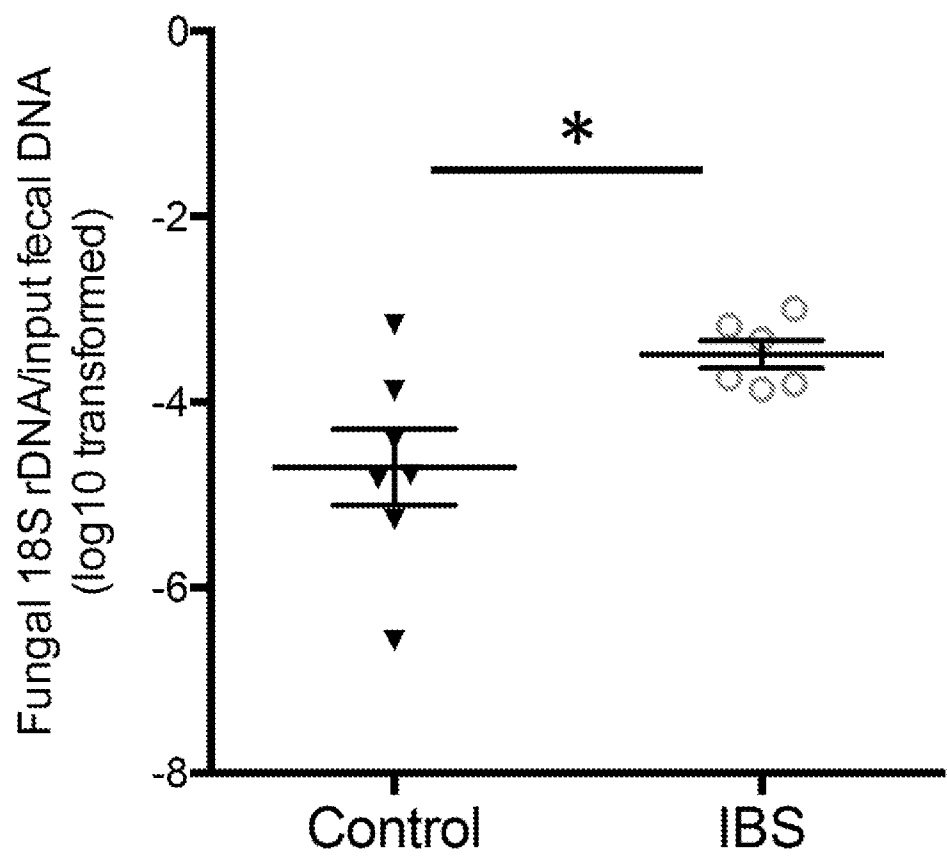


Fig. 20a

