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(54) Title: A COMMENSAL FUNGUS AND USES THEREOF

(57) Abstract: There is provided an isolated fungus belonging to the genus *Kazachstania*, a composition comprising same, and methods of using same, such as for preventing or treating an infection, a disease, or both.



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A COMMENSAL FUNGUS AND USES THEREOF**REFERENCE TO AN ELECTRONIC SEQUENCE LISTING**

[001] The contents of the electronic sequence listing (YEDA-P-012-PCT ST26.xml; size: 8,981,623 bytes; and date of creation: May 02, 2023) is herein incorporated by reference in its entirety.

CROSS-REFERENCE TO RELATED APPLICATION

[002] This application claims the benefit of priority of U.S. Provisional Patent Application No. 63/339,570, filed May 9, 2022, and of U.S. Provisional Patent Application No. 63/450,024 both titled "COMMENSAL FUNGUS AND USES THEREOF", filed March 5, 2023, all of which are hereby incorporated by reference in their entirety.

FIELD OF INVENTION

[003] The present invention relates to *inter alia* an isolated fungus, composition comprising same, and a method of using same, such as for preventing or treating a disease, an infection, or both.

BACKGROUND

[004] The genetic information that defines human beings includes the genomes of the human cells but also of the commensal microbiome. While the analysis of this metagenome has focused mainly on the abundant bacterial commensals, mucosal surfaces are also inhabited by fungi. However, the role of the mycobiota and their contributions to host fitness are less well understood. This is partially due to the underrepresentation of fungi in the microbiome of animal models that are kept under strict hygienic conditions.

[005] As one of the most common human fungal pathogens, *Candida albicans* causes hundreds of millions of symptomatic infections each year. Pathologies are frequently associated with immuno-deficiencies and range from superficial irritations of the skin and mucosae to life-threatening invasive infections of internal organs. In addition, inborn errors of IL-17 immunity are strongly linked to chronic mucocutaneous candidiasis (CMC). Fungal dissemination, leading to systemic infection, is believed to originate from the gut, where *C. albicans* normally reside as a harmless commensal. Candidiasis has been linked to filamentation of the fungus, which is strictly associated with the expression of the cytolytic

peptide toxin candidalysin that promotes barrier damage. Human individuals are colonized in childhood, and clonal fungal populations persist over their lifetime, mostly without any symptoms. Emerging evidence suggests that fungal colonization in fact rather benefits, than harms the host and that the gut mycobiota contributes to improving mammalian immunity. Commensal fungi, specifically *C. albicans*, were shown to affect the composition of the myeloid innate immune compartment and elicit cellular and humoral immunity. Insights into the diverse interactions of fungi with the mammalian hosts and other fungi, but also communication with bacterial commensals, could significantly aid the understanding of host physiology. Moreover, a better understanding might allow harnessing the impact of commensal fungi on human immunity for therapeutic purposes. The host-fungi interface remains, however, incompletely understood, not the least due to the lack of suitable experimental animal models and our limited insight into fungal commensalism.

[006] The dominant human fungal commensal, *C. albicans*, has also been reported as part of the mycobiota of wild mice. However, animals kept under special pathogen-free (SPF) conditions mostly lack *C. albicans* and generally harbor poorly developed mycobiota that also differ considerably between vendors. Laboratory mice generally even resist *C. albicans* colonization unless subjected to antibiotics (Abx), which are believed to be required to neutralize inhibiting bacteria, including *Lactobacillae*.

[007] There is still a great need for effective and safe treatment for *C. albicans* infections, such as by utilizing commensal fungi.

SUMMARY

[008] The present invention, in some embodiments, is based, at least in part, on the serendipitous identification of a novel fungal commensal of the *Kazachstania* genus that efficiently colonizes laboratory animals kept in SPF facilities without prior Abx conditioning. The isolated commensal fungus, disclosed herein, is termed *K. weizmannii*, and was shown to outcompete *C. albicans* during competitive seeding and even expelled *C. albicans* from stably colonized animals. Further, the current invention, in some embodiments, is based, at least in part, on the finding that unlike *C. albicans*, the non-filamenting *K. weizmannii* fungus did not disseminate or cause pathology in immune-suppressed animals. In fact, the inventors showed that *K. weizmannii* posed an inter-fungal competition that reduced the intestinal *C. albicans* load of mice mitigated fatal candidiasis.

WO 2023/218450 [007] The present invention, in some embodiments, is further based, at least in part, on the findings that colonization of fungi of the *Kazachstania* clade was found in some individuals to show inverse correlation with *C. albicans* abundance in the gut. PCT/IL2023/050470

[010] Therefore, the isolated fungus disclosed herein is suggested as a robust competitive commensal to *C. albicans*, and a method for mitigating systemic fungal pathology, is devised therewith.

[011] According to the first aspect, there is provided an isolated fungus belonging to the genus *Kazachstania* and deposited at the ATCC under the deposit number PTA-127318.

[012] According to another aspect, there is provided a composition comprising the isolated fungus, and a pharmaceutically acceptable carrier.

[013] According to another aspect, there is provided a composition comprising a fungus, wherein at least 90% of the fungus is the isolated fungus disclosed herein.

[014] According to another aspect, there is provided a method for preventing or treating an autoimmune disease in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising the isolated fungus disclosed herein, thereby preventing or treating an autoimmune disease in the subject.

[015] According to another aspect, there is provided a method for preventing or treating a fungal infection in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising the isolated fungus disclosed herein.

[016] According to another aspect, there is provided non-human animal subject comprising the isolated fungus disclosed herein.

[017] According to another aspect, there is provided a method for producing the non-human animal subject disclosed herein, comprising colonizing a gastrointestinal tract of a non-human animal subject with an effective amount of any one of: (a) the isolated fungus disclosed herein; and (b) the composition disclosed herein, thereby producing the non-human animal subject.

[018] In some embodiments, the composition is for use in the treatment or prevention of a disease in a subject in need thereof.

[019] In some embodiments, the disease comprises an autoimmune disease, an infectious disease or both.

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[020] In some embodiments, the autoimmune disease is a T helper 17 cells (Th17)-driven immunopathology.

[021] In some embodiments, the autoimmune disease is selected from the group consisting of: multiple sclerosis, psoriasis, and asthma.

[022] In some embodiments, the infectious disease is a fungal infection.

[023] In some embodiments, the fungal infection comprises a *Candida* infection.

[024] In some embodiments, the fungal infection comprises a *Candida albicans* infection.

[025] In some embodiments, the autoimmune disease is: (i) induced T helper 17 cells (Th17) activity; (ii) characterized by increased Th17 activity; or (iii) both (i) and (ii).

[026] In some embodiments, the Th17 activity is induced by a fungal infection.

[027] In some embodiments, the subject is afflicted with a fungal infection.

[028] In some embodiments, the treating comprises reducing the number or abundance of a fungus inducing said fungal infection in the gastrointestinal tract of said subject.

[029] In some embodiments, the reducing is: (i) by at least 30% compared to control; (ii) for at least 4 days; or (iii) both (i) and (ii).

[030] In some embodiments, the treating comprises reducing the activity, abundance, or both, of Th 17 cells in the subject.

[031] In some embodiments, the administering comprises colonizing the gut of the subject with the isolated fungus.

[032] In some embodiments, the non-human animal subject is gnotobiotic.

[033] In some embodiments, the non-human animal subject is a mouse.

[034] In some embodiments, the isolated fungus disclosed herein colonizes or is present in the gastrointestinal tract of the non-human animal subject.

[035] In some embodiments, the method further comprises rearing/culturing the non-human animal subject under pathogen free conditions.

[036] Unless otherwise defined, all technical and/or scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the invention, exemplary methods and/or materials are described below. In case of conflict, the patent specification,

WO 2023/218450 including definitions, will control. In addition, the materials, methods, and examples are PCT/IL2023/050470, are illustrative only and are not intended to be necessarily limiting.

[037] Further embodiments and the full scope of applicability of the present invention will become apparent from the detailed description given hereinafter. However, it should be understood that the detailed description and specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[038] **Figs. 1A-1H** include a scheme, graphs, fluorescent micrographs, and phylogenetic trees, showing the identification and characterization of *K. weizmannii*. **(1A)** Schematic non-limiting protocol of *C. albicans* colonization (adopted from Basu et al., 2000). **(1B)** Recoverable *C. albicans* in the feces of wt mice with Abx supplementation in the drinking water, 2 weeks after oral *C. albicans* inoculation. **(1C)** Recoverable fungi in feces of unexposed SPF mutant mice and *C. albicans* inoculated animals; stippled line, limit of detection. **(1D)** Recoverable *C. albicans* in the feces 2 weeks after oral inoculation - comparison of colonization between mutant and wt animals **(1E)** Flow cytometry and microscopical examination of fungal colonies recovered from mutant animals. **(1F)** Phylogenetic tree based on Maximum Parsimony of the 26S rDNA domains 1 and 2 (D1/D2) region of newly identified *K. weizmannii* in comparison to other yeast model species. **(1G)** Phylogenetic tree based on Maximum Parsimony of the 26S rDNA D1/D2 region of *K. weizmannii* in comparison to other *Kazachstania* species. **(1H)** Comparison of whole genome of newly identified *K. weizmannii* to whole genomes of *Kazachstania* species.

[039] **Figs. 2A-2F** include micrographs, graphs, a plot, and a curve showing *in vitro* culture characteristics of *K. weizmannii*. **(2A)** Representative images of *C. albicans* and *K. weizmannii* in liquid media in presence of filament-inducing conditions, n=3 independent experiments. **(2B)** A graph showing largest differences in growth between *K. weizmannii* and *C. albicans* with different carbon sources, based on a z-score > or < 2. Differences were calculated using the normalized area under the curve (AUC) of *K. weizmannii* and *C. albicans*. Normalization of the AUC are based on the growth of both species with glucose. Size of data points corresponds to the normalized AUC of *K. weizmannii* with the corresponding carbon source. All measurements were done using the Biolog Phenotype MicroArrays in biological triplicates. X-axis represents the difference in AUC between *K. weizmannii* and *C. albicans*,

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 while the x-axis shows the different carbon sources tested. The data points are color-coded according to the different types of carbon sources. **(2C)** A graph showing the largest differences in growth between *K. weizmannii* and *C. albicans* with different nitrogen sources, based on a z-score greater than or less than 2. Differences were calculated using AUC of *K. weizmannii* and *C. albicans* and normalized based on the growth of both species with glutamine. Size of data points corresponds to the normalized AUC of *K. weizmannii* with the corresponding carbon source. All measurements were performed using the Biolog Phenotype MicroArrays in biological triplicates. X-axis represents the difference in AUC between *K. weizmannii* and *C. albicans*, while the x-axis shows the different nitrogen sources tested. The data points are color-coded according to the different types of nitrogen sources. **(2D)** Plot of the largest differences in growth of *K. weizmannii* and *C. albicans* with different inhibitors to test their chemical sensitivity. Graph shows the largest differences in growth between *K. weizmannii* and *C. albicans* with different inhibitors, based on a z-score greater than or less than 2., calculated using AUC of *K. weizmannii* and *C. albicans*. AUC normalization was based on the growth of both species without any inhibitor at pH 5. The size of each data point corresponds to the AUC of *K. weizmannii* with the corresponding inhibitor. All measurements were done using the Biolog Phenotype MicroArrays for chemical sensitivity (PM21-PM25) in biological triplicates. **(2E)** Growth curves of *C. albicans*, *S. cerevisiae* and *K. weizmannii* under different Ph conditions in YPD medium; AUC, Area under curve, biological triplicates. **(2F)** Co-culture assay of *C. albicans* and *K. weizmannii*. GFP expressing *C. albicans* was detected by flowcytometry to discriminate between fungi, n=2 independent experiments.

[040] **Figs. 3A-3M** include fluorescent micrographs, graphs, and a scheme, showing *K. weizmannii* competition with *C. albicans* in colonized animals. **(3A)** A representative picture of a far-red fluorescent reporter *Kazachstania* strain (*K. weizmannii* ENO1-Mirf). **(3B-C)** Recoverable fecal *K. weizmannii* in colonized wild-type animals (without antibiotics) one and six months after single oral *K. weizmannii* ENO1-Mirf inoculation. **(3D)** Flow cytometric analysis of *C. albicans* SC5314 (ENO1-GFP), *K. weizmannii* (ENO1-Mirf), and mixed cultures used for oral inoculation **(3E)**. **(3E)** Representative plating analysis of feces of animals inoculated with 10^7 yeasts of *C. albicans* SC5314 (ENO1-GFP) or *K. weizmannii* (ENO1-Mirf), and mixed cultures (see **(3D)**) 3 weeks after administration. **(3F-3G)** Flow cytometric analysis of feces of animals orally inoculated with 10^7 yeasts of *C. albicans* SC5314 (ENO1-GFP), *K. weizmannii* (ENO1-Mirf), and mixed cultures (see **(D)**) 3 weeks after administration. Quantification (n=3-5 mice per group, 4 independent experiments) (see **(3D)**). **(3H)** Schematic non-limiting outline of a co-housing experiment. **(3I)** Representative flow cytometric feces analysis of colonized mice 4 weeks after initial colonization (before

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 [040] and 2 weeks after co-housing. (N=4 per group). (3J) Percentages of respective labelled fungi in feces of *C. albicans*, *K. weizmannii* colonized animals, before and after co-housing in Abx presence (except for day 48 and 58); antibiotics withdrawal on day 46 of cohousing, n=4 per group, representative of 2 independent experiments. (3K) Flow cytometric analysis of feces of germfree animals 1 week after oral inoculation of single fungi cultures or mixed *C. albicans* SC5314 (ENO1-GFP)/*K. weizmannii* (ENO1-Mirf) culture. (3L) Percentages and (3M) amount of *C. albicans* and *K. weizmannii* recovered from feces of germ-free animals colonized with *C. albicans* SC5314 (C), *K. weizmannii* (K), or mixed culture (C/K) compared to Abx-treated SPF animals 1 week after administration as determined by flow cytometry analysis (3K); representative of 2 independent experiments.

[041] **Figs. 4A-4F** include graphs showing humoral and cellular immune responses to *C. albicans* and *Kazachstania* sp. (4A) Flow cytometric analysis of myeloid blood cell compartment of mice 4 weeks colonized with *C. albicans* or *K. weizmannii*. Neutrophils, classical and non-classical monocytes are defined as Ly6G⁺ CD115⁻, Ly6C⁺ CD115⁺, and Ly6C⁺ CD115⁺ cells, respectively. N= 8-21 mice, pooled from 3 independent experiments. Gating strategy, left, results, right. (4B) Flow cytometric analysis of humoral anti-fungal reactivity. Gating strategy for the determination of serum immunoglobulin binding to cultured *C. albicans* or *K. weizmannii*. (4C) Anti- *C. albicans* and *K. weizmannii* IgA and IgG serum reactivity of single-colonized animals, measured as shown in (4B). Note that most but not all animals harbor cross-reactive sera. (4D) Representative gating strategy of RORγt⁺ T cells (left); percentage of RORγt⁺ cells among TCRβ CD4⁺ T cells 5 weeks upon *C. albicans* or *K. weizmannii* colonization, n=3-5 mice per group, 3 independent experiments (right). (4E) Representative gating strategy of RORγt⁺ T cells (left); percentage of RORγt⁺ cells among TCRβ CD4⁺ T cells 5 weeks upon *C. albicans* or *K. weizmannii* colonization, n=3-5 mice per group, 3 independent experiments (right). (4F) Epithelial cell (EC) coculture assay of *C. albicans*, *S. cerevisiae*, and *K. weizmannii*. EC damage was assessed using the LDH assay as described in (Allert et al., 2018).

[042] **Figs. 5A-5I** include a scheme, graphs, micrographs, and fluorescent micrographs, showing that commensal *C. albicans* but not *K. weizmannii* causes pathology in immunosuppressed animals. (5A) A non-limiting schematic outline of experimental set up for immunosuppression protocol. (5B) A weight curve of *C. albicans*-colonized mice and uncolonized controls upon cortisone 21-acetate injections, n=5 mice per group. (5C) Representative pictures of tongue candidiasis observed in *C. albicans*-colonized mice day 10 post immunosuppression. (5D) A representative picture of *C. albicans*-induced kidney

WO 2023/218450, pathology. (5L) A weight loss curve, comparison of *C. albicans* and *K. weizmannii* colonized mice, n=4-7 mice per group, 4 independent experiments, error bar represents SEM. (5F) Percent of initial weight in the endpoint of the experiment, graph represents mean with SD. (5G) Representative pictures of *C. albicans* and *K. weizmannii* recoverable by plating of feces (10 ng) and kidneys (10 mg) of *C. albicans* or *K. weizmannii* colonized animals (with Abx) 10 days after immunosuppression. (5H) Pathology score based on microscopic and macroscopic evaluation of kidneys, score scheme. (5I) Weight loss comparison between *K. weizmannii*- and non-colonized animals. n= 5 per group.

[043] **Figs. 6A-6F** include a scheme, graphs, micrographs, and fluorescent micrographs showing that *K. weizmannii* mitigates *C. albicans*-induced pathology in immunosuppressed mice. (6A) Recovered *C. albicans* in feces (during continuous Abx treatment), and upon Abx withdrawal, as analyzed by flow cytometry and cultivation. (6B) A weigh monitoring curve following immunosuppression of *C. albicans*-colonized animals kept on Abx or withdrawn from Abx. (6C) Representative pictures of recoverable *C. albicans* and *K. weizmannii* from feces of *C. albicans*-colonized animals with or without *K. weizmannii* supplementation in their drinking water, analyzed by flow cytometry and cultivation. (6D) A weight monitoring curve (% initial weight), comparison of immunosuppressed animals following *C. albicans*-colonization and *K. weizmannii*-outcompeted *C. albicans* colonization. (6E) Representative pictures of kidneys (PAS staining) of immunosuppressed animals following *C. albicans*-colonization and *K. weizmannii*-outcompeted *C. albicans* colonization and results of cultivations of 1 mg kidney homogenate. (6F) A weight monitoring curve (% initial weight) - *C. albicans* and *K. weizmannii*-outcompeted animals, error bar SEM.

[044] **Figs. 7A-7E** include schemes, diagrams, and graphs showing the presence of *Kazachstania* in human metagenomes. (7A) Schematic of bioinformatic screening strategy of metagenomes. Briefly (from right to left), comparative genome analysis identified nucleotides regions that are unique to the genomes of either *K. weizmannii* or *C. albicans* species, together with genus-level regions of their ITS sequences. These regions were used to screen thousands of shotgun metagenomics collected and assembled by the Global Microbial Gene Catalogue v1.0 (2) (see methods). (7B) Venn diagram. Number of human gut metagenomics samples positive for unique genomic markers of either *C. albicans* or *K. weizmannii* out of 7,059 metagenomics datasets. Overlap of positive samples is in mixed colors. (7C) Number of human-vagina metagenomes positive for species-specific sequences of either *C. albicans* or *K. weizmannii* (ITS or unique regions). (7D) Antigen-reactive T cell enrichment (ARTE) analysis of anti-fungal CD4+ T cell reactivity in blood of human individuals. (n= 10). (7E)

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ITS2 analysis of fecal samples of a cohort of 570 healthy individuals for presence of *Candida* and *Kazachstania* spp. PCT/IL2023/050470

[045] **Figs. 8A-8C** include micrographs and a table showing the identification of novel fungal isolate. **(8A)** A gel analysis of PCR products amplifying ITS1 region of *C. albicans* and fungal isolate. **(8B)** Identification of novel fungal sp. based on ITS1 sequence. **(8C)** A representative example of PCR sentinel screening using PCR primers for specific ITS1 of *K. weizmannii*.

[046] **Figs. 9A-9C** include a sequence, a micrograph and graphs showing the generation of a *K. weizmannii* reporter strain. **(9A)** A sequence of modified fluorescent reporter miRFP670 – further referred as 'Mirf'. **(9B)** PCR validation of proper insertion of Mirf into *K. weizmannii* genome and thus generation of ENO1-Mirf fusion protein. **(9C)** Representative pictures of recovery of *C. albicans* ENO1-GFP and *K. weizmannii* ENO1-Mirf from feces of single-colonized followed by cohousing (started as *Candida* or *Kazachstania*).

[047] **Figs. 10A-10D** include graphs and fluorescent micrographs showing the impact of *K. weizmannii* colonization on microbiome. **(10A)** Diversity and abundance of fecal bacterial genus and species of *K. weizmannii* colonized vs. non-colonized group (controls) determined by 16S sequencing, collapsed to genus and species levels. **(10B)** Normalized abundance of fecal *Lactobacillaceae* (divided into specific *Lactobacilli* species) and *Bifidobacteriaceae*, determined by 16S sequencing. **(10C-10D)** *In vivo* competition between *C. albicans* and *K. weizmannii* upon co-administration in Germ-free animals and in antibiotics treated wild type mice: FACS and cultivation analysis of culture inoculate for p.o. administration. Representative picture of recovery of *C. albicans* ENO1-GFP and *Kazachstania* ENO1-Mirf from feces of colonized animals using flow cytometry and cultivation.

[048] **Figs. 11A-11B** include graphs and micrographs showing analysis of *C. albicans*-colonized immunosuppressed animals. **(11A)** Absolute weight monitoring (grams) of individual mice in *C. albicans* colonization, *K. weizmannii* colonized and non-colonized group. **(11B)** Representative pictures of kidneys and tongues (PAS staining) of immunosuppressed non-colonized and *Kazachstania*-colonized animals in end time point (day 10).

[049] **Figs. 12A-12B** include graphs showing analysis of *K. weizmannii* and *C. albicans*-colonized immunosuppressed animals. **(12A)** Absolute weight (grams) monitoring, comparison of immunosuppressed animals following *C. albicans*-colonization and *K. weizmannii*-outcompeted *C. albicans* colonization, monitoring till the end of experiment (day 10). **(12B)** Absolute weight (grams) monitoring showing the prolonged course of

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immunosuppressive treatment (mice sacrificed upon >20% weight loss), comparison of immunosuppressed animals following *C. albicans*-colonization and *K. weizmannii*-outcompeted *C. albicans* colonization, *K. weizmannii* colonized and non-colonized animals.

[050] **Figs. 13A-13B** include tables showing metagenome analysis. **(13A)** Metadata and read counts of 22 metagenomes from human vaginal origin, in which either *K. weizmannii* or *C. albicans* were identified. **(13B)** Metadata and read counts of 32 metagenomes from human gut origin, in which *K. weizmannii* was identified. Number of mapped reads of *K. weizmannii* or *C. albicans* is noted, along with metadata information collected on the metagenomics projects from NCBI Sequence Read Archive (SRA) data.

DETAILED DESCRIPTION

Isolated fungus, and composition

[051] According to some embodiments, there is provided an isolated fungus deposited at the ATCC under the deposit number PTA-127318.

[052] According to some embodiments, there is provided an isolated fungus belonging to the genus *Kazachstania*.

[053] In some embodiments, the isolated fungus deposited at the ATCC under the deposit number PTA-127318 belongs to the genus *Kazachstania*.

[054] In some embodiments, the isolated fungus is a type of yeast.

[055] In some embodiments, the isolated fungus comprises at least one nucleic acid sequence as set forth in any one of SEQ ID Nos: 1-33.

[056] According to some embodiments, there is provided a composition comprising a fungus. In some embodiments, the fungus comprises the isolated fungus disclosed herein. In some embodiments, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 97%, at least 99%, or 100% of the fungus is the isolated fungus disclosed herein, or any value and range therebetween. Each possibility represents a separate embodiment of the invention.

[057] In some embodiments, 20-100%, 40-100%, 60-100%, 70-100%, or 80-100% of the fungus is the isolated fungus disclosed herein. Each possibility represents a separate embodiment of the invention.

[058] In some embodiments, there is provided a fungal composition, wherein the isolated fungus disclosed herein constitutes at least 20%, at least 30%, at least 40%, at least 50%, at

at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 97%, at least 99%, or 100% of fungi of the fungal composition, or any value and range therebetween. Each possibility represents a separate embodiment of the invention.

[059] In some embodiments, the isolated fungus disclosed herein constitutes 20-100%, 40-100%, 60-100%, 70-100%, or 80-100% of fungi of the fungal composition. Each possibility represents a separate embodiment of the invention.

[060] In some embodiments, the composition is for use in the treatment or prevention of a disease in a subject in need thereof.

[061] In some embodiments, the composition is for use in the preparation of a medicament or a product for the treatment or prevention of a disease in a subject in need thereof.

[062] In some embodiments, the disease comprises an autoimmune disease, an infectious disease or both.

[063] In some embodiments, an autoimmune disease comprises a T helper 17 cells (Th17)-derived immunopathology. In some embodiments, an autoimmune disease comprises: (i) induced T helper 17 cells (Th17) activity; (ii) characterized by increased Th17 activity; or (iii) both (i) and (ii).

[064] In some embodiments, Th17 activity is induced by a fungal infection. In some embodiments, Th17 activity comprises expression, translation, protein secretion, or any combination thereof, of at least one cytokine or a gene encoding thereof. In some embodiments, at least one cytokine comprises: interleukin (IL)-17A, IL-17F, IL-21, IL-22, CCL20, or any combination thereof.

