



US011638728B2

(12) **United States Patent**
Gajewski et al.

(10) **Patent No.:** **US 11,638,728 B2**
(45) **Date of Patent:** **May 2, 2023**

(54) **MICROBIOME BIOMARKERS
IMMUNOTHERAPY RESPONSIVENESS:
DIAGNOSTIC, PROGNOSTIC AND
THERAPEUTIC USES THEREOF**

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(71) Applicant: **The University of Chicago**, Chicago, IL (US)

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(73) Assignee: **The University of Chicago**, Chicago, IL (US)

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

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(21) Appl. No.: **16/618,700**

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(22) PCT Filed: **Jun. 5, 2018**

International Search Report and Written Opinion for PCT/US18/36052, dated Aug. 30, 2018. 7 pages.

(86) PCT No.: **PCT/US2018/036052**

§ 371 (c)(1),

(2) Date: **Dec. 2, 2019**

Andrews, FastQC: A quality control application for high throughput sequence data. Babraham Institute. Project page: <http://www.bioinformatics.babraham.ac.uk/projects/fastqc>, 2016. Retrieved May 11, 2022. 6 pages.

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(65) **Prior Publication Data**

US 2021/0177918 A1 Jun. 17, 2021

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Related U.S. Application Data

(60) Provisional application No. 62/515,366, filed on Jun. 5, 2017, provisional application No. 62/577,454, filed on Oct. 26, 2017.

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(51) **Int. Cl.**

A61K 35/745 (2015.01)

A61P 35/00 (2006.01)

A61K 35/747 (2015.01)

C07K 16/28 (2006.01)

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CPC **A61K 35/745** (2013.01); **A61K 35/747** (2013.01); **A61P 35/00** (2018.01); **C07K 16/2818** (2013.01); **C07K 16/2827** (2013.01)

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(58) **Field of Classification Search**

CPC A61K 39/00
USPC 424/9.2, 93.1, 93.3, 93.4, 234.1, 277.1, 424/278.1
See application file for complete search history.

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Primary Examiner — Oluwatosin A Ogunbiyi

(74) *Attorney, Agent, or Firm* — Casimir Jones SC; David W. Staple

(57) **ABSTRACT**

Provided herein are compositions and methods comprising microbiome biomarkers of responsiveness/resistance to immunotherapy (e.g., anti-PD1/PD-L1 therapy), and diagnostic, prognostic and therapeutic uses thereof. In particular, the amount, identity, presence, and/or ratio of microflora in the microbiome of a subject is used to determine the responsiveness/resistance of the subject to immunotherapy, and/or the microbiome of a subject is manipulated to enhance the responsiveness of the subject to various immunotherapies and co-therapies.

14 Claims, 29 Drawing Sheets
(19 of 29 Drawing Sheet(s) Filed in Color)
Specification includes a Sequence Listing.

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FIG. 1A

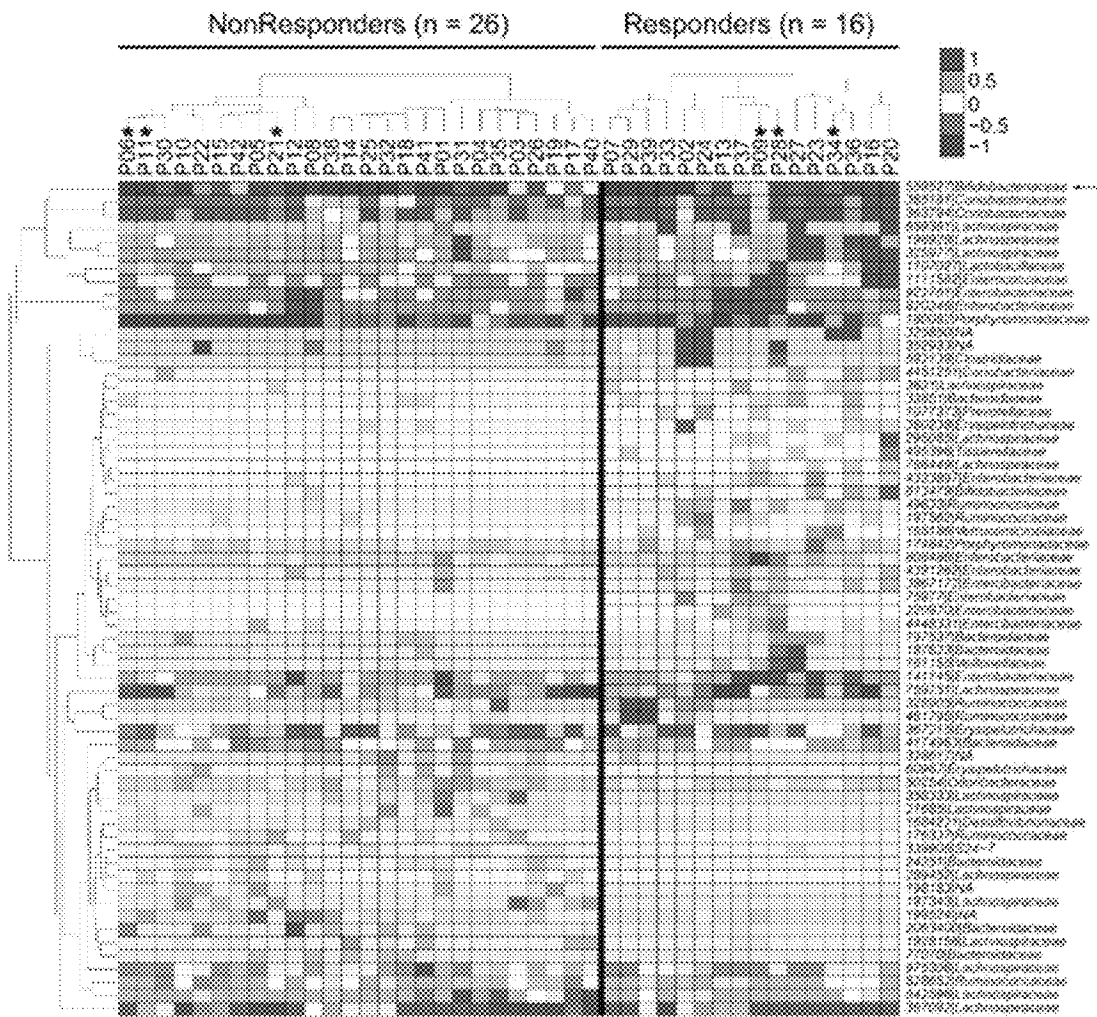


FIG. 1B

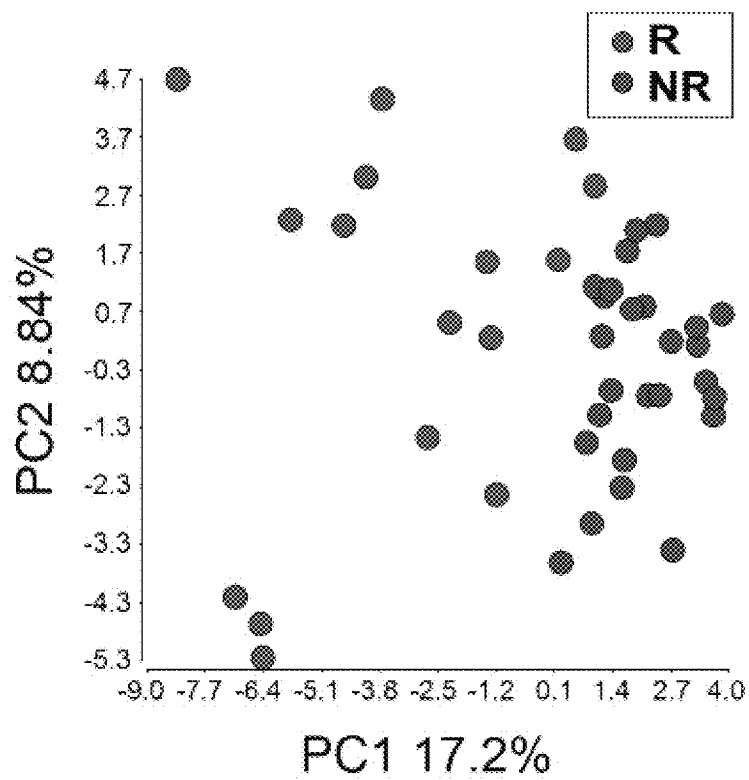


FIG. 2A

559527|Bifidobacteriaceae



FIG. 2B

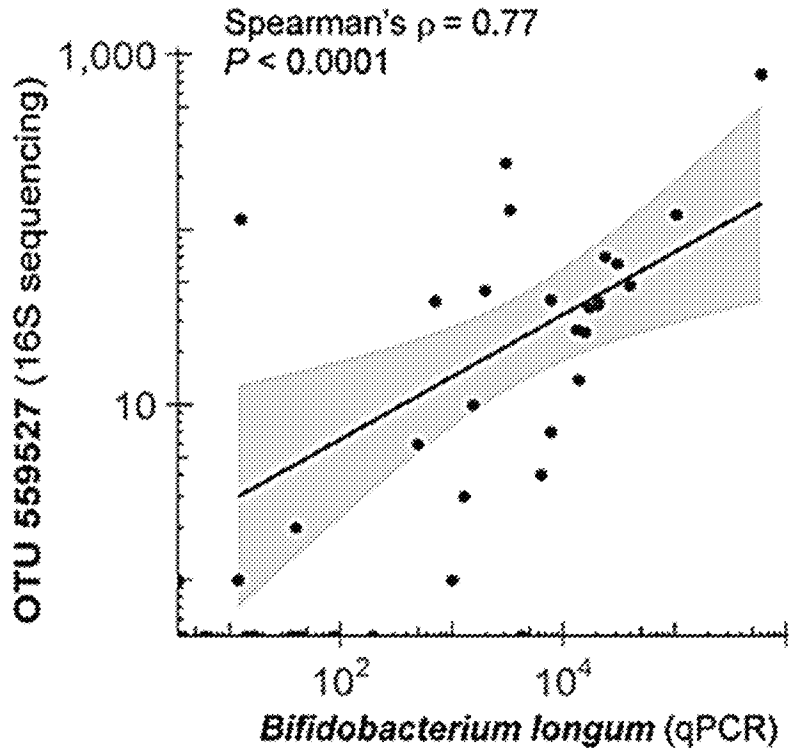
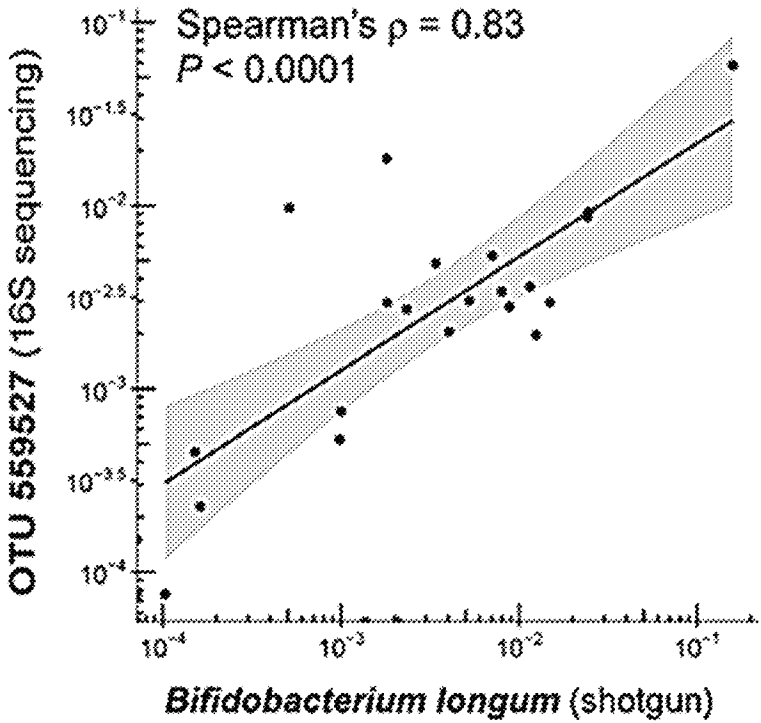


FIG. 2C

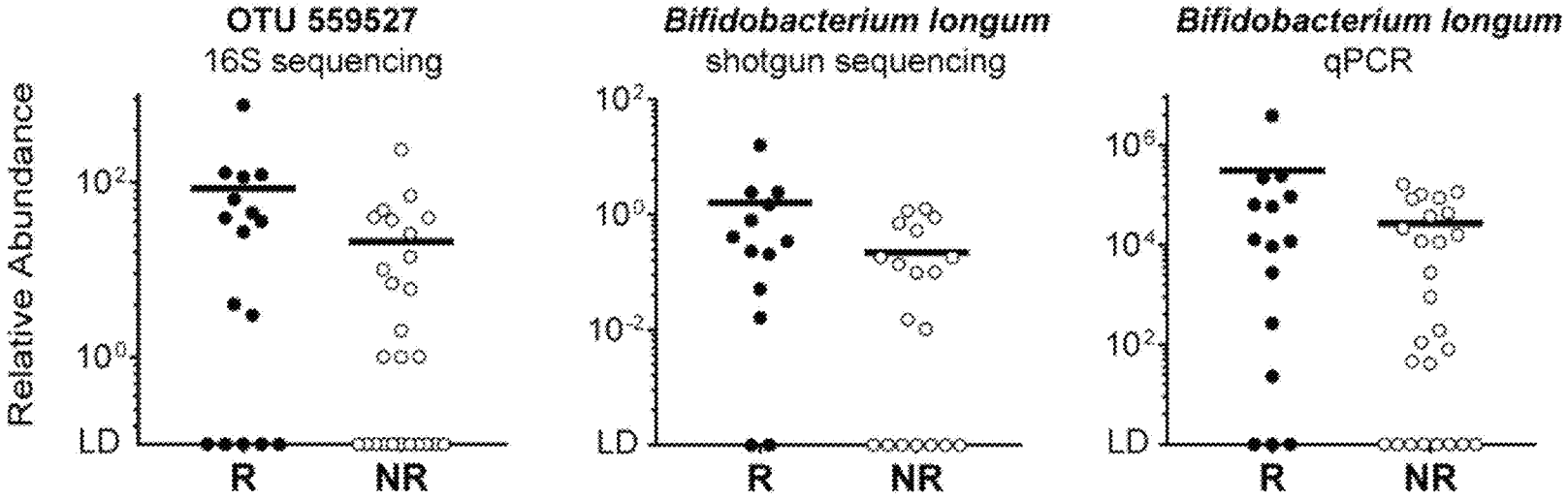


FIG. 2E

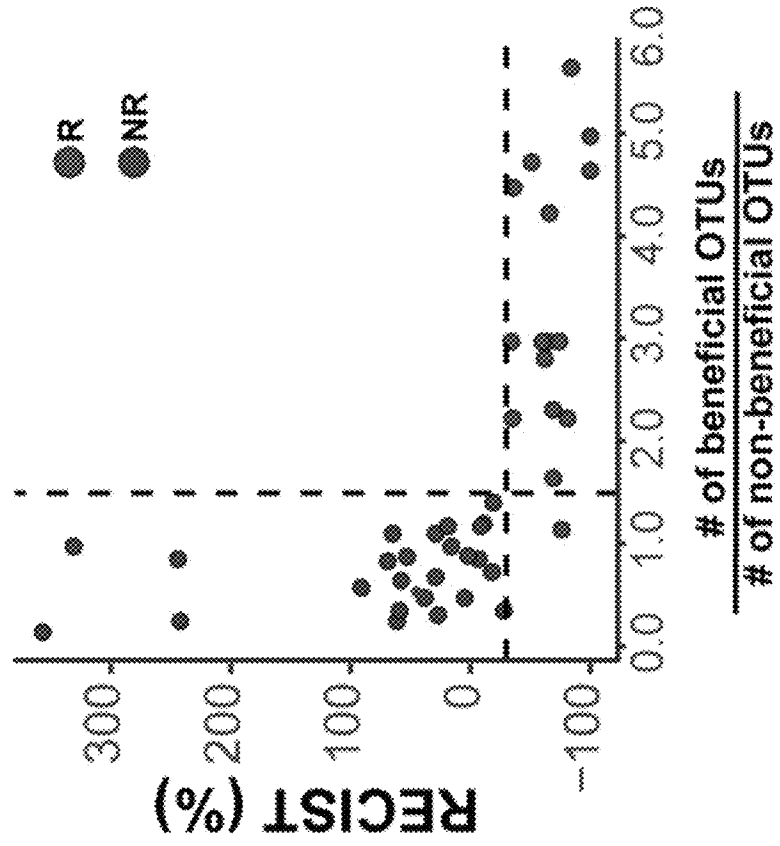


FIG. 2D

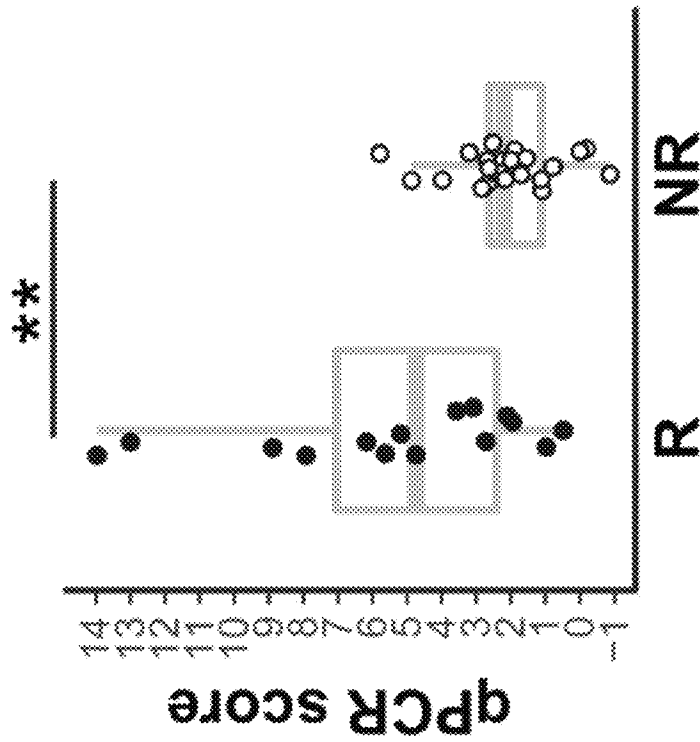


FIG. 3A

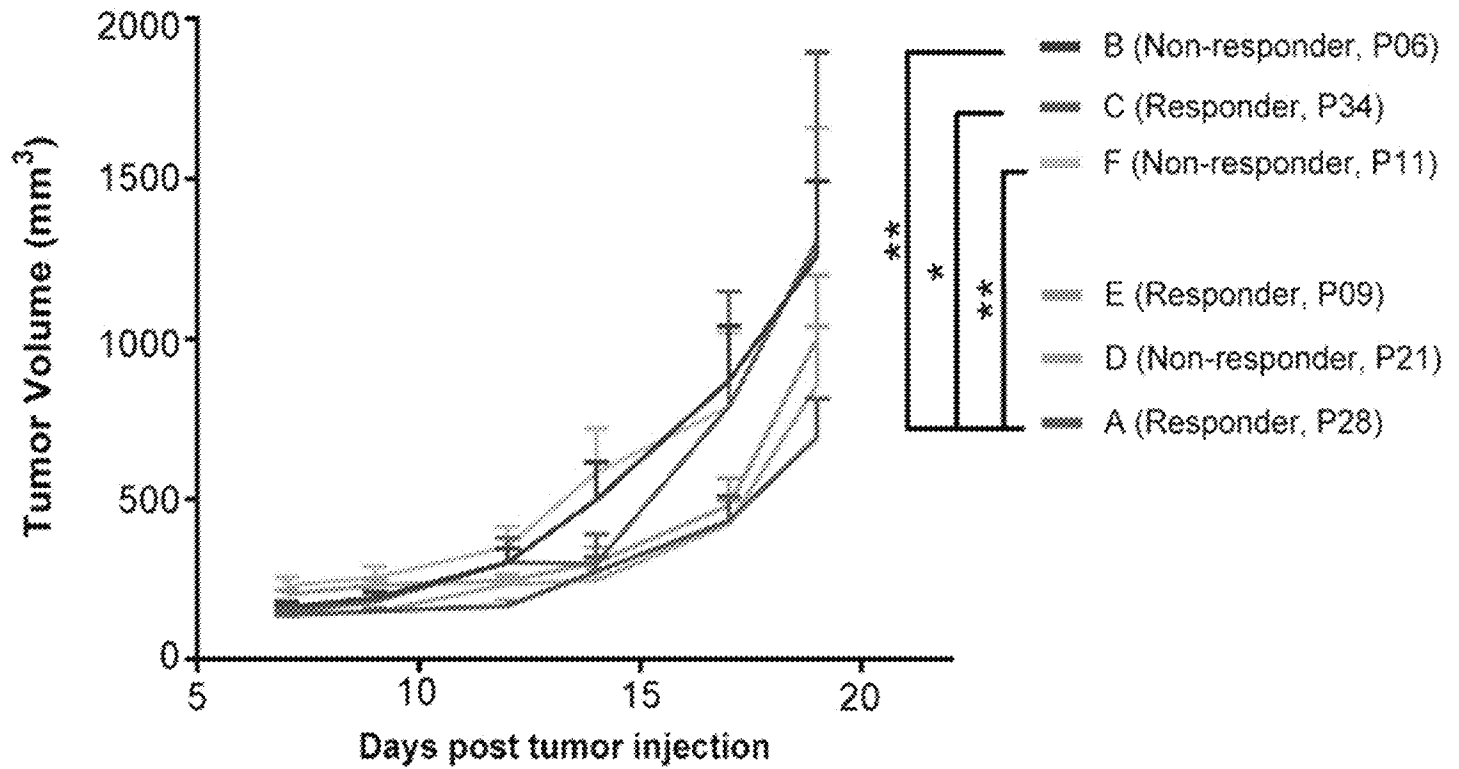


FIG. 3B

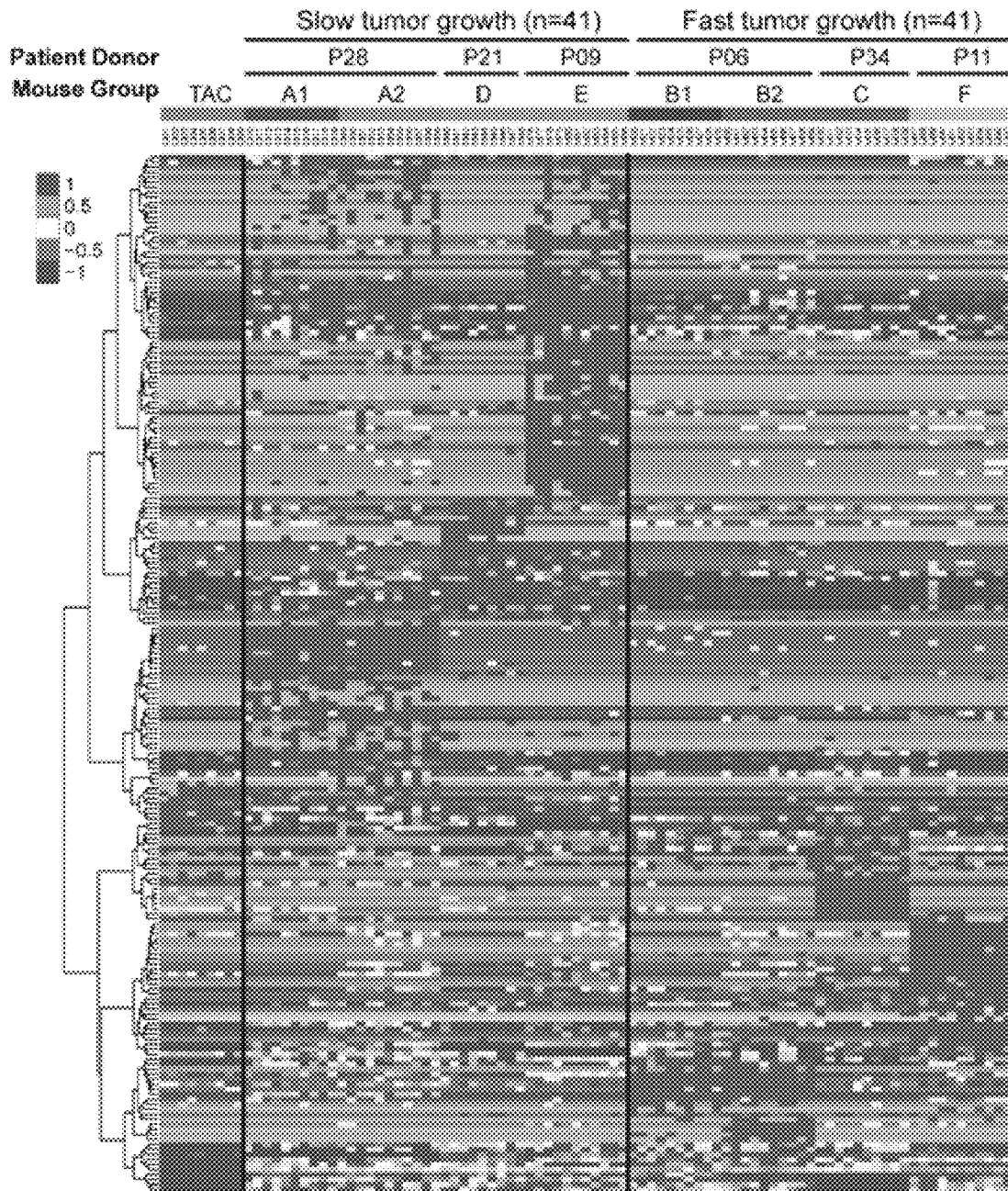


FIG. 3C

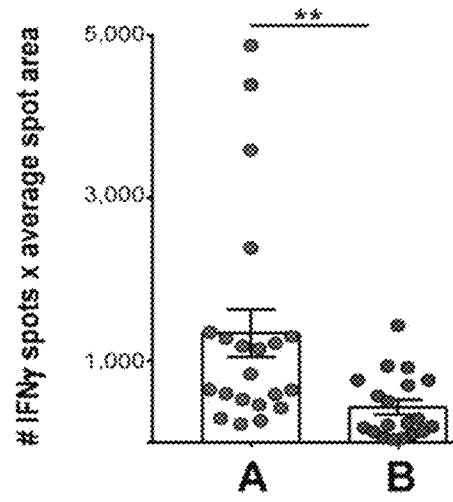


FIG. 3D

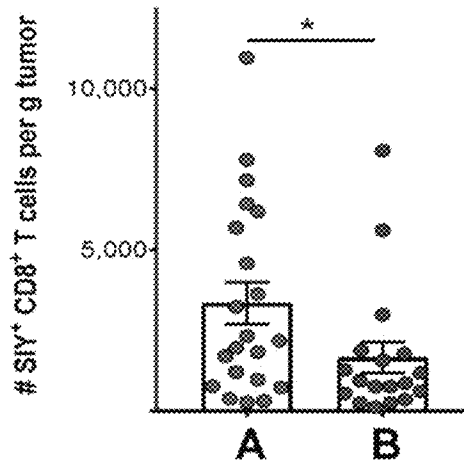


FIG. 3E

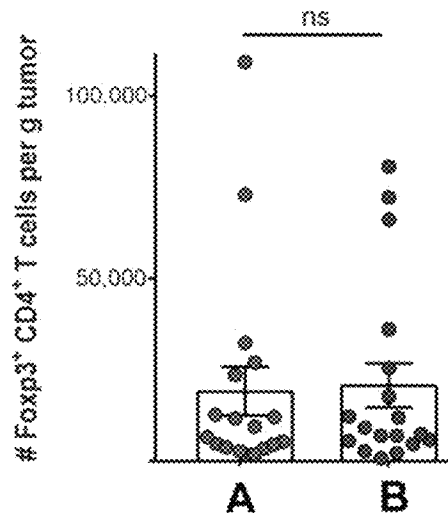


FIG. 3G

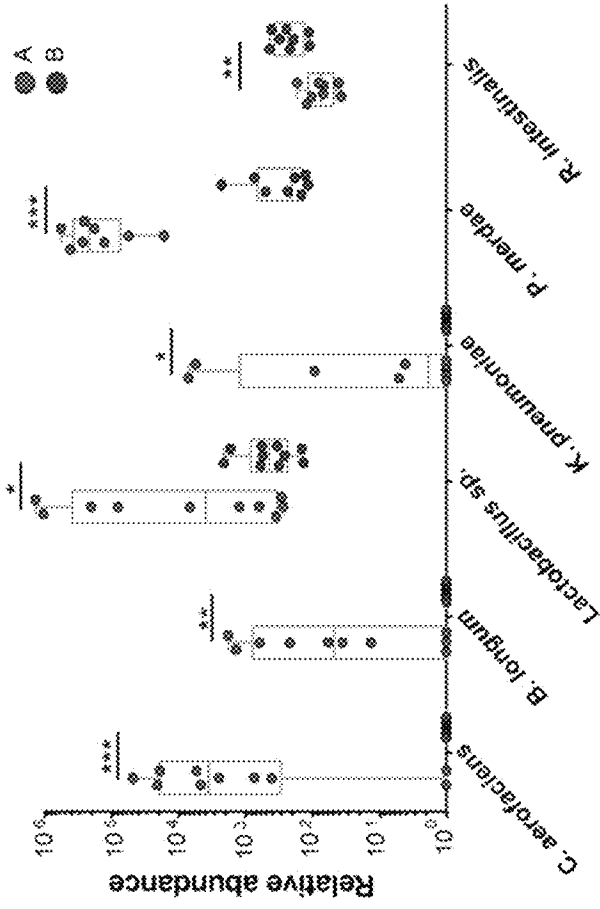


FIG. 3F

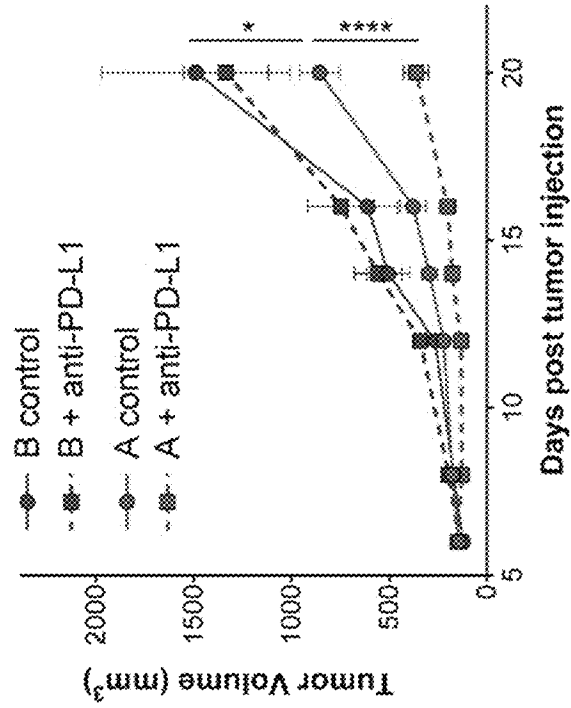
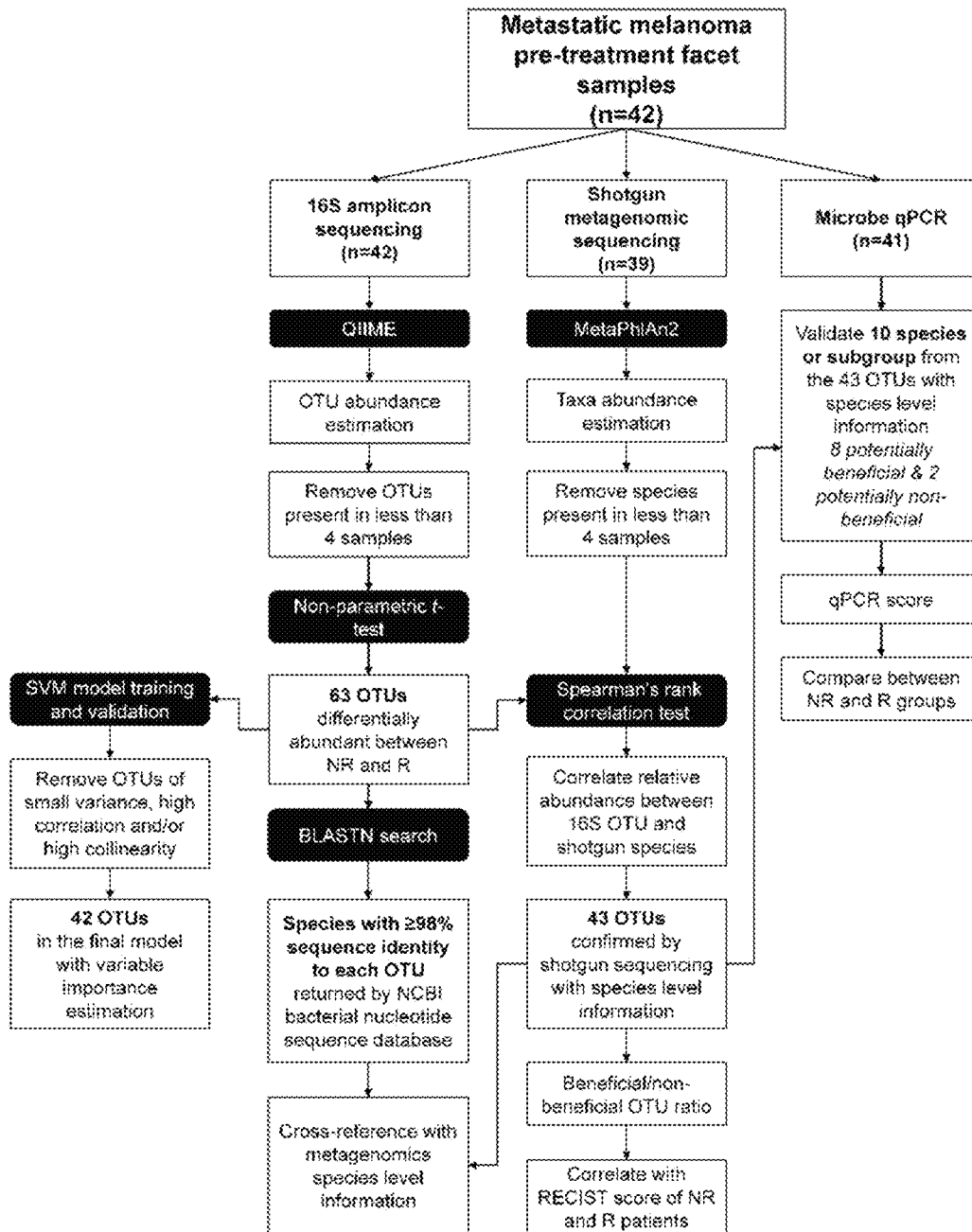
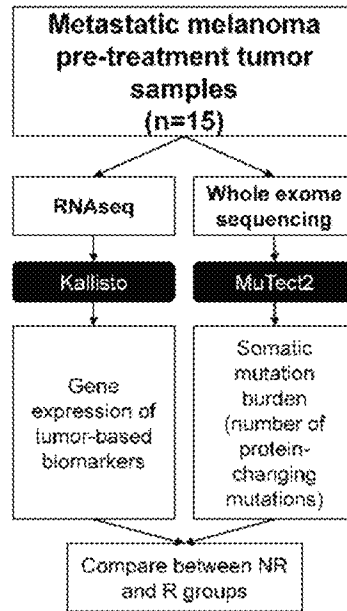


FIG. 4A



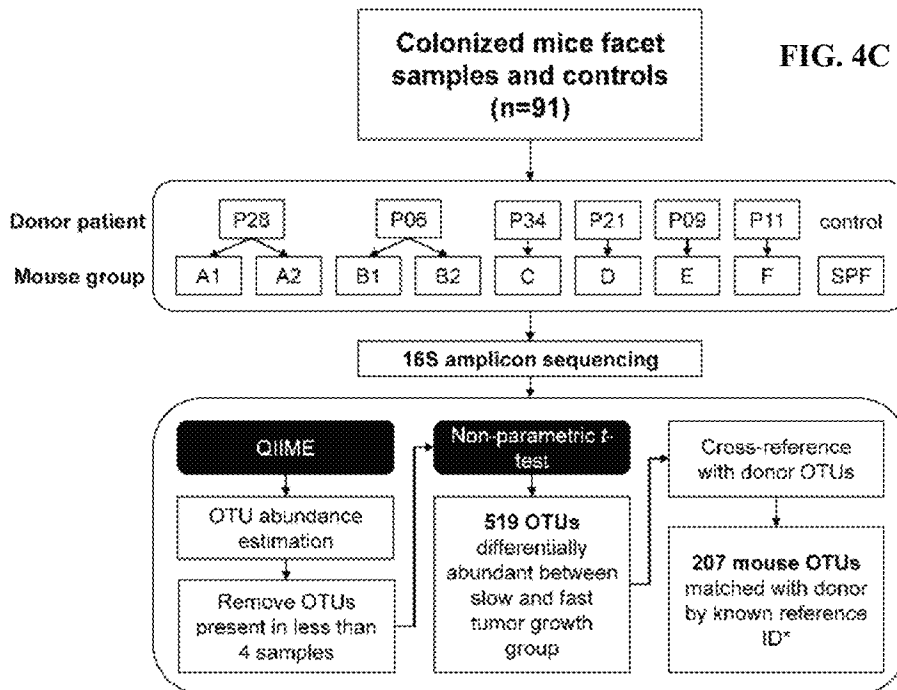
Human microbiota sequencing

FIG. 4B



Human tumor sequencing

FIG. 4C



* OTUs with new reference ID cannot be matched across cohorts.