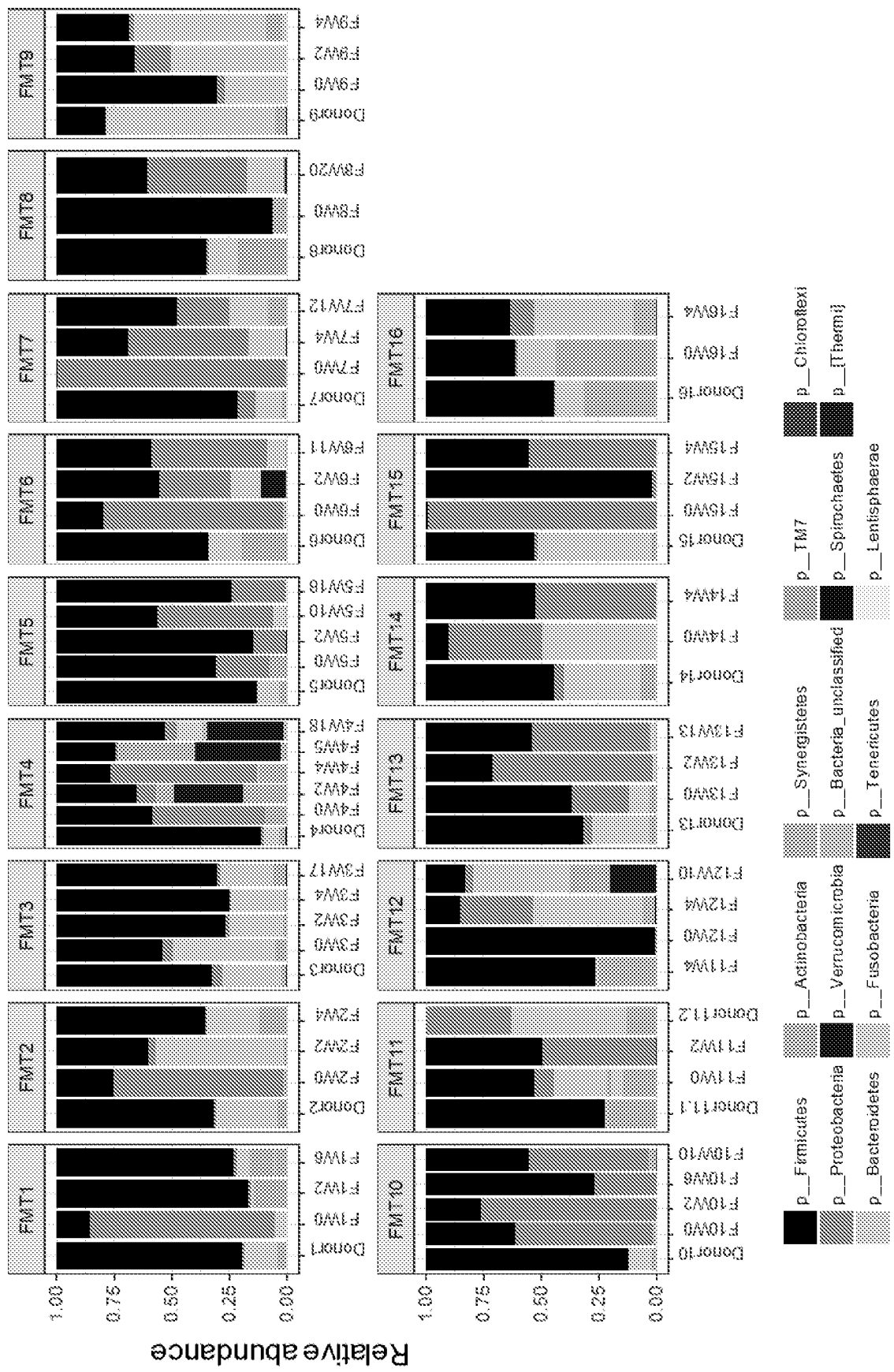


Fig. 20b