[065] In some embodiments, expression comprises gene expression, e.g., transcription of a gene to a messenger RNA (mRNA). In some embodiments, translation comprises production of a protein product encoded according to the gene as disclosed, or an mRNA transcript thereof.

[066] Methods for determining any one of expression, translation, protein secretion, are common and would be apparent to one of skill in the art. Such methods include, but are not limited to, polymerase chain reaction (PCR), quantitative PCR (qPCR), western blot, enzyme linked immunosorbent assay (ELISA), flow cytometry, and others, some of which are exemplified herein below.

[067] As used herein, the term "T helper 17 cells (Th17)-derived immunopathology" encompasses any disease, such as an autoimmune disease, that involves, is induced,

comprises, characterized, enhanced, exaggerated, propagated, incited, or any combination thereof, Th17 cells.

[068] In some embodiments, an autoimmune disease comprises multiple sclerosis, psoriasis, asthma, or any combination thereof.

[069] In some embodiments, an infectious disease comprises or is an infection. In some embodiments, the infectious disease is a fungal infection. In some embodiments, the disease involves, is induced, comprises, characterized, enhanced, exaggerated, propagated, incited, or any combination thereof, by a fungus.

[070] In some embodiments, a fungal infection comprises a *Candida* infection.

[071] In some embodiments, a fungal infection comprises a *Candida albicans* infection.

[072] In some embodiments, the composition is a synthetic or an artificial composition.

[073] In some embodiments, the composition is formulated for colonization of a gut of a subject. In some embodiments, the composition is formulated for rectal delivery, oral delivery, parenteral delivery, or any combination thereof.

[074] As used herein, the terms “synthetic” or “artificial” are interchangeable and refer to a manmade composition, such as *in vitro* grown, cultured, or formulated composition, e.g., in a lab or a comparable facility.

[075] In some embodiments, the composition further comprises an acceptable carrier. In some embodiments, the carrier comprises or is a pharmaceutically acceptable carrier.

[076] As used herein, the term “carrier”, “excipient”, or “adjuvant” refers to any component of a composition, that is not the active agent, such as, but not limited to the bacterial consortium disclosed herein.

[077] In some embodiments, the carrier is a physiologically acceptable carrier. In one embodiment, the phrases “physiologically acceptable carrier” and “pharmaceutically acceptable carrier” which be interchangeably used refer to a carrier or a diluent that does not cause significant irritation to an organism and does not abrogate the biological activity and properties of the administered compound. An adjuvant is included under these phrases. In one embodiment, “excipient” refers to an inert substance added to a pharmaceutical composition to further facilitate administration of an active ingredient. In one embodiment, excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.

WO 2023/218450 As used herein, the term "carrier", "excipient", or "adjuvant" refers to any component of a pharmaceutical composition that is not the active agent. As used herein, the term "pharmaceutically acceptable carrier" refers to non-toxic, inert solid, semi-solid liquid filler, diluent, encapsulating material, formulation auxiliary of any type, or simply a sterile aqueous medium, such as saline. Some examples of the materials that can serve as pharmaceutically acceptable carriers are sugars, such as lactose, glucose and sucrose, starches such as corn starch and potato starch, cellulose and its derivatives such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt, gelatin, talc; excipients such as cocoa butter and suppository waxes; oils such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol, polyols such as glycerin, sorbitol, mannitol and polyethylene glycol; esters such as ethyl oleate and ethyl laurate, agar; buffering agents such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline, Ringer's solution; ethyl alcohol and phosphate buffer solutions, as well as other non-toxic compatible substances used in pharmaceutical formulations. Some non-limiting examples of substances which can serve as a carrier herein include sugar, starch, cellulose and its derivatives, powdered tragacanth, malt, gelatin, talc, stearic acid, magnesium stearate, calcium sulfate, vegetable oils, polyols, alginic acid, pyrogen-free water, isotonic saline, phosphate buffer solutions, cocoa butter (suppository base), emulsifier as well as other non-toxic pharmaceutically compatible substances used in other pharmaceutical formulations. Wetting agents and lubricants such as sodium lauryl sulfate, as well as coloring agents, flavoring agents, excipients, stabilizers, antioxidants, and preservatives may also be present. Any non-toxic, inert, and effective carrier may be used to formulate the compositions contemplated herein. Suitable pharmaceutically acceptable carriers, excipients, and diluents in this regard are well known to those of skill in the art, such as those described in The Merck Index, Thirteenth Edition, Budavari et al., Eds., Merck & Co., Inc., Rahway, N.J. (2001); the CTFA (Cosmetic, Toiletry, and Fragrance Association) International Cosmetic Ingredient Dictionary and Handbook, Tenth Edition (2004); and the "Inactive Ingredient Guide," U.S. Food and Drug Administration (FDA) Center for Drug Evaluation and Research (CDER) Office of Management, the contents of all of which are hereby incorporated by reference in their entirety. Examples of pharmaceutically acceptable excipients, carriers and diluents useful in the present compositions include distilled water, physiological saline, Ringer's solution, dextrose solution, Hank's solution, and DMSO. These additional inactive components, as well as effective formulations and administration procedures, are well known in the art and are described in standard textbooks, such as Goodman and Gillman's:

WO 2023/218450 The Pharmacological Bases of Therapeutics, 8th Ed., Gilman et al. **PCT/IL2023/050470**, McGraw-Hill Press (1990); Remington's Pharmaceutical Sciences, 18th Ed., Mack Publishing Co., Easton, Pa. (1990); and Remington: The Science and Practice of Pharmacy, 21st Ed., Lippincott Williams & Wilkins, Philadelphia, Pa., (2005), each of which is incorporated by reference herein in its entirety. The presently described composition may also be contained in artificially created structures such as liposomes, ISCOMS, slow-releasing particles, and other vehicles which increase the half-life of the peptides or polypeptides in serum. Liposomes include emulsions, foams, micelles, insoluble monolayers, liquid crystals, phospholipid dispersions, lamellar layers and the like. Liposomes for use with the presently described peptides are formed from standard vesicle-forming lipids which generally include neutral and negatively charged phospholipids and a sterol, such as cholesterol. The selection of lipids is generally determined by considerations such as liposome size and stability in the blood. A variety of methods are available for preparing liposomes as reviewed, for example, by Coligan, J. E. et al, Current Protocols in Protein Science, 1999, John Wiley & Sons, Inc., New York, and see also U.S. Pat. Nos. 4,235,871, 4,501,728, 4,837,028, and 5,019,369.

Methods of use

[079] According to some embodiments, there is provided a method for preventing or treating an autoimmune disease in a subject in need thereof.

[080] According to some embodiments, there is provided a method for preventing or treating a fungal infection in a subject in need thereof.

[081] In some embodiments, the method comprises administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising the isolated fungus disclosed herein.

[082] In some embodiments, the subject is afflicted with a fungal infection.

[083] In some embodiments, the subject is a mammal subject, such as, but not limited to a human subject.

[084] In some embodiments, treating comprises reducing the number or abundance of a fungus inducing the fungal infection in the subject.

[085] In some embodiments, the number or abundance of a fungus inducing the fungal infection is reduced in the gastrointestinal tract (GI) of the subject.

[086] In some embodiments, reduction of the number or abundance of a fungus inducing the fungal infection is determined in a sample obtained or derived from the subject.

[007] In some embodiments, treating comprises reducing or inhibiting the activity of a fungus inducing the fungal infection in the subject. In some embodiments, reducing or inhibiting is of any one of: attachment, germination, penetration, host tissue colonization, growth, proliferation, metabolism, spore release, hyphae production, hyphae penetration, or any combination thereof, of a fungus inducing the fungal infection.

[088] In some embodiments, the method further comprises a step of determining a number or abundance of a fungus inducing the fungal infection in the subject.

[089] In some embodiments, the determining is in a sample obtained or derived from the subject. In some embodiments, the sample is obtained or derived from the GI of the subject.

[090] In some embodiments, reducing is by at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 99%, or 100% compared to a control, or any value and range therebetween. Each possibility represents a separate embodiment of the invention.

[091] In some embodiments, inhibiting comprises 100% reducing.

[092] In some embodiments, reducing is for at least 4 days, at least 7 days, at least 14 days, at least 28 days, at least 2 months, at least 4 months, at least 6 months, or at least 1 year, or any value and range therebetween. Each possibility represents a separate embodiment of the invention.

[093] In some embodiments, reducing is: (i) by at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 99%, or 100% compared to a control, or any value and range therebetween; and (ii) for at least 4 days, at least 7 days, at least 14 days, at least 28 days, at least 2 months, at least 4 months, at least 6 months, or at least 1 year, or any value and range therebetween. Each possibility represents a separate embodiment of the invention.

[094] In some embodiments, a control comprises a subject not being treated according to there herein disclosed method. In some embodiments, a control comprises the subject prior to being treated or administered according to the herein disclosed method.

[095] In some embodiments, treating comprises reducing the activity, abundance, or both, of Th 17 cells in the subject. In some embodiments, reducing is in the GI of the subject.

[096] In some embodiments, administering comprises colonizing the gut of the subject with the isolated fungus.

[0097] In some embodiments, administering comprises rectally administering. In some embodiments, administering comprises orally administering. In some embodiments, administering comprises rectally and orally administering.

[0098] As used herein the term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known manners, means, techniques and procedures by practitioners of the chemical, pharmacological, biological, biochemical, and medical arts.

[0099] As used herein, the term "treating" includes abrogating, substantially inhibiting, slowing or reversing the progression of a condition, substantially ameliorating clinical or aesthetical symptoms of a condition or substantially preventing the appearance of clinical or aesthetical symptoms of a condition. Further, treat, treatment, treating, as used herein, means any of the following: the reduction in severity of a hemostatic disorder; the prophylaxis of one or more symptoms associated with a hemostatic disorder, e.g., a bleeding episode; the reduction in the duration of a disease course of a hemostatic disorder; the amelioration of one or more symptoms associated with a hemostatic disorder; the reduction in duration of a bleeding episode associated with a hemostatic disorder; the provision of beneficial effects to a subject with a hemostatic disorder, without necessarily curing the hemostatic disorder.

[0100] As used herein, the terms "preventing" or "prevention" of a disease, disorder, or condition encompasses the delay, prevention, suppression, or inhibition of the onset of a disease, disorder, or condition. As used in accordance with the presently described subject matter, the term "prevention" relates to a process of prophylaxis in which a subject is exposed to the presently described compositions or composition prior to the induction or onset of the disease/disorder process. This could be done where an individual has a genetic pedigree indicating a predisposition toward occurrence of the disease/disorder to be prevented. For example, this might be true of an individual whose ancestors show a predisposition toward certain types of, for example, inflammatory disorders. The term "suppression" is used to describe a condition wherein the disease/disorder process has already begun but obvious symptoms of the condition have yet to be realized. Thus, the cells of an individual may have the disease/disorder, but no outside signs of the disease/disorder have yet been clinically recognized. In either case, the term prophylaxis can be applied to encompass both prevention and suppression. Conversely, the term "treatment" refers to the clinical application of active agents to combat an already existing condition whose clinical presentation has already been realized in a patient.

Non-human animal and a method for preparing same

[0102] According to some embodiments, there is provided a non-human animal subject. In some embodiments, the non-human animal subject comprises the isolated fungus disclosed herein.

[0103] In some embodiments, the non-human animal subject is gnotobiotic.

[0104] The term "gnotobiotic" would be apparent to one of skill in the art and encompasses any animal being characterized in a way that all microorganisms interacting with it are known and controlled.

[0105] In some embodiments, the non-human animal subject is a mammal. In some embodiments, the non-human animal subject is a rodent. In some embodiments, the non-human animal subject is mouse. In some embodiments, the non-human animal subject is a rat.

[0106] In some embodiments, the non-human animal subject comprises the isolated fungus disclosed herein, such as, in the gastrointestinal tract. In some embodiments, the gastrointestinal tract of the non-human animal subject is colonized with or comprises the isolated fungus disclosed herein. In some embodiments, the isolated fungus disclosed herein, colonizes or is present in the gastrointestinal tract of the non-human animal subject. In some embodiments, the isolated fungus disclosed herein, colonizes or is present in the gastrointestinal tract of the non-human animal subject, such that it constitutes at least 0.01%, 0.1%, 10% of fungus cells and/or fungal species in the gastrointestinal tract of the non-human animal subject, or any value and range therebetween. Each possibility represents a separate embodiment of the invention.

[0107] According to some embodiments, there is provided a method for producing the non-human animal subject disclosed herein.

[0108] In some embodiments, the method comprises colonizing a gastrointestinal tract of a non-human animal subject with an effective amount of the isolated fungus disclosed herein.

[0109] In some embodiments, the method comprises colonizing a gastrointestinal tract of a non-human animal subject with an effective amount of the composition disclosed herein.

[0110] In some embodiments, the method comprises colonizing a gastrointestinal tract of a non-human animal subject with an effective amount of the isolated fungus disclosed herein, and the composition disclosed herein.

WO 2023/218450 [0111] In some embodiments, colonizing comprises rectally administering, orally administering, parenterally administering, or any combination thereof. PCT/IL2023/050470

[0112] In some embodiments, the method further comprises rearing and/or culturing the non-human animal subject disclosed herein.

[0113] In some embodiments, the rearing and/or culturing is performed under pathogen free conditions, such as, specific pathogen free (SPF). In some embodiments, the rearing and/or culturing is performed under sterile conditions.

Kit and method for determination

[0114] According to another aspect, there is provided a kit for genotypic determination of the presence of the isolated fungus disclosed herein. In some embodiments, the determination is in a sample. In some embodiments, the determination is in vitro or ex vivo determination, e.g., in a tube, a plate, or any equivalent thereof being apparent to a person of skill in the art as means for genotypic determination. In another embodiment, the present invention further provides a pair of primers capable of amplifying a nucleic acid molecule comprising any one of SEQ ID Nos: 1-33 in a polymerase chain reaction (PCR). In another embodiment, the present invention further provides a probe capable of hybridizing to a nucleic acid molecule comprising any one of SEQ ID Nos: 1-33. In another embodiment, a kit comprises primers and PCR reagents. In another embodiment, a kit comprises the probe.

[0115] In another embodiment, the present invention further provides a method for determining the presence of isolated fungus disclosed herein in a sample, comprising the steps of: (a) extracting DNA from the sample, (b) contacting the extracted DNA from step (a) with a pair of primers capable of hybridizing to a nucleic acid molecule comprising any one of SEQ ID Nos: 1-33 in a PCR, and obtaining a PCR composition; (c) subjecting the PCR composition to PCR amplification and obtaining a product; and (d) screening the product of step (c) to the presence of the nucleic acid molecule comprising any one of SEQ ID Nos: 1-33; wherein the presence of the nucleic acid molecule comprising any one of SEQ ID Nos: 1-33 within the product indicates that an isolated fungus as disclosed herein is present in the sample, thereby determining the presence of the isolated fungus as disclosed herein in the sample. In another embodiment, the absence of the nucleic acid comprising any one of SEQ ID Nos: 1-33 indicates that the sample is devoid of the isolated fungus as disclosed herein.

[0116] In some embodiments, PCR comprises denaturing double-stranded DNA in a sample (to separate the complementary strands), annealing the primers to the dissociated DNA

WO 2023/218450 extension reaction from the primers catalyzed by **PCT/IL2023/050470** a thermostable DNA polymerase, the cycle is then repeated.

[0117] In some embodiments, a pair of DNA primers as described herein are specifically complementary to and hybridizing with opposite strands DNA with one to the left (5') and one to the right (3') of the target sequence within the nucleic acid sequence set forth in any one of SEQ ID Nos: 1-33, to be amplified. In some embodiments, the nucleic acid molecule set forth in any one of SEQ ID Nos: 1-33, is a specific marker of the isolated fungus disclosed herein. In some embodiments, the existence of a nucleic acid molecule comprising any one of SEQ ID Nos: 1-33 in a sample, or DNA extracted from a sample as described herein provides direct evidence for the presence of isolated fungus as described herein. In some embodiments, DNA is total DNA.

[0118] In one embodiment, a kit as described herein further comprises a DNA polymerase. In one embodiment, a kit as described herein further comprises a thermostable DNA polymerase.

[0119] As used herein, the term "screening" comprises identifying, isolating, enriching or any combination thereof. In one embodiment, methods for visualizing the nucleic acid molecule as described herein or the amplicons generated in the PCR is gel electrophoresis in polyacrylamide or agarose, followed by ethidium bromide staining. The observed sizes of the amplified target fragment – the nucleic acid molecule as described herein, should be identical to the predicted size based on the known nucleotide sequence as described and exemplified. In one embodiment, methods for visualizing the nucleic acid molecule as described herein or the amplicons generated in the PCR comprise Southern blot probing, dot-blot, or any known DNA hybridization technique wherein the nucleic acid molecule as described herein is utilized as a probe. In one embodiment, methods for visualizing the nucleic acid molecule as described herein or the amplicons generated in the PCR comprise a dissociation curve, a high-resolution melting curve, or any other DNA melting technique known in the art.

[0120] In some embodiments, the kit as described herein comprises a PCR buffer. In some embodiments, a PCR buffer comprises: 5 to 100 mM Tris-HCl and 20 to 100 mM KCl. In some embodiments, a PCR buffer further comprises 10 to 100 mM Magnesium Chloride. In some embodiments, the kit as described herein comprise a dNTP mixture. In some embodiments, the kit as described herein comprises DNA Polymerase such as but not limited to Taq DNA Polymerase. In some embodiments, the kit as described herein comprises distilled water.

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[0121] An example deposit of the *Kazachstania weizmannii* of budding yeast has been deposited under ATCC PTA-127318.

General

[0122] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

[0123] As used herein, the term "about" when combined with a value refers to plus and minus 10% of the reference value. For example, a length of about 1,000 nanometers (nm) refers to a length of 1,000 nm $\pm$ 100 nm.

[0124] It is noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a polynucleotide" includes a plurality of such polynucleotides and reference to "the polypeptide" includes reference to one or more polypeptides and equivalents thereof known to those skilled in the art, and so forth. It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as "solely," "only" and the like in connection with the recitation of claim elements or use of a "negative" limitation.

[0125] In those instances where a convention analogous to "at least one of A, B, and C, etc." is used, in general such a construction is intended in the sense one having skill in the art would understand the convention (e.g., "a system having at least one of A, B, and C" would include but not be limited to systems that have A alone, B alone, C alone, A and B together, A and C together, B and C together, and/or A, B, and C together, etc.). It will be further understood by those within the art that virtually any disjunctive word and/or phrase presenting two or more alternative terms, whether in the description, claims, or drawings, should be understood to contemplate the possibilities of including one of the terms, either of the terms, or both terms. For example, the phrase "A or B" will be understood to include the possibilities of "A" or "B" or "A and B".

[0126] It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single

embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination. All combinations of the embodiments pertaining to the invention are specifically embraced by the present invention and are disclosed herein just as if each and every combination was individually and explicitly disclosed. In addition, all sub-combinations of the various embodiments and elements thereof are also specifically embraced by the present invention and are disclosed herein just as if each and every such sub-combination was individually and explicitly disclosed herein.

[0127] Additional objects, advantages, and novel features of the present invention will become apparent to one ordinarily skilled in the art upon examination of the following examples, which are not intended to be limiting. Additionally, each of the various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below finds experimental support in the following examples.

[0128] Various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below find experimental support in the following examples.

DEPOSIT INFORMATION

[0129] A deposit of *Kazachstania weizmannii* budding yeast disclosed above and recited in the claims, has been made with the ATCC® Patent Depository (10801 University Boulevard, Manassas, Virginia 20110 USA) on June 22, 2022. The accession number for those deposited budding yeast is PTA-127318. Upon issuance of a patent, all restrictions upon the deposit will be removed. The deposit has been accepted under the Budapest Treaty and will be maintained in the depository for a period of 30 years, or 5 years after the last request, or for the effective life of the patent, whichever is longer, and will be replaced if necessary during that period.

EXAMPLES

[0130] Generally, the nomenclature used herein, and the laboratory procedures utilized in the present invention include molecular, biochemical, microbiological, and recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example, "Molecular Cloning: A laboratory Manual" Sambrook et al., (1989); "Current Protocols in Molecular Biology" Volumes I-III Ausubel, R. M., ed. (1994); Ausubel et al., "Current Protocols in Molecular Biology", John Wiley and Sons, Baltimore, Maryland

WO 2023/218450, PCT/IL2023/050470, "A Practical Guide to Molecular Cloning", John Wiley & Sons, New York (1982); Sambrook et al., "Molecular Cloning", Cold Spring Harbor Laboratory Press, New York (1989); Watson et al., "Recombinant DNA", Scientific American Books, New York; Birren et al. (eds) "Genome Analysis: A Laboratory Manual Series", Vols. 1-4, Cold Spring Harbor Laboratory Press, New York (1998); methodologies as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057; "Cell Biology: A Laboratory Handbook", Volumes I-III Cellis, J. E., ed. (1994); "Culture of Animal Cells - A Manual of Basic Technique" by Freshney, Wiley-Liss, N. Y. (1994), Third Edition; "Current Protocols in Immunology" Volumes I-III Coligan J. E., ed. (1994); Stites et al. (eds), "Basic and Clinical Immunology" (8th Edition), Appleton & Lange, Norwalk, CT (1994); Mishell and Shiigi (eds), "Strategies for Protein Purification and Characterization - A Laboratory Course Manual" CSHL Press (1996); all of which are incorporated by reference. Other general references are provided throughout this document.

Materials and Methods

Mice

[0131] This study involved wildtype and mutant mice (Cx3cr1Cre:Il23a^{fl/fl} (59, 60), all on C57BL/6 background. Unless indicated otherwise, animals were maintained in a specific-pathogen-free facility with chow and water provided ad libitum. Experiments were performed using sex- and age-matched controls. Animals were handled according to protocols approved by the Weizmann Institute Animal Care Committee as per international guidelines.

Microbes

[0132] The recombinant *C. albicans* SC5314 strain expressing ENO1-GFP fusion protein is as described in Gonia et al., 2017. *K. weizmannii* was first cultivated from feces of mice housed in the Weizmann Institute SPF facility. The fluorescent *K. weizmannii* strain with a fusion of ENO1 to the modified mRFP670 (herein *Kazachstania-Mirf*) was generated using Crispr/Cas9 targeted mutagenesis. *C. albicans* strain was cultured on solid YPD media at 30 °C for 24 h-36 h. *K. weizmannii* and *Kazachstania-Mirf* were cultured on solid YPD media at 37 °C.

Fungal gut colonization

[0133] To establish intestinal colonization with *C. albicans* and *K. weizmannii* the drinking water of mice was supplemented with ampicillin (1 mg/mL, Ampicillin sodium salt 5 G SIGMA cat. 9518) 2-3 days prior to oral fungal inoculation. Mice were maintained on Abx-supplemented drinking water throughout the whole experiment, unless stated otherwise. For oral inoculation, *C. albicans* and *K. weizmannii* were grown on solid YPD media 30 °C, or

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57 C respectively. Cultures were washed with PBS, and 10^7 yeast cells in 50 μ l PBS were administered dropwise into the mouths of mice.

Fungal-mediated pathology

[0134] Stably fungal-colonized mice were $5\times$ s.c injected with 225 mg·kg⁻¹ with cortisone 21-acetate, following the scheme of injection every other day as described previously (Solis and Filler, 2012). Mice were monitored for weight loss. Following sacrifice, organs and feces were collected for histological examination, flow cytometry and fungal cultivations). If the weight dropped to 20% of the starting weight, mice were sacrificed according to IACUC protocol.

Determination of CFU/g tissue/feces

[0135] For enumerating the number of recoverable *C. albicans* and *K. weizmannii* colony forming units, individual fecal pellets or each tissue from mice was sterilely dissected, weighed, and homogenized in sterile DDW. Serial dilutions on the organ homogenate were spread onto YPD media plates and the number of individual colonies enumerated after incubation at 37 °C for 24 hours.

Fecal DNA extraction for 16S rRNA gene sequencing

[0136] DNeasy Blood & Tissue Kit (cat. 69504) kit was used according to the manufacturer's instructions for fecal DNA isolation for 16S sequencing. Prior the kit isolation, frozen fecal samples were digested with proteinase K in ALT buffer of the kit at 56 °C, followed by bead-beating with sterile zirconia beads ($\varnothing$ 0.1 mm, BioSpec, Cat. No. 11079101).

16S rRNA gene sequencing and taxonomic assignment

[0137] Demultiplexed reads were uploaded into CLC genomics workbench (Quiagen) and analyzed using their 16S microbiome pipeline. The analysis workflow consisting of quality filtration of the sequence data, and operational taxonomic unit (OTU) clustering was performed with default parameter settings. The adaptor sequence was removed and the reads with a quality score lower than 25 or length <150 were discarded. The maximum number of acceptable ambiguous nucleotides was set to 2 and the length of the reads was fixed at 200–500 bp. Chimeric sequences and singletons were detected and discarded. The remaining unique reads were used for OTU clustering, which was performed by alignment to the SILVA database at 97% sequence similarity.