Mouse microbiota sequencing

FIG. 6A

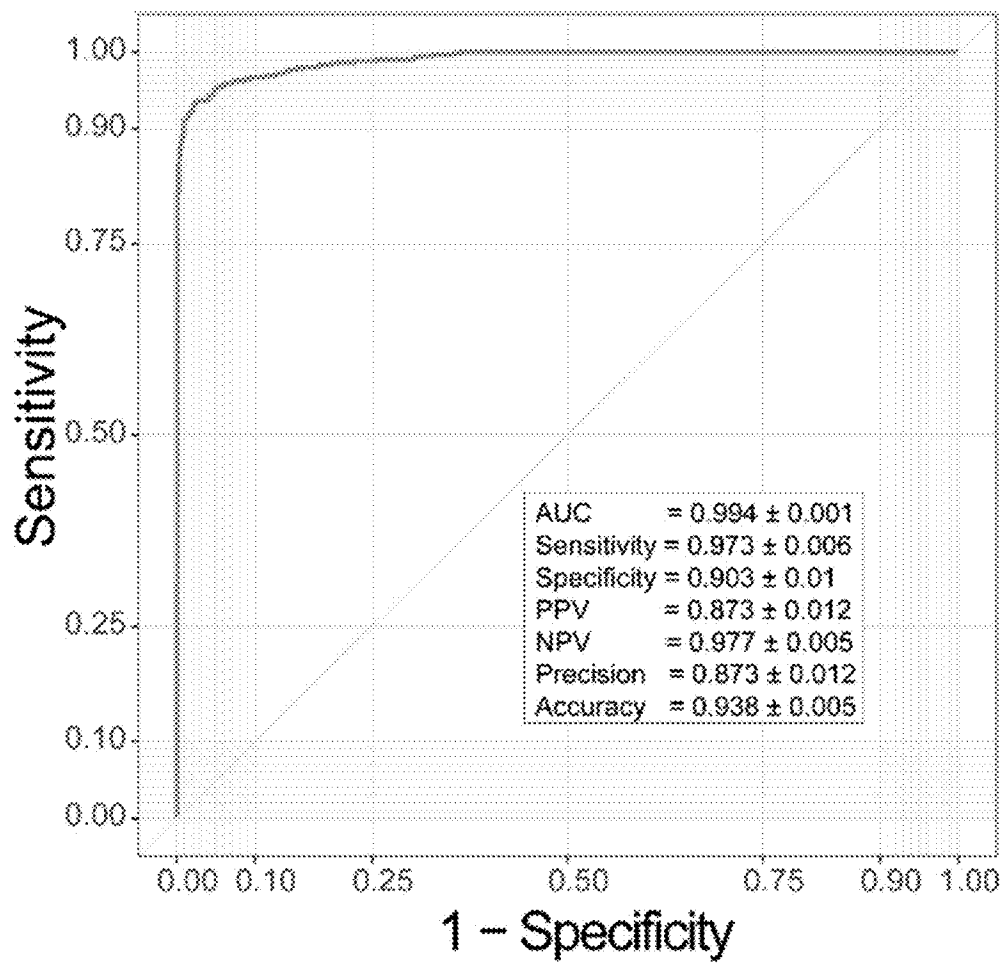


FIG. 6B

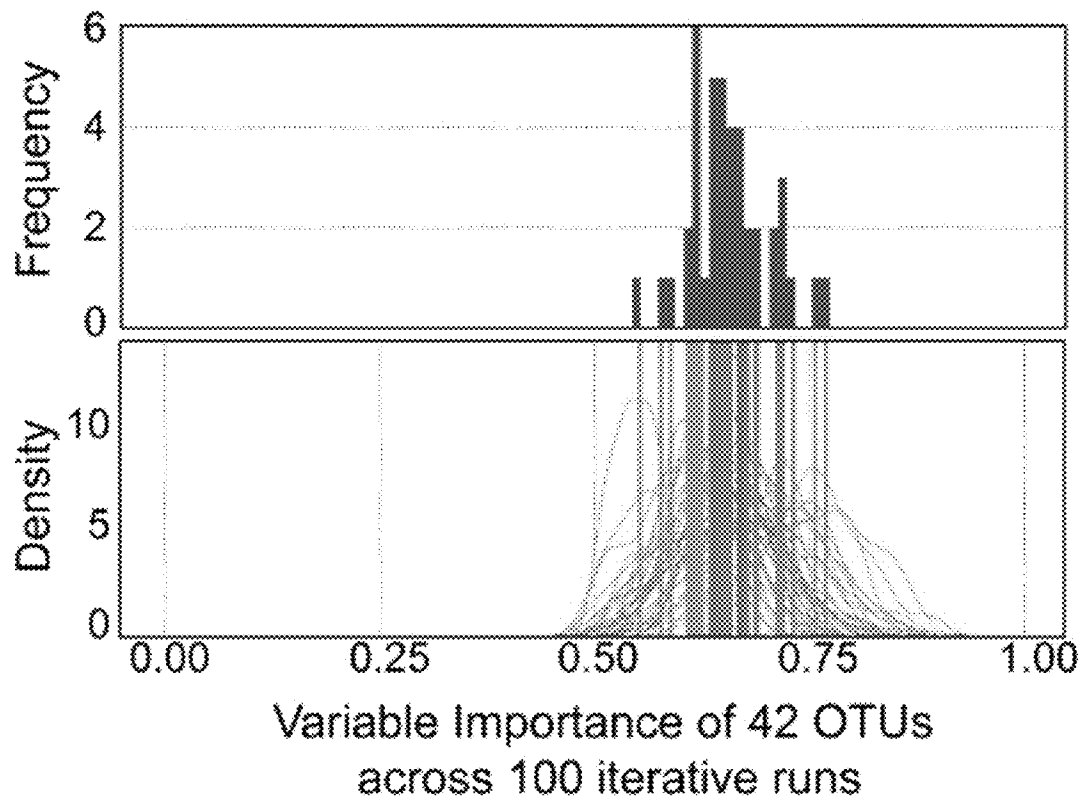


FIG. 8

A

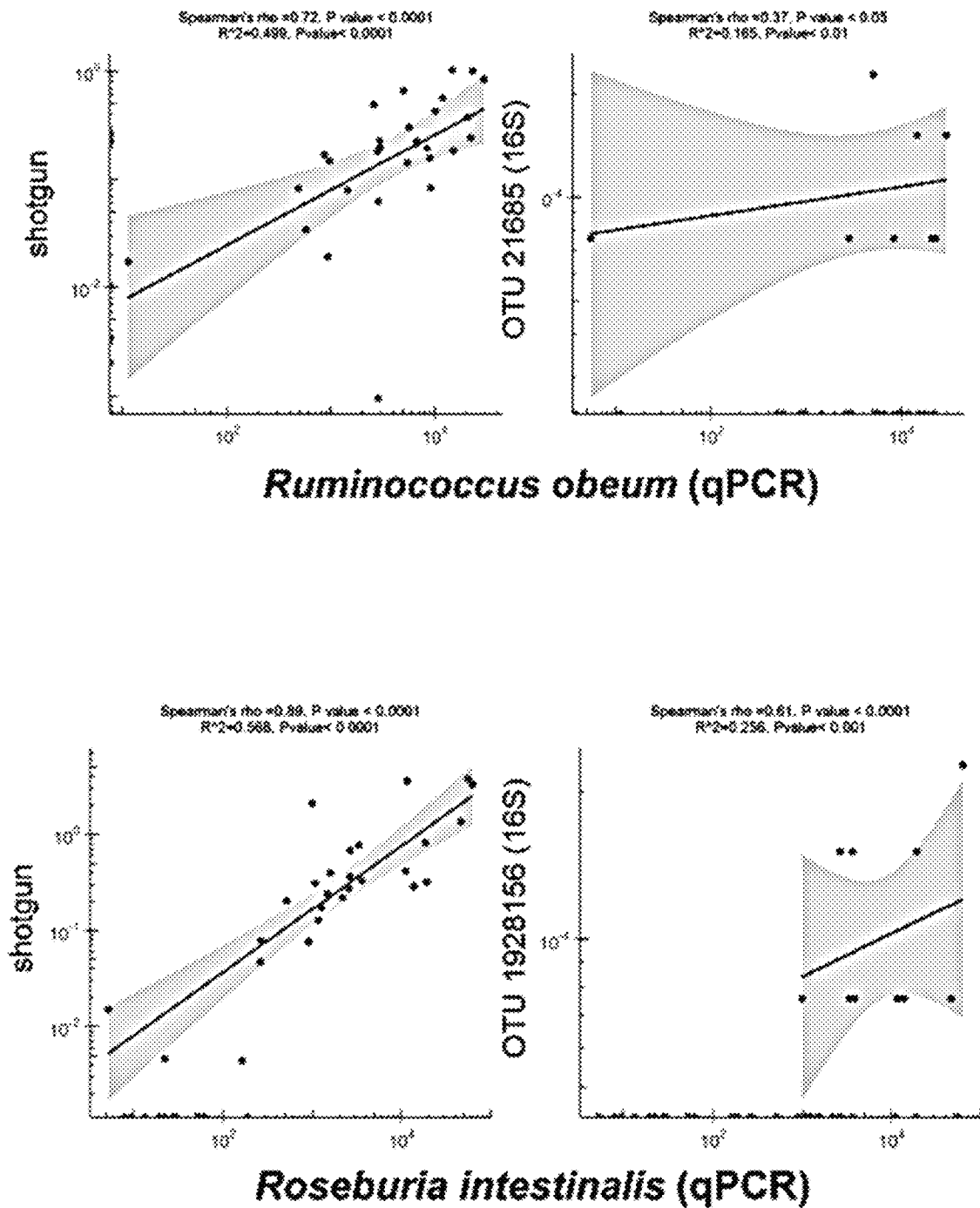


FIG. 8 (cont.)

B

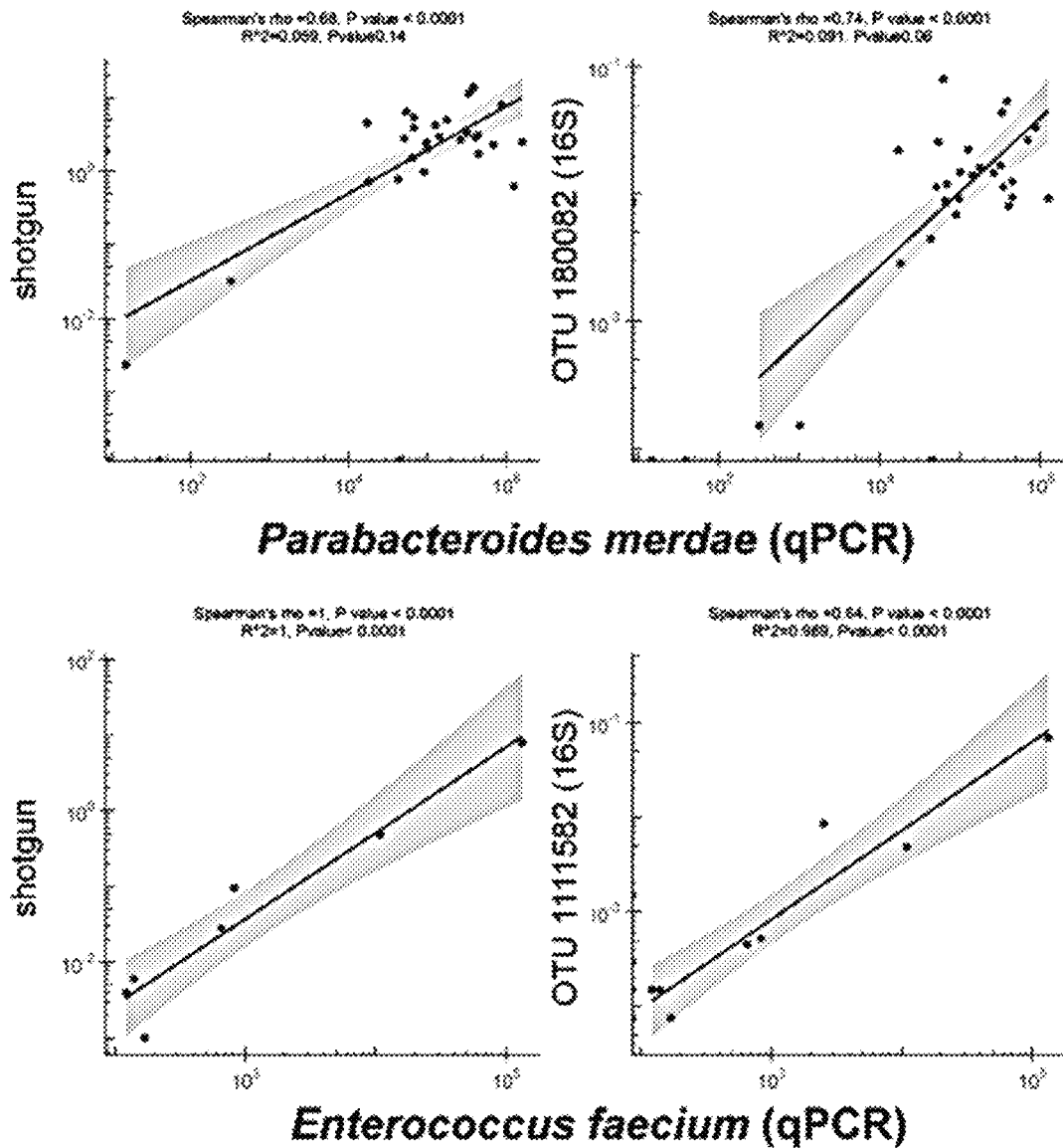


FIG. 8 (cont.)

B.

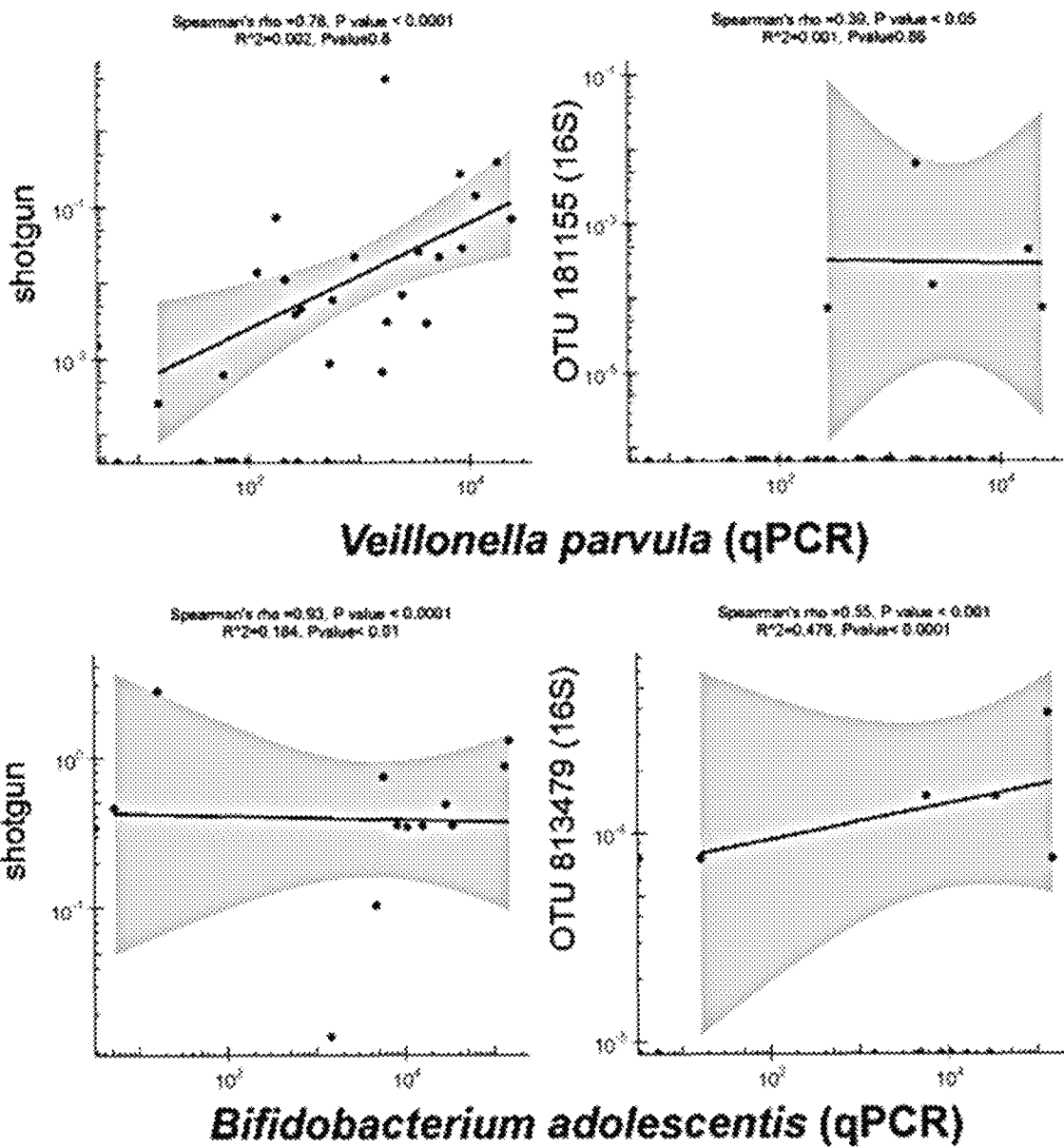


FIG. 8 (cont.)

B.