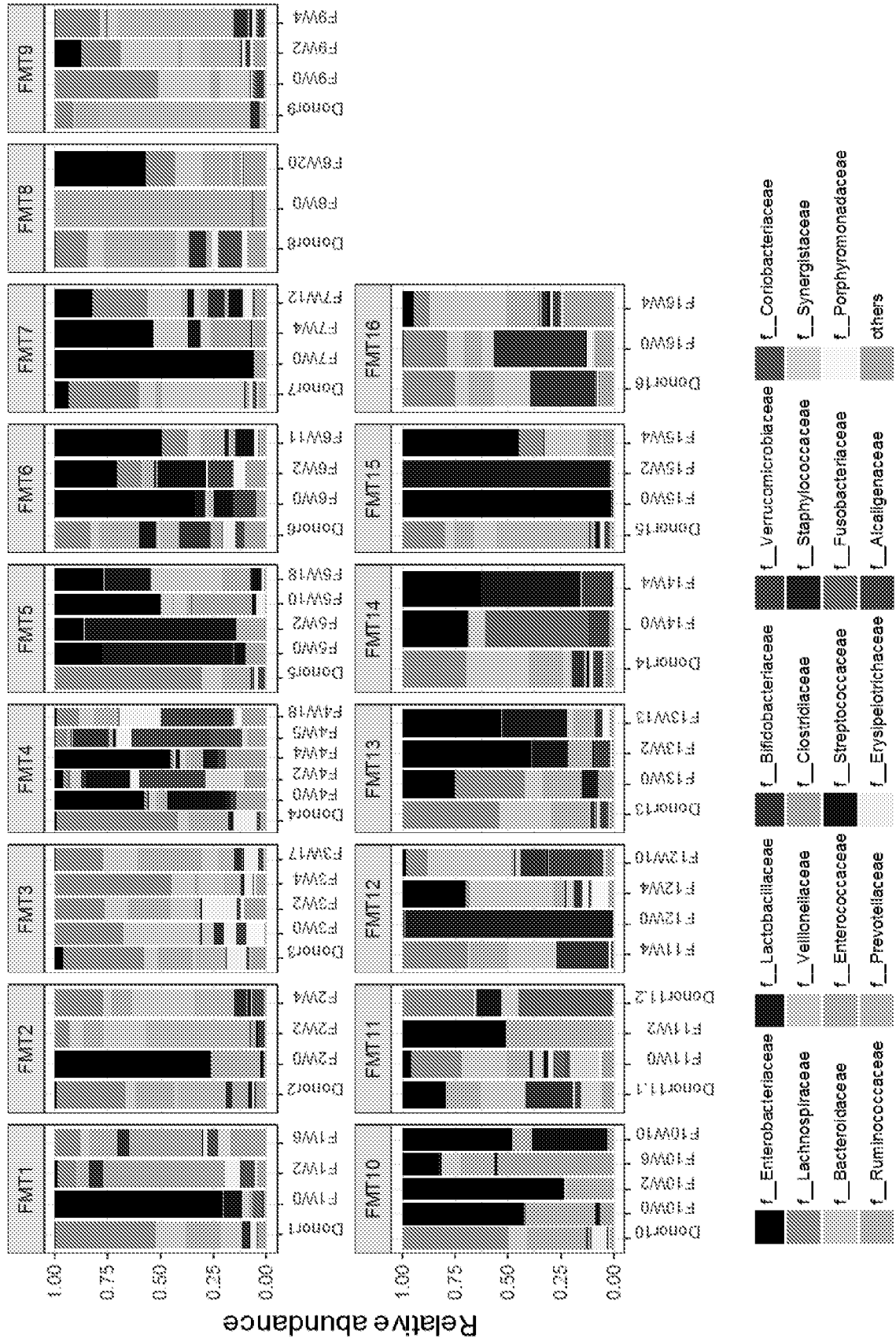


Fig. 20c