[0138] Visualization of OTU counts was done using the Marker Data profiling pipeline of MicrobiomeAnalyst. Counts were filtered to include OTUs with minimum 2 counts (mean abundance value) and scaled to library total sum. Abundance profiles were generated after merging small taxa with counts <10 based on their median counts. In order to explore uncultured species (D6 level), the annotations of 'uncultured bacteria' were concatenated to their family names (D4 level). Results were used for Alpha diversity analysis using Shannon diversity, T-test on filtered data, and Beta Diversity (PCoA using Bray-Curtis index) plots.

Bioinformatics screening of human shotgun metagenomics datasets to identify fungi species

Identification of genomic regions unique to K. weizmannii and C. albicans

[0139] In order to identify *K. weizmannii* and *C. albicans* in human metagenomics datasets, the inventors first selected a set of nucleotide regions that are unique to the genome sequence of either *K. weizmannii* or *C. albicans* and were used to specifically identify these fungi in the background of other fungi, bacteria, and other species in the metagenomes. The genome assemblies of *K. weizmannii* and *C. albicans* were compared to 22 genomes (genus *Kazachstania*) and 11 genomes (genus *Candida*), respectively, using the GView Server, with analysis type 'Unique genome' with default parameters except for the Genetic code, where 'Standard' was used. Nucleotide regions unique to each target genome were collected, and further compared to the NCBI nt database using BLAST (E-value <0.0001) in order to exclude regions that are also present in bacteria or other non-fungal organisms. This search resulted in 179 nucleotide sequences specific to *K. weizmannii*, and 904 nucleotide sequences specific to *C. albicans*.

Screening shotgun metagenomics datasets using unique Kazachstania sp. and C. albicans queries

[0140] A dataset of 13,174 metagenomes, collected from the Global Microbial Gene Catalog v1.0 (GMGC (2)) was screened to identify sequences that originate from either *Kazachstania* sp. or *C. albicans* genomes. Each GMGC metagenome was originally stratified into habitats, and its raw nucleotide sequences were assembled into thousands of contigs (provided to us by Luis Pedro Coelho). All assembled contigs from each metagenomics sample were used as a query and were searched against the set of unique *K. weizmannii* or *C. albicans* sequences constructed as described above, as a reference. Alignments were generated using bowtie2 algorithm (version 2.3.5.1, using local mode). In order to broaden the search to genus level, all metagenomics assemblies were also mapped to a set of genus-

WO 2023/218450 and ITS sequences, extracted from *K. weizmannii* **PCT/IL2023/050470** genomes (namely 25Sa, 18Sa, 5.8Sa, ITS1a, ITS2a, ETS1a, ETS2a). To avoid biases in the specificity of ITS searches, the inventors counted only sequences which mapped to ITS regions without mutations.

Genomic DNA isolation from fungal cultures

[0141] Fungal pellet was dissolved in 2 ml lysis buffer (100 mM Tris pH 8.0, 50 mM EDTA, 1% SDS) and sonicated for 10 s. Three hundred (300) μ l of the supernatant was transferred to 300 μ l 7 M ammonium acetate pH 7.0, vortexed and incubated 5min at 65 °C followed by 3 minutes on ice. Five hundred (500) μ l chloroform was added and the solution was mixed by inverting the tube. Samples were spun 10 min at 13,000 rpm at 4 °C. Three hundred (300) μ l of the upper phase was transferred to 400 μ l isopropanol-filled tubes and incubated for 5 min on ice. Samples were spun 10 min at 13,000rpm at 4 °C and pellet was washed with 800 μ l 70% EtOH, spun 1min at maximum speed, air dried and dissolved in DDW.

Genome sequencing, annotation, and comparison

[0142] Sequencing and hybrid (Nanopore and Illumina) assembly were performed by SeqCenter (Pittsburgh, PA), as follows: Illumina - Sample libraries were prepared using an Illumina DNA Prep kit and IDT 10bp UDI indices and sequenced on an Illumina NextSeq 2000 producing 2x151bp reads. The data was demultiplexed and adapters removed using bcl2fastq [2] (v2.20.0.445) (support.illumina.com/sequencing/sequencing_software/bcl2fastq-conversion-software.html). Nanopore - Samples were prepared for sequencing using Oxford Nanopore's "Genomic DNA by Ligation" kit (SQK-LSK109) and protocol. All samples were run on Nanopore R9 flow cells (R9.4.1) on a MinION. Basecalling was performed with Guppy (version 4.2.2), in high-accuracy mode (Default parameters +effbaf8). Quality control and adapter trimming was performed with porechop (<https://github.com/rrwick/Porechop>) version 0.2.2_seqan2.1.1 with the default parameters. Long read assembly with ONT reads was performed with flye (version 2.8). The long read assembly was polished with pilon (1.23). Annotation was performed with the Yeast Gene Annotation Pipeline (YGAP) with the Post-WGD settings, and Companion for Fungi with a reference organism of *Candida glabrata* CBS138. The output of both programs was compared with CD-HIT (version 4.8.1) with c=1, to reduce redundancy, and the remaining genes combined with YGAP as the base annotation, using in-house scripts. Whole genome comparison of 25 species of *Kazachstania* on the basis of *K. weizmannii* was performed with CCT (CGView Comparison Tool) (63), with a BlastN e-value of e^{-10} .

[0143] The d1d2 region of 26SrDNA of various species were aligned with both ClustalW2.1 and Muscle 3.8.31. Phylogenetic trees were constructed with Maximum likelihood and DNA parsimony, using PhyML 3.0, DNAML, DNAPARS and DNAPENNY in the Phylip package (3.697) (Felsenstein 2005). The Phylip trees were then processed through Consense. Trees with similar topologies were obtained, and the Muscle/DNAPENNY tree is shown. The tree was visualized with iTol version 6.

qPCR quantification

[0144] Twenty to twenty five (20-25) mg of feces or tissue were surgically resected including its content, homogenized using Lysing Matrix C (MP biomedical) with the Omni Bead Ruptor 24 (Omni international, inc) followed by DNA extraction using Quick-DNA plus kit (Zymo Research) according to manufacturer's instructions. 10ng of isolated DNA were used for quantitative PCR reaction using Fast SYBR green Master Mix (Thermo Fisher Scientific, cat 4385614). Reaction was performed on the Quantstudio 7 Flex Real-Time PCR system (Thermo Fisher Scientific) using the fast SYBR 10µl program. Fungal DNA content in the samples was calculated using standard curves and normalized to tissue weight. For detection of *C. albicans* the following primers were used: forward: 5'-GGTGTTGAGCAATACGAC-3' (SEQ ID NO: 34), reverse: 5'-AGACCTAAGCCATTGTC-3' (SEQ ID NO: 35). For the detection of *K. weizmannii* the following primers were used: forward: 5'-ATGCACGTTTTCTGGGTGC-3' (SEQ ID NO: 36) reverse: 5'-GTATCGCATTTTCGCTGCGTT-3' (SEQ ID NO: 37).

In vitro serum antibody binding assay

[0145] Blood was collected from mice at rested at RT for 1 hour, followed by centrifugation 2,000 g 10 min. Serum supernatant was collected and froze at -80 °C until use. Cultured fungi were normalized to OD600=1 in PBS supplemented with 1% bovine serum albumin and 0.01% sodium azide (PBA solution). Cultured fungi were incubated with 20× diluted mouse serum on ice for 45 minutes, then washed twice with PBA, followed by staining with anti-mouse IgA and IgG. Samples were recorded on Cytex Aurora and analyzed in FlowJo (Treestar). Antibody binding intensity was normalized to the negative controls. Isotype control, non-stained and no-serum were used as negative controls.

Tissue isolation for flow cytometry

[0146] Mesenteric and peripheral lymph nodes were aseptically resected out into sterile, ice-cold PBS and mashed manually using a 1 mL syringe plunger through a 40 µm nylon cell

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 One hundred to two hundred (100-200) μ L of mouse blood was collected from submandibular vein, resuspended in 15 μ L of heparin (Sigma) to prevent coagulation, followed by lysis with 1ml ACK buffer (8.29 g/ml NH_4Cl , and 1 g/l KHCO_3 , 37.2 mg/l Na-EDTA) for 5 min RT and subsequent centrifugation.

Cell staining, stimulation and flow cytometry and microscopy

[0147] Methods adhered to published guidelines (Cossarizza et al., 2019). Flow cytometry samples were recorded on BD LSR Fortessa 4 lasers or Cytex Aurora followed by data analysis using the FlowJo software (Treestar).

Immunohistochemistry and histology

[0148] Mice were euthanized and intestines, kidneys and tongue were excised and fixed overnight in 4% paraformaldehyde at 4 °C. Paraffin embedding and sectioning was performed by the institutional histology unit. For immunohistochemistry of gut samples, slides were deparaffinized by 2 washes of Xylene followed by 100%, 96%, 70% EtOH and three washes with PBS. Slides were incubated for 30 minutes in 1% hydrogen peroxide in PBS at room temperature in the dark. After washing three times in PBS, slides were boiled for 10 minutes in Citric acid buffer pH 6.0 (10 mM citric acid and 0.05% Tween-20 in DDW), then washed with PBS three times. Next, slides were blocked in BP buffer (0.3% Triton X-100, 2% horse serum and 1% bovine serum albumin in PBS) for 1h, then incubated overnight at 4 °C with a 1:200 dilution of the primary antibody (anti-Candida) in BP buffer. In the following day, slides were washed three times in PBS and conjugated with a secondary antibody diluted 1:200 (Cy3 anti-Rabbit) in BP buffer for 1h at 25 °C. Samples were washed three times with PBS and incubated for 5 min with DAPI (1:10,000), then mounted with Immu-Mount (Epredia). Sections were imaged using Zeiss 880 confocal laser scanning microscope. Images were processed using the Zeiss Zen blue software. For histopathology of typical tongue or kidney fungal lesions in the mouse model of immunosuppression, paraffin sections were stained with periodic acid-Schiff (PAS) or H&E and slides were captured using a Panoramic SCAN II (3DHISTECH) and analyzed using CaseViewer software (3DHISTECH).

REAGENT or RESOURCE	SOURCE	IDENTIFIER
IgA, PE	Invitrogen	Cat#12-4204-81
IgG, Alexa Fluor® 594	BioLegend	Cat#405326
CD45, Violet 450	Biogems	Cat#07512-40-108
CD4, Alexa Fluor® 647	BioLegend	Cat#100412

WO 2023/218450 TCR β chain, FITC	BioLegend	PCT/IL2023/050470 Cat#109209
IgG1 κ Isotype Ctrl, PE	BioLegend	Cat#400407
ROR gamma (t)	Invitrogen	Cat#12-69981-80
TCR β chain, PE/Cyanine5	BioLegend	Cat#109209
CD274, PE/Dazzle™ 594	BioLegend	Cat#124323
CD43, Alexa Fluor® 700	BioLegend	Cat#143214
Ly6C, Pacific Blue™	BioLegend	Cat#128014
Ly6G, FITC	BioLegend	Cat#127605
CD115, APC/Cyanine7	BioLegend	Cat#135532
CD11b, PE	BioLegend	Cat#101207
CD220, APC	BioLegend	Cat#103212
Zombie Aqua™ Fixable Viability Kit	BioLegend	Cat#423102
Anti-Candida albicans	Abcam	Cat# ab53891
Cy3-AffiniPure Donkey Anti-Rabbit	Jackson ImmunoResearch	Cat#711165152
purified CD16/32	BioLegend	Cat#101302
Calcofluor white		
Cell Activation Cocktail	BioLegend	Cat#423302
Brefeldin A Solution	BioLegend	Cat#420601
Fixation/Permeabilization Kit	BD Bioscience	Cat# 554714
True-Nuclear™ Transcription Factor Buffer Set	BioLegend	Cat#424401

Antigen-reactive T cell enrichment (ARTE)

[0149] PBMCs were freshly isolated from EDTA blood samples on the day of blood donation by density gradient centrifugation (Biocoll; Biochrom, Berlin, Germany). Antigen-reactive T cell enrichment (ARTE) was performed as follows. Briefly, 2×10^7 PBMCs were plated in RPMI-1640 medium (GIBCO), supplemented with 5% (v/v) human AB-serum (Sigma Aldrich, Schnellendorf, Germany) at a cell density of 1×10^7 PBMCs / 2 cm^2 in cell culture plates and stimulated with $40 \mu\text{g/ml}$ fungal lysates for 7 hr in presence of $1 \mu\text{g/ml}$ CD40 and $1 \mu\text{g/ml}$ CD28 pure antibody (both Miltenyi Biotec, Bergisch Gladbach, Germany). $1 \mu\text{g/ml}$ Brefeldin A (Sigma Aldrich) was added for the last 2 hr. Cells were labeled with

WO 2023/218450 followed by anti-Biotin MicroBeads (CD154 MicroBead Kit, Miltenyi Biotec) and magnetically enriched by two sequential MS columns (Miltenyi Biotec). Surface staining was performed on the first column, followed by fixation, permeabilization (Inside stain Kit, Miltenyi Biotec) and intracellular staining on the second column. The following antibodies were used: CD4-APC-Vio770 (M-T466), CD8-VioGreen (REA734), CD14-VioGreen (REA599), CD20-VioGreen (LT20), Integrin-b7-PE-Vio770 (REA441) (all Miltenyi Biotec); CD45RA-PE-Cy5 (HI100), IFN- γ -BV785 (clone: 4S.B3) (both Biolegend); IL-17A-BV650 (clone: N49-653), IL-22-PerCP-eFluor710 (clone: IL22JOP) (both BD Biosciences). Viability 405/520 Fixable Dye (Miltenyi Biotec) was used to exclude dead cells. Data were acquired on a LSR Fortessa (BD Bioscience, San Jose, CA, USA).

[0150] Frequencies of antigen-specific T cells were determined based on the total cell count of CD154+ T cells after enrichment, normalized to the total number of CD4+ T cells applied on the column. For each stimulation, background cells enriched from the non-stimulated control were subtracted.

ITS2 amplification and sequencing of human stool samples

[0151] ITS2 sequencing was used for fungal identification. Briefly, ITS2 sequencing applied to 570 human stool samples and 6 controls. PCR was performed on 10 ng of DNA per sample (or the maximum available). Three PCR batches were required with 2 wells left empty as library controls in each batch. Forward primer ITS86F 5'-759 GTGAATCATCGAATCTTTGAA-3' (SEQ ID NO: 38) and reverse primer ITS4 with rd2 Illumina adaptor 5'-AGACGTGTGCTCTTCCGATCTTCCTCCGCTTATTGATATGC-3' (SEQ ID NO: 39) were used for the first PCR amplification. PCR mix per sample contained 5 μ l sample DNA, 0.2 μ M per primer (primers purchased from Sigma), 0.02 unit/ μ l of Phusion Hot Start II DNA Polymerase (Thermo Scientific 763 F549), 10 μ l of $\times$ 5 Phusion HS HF buffer, 0.2 mM dNTPs (Larova GmbH), 31.5 μ l ultra-pure water, for a total reaction volume of 50 μ l. PCR conditions used were 98 °C 2min, (98 °C 10 sec, 55 °C 15 sec, 72 °C 35 sec) $\times$ 30, 72 °C 5 min. A second PCR was performed to attach Illumina adaptors and barcode per sample for 6 additional cycles. Samples from the 1st PCR were diluted 10-fold and added to the PCR mix as described above. Primers of second PCR included: forward primer P5-rd1-768 ITS86F 5' - AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGTGAATCATCGAATCTTTGAA-3' (SEQ ID NO: 40), and reverse primer 5'-CAAGCAGAAGACGGCATACGAGATNNNNNNNTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT-3' (SEQ ID NO: 41). Every 96 samples were combined for a single mix

by adding 14 μ l from each. Before mixing, an aliquot from each of the samples was run on an agarose gel. In cases where the amplified bands were very strong, samples were diluted between 5 and 20-fold before they were added to the mix. Each sample mix was cleaned with QIAquick PCR purification kit (QIAGEN, catalog # 28104). Two cleaned sample mixes were then combined into a single mix of 192 samples, and size selection was performed with Agencourt AMPure XP beads (Beckman Coulter #A63881) to remove any excess primers. The beads to sample ratio was 0.85 to 1. Samples were then run in three libraries on the Miseq v3 600 cycles paired-end with 30% PhiX.

ITS2 sequencing analysis

[0152] The ITS2 classification pipeline was built with Python 3.6. For each sequencing library, paired-end reads were joined using PEAR (version 0.9.10) followed by filtering of merged reads by minimum length of 80bp and trimming of primers from both ends with cutadapt (version 1.17). Within the QIIME 2 environment (version 2018.8), Dada2 was used to create amplicon sequence variants (ASVs), then ITSx (version 1.1b1) was used to delineate ASVs to ITS2 regions (removing preceding 5.8S and trailing 28S sequences). A taxonomic naive bayesian classifier in QIIME 2 (74) was trained on the UNITE database (version 8, dynamic, sh_taxonomy_qiime_ver8_dynamic_04.02.2020.txt) and used to classify the 180 processed ASVs. 91 percent of raw reads were classified to species level (**Table 1**).

	Number	Percent
Kingdom	29339412	100
Phylum	29339412	100
Class	29339412	100
Order	29339412	100
Family	28453602	96.98082
Genus	28423083	96.8768
Species	26935819	91.80763

[0153] Most of the downstream analysis and plots were performed with R version 4.1.1 and phyloseq 1.34.0. ASVs were filtered by the ITSx and UNITE classifications to include fungal reads only. 119 ASVs that were classified by ITSx as fungi were included in the downstream analysis, representing over 97.5% of reads. Out of the remaining 61 ASVs that were classified by ITSx as non-fungal (Tracheophyta (T), land plants), one (fid65) was included in the downstream analysis since its classification as fungi reached all the way to species level by

WO 2023/218450 and was validated by NCBI BLAST to be fungal. Next, the inventors introduced two types of data normalization: (1) Library normalization, where samples were normalized to account for the difference in the average number of reads/sample per library. (2) Dilution normalization: ASV reads were multiplied by the dilution factor per sample to reflect their true original load. Next, ASVs were aggregated based on UNITE classification, to species level when possible. ASVs that could not be classified to species level, were grouped together by the lowest known phylogenetic level, and labeled “Other”. A total of 55 species were detected, 13 of which were also detected in control samples, but with a maximum of 100 reads per sample demonstrating a very mild read leakage of the higher abundant species in the library, e.g., *Saccharomyces cerevisiae*. Therefore, flooring of species with less than 100 reads per sample was applied. Lastly, data were aggregated by summing all reads in each taxonomic level by the associated taxa in the level above it.

Biolog

[0154] Quantification of cytotoxicity (LDH assay). Differentiated C2BBel cells in 96-well plates were infected for a defined time period with 10^4 *C. albicans*, *S. cerevisiae* and *K. weizmannii*. cells/well. After coincubation, epithelial damage was quantified by measuring LDH release using a cytotoxicity detection kit (Roche) according to the manufacturer’s instructions.

Filamentation assay

[0155] *C. albicans* and *K. weizmannii* were tested for their ability to the filament in liquid filamentation conditions. Cells grown overnight in liquid YPD were centrifuged and washed 2× with PBS prior to the experiment and strains were diluted on the same OD. Yeast cells were incubated for 3 hr or 5 h in 30 °C, 37 °C or 42 °C with shaking, then directly fixed with 4% PFA in PBS for 30 min, followed by wash with PBS and images were captured by bright Zeiss field microscope.

Statistical analysis

[0156] In all experiments, data are presented as the mean $\pm$ SEM unless stated otherwise. Statistical significance was defined as $P < 0.05$. The number of animals is indicated as n. Animals of the same age, sex, and genetic background were randomly assigned to treatment groups.

Data availability

[0157] The data have been deposited to BioProject accession number PRJNA949686 in the NCBI BioProject database (ncbi.nlm.nih.gov/bioproject/).

Identification of a novel commensal fungus in laboratory mice

[0158] To probe for a role of myeloid cells in anti-fungal immunity, the inventors used mice that harbor a corresponding IL-23 deficiency and attempted to colonize them with *C. albicans*. Specifically, the inventors used a protocol involving prior conditioning of animals with antibiotics (Abx) (27) (**Fig. 1A**). Ampicillin-exposed wildtype (WT) mice could be readily and persistently seeded with *C. albicans* SC5314 harboring a GFP reporter (**Fig. 1B**). Surprisingly, however, the inventors consistently failed to colonize the mutant mice with *C. albicans* (**Fig. 1C**). Rather, following plating of the fecal microbiota of these animals, the inventors noted the growth of another yeast-like fungus of distinct morphology (**Figs. 1D-1E**). Sequencing of DNA isolated from the plated fungus using the internal transcribed spacer (ITS) as yeast barcode tentatively identified the fungus as a member of the *Kazachstania* clade (**Figs. 1A-1B**). This genus is composed of over 50 species found in both anthropic and non-anthropic environments and associated with sourdough production. Whole genome sequencing of the new fungus revealed the characteristic gene duplications of the Saccharomycetaceae family and established it as a novel *Kazachstania* strain, which the inventors term *Kazachstania weizmannii*. Phylogenetic analysis of 26S rDNA confirmed the assignment of *K. weizmannii* to the Saccharomycetaceae clade (**Fig. 1F**), and comparison to other *Kazachstania* species (**Fig. 1G**) placed it together with *K. heterogenica*, a fungus found in rodent feces, and in a sister clade with *K. sp. Y4206*, a species isolated from human feces, and *K. pintolopesii*, from guinea pig peritoneal fluid. Comparison of the *K. weizmannii* genome with other full length *Kazachstania* genomes (**Fig. 1H**) showed that *K. sp. Y4206* is the closest species, followed by *K. bovina*, *K. telluris*, and *K. slooffiae*.

[0159] Of note, although the inventors originally discovered the novel *Kazachstania* species in mutant mice that might have impaired anti-fungal immunity, sentinel screening in the animal facilities with the ITS assay revealed that *K. weizmannii* was widespread, irrespective of animal genotypes (**Fig. 8C**).

EXAMPLE 2

In vitro characterization of *K. weizmannii*

[0160] When cultured in a number of defined conditions frequently found in the human body, such as serum exposure or, neutral pH and or 37 °C, *C. albicans* forms hyphae the same challenges did not invoke the same morphology switch in *K. weizmannii* (**Fig. 2A**). Instead, the fungus continued to grow in a yeast-like morphology.

[0161] Comprehensive Biolog analysis revealed preferences for carbon and nitrogen sources of *C. albicans* and *K. weizmannii* (Figs. 2B-2C). *K. weizmannii* was for instance superior in growing with 2-Deoxy-D-Ribose and D-Glucosamine, as well as leucine dipeptides, while *C. albicans* could better thrive on maltose, D-galactose, urea and glycine dipeptides. The two fungi also displayed differential resistance to chemical inhibitors (Fig. 2D). Specifically, *K. weizmannii* showed as compared to *C. albicans* relative resistance to propiconazole and fucinazole, while the yeast was more sensitive to chlorides and bromides. When cultured in YPD media *C. albicans* and *K. weizmannii* displayed similar pH optima (Fig. 2E). Interestingly and in contrast to the observation for the gut, in *in vitro* cocultures, *C. albicans* and *K. weizmannii* grew together without interference (Fig. 2F).

EXAMPLE 3

***K. weizmannii* is a murine commensal that antagonizes *C. albicans* colonization**

[0162] To facilitate the comparative analysis of *K. weizmannii* and *C. albicans* SC5314, the inventors generated a *K. weizmannii* strain harboring a gene encoding a red fluorescent reporter in the enolase 1 (*ENO-1*) locus, mimicking the *C. albicans* SC5314-GFP configuration (Figs. 3A, 3C, and 9B-9C).

[0163] Colonization of WT animals by *K. weizmannii* did not require prior Abx treatment, in contrast to the colonization with *C. albicans* (Figs. 3B-3C). C57Bl/6 mice could be readily colonized by oral *K. weizmannii* inoculation or cohousing with animals bearing the fungus. Interestingly, and in line with the original observation disclosed herein, when Abx-treated mice were co-inoculated with a 1:1 mixture of *K. weizmannii* and *C. albicans*, *Candida* colonization was prevented (Figs. 3D-3G). To test if *K. weizmannii* would also outcompete established commensal *C. albicans*, the inventors stably colonized mice with *C. albicans* (on Abx) and then cohoused the animals with mice harboring *K. weizmannii* (Fig. 3H). Coprophagy led to the progressive rapid ousting of *C. albicans* by *K. weizmannii*, leaving only a residual *C. albicans* population. Colonization with *C. albicans* was further reduced upon Abx withdrawal (Figs. 3I-3J).

[0164] *C. albicans* colonization of mice is sensitive to the microbiome composition. The competition between the two fungi that the inventors observed could hence be due to alterations of the bacterial landscape. Comparison of the microbiome of *K. weizmannii* -colonized mice and non-colonized controls using 16S sequencing revealed, however, only minor consistent changes (Fig. 10A). Moreover, also the abundance of Lactobacillae that are known to compete with *C. albicans* and to impede colonization of the murine gut, was

WO 2023/218450 (Fig. 10B). To further probe the potential involvement of bacterial microbiota in fungal competition, the inventors orally inoculated germ-free animals with a mixture of the two fungi. Also in these mice, *K. weizmannii* prevented efficient *C. albicans* colonization (Figs. 3K-3M, and 10C-10D). Collectively, these data establish that *K. weizmannii* can outcompete *C. albicans* including previously colonized animals and that the observed inter-fungal competition is independent of bacterial components.