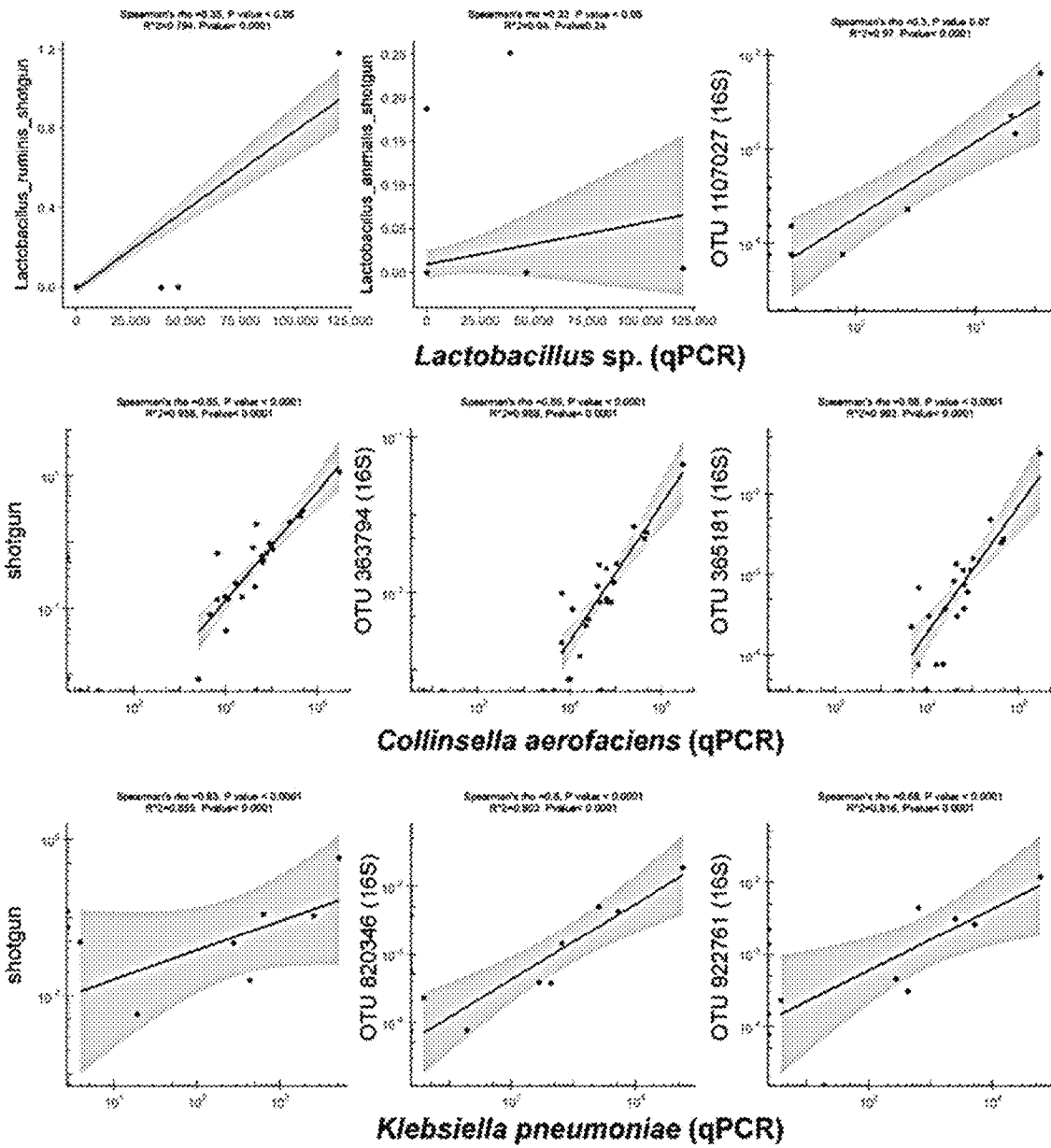


FIG. 9

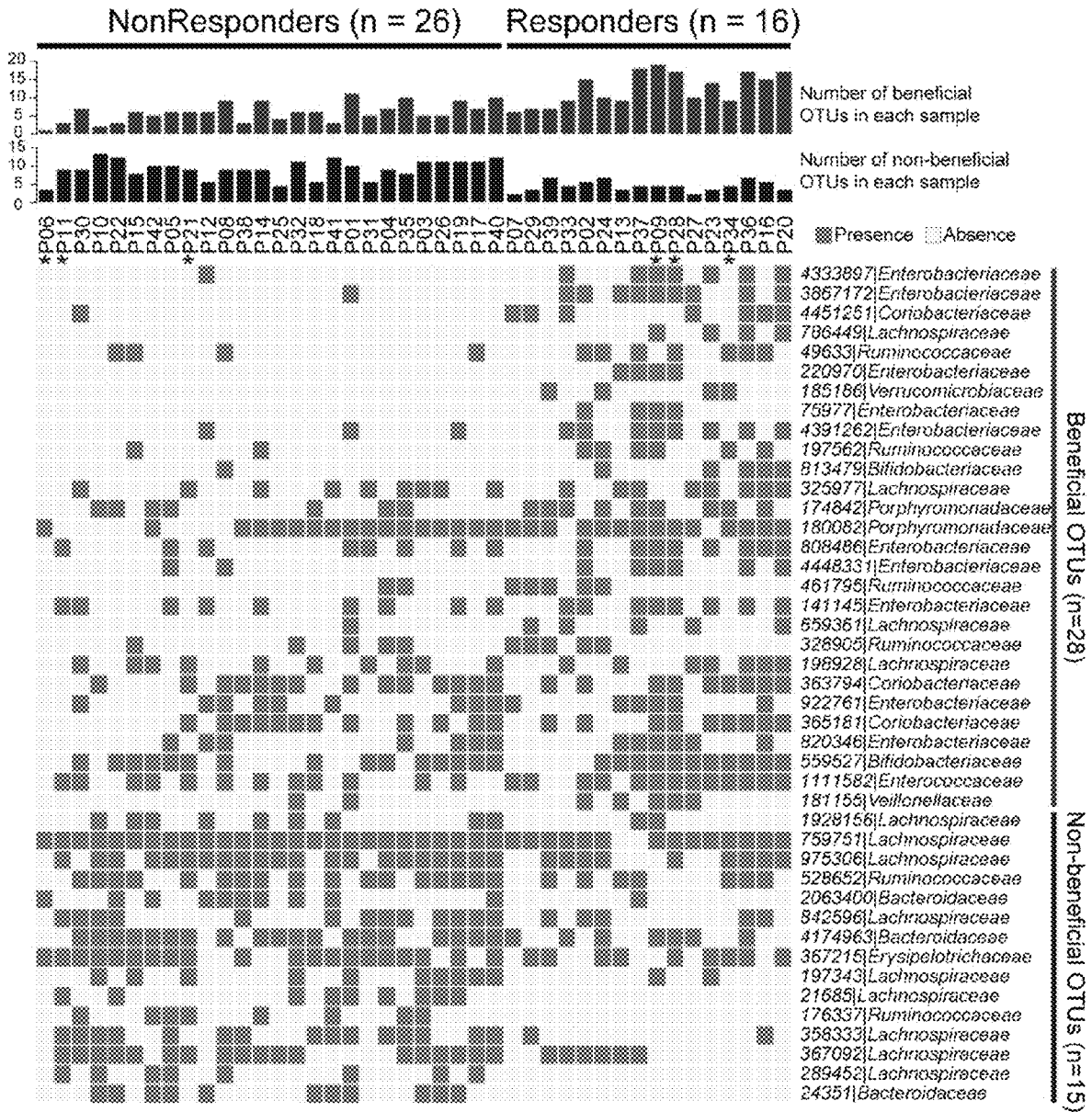


FIG. 10

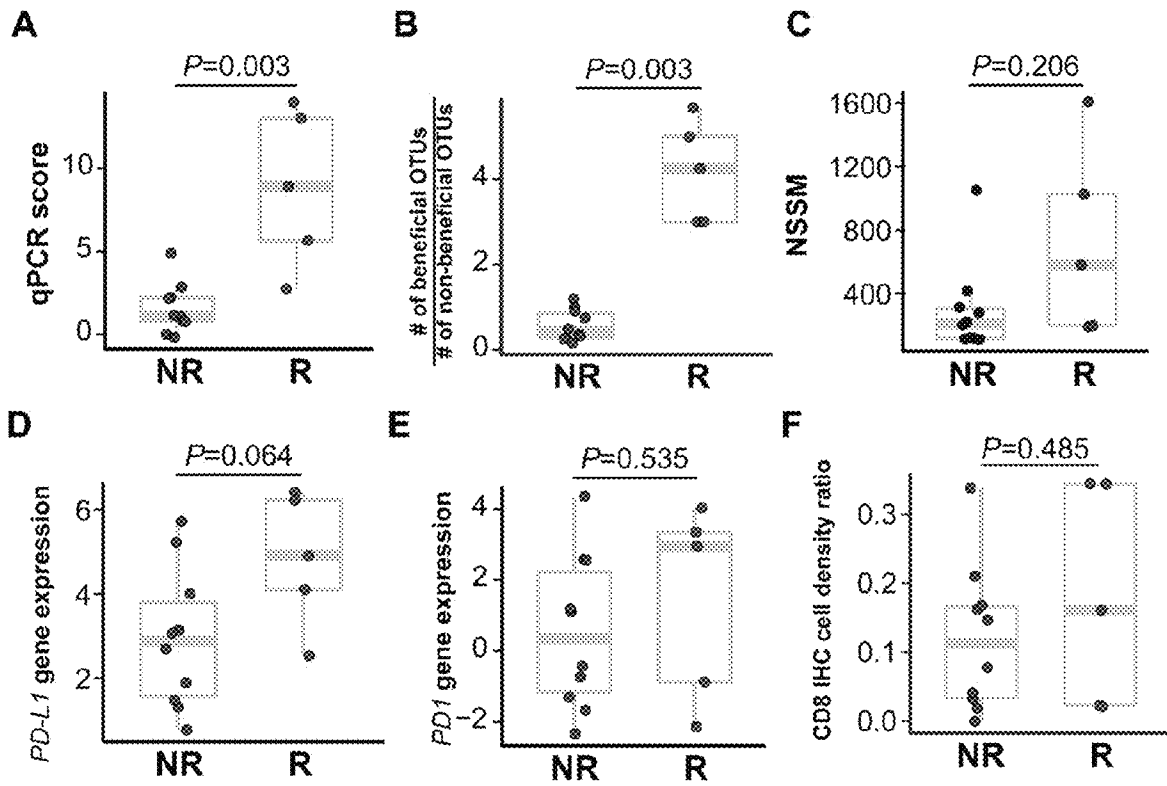


FIG. 11

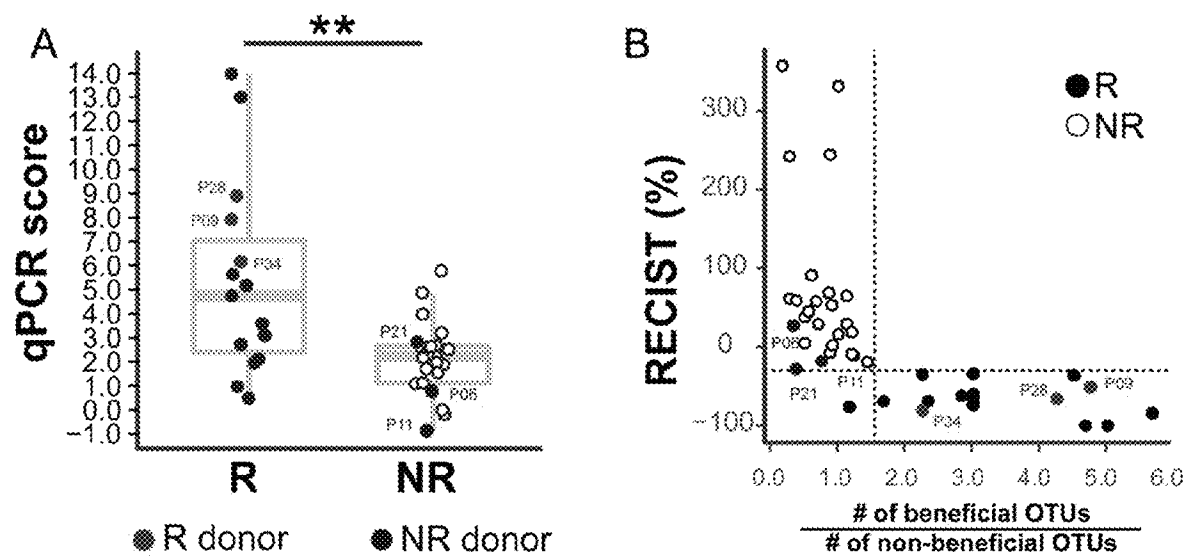


FIG. 12

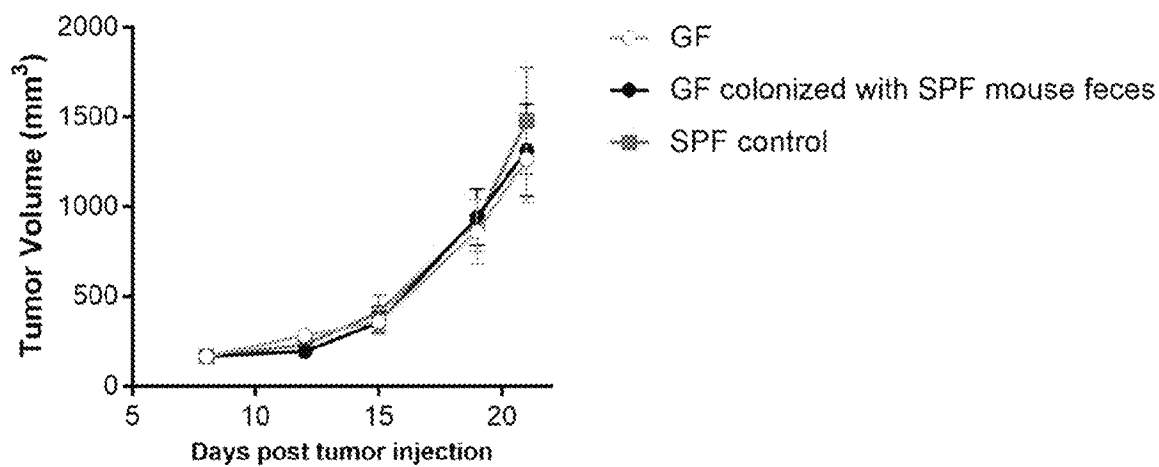


FIG. 13

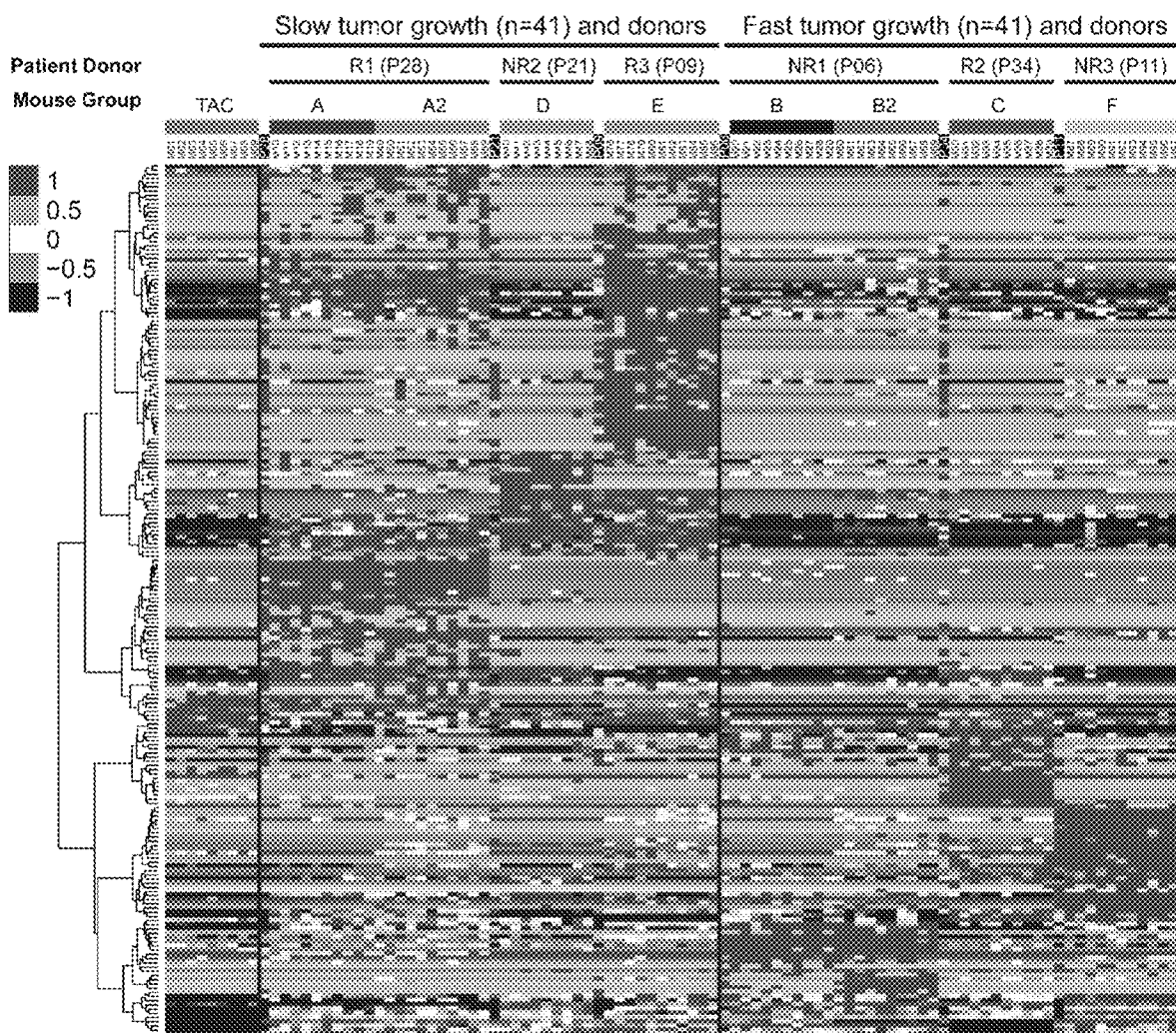


FIG. 14

ML workflow
Feature_selection

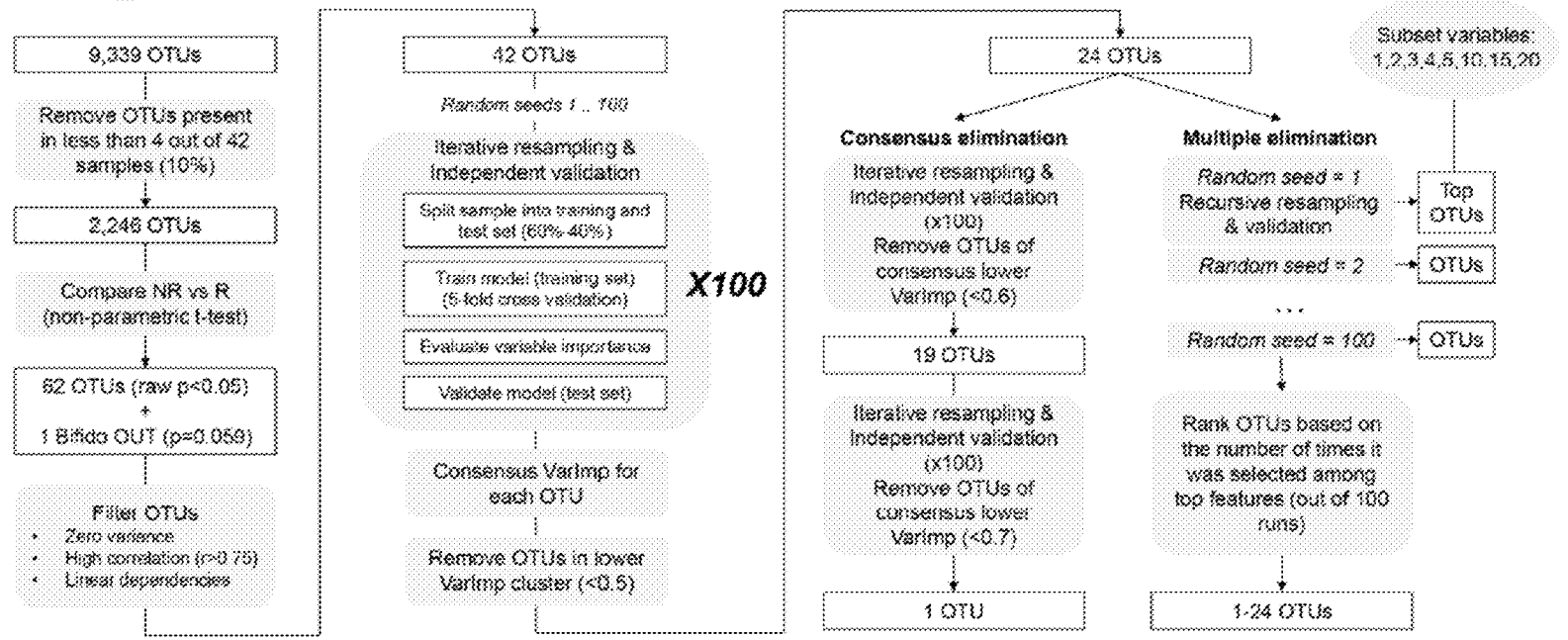
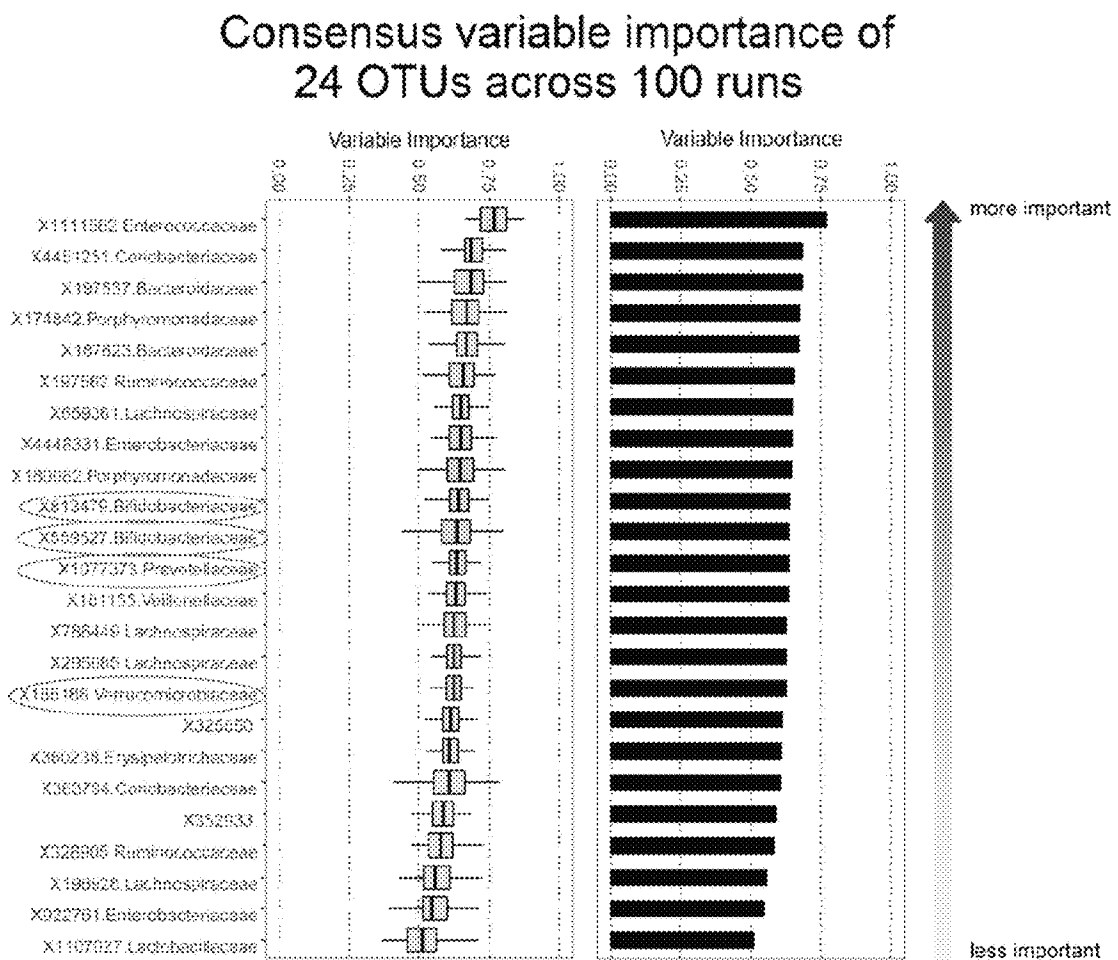


FIG. 15



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**MICROBIOME BIOMARKERS
IMMUNOTHERAPY RESPONSIVENESS:
DIAGNOSTIC, PROGNOSTIC AND
THERAPEUTIC USES THEREOF**

CROSS-REFERENCE TO RELATED
APPLICATION

The present application is a § 371 U.S. National Entry Application of PCT/US2018/036052, filed Jun. 5, 2018, which claims priority to U.S. Provisional Patent Application Ser. No. 62/515,366 filed Jun. 5, 2017, and U.S. Provisional Patent Application Ser. No. 62/577,454, filed Oct. 26, 2017, each of which is herein incorporated by reference in its entirety.

STATEMENT OF GOVERNMENT SUPPORT

This invention was made with government support under Grant Number CA210098 awarded by the National Institutes of Health. The government has certain rights in the invention.

SEQUENCE LISTING

The text of the computer readable sequence listing filed herewith, titled "35305-253_Sequence_Listing_ST25", created Dec. 2, 2019, having a file size of 7,000 bytes, is hereby incorporated by reference in its entirety.

FIELD

Provided herein are compositions and methods comprising microbiome biomarkers of responsiveness/resistance to immunotherapy (e.g., anti-PD1/PD-L1 therapy), and diagnostic, prognostic and therapeutic uses thereof. In particular, the amount, identity, presence, and/or ratio of microflora in the microbiome of a subject is used to determine the responsiveness/resistance of the subject to immunotherapy, and/or the microbiome of a subject is manipulated to enhance the responsiveness of the subject to various immunotherapies and co-therapies.

BACKGROUND

The responsiveness of patients to cancer immunotherapies, such as anti-CTLA-4 and anti-PD-1/PD-L1 antibodies (Hodi et al. The New England Journal of Medicine 363, 711-723 (2010); Hamid et al. The New England Journal of Medicine 369, 134-144 (2013); incorporated by reference in their entireties), is enhanced in patients who show evidence of an endogenous T cell response ongoing in the tumor microenvironment at baseline (Tumeh et al. Nature 515, 568-571 (2014); Spranger et al. Science Translational Medicine 5, 200ra116 (2013); Ji et al. Cancer Immunology, Immunotherapy: CII 61, 1019-1031 (2012); Gajewski et al. Cancer Journal 16, 399-403 (2010); herein incorporated by reference in their entireties). What is needed are biomarkers for characterizing a patient's responsiveness/resistance to immunotherapy and treatments for inducing T cell inflammation in the tumor microenvironment.

SUMMARY

Provided herein are compositions and methods comprising microbiome biomarkers of responsiveness/resistance to immunotherapy (e.g., anti-PD1/PD-L1 therapy), and diag-

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nostic, prognostic and therapeutic uses thereof. In particular, the amount, identity, presence, and/or ratio of microflora in the microbiome of a subject is used to determine the responsiveness/resistance of the subject to immunotherapy, and/or the microbiome of a subject is manipulated to enhance the responsiveness of the subject to various immunotherapies and co-therapies.

In some embodiments, provided herein are methods of treating or preventing cancer in a subject, comprising modulating levels of one or more commensal microbes within the subject to: (A) enhance an immune response by the subject, (B) inhibit the growth or spread of the cancer, (C) inhibit immune evasion by the cancer, and/or (D) enhance the efficacy of a therapeutic. In some embodiments, the levels of one or more commensal microbes are modulated within the gut of the subject. In some embodiments, modulating the levels of one or more commensal microbes comprises increasing and/or decreasing levels of bacteria strains, species, and/or families described herein. In some embodiments, the level is modulated of bacteria strains from one or more families selected from the group consisting of Coriobacteriaceae, Ruminococcaceae, Lachnospiraceae, Clostridiaceae, Bifidobacteriaceae, Enterobacteriaceae, and Erysipelotrichaceae. In some embodiments, the bacterial strains or species are selected from the OTU 365181 Coriobacteriaceae, 197562 Ruminococcaceae, 363794 Coriobacteriaceae, 295085 Lachnospiraceae, 659361 Lachnospiraceae, 461795 Ruminococcaceae, 592139 Clostridiaceae, 813479 Bifidobacteriaceae, OTU 325850, OTU 352933, 559527 Bifidobacteriaceae, 808486 Enterobacteriaceae, 830346 Enterobacteriaceae, 198928 Lachnospiraceae, 367215 Erysipelotrichaceae, 1973443 Lachnospiraceae, and 367092 Lachnospiraceae. In some embodiments, levels are modulated of a bacteria with variable importance score of 25 or greater (e.g., 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or greater, or ranges therebetween (e.g., 60 or greater). In some embodiments, levels are modulated of a bacteria identified on Table 6. In some embodiments, levels are modulated of a bacteria of the species *Enterococcus faecium*, *Collinsella aerofaciens*, *Bifidobacterium adolescentis*, *Klebsiella pneumoniae*, *Veillonella parvula*, *Parabacteroides merdae*, *Lactobacillus* sp. and/or *Bifidobacterium longum*.

In some embodiments, modulating the levels of one or more commensal microbes comprises administering a beneficial microbes to the subject. In some embodiments, the beneficial microbes are bacteria. In some embodiments, the bacteria are selected from the families Coriobacteriaceae, Ruminococcaceae, Lachnospiraceae, Clostridiaceae, Bifidobacteriaceae, Enterobacteriaceae, and Erysipelotrichaceae. In some embodiments, the bacteria are selected from the species *Enterococcus faecium*, *Collinsella aerofaciens*, *Bifidobacterium adolescentis*, *Klebsiella pneumoniae*, *Veillonella parvula*, *Parabacteroides merdae*, *Lactobacillus* sp. and/or *Bifidobacterium longum*. In some embodiments, the beneficial microbes are administered as a probiotic composition or via microflora transplant from a donor.

In some embodiments, modulating the levels of one or more commensal microbes comprises administering one or more antimicrobials. In some embodiments, the antimicrobial kills detrimental microbes. In some embodiments, the antimicrobial is an antibiotic. In some embodiments, methods further comprise administration of beneficial microbes to the subject (e.g., following antibiotic administration).

In some embodiments, methods herein comprise administering to a subject a bacterial formulation comprising bacteria described herein. In some embodiments, methods

further comprise administering to the subject a cancer therapy. In some embodiments, modulating levels of one or more commensal microbes within the subject (e.g., by administering a bacterial formulation, by administering an antibiotic, etc.) enhances an immune response by the subject and/or inhibits immune evasion by the cancer, and the cancer therapy is an immunotherapy. In some embodiments, the immunotherapy comprises administration of anti-CTLA-4 antibodies and/or anti-PD-L1 or anti-PD-1 antibodies. In some embodiments, modulating levels of one or more commensal microbes within the subject enhances the efficacy of a therapeutic, and the cancer therapy is said therapeutic. In some embodiments, the therapeutic comprises a chemotherapeutic. In some embodiments, methods further comprise testing the subject for immune evasion by the cancer. In some embodiments, methods further comprise surgical, radiation, and/or chemotherapeutic cancer intervention.

In some embodiments, provided herein are kits or compositions comprising a beneficial commensal microbe and a cancer therapeutic, said compositions or components of said kits formulated for therapeutic delivery to a subject.

In some embodiments, provided herein are beneficial commensal microbes for use as a medicament in the treatment of cancer, inhibition of immune evasion, and/or enhance immune response.

In some embodiments, provided herein are methods of treating or preventing cancer in a subject comprising administering to the subject a bacterial formulation comprising bacteria of the families Coriobacteriaceae, Ruminococcaceae, Lachnospiraceae, Clostridiaceae, Bifidobacteriaceae, Enterobacteriaceae, and/or Erysipelotrichaceae. In some embodiments, provided herein are methods of treating or preventing cancer in a subject comprising administering to the subject a bacterial formulation comprising bacteria selected from the species *Enterococcus faecium*, *Collinsella aerofaciens*, *Bifidobacterium adolescentis*, *Klebsiella pneumoniae*, *Veillonella parvula*, *Parabacteroides merdae*, *Lactobacillus* sp. and/or *Bifidobacterium longum*. In some embodiments, at least 50% of the bacteria in the bacterial formulation are of the families Coriobacteriaceae, Ruminococcaceae, Lachnospiraceae, Clostridiaceae, Bifidobacteriaceae, Enterobacteriaceae, and/or Erysipelotrichaceae. In some embodiments, at least 90% of the bacteria in the bacterial formulation are of the genera families Coriobacteriaceae, Ruminococcaceae, Lachnospiraceae, Clostridiaceae, Bifidobacteriaceae, Enterobacteriaceae, and/or Erysipelotrichaceae. In some embodiments, at least 50% of the bacteria in the bacterial formulation are selected from the species *Enterococcus faecium*, *Collinsella aerofaciens*, *Bifidobacterium adolescentis*, *Klebsiella pneumoniae*, *Veillonella parvula*, *Parabacteroides merdae*, *Lactobacillus* sp. and/or *Bifidobacterium longum*. In some embodiments, at least 90% of the bacteria in the bacterial formulation are selected from the species *Enterococcus faecium*, *Collinsella aerofaciens*, *Bifidobacterium adolescentis*, *Klebsiella pneumoniae*, *Veillonella parvula*, *Parabacteroides merdae*, *Lactobacillus* sp. and/or *Bifidobacterium longum*.

In some embodiments, the bacteria are selected from the group consisting of OTU 365181 Coriobacteriaceae, 197562 Ruminococcaceae, 363794 Coriobacteriaceae, 295085 Lachnospiraceae, 659361 Lachnospiraceae, 461795 Ruminococcaceae, 592139 Clostridiaceae, 813479 Bifidobacteriaceae, 325850, OTU 352933, OTU 559527 Bifidobacteriaceae, 808486 Enterobacteriaceae, 830346 Enterobacteriaceae, 198928 Lachnospiraceae, 367215 Erysipelotrichaceae, 1973443 Lachnospiraceae, and 367092 Lachnospiraceae.

In some embodiments, the bacteria are selected from the group consisting of *Enterococcus faecium*, *Collinsella aerofaciens*, *Bifidobacterium adolescentis*, *Klebsiella pneumoniae*, *Veillonella parvula*, *Parabacteroides merdae*, *Lactobacillus* sp. and/or *Bifidobacterium longum*.

In some embodiments, provided herein are methods of treating cancer in a human subject comprising administering to the subject an immune checkpoint inhibitor and a bacterial formulation comprising bacteria of the species *Enterococcus faecium*, *Collinsella aerofaciens*, *Bifidobacterium adolescentis*, *Klebsiella pneumoniae*, *Veillonella parvula*, *Parabacteroides merdae*, *Lactobacillus* sp. and/or *Bifidobacterium longum*.

In some embodiments, provided herein are methods of characterizing the degree of responsiveness/non-responsiveness of a tumor within a subject to immunotherapy treatment, comprising determining the presence, absence, or level of one or more bacteria, wherein increased levels of bacteria of the species *Enterococcus faecium*, *Collinsella aerofaciens*, *Bifidobacterium adolescentis*, *Klebsiella pneumoniae*, *Veillonella parvula*, *Parabacteroides merdae*, *Lactobacillus* sp. and/or *Bifidobacterium longum* in a subject are indicative of tumor responsiveness to immunotherapy treatment, and wherein increased levels of bacteria of the species *Ruminococcus obeum* and/or *Roseburia intestinalis* in a subject are indicative of tumor non-responsiveness to immunotherapy treatment.

In some embodiments, provided herein are methods of treating a subject with cancer by administering an immunotherapy to a subject with increased levels of *Enterococcus faecium*, *Collinsella aerofaciens*, *Bifidobacterium adolescentis*, *Klebsiella pneumoniae*, *Veillonella parvula*, *Parabacteroides merdae*, *Lactobacillus* sp. and/or *Bifidobacterium longum* bacteria.

In some embodiments, provided herein are methods of treating a subject with cancer by administering an immunotherapy and a bacterial formulation comprising one or more of *Enterococcus faecium*, *Collinsella aerofaciens*, *Bifidobacterium adolescentis*, *Klebsiella pneumoniae*, *Veillonella parvula*, *Parabacteroides merdae*, *Lactobacillus* sp. and/or *Bifidobacterium longum* bacteria, wherein prior to treatment the subject has increased levels of *Ruminococcus obeum* and/or *Roseburia intestinalis* bacteria and/or decreased levels of *Enterococcus faecium*, *Collinsella aerofaciens*, *Bifidobacterium adolescentis*, *Klebsiella pneumoniae*, *Veillonella parvula*, *Parabacteroides merdae*, *Lactobacillus* sp. and/or *Bifidobacterium longum* bacteria.

In some embodiments, provided herein are methods of treating a subject by administering an immunotherapy wherein the subject has a ratio of beneficial:nonbeneficial microbes greater than 1.0. In some embodiments, the ratio is equal to or greater than 1.5.

In some embodiments, the cancer is selected from the group consisting of acute nonlymphocytic leukemia, chronic lymphocytic leukemia, acute granulocytic leukemia, chronic granulocytic leukemia, acute promyelocytic leukemia, adult T-cell leukemia, aleukemic leukemia, aleukocythemetic leukemia, basophilic leukemia, blast cell leukemia, bovine leukemia, chronic myelocytic leukemia, leukemia cutis, embryonal leukemia, eosinophilic leukemia, Gross' leukemia, Rieder cell leukemia, Schilling's leukemia, stem cell leukemia, subleukemic leukemia, undifferentiated cell leukemia, hairy-cell leukemia, hemoblastic leukemia, hemocytoblastic leukemia, histiocytic leukemia, stem cell leukemia, acute monocytic leukemia, leukopenic leukemia, lymphatic leukemia, lymphoblastic leukemia, lymphocytic leukemia, lymphogenous leukemia, lymphoid leukemia, lymphosar-

coma cell leukemia, mast cell leukemia, megakaryocytic leukemia, micromyeloblastic leukemia, monocytic leukemia, myeloblastic leukemia, myelocytic leukemia, myeloid granulocytic leukemia, myelomonocytic leukemia, Naegeli leukemia, plasma cell leukemia, plasmacytic leukemia, promyelocytic leukemia, acinar carcinoma, acinous carcinoma, adenocystic carcinoma, adenoid cystic carcinoma, carcinoma adenomatosum, carcinoma of adrenal cortex, alveolar carcinoma, alveolar cell carcinoma, basal cell carcinoma, carcinoma basocellulare, basaloid carcinoma, basosquamous cell carcinoma, bronchioalveolar carcinoma, bronchiolar carcinoma, bronchogenic carcinoma, cerebriiform carcinoma, cholangiocellular carcinoma, chorionic carcinoma, colloid carcinoma, comedo carcinoma, corpus carcinoma, cribriform carcinoma, carcinoma en cuirasse, carcinoma cutaneum, cylindrical carcinoma, cylindrical cell carcinoma, duct carcinoma, carcinoma durum, embryonal carcinoma, encephaloid carcinoma, epienoid carcinoma, carcinoma epitheliale adenoides, exophytic carcinoma, carcinoma ex ulcere, carcinoma fibrosum, gelatiniform carcinoma, gelatinous carcinoma, giant cell carcinoma, signet-ring cell carcinoma, carcinoma simplex, small-cell carcinoma, solanoid carcinoma, spheroidal cell carcinoma, spindle cell carcinoma, carcinoma spongiosum, squamous carcinoma, squamous cell carcinoma, string carcinoma, carcinoma telangiectaticum, carcinoma telangiectodes, transitional cell carcinoma, carcinoma tuberosum, tuberosus carcinoma, verrucous carcinoma, carcinoma villosum, carcinoma gigantocellulare, glandular carcinoma, granulosa cell carcinoma, hair-matrix carcinoma, hematoid carcinoma, hepatocellular carcinoma, Hurthle cell carcinoma, hyaline carcinoma, hypernephroid carcinoma, infantile embryonal carcinoma, carcinoma in situ, intraepidermal carcinoma, intraepithelial carcinoma, Krompecher's carcinoma, Kulchitzky-cell carcinoma, large-cell carcinoma, lenticular carcinoma, carcinoma lenticulare, lipomatous carcinoma, lymphoepithelial carcinoma, carcinoma medullare, medullary carcinoma, melanotic carcinoma, carcinoma molle, mucinous carcinoma, carcinoma muciparum, carcinoma mucocellulare, mucoepidermoid carcinoma, carcinoma mucosum, mucous carcinoma, carcinoma myxomatodes, nasopharyngeal carcinoma, oat cell carcinoma, carcinoma ossificans, osteoid carcinoma, papillary carcinoma, periportal carcinoma, preinvasive carcinoma, prickle cell carcinoma, pultaceous carcinoma, renal cell carcinoma of kidney, reserve cell carcinoma, carcinoma sarcomatodes, schneiderian carcinoma, scirrhous carcinoma, carcinoma scroti, chondrosarcoma, fibrosarcoma, lymphosarcoma, melanosarcoma, myxosarcoma, osteosarcoma, endometrial sarcoma, stromal sarcoma, Ewing's sarcoma, fascial sarcoma, fibroblastic sarcoma, giant cell sarcoma, Abemethy's sarcoma, adipose sarcoma, liposarcoma, alveolar soft part sarcoma, ameloblastic sarcoma, botryoid sarcoma, chloroma sarcoma, chorio carcinoma, embryonal sarcoma, Wilms' tumor sarcoma, granulocytic sarcoma, Hodgkin's sarcoma, idiopathic multiple pigmented hemorrhagic sarcoma, immunoblastic sarcoma of B cells, lymphoma, immunoblastic sarcoma of T-cells, Jensen's sarcoma, Kaposi's sarcoma, Kupffer cell sarcoma, angiosarcoma, leukosarcoma, malignant mesenchymoma sarcoma, parosteal sarcoma, reticulocytic sarcoma, Rous sarcoma, serocystic sarcoma, synovial sarcoma, telangiectaltic sarcoma, Hodgkin's Disease, Non-Hodgkin's Lymphoma, multiple myeloma, neuroblastoma, breast cancer, ovarian cancer, lung cancer, rhabdomyosarcoma, primary thrombocytosis, primary macroglobulinemia, small-cell lung tumors, primary brain tumors, stomach cancer, colon cancer, malignant pancreatic insulanoma, malignant

carcinoid, premalignant skin lesions, testicular cancer, lymphomas, thyroid cancer, neuroblastoma, esophageal cancer, genitourinary tract cancer, malignant hypercalcemia, cervical cancer, endometrial cancer, adrenal cortical cancer, 5 Harding-Passey melanoma, juvenile melanoma, lentigo maligna melanoma, malignant melanoma, acral-lentiginous melanoma, amelanotic melanoma, benign juvenile melanoma, Cloudman's melanoma, S91 melanoma, nodular melanoma subungal melanoma, and superficial spreading melanoma. 10

In some embodiments, the subject is human. In some embodiments, the bacterial formulation is administered by oral administration, rectal administration, topical administration, inhalation or injection. In some embodiments, the bacterial formulation is a food product. In some embodiments, the bacterial formulation comprises at least about 5×10^6 CFU of bacteria. In some embodiments, the bacterial formulation is administered to the subject in two or more doses. In some embodiments, the administration of at least two of the two or more doses are separated by at least 1 day. In some embodiments, the administration of at least two of the two or more doses are separated by at least 1 week. 15

In some embodiments, methods further comprise administering to the subject an antibiotic. In some embodiments, the antibiotic is administered to the subject before the bacterial formulation. In some embodiments, the antibiotic is administered to the subject at least 1 day before the bacterial formulation is administered to the subject. 20

In some embodiments, methods further comprise administering to the subject an immune checkpoint inhibitor. In some embodiments, the immune checkpoint inhibitor is a protein or polypeptide that specifically binds to an immune checkpoint protein. In some embodiments, the immune checkpoint protein is selected from the group consisting of CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3 or VISTA. In some embodiments, the polypeptide or protein is an antibody or antigen-binding fragment thereof. In some embodiments, the immune checkpoint inhibitor is an interfering nucleic acid molecule. In some embodiments, the interfering nucleic acid molecule is a siRNA molecule, a shRNA molecule or an antisense RNA molecule. In some embodiments, the immune checkpoint inhibitor is selected from the group consisting of nivolumab, pembrolizumab, pidilizumab, AMP-224, AMP-514, STI-A1110, TSR-042, RG-7446, BMS-936559, BMS-936558, MK-3475, CT 011, MPDL3280A, MEDI-4736, MSB-0020718C, AUR-012 and STI-A1010. In some embodiments, the immune checkpoint inhibitor is administered before the bacterial formulation. In some embodiments, the immune checkpoint inhibitor is administered at least one day before the bacterial formulation. In some embodiments, the immune checkpoint is administered at about the same time as the bacterial formulation. In some embodiments, the immune checkpoint inhibitor is administered on the same day as the bacterial formulation. In some embodiments, the immune checkpoint inhibitor is administered after the bacterial formulation. In some embodiments, the immune checkpoint inhibitor is administered at least one day after the bacterial formulation. In some embodiments, the immune checkpoint inhibitor is administered by injection. In some embodiments, the injection is an intravenous, intramuscular, intratumoral or subcutaneous injection. 25 30 35 40 45 50 55 60

In some embodiments, provided herein are methods of treating cancer in a human subject comprising administering to the subject an immune checkpoint inhibitor and a bacterial formulation comprising bacteria of the families Corio-

bacteriaceae, Ruminococcaceae, Lachnospiraceae, Clostridiaceae, Bifidobacteriaceae, Enterobacteriaceae, and/or Erysipelotrichaceae. In some embodiments, the bacteria are selected from the OTU group consisting of 365181 Coriobacteriaceae, 197562 Ruminococcaceae, 363794 Coriobacteriaceae, 295085 Lachnospiraceae, 659361 Lachnospiraceae, 461795 Ruminococcaceae, 592139 Clostridiaceae, 813479 Bifidobacteriaceae, OTU 325850, OTU 352933, 559527 Bifidobacteriaceae, 808486 Enterobacteriaceae, 830346 Enterobacteriaceae, 198928 Lachnospiraceae, 367215 Erysipelotrichaceae, 1973443 Lachnospiraceae, and 367092 Lachnospiraceae. In some embodiments, at least 50% (e.g., 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, or more, or ranges therebetween) of the bacteria in the bacterial formulation are of the families Coriobacteriaceae, Ruminococcaceae, Lachnospiraceae, Clostridiaceae, Bifidobacteriaceae, Enterobacteriaceae, and/or Erysipelotrichaceae. In some embodiments, at least 90% (e.g., 90%, 95%, 99%, 99.9%, 99.99%, or more or ranges therebetween) of the bacteria in the bacterial formulation are of the families Coriobacteriaceae, Ruminococcaceae, Lachnospiraceae, Clostridiaceae, Bifidobacteriaceae, Enterobacteriaceae, and/or Erysipelotrichaceae. In some embodiments, the bacteria of the families Coriobacteriaceae, Ruminococcaceae, Lachnospiraceae, Clostridiaceae, Bifidobacteriaceae, Enterobacteriaceae, and/or Erysipelotrichaceae comprise bacteria of the strains or species from the OTU 365181 Coriobacteriaceae, 197562 Ruminococcaceae, 363794 Coriobacteriaceae, 295085 Lachnospiraceae, 659361 Lachnospiraceae, 461795 Ruminococcaceae, 592139 Clostridiaceae, 813479 Bifidobacteriaceae, OTU 325850, OTU 352933, 559527 Bifidobacteriaceae, 808486 Enterobacteriaceae, 830346 Enterobacteriaceae, 198928 Lachnospiraceae, 367215 Erysipelotrichaceae, 1973443 Lachnospiraceae, and 367092 Lachnospiraceae.

In some embodiments, provided herein are methods of treating cancer in a human subject comprising administering to the subject an immune checkpoint inhibitor and a bacterial formulation comprising bacteria of the species *Enterococcus faecium*, *Collinsella aerofaciens*, *Bifidobacterium adolescentis*, *Klebsiella pneumoniae*, *Veillonella parvula*, *Parabacteroides merdae*, *Lactobacillus* sp. and/or *Bifidobacterium longum*. In some embodiments, at least 50% (e.g., 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, or more, or ranges therebetween) of the bacteria in the bacterial formulation are of the species *Enterococcus faecium*, *Collinsella aerofaciens*, *Bifidobacterium adolescentis*, *Klebsiella pneumoniae*, *Veillonella parvula*, *Parabacteroides merdae*, *Lactobacillus* sp. and/or *Bifidobacterium longum*. In some embodiments, at least 90% (e.g., 90%, 95%, 99%, 99.9%, 99.99%, or more or ranges therebetween) of the bacteria in the bacterial formulation are of the species *Enterococcus faecium*, *Collinsella aerofaciens*, *Bifidobacterium adolescentis*, *Klebsiella pneumoniae*, *Veillonella parvula*, *Parabacteroides merdae*, *Lactobacillus* sp. and/or *Bifidobacterium longum*.

In some embodiments, the bacterial formulation is administered by oral administration or rectal administration. In some embodiments, the bacterial formulation is administered by oral administration. In some embodiments, the bacterial formulation comprises at least 5×10^6 CFU (e.g., 5×10^6 CFU, 1×10^7 CFU, 2×10^7 CFU, 5×10^7 CFU, 1×10^8 CFU, 2×10^8 CFU, 5×10^8 CFU, 1×10^9 CFU, 2×10^9 CFU, 5×10^9 CFU, 1×10^{10} CFU, 2×10^{10} CFU, 5×10^{10} CFU, 1×10^{11} CFU, 2×10^{11} CFU, 5×10^{11} CFU, 1×10^{12} CFU, 2×10^{12} CFU, 5×10^{12} CFU, or more or ranges therebetween) of bacteria of the species described herein. In some embodiments, the

bacterial formulation is administered to the subject in two or more doses (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more, or ranges therebetween). In some embodiments, the administration of doses are separated by at least 1 week. In some embodiments, methods further comprise administering to the subject an antibiotic prior to the administration of the bacterial formulation. In some embodiments, the antibiotic is administered to the subject at least 1 day before the bacterial formulation is administered to the subject. In some embodiments, the immune checkpoint inhibitor is a protein or polypeptide that binds to an immune checkpoint protein. In some embodiments, the immune checkpoint inhibitor is an antibody or antigen binding fragment thereof that binds to an immune checkpoint protein. In some embodiments, the immune checkpoint protein is CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3 or VISTA. In some embodiments, the immune checkpoint protein is PD-1 or PD-L1. In some embodiments, the immune checkpoint inhibitor is nivolumab, pembrolizumab, pidilizumab, AMP-224, AMP-514, STI-A1110, TSR-042, RG-7446, BMS-936559, BMS-936558, MK-3475, CT 011, MPDL3280A, MEDI-4736, MSB-0020718C, AUR-012 and STI-A1010. In some embodiments, the immune checkpoint inhibitor is administered by intravenous injection, intramuscular injection, intratumoral injection or subcutaneous injection.