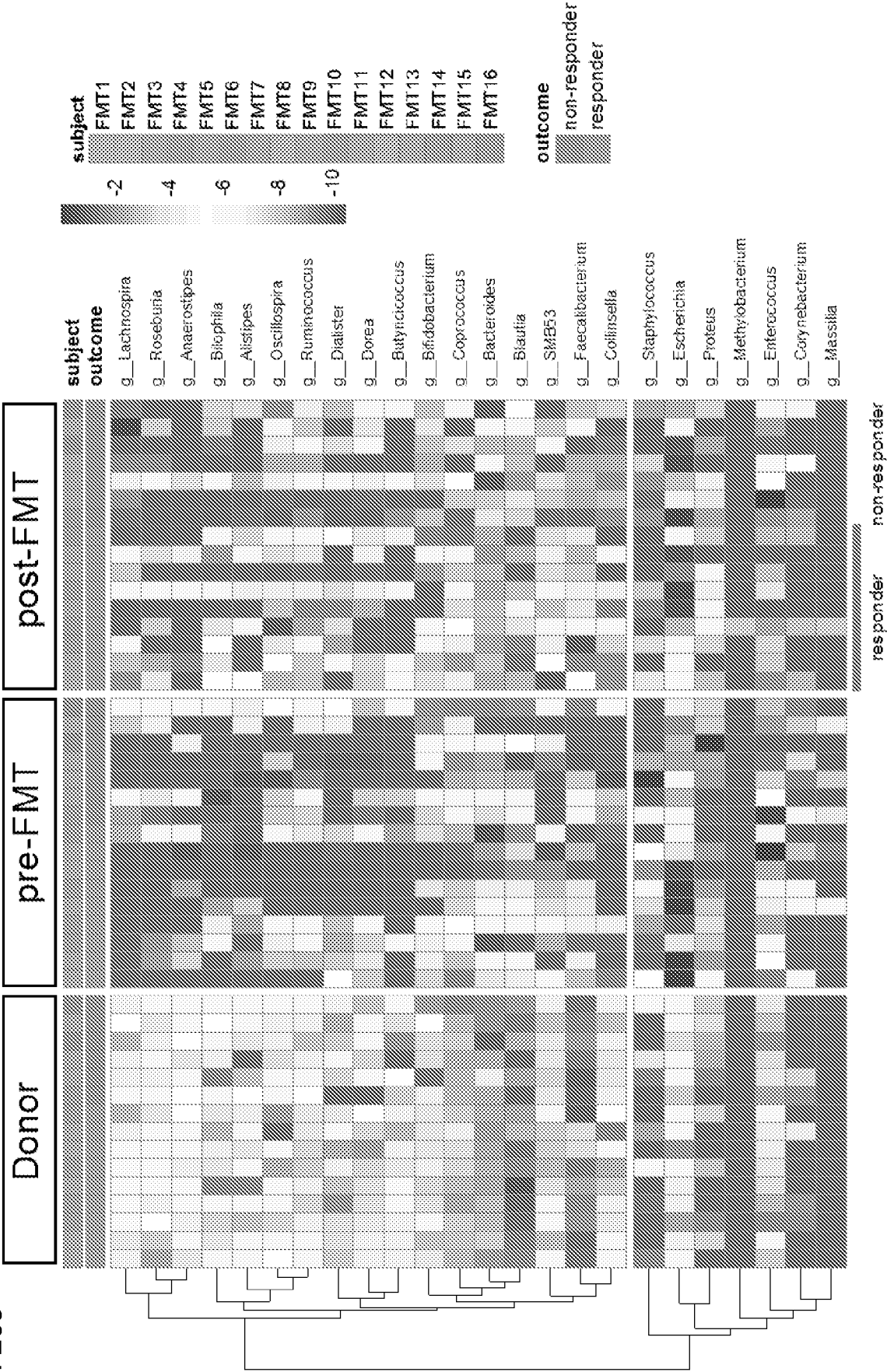


Fig. 20d

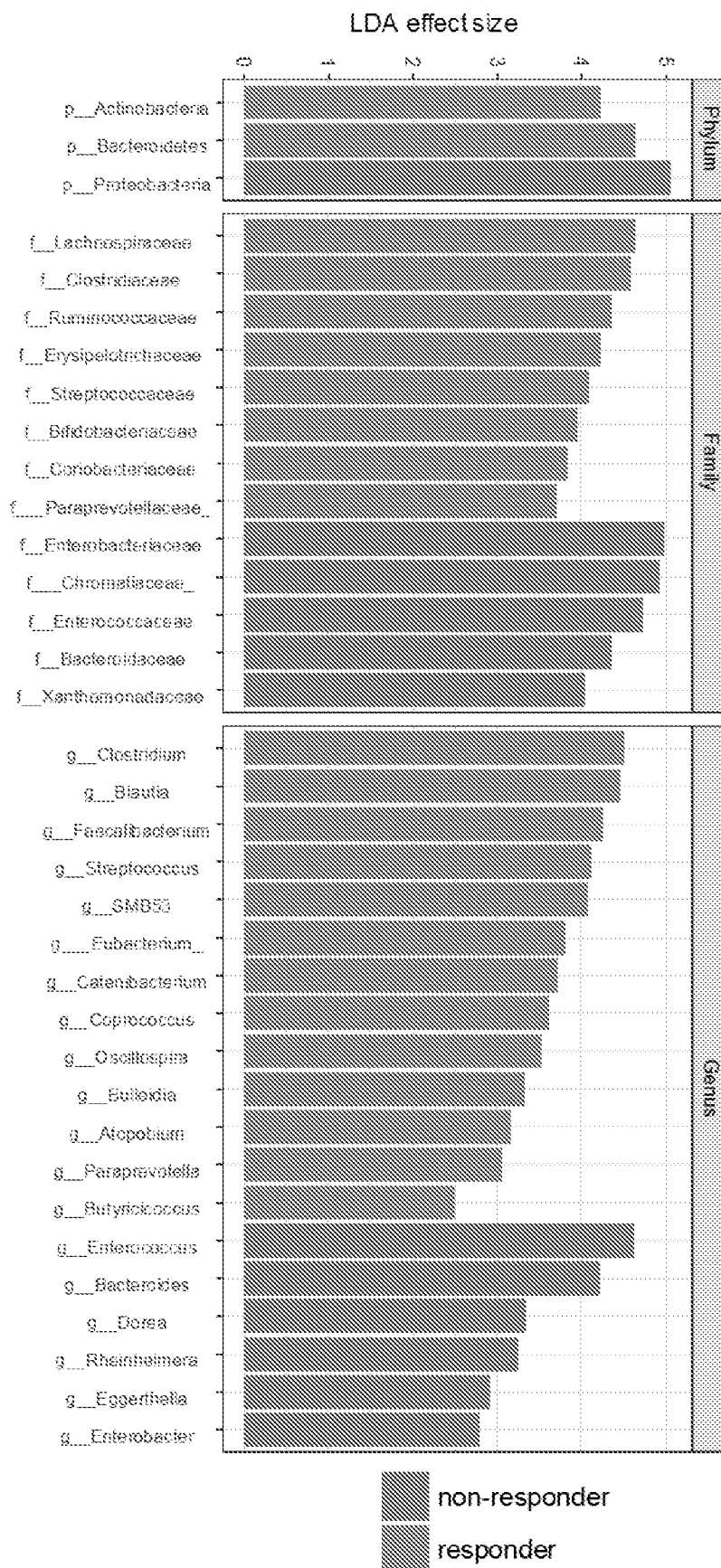