EXAMPLE 4

Comparative analysis of the host immune response to *C. albicans* and *K. weizmannii*

[0165] Fungi are known to affect host granulopoiesis and induce both humoral and cellular immunity. In line with these reports, Abx-treated animals colonized with *C. albicans* showed an expanded neutrophil compartment in the blood. In contrast, *K. weizmannii*-colonized animals displayed no significantly altered abundance of granulocytes, classical or non-classical monocytes (Fig. 4A).

[0166] Intestinal IgA responses to *C. albicans* were proposed to balance commensalism vs. pathogenicity by controlling the critical morphological hyphae-to-yeast switch of the fungus. To assess humoral immunity against the two fungal commensals, the inventors analyzed sera of colonized animals for reactivity to cultured *K. weizmannii* or *C. albicans*. Colonization with either yeast induced robust anti-fungal serum IgA and IgG titers in most animals. (Figs. 4B-4C). The induced antibodies were mostly, but not always, cross-reactive between the two fungal species.

[0167] Mucosa-associated fungi, and specifically *C. albicans* have been shown to induce Th17 type cellular immune response. Accordingly, *C. albicans*-colonized animals displayed an expansion of Th17 cells in gut mucosa-associated mesenteric and peripheral lymph nodes (Figs. 4D-4E). In contrast, even after extended colonization, no Th17 cell expansion was observed in *K. weizmannii*-colonized animals.

[0168] To directly gauge the impact of *K. weizmannii* on host cells the inventors performed an endothelial cell (EC) co-culture assay. EC exposure to *C. albicans* resulted in EC damage as measured by LDH release. In contrast and similar to co-culture with *S. cerevisiae*, *K. weizmannii* did not affect EC viability (Fig. 4F). However, co-infection of EC cultures with *K. weizmannii* and *C. albicans* did not prevent EC damage.

WO 2023/218450 [0169] *Kazachnia* together, colonization of Abx-treated animals with *C. albicans* and *K. weizmannii* induced largely cross-reactive humoral immune responses reflected by serum IgA and IgG titers. The characteristic anti-fungal Th17 response was restricted to *C. albicans*-colonized mice. In combination with the *in vitro* culture results, these data suggest that *K. weizmannii* is an innocuous commensal.

EXAMPLE 5

Commensal *C. albicans*, but not *K. weizmannii* causes pathology in immune-suppressed animals

[0170] Invasive candidiasis is widely recognized as a major cause of morbidity and mortality in the healthcare environment, often associated with an underlying immune-compromised state. Candidiasis can be induced in otherwise resistant, orally *C. albicans*-challenged animals by immune-suppression. To test whether corticosteroid treatment would cause *C. albicans* and *K. weizmannii* to spread from established commensal reservoirs and cause systemic pathology, the inventors treated mice that were stably colonized with the respective fungi with cortisone 21-acetate boli (225 mg/kg s.c) every other day (**Fig. 5A**). Unlike control mice, *C. albicans*-harboring animals lost significant weight after one week of treatment and became moribund (**Fig. 5B**). Animals displayed prominent tongue candidiasis and fungal growth in the kidneys (**Figs. 5C-5D**). In stark contrast, immuno-suppressed animals colonized with *K. weizmannii* showed neither weight loss nor evidence of fungal spread (**Figs. 5E-5I**, and **11A-11B**). These data establish that in mice, *K. weizmannii* is innocuous and even in immunosuppressed animals neither breaches the intestinal barrier to spread systemically nor causes other pathologies.

EXAMPLE 6

Competitive commensalism mitigates Candidiasis

[0171] Since *K. weizmannii* exposure during competitive seeding and cohousing significantly reduces the commensal *C. albicans* burden in colonized animals (**Fig. 3J**), the inventors next examined whether this commensal competition would also mitigate candidiasis pathology. To simplify the mode of *Kazachstania* administration, the inventors treated *Candida* colonized animals with *Kazachstania*-supplemented drinking water (**Fig. 6A**) prior to immunosuppression. As expected, also in this setting *Kazachstania* efficiently expelled *C. albicans* from colonized animals (**Fig. 6B**). Indeed, exposure of immunosuppressed *C. albicans*-colonized animals to *K. weizmannii* significantly delayed

WO 2023/218450 and systemic yeast spread, as indicated by the absence of *K. weizmannii* colonization (Figs. 6C-6E, and 12A). As to expect, the animals were, however, not permanently protected. Rather, their residual commensal *C. albicans* burden eventually disseminated and caused pathology (Figs. 6F-6G, and 12B). Collectively these results establish that *K. weizmannii* out-competes *C. albicans* from the commensal microbiome and thereby reducing the cause of pathology, mitigates candidiasis and improves the health status of immunosuppressed animals.

EXAMPLE 7

Kazachstania presence in human metagenomes

[0172] *Candida* species are a major component of the human mycobiota, with *C. albicans* being the most prevalent. Despite their established role in dough fermentation, *Kazachstania* spp. presence in healthy humans or during pathology-associated dysbiosis has very rarely been reported. To gauge the abundance of *Kazachstania* spp., and in particular, the newly identified *K. weizmannii* disclosed herein in human microbiota, the inventors designed a bioinformatic screen to detect genomic regions specific to these fungi, out of shotgun sequence information from a collection of 13,174 published metagenomics data sets (Fig. 7A). Among 7,059 human stool samples analyzed, the inventors identified 32 metagenomes that displayed specific evidence for *K. weizmannii* and 9,236 reads that harbored *Kazachstania* spp. sequences (26S). 7,167 samples displayed evidence for *C. albicans* (Fig. 7B). Likewise, among 173 vaginal metagenomes, the inventors found 22 samples with evidence for *Kazachstania* and *Candida* sequences, including presence of *K. weizmannii* (Figs. 7C, and 13A). These samples had a wide range of biogeographical distribution (Fig. 13B). Collectively, the current data support the notion that *Kazachstania* spp. are a common and integral part of the human microbiome. The inventors corroborated this notion by an antigen-reactive T cell enrichment (ARTE) analysis of peripheral blood of a limited number of healthy individuals. As shown earlier (Bacher et al., 2019), ARTE revealed T cell reactivity directed against *C. albicans* extracts, but also against *K. weizmannii* (Fig. 7D). *C. albicans*-reactive T cells were polarized towards IL-17- and IL22-producing Th17 fates. In contrast, *K. weizmannii*-reactive T cells were of the Th1 type, like T cells which reacted to *S. cerevisiae*. Of note, CD154⁺ memory T cells responsive to Saccharomycetaceae extracts also expressed b7 integrin indicative of their generation in the gut mucosa.

[0173] In mice, abundance of *C. albicans* and *K. weizmannii* anti-correlated, suggesting that these yeasts compete for similar niches. To investigate the relative distribution of *Candida* and *Kazachstania* species in the human microbiome, the inventors performed a sensitive ITS2 analysis of fecal samples of a cohort of 570 healthy individuals (**Fig. 7E**). Since the *Kazachstania* genus had not been widely detected in prior ITS2 sequencing initiatives the inventors anticipated that it would be a less prevalent genus with lower abundance when compared with *Candida* and other prominent genera. Therefore, the inventors added empty control samples which underwent the same amplification and library preparation processes to enable the application of a novel ITS2 processing pipeline previously applied to analyze the low biomass environment of the tumor mycobiome. Indeed, 13 fungal species could be detected in control samples with read numbers ranging from 1-100 reads per species per sample. The inventors, therefore, applied an aggressive cutoff by flooring all species which obtained less than 100 reads in a given sample to zero in that sample. This process yielded 215 samples with ITS2 sequence evidence of either *Candida* or *Kazachstania* species.

[0174] *C. albicans*, was the most prominent *Candida* species found in 120 samples, followed by *C. parapsilosis* and *C. tropicalis*, with 29 and 15 samples respectively. Two *Kazachstania* species were detected across 37 samples prior to flooring, and were completely absent from negative control samples. Still, these species were floored in samples where they did not reach 100 reads leaving 12 samples with *Kazachstania servazii* and 3 samples with *Kazachstania exigua*, close relatives of the novel *K. weizmannii* species. The inventors did not detect *K. weizmannii* specifically in this limited cohort. Notably, the majority of individuals harboring *Kazachstania* species displayed mutual exclusive presence with *Candida* spp. (**Fig. 7E**) suggesting competitive fungal commensalism, as in mice. Collectively, these data identify *Kazachstania* spp. as part of the human microbiome and the current data suggest an inverse correlation of these fungi and *Candida* spp. warranting further studies on a larger scale.

[0175] Taken together the herein disclosed *K. weizmannii* is identified as an innocuous fungal commensal in men and mice. By its virtue to successfully compete with *C. albicans* in the murine gut for to-be-defined niches, *K. weizmannii* lowered the pathobiont burden and mitigated candidiasis development in immunosuppressed animals. This competitive fungal commensal is thus suggested as a potential therapeutic for the management of *C. albicans*-mediated diseases.

[0176] Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications, and variations will be apparent to

WO 2023/218450 in the art. Accordingly, it is intended to embrace **PCT/IL2023/050470** ~~and such alternatives,~~ modifications and variations that fall within the spirit and broad scope of the appended claims.

What is claimed is:

1. An isolated fungus belonging to the genus *Kazachstania* and deposited at the ATCC under the deposit number PTA-127318.
2. A composition comprising the isolated fungus of claim 1, and a pharmaceutically acceptable carrier.
3. A composition comprising a fungus, wherein at least 90% of said fungus is the isolated fungus of claim 1.
4. The composition of claim 2 or 3, for use in the treatment or prevention of a disease in a subject in need thereof.
5. The composition of claim 4, wherein said disease comprises, an autoimmune disease, an infectious disease or both.
6. The composition of claim 5, wherein said autoimmune disease is a T helper 17 cells (Th17)-driven immunopathology.
7. The composition of claim 5 or 6, wherein said autoimmune disease is selected from the group consisting of: multiple sclerosis, psoriasis, and asthma.
8. The composition of any one of claims 5 to 7, wherein said infectious disease is a fungal infection.
9. The composition of claim 8, wherein said fungal infection comprises a *Candida* infection.
10. The composition of claim 8 or 9, wherein said fungal infection comprises a *Candida albicans* infection.
11. A method for preventing or treating an autoimmune disease in a subject in need thereof, the method comprising administering to said subject a therapeutically effective amount of a pharmaceutical composition comprising the isolated fungus of claim 1, thereby preventing or treating an autoimmune disease in the subject.
12. The method of claim 11, wherein said autoimmune disease is: (i) induced T helper 17 cells (Th17) activity; (ii) characterized by increased Th17 activity; or (iii) both (i) and (ii).

14. The method of any one of claims 11 to 13, wherein said subject is afflicted with a fungal infection.

15. The method of any one of claims 11 to 14, wherein said autoimmune disease is selected from the group consisting of: multiple sclerosis, psoriasis, and asthma.

16. A method for preventing or treating a fungal infection in a subject in need thereof, the method comprising administering to said subject a therapeutically effective amount of a pharmaceutical composition comprising the isolated fungus of claim 1.

17. The method of any one of claims 11 to 16, wherein said fungal infection comprises a *Candida* infection.

18. The method of any one of claims 11 to 17, wherein said fungal infection comprises a *Candida albicans* infection.

19. The method of any one of claims 16 to 18, wherein said treating comprises reducing the number or abundance of a fungus inducing said fungal infection in the gastrointestinal tract of said subject.

20. The method of claim 19, wherein said reducing is: (i) by at least 30% compared to control; (ii) for at least 4 days; or (iii) both (i) and (ii).

21. The method of any one of claims 16 to 20, wherein said treating comprises reducing the activity, abundance, or both, of Th 17 cells in said subject.

22. The method of any one of claims 11 to 21, wherein said administering comprises colonizing the gut of said subject with said isolated fungus.

23. A non-human animal subject comprising the isolated fungus of claim 1.

24. The non-human animal subject of claim 23, wherein said non-human animal subject is gnotobiotic.

25. The non-human animal subject of claim 23 or 24, being a mouse.

WO 2023/218450 ~~20.~~ The non-human animal subject of any one of claims 23 to 25, **PCT/IL2023/050470** ~~25,~~ wherein the isolated fungus of claim 1 colonizes or is present in the gastrointestinal tract of said non-human animal subject.

27. A method for producing the non-human animal subject of any one of claims 23 to 26, comprising colonizing a gastrointestinal tract of a non-human animal subject with an effective amount of any one of:

- a. the isolated fungus of claim 1; and
- b. the composition of claim 2 or 3,

thereby producing the non-human animal subject.

28. The method of claim 27, further comprising rearing/culturing said non-human animal subject under pathogen free conditions.

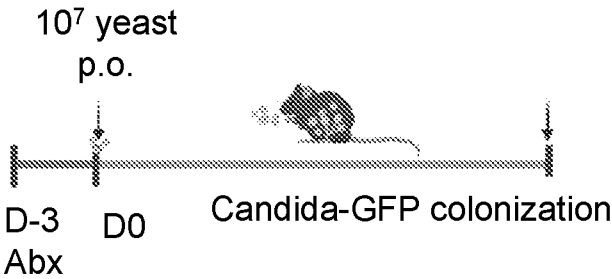


FIGURE 1A

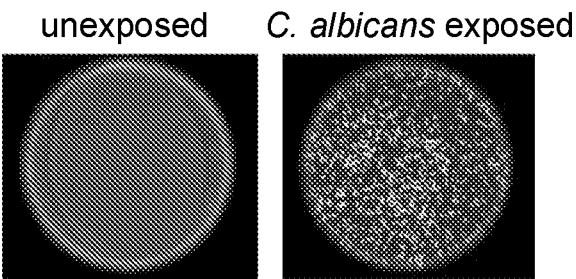


FIGURE 1B

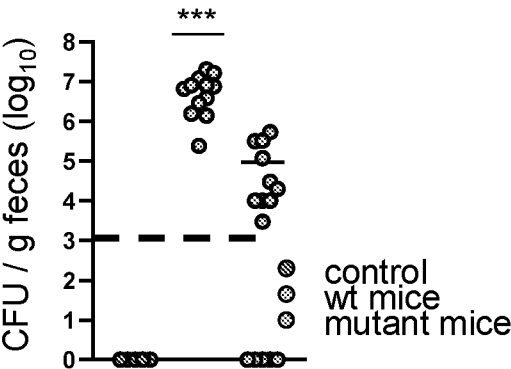


FIGURE 1C

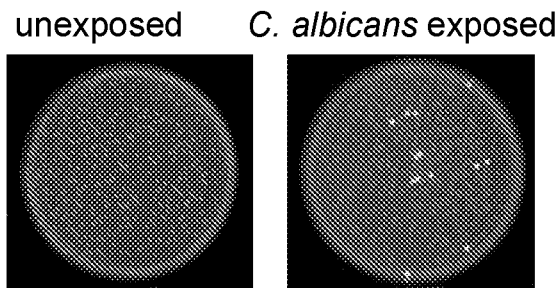


FIGURE 1D

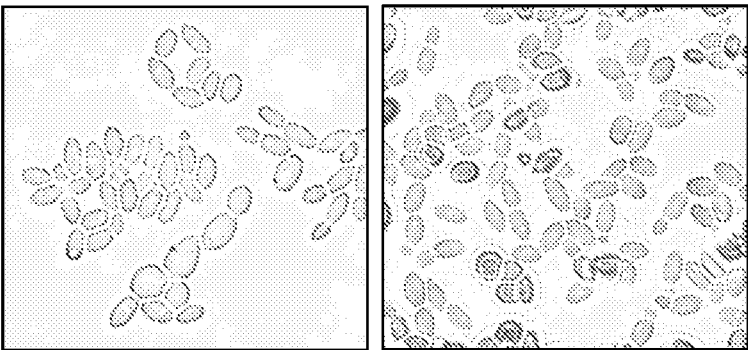
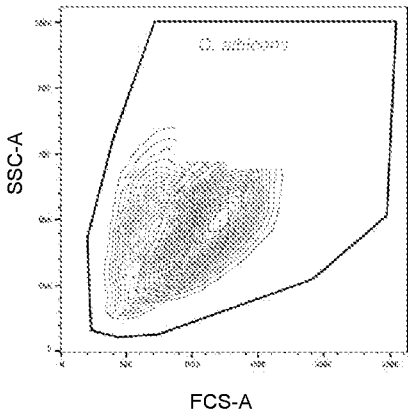


FIGURE 1E

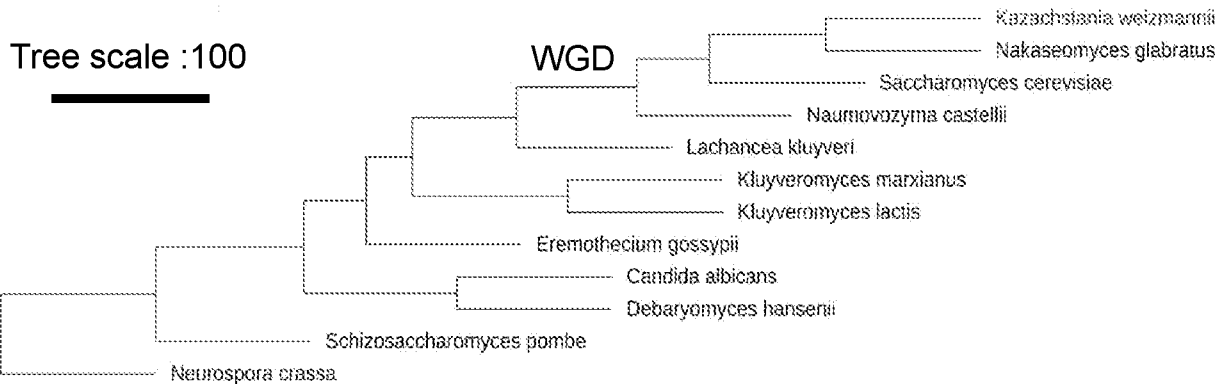


FIGURE 1F

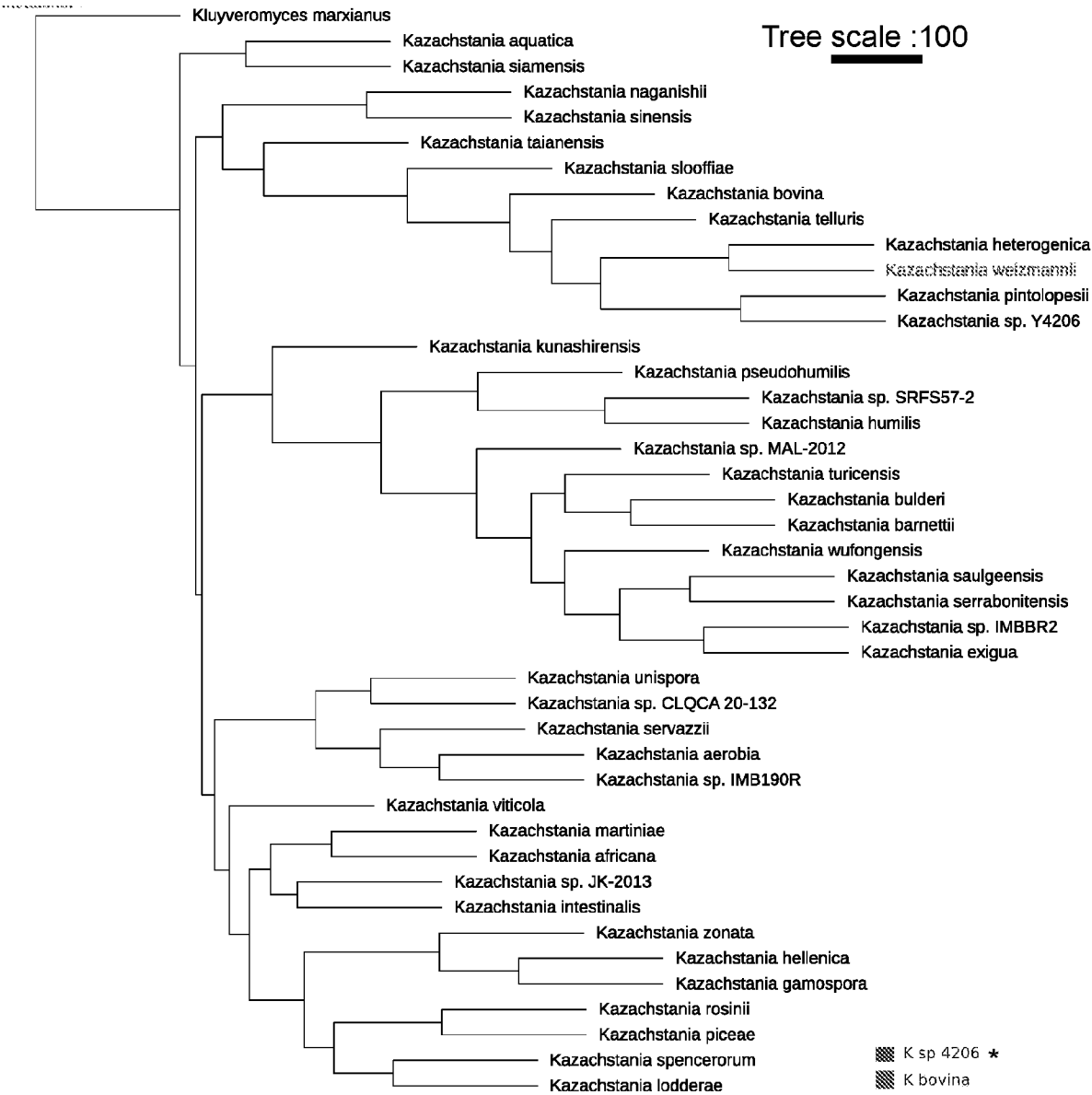


FIGURE 1G

*Kazachstania sp. Y4206, whole
genome shotgun sequencing project
GenBank: JAMAJF000000000.1,
human

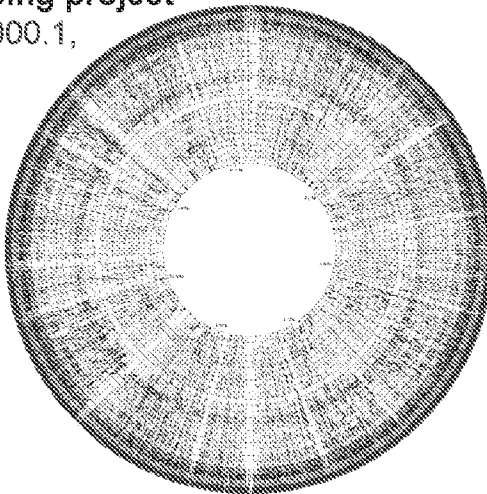


FIGURE 1H

- K sp 4206 *
- K bovina
- K telluris
- K slooffiae
- K unispora
- K aerobia
- K saulgeensis
- K solicola
- K turicensis
- K viticola
- K servazzii
- K siamensis
- K barnettii
- K bulderi
- K humilis
- K kunashirensis
- K martiniae
- K africana
- K exigua
- K bromeliacearum
- K spencerorum
- K rosinii
- K taianensis
- K naganishii
- K intestinalis