In some embodiments, provided herein are methods of treating cancer in a human subject comprising administering to the subject a bacterial formulation comprising at least 5×10^6 CFU (e.g., 5×10^6 CFU, 1×10^7 CFU, 2×10^7 CFU, 5×10^7 CFU, 1×10^8 CFU, 2×10^8 CFU, 5×10^8 CFU, 1×10^9 CFU, 2×10^9 CFU, 5×10^9 CFU, 1×10^{10} CFU, 2×10^{10} CFU, 5×10^{10} CFU, 1×10^{11} CFU, 2×10^{11} CFU, 5×10^{11} CFU, 1×10^{12} CFU, 2×10^{12} CFU, 5×10^{12} CFU, or more or ranges therebetween) of bacteria of these species described herein. In some embodiments, at least 90% (e.g., 90%, 95%, 99%, 99.9%, 99.99%, or more or ranges therebetween) of the bacteria in the bacterial formulation are of the species described herein. In some embodiments, the bacterial formulation is administered by oral administration or rectal administration. In some embodiments, the bacterial formulation is administered by oral administration. In some embodiments, the bacterial formulation is administered to the subject in two or more doses (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more, or ranges therebetween). In some embodiments, methods further comprise administering to the subject an antibiotic before the bacterial formulation is administered to the subject. In some embodiments, methods further comprise administering to the subject an immune checkpoint inhibitor. In some embodiments, the immune checkpoint inhibitor is an antibody or antigen binding fragment thereof that binds to CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3 or VISTA. In some embodiments, the immune checkpoint inhibitor is an antibody or antigen binding fragment thereof that binds to PD-1 or PD-L1. In some embodiments, the immune checkpoint inhibitor is nivolumab, pembrolizumab, pidilizumab, AMP-224, AMP-514, STI-A1110, TSR-042, RG-7446, BMS-936559, BMS-936558, MK-3475, CT 011, MPDL3280A, MEDI-4736, MSB-0020718C, AUR-012 and STI-A1010.

In some embodiments, provided herein are methods of characterizing the degree of responsiveness/non-responsiveness of a tumor within a subject to immunotherapy treatment, comprising determining the presence, absence, or level of one or more bacteria described herein. In some embodiments, bacteria are of the families Coriobacteriaceae, Ruminococcaceae, Lachnospiraceae, Clostridiaceae, Bifido-

bacteriacea, Enterobacteriacea, and/or Erysipelotrichaceae, wherein the bacteria in a subject are indicative of tumor responsiveness or non-responsiveness to immunotherapy treatment. In some embodiments, bacteria of the species *Enterococcus faecium*, *Collinsella aerofaciens*, *Bifidobacterium adolescentis*, *Klebsiella pneumoniae*, *Veillonella parvula*, *Parabacteroides merdae*, *Lactobacillus* sp. and/or *Bifidobacterium longum*. In some embodiments, the bacteria are selected from the bacteria listed in Table 6. In some embodiments, the bacteria comprise strains or species selected from the group consisting of 365181 Coriobacteriaceae, 197562 Ruminococcaceae, 363794 Coriobacteriaceae, 295085 Lachnospiraceae, 659361 Lachnospiraceae, 461795 Ruminococcaceae, 592139 Clostridiaceae, 813479 Bifidobacteriaceae, OTU 325850, OTU 352933, 559527 Bifidobacteriaceae, 808486 Enterobacteriaceae, 830346 Enterobacteriaceae, 198928 Lachnospiraceae, 367215 Erysipelotrichaceae, 1973443 Lachnospiraceae, and 367092 Lachnospiraceae. In some embodiments, methods provide a diagnosis (e.g., T-cell inflamed tumor, non-T-cell-inflamed tumor, etc), prognosis (e.g., tumor will respond (or likely to respond) to immunotherapy, tumor will not respond (or unlikely to respond) to immunotherapy), or treatment course of action (e.g., proceed with immunotherapy, proceed with co-therapy or immunotherapy and beneficial bacteria, proceed by manipulating microbiome to achieve responsive tumor microenvironment, etc). In some embodiments, such methods find use with other embodiments (e.g., cancers, treatments, etc.) described herein.

In some embodiments, provided herein are methods of predicting a clinical response of a subject to a cancer treatment comprising: (a) characterizing the make-up of the gut microflora of the subject, wherein characterizing the make-up of the gut microflora comprising determining the amounts of various beneficial bacterial and non-beneficial bacteria in the gut of the subject; (b) determining whether the subject is a likely responder or likely non-responder to the cancer treatment based on the relative amounts of the beneficial and non-beneficial bacteria in the gut of the subject. In some embodiments, methods comprise calculating a ratio of beneficial bacterial to non-beneficial bacteria in the gut of the subject. In some embodiments, methods comprise determining that the subject is a likely responder if the ratio of beneficial bacterial to non-beneficial bacteria is above a threshold value. In some embodiments, the threshold value is 1.0 or greater (e.g., 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, or greater, or ranges therebetween (e.g., 1.5 or greater)). In some embodiments, the cancer treatment is an immunotherapy. In some embodiments, provided herein are methods of treating a subject with cancer by comprising: (a) predicting a clinical response of a subject to a cancer treatment by the methods herein; and (b) if the subject is determined to be a likely responder to the cancer treatment, administering the cancer treatment of the subject.

BRIEF DESCRIPTION OF THE DRAWINGS

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawings will be provided by the Office upon request and payment of the necessary fee.

FIG. 1A-B. Distinct commensal microbial communities in anti-PD-1 responders and non-responders as assessed by 16S rRNA sequencing. (A) Relative abundance of differentially abundant taxa in responders vs. non-responders; 62 OTUs were identified as different with $P < 0.05$ (unadjusted,

non-parametric t test). An additional OTU 559527 (arrow) identified as Bifidobacteriaceae approached significance ($P < 0.058$). Supervised hierarchical clustering of the differential taxa was performed based on clinical outcome. Individual samples are organized in columns, labeled with patient identification number. Asterisks indicate samples used in further in-vivo experiments. (B) Principal component analysis of relative abundance of the 63 OTUs shown in FIG. 1A.

FIG. 2A-E. Integration of sequencing methods and qPCR for the identification of commensal bacterial species associated with clinical response to anti-PD-1 therapy. (A) Ranked Spearman's correlation coefficients between the relative abundances of Bifidobacteriaceae OTU 559527 from the 16S data set and species-level identities predicted by shotgun sequencing. The species profiled with shotgun sequencing were compared to the taxonomy of OTUs generated from 16S sequencing at family level. (B) Spearman's correlation between abundance of OTU 559527 from the 16S dataset and *B. longum* identified by metagenomics shotgun sequencing analysis (left) and qPCR (right). Shaded band indicates 95% CI of the values fitted by linear regression. (C) Relative abundance in responders (R) vs. non-responders (NR) of OTU 559527 (16S sequencing; left), *Bifidobacterium longum* (shotgun sequencing; middle), and *Bifidobacterium longum* (qPCR; right). (D) qPCR score representing an aggregate data for the relative abundances of 10 species correlated to OTUs with differential abundance in responders vs. non-responders. (E) Ratio of beneficial to non-beneficial OTU numbers for each patient vs. the patient's RECIST aggregate tumor measurement change. Dashed lines label RECIST % = -30 and ratio = 1.5. Only the 43 16S OTUs confirmed by shotgun metagenomic sequencing were included.

FIG. 3A-G. Human commensal communities modulate anti-tumor immunity in a mouse melanoma model. Germ-free mice were gavaged with fecal material from 3 responder (P28, P34, P09) and 3 non-responder (P06, P21, P11) patient donors. (A) B16.SIY melanoma was injected subcutaneously 2 weeks post-gavage; tumor growth data is from one (groups C, D, E, and F) or two experiments (groups A and B) with 7-11 mice per group per experiment. Error bars represent Mean+SEM. (B) Relative abundance of 207 OTUs from patient donors that colonized in mice, and were differentially abundant between slow and fast tumor growth groups. Columns depict individual mice arranged in groups A through F. Groups A, B, A2, and B2 are from 2 independent duplicate experiments. Rows indicate individual OTUs with exact reference ID match between human and mouse 16S rRNA data sets. (C) In groups A and B, 20 days after B16.SIY injection, ex-vivo activation of splenocytes by SIY peptide was measured with IFN- γ ELISPOT 3 weeks after tumor injection. Tumor-infiltrating SIY-specific CD8⁺ T cells (D) and FoxP3⁺ regulatory T cells (E) were enumerated with flow cytometry. (F) Efficacy of anti-PD-L1 therapy was determined in groups A and B. Data are from one experiment with 7-8 mice per group. (G) Relative abundance in mouse groups A and B of key species validated for qPCR scoring. Six out of the ten species are shown that gave positive PCR signals. Tumor growth curves were analyzed with two-way ANOVA using Tukey's multiple comparisons post-test, flow cytometry data was analyzed using unpaired, two-tailed Student's t-test, and qPCR data was analyzed using non-parametric Mann-Whitney U test. $P < 0.05$ was considered statistically significant; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

FIG. 4A-C. Workflow schematic indicating (A) the integration of methods for microbial identification, (B) analyses of tumor-associated biomarkers, and (C) 16S rRNA sequencing-based identification of human fecally-derived bacteria with a potential role in modulating anti-tumor immunity in a mouse melanoma model.

FIG. 5. Segregation of responder and non-responder samples based on relative abundance data for the 63 differentially abundant OTUs determined with 16S sequencing using unadjusted, non-parametric t test. (62 OTUs were significantly different with $P < 0.05$; 1 OTU, Bifidobacteriaceae OTU 559527 indicated with arrow, approached significance with $P < 0.058$). Columns depict individual patients clustered using unsupervised hierarchical clustering with Euclidean distance. Asterisks indicate samples used in further in-vivo experiments. Annotation bar above the heatmap indicates clinical response to immunotherapy. The ID of de-novo assembled OTUs (new clean-up reference OTUs picked by QIIME) were abbreviated to show only the unique identifier digits, and the full OTU IDs are provided in Table 2.

FIG. 6A-B. Performance assessment of the support vector machine (SVM) final models and relative variable importance of 42 OTU predictors. (A) Combined ROC curve produced by 100 iterative runs of model training and testing. The model performance metrics are shown as the Mean \pm SEM for area under curve (AUC), sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), precision, and balanced accuracy. (B) Variable importance estimation of the predictors across 100 iterative runs. The upper panel represents a histogram of the mean variable importance of each predictor. The bottom panel shows the variable importance distribution, with each line representing one predictor, and vertical line representing the mean variable importance for each predictor. Different colors indicate different predictors.

FIG. 7A-B. Ranked species-level identities of 16S OTUs predicted with shotgun sequencing. OTUs picked by 16S sequencing analysis were first matched to species identified by shotgun sequencing at the family level. Then, pairwise tie-corrected Spearman's correlation was computed for each matching pair and the species matched to each OTU were ranked based on the p value. A complete list of the 63 OTU-to-species matching between the 16S and shotgun sequencing datasets is included in Table S4.

FIG. 8A-B. Use of species-specific qPCR for additional confirmation of the OTU-to-species matches determined by 16S and shotgun sequencing data comparisons. OTUs and their best-match species as measured with 16S and shotgun sequencing, respectively, were correlated by Spearman's test against the relative abundance of the corresponding species measured with qPCR. Depicted are correlations for OTUs (and their best-match species), which are more abundant in non-responders (A) or in responders (B) and are used for computation of the qPCR score. OTU 1107027 (identified as *Lactobacillus ruminis* with 16S sequencing analysis) was best matched to *Lactobacillus animalis* (from the shotgun sequencing data set) with $P < 0.1$ (Table 3B) and was included in the qPCR score, because a primer set with a broader *Lactobacillus* sp. specificity was used (Table 4A-B).

FIG. 9. Visual representation of the presence/absence-based ratio of beneficial/non-beneficial OTUs. The bar graphs represent the total number of potentially beneficial OTUs (more abundant in responders; depicted in red) and potentially non-beneficial OTUs (more abundant in non-responders; depicted in blue) in each patient. The grid map represents presence (green) or absence (white) of beneficial

and non-beneficial OTUs in each patient sample. Columns depict individual patients grouped based on clinical response to immunotherapy in the same order as in FIG. 1A. Rows indicate the 43 OTUs from 16S sequencing that were confirmed by shotgun sequencing (Table. 3). Asterisks indicate samples used in further in-vivo experiments. The ID of de novo assembled OTUs (new clean-up reference OTUs picked by QIIME) were abbreviated to show only the unique identifier digits, and the full OTU IDs are provided in Table 2.

FIG. 10A-F. The qPCR score and the beneficial/non-beneficial OTU ratio as candidate predictors of clinical response to immunotherapy. The qPCR score (A) and the ratio of beneficial-to-non-beneficial OTUs (B) were more distinct between non-responders (NR) and responders (R), compared to the non-synonymous somatic mutational (NSSM) load (C), expression levels of PD-L1 (D) and PD-1 (E), as determined by whole transcriptome sequencing of tumor samples, and intra-tumoral CD8 T cell infiltration (F) as determined with immunohistochemistry of tumor samples. This analysis was limited to subset of 5 responders and 10 non-responders from the original 42 patient cohort, whose samples passed quality control for RNA sequencing. Wilcoxon-Mann-Whitney test (non-parametric) was used for comparing qPCR score, OTU ratio, and NSSM in NR and R groups, which does not assume data follow normal distribution. Student's t-test was used for the rest of the markers.

FIG. 11A-B. Donor samples used in mouse colonization experiments are representative of the responder and non-responder patient groups with respect to qPCR score (A) and ratio of beneficial to non-beneficial OTUs (B) as in FIG. 2.

FIG. 12. Germ-free mice and Taconic SPF mice show similar tumor growth rates. Standard specific pathogen-free (SPF) mice were purchased from Taconic. Germ-free (GF) mice, originally purchased from Taconic were bred in the University of Chicago gnotobiotic facility. The GF mice were divided into two groups, and housed in two separate isolators in the same room. One group (black line; $n=11$) was colonized by oral gavage with fecal material from the SPF mice. The other group remained GF (grey line; $n=10$). Standard Taconic SPF mice were housed in ventilated cages in a standard barrier facility. All mice were maintained on the same diet. Two weeks later, the mice were injected with B16.SIY melanoma and tumor growth was measured.

FIG. 13. Relative abundance of 207 OTUs from patient donors that colonized in mice, and were differentially abundant between slow and fast tumor growth groups. Columns depict individual mice arranged in groups A through F, with donor patient samples added at the beginning of each group. Rows indicate individual OTUs with exact reference ID match between human and mouse 16S rRNA data sets.

FIG. 14. Exemplary data-driven feature (e.g., OTUs) selection workflow. Experiments were conducted during development of embodiments herein to select important features (e.g., OTUs) in a data-driven manner. The goal was to build a model with the least number of features possible (to reduce the likelihood of overfitting), at the cost of none or limited performance decrease. Models were validated using independent test set, to evaluate performance and to identify potential overfitting problems. 100 random seeds were tested to verify: (i) the stability of variable importance (e.g., that it is not tied to certain "lucky" or "unlucky" seeds), (ii) the stability of model performance (e.g., that it is not tied to a "good" or "bad" resampling; evaluation metric="ROC"), and (iii) consistent performance. Features were selected by iterative resampling and validation. First,

consensus elimination is used, running multiple runs (e.g., 100 runs), changing random seeds for different resampling. In each run, model training & validation are performed, variable importance is estimated. After all runs are finished, features are eliminated based on consensus variable importance across the runs.

FIG. 15. Consensus variable important of 24 OTUs across 100 runs.

DEFINITIONS

The terminology used herein is for the purpose of describing the particular embodiments only, and is not intended to limit the scope of the embodiments described herein. Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. However, in case of conflict, the present specification, including definitions, will control. Accordingly, in the context of the embodiments described herein, the following definitions apply.

As used herein and in the appended claims, the singular forms “a”, “an” and “the” include plural reference unless the context clearly dictates otherwise. Thus, for example, reference to “a microbiome biomarker” is a reference to one or more microbiome biomarkers of resistance to immunotherapy and equivalents thereof known to those skilled in the art, and so forth.

As used herein, the term “comprise” and linguistic variations thereof denote the presence of recited feature(s), element(s), method step(s), etc. without the exclusion of the presence of additional feature(s), element(s), method step(s), etc. Conversely, the term “consisting of” and linguistic variations thereof, denotes the presence of recited feature(s), element(s), method step(s), etc. and excludes any unrecited feature(s), element(s), method step(s), etc., except for ordinarily-associated impurities. The phrase “consisting essentially of” denotes the recited feature(s), element(s), method step(s), etc. and any additional feature(s), element(s), method step(s), etc. that do not materially affect the basic nature of the composition, system, or method. Many embodiments herein are described using open “comprising” language. Such embodiments encompass multiple closed “consisting of” and/or “consisting essentially of” embodiments, which may alternatively be claimed or described using such language.

As used herein, the term “microbe” refers to cellular microorganisms including bacteria, fungi, and archaea, and encompasses both individual organisms and populations comprising any number of the organisms.

As used herein, the term “tumor microenvironment” refers to the tissues, cells, molecules, and blood vessels that surround and feed a tumor cell. A tumor’s microenvironment is dynamic and a tumor can change its microenvironment, and the microenvironment can affect how a tumor grows and spread.

As used herein, the terms “microbiota” and “microflora” refer to an assemblage of microorganisms localized to a distinct environment. Microflora may include, for example, populations of various bacteria, fungi, and/or archaea that inhabit a particular environment. For example, “gut microflora,” “vaginal microbiota,” and “oral microflora” are an assemblage of one or more species of microorganisms that are localized to, or found in, the gut, vagina, or mouth, respectively. “Normal microflora” refers to a population of microorganisms that localize in a particular environment in a normal, non-pathological state (e.g., a sample of gut

microflora from a subject without cancer). “Pathologic microflora” refers to a population of various microorganisms that localize in a particular environment in pathological state and differs from normal microflora in terms of identify, absolute amount, or relative amount of the various microbes.

As used herein, the term “commensal microbe” refers to a microorganism that is non-pathogenic to a host and is part of the normal microflora of the host.

As used herein, the term “co-administration” refers to the administration of at least two agents (e.g., commensal microflora and a cancer therapy) or therapies to a subject. In some embodiments, the co-administration of two or more agents/therapies is concurrent. In other embodiments, the co-administration of two or more agents/therapies is sequential (e.g., a first agent/therapy is administered prior to a second agent/therapy).

As used herein, the term “beneficial microbe” refers to a microbe (e.g., bacterium) strain or species that inhibits the growth of cancer/tumor cells and/or facilitates treatment of cancer/tumor cells (e.g., inhibits immune evasion). Beneficial microbes may function by, for example, creating an anti-cancer/anti-tumor environment, microenvironment and/or metabolome, and/or by creating an environment, microenvironment and/or metabolome that inhibits immune evasion or other mechanisms by which cancer cells resist therapy.

As used herein, the term “detrimental microbe” refers to a microbe (e.g., bacterium) strain or species that facilitates the growth of cancer/tumor cells and/or prevents or reduces the effectiveness of treatment of cancer/tumor cells. Detrimental microbes may function by, for example, creating an environment, microenvironment and/or metabolome that facilitates immune evasion or other mechanisms by which cancer cells resist therapy and/or enhance cancer/tumor growth.

As used herein, the term “pharmaceutical agent” refers to a compound, macromolecule, or other chemical/non-biological entity that is administered to a subject to elicit a desired biological response. A pharmaceutical agent may be a “drug” or another entity which is biologically active in a human being or other mammal, locally and/or systemically. Examples of drugs are disclosed in the Merck Index and the Physicians Desk Reference, the entire disclosures of which are incorporated by reference herein for all purposes.

As used herein, the terms “microbial agent,” “commensal microbial agent,” and “probiotic” refer to compositions comprising a microbe or population of multiple different microbes for administration to a subject.

As used herein, the term “antimicrobial agent” is used to describe a therapeutic compound or bioactive agent which treats a microbial infection, for example, an infection caused by a bacteria, virus, protozoa or fungus. The antimicrobial agent may be an antibiotic, an antifungal agent, an antiviral or an antiprotozoal or antiparasitic agent (which may also be used to treat multicellular parasites).

As used herein, the terms “antibiotic” and “antibacterial agent” refer to a chemical agent which is active against bacteria. In common usage, an antibiotic is a substance or compound that kills or inhibits the growth of bacteria. Anti-bacterial antibiotics can be categorized based on their target specificity: “narrow-spectrum” antibiotics target particular types of bacteria, such as Gram-negative or Gram-positive bacteria, while broad-spectrum antibiotics affect a wide range of bacteria. Antibiotics which target the bacterial cell wall (e.g., penicillins, cephalosporins, cepheids), or cell membrane (e.g., polymyxins), or interfere with essential bacterial enzymes (e.g., quinolones, sulfonamides) usually

are bactericidal in nature. Those which target protein synthesis such as the aminoglycosides, macrolides and tetracyclines are usually bacteriostatic. Three newer classes of antibiotics include: cyclic lipopeptides (e.g., daptomycin), glycyclines (e.g., tigecycline), and oxazolidinones (e.g., linezolid). Tigecycline is a broad-spectrum antibiotic, while the two others are useful for Gram-positive infections.

As used herein, the term “antiviral agent” refers to a chemical agent which is used to treat a viral infection. Antiviral drugs are a class of medication used specifically for treating viral infections, specific antivirals are useful for treating infection by specific viruses. Antivirals typically only inhibit virus development.

As used herein, the term “antifungal agent” refers to a therapeutic compound or bioactive agent which may be used to treat a fungal infection in a patient. An antifungal drug is a medication used to treat fungal infections such as athlete’s foot, ringworm, candidiasis (thrush), serious systemic infections such as cryptococcal meningitis, and related fungal infections. Antifungal agents include, for example, polyene antifungals, imidazole, triazole and thiazole antifungals, allylamines, echinocandins, griseofulvin, flucytosine, undecylenic acid, among others.

As used herein, the term “antiparasitic agent” refers to a therapeutic compound or bioactive agent that is used to treat parasitic diseases including nematodes, cestodes, trematodes, infectious protozoa, and amoebas. Exemplary antiparasitic agents include: antinematodes (e.g., mebendazole, pyrantel pamoate, thiabendazole, diethylcarbazine), anticestodes (e.g., niclosamide, praziquantel), antitremitodes (e.g., praziquantel), antiamoebics (e.g., rifampin and amphotericin B), antiprotozoals (e.g., melarsoprol, eflornithine, metronidazole and tinidazole), among others.

As used herein, the term “pharmaceutical formulation” refers to at least one pharmaceutical agent and/or microbial agent in combination with one or more additional components that assist in rendering the agent(s) suitable for achieving the desired effect upon administration to a subject. The pharmaceutical formulation may include one or more additives, for example pharmaceutically acceptable excipients, carriers, penetration enhancers, coatings, stabilizers, buffers or other materials physically associated with the pharmaceutical/microbial agent to enhance the administration, release (e.g., timing of release), deliverability, bioavailability, effectiveness, etc. of the dosage form. The formulation may be, for example, a liquid, a suspension, a solid, a nanoparticle, emulsion, micelle, ointment, gel, emulsion, coating, etc. A pharmaceutical formulation may contain a single agent or multiple agents (e.g., microbial agent and pharmaceutical agent).

As used herein, the term “subject” broadly refers to any animal, including but not limited to, human and non-human animals (e.g., dogs, cats, cows, horses, sheep, poultry, fish, crustaceans, etc.). As used herein, the term “patient” typically refers to a subject that is being treated for a disease or condition (e.g., cancer, solid tumor cancer, non-T cell-infiltrated tumor cancer, etc.).

As used herein, an “immune response” refers to the action of a cell of the immune system (e.g., T lymphocytes, B lymphocytes, natural killer (NK) cells, macrophages, eosinophils, mast cells, dendritic cells, neutrophils, etc.) and soluble macromolecules produced by any of these cells or the liver (including Abs, cytokines, and complement) that results in selective targeting, binding to, damage to, destruction of, and/or elimination from a subject of invading pathogens, cells or tissues infected with pathogens, or cancerous or other abnormal cells.

As used herein, the term “immunoregulator” refers to an agent or a signaling pathway (or a component thereof) that regulates an immune response. “Regulating,” “modifying” or “modulating” an immune response refers to any alteration of the immune system or in the activity of such cell. Such regulation includes stimulation or suppression of the immune system which may be manifested by an increase or decrease in the number of various cell types, an increase or decrease in the activity of these cells, or any other changes which can occur within the immune system. Both inhibitory and stimulatory immunoregulators have been identified, some of which may have enhanced function in a cancer microenvironment.

As used herein, the term “immune evasion” refers to inhibition of a subject’s immune system or a component thereof (e.g., endogenous T cell response) by a cancer or tumor cell in order to maximize or allow continued growth or spread of the cancer/tumor.

As used herein, the term “immunotherapy” refers to the treatment or prevention of a disease or condition (e.g., cancer) by a method comprising inducing, enhancing, suppressing or otherwise modifying an immune response.

As used herein, “potentiating an endogenous immune response” means increasing the effectiveness or potency of an existing immune response in a subject. This increase in effectiveness and potency may be achieved, for example, by overcoming mechanisms that suppress the endogenous host immune response or by stimulating mechanisms that enhance the endogenous host immune response.

As used herein, the term “antibody” refers to a whole antibody molecule or a fragment thereof (e.g., fragments such as Fab, Fab’, and F(ab’)2), it may be a polyclonal or monoclonal antibody, a chimeric antibody, a humanized antibody, a human antibody, etc.

A native antibody typically has a tetrameric structure. A tetramer typically comprises two identical pairs of polypeptide chains, each pair having one light chain (in certain embodiments, about 25 kDa) and one heavy chain (in certain embodiments, about 50-70 kDa). In a native antibody, a heavy chain comprises a variable region, VH, and three constant regions, CH1, CH2, and CH3. The VH domain is at the amino-terminus of the heavy chain, and the CH3 domain is at the carboxy-terminus. In a native antibody, a light chain comprises a variable region, VL, and a constant region, CL. The variable region of the light chain is at the amino-terminus of the light chain. In a native antibody, the variable regions of each light/heavy chain pair typically form the antigen binding site. The constant regions are typically responsible for effector function.

In a native antibody, the variable regions typically exhibit the same general structure in which relatively conserved framework regions (FRs) are joined by three hypervariable regions, also called complementarity determining regions (CDRs). The CDRs from the two chains of each pair typically are aligned by the framework regions, which may enable binding to a specific epitope. From N-terminus to C-terminus, both light and heavy chain variable regions typically comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The CDRs on the heavy chain are referred to as H1, H2, and H3, while the CDRs on the light chain are referred to as L1, L2, and L3. Typically, CDR3 is the greatest source of molecular diversity within the antigen-binding site. H3, for example, in certain instances, can be as short as two amino acid residues or greater than 26. The assignment of amino acids to each domain is typically in accordance with the definitions of Kabat et al. (1991) Sequences of Proteins of Immunological Interest (National

Institutes of Health, Publication No. 91-3242, vols. 1-3, Bethesda, Md.); Chothia, C., and Lesk, A. M. (1987) *J. Mol. Biol.* 196:901-917; or Chothia, C. et al. *Nature* 342:878-883 (1989). In the present application, the term "CDR" refers to a CDR from either the light or heavy chain, unless otherwise specified.

As used herein, the term "heavy chain" refers to a polypeptide comprising sufficient heavy chain variable region sequence to confer antigen specificity either alone or in combination with a light chain.

As used herein, the term "light chain" refers to a polypeptide comprising sufficient light chain variable region sequence to confer antigen specificity either alone or in combination with a heavy chain.

As used herein, when an antibody or other entity "specifically recognizes" or "specifically binds" an antigen or epitope, it preferentially recognizes the antigen in a complex mixture of proteins and/or macromolecules, and binds the antigen or epitope with affinity which is substantially higher than to other entities not displaying the antigen or epitope. In this regard, "affinity which is substantially higher" means affinity that is high enough to enable detection of an antigen or epitope which is distinguished from entities using a desired assay or measurement apparatus. Typically, it means binding affinity having a binding constant (K_a) of at least $10^7 M^{-1}$ (e.g., $>10^7 M^{-1}$, $>10^8 M^{-1}$, $>10^9 M^{-1}$, $>10^{10} M^{-1}$, $>10^{11} M^{-1}$, $>10^{12} M^{-1}$, $>10^{13} M^{-1}$, etc.). In certain such embodiments, an antibody is capable of binding different antigens so long as the different antigens comprise that particular epitope. In certain instances, for example, homologous proteins from different species may comprise the same epitope.

As used herein, the term "monoclonal antibody" refers to an antibody which is a member of a substantially homogeneous population of antibodies that specifically bind to the same epitope. In certain embodiments, a monoclonal antibody is secreted by a hybridoma. In certain such embodiments, a hybridoma is produced according to certain methods known to those skilled in the art. See, e.g., Kohler and Milstein (1975) *Nature* 256: 495-499; herein incorporated by reference in its entirety. In certain embodiments, a monoclonal antibody is produced using recombinant DNA methods (see, e.g., U.S. Pat. No. 4,816,567). In certain embodiments, a monoclonal antibody refers to an antibody fragment isolated from a phage display library. See, e.g., Clackson et al. (1991) *Nature* 352: 624-628; and Marks et al. (1991) *J. Mol. Biol.* 222: 581-597; herein incorporated by reference in their entirety. The modifying word "monoclonal" indicates properties of antibodies obtained from a substantially-homogeneous population of antibodies, and does not limit a method of producing antibodies to a specific method. For various other monoclonal antibody production techniques, see, e.g., Harlow and Lane (1988) *Antibodies: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.); herein incorporated by reference in its entirety.

As used herein, the term "antibody fragment" refers to a portion of a full-length antibody, including at least a portion antigen binding region or a variable region. Antibody fragments include, but are not limited to, Fab, Fab', F(ab')₂, Fv, scFv, Fd, diabodies, and other antibody fragments that retain at least a portion of the variable region of an intact antibody. See, e.g., Hudson et al. (2003) *Nat. Med.* 9:129-134; herein incorporated by reference in its entirety. In certain embodiments, antibody fragments are produced by enzymatic or chemical cleavage of intact antibodies (e.g., papain diges-

tion and pepsin digestion of antibody) produced by recombinant DNA techniques, or chemical polypeptide synthesis.

For example, a "Fab" fragment comprises one light chain and the CH1 and variable region of one heavy chain. The heavy chain of a Fab molecule cannot form a disulfide bond with another heavy chain molecule. A "Fab" fragment comprises one light chain and one heavy chain that comprises additional constant region, extending between the CH1 and CH2 domains. An interchain disulfide bond can be formed between two heavy chains of a Fab' fragment to form a "F(ab')₂" molecule.

An "Fv" fragment comprises the variable regions from both the heavy and light chains, but lacks the constant regions. A single-chain Fv (scFv) fragment comprises heavy and light chain variable regions connected by a flexible linker to form a single polypeptide chain with an antigen-binding region. Exemplary single chain antibodies are discussed in detail in WO 88/01649 and U.S. Pat. Nos. 4,946, 778 and 5,260,203; herein incorporated by reference in their entirety. In certain instances, a single variable region (e.g., a heavy chain variable region or a light chain variable region) may have the ability to recognize and bind antigen.

Other antibody fragments will be understood by skilled artisans.

As used herein, the term "chimeric antibody" refers to an antibody made up of components from at least two different sources. In certain embodiments, a chimeric antibody comprises a portion of an antibody derived from a first species fused to another molecule, e.g., a portion of an antibody derived from a second species. In certain such embodiments, a chimeric antibody comprises a portion of an antibody derived from a non-human animal fused to a portion of an antibody derived from a human. In certain such embodiments, a chimeric antibody comprises all or a portion of a variable region of an antibody derived from a non-human animal fused to a constant region of an antibody derived from a human.

A "humanized" antibody refers to a non-human antibody that has been modified so that it more closely matches (in amino acid sequence) a human antibody. A humanized antibody is thus a type of chimeric antibody. In certain embodiments, amino acid residues outside of the antigen binding residues of the variable region of the non-human antibody are modified. In certain embodiments, a humanized antibody is constructed by replacing all or a portion of a complementarity determining region (CDR) of a human antibody with all or a portion of a CDR from another antibody, such as a non-human antibody, having the desired antigen binding specificity. In certain embodiments, a humanized antibody comprises variable regions in which all or substantially all of the CDRs correspond to CDRs of a non-human antibody and all or substantially all of the framework regions (FRs) correspond to FRs of a human antibody. In certain such embodiments, a humanized antibody further comprises a constant region (Fc) of a human antibody.

The term "effective dose" or "effective amount" refers to an amount of an agent, e.g., an antibody, that results in the reduction of symptoms in a patient or results in a desired biological outcome. In certain embodiments, an effective dose or effective amount is sufficient to treat or reduce symptoms of a disease or condition.

DETAILED DESCRIPTION

Provided herein are compositions and methods comprising microbiome biomarkers of responsiveness/resistance to

immunotherapy (e.g., anti-PD1/PD-L1 therapy), and diagnostic, prognostic and therapeutic uses thereof. In particular, the amount, identity, presence, and/or ratio of microflora in the microbiome of a subject is used to determine the responsiveness/resistance of the subject to immunotherapy, and/or the microbiome of a subject is manipulated to enhance the responsiveness of the subject to various immunotherapies and co-therapies.

In some embodiments, the effectiveness of an endogenous immune response, immunotherapy, chemotherapeutic, or other treatment (e.g., surgery, radiation, etc.) in the treatment or prevention of reoccurrence of cancer and/or tumor is dependent upon conditions within the subject (e.g., the tumor microenvironment). In particular, the identity or characteristics (e.g., concentration or level) of the microflora within a subject affects the effectiveness of cancer treatments (e.g., generally or specific treatments) and/or the effectiveness of the subject's own immune response to cancer.

In some embodiments, the presence or increased level of one or more microbes (e.g., one or more types of bacteria) in a subject potentiates cancer/tumor growth, spread (e.g., malignancy), and/or evasion of treatment/immune response. In some embodiments, the presence or increased level of one or more microbes (e.g., one or more types of bacteria) in a subject inhibits treatment (e.g., immunotherapy, chemotherapy, etc.) and/or the subject's endogenous immune response to cancer and/or tumor cells. In some embodiments, the absence and/or decreased level of one or more microbes (e.g., one or more types of bacteria) in a subject potentiates cancer/tumor growth, spread, and/or evasion of treatment/immune response. In some embodiments, the absence or decreased level of one or more microbes (e.g., one or more types of bacteria) in a subject inhibits treatment (e.g., immunotherapy, chemotherapy, etc.) and/or the subject's endogenous immune response to cancer and/or tumor cells.

In some embodiments, the presence or increased level of one or more microbes (e.g., one or more types of bacteria) in a subject discourages cancer/tumor growth, spread, and/or evasion of treatment/immune response. In some embodiments, the presence or increased level of one or more microbes (e.g., one or more types of bacteria) in a subject facilitates treatment (e.g., immunotherapy, chemotherapy, etc.) and/or the subject's endogenous immune response to cancer and/or tumor cells. In some embodiments, the absence and/or decreased level of one or more microbes (e.g., one or more types of bacteria) in a subject discourages cancer/tumor growth, spread, and/or evasion of treatment/immune response. In some embodiments, the absence or decreased level of one or more microbes (e.g., one or more types of bacteria) in a subject facilitates treatment (e.g., immunotherapy, chemotherapy, etc.) and/or the subject's endogenous immune response to cancer and/or tumor cells.

In some embodiments, the presence of beneficial microbes (e.g., microbes that facilitate cancer treatment) in a subject creates an environment or microenvironment (e.g., metabolome) that is conducive to the treatment of cancer and/or inhibits cancer/tumor growth. In some embodiments, the presence of detrimental microbes (e.g., microbes that facilitate cancer/tumor growth and/or prevent treatment) in a subject creates an environment or microenvironment (e.g., metabolome) that is conducive to the treatment of cancer and/or inhibits cancer/tumor growth.