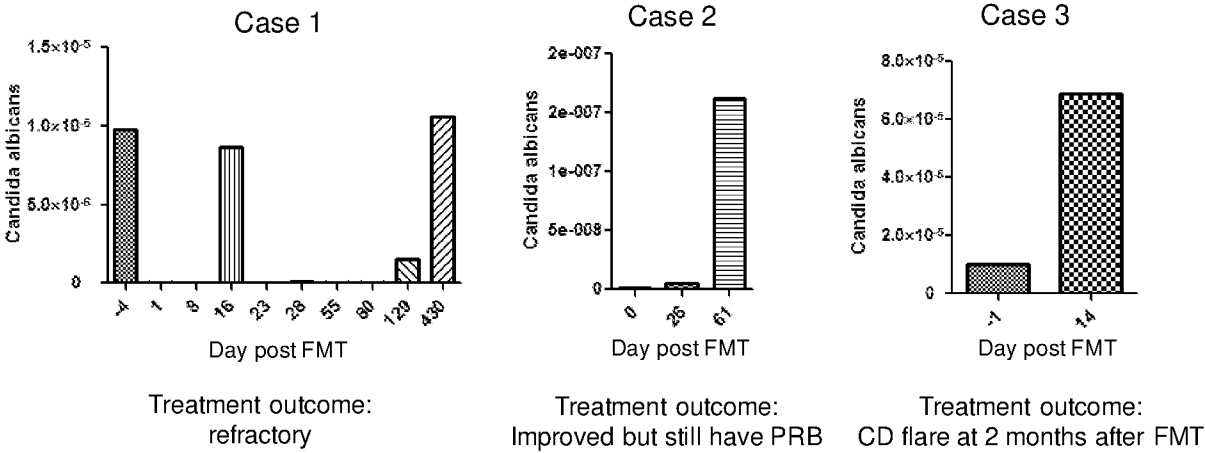