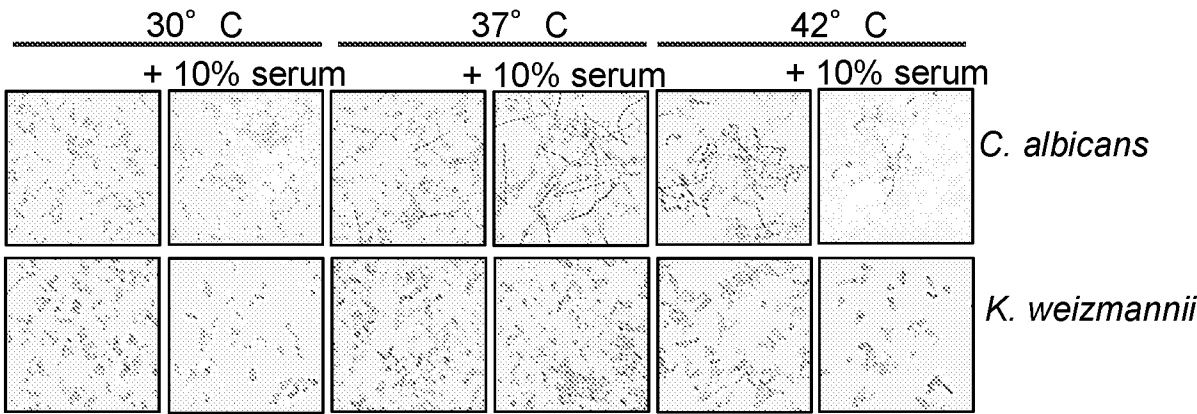


FIGURE 2A

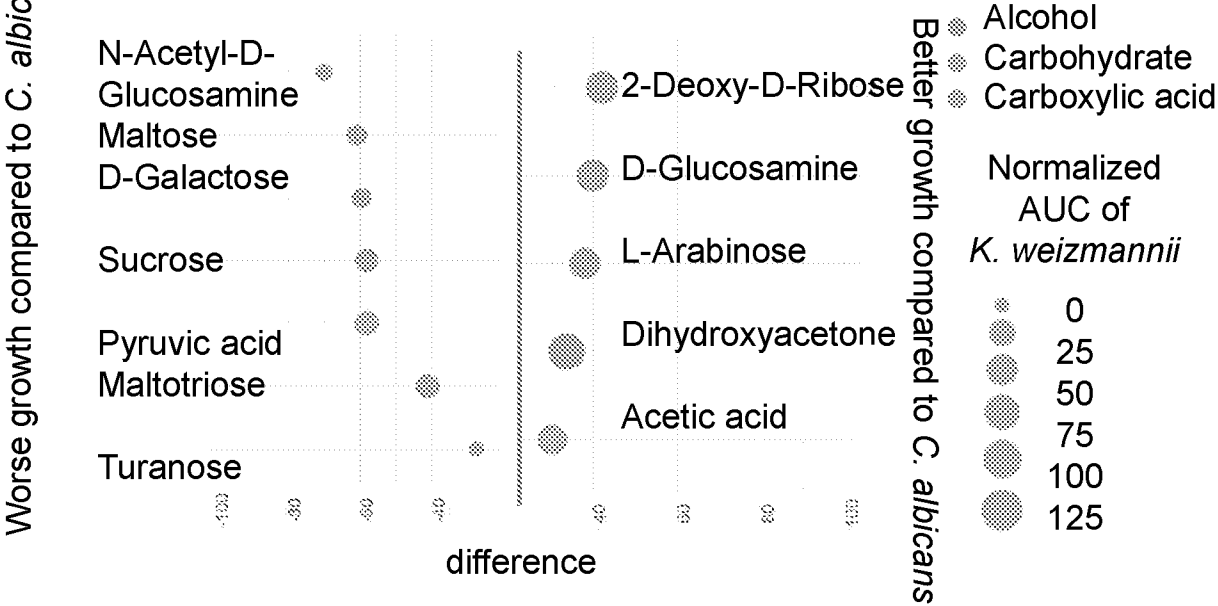


FIGURE 2B

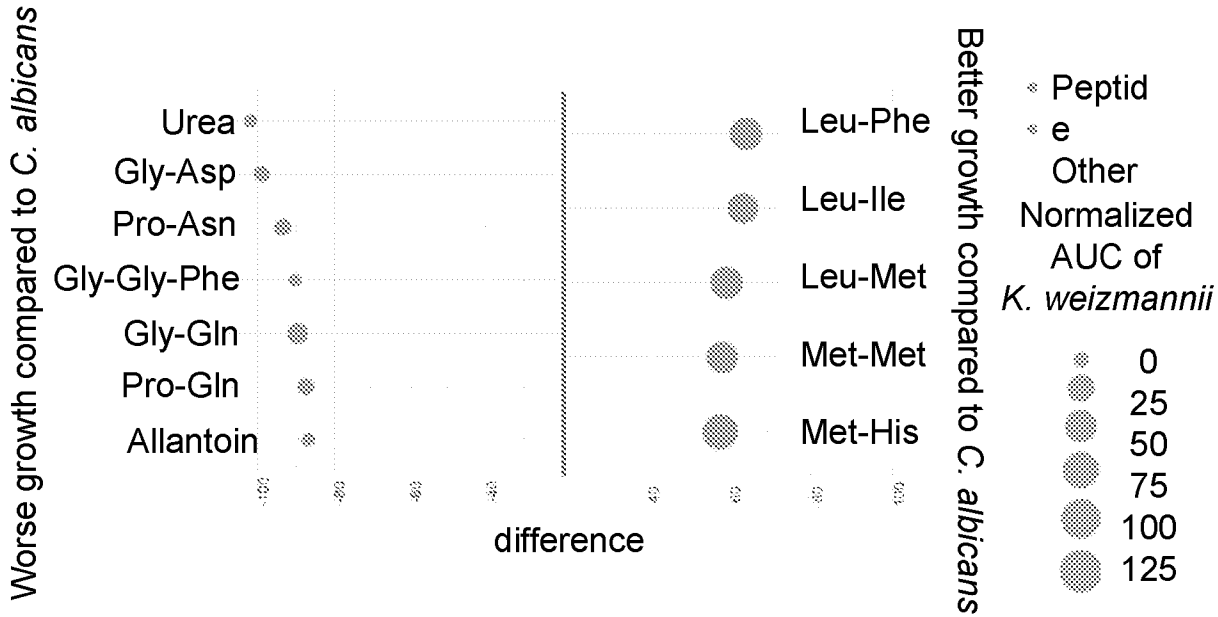


FIGURE 2C

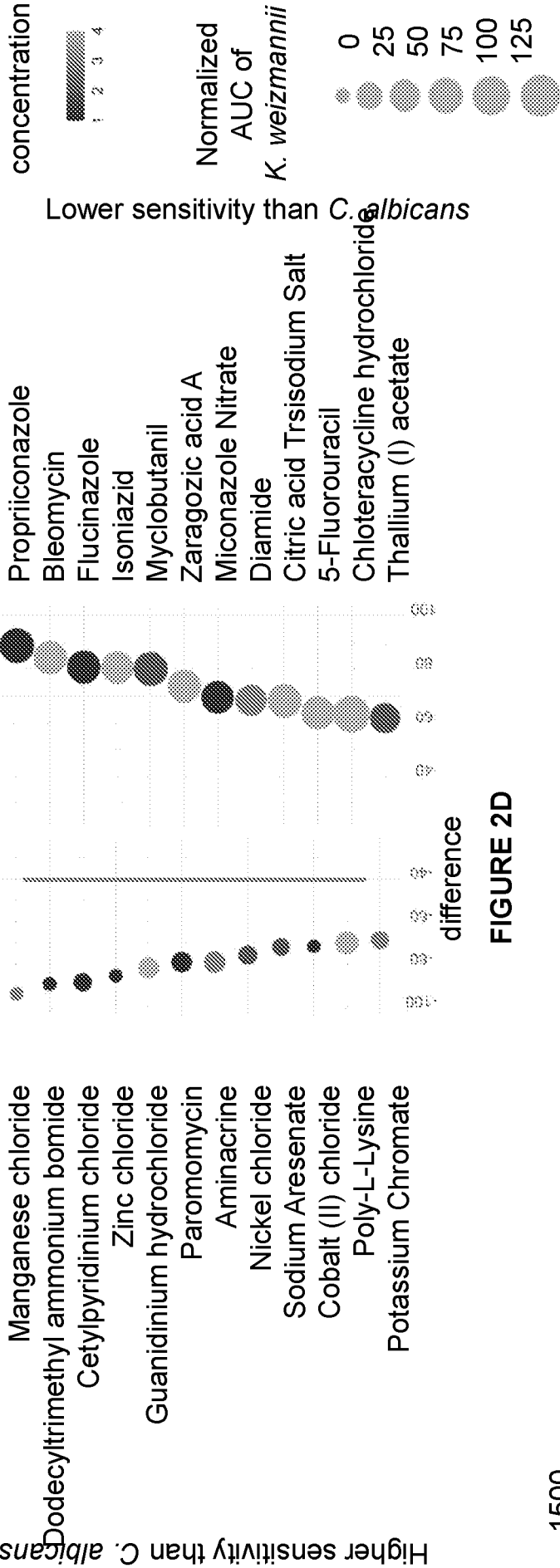


FIGURE 2D

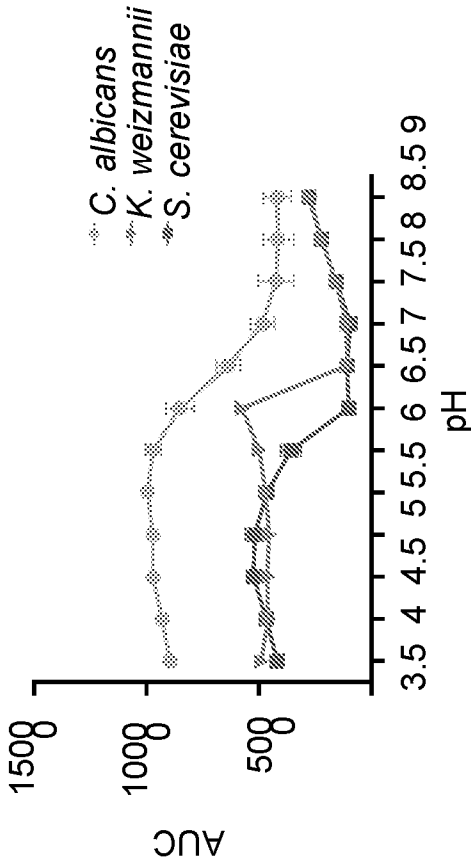


FIGURE 2E

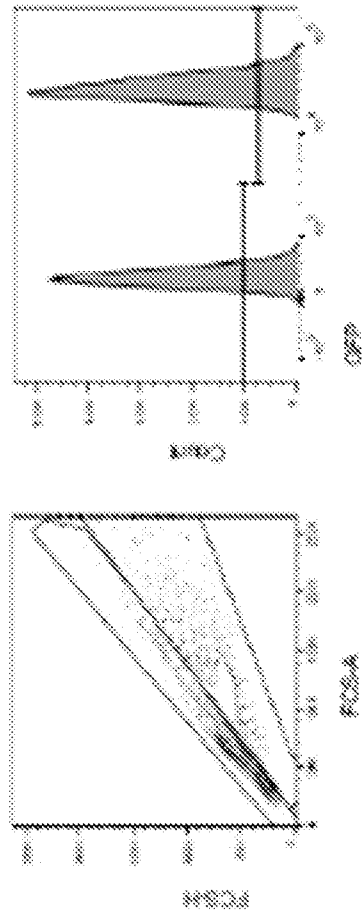


FIGURE 2F

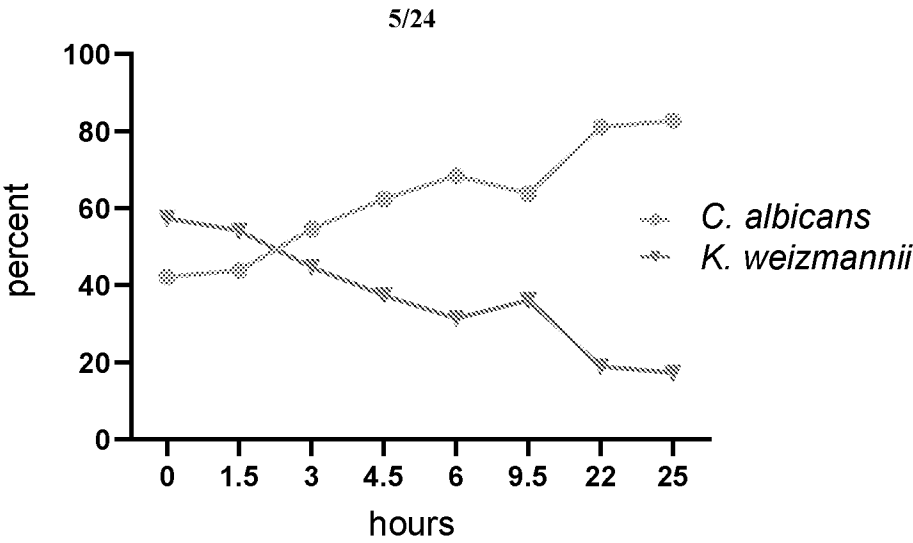


FIGURE 2F – Continue

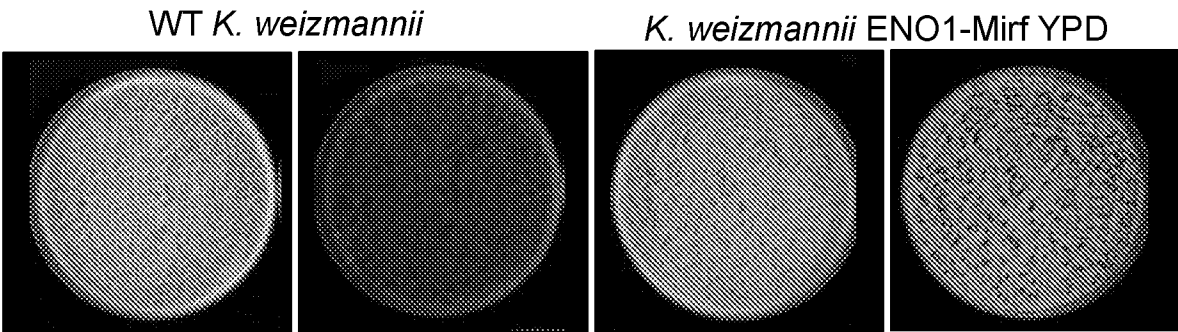


FIGURE 3A

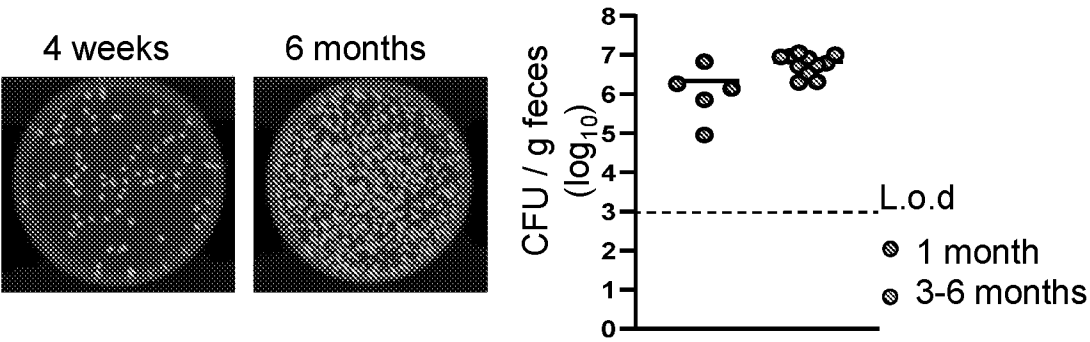


FIGURE 3B

FIGURE 3C

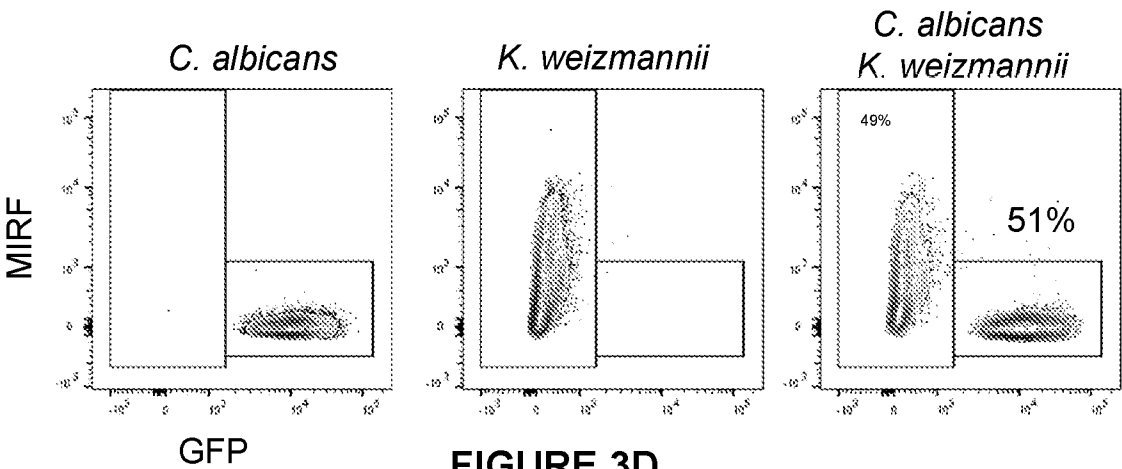


FIGURE 3D

colonization

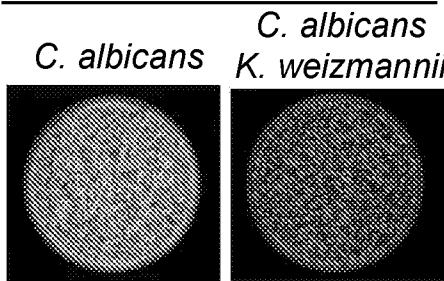


FIGURE 3E

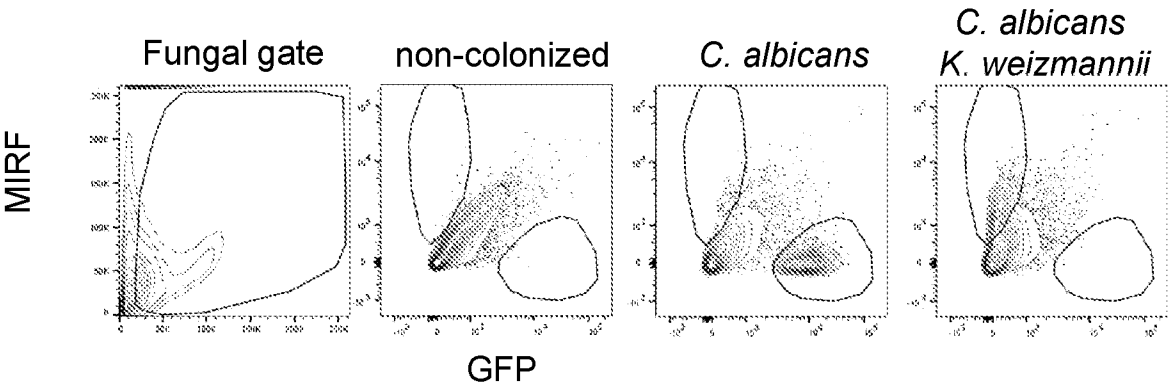


FIGURE 3F

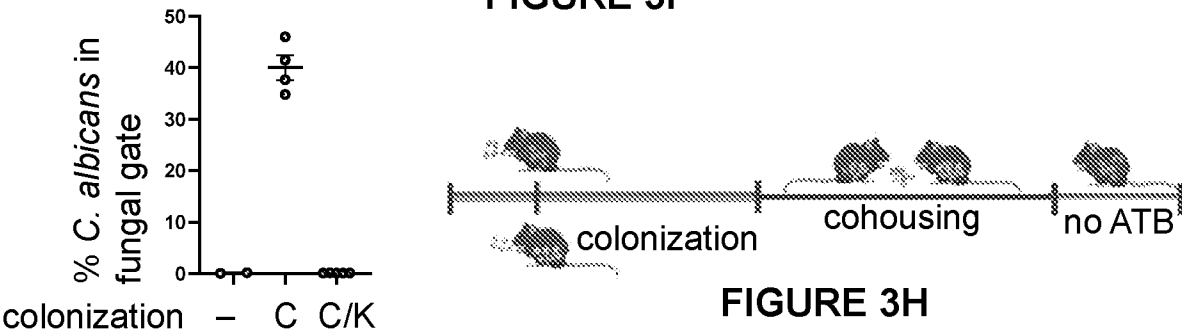


FIGURE 3H

FIGURE 3G

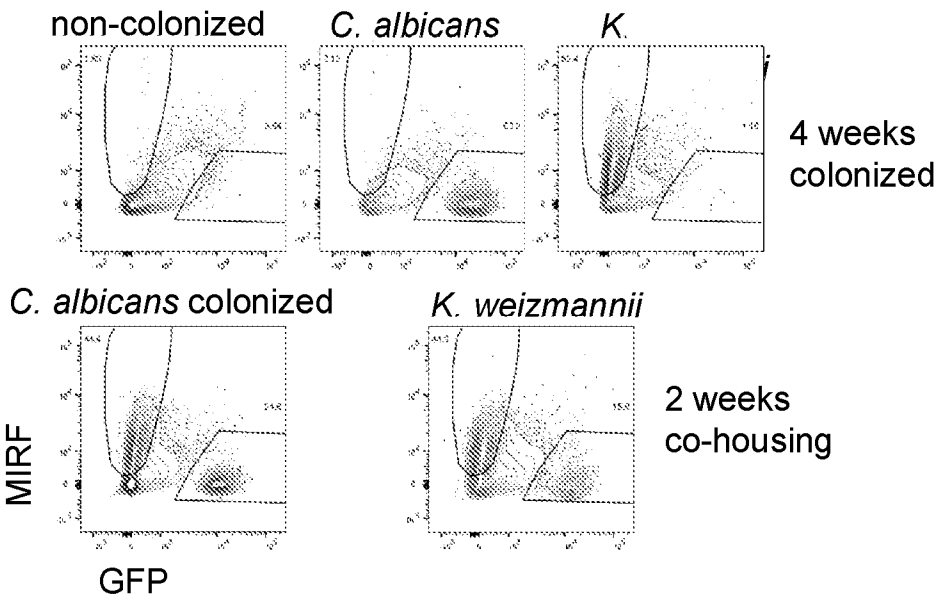


FIGURE 3I

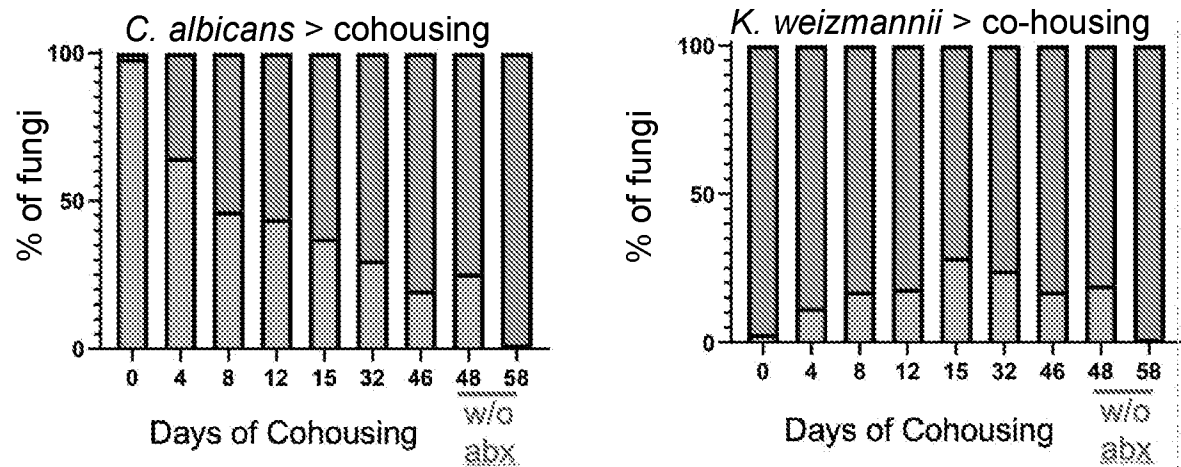


FIGURE 3J

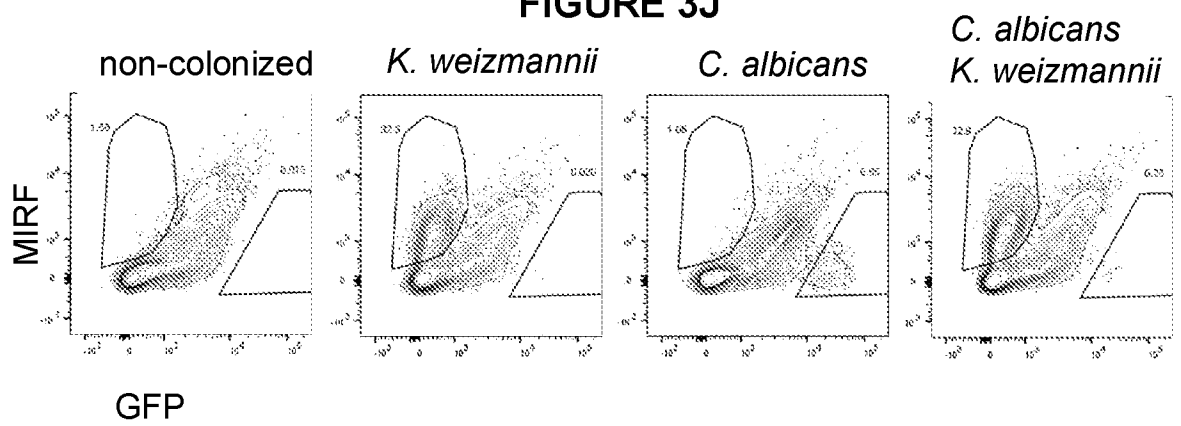


FIGURE 3K

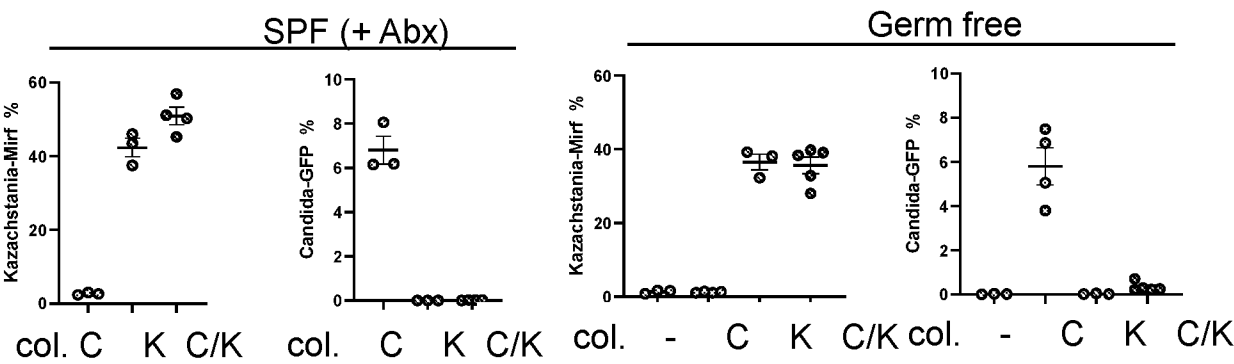


FIGURE 3L

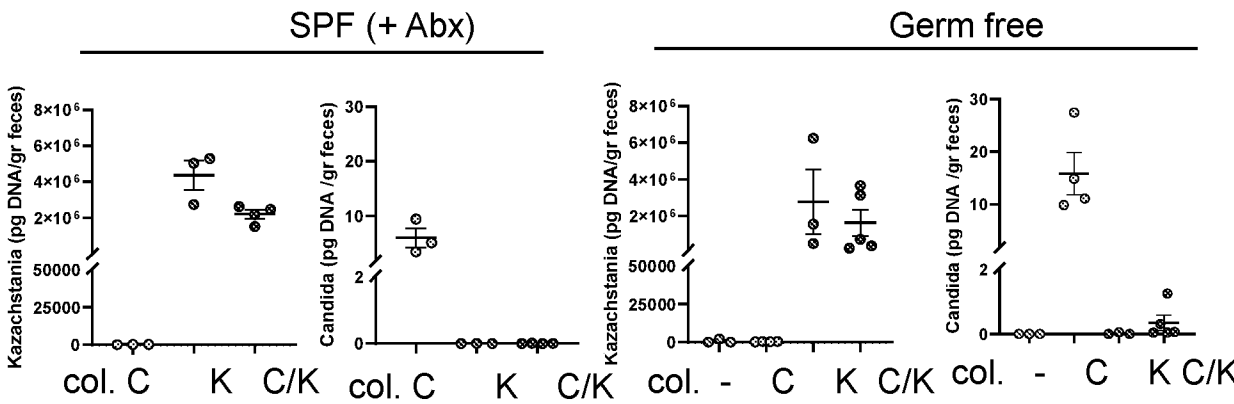


FIGURE 3M

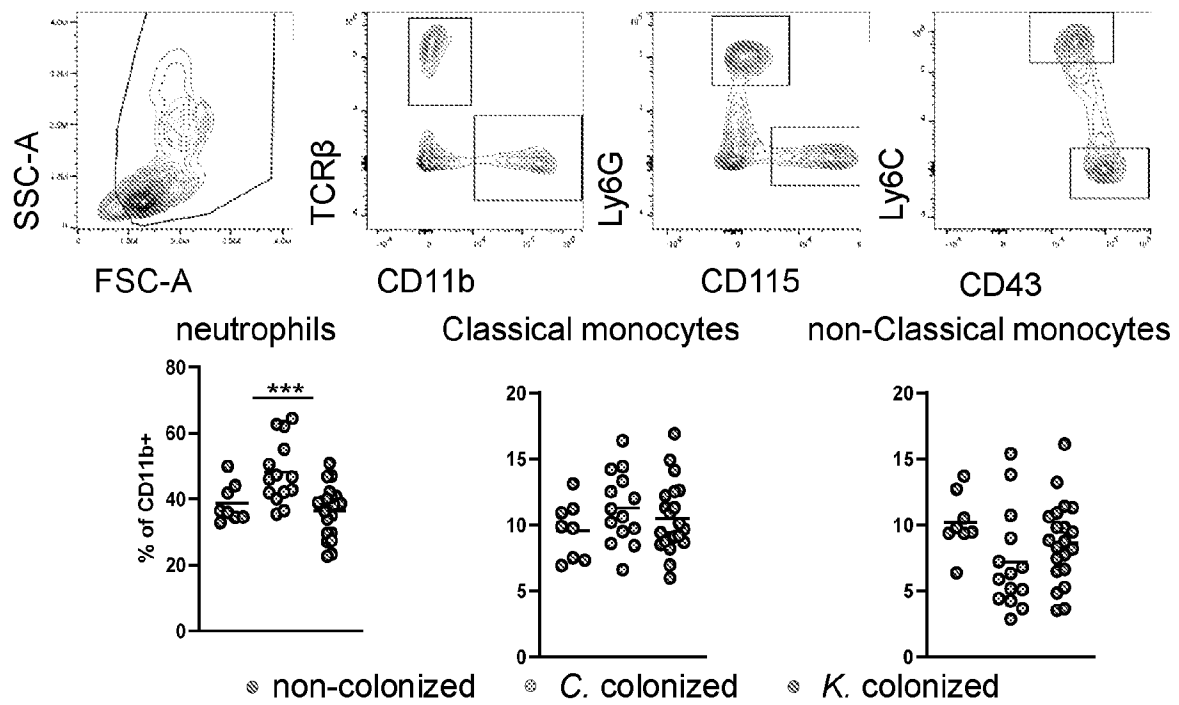


FIGURE 4A

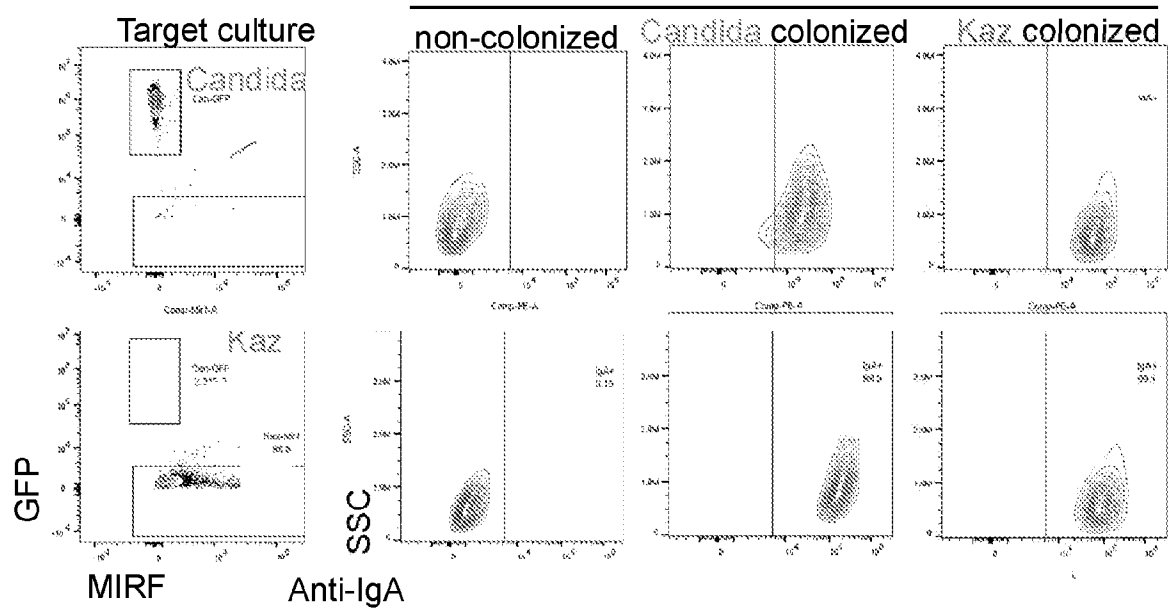


FIGURE 4B

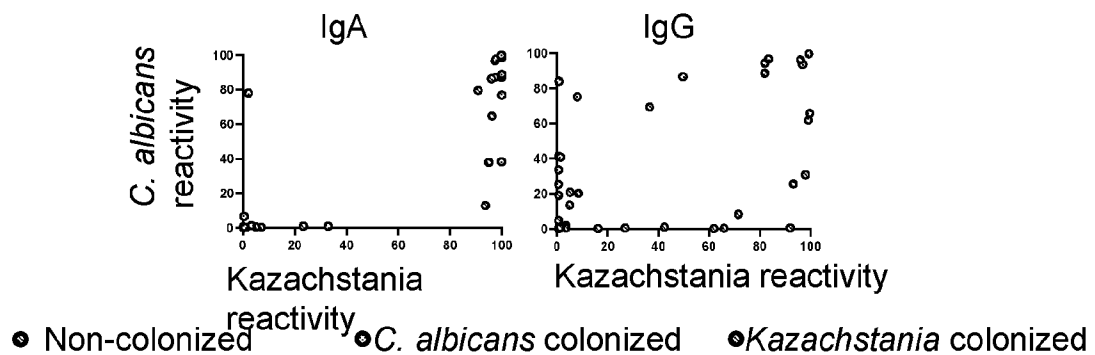


FIGURE 4C

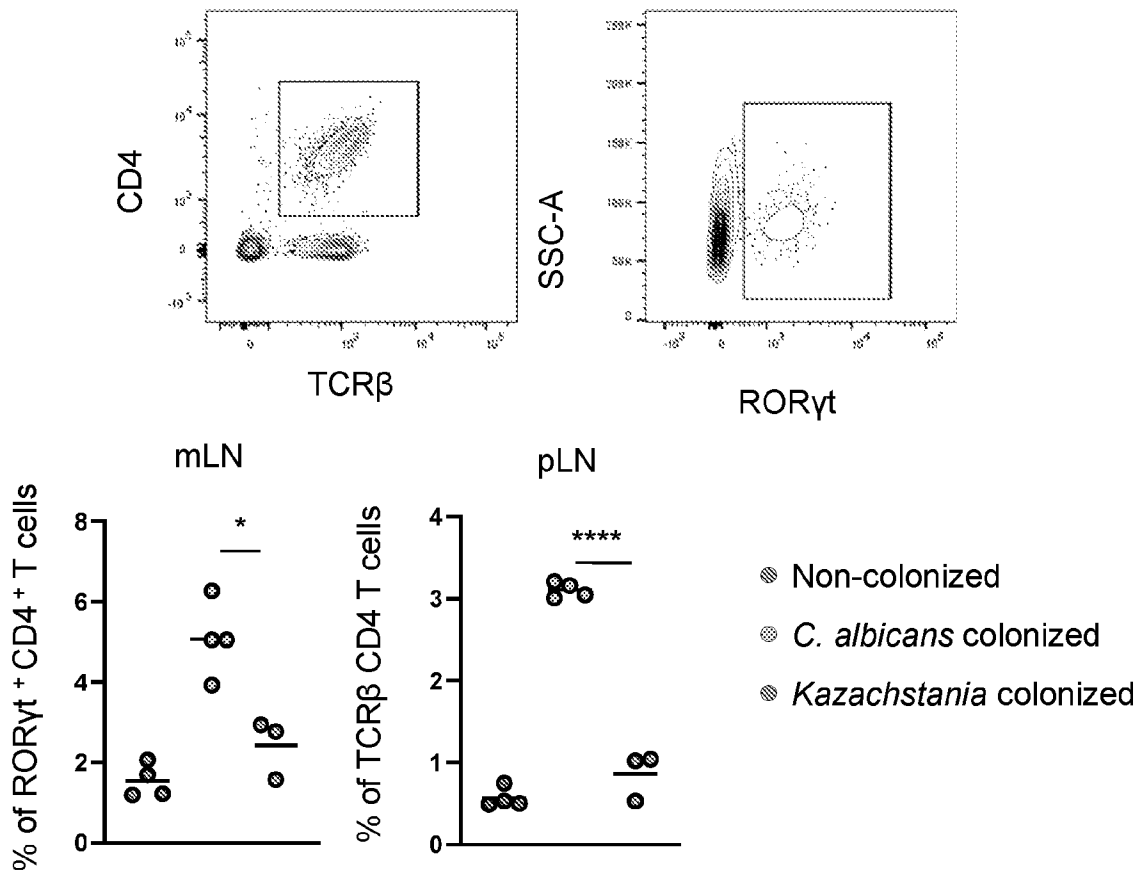


FIGURE 4D

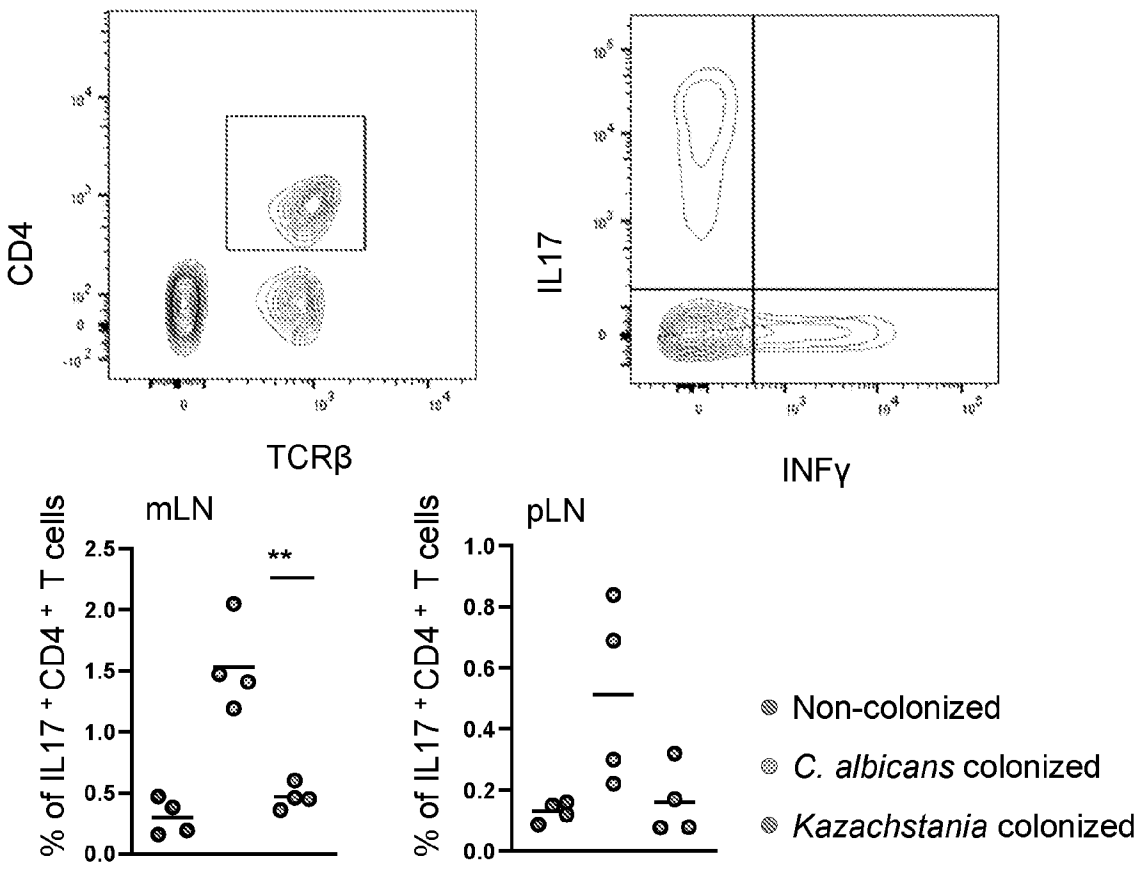