Experiments conducted during development of embodiments described herein demonstrate that the responsiveness/resistance of a tumor to immunotherapies can be assessed by

assaying the bacteria present in the microbiome of a subject. Particular microbes identified in experiments herein (e.g., bacteria of Table 6, bacteria of the families Coriobacteriaceae, Ruminococcaceae, Lachnospiraceae, Clostridiaceae, Bifidobacteriaceae, Enterobacteriaceae, and/or Erysipelotrichaceae, bacteria are selected from the OTU group consisting of 365181 Coriobacteriaceae, 197562 Ruminococcaceae, 363794 Coriobacteriaceae, 295085 Lachnospiraceae, 659361 Lachnospiraceae, 461795 Ruminococcaceae, 592139 Clostridiaceae, 813479 Bifidobacteriaceae, OTU 325850, OTU 352933, 559527 Bifidobacteriaceae, 808486 Enterobacteriaceae, 830346 Enterobacteriaceae, 198928 Lachnospiraceae, 367215 Erysipelotrichaceae, 1973443 Lachnospiraceae, and 367092 Lachnospiraceae; etc.) correlate, are indicative of, contribute to, and/or are causative of responsiveness or resistance of tumors to immunotherapies. Characterization of the identity of microbes with a subject finds use in determining the likelihood that a tumor within the subject will respond to particular immunotherapies. In some embodiments, treatment course of action (e.g., immunotherapy, chemotherapy, microbial therapy, combinations thereof, etc.) is determined based on the methods herein.

Experiments conducted during development of embodiments described herein demonstrate that modulation of levels and/or identity of the microflora in a subject facilitates treatment of cancer/tumor within the subject, enhances the endogenous immune response, decreases immune evasion or other inhibitory mechanisms to treatment of endogenous immune response, and/or improves cancer outcomes for the subject. Modulation of microflora levels and/or identity may comprise encouraging or facilitating growth of one or more types of beneficial microbes (e.g., microbes that facilitate cancer treatment), discouraging or inhibiting growth of one or more types of detrimental microbes (e.g., microbes that facilitate cancer/tumor growth and/or prevent treatment), administering one or more types of beneficial microbes (e.g., microbes that facilitate cancer treatment) to the subject, and/or combinations thereof. Embodiments within the scope herein are not limited by the mechanisms for introducing one or more microbes (e.g., fecal transplant, probiotic administration, etc.), encouraging growth of beneficial microbes (e.g., administering agents that skew the environment within the subject toward growth conditions for the beneficial microbes), discouraging or inhibiting growth of detrimental microbes (e.g., administering agents that skew the environment within the subject away from growth conditions for the detrimental microbes, administration of antimicrobial(s), etc.), and combinations thereof.

In some embodiments, methods are provided for the treatment or prevention of cancer by the manipulation of the presence, amount, or relative ratio of commensal microflora (e.g., gut microflora). In some embodiments, the presence, amount, or relative ratio of particular bacteria, fungi, and/or archaea within a subject is manipulated. In some embodiments, the levels of one or more bacterial list in Table 6 are manipulated. For example, in some embodiments, the presence, amount, or relative ratio of one or more bacteria from the families Coriobacteriaceae, Ruminococcaceae, Lachnospiraceae, Clostridiaceae, Bifidobacteriaceae, Enterobacteriaceae, and/or Erysipelotrichaceae are manipulated. In some embodiments, the presence, amount, or relative ratio of one or more of 365181 Coriobacteriaceae, 197562 Ruminococcaceae, 363794 Coriobacteriaceae, 295085 Lachnospiraceae, 659361 Lachnospiraceae, 461795 Ruminococcaceae, 592139 Clostridiaceae, 813479 Bifidobacteriaceae, OTU 325850, OTU 352933, 559527 Bifidobacteriaceae, 808486 Enterobacteriaceae, 830346 Enterobacteriaceae,

198928 Lachnospiraceae, 367215 Erysipelotrichaceae, 1973443 Lachnospiraceae, and 367092 Lachnospiraceae are manipulated.

In some embodiments, the presence and/or levels of one or more commensal microbes are manipulated in a subject suffering from cancer, at heightened risk of cancer, and/or receiving treatment for cancer. Exemplary commensal microbes include *Lactococcus* (e.g., *Lactococcus cremoris* and *Lactococcus lactis*), *Lactobacillus* (e.g., *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus kefir*, *Lactobacillus bifidus*, *Lactobacillus brevis*, *Lactobacillus helveticus*, *Lactobacillus paracasei*, *Lactobacillus rhamnosus*, *Lactobacillus salivarius*, *Lactobacillus curvatus*, *Lactobacillus bulgaricus*, *Lactobacillus sakei*, *Lactobacillus reuteri*, *Lactobacillus fermentum*, *Lactobacillus farciminis*, *Lactobacillus lactis*, *Lactobacillus delbrueckii*, *Lactobacillus plantarum*, *Lactobacillus paraplantarum*, *Lactobacillus crispatus*, *Lactobacillus gasseri*, *Lactobacillus johnsonii* and *Lactobacillus jensenii*), *Leuconostoc*, *Carnobacterium*, *Enterococcus*, *Propionibacterium*, *Pediococcus*, *Bifidobacterium* (e.g., *Bifidobacterium lactis*, *Bifidobacterium bifidum*, *Bifidobacterium longum*, *Bifidobacterium animalis*, *Bifidobacterium breve*, *Bifidobacterium infantis*, *Bifidobacterium catenulatum*, *Bifidobacterium pseudocatenulatum*, *Bifidobacterium adolescentis*, *Bifidobacterium angulatum*, etc.), *Streptococcus* (e.g., *Streptococcus thermophiles*, *Streptococcus salivarius*, *Streptococcus oralis*, *Streptococcus uberis*, *Streptococcus rattus*, etc.); *Escherichia coli*, *Bacillus coagulans*, *Bacillus lantii*, Yeast (e.g., *Saccharomyces cerevisiae*, *Saccharomyces boulardii*, etc.); and combinations thereof.

In experiments conducted during development of embodiments herein, baseline stool samples were analyzed from metastatic melanoma patients prior to immunotherapy treatment, through an integration of 16S rRNA sequencing, metagenomic shotgun sequencing, and quantitative PCR for selected bacteria. A significant association between commensal microbial composition and clinical response was observed. Bacterial species more abundant in responders included *Enterococcus faecium*, *Collinsella aerofaciens*, *Bifidobacterium adolescentis*, *Klebsiella pneumoniae*, *Veillonella parvula*, *Parabacteroides merdae*, *Lactobacillus* sp. and *Bifidobacterium longum*. Bacterial species more abundant in non-responders included *Ruminococcus obeum* and *Roseburia intestinalis*. The experiments indicate that reconstitution of germ-free subjects with fecal material from responding patients provides improved tumor control, augmented T cell responses, and/or greater efficacy of immunotherapy (e.g., anti-PD-L1 therapy). The experimental results demonstrate a mechanistic impact of the commensal microbiota on anti-tumor immunity in cancer.

In some embodiments, the presence and/or levels of one or more commensal microbes are manipulated in a subject suffering from cancer, at heightened risk of cancer, and/or receiving treatment for cancer.

In some embodiments, one or more species, genera, and/or types of microbes are administered and/or the growth thereof is facilitated. In some embodiments, the growth of one or more species, genera, and/or types of microbes is inhibited. In some embodiments, one or more species, genera, and/or types of microbes are administered and/or the growth thereof is facilitated; and the growth of one or more other species, genera, and/or types of microbes is inhibited.

In some embodiments, the level or presence of one or more beneficial microbes (e.g., microbes that inhibit cancer/

tumor growth or spread, enhance cancer/tumor treatment, etc.) is modulated by the administration of such microbes to a subject.

In some embodiments, microflora-modulation utilizes prepared probiotic compositions for administration to/by a subject. Probiotic compositions comprise one or more beneficial microbes (e.g., bacteria) formulated such that administration of the probiotic (e.g., orally, rectally, by inhalation, etc.) results in population of the subject by the beneficial microbes.

In some embodiments, probiotic compositions comprise cultured microbes that are combined and/or formulated for administration to a subject. In some embodiments, probiotics contain microbes of known genera, species, etc. and/or at known concentrations (cfus). Probiotic compositions may be in the form of a pharmaceutical-type composition (e.g., capsule, tables, liquid, aerosol, etc.) or in the form of a food supplement.

In some embodiments, probiotic microbes (e.g., bacteria) are formulated in a pharmaceutically acceptable composition for delivery to a subject. In some embodiments, probiotics are formulated with a pharmaceutically acceptable carrier suitable for a solid or semi-solid formulation. In some embodiments, probiotic microbes are formulated with a pharmaceutically acceptable carrier suitable for a liquid or gel formulation. Probiotic formulations may be formulated for enteral delivery, e.g., oral delivery, or delivery as a suppository, but can also be formulated for parenteral delivery, e.g., vaginal delivery, inhalational delivery (e.g., oral delivery, nasal delivery, and intrapulmonary delivery), and the like.

The probiotic compositions that find use in embodiments described herein may be formulated in a wide variety of oral administration dosage forms, with one or more pharmaceutically acceptable carriers. The pharmaceutically acceptable carriers can be either solid or liquid. Solid form preparations include powders, tablets, pills, capsules, cachets, suppositories, and dispersible granules. A solid carrier can be one or more substances which may also act as diluents, flavoring agents, solubilizers, lubricants, suspending agents, binders, preservatives, tablet disintegrating agents, or an encapsulating material. In powders, the carrier is a finely divided solid which is a mixture with the probiotic microbes. In tablets, the microbes are mixed with the carrier having the necessary binding capacity in suitable proportions and compacted in the shape and size desired. Suitable carriers are magnesium carbonate, magnesium stearate, talc, sugar, lactose, pectin, dextrin, starch, gelatin, tragacanth, methylcellulose, sodium carboxymethylcellulose, a low melting wax, cocoa butter, and the like. Other forms suitable for oral administration include liquid form preparations such as emulsions, syrups, elixirs, aqueous solutions, aqueous suspensions, or solid form preparations which are intended to be converted shortly before use to liquid form preparations. Aqueous suspensions can be prepared by dispersing the probiotic microbes in water with viscous material, such as natural or synthetic gums, resins, methylcellulose, sodium carboxymethylcellulose, and other well-known suspending agents.

The probiotic compositions (e.g., microbes (e.g., bacteria)) may be formulated for administration as suppositories. A low melting wax, such as a mixture of fatty acid glycerides or cocoa butter is first melted and the probiotic microbes are dispersed homogeneously, for example, by stirring. The molten homogeneous mixture is then poured into conveniently sized molds, allowed to cool, and to solidify.

The probiotic compositions (e.g., microbes (e.g., bacteria)) may be formulated for vaginal administration. Pessar-

suitable ranges therein (e.g., from about 10^2 cfu to about 10^{13} cfu, about 1×10^4 to about 1×10^{11} cfu, about 1×10^6 to about 1×10^9 cfu, about 1×10^{10} to about 1×10^{12} cf, etc.), etc.).

The dose can be administered in a single unit dose administered at any time during a day. Alternatively the loading dose can be administered in two or more doses administered at a single time of day or at two or more separate times of day.

Over the course of multiple dosing periods, the dose can be tapered from an initial dose to a higher dose (or increased from an initial dose to a higher dose), on predetermined timing or by the when the subject and/or clinician based on the results of the treatment. The appropriate dosage amount will vary by, for example, an individual subject's age, weight, condition or disease, severity of disease, etc.

In some embodiments, microbes for probiotic compositions are obtained from culture. In some embodiments, strains of beneficial microbes are genetically engineered to enhance one or more of production (e.g., at scale), formulation, delivery, or the biological effect of the microbe. In some embodiments, microbes are engineered to express a detectable marker that allows tracking of the microbes within a subject, or confirmation that the microbe has integrated into a subject's microflora. In some embodiments, microbes are engineered to express a cancer therapeutic (e.g., chemotherapeutic, immunotherapeutic, antibodies, etc.), anti-inflammatory agent, of other drug.

In some embodiments, one or more prebiotics are administered to a subject as an independent treatment (e.g., to increase the level of a beneficial microbe) or in conjunction with other treatments described herein. Prebiotics are agents that increase the in vivo growth rate or activity of commensal microbes. In some embodiments, prebiotics are soluble fiber sources. In some embodiments, when prebiotics are administered (e.g., fed) to a subject they are not digested or are not fully digested by the subject's digestive enzymes, but rather support the intestinal health of the subject and provide an energy source for the beneficial microbes and enhance the growth thereof. Prebiotics include, for example, naturally occurring lecithins and/or oleic acid, and are described, for example in U.S. Pat. No. 8,449,878 which is herein incorporated by reference in its entirety.

In some embodiments, the level or presence of one or more detrimental microbes (e.g., microbes that facilitate cancer/tumor growth or spread, inhibit cancer/tumor treatment, etc.) is modulated, for example, by the administration of one or more antimicrobial agents to a subject or modulation of conditions within the subject to disfavor growth of the detrimental microbes. In some embodiments, antimicrobial agents are administered.

In some embodiments, the antimicrobial agent is an antibiotic. Exemplary antibiotics that may find use in some embodiments include, but are not limited to: amikacin, gentamicin, kanamycin, neomycin, netilmicin, streptomycin, tobramycin, paromycin, geldanamycin, herbimycin, loracarbef, ertapenem, doripenem, imipenem, meropenem, cefaclor, cefamandole, cefotaxim, cefprozil, cefuroxime, cefixime, cefdinir, cefditoren, cefpodoxime, ceftazidime, ceftibuten, ceftizoxime, ceftriaxone, cefepime, ceftobiprole, vancomycin, azithromycin, clarithromycin, dirithromycin, erythromycin, roxithromycin, troleandomycin, telithromycin, spectinomycin, aztreonam, amoxicillin, ampicillin, azociling, carbenicillin, cloxacillin, dicloxacillin, flucloxacillin, mezlocillin, meticillin, nafcillin, oxacillin, peperacillin, ticarcillin, bacitracin, colistin, polymyxin B, ciprofloxacin, clavulanic acid, enoxacin, gatifloxacin, levofloxacin, lomefloxacin, moxifloxacin, nonfloxacin, ofloxa-

cin, trovafloxacin, grepafloxacin, sparfloxacin, AL-15469A, AL-38905, OP-145, afenide, prontosil, sulfacetamide, sulfamethiazole, sulfanamide, sulfasalazine, sulfisoxazole, trimethoprim, cotrimoxazole, demeclocycline, doxycycline, minocycline, oxytetracycline, tetracycline, linezolid, arsogebanubem chloramphenicol, clindamycin, lincomycin, ethambutol, fosfomycin, fusidic acid, furazolidone, isoniazid, linezolid, metronidazole, mupirocin, nitrofurantoin, rifampicin, thiamphenicol, tinidazole, amoxicillin+clavulanic acid, Maximin H5, Dermcidin, Cecropins, andropin, moricin, ceratotoxin, melittin, Magainin, dermasseptin, bombinin, brevinin-1,esculentins and buforin II, CAP 18, LL37, abaeicin, apidaecins, prophenin, indolicidin, brevinins, protegrin, tachyplesins, defensins, drosomycin, alamethicin, pexiganan or MSI-78, MSI-843, MSI-594, polyphemusin, colicin, pyocin, klebicin, subtilin, epidermin, herbicolacin, brevicin, halocin, agrocin, alveicin, carnocin, curvaticin, divercin, enterocin, enterolysin, erwiniocin, glycinecin, lactococin, lacticin, leuococin, mesentericin, pediocin, plantaricin, sakacin, sulfolobacin, vibriocin, warnerinand, nisin, or a salt or cocrystal, or prodrug or solvate thereof, or a combination thereof.

In some embodiments, the antimicrobial is an antifungal agent. Exemplary antifungals that may find use in some embodiments include, but are not limited to: amroline, utenafine, naftifine, terbinafine, flucytosine, fluconazole, itraconazole, ketoconazole, posaconazole, ravuconazole, voriconazole, clotrimazole, econazole, miconazole, oxiconazole, sulconazole, terconazole, tioconazole, nikkomyacin Z, caspofungin, micafungin, anidulafungin, amphotericin B, liposomal nystatin, pimaricin, griseofulvin, ciclopirox olamine, haloprogin, tolnaftate, undecylenate, clioquinol, and combinations thereof.

In some embodiments, the antimicrobial is an antiparasitic. Exemplary antiparasitics that may find use in some embodiments include, but are not limited to: amitraz, amoscanate, avermectin, carbadox, diethylcarbamazine, dimetridazole, diminazene, ivermectin, macrofilaricide, malathion, mitaban, oxamniquin, permethrin, praziquantel, prantel pamoate, selamectin, sodium stibogluconate, thiabendazole, and combinations thereof.

In some embodiments, methods and compositions for reduction of detrimental microbe levels are co-administered (e.g., serially, concurrently, etc.) with methods and compositions for increasing beneficial microbe levels. In some embodiments, by reducing overall microbe levels or by reducing the levels of specific microbes (e.g., detrimental microbes, high population microbes, etc.), the population of beneficial microbes can more effectively be modulated (e.g., increased).

In some embodiments, in order to develop a microflora population within a subject that facilitates cancer treatment or inhibits cancer growth/spread, antimicrobial agents are first administered to eliminate or reduce the microflora within the subject, and then the microflora population is reestablished using the methods and compositions described herein (e.g., administration of beneficial microbes). In some embodiments, antimicrobials (e.g., antibiotics) that reduce the microbe (e.g., bacteria) population generally are employed. In some embodiments, antimicrobials that target detrimental microbes preferentially are employed.

In some embodiments, modulating the microflora composition is sufficient on its own to allow the endogenous immune system of a subject to respond to the presence of cancer cells and or tumor growth. However, in other embodiments, microflora composition is manipulated along with one or more other cancer therapies. In some embodi-

ments, manipulation of the microflora composition (e.g., identity and/or level) treats cancer by a mechanism independent of one or more additional cancer treatments. In other embodiments, modulation of microflora composition facilitates (e.g., increases the effectiveness of) the cancer treatment. In some embodiments, one or more cancer treatments enhance the effectiveness of the modulation of microflora composition. Embodiments herein are not limited by the types of cancer treatments (e.g., surgery, radiation, immunotherapy, chemotherapeutic, etc.) unless specifically noted.

In some embodiments, immunotherapeutic cancer treatment encompasses blockade of immune-inhibitory receptors, for example using monoclonal antibodies (mAbs) against CTLA-4 and PD-1/PD-L1 (Wolchok, J. D. et al. *The New England Journal of Medicine* 369, 122-133 (2013); Topalian, S. L. et al. *Journal of Clinical Oncology* 32, 1020-1030 (2014); Topalian, S. L. et al. *The New England Journal of Medicine* 366, 2443-2454 (2012); Hodi, F. S. et al. *The New England Journal of Medicine* 363, 711-723 (2010); herein incorporated by reference in their entireties).

In some embodiments, the immunotherapy includes the administration of an immune checkpoint inhibitor. Immune checkpoint inhibition broadly refers to inhibiting the checkpoint markers that cancer cells can produce to prevent or downregulate an immune response. Examples of immune checkpoint proteins include, but are not limited to, CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3, IDO or VISTA. Immune checkpoint inhibitors can be antibodies or antigen binding fragments thereof that bind to and inhibit an immune checkpoint protein. Examples of immune checkpoint inhibitors include, but are not limited to, nivolumab, pembrolizumab, pidilizumab, AMP-224, AMP-514, STI-A1110, TSR-042, RG-7446, BMS-936559, BMS-936558, MK-3475, CT 011, MPDL3280A, MEDI-4736, MSB-0020718C, AUR-012 and STI-A1010. In some embodiments, the immune checkpoint inhibitor may be administered via injection (e.g., intravenously, intratumorally, subcutaneously, or into lymph nodes), but may also be administered orally, topically, or via aerosol.

In some embodiments, the compositions for and/or methods of modulating microflora in a subject overcome immune invasion of cancer cells, tumor, tumor microenvironment, etc. In some embodiments, one or more additional cancer immunotherapies are employed (e.g., concurrently or serially) to make use of the induced immune-responsiveness treated cells/tumor. Suitable immunotherapies may include, but are not limited to: cell-based therapies (e.g., dendritic cell or T cell therapy, etc.), monoclonal antibody (mAb) therapy (e.g., naked mAbs, conjugated mAbs), cytokine therapy (e.g., interferons, interleukins, etc.), adjuvant treatment (e.g., polysaccharide-K, CpG oligonucleotides), etc.

Examples of antibodies that may find use in the compositions and methods disclosed herein, particularly for use in immunotherapies include, but are not limited, to antibodies such as trastuzumab (anti-HER2/neu antibody); pertuzumab (anti-HER2 mAb); cetuximab (chimeric monoclonal antibody to epidermal growth factor receptor EGFR); panitumumab (anti-EGFR antibody); nimotuzumab (anti-EGFR antibody); zalutumumab (anti-EGFR mAb); Necitumumab (anti-EGFR mAb); MDX-210 (humanized anti-HER-2 bispecific antibody); MDX-210 (humanized anti-HER-2 bispecific antibody); MDX-447 (humanized anti-EGF receptor bispecific antibody); rituximab (chimeric murine/human anti-CD20 mAb); obinutuzumab (anti-CD20 mAb); ofatumumab (anti-CD20 mAb); tositumumab-1131 (anti-CD20

mAb); ibritumomab tiuxetan (anti-CD20 mAb); bevacizumab (anti-VEGF mAb); ramucirumab (anti-VEGFR2 mAb); ranibizumab (anti-VEGF mAb); aflibercept (extracellular domains of VEGFR1 and VEGFR2 fused to IgG1 Fc); AMG386 (angiopoietin-1 and -2 binding peptide fused to IgG1 Fc); dalotuzumab (anti-IGF-1R mAb); gemtuzumab ozogamicin (anti-CD33 mAb); alemtuzumab (anti-Campath-1/CD52 mAb); brentuximab vedotin (anti-CD30 mAb); catumaxomab (bispecific mAb that targets epithelial cell adhesion molecule and CD3); naptumomab (anti-5T4 mAb); girentuximab (anti-Carbonic anhydrase ix); or farletuzumab (anti-folate receptor). Other examples include antibodies such as Panorex™ (17-1A) (edrecolomab, murine monoclonal antibody); BEC2 (ami-idiotypic mAb, mimics the GD epitope) (with BCG); Oncolym (Lym-1 monoclonal antibody); SMART M195 Ab, humanized 13' 1 LYM-1 (Oncolym); Ovarex (B43.13, anti-idiotypic mouse mAb); 3622W94 mAb that binds to EGP40 (17-1A) pancreatic carcinoma antigen on adenocarcinomas; Zenapax (SMART Anti-Tac (IL-2 receptor); SMART M195 Ab, humanized Ab, humanized); NovoMab-G2 (pancarcinoma specific Ab); TNT (chimeric mAb to histone antigens); Gliomab-H (Monoclonals—Humanized Abs); GNI-250 Mab; EMD-72000 (chimeric-EGF antagonist); LymphoCide (humanized IL.L.2 antibody); and MDX-260 bispecific, targets GD-2, ANA Ab, SMART IDIO Ab, SMART ABL 364 Ab, or ImmuRAIT-CEA.

In some embodiments, an immunotherapy, utilized as a co-therapy with the microflora modulation described herein, directly or indirectly targets one of more of: a regulatory T cell, myeloid suppressor cell, or dendritic cell. In another aspect, an immunotherapy specifically targets one of the following molecules: CD4; CD25 (IL-2 α receptor; IL-2 α R); cytotoxic T-lymphocyte antigen-4 (CTLA-4; CD152); Interleukin-10 (IL-10); Transforming growth factor-beta receptor (TGF- β R); Transforming growth factor-beta (TGF- β); Programmed Death-1 (PD-1); Programmed death-1 ligand (PD-L1 or PD-L2); Receptor activator of nuclear factor-KB (RANK); Receptor activator of nuclear factor-KB (RANK) ligand (RANKL); LAG-3; glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; TNFRSF18); indoleamine-pyrrole 2,3-dioxygenase (IDO) or Interleukin-4 receptor (IL-4R). In some embodiments, the immunotherapy acts as an agonist that increases the function of the targeted molecule. In other embodiments, the immunotherapy is an antagonist that inhibits the function of the targeted molecule.

In some embodiments, an immunotherapy, utilized as a co-therapy with the microflora modulation described herein, directly or indirectly targets one of more of a specific cytokine, cytokine receptor, co-stimulatory molecule, co-inhibitory molecule, or immunomodulatory receptor that modulates the immune system. In another aspect, one of the following molecules are targeted by co-treatment with microflora modulation: tumor necrosis factor (TNF) superfamily; tumor necrosis factor- α (TNF- α); tumor necrosis factor receptor (TNFR) superfamily; Interleukin-12 (IL-12); IL-12 receptor; 4-IBB (CD137); 4-IBB ligand (4-1BBL; CD137L); OX40 (CD134; TNR4); OX40 ligand (OX40L; CD40); CD40 ligand (CD40L); CTLA-4; Programmed death-1 (PD-1); PD-1 ligand I (PD-L1; B7-H1); or PD-1 ligand 2 (PD-L2; B7-DC); B7 family; B7-1 (CD80); B7-2 (CD86); B7-H3; B7-H4; GITR/AITR: GITRL/AITRL; BTLA; CD70; CD27; LIGHT; HVEM: Toll-like receptor (TLR) (TLR 1, 2, 3, 4, 5, 6, 7, 8, 9, 10).

In some embodiments, the compositions for and/or methods of modulating microflora in a subject sensitize the

cancer cells and/or tumor to treatment by one or more chemotherapeutic agents. In some embodiments, one or more chemotherapies are employed in addition to microflora modulation (e.g., concurrently or serially) to make use of the induced chemotherapeutic sensitivity. In other embodi-

ments, one or more chemotherapeutics are provided as co-therapies with microflora modulation, with or without (known) synergism between the microflora modulation and the chemotherapy.

In some embodiments, exemplary anticancer agents suitable for use in compositions and methods described herein (e.g., co-administered with a β -catenin inhibitor) include, but are not limited to: 1) alkaloids, including microtubule inhibitors (e.g., vincristine, vinblastine, and vindesine, etc.), microtubule stabilizers (e.g., paclitaxel (Taxol), and docetaxel, etc.), and chromatin function inhibitors, including topoisomerase inhibitors, such as epipodophylotoxins (e.g., etoposide (VP-16), and teniposide (VM-26), etc.), and agents that target topoisomerase I (e.g., camptothecin and irinotecan (CPT-11), etc.); 2) covalent DNA-binding agents (alkylating agents), including nitrogen mustards (e.g., mechlorethamine, chlorambucil, cyclophosphamide, ifosfamide, and busulfan (MYLERAN), etc.), nitrosoureas (e.g., carmustine, lomustine, and semustine, etc.), and other alkylating agents (e.g., dacarbazine, hydroxymethylmelamine, thiotepa, and mitomycin, etc.); 3) noncovalent DNA-binding agents (antitumor antibiotics), including nucleic acid inhibitors (e.g., dactinomycin (actinomycin D), etc.), anthracyclines (e.g., daunorubicin (daunomycin, and cerubidine), doxorubicin (adriamycin), and idarubicin (idamycin), etc.), anthracenediones (e.g., anthracycline analogues, such as mitoxantrone, etc.), bleomycins (BLENOXANE), etc., and plicamycin (mithramycin), etc.); 4) antimetabolites, including antifolates (e.g., methotrexate, FOLEX, and MEXATE, etc.), purine antimetabolites (e.g., 6-mercaptopurine (6-MP, PURINETHOL), 6-thioguanine (6-TG), azathioprine, acyclovir, ganciclovir, chlorodeoxyadenosine, 2-chlorodeoxyadenosine (CdA), and 2'-deoxycoformycin (pentostatin), etc.), pyrimidine antagonists (e.g., fluoropyrimidines (e.g., 5-fluorouracil (ADRUCIL), 5-fluorodeoxyuridine (FdUrd) (floxuridine)) etc.), and cytosine arabinosides (e.g., CYTOSAR (ara-C) and fludarabine, etc.); 5) enzymes, including L-asparaginase, and hydroxyurea, etc.; 6) hormones, including glucocorticoids, antiestrogens (e.g., tamoxifen, etc.), nonsteroidal antiandrogens (e.g., flutamide, etc.), and aromatase inhibitors (e.g., anastrozole (ARIMIDEX), etc.); 7) platinum compounds (e.g., cisplatin and carboplatin, etc.); 8) monoclonal antibodies (e.g., conjugated with anticancer drugs, toxins, and/or radionuclides, etc; neutralizing antibodies; etc.); 9) biological response modifiers (e.g., interferons (e.g., IFN- α), etc.) and interleukins (e.g., IL-2, etc.), etc.); 10) adoptive immunotherapy; 11) hematopoietic growth factors; 12) agents that induce tumor cell differentiation (e.g., all-trans-retinoic acid, etc.); 13) gene therapy techniques; 14) antisense therapy techniques; 15) tumor vaccines; 16) therapies directed against tumor metastases (e.g., batimastat, etc.); 17) angiogenesis inhibitors; 18) proteasome inhibitors (e.g., VELCADE); 19) inhibitors of acetylation and/or methylation (e.g., HDAC inhibitors); 20) modulators of NF kappa B; 21) inhibitors of cell cycle regulation (e.g., CDK inhibitors); and 22) modulators of p53 protein function.

In some embodiments, compositions and methods herein comprise multiple modes for the treatment and/or prevention of cancer. In some embodiments, beneficial microbes are provided/administered (e.g., by a probiotic composition, fecal transplant, etc.) with prebiotics and/or other agents that

facilitate the growth of the beneficial microbes. In some embodiments, beneficial microbes are provided/administered (e.g., by a probiotic composition, fecal transplant, etc.) with antimicrobial(s) (e.g., antibiotics) directed to kill or inhibit the growth of detrimental microbes. In some embodi-

ments, prebiotics and/or other agents that facilitate the growth of the beneficial microbes are provided/administered with antimicrobial(s) (e.g., antibiotics) directed to kill or inhibit the growth of detrimental microbes. In some embodiments, beneficial microbes, prebiotics and/or other agents that facilitate the growth of the beneficial microbes, and an antimicrobial(s) (e.g., antibiotics) directed to kill or inhibit the growth of detrimental microbes are all co-administered.

In some embodiments, the co-administered agents are formulated into a single dose and/or composition. In some embodiments, the co-administered agents are in separate doses and/or compositions. In some embodiments in which separate doses and/or compositions are administered, the doses and/or compositions are administered simultaneously, consecutively, or spaced over a time span (e.g., <30 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 1 week, or more, or any suitable ranges therebetween).

In some embodiments, beneficial microbes, prebiotics and/or other agents that facilitate the growth of the beneficial microbes, antimicrobial(s) (e.g., antibiotics) directed to kill or inhibit the growth of detrimental microbes, or any of the above mentioned combinations thereof are administered with a treatment for cancer. In some embodiments, in which the modulation of microflora itself provides treatment for cancer, suitable co-treatments include immunotherapy, chemotherapy, surgery (e.g., tumor removal), radiation, etc. In other embodiments, in which the modulation of microflora sensitizes a subject or the tumor microenvironment to a particular cancer therapy (e.g., an immunotherapy, a chemotherapy, etc.), the particular cancer therapy is administered (e.g., optionally in addition to one or more other cancer therapies to which the subject is not directly sensitized to by the modulation).

In some embodiments, microflora modulation is provided as a co-therapy (e.g., chemotherapy, immunotherapy, etc.) with one or more additional therapies that target and/or bind to specific cancer or tumor cell markers. Such markers may be selected from the group including but not limited to, epidermal growth factor receptor (EGFR, EGFR1, ErbB-1, HER1). ErbB-2 (HER2/neu), ErbB-3/HER3, ErbB-4/HER4, EGFR ligand family; insulin-like growth factor receptor (IGFR) family, IGF-binding proteins (IGFBPs), IGF ligand family (IGF-1R); platelet derived growth factor receptor (PDGFR) family, PDGFR ligand family; fibroblast growth factor receptor (FGFR) family, FGFR ligand family, vascular endothelial growth factor receptor (VEGFR) family, VEGF family; HGF receptor family; TRK receptor family; ephrin (EPH) receptor family; AXL receptor family; leukocyte tyrosine kinase (LTK) receptor family; TIE receptor family, angiopoietin 1, 2; receptor tyrosine kinase-like orphan receptor (ROR) receptor family; discoidin domain receptor (DDR) family; RET receptor family; KLG receptor family; RYK receptor family; MuSK receptor family; Transforming growth factor alpha (TGF- α), TGF- α receptor; Transforming growth factor-beta (TGF- β), TGF- β receptor; Interleukin β receptor alpha2 chain (IL13Ralpha2), Interleukin-6 (IL-6), IL-6 receptor, interleukin-4, IL-4 receptor, Cytokine receptors, Class I (hematopoietin family) and Class II (interferon/IL-10 family) receptors, tumor necrosis factor (TNF) family, TNF- α , tumor necrosis factor (TNF) receptor superfamily (TNRSF), death receptor family,

TRAIL-receptor; cancer-testis (CT) antigens, lineage-specific antigens, differentiation antigens, alpha-actinin-4, ARTC1, breakpoint cluster region-Abelson (Bcr-abl) fusion products, B-RAF, caspase-5 (CASP-5), caspase-8 (CASP-8), beta-catenin (CTNNB1), cell division cycle 27 (CDC127), cyclin-dependent kinase 4 (CDK4), CDKN2A, COA-1, dek-can fusion protein, EFTUD-2, Elongation factor 2 (ELF2), Ets variant gene 6/acute myeloid leukemia 1 gene ETS (ETC6-AML1) fusion protein, fibronectin (FN), GPNMB, low density lipid receptor/GDP-L fucose: beta-Dgalactose 2-alpha-L-fucosyltransferase (LDLR/FUT) fusion protein, HLA-A2, MLA-A11, heat shock protein 70-2 mutated (HSP70-2M), KIAA0205, MART2, melanoma ubiquitous mutated 1, 2, 3 (MUM-1, 2, 3), prostatic acid phosphatase (PAP), neo-PAP, Myosin class 1, NFYC, OGT, OS-9, pml-RARalpha fusion protein, PRDXS, PTPRK, K-ras (KRAS2), N-ras (NRAS), HRAS, RBAF600, SIRT12, SNRPD1, SYT-SSX1 or -SSX2 fusion protein, Triosephosphate Isomerase, BAGE, BAGE-1, BAGE-2, 3, 4, 5, GAGE-1, 2, 3, 4, 5, 6, 7, 8, GnT-V (aberrant N-acetyl glucosaminyl transferase V, MGATS), HERV-K MEL, KKL-C, LAGE, LAGE-1, CTL-recognized antigen on melanoma (CAMEL), MAGE-A1 (MAGE-1). MAGE-A2, MAGE-A3, MAGE-A4, MAGE-AS, MAGE-A6, MAGE-A8, MAGE-A9, MAGE-A10. MAGE-A11, MAGE-A12, MAGE-3, MAGE-B1, MAGE-B2, MAGE-B5. MAGE-B6, MAGE-C1, MAGE-C2, mucin 1 (MUC1), MART-1/Melan-A (MLANA), gp100, gp100/Pme117 (SILV), tyrosinase (TYR), TRP-1, HAGE, NA-88, NY-ESO-1, NY-ESO-1/LAGE-2, SAGE, Sp17. SSX-1, 2, 3, 4, TRP2-INT2, carcino-embryonic antigen (CEA), Kallikrein 4, mammaglobin-A, OA1, prostate specific antigen (PSA), prostate specific membrane antigen, TRP-1/, 75. TRP-2 adipophilin, interferon inducible protein absent in melanoma 2 (AIM-2). BING-4, CPSF, cyclin D1, epithelial cell adhesion molecule (Ep-CAM), EpbA3, fibroblast growth factor-5 (FGF-5), glycoprotein 250 (gp250intestinal carboxyl esterase (iCE), alpha-feto protein (AFP), M-CSF, mdm-2, MUC1, p53 (TP53), PBF, PRAME, PSMA, RAGE-1, RNF43, RU2AS, SOX10, STEAP1, survivin (BIRCS), human telomerase reverse transcriptase (hTERT), telomerase, Wilms' tumor gene (WT1), SYCP1, BRDT, SPANX, XAGE, ADAM2, PAGE-5, LIPI, CTAGE-1, CSAGE, MMA1, CAGE, BORIS, HOM-TES-85, AF15q14, HCA66I, LDHC, MORC, SGY-1, SPO11, TPX1, NY-SAR-35, FTHL17, NXF2 TDRD1, TEX 15, FATE, TPTE, immunoglobulin idiotypes, Bence-Jones protein, estrogen receptors (ER), androgen receptors (AR), CD40, CD30, CD20, CD19, CD33, CD4, CD25, CD3, cancer antigen 72-4 (CA 72-4), cancer antigen 15-3 (CA 15-3), cancer antigen 27-29 (CA 27-29), cancer antigen 125 (CA 125), cancer antigen 19-9 (CA 19-9), beta-human chorionic gonadotropin, 1-2 microglobulin, squamous cell carcinoma antigen, neuron-specific enolase, heat shock protein gp96. GM2, sargramostim, CTLA-4, 707 alanine proline (707-AP), adenocarcinoma antigen recognized by T cells 4 (ART-4), carcinoembryonic antigen peptide-1 (CAP-1), calcium-activated chloride channel-2 (CLCA2), cyclophilin B (Cyp-B), human signet ring tumor-2 (HST-2), etc.