Fig. 21



INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2019/074353

A. CLASSIFICATION OF SUBJECT MATTER

C12Q 1/68(2018.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CNABS, VEN, WOTXT, USTXT, EPTXT, SIPOABS, DWPI, CNKI, GOOGLE, PubMed, ISI Web of Knowledge: THE CHINESE UNIVERSITY OF HONG KONG, ZUO TAO, fecal microbiota transplantation, FMT, fecal transplant, Clostridium difficile, C.difficile, CD, recipient, efficacy, effective, antifungal agent

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2015170979 A1 (IS-DIAGNOSTICS LTD.) 12 November 2015 (2015-11-12) description example 20, page 21 paragraph 4 to page 22 paragraph 1	1-25,29
Y	WO 2015170979 A1 (IS-DIAGNOSTICS LTD.) 12 November 2015 (2015-11-12) description example 20, page 21 paragraph 4 to page 22 paragraph 1	26-28
Y	WO 2017210428 A1 (CRESTOVO LLC) 07 December 2017 (2017-12-07) description paragraph [0009]	26-28
A	WO 2017060178 A1 (DA VOLTERRA) 13 April 2017 (2017-04-13) the whole document	1-29
A	WO 2017103550 A1 (MAAT PHARMA ET AL.) 22 June 2017 (2017-06-22) the whole document	1-29
A	ALLEGRETTI, J.R.et al. "Restoring the gut microbiome for the treatment of inflammatory bowel diseases" <i>World Journal of Gastroenterology</i> , Vol. 20, No. 13, 07 April 2014 (2014-04-07), pages 3468-3474	1-29

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

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Date of the actual completion of the international search

09 April 2019

Date of mailing of the international search report

28 April 2019

Name and mailing address of the ISA/CN

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Facsimile No. (86-10)62019451

Telephone No. 86-(010)-53961975

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2019/074353

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	OLDFIELD, E.C.et al. "Clinical update for the diagnosis and treatment of Clostridium difficile infection" <i>World Journal of Gastrointestinal Pharmacology and Therapeutics</i> , Vol. 05, No. 01, 06 February 2014 (2014-02-06), pages 1-26	1-29
A	ROSSEN, N.G.et al. "Fecal microbiota transplantation as novel therapy in gastroenterology:A systematic review" <i>World Journal of Gastroenterology</i> , Vol. 21, No. 17, 07 May 2015 (2015-05-07), pages 5359-5371	1-29

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/CN2019/074353

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
WO	2015170979	A1	12 November 2015	US	2017159108	A1	08 June 2017
				CA	2948134	A1	12 November 2015
				EP	3140424	A1	15 March 2017
WO	2017210428	A1	07 December 2017	US	2017348360	A1	07 December 2017
				AU	2017274416	A1	03 January 2019
				CA	3026414	A1	07 December 2017
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				CN	108135927	A	08 June 2018
				EP	3359165	A1	15 August 2018
				US	2018303867	A1	25 October 2018
WO	2017103550	A1	22 June 2017	JP	2019501156	A	17 January 2019
				CN	108430481	A	21 August 2018
				US	2018369147	A1	27 December 2018
				AU	2016370600	A1	28 June 2018
				EP	3389679	A1	24 October 2018
				KR	20180086506	A	31 July 2018
				CA	3007289	A1	22 June 2017
				IL	259888	D0	31 July 2018
				FR	3045383	A1	23 June 2017