FIGURE 4E

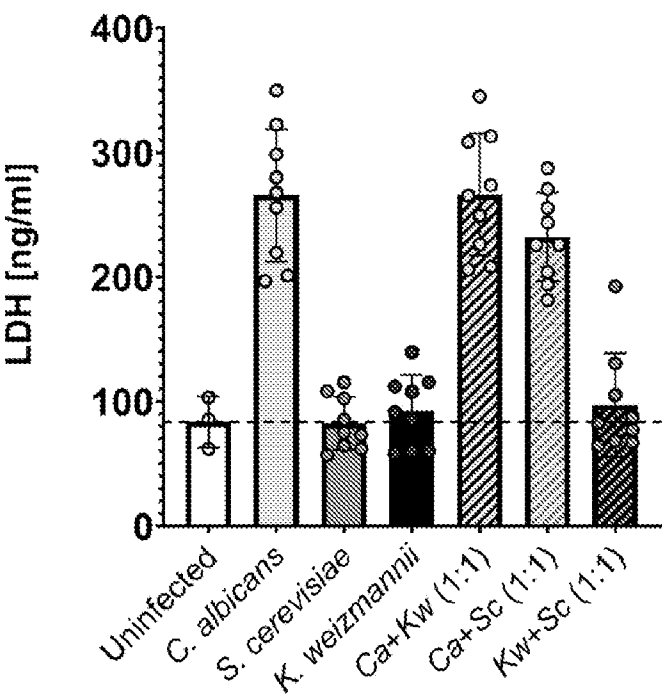


FIGURE 4F

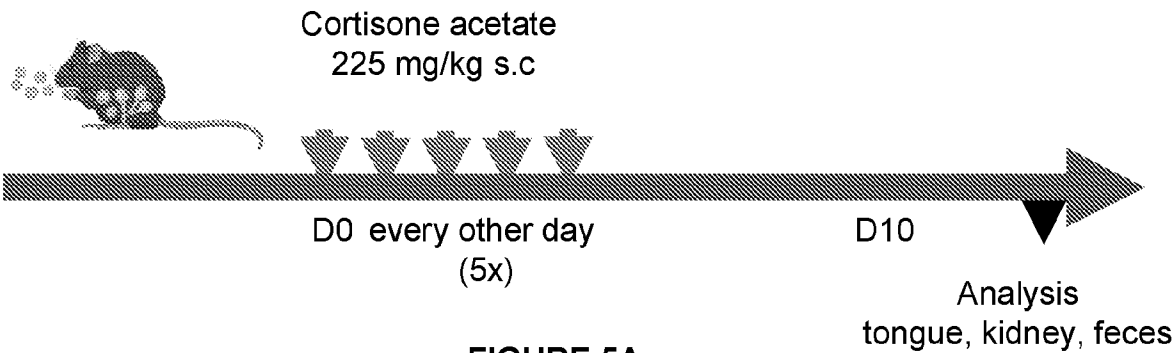


FIGURE 5A

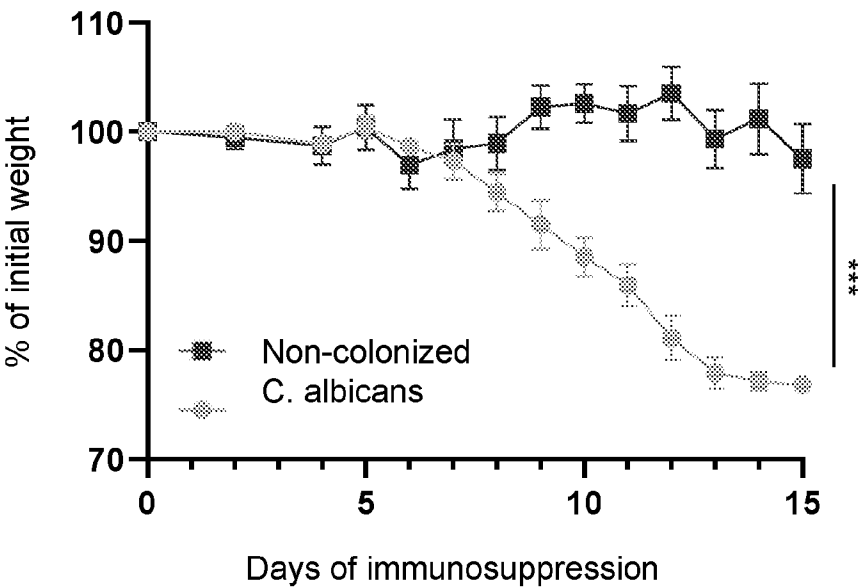


FIGURE 5B

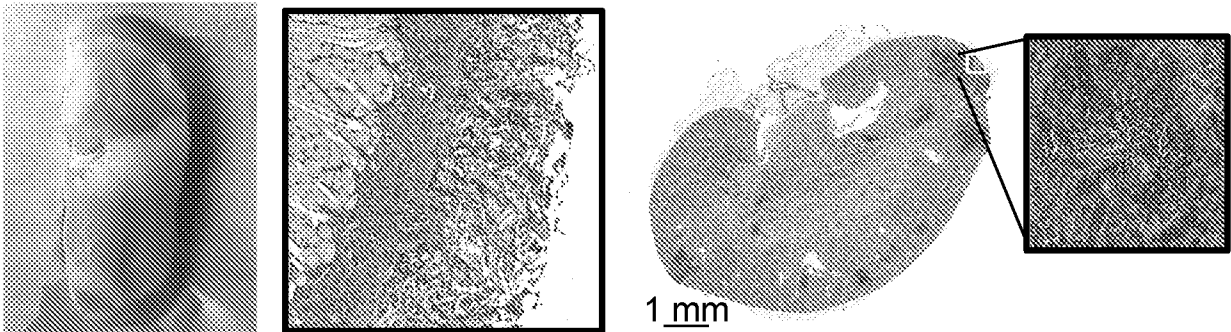


FIGURE 5C

FIGURE 5D

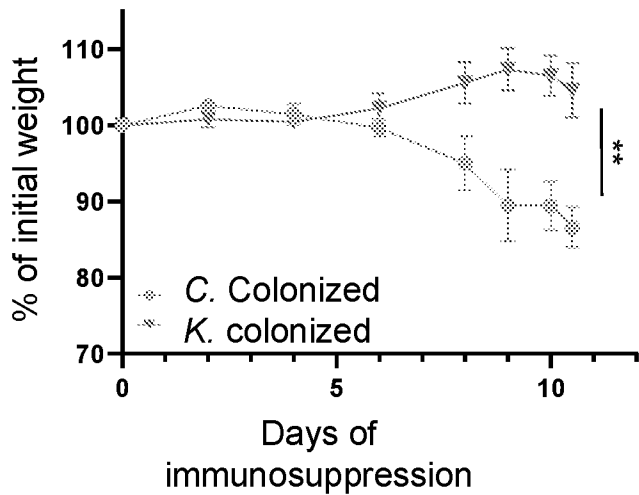


FIGURE 5E

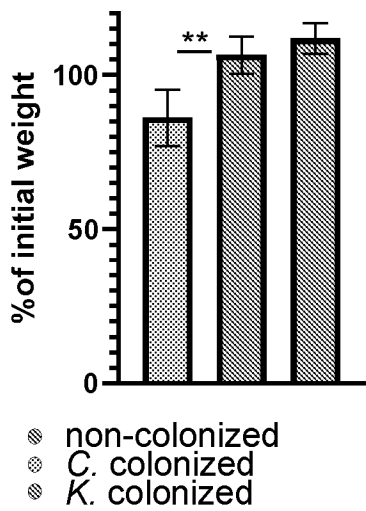


FIGURE 5F

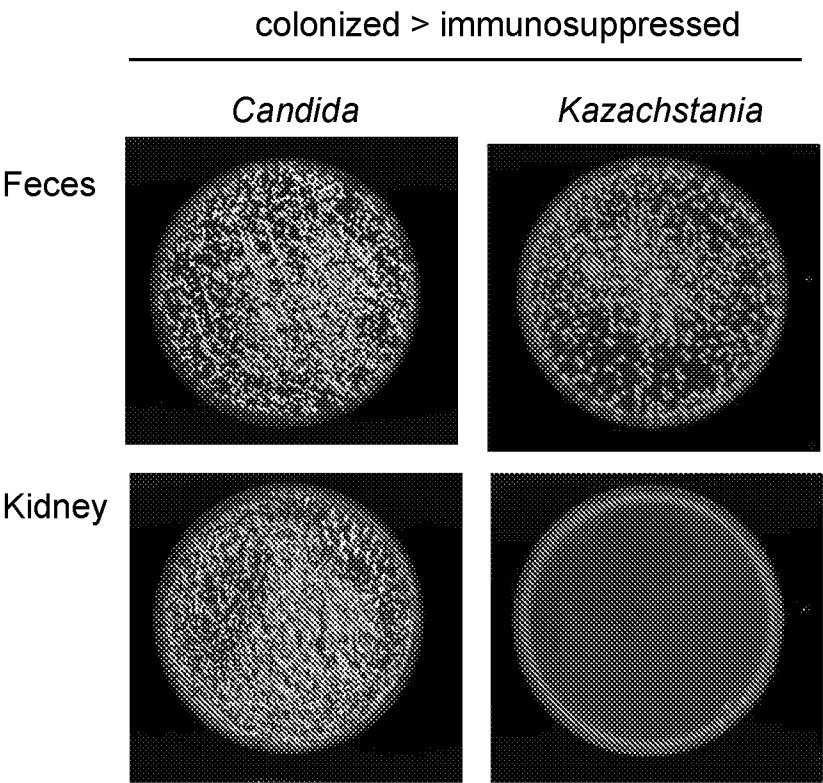


FIGURE 5G

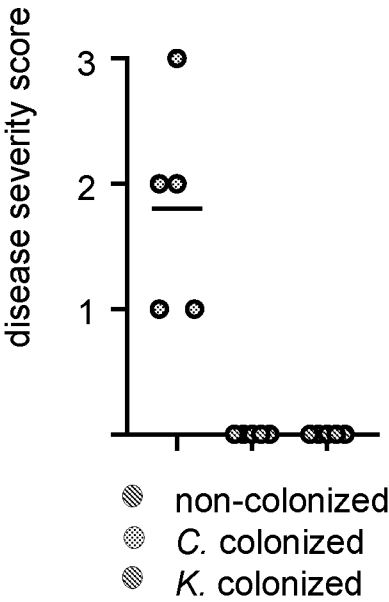


FIGURE 5H

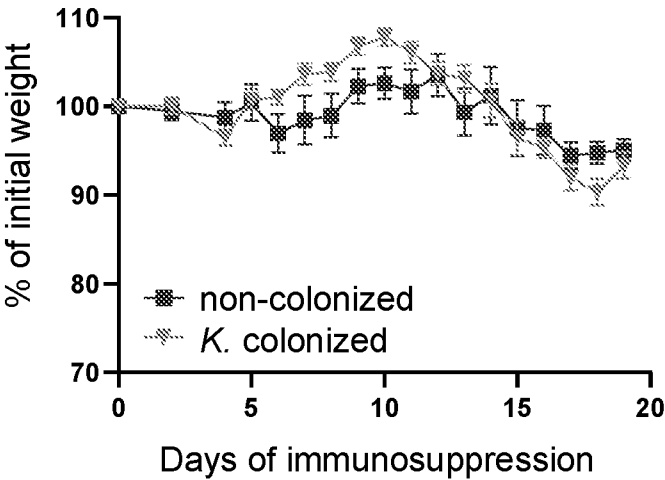


FIGURE 5I

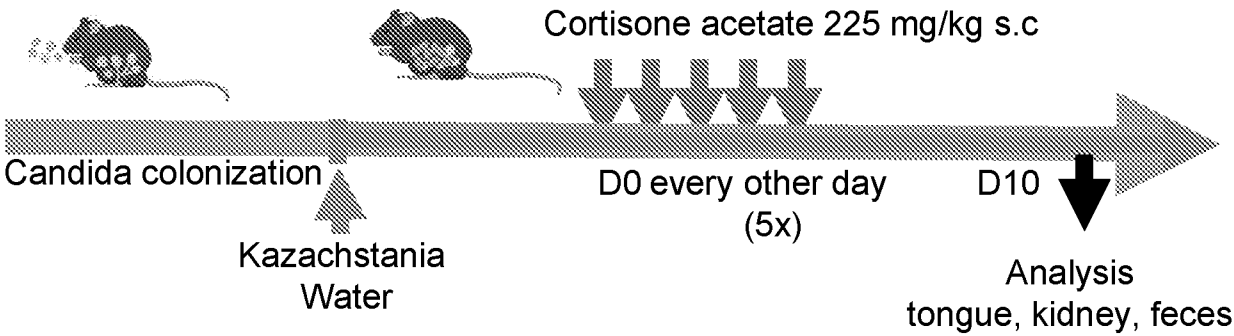


FIGURE 6A

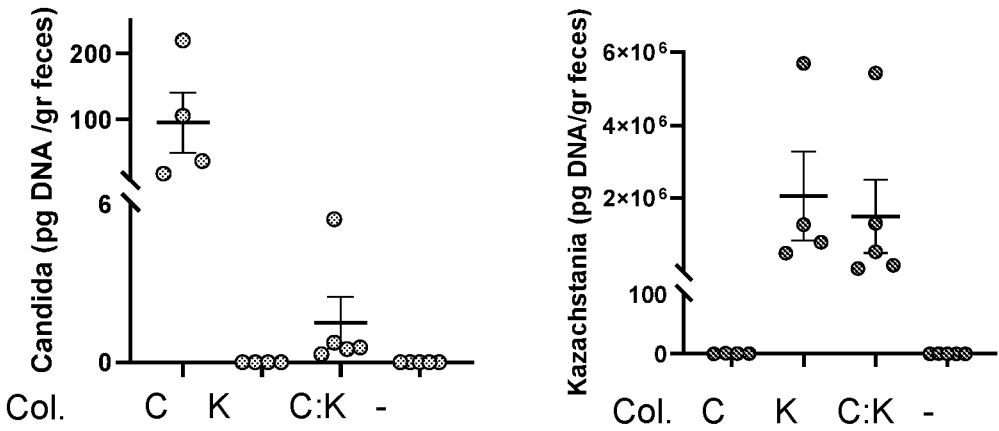


FIGURE 6B

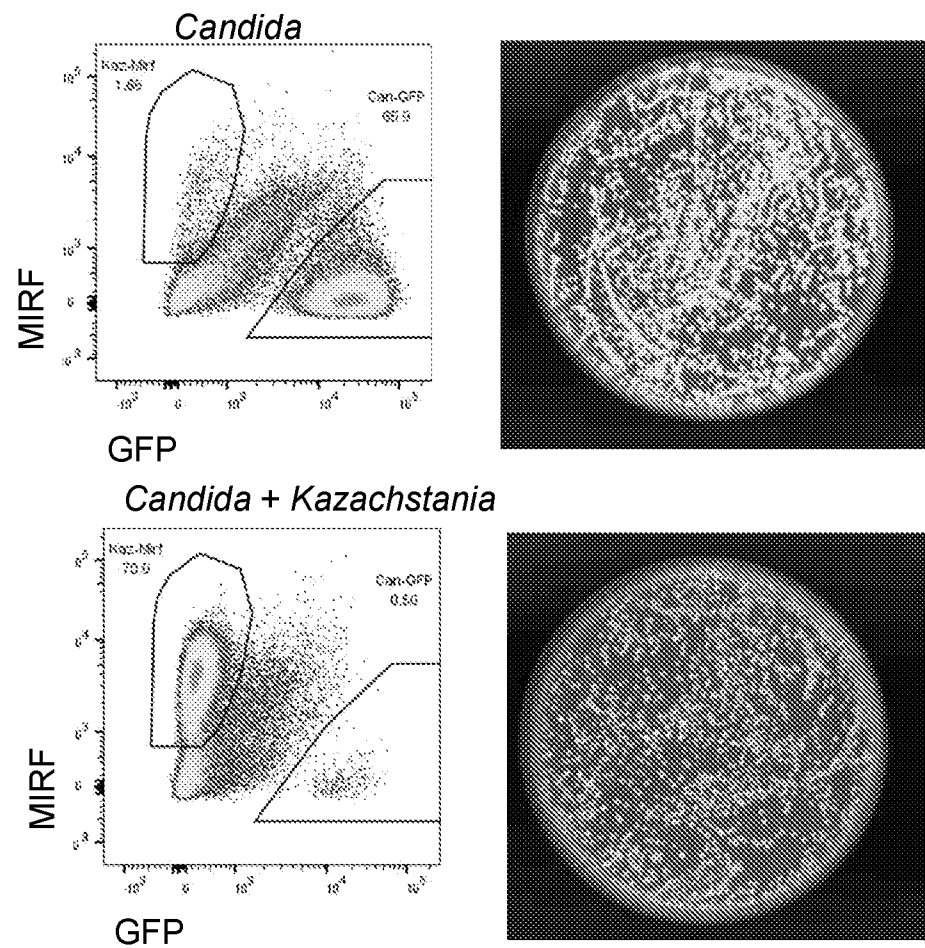


FIGURE 6C

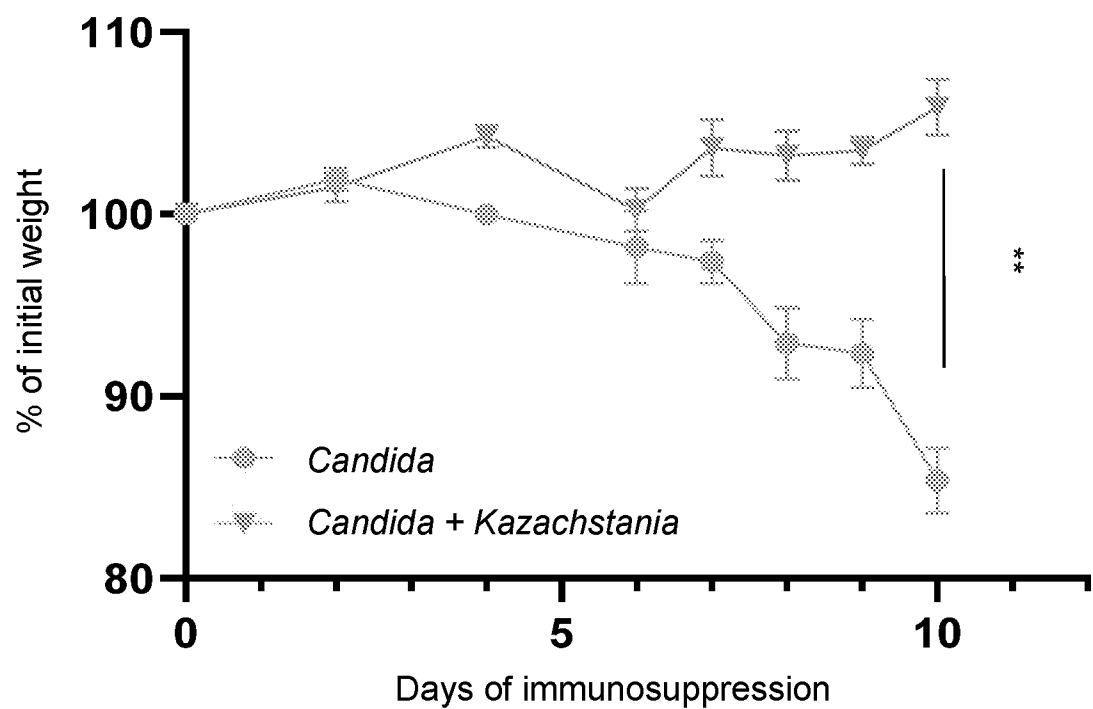


FIGURE 6D

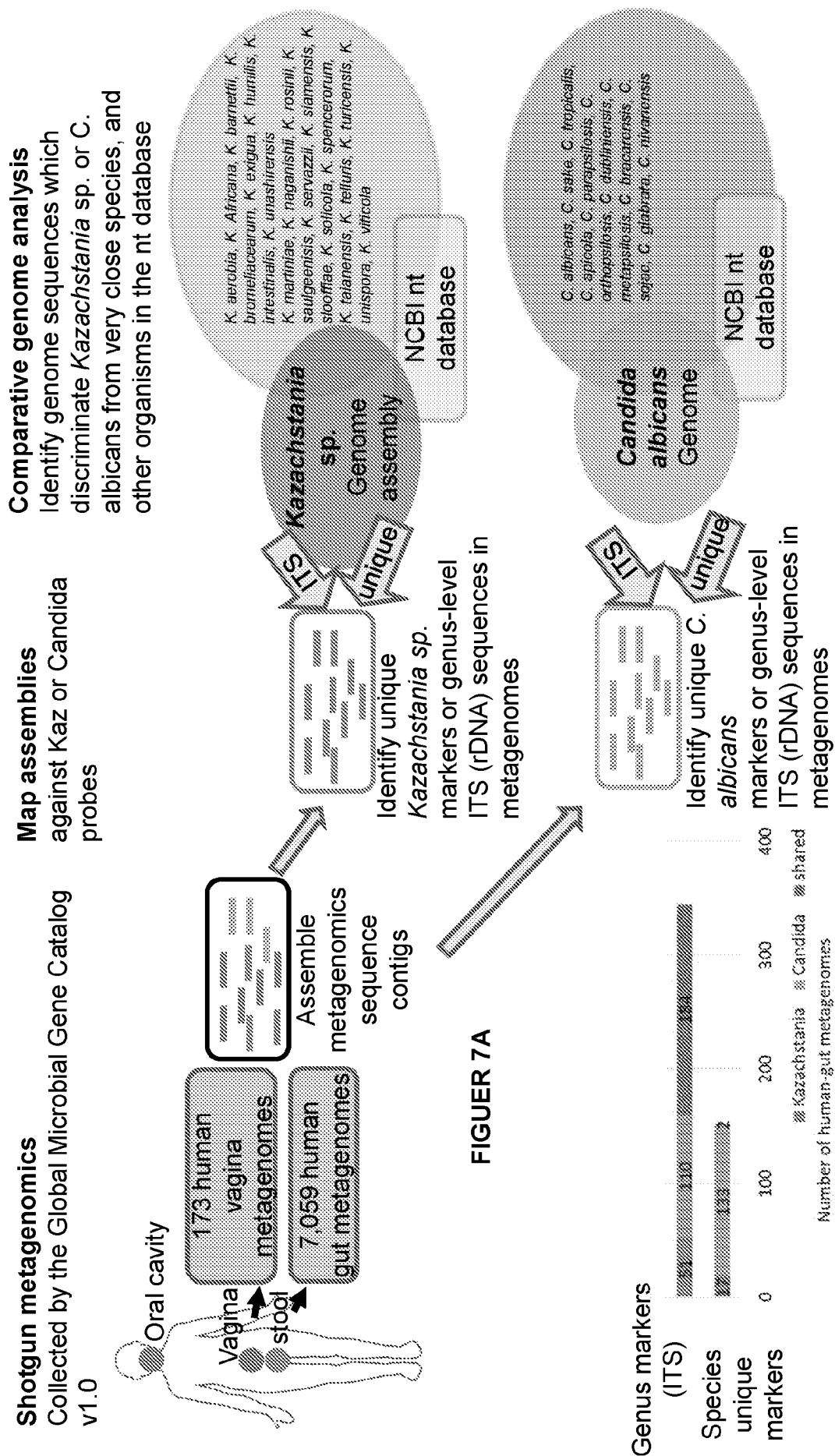
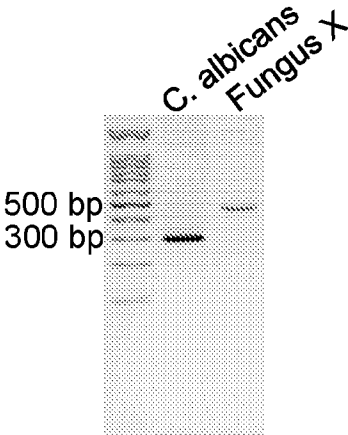
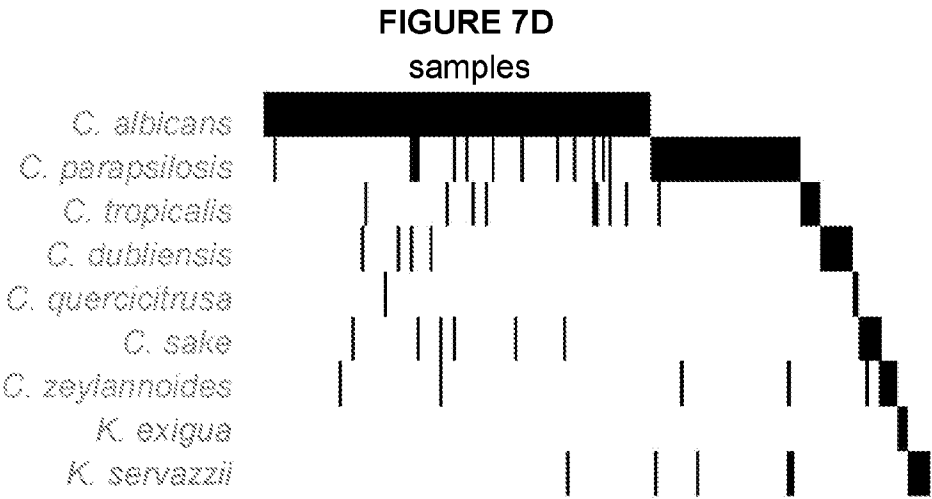
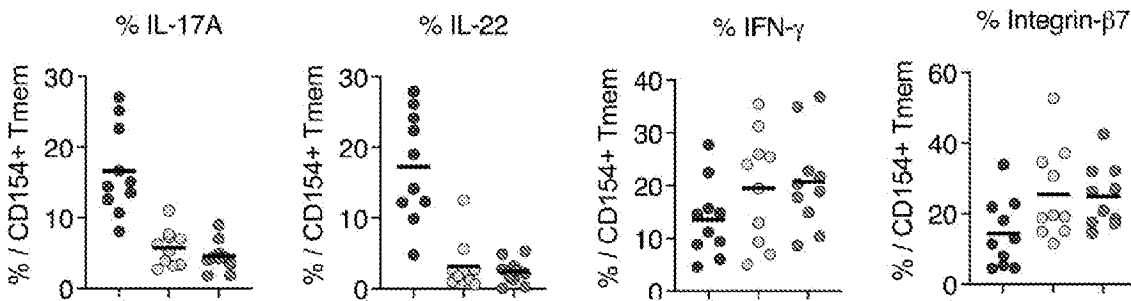
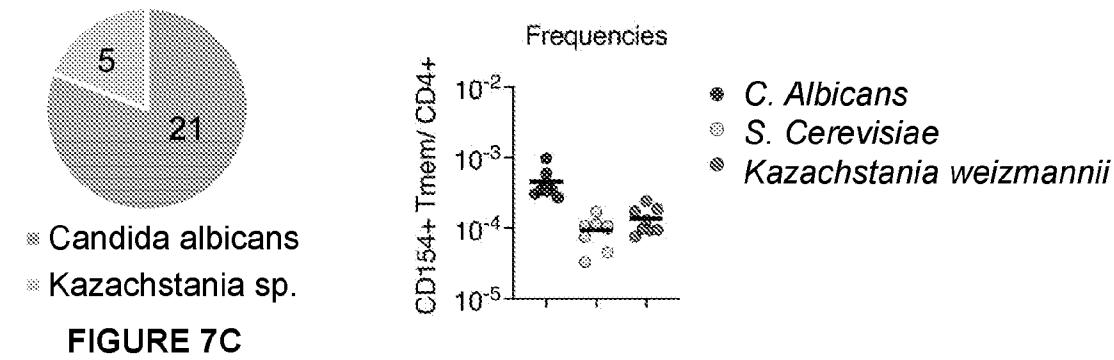


FIGURE 7B



Description	Max Score	Total Score	Query Cover	E value	Per. Ident
<i>Kazachstanella telluris</i> isolate 1.68 internal transcribed spacer 1, partial sequence, 5.3S ribosomal RNA gene, complete sequence, and internal tra	719	719	100%	0.0	100.00%
<i>Kazachstanella heterogenica</i> culture CBS 2774 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosoma	713	713	100%	0.0	99.74%