Non-limiting examples of cancers that may be treated with the compositions and methods described herein include, but are not limited to: cancer cells from the bladder, blood, bone, bone marrow, brain, breast, colon, esophagus, gastrointestinal, gum, head, kidney, liver, lung, nasopharynx, neck, ovary, prostate, skin, stomach, testis, tongue, or uterus. In addition, the cancer may specifically be of the following histological type, though it is not limited to these: neoplasm,

malignant; carcinoma; carcinoma, undifferentiated; giant and spindle cell carcinoma; small cell carcinoma; papillary carcinoma; squamous cell carcinoma; lymphoepithelial carcinoma; basal cell carcinoma; pilomatrix carcinoma; transitional cell carcinoma; papillary transitional cell carcinoma; adenocarcinoma; gastrinoma, malignant; cholangiocarcinoma; hepatocellular carcinoma; combined hepatocellular carcinoma and cholangiocarcinoma; trabecular adenocarcinoma; adenoid cystic carcinoma; adenocarcinoma in adenomatous polyp; adenocarcinoma, familial polyposis coli; solid carcinoma; carcinoid tumor, malignant; branchioloalveolar adenocarcinoma; papillary adenocarcinoma; chromophobe carcinoma; acidophil carcinoma; oxyphilic adenocarcinoma; basophil carcinoma; clear cell adenocarcinoma; granular cell carcinoma; follicular adenocarcinoma; papillary and follicular adenocarcinoma; nonencapsulating sclerosing carcinoma; adrenal cortical carcinoma; endometrioid carcinoma; skin appendage carcinoma; apocrine adenocarcinoma; sebaceous adenocarcinoma; ceruminous adenocarcinoma; mucoepidermoid carcinoma; cystadenocarcinoma; papillary cystadenocarcinoma; papillary serous cystadenocarcinoma; mucinous cystadenocarcinoma; mucinous adenocarcinoma; signet ring cell carcinoma; infiltrating duct carcinoma; medullary carcinoma; lobular carcinoma; inflammatory carcinoma; paget's disease, mammary; acinar cell carcinoma; adenosquamous carcinoma; adenocarcinoma w/squamous metaplasia; thymoma, malignant; ovarian stromal tumor, malignant; thecoma, malignant; granulosa cell tumor, malignant; and roblastoma, malignant; sertoli cell carcinoma; leydig cell tumor, malignant; lipid cell tumor, malignant; paraganglioma, malignant; extramammary paraganglioma, malignant; pheochromocytoma; glomangiosarcoma; malignant melanoma; amelanotic melanoma; superficial spreading melanoma; malig melanoma in giant pigmented nevus; epithelioid cell melanoma; blue nevus, malignant; sarcoma; fibrosarcoma; fibrous histiocytoma, malignant; myxosarcoma; liposarcoma; leiomyosarcoma; rhabdomyosarcoma; embryonal rhabdomyosarcoma; alveolar rhabdomyosarcoma; stromal sarcoma; mixed tumor, malignant; mullerian mixed tumor; nephroblastoma; hepatoblastoma; carcinosarcoma; mesenchymoma, malignant; brenner tumor, malignant; phyllodes tumor, malignant; synovial sarcoma; mesothelioma, malignant; dysgerminoma; embryonal carcinoma; teratoma, malignant; struma ovarii, malignant; choriocarcinoma; mesonephroma, malignant; hemangiosarcoma; hemangioendothelioma, malignant; kaposi's sarcoma; hemangiopericytoma, malignant; lymphangiosarcoma; osteosarcoma; juxtacortical osteosarcoma; chondrosarcoma; chondroblastoma, malignant; mesenchymal chondrosarcoma; giant cell tumor of bone; ewing's sarcoma; odontogenic tumor, malignant; ameloblastic odontosarcoma; ameloblastoma, malignant; ameloblastic fibrosarcoma; pinealoma, malignant; chordoma; glioma, malignant; ependymoma; astrocytoma; protoplasmic astrocytoma; fibrillary astrocytoma; astroblastoma; glioblastoma; oligodendroglioma; oligodendroblastoma; primitive neuroectodermal; cerebellar sarcoma; ganglioneuroblastoma; neuroblastoma; retinoblastoma; olfactory neurogenic tumor; meningioma, malignant; neurofibrosarcoma; neurilemmoma, malignant; granular cell tumor, malignant; malignant lymphoma; Hodgkin's disease; Hodgkin's lymphoma; paragranuloma; malignant lymphoma, small lymphocytic; malignant lymphoma, large cell, diffuse; malignant lymphoma, follicular; mycosis fungoides; other specified non-Hodgkin's lymphomas; malignant histiocytosis; multiple myeloma; mast cell sarcoma; immunoproliferative small intestinal disease; leukemia; lymphoid leukemia; plasma

cell leukemia; erythroleukemia; lymphosarcoma cell leukemia; myeloid leukemia; basophilic leukemia; eosinophilic leukemia; monocytic leukemia; mast cell leukemia; megakaryoblastic leukemia; myeloid sarcoma; and hairy cell leukemia. In some embodiments, the cancer is a melanoma (e.g., metastatic malignant melanoma), renal cancer (e.g. clear cell carcinoma), prostate cancer (e.g. hormone refractory prostate adenocarcinoma), pancreatic cancer (e.g., adenocarcinoma), breast cancer, colon cancer, gallbladder cancer, lung cancer (e.g. non-small cell lung cancer), esophageal cancer, squamous cell carcinoma of the head and neck, liver cancer, ovarian cancer, cervical cancer, thyroid cancer, glioblastoma, glioma, leukemia, lymphoma, and other neoplastic malignancies. In some embodiments, the cancer is a solid tumor cancer.

In some embodiments, the methods provided herein relate to the treatment and/or prevention of a leukemia. The term "leukemia" is meant broadly progressive, malignant diseases of the hematopoietic organs/systems and is generally characterized by a distorted proliferation and development of leukocytes and their precursors in the blood and bone marrow. Non-limiting examples of leukemia diseases include, acute nonlymphocytic leukemia, chronic lymphocytic leukemia, acute granulocytic leukemia, chronic granulocytic leukemia, acute promyelocytic leukemia, adult T-cell leukemia, aleukemic leukemia, a leukocythemic leukemia, basophilic leukemia, blast cell leukemia, bovine leukemia, chronic myelocytic leukemia, leukemia cutis, embryonal leukemia, eosinophilic leukemia, Gross' leukemia, Rieder cell leukemia, Schilling's leukemia, stem cell leukemia, subleukemic leukemia, undifferentiated cell leukemia, hairy-cell leukemia, hemoblastic leukemia, hemocytoblastic leukemia, histiocytic leukemia, stem cell leukemia, acute monocytic leukemia, leukopenic leukemia, lymphatic leukemia, lymphoblastic leukemia, lymphocytic leukemia, lymphogenous leukemia, lymphoid leukemia, lymphosarcoma cell leukemia, mast cell leukemia, megakaryocytic leukemia, micromyeloblastic leukemia, monocytic leukemia, myeloblastic leukemia, myelocytic leukemia, myeloid granulocytic leukemia, myelomonocytic leukemia, Naegeli leukemia, plasma cell leukemia, plasmacytic leukemia, and promyelocytic leukemia.

In some embodiments, the methods provided herein relate to the treatment and/or prevention of a carcinoma. The term "carcinoma" refers to a malignant growth made up of epithelial cells tending to infiltrate the surrounding tissues, and/or resist physiological and non-physiological cell death signals and gives rise to metastases. Non-limiting exemplary types of carcinomas include, acinar carcinoma, acinous carcinoma, adenocystic carcinoma, adenoid cystic carcinoma, carcinoma adenomatousum, carcinoma of adrenal cortex, alveolar carcinoma, alveolar cell carcinoma, basal cell carcinoma, carcinoma basocellulare, basaloid carcinoma, basosquamous cell carcinoma, bronchioalveolar carcinoma, bronchiolar carcinoma, bronchogenic carcinoma, cerebri-
form carcinoma, cholangiocellular carcinoma, chorionic carcinoma, colloid carcinoma, comedo carcinoma, corpus carcinoma, cribriform carcinoma, carcinoma en cuirasse, carcinoma cutaneum, cylindrical carcinoma, cylindrical cell carcinoma, duct carcinoma, carcinoma durum, embryonal carcinoma, encephaloid carcinoma, epienoid carcinoma, carcinoma epitheliale adenoides, exophytic carcinoma, carcinoma ex ulcere, carcinoma fibrosum, gelatiniform carcinoma, gelatinous carcinoma, giant cell carcinoma, signet-ring cell carcinoma, carcinoma simplex, small-cell carcinoma, solanoid carcinoma, spheroidal cell carcinoma, spindle cell carcinoma, carcinoma spongiosum, squamous

carcinoma, squamous cell carcinoma, string carcinoma, carcinoma telangiectaticum, carcinoma telangiectodes, transitional cell carcinoma, carcinoma tuberosum, tuberous carcinoma, verrucous carcinoma, carcinoma villosum, carcinoma gigantocellulare, glandular carcinoma, granulosa cell carcinoma, hair-matrix carcinoma, hematoid carcinoma, hepatocellular carcinoma, Hurthle cell carcinoma, hyaline carcinoma, hypernephroid carcinoma, infantile embryonal carcinoma, carcinoma in situ, intraepidermal carcinoma, intraepithelial carcinoma, Krompecher's carcinoma, Kulchitzky-cell carcinoma, large-cell carcinoma, lenticular carcinoma, carcinoma lenticulare, lipomatous carcinoma, lymphoepithelial carcinoma, carcinoma medullare, medullary carcinoma, melanotic carcinoma, carcinoma molle, mucinous carcinoma, carcinoma muciparum, carcinoma mucocellulare, mucocoepermoid carcinoma, carcinoma mucosum, mucous carcinoma, carcinoma myxomatodes, nasopharyngeal carcinoma, oat cell carcinoma, carcinoma ossificans, osteoid carcinoma, papillary carcinoma, periportal carcinoma, preinvasive carcinoma, prickle cell carcinoma, pultaceous carcinoma, renal cell carcinoma of kidney, reserve cell carcinoma, carcinoma sarcomatodes, schneiderian carcinoma, scirrhus carcinoma, and carcinoma scroti.

In some embodiments, the methods provided herein relate to the treatment and/or prevention of a sarcoma. The term "sarcoma" generally refers to a tumor which is made up of a substance like the embryonic connective tissue and is generally composed of closely packed cells embedded in a fibrillar, heterogeneous, or homogeneous substance. Sarcomas include, but are not limited to, chondrosarcoma, fibrosarcoma, lymphosarcoma, melanosarcoma, myxosarcoma, osteosarcoma, endometrial sarcoma, stromal sarcoma, Ewing's sarcoma, fascial sarcoma, fibroblastic sarcoma, giant cell sarcoma, Abemethy's sarcoma, adipose sarcoma, liposarcoma, alveolar soft part sarcoma, ameloblastic sarcoma, botryoid sarcoma, chloroma sarcoma, chorio carcinoma, embryonal sarcoma, Wilms' tumor sarcoma, granulocytic sarcoma, Hodgkin's sarcoma, idiopathic multiple pigmented hemorrhagic sarcoma, immunoblastic sarcoma of B cells, lymphoma, immunoblastic sarcoma of T-cells, Jensen's sarcoma, Kaposi's sarcoma, Kupffer cell sarcoma, angiosarcoma, leukosarcoma, malignant mesenchymoma sarcoma, parosteal sarcoma, reticulocytic sarcoma, Rous sarcoma, serocystic sarcoma, synovial sarcoma, and telangiectaltic sarcoma.

Additional exemplary neoplasias that can be treated and/or prevented using the methods described herein include Hodgkin's Disease, Non-Hodgkin's Lymphoma, multiple myeloma, neuroblastoma, breast cancer, ovarian cancer, lung cancer, rhabdomyosarcoma, primary thrombocytosis, primary macroglobulinemia, small-cell lung tumors, primary brain tumors, stomach cancer, colon cancer, malignant pancreatic insulanoma, malignant carcinoid, premalignant skin lesions, testicular cancer, lymphomas, thyroid cancer, neuroblastoma, esophageal cancer, genitourinary tract cancer, malignant hypercalcemia, cervical cancer, endometrial cancer, and adrenal cortical cancer.

In some embodiments, the cancer treated and/or prevented is a melanoma. The term "melanoma" is taken to mean a tumor arising from the melanocytic system of the skin and other organs. Non-limiting examples of melanomas are Harding-Passey melanoma, juvenile melanoma, lentigo maligna melanoma, malignant melanoma, acral-lentiginous melanoma, amelanotic melanoma, benign juvenile melanoma, Cloudman's melanoma, S91 melanoma, nodular melanoma subungal melanoma, and superficial spreading melanoma.

Particular categories of tumors that can be treated and/or prevented using methods described herein include lymphoproliferative disorders, breast cancer, ovarian cancer, prostate cancer, cervical cancer, endometrial cancer, bone cancer, liver cancer, stomach cancer, colon cancer, pancreatic cancer, cancer of the thyroid, head and neck cancer, cancer of the central nervous system, cancer of the peripheral nervous system, skin cancer, kidney cancer, as well as metastases of all the above. Particular types of tumors include hepatocellular carcinoma, hepatoma, hepatoblastoma, rhabdomyosarcoma, esophageal carcinoma, thyroid carcinoma, ganglioblastoma, fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, Ewing's tumor, leiomyosarcoma, rhabdotheriosarcoma, invasive ductal carcinoma, papillary adenocarcinoma, melanoma, pulmonary squamous cell carcinoma, basal cell carcinoma, adenocarcinoma (well differentiated, moderately differentiated, poorly differentiated or undifferentiated), bronchioloalveolar carcinoma, renal cell carcinoma, hypernephroma, hypernephroid adenocarcinoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, testicular tumor, lung carcinoma including small cell, non-small and large cell lung carcinoma, bladder carcinoma, glioma, astrocyoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, retinoblastoma, neuroblastoma, colon carcinoma, rectal carcinoma, hematopoietic malignancies including all types of leukemia and lymphoma including: acute myelogenous leukemia, acute myelocytic leukemia, acute lymphocytic leukemia, chronic myelogenous leukemia, chronic lymphocytic leukemia, mast cell leukemia, multiple myeloma, myeloid lymphoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma.

Cancers prevented and/or treated in certain embodiments also include precancerous lesions, e.g. actinic keratosis (solar keratosis), moles (dysplastic nevi), actinic cheilitis (farmer's lip), cutaneous horns, Barrett's esophagus, atrophic gastritis, dyskeratosis congenita, sideropenic dysphagia, lichen planus, oral submucous fibrosis, actinic (solar) elastosis and cervical dysplasia.

Cancers prevented and/or treated in some embodiments include non-cancerous or benign tumors, e.g. of endodermal, ectodermal or mesenchymal origin, including, but not limited to cholangioma, colonic polyp, adenoma, papilloma, cystadenoma, liver cell adenoma, hydatidiform mole, renal tubular adenoma, squamous cell papilloma, gastric polyp, hemangioma, osteoma, chondroma, lipoma, fibroma, lymphangioma, leiomyoma, rhabdomyoma, astrocytoma, nevus, meningioma, and ganglioneuroma.

Some embodiments described herein are particularly useful for the treatment of tumors that do not otherwise respond to immunotherapeutic approaches. In some embodiments, such tumors are non-responsive (or have a reduced response) to T cells (e.g., prevent infiltration of one or more T cell types (e.g., CD8⁺ T cells) or antigen presenting cells (e.g., dendritic cells (e.g., CD103⁺DCs, etc.), etc.). In some embodiments, compositions and methods described herein find use in the treatment of cancers in which T cells are not appropriately primed against tumor-associated antigens.

In some embodiments, methods are provided for testing sample (e.g., cell, tissue, population of cells, tumor, blood, urine, saliva, etc.) from a subject for one or more biomarkers of cancer, immune evasion, cancer promoting microenvironment, malignancy-promoting microenvironment, etc. Such biomarkers may comprise nucleic acids, small molecules, proteins, peptides, etc., and may be detected using any suitable assay of technique. In some embodiments,

provided herein are DNA-, RNA-, small molecule, and/or protein-based diagnostic methods that either directly or indirectly detect the biomarkers of the evasion of immune response or immunotherapy by cancer cells or tumors. The present invention also provides compositions, reagents, and kits for such diagnostic purposes.

In some embodiments, biomarkers are detected at the nucleic acid (e.g., RNA, 16s rRNA) level. For example, the presence or amount of biomarker nucleic acid (e.g., mRNA) in a sample is determined (e.g., to determine the presence or level of biomarker expression). Biomarker nucleic acid (e.g., RNA, amplified cDNA, etc.) may be detected/quantified using a variety of nucleic acid techniques known to those of ordinary skill in the art, including but not limited to nucleic acid sequencing, nucleic acid hybridization, nucleic acid amplification (e.g., by PCR, RT-PCR, qPCR, etc.), microarray, Southern and Northern blotting, sequencing, etc. Non-amplified or amplified nucleic acids can be detected by any conventional means. For example, in some embodiments, nucleic acids are detected by hybridization with a detectably labeled probe and measurement of the resulting hybrids. Nucleic acid detection reagents may be labeled (e.g., fluorescently) or unlabeled, and may be free in solution or immobilized (e.g., on a bead, well, surface, chip, etc.).

In some embodiments, biomarkers are detected at the protein level. For example, the presence or amount of biomarker protein in a sample is determined (e.g., to determine the presence or level of biomarker expression or localization). In some embodiments, reagents are provided for the detection and/or quantification of biomarker proteins. Suitable reagents include primary antibodies (e.g., that bind to the biomarkers), secondary antibodies (e.g., that bind primary antibodies), antibody fragments, aptamers, etc. Protein detection reagents may be labeled (e.g., fluorescently) or unlabeled, and may be free in solution or immobilized (e.g., on a bead, well, surface, chip, etc.).

In particular embodiments, biomarkers are microbiome biomarkers. In some embodiments, the microbiome of a subject is assayed and depending on the particular microbial population, the responsiveness/resistance of the subject to immunotherapy is characterized. In some embodiments, the presence, absence, or level of one or more bacteria determined during experiments conducted during development of embodiments herein to be indicative and/or causative of immunotherapy responsiveness/resistance (e.g., indicative and/or causative of T cell inflammation) is determined. In some embodiments, bacteria of the species *Enterococcus faecium*, *Collinsella aerofaciens*, *Bifidobacterium adolescentis*, *Klebsiella pneumoniae*, *Veillonella parvula*, *Parabacteroides merdae*, *Lactobacillus* sp. and *Bifidobacterium longum* indicate responsiveness (e.g., levels above a threshold). In some embodiments, bacteria of the species *Ruminococcus obeum* and *Roseburia intestinalis* indicate non-responsiveness (e.g., levels above a threshold).

In some embodiments, samples are obtained from a subject (e.g., a patient or a subject in need of treatment according to the technology provided herein) at any suitable interval of time, varying from minutes to hours apart, days to weeks apart, or even weeks to months apart. Biomarker samples may be obtained multiple times a day, week, month or year. The duration of sampling can also vary. For example, the duration of sampling may be for about a month, about 6 months, about 1 year, about 2 years, about 3 years, about 4 years, about 5 years, about 6 years, about 7 years, about 8 years, about 9 years, about 10 years, about 11 years, about 12 years, about 13 years, about 14 years, about 15 years, about 16 years, about 17 years, about 18 years, about

19 years, about 20 years, about 30 years, or more. In some embodiments, the sampling is taken prior to or after administration of treatment according to the technology provided herein.

In one embodiment, the sample is taken from a subject being treated for cancer with an immunotherapy. Based on the level of bacteria in the sample, the subject may receive a second immunotherapy that is different from the first immunotherapy. Additionally, the subject may be treated with a mixture of bacteria including but not limited to *Enterococcus faecium*, *Collinsella aerofaciens*, *Bifidobacterium adolescentis*, *Klebsiella pneumoniae*, *Veillonella parvula*, *Parabacteroides merdae*, *Lactobacillus* sp. and *Bifidobacterium longum* prior to administration of the first or second immunotherapy.

In some embodiments, the subject's biomarker (e.g., bacteria) level is compared to a threshold wherein the threshold is determined by the average level of such biomarkers within a population of patients. In particular, the patient population size may include 50, 100, 200, 300, 400, 500, 1000, 2000, 3000, 4000, 5000 and ranges in between of patients who have responded or have not responded to an immunotherapy treatment.

In some embodiments, kits are provided comprising, for example, the probiotic or microflora transplant compositions described herein. Kits may further comprise instructions, cancer treatments, other probiotics, agents to enhance integration of microbes into the subject's microflora, etc.

EXPERIMENTAL

Example 1

Materials and Methods

Patient Fecal Sample Collection

Stool samples were collected from 42 metastatic melanoma patients prior to immunotherapy initiation. Eligible patients were provided an EasySampler Collection Kit (ALPCO) to collect stool sample at home. The stool samples were brought to the lab within 24 hours after collection. DNA was immediately isolated from 250 mg of stool and the rest of the sample was aliquoted and stored at -80°C . Of the 42 patients 38 were thereafter subjected to anti-PD-1 therapy and 4 were given anti-CTLA-4 therapy. Exclusion of the latter 4 patients did not change the downstream conclusions, so they were retained in the analysis.

Microbial DNA Isolation

Patient stool samples were handled under BSL2 laminar flow hood using sterile technique. The technician wore gloves, gown, face mask and hair net to prevent contamination of the samples. Isolation of microbial DNA from patient and mouse fecal samples was performed using QIAamp PowerFecal DNA Kit and QIAamp DNA Stool Mini Kit, respectively (Qiagen, Germantown, Md.). DNA concentration was measured using a Nanodrop-nd1000 and the DNA was stored at -80°C .

16S rRNA Gene Amplicon Library Preparation and Sequencing

16S rRNA gene amplicon library preparation and sequencing was performed at the Argonne National Laboratory. PCR amplicon libraries targeting the 16S rRNA encoding gene were produced using a barcoded primer set adapted for the Illumina HiSeq2000 and MiSeq (Ref 9A; herein incorporated by reference in its entirety). DNA sequence data were then generated using Illumina paired-end sequencing at the Environmental Sample Preparation and Sequencing Facility (ESPSF) at Argonne National

Laboratory. Specifically, the V4 region of the 16S rRNA gene (515F-806R) was PCR-amplified with region-specific primers that include sequencer adapter sequences used in the Illumina flowcell (refs. 9A, 10A; herein incorporated by reference in their entireties). The forward amplification primer also contains a twelve-base barcode sequence that supports pooling of up to 2,167 different samples in each lane. Each 25 μL PCR reaction contained 9.5 μL of MO BIO PCR Water (Certified DNA-Free), 12.5 μL of QuantaBio's AccuStart II PCR ToughMix (2 \times concentration, 1 \times final), 1 μL Golay barcode tagged Forward Primer (5 μM concentration, 200 pM final), 1 μL Reverse Primer (5 μM concentration, 200 pM final), and 1 μL of template DNA. The conditions for PCR were as follows: 94°C . for 3 minutes to denature the DNA, with 35 cycles at 94°C . for 45 s, 50°C . for 60 s, and 72°C . for 90 s; with a final extension of 10 min at 72°C . to ensure complete amplification. Amplicons were then quantified using PicoGreen (Invitrogen) and a plate reader (Infinite[®] 200 PRO, Tecan). Once quantified, volumes of each of the products were pooled into a single tube so that each amplicon was represented in equimolar amounts. This pool was then cleaned up using AMPure XP Beads (Beckman Coulter) and quantified using a fluorometer (Qubit, Invitrogen). The pool was diluted down to 2 nM, denatured, and further diluted to a final concentration of 6.75 pM with a 10% PhiX spike for sequencing on the Illumina MiSeq. Amplicons were sequenced on a 151 bp \times 12 bp \times 151 bp MiSeq run using previously described sequencing primers and procedures. The average sequencing depth for the patient samples was 51,029, ranging from 28,040 to 68,928 reads; the average sequencing depth for mouse samples was 158,728, ranging from 54,632 to 327,216 reads per sample. Microbial 16S rRNA Gene Amplicon Analysis

The microbial 16S rRNA gene amplicon sequencing data from human and mouse fecal collections were processed separately using Quantitative Insights Into Microbial Ecology (QIIME) (version 1.91) (Refs. 3A, 11A; herein incorporated by reference in their entireties). Raw reads were trimmed to remove low quality bases and paired-end 3' overlapping mates were merged using SeqPrep (github.com/jstjohn/SeqPrep). The open reference OTU picking protocol was used at 97% sequence identity against the Greengenes database (08/2013 release) (Ref 12A; herein incorporated by reference in its entirety). PyNAST was used to align sequences (Ref 13A; herein incorporated by reference in its entirety) and RDP Classifier was used for taxonomic assignment (Ref 14A; herein incorporated by reference in its entirety).

Analysis of the mouse 16S dataset revealed 519 OTUs differentially abundant between the fast and slow tumor growth groups at FDR-adjusted $p < 0.05$. Among these, 298 OTUs were assigned with known reference IDs and 221 with new reference ID. The new reference OTU IDs are not comparable between different cohorts, hence we focused on the OTUs with known reference IDs. Out of 298 OTUs, 207 OTUs were matched with human donors and used for generation of the heatmap depicted in FIG. 3B. In addition, binary Bray-Curtis dissimilarity index was computed for each donor-mouse sample pair based on presence/absence of matched OTUs. For each pair, OTUs of relative abundance > 0.0001 in the donor or the mouse sample was included for the calculation.

BLASTN Methodology

To investigate the identity of the OTUs differentially abundant between responders and non-responder patients, the assembled 16S rRNA gene amplicon sequences were characterized by a BLAST search against NCBI bacterial

nucleotide sequence database. Using the blastn command line tool and the “megablast” program selection method, the top hits with $\geq 98\%$ identity to the query sequence were returned from the nucleotide collection database restricted to bacteria, and excluding environmental or uncultured sample sequences. Results are shown in Table 2. For some OTUs there were no hits with $\geq 98\%$ identity and the top 10 hits are listed with regardless of the % identity value.

Metagenomic Shotgun Sequencing

Metagenomic shotgun sequencing was performed at the Marine Biological Laboratory affiliated with the University of Chicago. The quantity of the DNA sample was measured using Picogreen (Invitrogen). DNA was then sheared using a Covaris and the libraries were constructed with the Nugen Ovation Rapid DR Multiplex System (PCR-free). The aimed insert size is between 400-600 bp. Amplified libraries were visualized on an Agilent DNA1000 chip or Caliper HiSens Bioanalyzer assay, pooled at equimolar concentrations and size selected using a Sage PippinPrep 2% cassette. The library pool was quantified using a Kapa Biosystems qPCR library quantification protocol, then sequenced on the Illumina NextSeq in a 2x150 paired-end sequencing run using dedicated read indexing. The samples were demultiplexed with bcl2fastq. An average of 80.4 million reads were generated per sample, ranging from 38.9 to 156.7 million reads.

Microbial Shotgun Metagenomics Analysis

The microbial shotgun metagenome sequencing data from human facet collections were taxonomically profiled using Metagenomic Phylogenetic Analysis (MetaPhlan 2) (Ref 15A; herein incorporated by reference in its entirety). The average sequencing depth was 80369403 (± 33712841), ranging from 38841706 to 156677784 reads per sample. Species-level taxonomic relative abundances were inferred for all samples (Ref 16A; herein incorporated by reference in its entirety). Metagenomic reads were mapped against a catalog of ~ 1 million clade-specific marker sequences identified from 17000 reference genomes currently spanning bacteria, archaea, eukaryotes and virus phylogenies to assign reads to microbial clades. The relative abundance of each taxonomic unit in each sample was estimated by normalizing read counts assigned to each clade by the nucleotide length of its markers and by the sum of all weighted read counts in this clade including all subclades. To compare species identified from 16S and shotgun sequencing, the profiled bacterial species were then compared to the taxonomy of OTUs generated from 16S sequencing at family level, and the statistical dependence between the relative abundance of 16S OTUs and each matched shotgun species was determined using Spearman’s rank correlation tests, followed by filtering for those with positive correlation and at $P < 0.05$.

Model Training and Validation

To evaluate the predictive power of 63 OTUs differentially present between NR ($n=26$) and R ($n=16$) groups, a support vector machine (SVM) model with radial basis function (RBF) kernel and estimated the variable importance of each predictor using Caret (version 6.0-76) (CRAN.R-project.org/package=caret) was built. 21 OTUs with near zero-variance, highly correlated (Spearman’s $\rho > 0.75$), and/or of potential linear dependencies were identified and removed using functions nearZeroVar, findCorrelation, and findLinearCombs, respectively. 42 OTUs were carried on for further analysis. The 42 patient samples were randomly split into training and test sets by 60% ($n=25$) to 40% ratio ($n=17$). The mean and standard deviation of the training set was computed and used to center and scale the training and

test data separately by the preProcess function. The training set was then used to tune the parameters and select the best model using 5-fold cross validation with ROC metrics to evaluate model performance. Relative importance of each predictor was estimated by ROC curve analysis using function filterVarImp. After training, the test set was used to independently assess the performance of the final model, and metrics such as balanced accuracy, sensitivity, specificity, positive prediction value (PPV), negative prediction value (NPV), and area under the curve (AUC) were computed using the confusionMatrix function from caret package. The above model training and testing procedure was performed iteratively 100 times with 100 different random seeds to evaluate the robustness and stability of the OTU predictors in the classification of NR and R groups from the study cohort.

qPCR Validation of Metagenomic and 16S rRNA Gene Sequencing of Fecal Samples

The abundance of some of the bacterial species identified with the metagenomic and 16S rRNA gene amplicon sequencing approaches were further measured by qPCR using previously validated subgroup- or species-specific primers and probes (Refs. 17A-29A; herein incorporated by reference in their entirety) and SYBR Green or TaqMan PCR master mix (Applied Biosystems). The primers and probes were synthesized by Integrated DNA Technologies (Coralville, Iowa) and Life Technologies, respectively. qPCR was performed on StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, Calif.) and analyzed with StepOnePlus Software. The primer concentrations were as previously described (Table 5). The cycling conditions for the TaqMan-based reactions were 50° C. for 2 min, 95° C. for 10 min, 40 cycles of 95° for 15 secs, 60-65° C. for 1 min, with varying annealing temperatures depending on the primer pair. The cycling conditions for the SYBR Green-based reactions were 95° C. for 10 min, 40 cycles of 95° for 15 sec, 60-75° C. for 10-40 sec, 72° C. for 20-50 sec, with varying annealing temperatures and times depending on the primer pair. Fluorescence signal was detected at the end of each cycling stage. For some reactions, fluorescence detection was done during an additional 15 sec step at a higher temperature to minimize signal from primer dimers and minor non-target products (Ref 18A; herein incorporated by reference in its entirety). Melt curve analysis was performed to confirm amplification specificity. The results were expressed as relative abundance normalized to the total bacterial load. Specifically, to calculate the total bacterial load, qPCR was performed using previously described universal bacterial primers (Ref 30A; herein incorporated by reference in its entirety). A standard curve was generated using the PCR blunt vector (Invitrogen) containing a single copy of the 16S rRNA gene derived from a member of the Porphyromonadaceae family (Ref 31A; herein incorporated by reference in its entirety) and the total 16S rRNA gene copies per ng DNA was calculated for each sample. Relative abundance for each species was expressed as 2^{-Ct} normalized to the number of total 16S rRNA gene copies per ng DNA in each sample. A summation qPCR score was computed per individual sample taking into consideration the abundance of 10 validated qPCR targets (*Enterococcus faecium*, *Collinsella aerofaciens*, *Bifidobacterium adolescentis*, *Klebsiella pneumoniae*, *Veillonella* sp., *Parabacteroides merdae*, *Lactobacillus* sp., *Bifidobacterium longum*, *Ruminococcus obeum* and *Roseburia intestinalis*). First, data transformation was applied on the relative abundance to bring signal close to Gaussian distribution. The relative abundance of each species was multiplied by a constant

(7.3×10^{-19}) to bring all values larger than 1, log₁₀ transformed, and scaled by dividing the value by their root mean square across samples. The abundance of *Ruminococcus obeum* and *Roseburia intestinalis* (more abundant in non-responders) were multiplied by (-1). The sum of the transformed abundance of the 10 qPCR results was calculated to generate the score, and compared between groups of interest using two-sided Student's t-test.

RNAseq of Tumor Samples and Data Analysis

RNA was isolated from tumor samples using the QIAGEN AllPrep DNA/RNA FFPE kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The quality of RNA was measured on Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, USA). cDNA was reverse transcribed from RNA and used for library preparation following dUTP strand-specific protocol by the University of Chicago Genomics Core Facility. Ribosome RNA was removed using the Ribo-Zero rRNA Removal Kit (Human) (Illumina, San Diego, USA). Sequence reads were generated on an Illumina HiSeq 4000 instrument at the Functional Genomics Facility. An average of 133.3 million 2x100 bp paired-end (PE) reads were generated for each sample, ranging from 93.2 to 208.0 million reads. The quality of raw reads was assessed by FastQC (Ref 32A; herein incorporated by reference in its entirety) (v0.11.5). Reads were aligned to human reference transcriptome with Gencode gene annotation (v26, GRCh38) by Kallisto (Ref 33A; herein incorporated by reference in its entirety) (v0.43.1) with the strand-specific mode, which implements kmer-based pseudoalignment algorithm for accurate quantification of transcripts from RNAseq data and is robust to errors in the reads. Transcript abundance was quantified at transcript level specifying strand-specific protocol, summarized into gene level using tximport (Ref 34A; herein incorporated by reference in its entirety) (v1.4.0), normalized by trimmed mean of M values (TMM) method, and log₂-transformed for further analysis. Selected transcripts (PD-L1 and PD-1) were compared between responders and non-responders.

Whole Exome+UTR Sequencing of Tumor Samples and Data Analysis

Tumor DNA were isolated from tumor samples using the QIAGEN AllPrep DNA/RNA FFPE kit (Qiagen, Hilden, Germany), and the integrity and quantification were evaluated on an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, USA) and qubit (Thermo Fisher, Waltham, USA), respectively. 200 ng of DNA was used for whole exome+UTR capture using the Agilent SureSelect Human All Exon V6 plus UTR kit (Agilent Technologies, Santa Clara, USA). Sequence reads were generated on an Illumina NextSeq 500 instrument (Illumina, San Diego, USA) at the University of Chicago Functional Genomics Facility. An average of 62.2 million 2x100 bp paired-end (PE) reads were generated for each sample, ranging from 51.9 to 70.9 million reads.