<i>Kazachstanella heterogenica</i> CBS 2676 ITS region, from TYPE material	706	706	100%	0.0	99.49%
<i>Kazachstanella heterogenica</i> culture CBS 2675 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1 and 5.8S ribosa	706	706	100%	0.0	99.49%
<i>Kazachstanella putidopacii</i> culture CBS 2676 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal	616	616	100%	4e-172	95.14%

FIGURE 8B

ATGGCTAACTTGGACGAAATGTTGCAAACTA
CCGTTGAAGAAGTCAGACAATCTTGCAAGT
TAATCGTGCTTGGTGTCCCAATTTGAAGAG
GATTATCCGGTGTGTTGTTGTCGAAAGCTG
TTGATGATAGATGGCAATCTATTTGGGTACT
CAAGTCAGAGACAGGTACTTCATGGAAACTA
GAGGTGAAGAATACTCCCATGGTAGATATCA
AGCTATTGCCGATATCTACACTGCTAACTTGA
CTGAATGCTACAGAGAGTTGTTGGAACAATT
TCAAGTTAGAGCCATTTTGGCCGTTCCAATC
TTGCAAGGTAATCAATTGTGGGGTTTGTGGA
TTGCCCATCAATTGTCTGCTCCAAGACAATG
GCAACCATGGGAAATTGATTCTTTGCAACAA
ATTGCTACCCCAATTGGCCATTGCTATTCAACA
AGCT (SEQ ID NO: 42)

FIGURE 9A

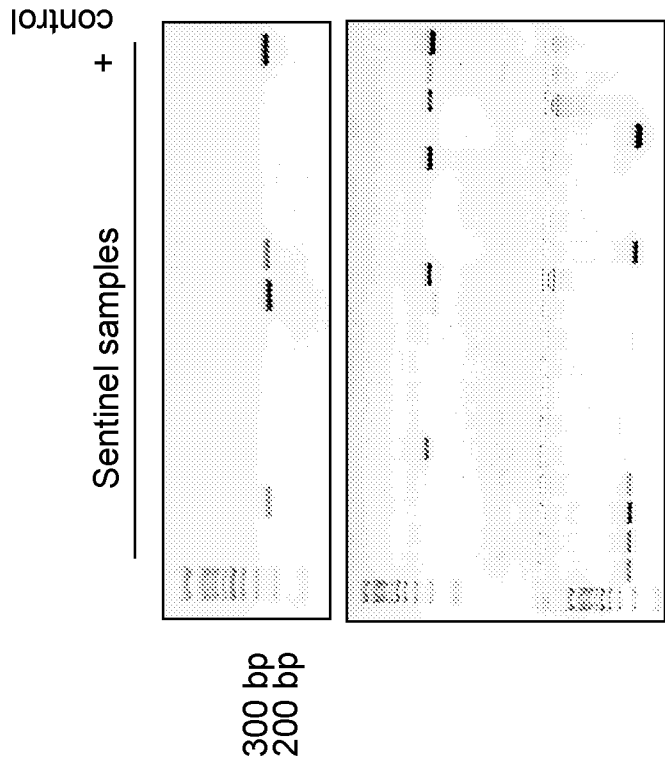
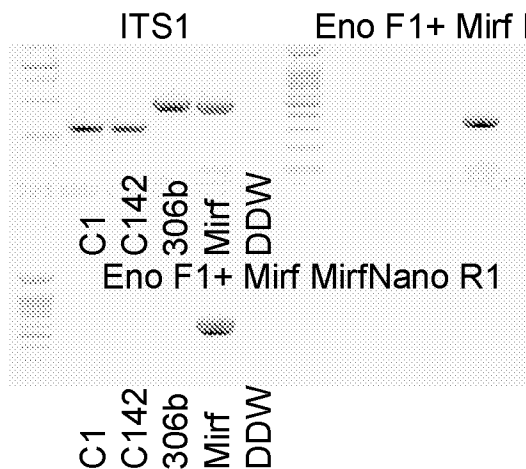


FIGURE 8C

Validation of ENO1-Mirf fusion protein



PCR

ITS1 F+R DNA quality controls

Eno F1+ Mirf R screen only Mirf

inserted into ENO1 locus will amplify (350 bp)

Eno F1+ MirfNano R1

only Mirf inserted into ENO1 locus will amplify (600 bp)

FIGURE 9B

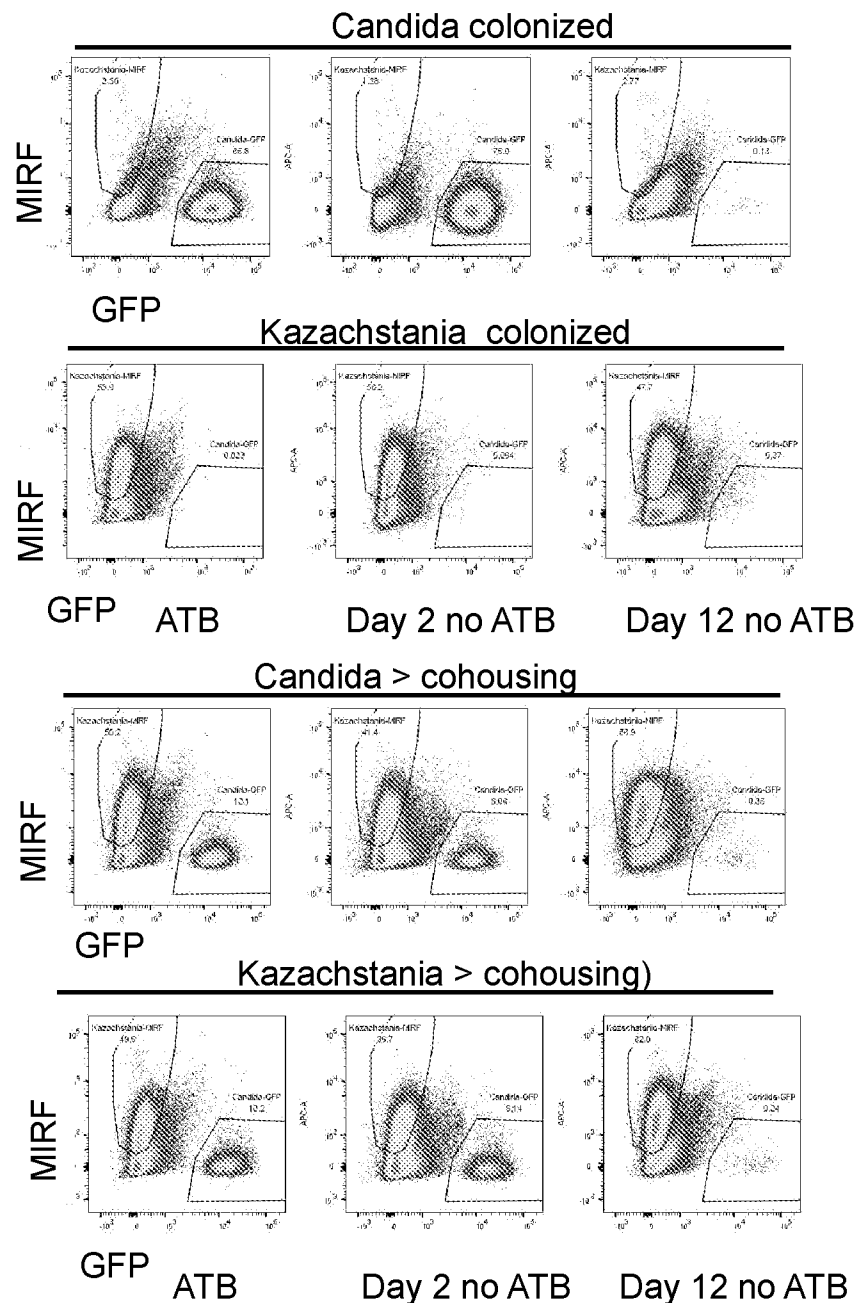


FIGURE 9C

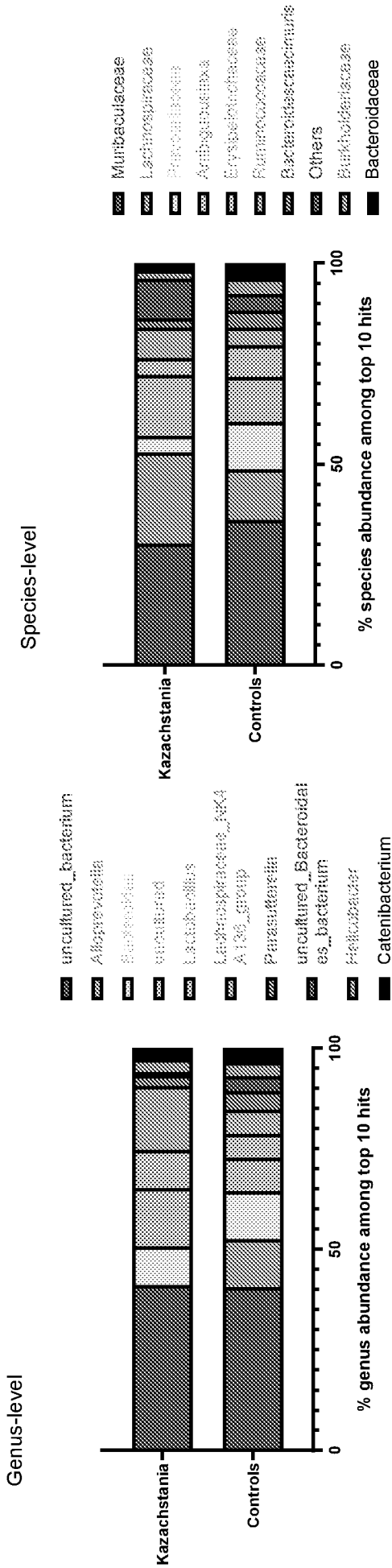


FIGURE 10A

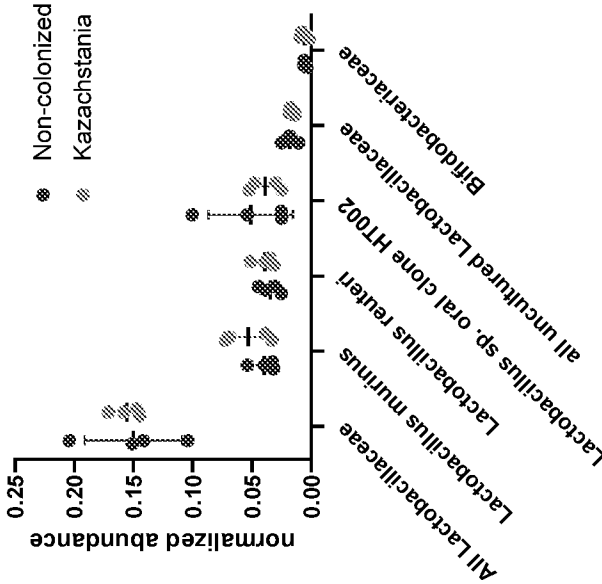
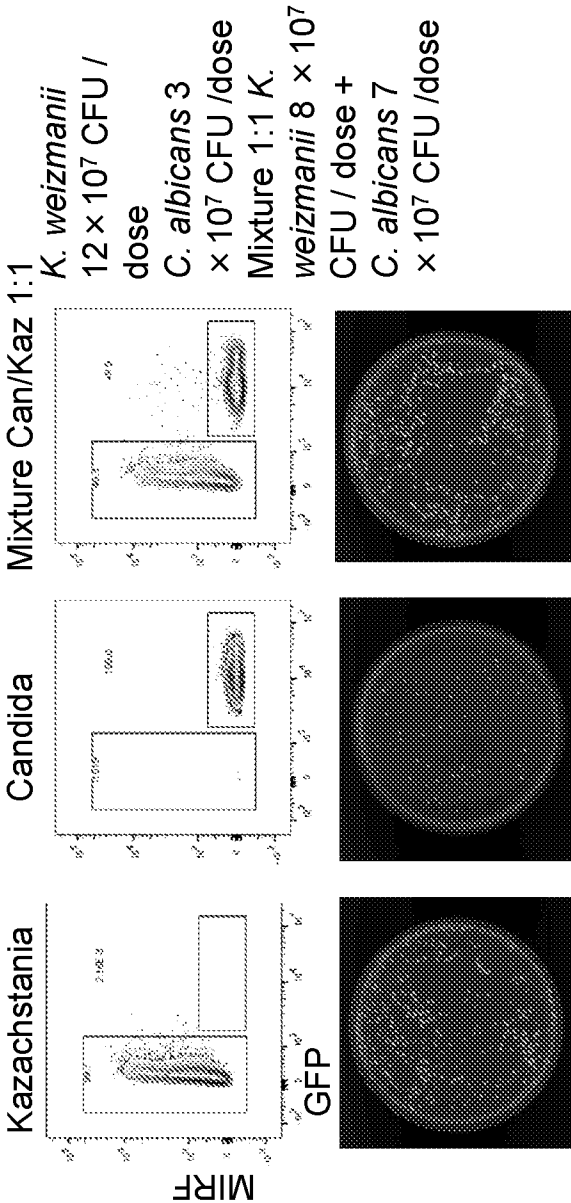


FIGURE 10B



Ampicillin treated SPF animals

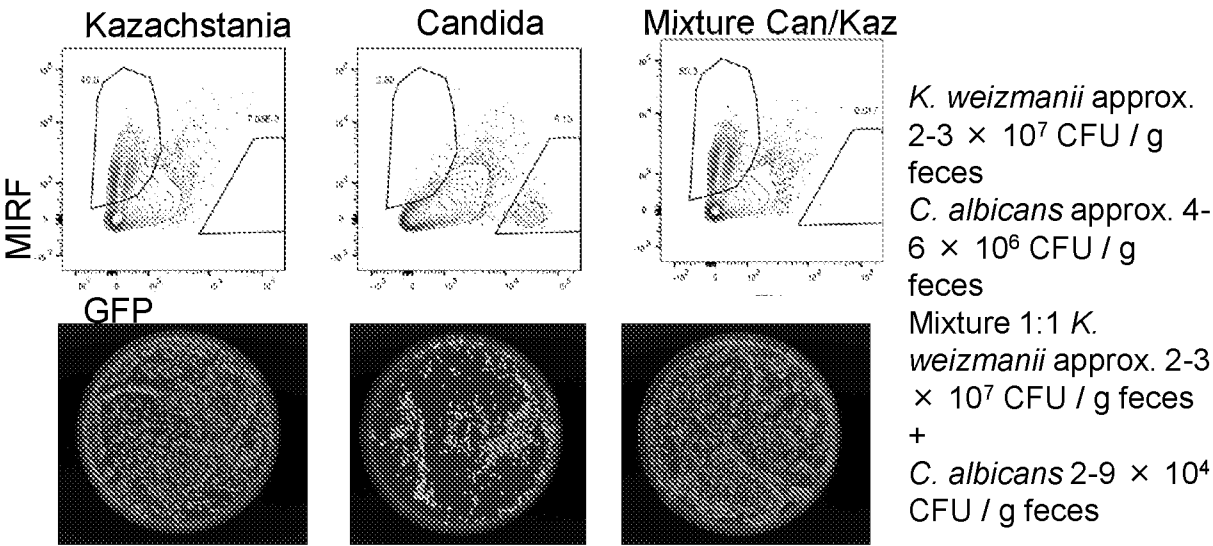
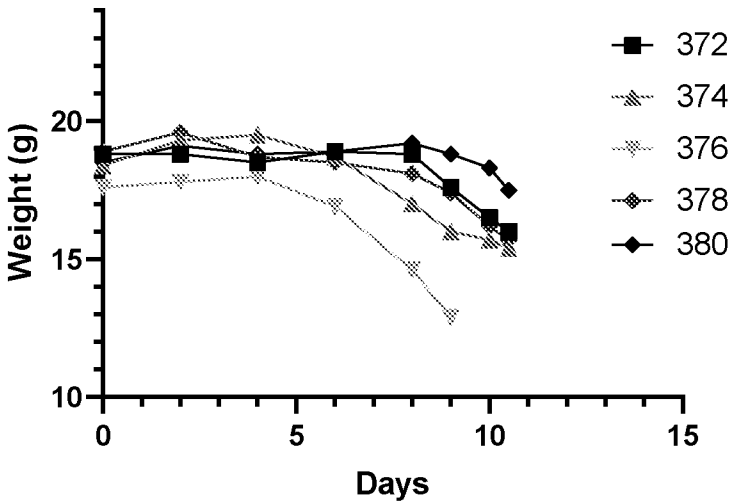


FIGURE 10D

Candida colonized mice



Kazachstania colonized mice

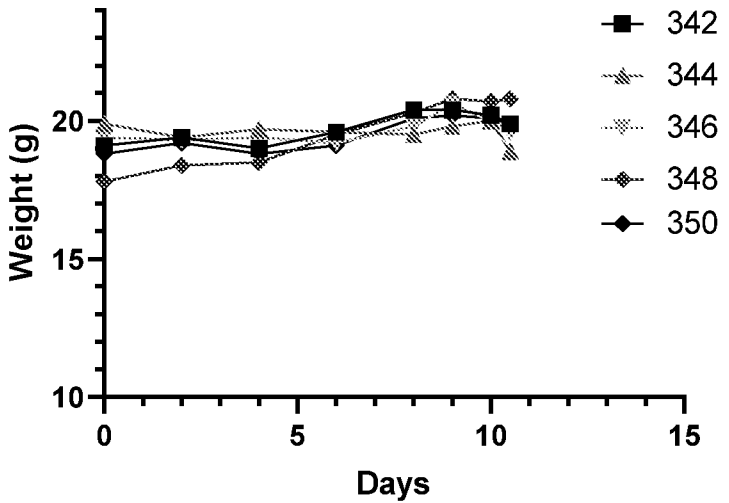


FIGURE 11A

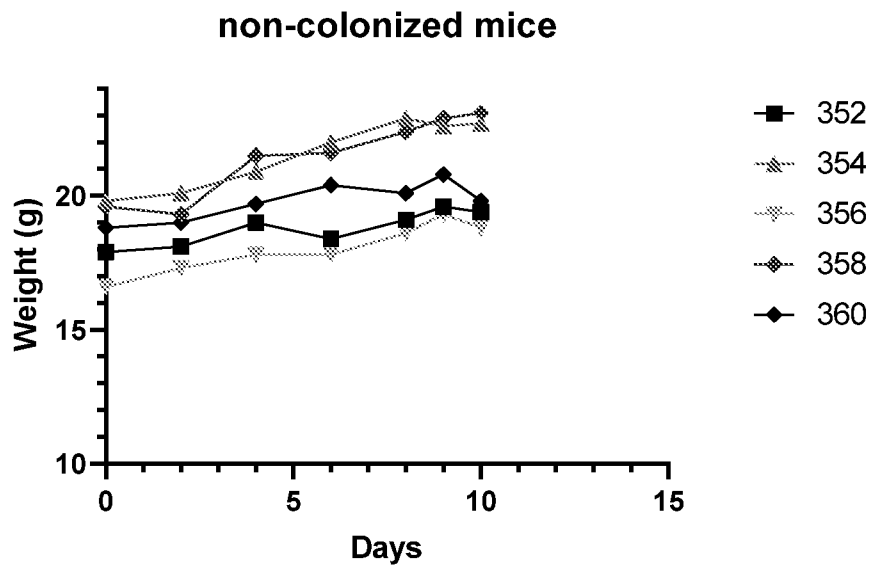


FIGURE 11A – Continue

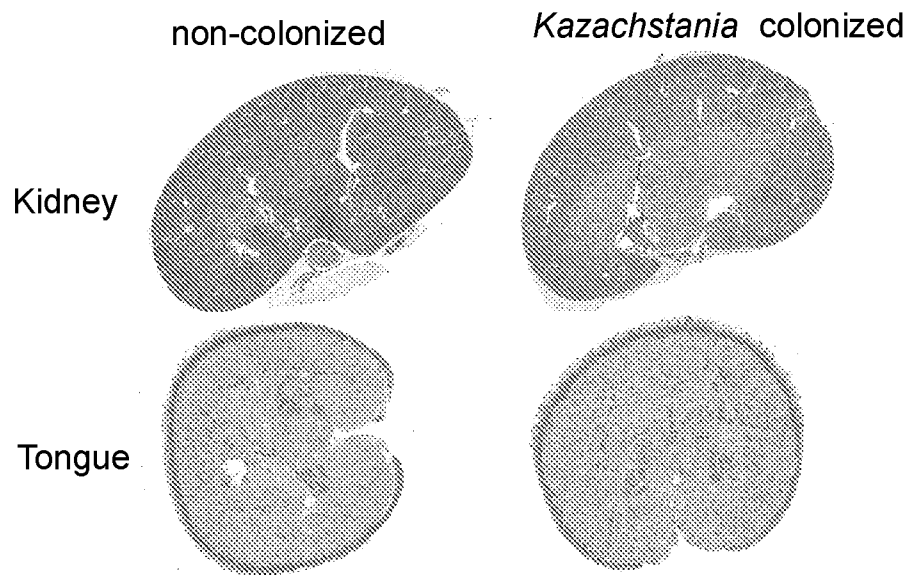


FIGURE 11B

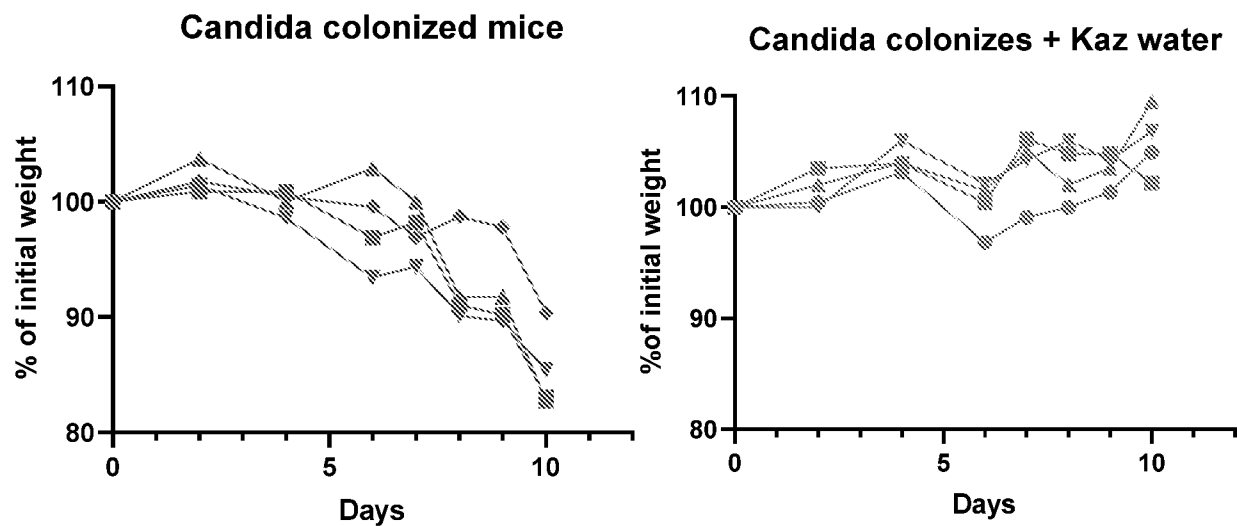


FIGURE 12A

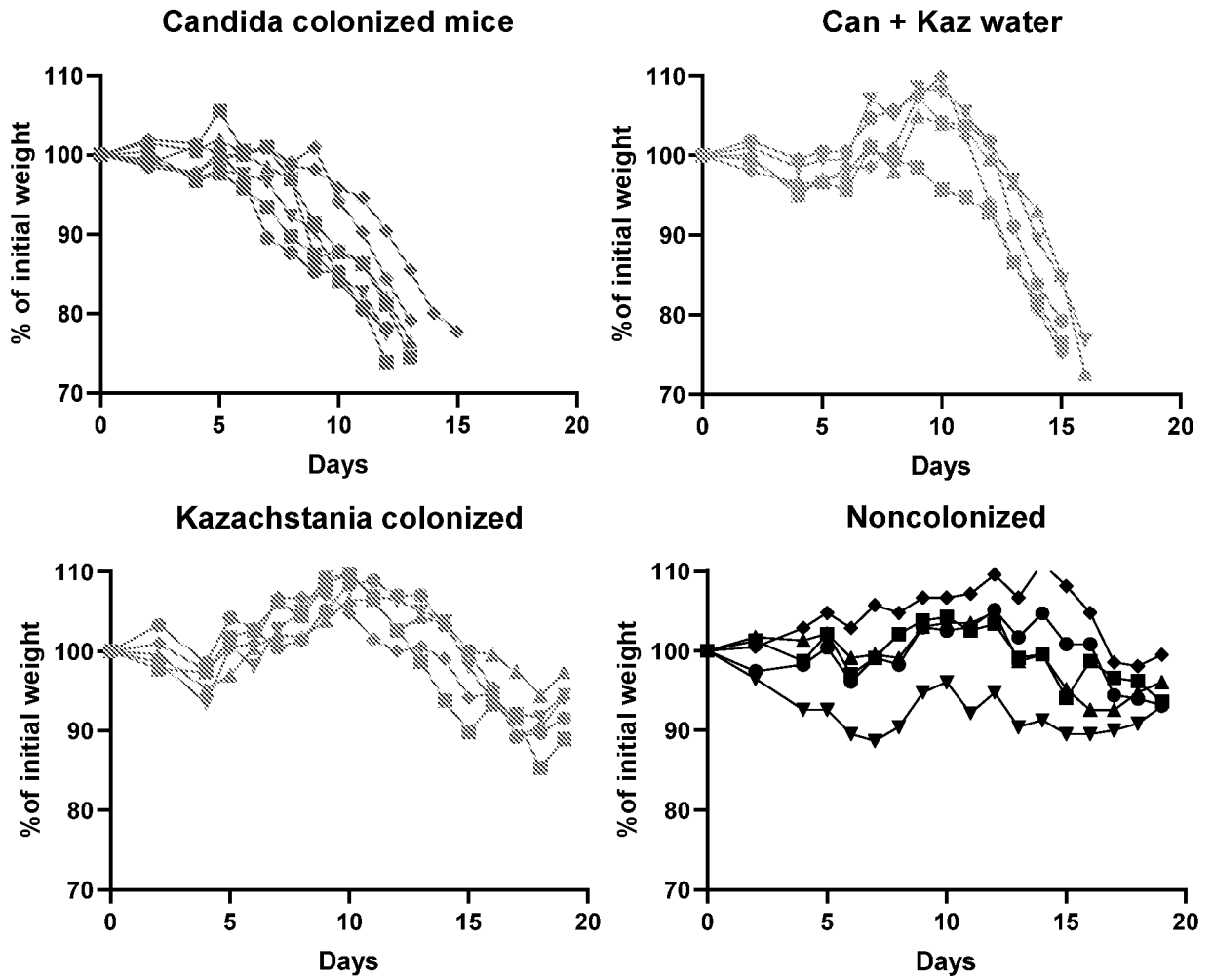


FIGURE 12B

Metagenome source	Habitat	No. of reads mapped on K. weizmannii unique-JTS markers	No. of reads mapped on C. albicans unique markers	Total number of contigs screened	Geographical Location
SAMN04126284	Human gut	233	54	305,514	Canada:Montreal
SAMN04126280	Human gut	62	9	96,512	Canada:Montreal
SAMN05545065	Human gut	61	0	157,550	USA: Baltimore
SAMN04126316	Human gut	56	13	37,032	Canada:Montreal
SAMN05545049	Human gut	54	0	153,220	USA: Baltimore
SAMN05545064	Human gut	50	1	114,680	USA: Baltimore
SAMN05545009	Human gut	50	1	117,558	USA: Baltimore
SAMN04126282	Human gut	48	11	87,156	Canada:Montreal
SAMN04126272	Human gut	43	5	41,148	Canada:Montreal
SAMN05545062	Human gut	35	0	68,574	USA: Baltimore
SAMN02675504	Human gut	34	229	1,169,572	USA
SAMN05545045	Human gut	34	1	212,946	USA: Baltimore
SAMN02334020	Human gut	26	0	523,358	
SAMN04126287	Human gut	21	0	91,130	Canada:Montreal
SAMEA2467006	Human gut	12	0	220,102	France
SAMN02333976	Human gut	9	0	174,448	
SAMN02334152	Human gut	9	0	159,108	
SAMN01828410	Human gut	7	0	21,816	USA
SAMN02676195	Human gut	7	1	183,562	USA
SAMN01795958	Human gut	6	0	34,840	
SAMEA3664944	Human gut	4	0	240,474	Israel
SAMN02334061	Human gut	4	0	700,766	
SAMN04126311	Human gut	2	770	40,596	Canada:Montreal
SAMEA2143074	Human gut	2	0	173,892	Denmark
SAMEA3449186	Human gut	2	1	332,418	United Kingdom
SAMEA3541530	Human gut	1	0	244,606	China
SAMEA3879608	Human gut	1	0	288,780	Washington, DC, USA
SAMEA2042558	Human gut	1	0	108,620	Denmark
SAMEA2163351	Human gut	1	0	108,224	Denmark
SAMEA3664845	Human gut	1	0	272,902	Israel
SAMN04126109	Human gut	1	0	86,446	Canada:Montreal
SAMN05525378	Human gut	1	0	212,452	USA:Houston

FIGURE 13A

Metagenome source	Habitat	No. of reads mapped on <i>C. albicans</i> ITS, allowing mismatches (in parenthesis without mismatches)	No. of reads mapped on <i>C. albicans</i> unique markers	No. of reads mapped on <i>K. weitzmannii</i> ITS (allowing mismatches)	No. of reads mapped on <i>K. weitzmannii</i> unique markers (in parenthesis: high confidence markers)
SRS042428	human vagina	3 (3)	28	2	0
SRS1041155	human vagina	6 (2)	3	6	9
SRS1055021	human vagina	5	3	6	24 (2)
SRS1055028	human vagina	4	10	4	16 (1)
SRS1041185	human vagina	3	9	4	15 (1)
SRS1041175	human vagina	4	7	4	10 (1)
SRS1055082	human vagina	4	9	4	6 (1)
SRS1041160	human vagina	4	14	4	40
SRS1055094	human vagina	4	11	4	24
SRS893364	human vagina	7	14	7	21
SRS1055044	human vagina	6	10	6	19
SRS893344	human vagina	5	8	5	17
SRS1055066	human vagina	3	4	4	14
SRS1041163	human vagina	5	5	5	13
SRS1055075	human vagina	6	3	6	10
SRS893357	human vagina	3	4	4	6
SRS1055051	human vagina	6	5	5	5
SRS893262	human vagina	5	6	5	5
SRS1041127	human vagina	4	4	5	5
SRS893320	human vagina	4	4	5	5
SRS893287	human vagina	4	2	5	2
SRS024428	human vagina	2	0	2	0

FIGURE 13B

INTERNATIONAL SEARCH REPORT

International application No

PCT/IL2023/050470

A. CLASSIFICATION OF SUBJECT MATTER

INV. C12N1/16 A61K36/064

ADD.

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)

C12N A61K C12R

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)

EPO-Internal, BIOSIS, COMPENDEX, EMBASE, FSTA, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KIM JONG-HWA ET AL: "Kazachstania turicensis CAU Y1706 ameliorates atopic dermatitis by regulation of the gut-skin axis", JOURNAL OF DAIRY SCIENCE, AMERICAN DAIRY SCIENCE ASSOCIATION, US , vol. 102, no. 4 31 March 2019 (2019-03-31), pages 2854-2862, XP009519690, ISSN: 0022-0302, DOI: 10.3168/JDS.2018-15849 Retrieved from the Internet: URL:https://www.sciencedirect.com/science/article/pii/S002203021930133X the whole document ----- -/--	1-28



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

2 August 2023

Date of mailing of the international search report

09/08/2023

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2

NL - 2280 HV Rijswijk

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Authorized officer

Lejeune, Robert

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IL2023/050470

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed.
 - b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.
2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No

PCT/IL2023/050470

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SUMMERS KATIE LYNN ET AL: "Characterization of Kazachstania slooffiae, a Proposed Commensal in the Porcine Gut", JOURNAL OF FUNGI, vol. 7, no. 2, 17 February 2021 (2021-02-17), page 146, XP093065470, DOI: 10.3390/jof7020146 Retrieved from the Internet: URL:https://www.ncbi.nlm.nih.gov/pmc/artic les/PMC7922399/pdf/jof-07-00146.pdf> the whole document -----	1-28
A	US 2019/183950 A1 (WAY SING SING [US]) 20 June 2019 (2019-06-20) the whole document -----	1-28

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/IL2023/050470

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2019183950	A1	20-06-2019	NONE