The raw sequencing data were analyzed by an in-house pipeline constructed for WES analyses of paired or unpaired cancer genomes. The quality of raw reads is assessed by FastQC (ref 32A; herein incorporated by reference in its entirety) (v0.11.5), and preprocessed to trim adaptors and merge 3' overlapping mates using SeqPrep (v1.2). Reads were aligned to human reference genome (GRCh37) using BWA-MEM (Ref 35A; herein incorporated by reference in its entirety) (v0.7.15) with soft-clipping option activated by default. Read duplicates were marked using Sambamba (Ref 36A; herein incorporated by reference in its entirety) (v0.6.3) and alignments of mapping quality <30 were

removed. Reads alignment was further refined using insertions/deletions realignment and base quality score recalibration (BQSR) using GATK (Ref 37A; herein incorporated by reference in its entirety) (v3.8.0). Callable loci were collected from the alignment using GATK CallableLoci program, and merged with Agilent V6+UTR exome capture target regions provided by the vendor. Putative somatic mutations were detected by MuTect2 (Ref 38A; herein incorporated by reference in its entirety) (v3.8.0), which identifies somatic single nucleotide variants (SNVs) and indels from high-quality bases using the tumor-only mode. Stringent filters were applied on variants that passed the default setting of the caller to further remove potential germline variants identified as those present in dbSNP database, or at allele frequency (AF) ≥ 0.0001 in 1000 Genomes Project (G1000) (Ref 39A; herein incorporated by reference in its entirety), the NHLBI Grand Opportunity Exome Sequencing Project (ESP) (Ref 40A; herein incorporated by reference in its entirety), or the Exome Aggregation Consortium (ExAC) (Ref 42A; herein incorporated by reference in its entirety) on non-TCGA samples. Variants that passed all filters were carried on for annotation using ANNOVAR. The somatic mutation burden was calculated by the total number of mutations that were predict to cause protein sequencing change, including non-synonymous, stopgain, and stoploss SNVs, frameshift and non-frameshift indels, and variants that modify splicing sites.

Immunohistochemistry of Tumor Samples

Tissue sections were prepared from paraffin-embedded tumor samples from 5 responders and 10 non-responder patients. The slides were stained using Leica Bond RX automatic stainer. Bond™ Epitope Retrieval Solution I (Leica Biosystems, CatNo: AR9961) was applied for 20 minutes. A primary anti-CD8 antibody (clone C8/144B from Dako; 1:400 dilution) was applied for 25 minutes. The primary antibody was then detected with Bond™ Polymer Refine Detection kit (Leica Biosystems, CatNo: DS9800). The CD8⁺ cell density was expressed as a ratio of CD8⁺ cells/pixel to total cells/pixel using inForm Cell Analysis software (PerkinElmer).

Animals, Fecal Transfer, and Tumor Model

Specific pathogen-free (SPF) C57BL/6 mice were obtained from Taconic Biosciences (Hudson, N.Y.). SPF mice were fed Teklad irradiated 2918 diet (Envigo), or in some cases autoclaved 5K67 diet (Lab Diet, St. Louis, Mo.), and housed in the University of Chicago SPF animal facility. Germ-free (GF) C57BL/6 mice were initially purchased from Taconic biosciences, then bred and housed in flexible-film isolators in the University of Chicago Gnotobiotic Research Animal Facility and fed autoclaved 5K67 diet. Some GF mice were gifted by Dr. Eugene Chang at the University of Chicago. For all experiments, 6-8-week-old mice were used. The C57BL/6-derived melanoma cell line B16.F10.SIY (henceforth referred to as B16.SIY) was generated (Ref 43A; herein incorporated by reference in its entirety). For tumor growth experiments, some GF mice were colonized with fecal microbiota from 3 responders and 3 non-responder patients, or microbiota from SPF mice by oral gavage. 200 mg of human stool was thawed and suspended in 3 ml of PBS or mouse fecal pellets were collected fresh and suspended in 1 ml of PBS per pellet. After settling of the particulate material, each mouse was gavaged with 10 ml/kg body weight (approximately 200 μ l per mouse) of the fecal supernatant. Two weeks after gavage, the colonized mice were injected subcutaneously with 1×10^6 B16.SIY tumor cells. Some mice were injected i.p. 7, 10, 13, and 16 days after tumor inoculation with 100 μ g of

anti-PD-L1 monoclonal antibody (BioXCell, 10F.9G2). Tumor size was measured three times per week until the endpoint and tumor volume was determined as length \times width \times 0.5. Microbiota composition of the colonized mice was assessed with 16S rRNA gene amplicon sequencing of DNA extracted from fecal samples collected 4 weeks after colonization. Taconic SPF mice were used as a reference control. The experimental animal procedures were approved by the University of Chicago Animal Care and Use Committee.

SIY Pentamer Analyses

For immune profiling, cells were labeled with a PE-MHC class I pentamer (Proimmune) consisting of murine H-2K^b complexed to SIYRYYYGL (SIY) peptide or to an irrelevant SIINFEKL peptide. Tumor cell suspensions were subsequently stained with CD3-AX700 (Ebioscience, 17A2), CD8 α -Pacific Blue (Biolegend, 53-6.7), CD4-BV711 (Biolegend, RM4-5), CD44-FITC (BD, IM7), and Fixable Viability Dye-eFluor780 (Ebioscience). Once stained, cells were fixed with 1% paraformaldehyde and analyzed on LSRFortessa flow cytometer with FACSDiva software (BD). Data analysis was performed using FlowJo software (Tree Star).

IFN- γ ELISPOT

ELISPOT was carried out using anti-IFN- γ capture/detection antibody pair from BD Biosciences. ELISPOT plates (Millipore, MAIP 54510) were coated with capture antibody (CatNo: 51-2525KD) overnight at 4 $^{\circ}$ C. and then blocked with DMEM+10% FBS for 2 hours at room temperature. Splenocytes were enumerated using flow cytometry, plated at 10⁵ cells per well and stimulated with 160 nM SIY peptide or irrelevant OVA peptide as negative control, or 500 ng/ml ionomycin+50 ng/ml PMA as positive control, overnight at 37 $^{\circ}$ C. The following day, IFN- γ spots were detected with biotinylated detection antibody (CatNo: 51-1818KZ), followed by streptavidin-HRP and AEC substrate kit (all from BD Biosciences). The spot number and size were quantified

using an Immunospot Series 3 Analyzer and ImmunoSpot software (Cellular Technology).

Statistical Analysis

Tumor growth curves were analyzed using two-way ANOVA with Tukey's multiple comparisons post-test using GraphPad PRISM. For other comparisons between two groups, including evaluating significance in immune profiling or quantitative PCRs, unpaired, two-tailed Student's t-test or non-parametric Mann-Whitney U test was used as indicated in the figure legends. Microbial composition comparisons were performed using non-parametric t-tests. For multiple comparisons, p-value was adjusted using Benjamini-Hochberg FDR correction (Ref 44A; herein incorporated by reference in its entirety). Spearman's rank correlation coefficient ρ was used for measuring statistical dependence between relative abundance of bacteria produced by different platforms. P<0.05 was considered statistically significant and denoted as follows: *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. Statistical analysis was performed using GraphPad PRISM and R.

Results

To evaluate whether commensal bacterial composition might be associated with clinical efficacy of checkpoint blockade immunotherapy, stool samples were collected from 42 patients prior to treatment as part of a multi-dimensional biomarker analysis in metastatic melanoma. Clinical response rate was determined in a blinded fashion from biomarker results, using Response Evaluation Criteria In Solid Tumors (RECIST) version 1.1. There were 16 responders (from here on, referred to as R) and 26 non-responders (NR), giving a response rate of 38% in line with published clinical data with anti-PD-1 agents in metastatic melanoma (Refs. 1B, 2B; herein incorporated by reference in their entireties). No major differences in patient characteristics were observed in R vs. NR, except a borderline difference in prior (but not current) smoking history (Table 1).

TABLE 1

		Baseline patient characteristics with Responders Vs. Non Responders				
		Total (N = 42)	Responders	Non Responders	P-value	OR (95% CI)
Gender	Female	22	8	14	1.00	0.86 (0.21, 3.56)
	Male	20	8	12	1.00	1.16 (0.28, 4.84)
Race	White	39	15	24	1.00	1.24 (0.06, 78.56)
	Other Race ^f	3	1	2	1.00	0.8 (0.01, 16.73)
Age	<50	4	2	2	0.63	1.69 (0.11, 25.78)
	51-70	28	12	16	0.51	1.85 (0.4, 10.11)
	71+	10	2	8	0.27	0.33 (0.03, 2.03)
BMI*	Underweight	1	0	1	1.00	0 (0, 63.31)
	Normal	14	5	9	1.00	0.86 (0.18, 3.85)
	Overweight	17	6	11	1.00	0.82 (0.18, 3.46)
Smoking	Obese	10	5	5	0.46	1.88 (0.35, 10.22)
	Never	25	7	18	0.12	0.36 (0.08, 1.5)
	Former	13	8	5	0.05	4.04 (0.87, 21.18)
EtOH**	Current	4	1	3	1.00	0.52 (0.01, 7.18)
	No Current Use	18	7	11	1.00	1.06 (0.25, 4.42)
	Occasional	17	6	11	1.00	0.82 (0.18, 3.46)
ECOG PS	Moderate	6	2	4	1.00	0.79 (0.06, 6.4)
	Heavy	1	1	0	0.38	NE (0.04, NE)
	0	25	9	16	0.76	0.81 (0.19, 3.45)
Prior Therapy	1	17	7	10	0.76	1.24 (0.29, 5.24)
	None	27	11	16	0.75	1.36 (0.31, 6.59)
	1	10	4	6	1.00	1.11 (0.19, 5.85)
LDH***	> 1	5	1	4	0.63	0.37 (0.01, 4.29)
	Normal	32	11	21	0.46	0.53 (0.1, 2.86)
	Elevated	10	5	5	0.46	1.88 (0.35, 10.22)
Melanoma sub-type	Cutaneous	35	14	21	0.69	1.65 (0.23, 19.6)
	Mucosal	6	2	4	1.00	0.79 (0.06, 6.4)
	Uveal	1	0	1	1.00	0 (0, 63.31)

TABLE 1-continued

Baseline patient characteristics with Responders Vs. Non Responders						
		Total (N = 42)	Responders	Non Responders	P-value	OR (95% CI)
Mutation****	BRAF	16	6	10	1.00	0.96 (0.21, 4.1)
	RAS	9	4	5	0.71	1.39 (0.23, 7.93)
	Other	4	1	3	1.00	0.52 (0.01, 7.18)
	None	10	4	6	1.00	1.11 (0.19, 5.85)
	Unknown	3	1	2	1.00	0.8 (0.01, 16.73)
M stage	M1a	7	3	4	1.00	1.26 (0.16, 8.81)
	M1b	11	3	8	0.49	0.53 (0.08, 2.77)
	M1c	24	10	14	0.75	1.42 (0.34, 6.29)
Sites of metastatic disease	≥3	10	6	4	0.14	3.2 (0.6, 19.19)
	<3	32	10	22	0.14	0.31 (0.05, 1.66)
Prior brain metastases	Yes	4	1	3	1.00	0.52 (0.01, 7.18)
	No	38	15	23	1.00	1.93 (0.14, 109.39)

ECOG; Eastern Cooperative Oncology Group,

LDH; Lactate Dehydrogenase;

PS; Performance status

*BMI; Body Mass Index, Underweight (<18.5), Normal (18.5 to 24.9), Overweight (25 to 29.9), Obese (>30)

**EtOH, alcohol consumption reported; occasional (0.1-4), moderate (5-11), heavy (12+) drinks per week

***LDH institutional upper limit of normal is 245 units/liter

****Other mutations include: NF1, KIT, GNAQ, GNA11

[†]Other Race Than White include: Black, African-American, American Indian or Alaska Native, Patient Decline Information

NE = not evaluable.

To determine whether the composition of the commensal microbiota is associated with clinical response, three methods for DNA sequence-based bacterial identification were integrated (FIG. 4A). First, using 16S rRNA sequencing, genus-level taxa present at different abundance in R vs. NR we identified (Table 2). A BLAST search of the 16S rRNA sequences against the NCBI database was utilized to reveal species-level identities. Further level of confidence in species identification was gained by matching the genus-level taxa from the 16S rRNA dataset to species-level identities

revealed by metagenomic shotgun sequencing (Table 3A-B). Species-specific qPCR was employed for those candidate species having previously validated primers (Table 4A-B). Compared to the 16S rRNA analysis, the metagenomic sequencing yielded a smaller number of species differentially represented in R vs. NR, which significantly overlapped with the 16S results (Table 5). Treating these assays as a screen for maximizing the number of candidate species, the 16S rRNA sequencing method was utilized as a starting point in analysis.

TABLE 2

16S rRNA sequencing					
OTU	Test-Statistic	P value	NonResponder_mean	Responder_mean	taxonomy
1111582	-1.591732208	0.000999001	0.576923077	71.3125	k_Bacteria; p_Firmicutes; c_Bacilli; o_Lactobacillales; f_Enterococaceae; g_Enterococcus; s_
4333897	-3.274533619	0.001998002	0.038461538	0.625	k_Bacteria; p_Proteobacteria; c_Gammaproteobacteria; o_Enterobacteriales; f_Enterobacteriaceae; g_;
363794	-1.839972439	0.003996004	4.846153846	56.9375	s_
3867172	-3.115289034	0.005994006	0.115384615	0.9375	k_Bacteria; p_Actinobacteria; c_Coriobacteriia; o_Coriobacteriales; f_Coriobacteriaceae; g_Collinsella; s_aerofaciens
New.CleanUp.ReferenceOTU49633	-2.863148132	0.005994006	0.153846154	0.875	k_Bacteria; p_Proteobacteria; c_Gammaproteobacteria; o_Enterobacteriales; f_Enterobacteriaceae; g_;
					s_

TABLE 2-continued

16S rRNA sequencing					
OTU	Test-Statistic	P value	NonResponder_mean	Responder_mean	taxonomy
365181	-1.719135039	0.006993007	3.423076923	37.625	k_Bacteria; p_Actinobacteria; c_Coriobacteriia; o_Coriobacteriales; f_Coriobacteriaceae; g_Collinsella; s_aerofaciens
4451251	-2.958594851	0.006993007	0.076923077	0.625	k_Bacteria; p_Actinobacteria; c_Coriobacteriia; o_Coriobacteriales; f_Coriobacteriaceae; g_Atopobium; s_
1684221	2.903506373	0.007992008	0.692307692	0.1875	k_Bacteria; p_Proteobacteria; c_Deltaproteobacteria; o_Desulfovibrionales; f_Desulfovibrionaceae; g_Desulfovibrio; s_
813479	-2.424212194	0.007992008	0.038461538	0.6875	k_Bacteria; p_Actinobacteria; c_Actinobacteria; o_Bifidobacteriales; f_Bifidobacteriaceae; g_Bifidobacterium; s_
New.CleanUp.ReferenceOTU24351	3.071336285	0.00999001	0.5	0	k_Bacteria; p_Bacteroidetes; c_Bacteroidia; o_Bacteroidales; f_Bacteroidaceae; g_Bacteroides; s_
659361	-2.029898573	0.010989011	0.153846154	20.5	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_Dorea; s_
187623	-2.236097332	0.011988012	0.192307692	1.1875	k_Bacteria; p_Bacteroidetes; c_Bacteroidia; o_Bacteroidales; f_Bacteroidaceae; g_Bacteroides; s_
198183	2.53390364	0.011988012	0.692307692	0.0625	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_; g_;
358333	2.414385239	0.011988012	1.038461538	0.125	s_ k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_Blautia; s_
325977	-2.399267823	0.012987013	1.384615385	9.8125	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_[Ruminococcus]; s_
197562	-2.464382476	0.013986014	0.076923077	0.625	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Ruminococcaceae; g_;
289452	2.602400195	0.013986014	0.307692308	0	s_ k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_;
461795	-2.046964459	0.014985015	0.076923077	2.0625	s_ k_Bacteria; p_Firmicutes; c_Clostridia;

TABLE 2-continued

16S rRNA sequencing					
OTU	Test-Statistic	P value	NonResponder_mean	Responder_mean	taxonomy
185186	-2.659855246	0.015984016	0	0.5	o_Clostridiales; f_Ruminococcaceae; g_Oscillospira; s_ k_Bacteria; p_Verrucomicrobia; c_Verrucomicrobiae; o_Verrucomicrobiales; f_Verrucomicrobiaceae; g_Akkermansia; s_muciniphila k_Bacteria;
220970	-2.681219889	0.015984016	0	0.375	p_Proteobacteria; c_Gammaproteobacteria; o_Enterobacteriales; f_Enterobacteriaceae; g_Klebsiella; s_ k_Bacteria;
4391262	-2.544079887	0.015984016	0.192307692	0.75	p_Proteobacteria; c_Gammaproteobacteria; o_Enterobacteriales; f_Enterobacteriaceae; g_; s_ k_Bacteria;
786449	-2.872972025	0.015984016	0	0.25	p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_;
1077373	-2.667487558	0.016983017	0	0.3125	s_ k_Bacteria; p_Bacteroidetes; c_Bacteroidia; o_Bacteroidales; f_Prevotellaceae; g_Prevotella; s_ k_Bacteria;
295085	-2.031498001	0.017982018	0	0.5	p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_Blautia; s_ k_Bacteria;
181155	-1.44077663	0.01998002	0.076923077	6	p_Firmicutes; c_Clostridia; o_Clostridiales; f_Veillonellaceae; g_Veillonella; s_dispar k_Bacteria;
325850	-2.480422027	0.01998002	0.076923077	17.125	p_Proteobacteria; c_Alphaproteobacteria; o_RF32; f_; g_; s_ k_Bacteria;
367092	2.416382368	0.01998002	13.88461538	2.625	p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae k_Bacteria;
New.CleanUp.ReferenceOTU75977	-2.659855246	0.01998002	0	0.5	p_Proteobacteria; c_Gammaproteobacteria; o_Enterobacteriales; f_Enterobacteriaceae; g_; s_ k_Bacteria;
174842	-2.370319725	0.020979021	0.423076923	1.3125	p_Bacteroidetes; c_Bacteroidia; o_Bacteroidales; f_Porphyrimonadaceae; g_Parabacteroides; s_distasonis k_Bacteria;
180082	-2.219337047	0.021978022	109.3846154	253.75	p_Bacteroidetes; c_Bacteroidia; o_Bacteroidales; f_Porphyrimonadaceae; g_Parabacteroides; s_

TABLE 2-continued

16S rRNA sequencing					
OTU	Test-Statistic	P value	NonResponder_mean	Responder_mean	taxonomy
808486	-2.189498747	0.021978022	0.5	1.875	k_Bacteria; p_Proteobacteria; c_Gammaproteobacteria; o_Enterobacteriales; f_Enterobacteriaceae; g_ ; s_
339905	2.407682083	0.022977023	0.384615385	0.0625	k_Bacteria; p_Bacteroidetes; c_Bacteroidia; o_Bacteroidales; f_S24-7; g_ ; s_
592139	-1.321911854	0.024975025	0.115384615	40.875	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Clostridiaceae; g_Clostridium; s_
922761	-1.755181534	0.024975025	3.653846154	47.875	k_Bacteria; p_Proteobacteria; c_Gammaproteobacteria; o_Enterobacteriales; f_Enterobacteriaceae; g_ ; s_
New.CleanUp.ReferenceOTU3631	-2.585685376	0.024975025	0.038461538	0.375	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_Blautia; s_
199524	2.384248595	0.027972028	1.269230769	0.1875	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales
2063400	2.065375831	0.02997003	0.923076923	0.0625	k_Bacteria; p_Bacteroidetes; c_Bacteroidia; o_Bacteroidales; f_Bacteroidaceae; g_Bacteroides; s_
4448331	-2.143054695	0.031968032	0.115384615	0.625	k_Bacteria; p_Proteobacteria; c_Gammaproteobacteria; o_Enterobacteriales; f_Enterobacteriaceae; g_ ; s_
352933	1.836190984	0.032967033	0.807692308	11.6875	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_ ; g_ ; s_
New.CleanUp.ReferenceOTU50254	2.394751585	0.032967033	1.192307692	0.3125	k_Bacteria; p_Bacteroidetes; c_Bacteroidia; o_Bacteroidales; f_[Odoribacteraceae]; g_Odoribacter; s_
176337	2.39665494	0.034965035	0.423076923	0	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Ruminococcaceae; g_ ; s_
842596	2.08340893	0.034965035	3.307692308	0.5	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_Coprococcus; s_
New.CleanUp.ReferenceOTU21685	2.172568314	0.034965035	0.653846154	0	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_ ; s_

TABLE 2-continued

16S rRNA sequencing					
OTU	Test-Statistic	P value	NonResponder_mean	Responder_mean	taxonomy
New.CleanUp.ReferenceOTU60967	2.223328377	0.035964036	0.423076923	0	k_Bacteria; p_Firmicutes; c_Erysipelotrichi; o_Erysipelotrichales; f_Erysipelotrichaceae; g_;
198928	-1.841787835	0.038961039	1.461538462	7.8125	s_ k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_[Ruminococcus]; s_
197343	2.136989115	0.041958042	0.961538462	0.125	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_;
328617	2.04397235	0.041958042	0.807692308	0.1875	s_ k_Bacteria; p_Bacteroidetes; c_Bacteroidia; o_Bacteroidales; f_Bacteroidaceae; g_Bacteroides
328905	-1.885076484	0.041958042	0.5	8.125	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Ruminococcaceae; g_Oscillospira; s_
759751	1.863649835	0.041958042	43.19230769	17.125	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_Dorea; s_
New.CleanUp.ReferenceOTU77070	2.168565819	0.041958042	0.653846154	0.1875	k_Bacteria; p_Bacteroidetes; c_Bacteroidia; o_Bacteroidales; f_Bacteroidaceae; g_Bacteroides; s_
4174963	2.083768333	0.043956044	2.307692308	0.875	k_Bacteria; p_Bacteroidetes; c_Bacteroidia; o_Bacteroidales; f_Bacteroidaceae; g_Bacteroides; s_
New.CleanUp.ReferenceOTU33851	-2.345446101	0.043956044	0.115384615	0.5	k_Bacteria; p_Bacteroidetes; c_Bacteroidia; o_Bacteroidales; f_Bacteroidaceae; g_Bacteroides; s_ovatus
367215	2.128877321	0.044955045	6	2.625	k_Bacteria; p_Firmicutes; c_Erysipelotrichi; o_Erysipelotrichales; f_Erysipelotrichaceae; g_Holdmania; s_
975306	1.868365208	0.044955045	3.615384615	1.125	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_;
1928156	1.830574658	0.045954046	0.538461538	0.125	s_ k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_Roseburia; s_
495396	-2.024760012	0.045954046	0	0.3125	k_Bacteria; p_Firmicutes; c_Clostridia;

TABLE 2-continued

16S rRNA sequencing					
OTU	Test-Statistic	P value	NonResponder_mean	Responder_mean	taxonomy
820346	-1.654631254	0.046953047	4.692307692	72.125	o_Clostridiales; f_[Tissierellaceae]; g_Anaerococcus; s_ k_Bacteria; p_Proteobacteria; c_Gammaproteobacteria; o_Enterobacteriales; f_Enterobacteriaceae; g_; s_ k_Bacteria; p_Firmicutes; c_Erysipelotrichi; o_Erysipelotrichales; f_Erysipelotrichaceae; g_; s_ k_Bacteria; p_Firmicutes; c_Bacilli; o_Lactobacillales; f_Lactobacillaceae; g_Lactobacillus; s_ruminis k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Ruminococcaceae; g_ s_ k_Bacteria; p_Proteobacteria; c_Gammaproteobacteria; o_Enterobacteriales; f_Enterobacteriaceae; g_; s_ k_Bacteria; p_Bacteroidetes; c_Bacteroidia; o_Bacteroidales; f_Bacteroidaceae; g_Bacteroides; s_ k_Bacteria; p_Actinobacteria; c_Actinobacteria; o_Bifidobacteriales; f_Bifidobacteriaceae; g_Bifidobacterium; s_
360238	-1.903434004	0.047952048	0.076923077	0.5625	
1107027	-1.830397468	0.048951049	0.884615385	8.75	
528652	2.047565304	0.048951049	1.884615385	0.75	
141145	-2.039936818	0.04995005	1.730769231	5.125	
197537	-1.994647776	0.04995005	0.346153846	0.9375	
559527	-1.651499936	0.057942058	20.80769231	84.6875	

TABLE 3A

16S rRNA data				
Family 16S	Species 16S	Direction of change	Non-parametric T test statistic	Non-parametric T test P value
Enterobacteriaceae	NA	UP in R	-3.274534	0.001998
Enterobacteriaceae	NA	UP in R	-3.115289	0.005994
Coriobacteriaceae	NA	UP in R	-2.958595	0.006993
Lachnospiraceae	NA	UP in R	-2.872972	0.015984
Ruminococcaceae	NA	UP in R	-2.863148	0.005994
Enterobacteriaceae	NA	UP in R	-2.681220	0.015984
Prevotellaceae	NA	UP in R	-2.667488	0.016983
Verrucomicrobiaceae	<i>Akkermansia_muciniphila</i>	UP in R	-2.659855	0.015984
Enterobacteriaceae	NA	UP in R	-2.659855	0.019980
Lachnospiraceae	NA	UP in R	-2.585685	0.024975
Enterobacteriaceae	NA	UP in R	-2.544080	0.015984
NA	NA	UP in R	-2.480422	0.019980
Ruminococcaceae	NA	UP in R	-2.464382	0.013986
Bifidobacteriaceae	NA	UP in R	-2.424212	0.007992
Lachnospiraceae	NA	UP in R	-2.399268	0.012987
Porphyromonadaceae	<i>Parabacteroides_distasonis</i>	UP in R	-2.370320	0.020979
Bacteroidaceae	<i>Bacteroides_ovatus</i>	UP in R	-2.345446	0.043956

TABLE 3A-continued

16S rRNA data				
Family 16S	Species 16S	Direction of change	Non-parametric T test statistic	Non-parametric T test P value
Bacteroidaceae	NA	UP in R	-2.236097	0.011988
Porphyromonadaceae	NA	UP in R	-2.219337	0.021978
Enterobacteriaceae	NA	UP in R	-2.189499	0.021978
Enterobacteriaceae	NA	UP in R	-2.143055	0.031968
Ruminococcaceae	NA	UP in R	-2.046964	0.014985
Enterobacteriaceae	NA	UP in R	-2.039937	0.049950
Lachnospiraceae	NA	UP in R	-2.031498	0.017982
Lachnospiraceae	NA	UP in R	-2.029899	0.010989
Tissierellaceae	NA	UP in R	-2.024760	0.045954
Bacteroidaceae	NA	UP in R	-1.994648	0.049950
Erysipelotrichaceae	NA	UP in R	-1.903434	0.047952
Ruminococcaceae	NA	UP in R	-1.885076	0.041958
Lachnospiraceae	NA	UP in R	-1.841788	0.038961
Coriobacteriaceae	<i>Collinsella_aerofaciens</i>	UP in R	-1.839972	0.003996
NA	NA	UP in R	-1.836191	0.032967
Lactobacillaceae	<i>Lactobacillus_ruminis</i>	UP in R	-1.830397	0.048951
Enterobacteriaceae	NA	UP in R	-1.755182	0.024975
Coriobacteriaceae	<i>Collinsella_aerofaciens</i>	UP in R	-1.719135	0.006993
Enterobacteriaceae	NA	UP in R	-1.654631	0.046953
Bifidobacteriaceae	NA	UP in R	-1.651500	0.057942
Enterococcaceae	NA	UP in R	-1.591732	0.000999
Veillonellaceae	<i>Veillonella_dispar</i>	UP in R	-1.440777	0.019980
Clostridiaceae	NA	UP in R	-1.321912	0.024975
Lachnospiraceae	NA	UP in NR	1.830575	0.045954
Lachnospiraceae	NA	UP in NR	1.863650	0.041958
Lachnospiraceae	NA	UP in NR	1.868365	0.044955
Bacteroidaceae	NA	UP in NR	2.043972	0.041958
Ruminococcaceae	NA	UP in NR	2.047565	0.048951
Bacteroidaceae	NA	UP in NR	2.065376	0.029970
Lachnospiraceae	NA	UP in NR	2.083409	0.034965
Bacteroidaceae	NA	UP in NR	2.083768	0.043956
Erysipelotrichaceae	NA	UP in NR	2.128877	0.044955
Lachnospiraceae	NA	UP in NR	2.136989	0.041958
Bacteroidaceae	NA	UP in NR	2.168566	0.041958
Lachnospiraceae	NA	UP in NR	2.172568	0.034965
Erysipelotrichaceae	NA	UP in NR	2.223328	0.035964
NA	NA	UP in NR	2.384249	0.027972
Odoribacteraceae	NA	UP in NR	2.394752	0.032967
Ruminococcaceae	NA	UP in NR	2.396655	0.034965
S24-7	NA	UP in NR	2.407682	0.022977
Lachnospiraceae	NA	UP in NR	2.414385	0.011988
Lachnospiraceae	NA	UP in NR	2.416382	0.019980
NA	NA	UP in NR	2.533904	0.011988
Lachnospiraceae	NA	UP in NR	2.602400	0.013986
Desulfovibrionaceae	NA	UP in NR	2.903506	0.007992
Bacteroidaceae	NA	UP in NR	3.071336	0.009990

TABLE 3B

Shotgun sequencing data.					
Species shotgun	Spearman correlation rho	Spearman correlation P value	Confirmed by shotgun sequencing	BLAST agreement	Inclusion in the SVM model and validation
<i>Escherichia_coli</i>	0.550530	0.000282	Yes	Yes	Removed
<i>Escherichia_coli</i>	0.629962	0.000017	Yes	No	Yes
<i>Atopobium_parvulum</i>	0.576876	0.000121	Yes	Yes	Removed
<i>Dorea_formicigenerans</i>	0.334512	0.037394	Yes	No	Yes
<i>Faecalibacterium_prausnitzii</i>	0.414561	0.008692	Yes	Yes	Removed
<i>Klebsiella_pneumoniae</i>	0.533469	0.000470	Yes	Yes	Yes
NA	NA	NA	NE	Yes	Yes
<i>Akkermansia_muciniphila</i>	0.410041	0.009528	Yes	Yes	Yes
<i>Klebsiella_oxytoca</i>	0.413549	0.008873	Yes	Yes	Removed
<i>Anaerostipes_caccaae</i>	0.311151	0.053845	NE	Yes	Removed
<i>Escherichia_coli</i>	0.646825	0.000009	Yes	Yes	Removed

TABLE 3B-continued

Shotgun sequencing data.					
Species shotgun	Spearman correlation rho	Spearman correlation P value	Confirmed by shotgun sequencing	BLAST agreement	Inclusion in the SVM model training and validation
NA	NA	NA	NE	NA	Yes
<i>Faecalibacterium_prausnitzii</i>	0.362051	0.023519	Yes	NA	Yes
<i>Bifidobacterium_adolescentis</i>	0.566864	0.000168	Yes	No	Yes
Lachnospiraceae_bacterium_6_1_63FAA	0.536065	0.000436	Yes	Yes	Removed
<i>Parabacteroides_distasonis</i>	0.398512	0.011978	Yes	No	Yes
<i>Bacteroides_eggerthii</i>	0.301822	0.061851	NE	No	Removed
<i>Bacteroides_xylanisolvans</i>	0.270799	0.095427	NE	NA	Yes
<i>Parabacteroides_merdae</i>	0.899720	6.86E-15	Yes	No	Yes
<i>Escherichia_coli</i>	0.644850	0.000009	Yes	No	Removed
<i>Escherichia_unclassified</i>	0.496450	0.001307	Yes	NA	Yes
<i>Anaerotruncus_unclassified</i>	0.420373	0.007710	Yes	No	Yes
<i>Escherichia_coli</i>	0.755701	2.68E-08	Yes	Yes	Removed
Lachnospiraceae_bacterium_8_1_57FAA	0.250059	0.124705	NE	Yes	Yes
Lachnospiraceae_bacterium_9_1_43BFAA	0.455202	0.003594	Yes	Yes	Yes
NA	NA	NA	NE	Yes	Removed
<i>Bacteroides_ovatus</i>	0.279476	0.084877	NE	Yes	Yes
<i>Solobacterium_moorei</i>	0.311151	0.053845	NE	Yes	Yes
<i>Ruminococcus_callidus</i>	0.498926	0.001225	Yes	Yes	Removed
Lachnospiraceae_bacterium_6_1_63FAA	0.493379	0.001420	Yes	Yes	Removed
<i>Collinsella_aerofaciens</i>	0.909381	1.15E-15	Yes	Yes	Removed
NA	NA	NA	NE	No	Yes
<i>Lactobacillus_animalis</i>	0.269342	0.097300	NE	Yes	Yes
<i>Klebsiella_pneumoniae</i>	0.681088	0.000002	Yes	No	Removed
<i>Collinsella_aerofaciens</i>	0.884610	8.07E-14	Yes	Yes	Yes
<i>Klebsiella_pneumoniae</i>	0.776955	6.05E-09	Yes	Yes	Yes
<i>Bifidobacterium_longum</i>	0.827885	7.96E-11	Yes	Yes	Yes
<i>Enterococcus_faecium</i>	0.540876	0.000378	Yes	Yes	Yes
<i>Veillonella_parvula</i>	0.466233	0.002780	Yes	Yes	Yes
<i>Clostridium_perfringens</i>	0.153504	0.351000	NE	No	Removed
<i>Roseburia_intestinalis</i>	0.541337	0.000373	Yes	No	Yes
<i>Dorea_unclassified</i>	0.483302	0.001830	Yes	Yes	Yes
<i>Ruminococcus_obeum</i>	0.452795	0.003799	Yes	Yes	Yes
NA	NA	NA	NE	NA	Yes
<i>Faecalibacterium_prausnitzii</i>	0.419746	0.007811	Yes	No	Yes
<i>Bacteroides_thetaiotaomicron</i>	0.485656	0.001720	Yes	NA	Yes
<i>Marvinbryantia_formatexigens</i>	0.325481	0.043184	Yes	NA	Yes
<i>Bacteroides_vulgatus</i>	0.492837	0.001435	Yes	Yes	Yes
<i>Holdemanian_filiformis</i>	0.684482	0.000002	Yes	NA	Yes
<i>Dorea_longicatena</i>	0.558706	0.000218	Yes	Yes	Yes
<i>Bacteroides_finegoldii</i>	0.281691	0.082334	NE	NA	Removed
<i>Ruminococcus_obeum</i>	0.510578	0.000897	Yes	No	Removed
<i>Holdemanian_unclassified</i>	0.151990	0.355654	NE	No	Removed
NA	NA	NA	NE	NA	Yes
NA	NA	NA	NE	Yes	Removed
<i>Ruminococcus_sp_5_1_39BFAA</i>	0.338589	0.034995	Yes	NA	Yes
NA	NA	NA	NE	Yes	Yes
Lachnospiraceae_bacterium_9_1_43BFAA	0.378586	0.017475	Yes	Yes	Yes
Lachnospiraceae_bacterium_5_1_63FAA	0.680934	0.000002	Yes	No	Yes
NA	NA	NA	NE	No	Yes
<i>Roseburia_inulinivorans</i>	0.432282	0.005991	Yes	Yes	Yes
NA	NA	NA	NE	No	Yes
<i>Bacteroides_salysersiae</i>	0.355278	0.026453	Yes	No	Removed

TABLE 4A

qPCR data											
Sample Name	R/NR	Up in R <i>V. parvula</i>	Up in R <i>E. faecium</i>	Up in R <i>C. aerofaciens</i>	Up in R <i>B. adolescentis</i>	Up in R <i>B. longum</i>	Up in R <i>K. pneumoniae</i>	Up in R <i>L. ruminis</i> subgroup	Up in R <i>P. merdae</i>	Up in NR <i>R. intestinalis</i>	Up in NR <i>R. obeum</i>
P01	NR	11,475	0	62,725	0	0	0	0	1,597,873	13	0
P02	R	24,078	0	22,452	0	0	0	5,513,956	97,020	516	482
P03	NR	36	168	16	0	15,995	0	0	260,671	45,708	0
P04	NR	2,433	0	0	8,003	102,209	0	0	42,860	3,849	9,634
P05	NR	15	0	0	10,480	82,559	0	132,1112	0	13,676	9,072
P06	NR	0	0	0	0	111	0	0	319	2,141	29,679

TABLE 4A-continued

qPCR data											
Sample Name	R/NR	Up in R <i>F. parvula</i>	Up in R <i>E. faecium</i>	Up in R <i>C. aerofaciens</i>	Up in R <i>B. adolescentis</i>	Up in R <i>B. longum</i>	Up in R <i>K. pneumoniae</i>	Up in R <i>L. ruminis</i> subgroup	Up in R <i>P. merdae</i>	Up in NR <i>R. intestinalis</i>	Up in NR <i>R. obeum</i>
P07	R	1,779	0	0	0	0	0	0	316,456	2,539	5,416
P08	NR	4,103	0	11,231	16	43,243	0	0	0	10,940	20,678
P09	NR	945	0	0	0	0	0	78,39282	346,039	9	0
P10	NR	543	0	10,150	0	0	0	0	15	23	2,797
P11	NR	0	0	0	0	193	0	0	0	54,846	860
P12	NR	177	0	44,697	0	0	5,184	0	0	11,575	2,836
P13	R	0	0	0	0	0	ND	0	0	0	ND
P14	NR	5,340	0	16,232	0	11,295	0	0	50,976	1,170	2,850
P15	NR	119	0	0	0	88,445	0	34,14117	39	28	0
P16	R	1,587	133,010	246,723	145,416	9,352	435	120019	0	2,647	925
P17	NR	0	0	64,284	0	164,311	0	0	67,195	1,441	0
P18	NR	71	0	4,493	0	0	0	0	126,522	29	11
P19	NR	576	0	9,559	4,611	909	4	0	175,857	8	0
P20	R	212	10,831	2,967,175	128,639	3,901,078	0	46600.89	17,967	1,581	0
P21	NR	55	0	39,673	28,122	12,003	0	0	0	3,341	5,039
P22	NR	209	0	0	0	42	0	58,23432	0	19	8,365
P23	R	6	0	477,784	33,745	243,696	0	0	43,826	2,597	6,693
P24	R	95	648	7	0	59,624	0	0	99,812	164	22,048
P25	NR	0	0	62,221	0	80	0	8,494507	440,357	18,236	15,102
P26	NR	914	0	64,251	15,483	37,583	0	9,874153	142,228	184	6,696
P27	R	1,737	820	0	0	218,452	0	0	330,927	266	2,965
P28	R	17,842	0	103,390	5	92,534	2,532	38943.37	64,752	3,582	5
P29	R	8,259	0	0	0	23	0	0	686,549	62,125	11,876
P30	NR	64	139	2,530	ND	2,788	ND	ND	0	1,253	14,685
P31	NR	36	0	0	0	0	0	0	869,370	1,066	22,889
P32	NR	272	0	45,490	0	46	0	0	1,247,815	19,038	8,921
P33	R	3,458	0	0	0	0	0	0	0	258	959
P34	R	512	121	43,590	1,419	64,464	0	8,103439	89,046	63	2,981
P35	NR	58	0	79,197	0	0	19	0	442,665	61	10,102
P36	R	265	0	6,651	5,618	12,852	0	0	384,166	22	571
P37	R	300	0	0	0	2,794	638	0	67,680	5	2,590
P38	NR	74	0	6,556	0	0	0	0	402,480	904	0
P39	R	0	0	423,770	0	265	0	0	17,056	53	0
P40	NR	8,479	0	91,856	0	21,494	278	8,091473	53,714	3,553	5,657
P41	R	2,425	2,501	25,124	0	11,689	58,503	0	61,827	984	1,441
P42	NR	1,036	0	0	0	115,017	0	718.3709	1,012	11,668	17,655

TABLE 4B

qPCR primers						
Target species	Primer sequence (5'->3')	SEQ ID NO:	SYBR/TaqMan	Annealing	Extension and fluorescence detection	
<i>Akkermansia muciniphila</i>	Forward: CAGCACGTGAAGGTGGGGAC	1	SYBR	60° C./40 s	72° C./30 s	
	Reverse: CCTTGCGGTTGGCTTCAGAT	2				
<i>Bacteroides vulgatus</i>	Forward: AAGGGAGCGTAGATGGATGTTA	3	TaqMan	65° C./60 s		
	Reverse: CGAGCCTCAATGTCAGTTGC	4				
	Probe (FAM/TAMRA): CCTGCCTCAACTGCACTCAAGATATCCAGTA	5				
<i>Bifidobacterium adolescentis</i>	Forward: CTCCGCCGTGATCCGGAAGTCG	6	SYBR	75° C./15 s	72° C./15 s	
	Reverse: AACCAACTCGGCGATGTGGACGACA	7			83° C./15 s	
<i>Bifidobacterium longum</i>	Forward: TTCCAGTTGATCGCATGGTC	8	SYBR	60° C./30 s	85° C./60 s	
	Reverse: TC(G/C)CGCTTGCTCCCGAT	9				
<i>Collinsella aerofaciens</i>	Forward: CCCGACGGGAGGGGAT	10	SYBR	60° C./40 s	72° C./30 s	
	Reverse: CTTCTGACGTACAGTCTTGA	11				
<i>Enterococcus faecium</i>	Forward: CGAGAAGAGCTGCAAAAATGCTTTAGC ***	12	SYBR	60° C./40 s	72° C./30 s	
	Reverse: GCGCGCTTCAATTCCTTGT	13				
<i>Faecalibacterium prausnitzii</i>	Forward: CCCTTCAGTGCCGACGT	14	SYBR	61° C./40 s	72° C./30 s	
	Reverse: GTCGCAGGATGTCAAGAC	15				
<i>Klebsiella pneumoniae</i>	Forward: GCG TGG CGG TAG ATC TAA GTC ATA	16	SYBR	58° C./10 s	72° C./40 s	
	Reverse: TTC AGC TCC GCC ACA AAG GTA	17				
<i>Lactobacillus ruminis</i>	Forward: CACCGAATGCTTGCAAYTCACC	18	SYBR	60° C./20 s	72° C./50 s	
	Reverse: GCCGCGGGTCCATCCAAAA	19				
subgroup **						
<i>Parabacteroides distasonis</i>	Forward: TGCTATCAGAGGGGATAAC	20	TaqMan	60° C./60 s		
	Reverse: GCAAATATTCATGCGGGAT	21				
	Probe (FAM/TAMRA): CGAAAGTCGACTAATACCGCATGAAGC	22				

TABLE 4B-continued

qPCR primers					
Target species	Primer sequence (5'->3')	SEQ ID NO:	SYBR/TaqMan	Annealing	Extension and fluorescence detection
<i>Parabacteroides merdae</i>	Forward: AGGGTGGTAGGTGGTGAT	23	TaqMan		65/60 s
	Reverse: TTCACCGCTACACCACGC	24			
	Probe (FAM/TAMRA): TTACTTGAGTGTGTTTGGAGGTAGGCGG	25			
<i>Roseburia intestinalis</i>	Forward: TTCGCAGCTCAGTCTATCGC ***	26	SYBR	55° C./30 s	72° C./30 s
	Reverse: GCAATCCCCGGGAAGTCATT ***	27			
<i>Ruminococcus obeum</i>	Forward: GCAGATTTGGTCTGTTC	28	TaqMan		60° C./60 s
	Reverse: CGGTATTAGCAACCAATTC	29			
	Probe (FAM/TAMRA): CTGTATAAGGCAGGTTACCCACGC	30			
<i>Veillonella</i> spp.*	Forward: A(C/T)CAACCTGCCCTTCAGA	31	SYBR	60° C./40 s	72° C./30 s
	Reverse: CGTCCCATTACAGAGCTT	32			
Universal bacterial primers	Forward: AGAGTTTGATCCTGGCTCAG	33	SYBR	52° C./30 s	72° C./60 s
	Reverse: TGCTGCCTCCCGTAGGAG	34			

*Target species include *V. parvula*, *V. dispar*, *V. atypica*, *V. ratti*, *V. criceti*, *V. rodentium*, *V. caviae*

** This subgroup was specified based on similarity in 16S rRNA gene sequence in the cited study. The primers amplify *L. ruminis*, *L. animalis*, *L. mali*, *L. salivarius*, *L. satsumensis*, *L. graminis*, *L. panis*

*** The sequence of this primer was modified from the originally reported sequence

TABLE 5

Metagenomic sequencing.				
Species shotgun	Direction of change	Non-parametric T test statistic	Non-parametric T test P value	Overlap with the 63 16S OTUs
<i>Enterococcus faecalis</i>	Up in R	-2.392458368	0.014985015	.
<i>Escherichia coli</i>	Up in R	-2.210901572	0.016983017	Yes
<i>Escherichia unclassified</i>	Up in R	-2.14017738	0.032967033	Yes
<i>Bacteroides ovatus</i>	Up in R	-2.005216423	0.043956044	NE
<i>Turicibacter sanguinis</i>	Up in R	-1.857270068	0.033966034	.
<i>Collinsella aerofaciens</i>	Up in R	-1.833005718	0.017982018	Yes
<i>Clostridium scindens</i>	Up in R	-1.811319782	0.076923077	.
<i>Clostridium nexile</i>	Up in R	-1.801806225	0.007992008	.
<i>Actinomyces graevenitzi</i>	Up in R	-1.741014848	0.091908092	.
<i>Eubacterium siraeum</i>	Up in R	-1.729111828	0.091908092	.
<i>Lachnospiraceae bacterium_7_1_58FAA</i>	Up in R	-1.716806138	0.095904096	.
<i>Bifidobacterium longum</i>	Up in R	-1.701918723	0.023976024	Yes
<i>Haemophilus parainfluenzae</i>	Up in R	-1.669086484	0.071928072	.
<i>Veillonella parvula</i>	Up in R	-1.502989879	0.011988012	Yes
<i>Lachnospiraceae bacterium_6_1_63FAA</i>	Up in R	-1.42054428	0.084915085	Yes
<i>Klebsiella oxytoca</i>	Up in R	-1.401188635	0.046953047	Yes
<i>Enterococcus faecium</i>	Up in R	-1.378987414	0.000999001	Yes
<i>Campylobacter gracilis</i>	Up in R	-1.330045919	0.043956044	.
<i>Burkholderiales bacterium_1_1_47</i>	Up in NR	1.831387273	0.051948052	.
<i>Bacteroides intestinalis</i>	Up in NR	1.864425356	0.064935065	.
<i>Adlercreutzia equolifaciens</i>	Up in NR	1.93586273	0.062937063	.
<i>Holdemanella filiformis</i>	Up in NR	2.071359703	0.056943057	Yes
<i>Coprococcus comes</i>	Up in NR	2.097221049	0.027972028	.

The 16S rRNA sequencing revealed 62 Operational Taxonomic Units (OTUs) of different abundance in R vs. NR (Table 2). Unsupervised hierarchical clustering based on abundance similarity of these OTUs revealed that most samples were accurately grouped according to clinical response (FIG. 2). Supervised clustering according to clinical outcome is depicted in FIG. 1A. Thirty-nine OTUs were more abundant in R and twenty-three were more abundant in NR. One Bifidobacteriaceae OTU was significantly more abundant in R and a second Bifidobacteriaceae OTU (559527) had borderline significance and was included in the analyses (total=63 OTUs). This observation recapitulates the previous results that associated Bifidobacteriaceae family members with improved immune-mediated tumor control and efficacy of anti-PD-L1 therapy in mice (Ref. 3B; herein incorporated by reference in its entirety).

A principal component analysis (PCA) of the 63 OTUs revealed separation of R from NR (FIG. 1B). Predictive modeling was performed to assess the robustness of the data. The 63 OTUs (predictors) were evaluated to remove those of small variance, high correlation, and/or high collinearity, leaving 42 OTUs for the predictive modeling analysis (Table 3). Iterative modeling was performed by randomly splitting the samples into 60% as a training set model selection with 5-fold cross-validation, and 40% as a test set for independent assessment of model performance. A receiver-operator characteristic curve revealed an average of 87% positive and 98% negative predictive value for response as a function of 16S OTU predictors (FIG. 6A). A frequency plot of each of the 42 OTUs across these iterative runs revealed an overlapping range of importance of each of the features without any OTU dominating the model (FIG. 6B). These results

indicate that stability of the data is not skewed based on disproportionate influence of a small number of elements.

A BLAST search of the 63 OTUs against the NCBI database of bacterial sequences returned multiple species corresponding to each OTU with $\geq 98\%$ identity. To gain more accurate species-level characterization, the same samples were subjected to metagenomic shotgun sequencing. Illumina paired-end reads were assigned to microbial clades and analyzed for closest matches to the 63 OTUs identified by 16S sequencing. Potential species matches were identified for 43 of the original 63 OTUs (Table 3). Species-specific qPCR assays were performed as an additional approach to assess the identity of species, for which sufficiently validated qPCR primers were available (Table 4A-B). Thus, integration of the three methods led to the selection of 10 species differentially enriched in R vs. NR. Eight of these were more abundant in R: *Enterococcus faecium*, *Collinsella aerofaciens*, *Bifidobacterium adolescentis*, *Klebsiella pneumoniae*, *Veillonella parvula*, *Parabacteroides merdae*, *Lactobacillus* sp. and *Bifidobacterium longum*, whereas 2 were more abundant in NR: *Ruminococcus obeum* and *Roseburia intestinalis*. As an example, the integrative analysis for *B. longum* (OTU 559627) is depicted in FIG. 2A-C. Similar correlation analyses for the remaining 9 species are depicted in FIGS. 7 and 8. PCR results for these 10 species were integrated into a summation PCR score for each patient, which was significantly higher in responders ($P=0.004$; FIG. 2D). The list of species is likely an underestimate of the total number of entities showing differential abundance in R vs. NR, because of the stringency of this composite analysis. For example, *Akkermansia muciniphila* was detected in 4 patients by 16S sequencing and all were responders, but statistical analysis of the entire cohort did not reach significance. As an alternative way to represent the aggregate data towards development of a candidate predictive biomarker, the total numbers of potentially “beneficial” and “non-beneficial” OTUs were scored for each patient (FIG. 9), and a ratio was calculated). When plotted against the absolute change in tumor size as assessed by RECIST, a clean correlation was observed such that patients with a ratio over 1.5 all showed clinical response (FIG. 2E). These results suggest that the commensal microbiota composition might be useful as a biomarker to predict response to checkpoint blockade therapy, which motivated comparison to other candidate predictive biomarkers. Archived pre-treatment tumor specimens that passed quality control were available for 15 patients (5 R, 10 NR). Microbial composition remained significantly different in R vs. NR for this subset (FIGS. 10A and B). Exome sequencing followed by enumeration of non-synonymous somatic mutations (NSSM) showed a trend of higher frequency in R, as did levels of PD-L1 and PD-1 mRNA (FIG. 10C-E) and enumeration of baseline CD8⁺ T cells by immunohistochemistry (FIG. 10F). While these statistical trends not meeting significance were likely limited by sample size, it is noteworthy that the microbiota parameters still markedly separated responders and non-responders.

The strong correlation between commensal bacteria and clinical response to immunotherapy indicates a causal effect, in light of data demonstrating an immune-potentiating impact of the microbiome in mouse tumor models (Refs. 3B, 5B, 6B; herein incorporated by reference in their entireties). To investigate the capability of human commensal microbes to potentiate anti-tumor T cell responses, germ-free (GF) mice were employed as recipients. In setting up this model, it was found that tumor growth in GF mice was similar to that in Taconic SPF mice and in GF mice colonized with

Taconic feces (FIG. 12), indicating reduced spontaneous immune-mediated tumor control in GF mice as had been seen in Taconic compared to Jackson mice previously (ref. 3B; herein incorporated by reference in its entirety). Improved tumor control had been achieved previously with transfer of Jackson fecal material, suggesting that GF mice are suitable hosts for human-derived microbiota with an opportunity to similarly detect improved anti-tumor immunity. Fecal material was transferred from 3 R and 3 NR (indicated in FIGS. 1A, 5, 9, and 11) into cohorts of GF mice, followed by implantation of B16.SIY melanoma cells. The human microbiota-colonized mouse groups segregated into 2 phenotypes with respect to tumor growth rate—a faster growing group and a slower growing group (FIG. 3A). Two of three mouse cohorts reconstituted with R fecal material had slower tumor growth, and two of the three cohorts reconstituted from NR showed faster tumor growth. Thus, the ability of the human microbiota to support improved tumor control in mice usually, but not always, paralleled the clinical response to anti-PD-1 seen in the donor patient. Achieving slower tumor growth with fecal transplant alone is similar to previous mouse studies, in which transfer of feces from Jackson into Taconic mice was sufficient for a partial therapeutic effect (Ref. 3; herein incorporated by reference in its entirety).

Patterns of bacteria that successfully reconstituted mice and fidelity to the original human donor were assessed with 16S rRNA sequencing. There were significant differences in microbiota composition between the slow and fast tumor growth phenotypes, which were both distinct from Taconic mice (FIG. 3B). Groups C and D, which did not show the same pattern of tumor control as the original human donors, showed a large degree of difference of microbiota composition from the original human donors (FIG. 13). In agreement, a Bray-Curtis dissimilarity index for each donor/recipient pair was highest, at 0.7, for cohorts C and D vs. 0.5-0.6 for the rest of the groups.

Mouse groups A and B were used for further mechanistic studies. There was a high level of consistency between repeated experiments, both with respect to tumor growth rate and microbial colonization (A vs. A2 and B vs. B2 comparisons in FIG. 3B). To determine whether the difference in tumor control could be attributed to host immunity, IFN- γ ELISPOT of ex-vivo SIY-stimulated splenocytes was performed and indicated an increased frequency of activated T cells from R microbiota-reconstituted mice 3 weeks after inoculation with B16.SIY melanoma cells (FIG. 3C). Analysis of the tumor microenvironment also showed a significantly greater number of SIY-specific CD8⁺ T cells, but not of FoxP3⁺CD4⁺ regulatory T cells, in these mice (FIGS. 3D and E), consistent with increased priming of tumor antigen-specific CD8⁺ T cells. Anti-PD-L1 was markedly therapeutic in mice colonized with R microbiota, yet completely ineffective in NR-derived mice (FIG. 3F), demonstrating a profound impact of the commensal microbiota on immunotherapy efficacy in vivo. qPCR interrogation of fecal DNA from these mice recapitulated the results from the analysis of patients. Of the 10 PCR reactions validated in patients, 6 gave a signal in reconstituted mice, with the same pattern of being enriched in R or NR recipient mice as was seen in the original patients (FIG. 3G).

The experiments conducted during development of embodiments herein indicate that the composition of the commensal microbiota in patients is associated with therapeutic efficacy of anti-PD-1 mAb. While *Bifidobacterium longum* was one commensal identified in the current study that had also been found in mouse models to be associated

with improved immune-mediated tumor control (Ref. 3B; herein incorporated by reference in its entirety), it is likely that multiple specific bacteria may contribute to improved anti-tumor immunity in patients. In addition to the panel of bacteria over-represented in responders, several OTUs were over-represented in non-responders, and prior work in mice has indicated that some commensals have the potential to be immune-inhibitory, for example through the induction of FoxP3+ regulatory T cells (Refs. 7B, 8B; herein incorporated by reference in their entireties) In addition, in the current cohort, a ratio of “beneficial” OTUs to “non-beneficial” OTUs was the strongest predictor of clinical response, indicating that a higher frequency of beneficial bacteria combined with a lower frequency of bacteria of negative impact may combine for the most favorable clinical outcome.

Several of the bacterial species identified in the current study to be differentially abundant in responding vs. non-responding patients have been examined previously for mechanistic impact on host immune responses in germ-free mice *in vivo* (Ref. 9B; herein incorporated by reference in its entirety). Monocolonization with several species found to be at increased frequency in our responders, including *Enterococcus faecium*, *Collinsella aerofaciens*, *Bifidobac-*

terium adolescentis, and *Parabacteroides merdae*, were reported to result in a decreased frequency of peripherally-derived colonic regulatory T cells compared to other bacterial species. An increased frequency of the Batf3-lineage DCs and greater Th1 responses were also found with bacteria currently identified to be more abundant in responders (Ref. 9B; herein incorporated by reference in its entirety). Decreased Tregs, increased Batf3 DCs, and augmented Th1 responses would all be expected to improve immune-mediated tumor control.

Example 2

Data were generated by 16S rRNA sequencing of patient stool samples (pre-treatment). The reads were assigned to microbe taxa using open-reference OTU picking protocol from QIIME package. About 10,000 OTUs were generated; after filtering those not present in more than 10% of the samples, 2000 OTUs remained. The abundance of OTUs in each sample was estimated, normalized and compared between non-responder (NR) and responder (R) groups. 62 significant OTUs were identified to be differentially present in NR vs R ($p < 0.05$), plus one *Bifidobacterium* OTU with $p = 0.59$ were include this for subsequent analysis. OTUs identified by the 16S sequencing are listed in Table 6.

TABLE 6

Responder/non-responder bacteria identified by 16S sequencing OTUs.		
Family	Subject Seq-ID	Source
Enterococcaceae	gi 1158620683 gb KY129997.1	<i>Enterococcus faecium</i> strain LM13 16S ribosomal RNA gene, partial sequence
Enterococcaceae	gi 1158620682 gb KY129996.1	<i>Enterococcus faecium</i> strain LM5 16S ribosomal RNA gene, partial sequence
Enterococcaceae	gi 1158620681 gb KY129995.1	<i>Enterococcus faecium</i> strain LM2 16S ribosomal RNA gene, partial sequence
Enterococcaceae	gi 1153161578 gb KY697085.1	<i>Enterococcus faecalis</i> strain Cp5 16S ribosomal RNA gene, partial sequence
Enterococcaceae	gi 1153161577 gb KY697084.1	<i>Enterococcus faecalis</i> strain Cp4 16S ribosomal RNA gene, partial sequence
Enterococcaceae	gi 1153161576 gb KY697083.1	<i>Enterococcus faecalis</i> strain Cp3 16S ribosomal RNA gene, partial sequence
Enterococcaceae	gi 1153161575 gb KY697082.1	<i>Enterococcus faecalis</i> strain Cp2 16S ribosomal RNA gene, partial sequence
Enterococcaceae	gi 1153161574 gb KY697081.1	<i>Enterococcus faecalis</i> strain Cp1 16S ribosomal RNA gene, partial sequence
Enterococcaceae	gi 1158645201 gb CP019992.1	<i>Enterococcus faecium</i> isolate 2014-VREF-268, complete genome
Enterococcaceae	gi 1158642123 gb CP019988.1	<i>Enterococcus faecium</i> isolate 2014-VREF-63, complete genome
Enterococcaceae	gi 1154289756 gb CP019970.1	<i>Enterococcus faecium</i> isolate 2014-VREF-114, complete genome
Enterococcaceae	gi 1154289756 gb CP019970.1	<i>Enterococcus faecium</i> isolate 2014-VREF-114, complete genome
Enterococcaceae	gi 1150406800 gb CP019208.1	<i>Enterococcus faecium</i> strain 2014-VREF-41, complete genome
Enterococcaceae	gi 1149032865 gb KY486862.1	<i>Enterococcus faecium</i> strain C52 16S ribosomal RNA gene, partial sequence
Enterococcaceae	gi 1149032864 gb KY486861.1	<i>Enterococcus faecium</i> strain C7 16S ribosomal RNA gene, partial sequence
Enterococcaceae	gi 1149032863 gb KY486860.1	<i>Enterococcus faecium</i> strain C100 16S ribosomal RNA gene, partial sequence
Enterococcaceae	gi 1149032562 gb KX575840.1	<i>Enterococcus faecalis</i> strain DES-1 16S ribosomal RNA gene, partial sequence
Enterococcaceae	gi 1148879018 gb KY630672.1	<i>Enterococcus faecalis</i> strain UFVCC1189 16S ribosomal RNA gene, partial sequence
Enterococcaceae	gi 1148879014 gb KY630668.1	<i>Enterococcus faecalis</i> strain UFVCC1181 16S ribosomal RNA gene, partial sequence
Enterococcaceae	gi 1148879008 gb KY630662.1	<i>Enterococcus faecalis</i> strain UFVCC1180 16S ribosomal RNA gene, partial sequence

TABLE 6-continued

Responder/non-responder bacteria identified by 16S sequencing OTUs.		
Family	Subject Seq-ID	Source
Enterococcaceae	gi 1148303219 gb KX832370.1	<i>Enterococcus faecium</i> strain 27321 16S ribosomal RNA gene, partial sequence
Coriobacteriaceae	gi 1005392054 gb KU851139.1	<i>Atopobium parvulum</i> strain R42.12 16S ribosomal RNA gene, partial sequence
Coriobacteriaceae	gi 1139267198 emb LT681140.1	<i>Shuttleworthia satelles</i> partial 16S rRNA gene, isolate 523N_6554
Coriobacteriaceae	gi 158263915 gb EU186380.1	<i>Atopobium</i> sp. DMCT15023 16S ribosomal RNA gene, partial sequence
Coriobacteriaceae	gi 1139287456 emb LT699560.1	<i>Prevotella melaninogenica</i> partial 16S rRNA gene, isolate R161T_24978
Coriobacteriaceae	gi 1139268302 emb LT682126.1	<i>Prevotella melaninogenica</i> partial 16S rRNA gene, isolate 244T_7541
Coriobacteriaceae	gi 788262483 gb KP192308.1	<i>Atopobium deltae</i> strain DNF00019 16S ribosomal RNA gene, partial sequence
Coriobacteriaceae	gi 784501155 dbj LC037224.1	<i>Atopobium fossor</i> gene for 16S ribosomal RNA, partial sequence, strain: JCM 9981
Coriobacteriaceae	gi 775465093 dbj LC036309.1	<i>Atopobium minutum</i> gene for 16S ribosomal RNA, partial sequence, strain: JCM 1118
Coriobacteriaceae	gi 1173202442 emb LT598591.2	<i>Olsenella</i> sp. Marseille-P3256 partial 16S rRNA gene, strain Marseille-P3256
Coriobacteriaceae	gi 1182956107 ref NR_146815.1	<i>Olegusella massiliensis</i> strain KHD7 16S ribosomal RNA, partial sequence
Coriobacteriaceae	gi 1179666524 dbj LC258149.1	<i>Olsenella profusa</i> gene for 16S ribosomal RNA, partial sequence, strain: JCM 14553
Coriobacteriaceae	gi 1152067376 emb LT797539.1	<i>Olsenella</i> sp. Marseille-P3359 partial 16S rRNA gene, strain Marseille-P3359
Coriobacteriaceae	gi 33089980 gb AY269023.1	<i>Atopobium vaginae</i> clone FX102-1 16S ribosomal RNA gene, partial sequence
Coriobacteriaceae	gi 1005651670 gb KU726641.1	<i>Atopobium vaginae</i> strain DNF00180 16S ribosomal RNA gene, partial sequence
Coriobacteriaceae	gi 647404812 emb LK021119.1	Bacterium OL-1 partial 16S rRNA gene, isolate OL-1
Coriobacteriaceae	gi 33089990 gb AY269033.1	<i>Atopobium vaginae</i> clone FX162-5 16S ribosomal RNA gene, partial sequence
Coriobacteriaceae	gi 33089988 gb AY269031.1	<i>Atopobium vaginae</i> clone FX119-5 16S ribosomal RNA gene, partial sequence
Coriobacteriaceae	gi 33089986 gb AY269029.1	<i>Atopobium vaginae</i> clone FX184-1 16S ribosomal RNA gene, partial sequence
Coriobacteriaceae	gi 33089984 gb AY269027.1	<i>Atopobium vaginae</i> clone FX135-1 16S ribosomal RNA gene, partial sequence
Coriobacteriaceae	gi 33089981 gb AY269024.1	<i>Atopobium vaginae</i> clone FX103-1 16S ribosomal RNA gene, partial sequence
Bacteroidaceae	gi 1167601288 gb KX658684.1	<i>Bacteroides xylanisolvens</i> strain A3 16S ribosomal RNA gene, partial sequence
Bacteroidaceae	gi 1181557329 gb KY950632.1	<i>Bacteroides acidifaciens</i> strain V 16S ribosomal RNA gene, partial sequence
Bacteroidaceae	gi 1173512652 gb CP015401.2	<i>Bacteroides caecimuris</i> strain I48, complete genome
Bacteroidaceae	gi 1153695773 gb KY703634.1	<i>Anaerostipes</i> sp. strain KFT8 16S ribosomal RNA gene, partial sequence
Bacteroidaceae	gi 1154066557 emb LT598566.3	<i>Bacteroides</i> sp. Marseille-P3132 partial 16S rRNA gene, strain Marseille-P3132
Bacteroidaceae	gi 1074125775 emb LT622246.1	<i>Bacteroides ovatus</i> V975 genome assembly, chromosome: I
Bacteroidaceae	gi 988570982 gb KR364742.1	<i>Bacteroides</i> sp. JJM0207-12 16S ribosomal RNA gene, partial sequence
Bacteroidaceae	gi 988570981 gb KR364741.1	<i>Bacteroides caecimuris</i> strain I48 16S ribosomal RNA gene, partial sequence
Bacteroidaceae	gi 988570980 gb KR364740.1	<i>Bacteroides acidifaciens</i> strain JJM0207-2 16S ribosomal RNA gene, partial sequence
Bacteroidaceae	gi 745311129 gb KP202688.1	<i>Bacteroides</i> sp. G25 16S ribosomal RNA gene, partial sequence
Bacteroidaceae	gi 728055098 gb KM396275.1	<i>Bacteroides ovatus</i> strain G19 16S ribosomal RNA gene, partial sequence
Bacteroidaceae	gi 723001884 dbj AB908394.1	<i>Bacteroides ovatus</i> gene for 16S ribosomal RNA, partial sequence, strain: EFEL003
Bacteroidaceae	gi 676402736 gb KM043747.1	Bacterium PIC8 16S ribosomal RNA gene, partial sequence

TABLE 6-continued

Responder/non-responder bacteria identified by 16S sequencing OTUs.		
Family	Subject Seq-ID	Source
Bacteroidaceae	gi 672228637 dbj AB908392.1	<i>Bacteroides xylanisolvens</i> gene for 16S ribosomal RNA, partial sequence, strain: EFEL001
Bacteroidaceae	gi 159159338 gb EU136694.1	<i>Bacteroides acidifaciens</i> strain JCM10556 16S ribosomal RNA gene, partial sequence
Bacteroidaceae	gi 636560121 refl NR_116181.1	<i>Bacteroides ovatus</i> strain JCM5824 16S ribosomal RNA gene, partial sequence<> <i>Bacteroides ovatus</i> strain JCM5824 16S ribosomal RNA gene, partial sequence
Bacteroidaceae	gi 636559244 refl NR_115301.1	<i>Bacteroides ovatus</i> strain CIP 103756 16S ribosomal RNA gene, partial sequence<> <i>Bacteroides ovatus</i> 16S ribosomal RNA gene, partial sequence
Bacteroidaceae	gi 148791577 gb EF608211.1	<i>Bacteroides</i> sp. EBA5-17 16S ribosomal RNA gene, partial sequence
Bacteroidaceae	gi 343201773 refl NR_042499.1	<i>Bacteroides xylanisolvens</i> strain XB1A 16S ribosomal RNA gene, partial sequence<> <i>Bacteroides xylanisolvens</i> partial 16S rRNA gene, type strain XB1AT
Bacteroidaceae	gi 46370580 gb AY538687.1	Bacteroidaceae bacterium Smarlab 3301643 16S ribosomal RNA gene, partial sequence
Porphyromonadaceae	gi 1181557324 gb KY950627.1	<i>Parabacteroides distasonis</i> strain Q 16S ribosomal RNA gene, partial sequence
Porphyromonadaceae	gi 1153695770 gb KY703631.1	<i>Parabacteroides</i> sp. strain CT06 16S ribosomal RNA gene, partial sequence
Porphyromonadaceae	gi 1153695769 gb KY703630.1	<i>Parabacteroides</i> sp. strain AT13 16S ribosomal RNA gene, partial sequence
Porphyromonadaceae	gi 159159325 gb EU136681.1	<i>Parabacteroides distasonis</i> strain JCM5825 16S ribosomal RNA gene, partial sequence
Porphyromonadaceae	gi 149935098 gb CP000140.1	<i>Parabacteroides distasonis</i> ATCC 8503, complete genome
Porphyromonadaceae	gi 89191772 dbj AB238923.1	<i>Parabacteroides distasonis</i> gene for 16S ribosomal RNA, partial sequence, strain: JCM 13400
Porphyromonadaceae	gi 343200655 refl NR_041342.1	<i>Parabacteroides distasonis</i> strain JCM 5825 16S ribosomal RNA gene, partial sequence<> <i>Parabacteroides distasonis</i> gene for 16S ribosomal RNA, partial sequence, strain: JCM 5825
Porphyromonadaceae	gi 173915 gb M86695.1 BNRRR16S	<i>Bacteroides distasonis</i> 16S ribosomal RNA<> <i>Parabacteroides</i> sp. MC_17 partial 16S rRNA gene, strain DSM-20701, isolate MC_17
Porphyromonadaceae	gi 988571022 gb KR364782.1	<i>Parabacteroides distasonis</i> strain SAB-131-CoC-3 16S ribosomal RNA gene, partial sequence
Porphyromonadaceae	gi 89191775 dbj AB238926.1	<i>Parabacteroides distasonis</i> gene for 16S ribosomal RNA, partial sequence, strain: JCM 13403<> <i>Parabacteroides distasonis</i> gene for 16S ribosomal RNA, partial sequence, strain: JCM 13404
Porphyromonadaceae	gi 89191774 dbj AB238925.1	<i>Parabacteroides distasonis</i> gene for 16S ribosomal RNA, partial sequence, strain: JCM 13402
Porphyromonadaceae	gi 89191773 dbj AB238924.1	<i>Parabacteroides distasonis</i> gene for 16S ribosomal RNA, partial sequence, strain: JCM 13401
Porphyromonadaceae	gi 1154838059 emb LT598573.4	<i>Parabacteroides</i> sp. Marseille-P3236 partial 16S rRNA gene, strain Marseille-P3136
Porphyromonadaceae	gi 1046811522 gb KX462878.1	<i>Parabacteroides</i> sp. strain B3181 16S ribosomal RNA gene, partial sequence
Porphyromonadaceae	gi 1024389103 emb LT558809.1	<i>Bacillus nealsonii</i> partial 16S rRNA gene, strain Marseille-P2085
Porphyromonadaceae	gi 1008904221 emb LT223609.1	<i>Parabacteroides faecis</i> partial 16S rRNA gene, strain Marseille-IHU_AA00074
Porphyromonadaceae	gi 1142726810 emb LT725663.1	<i>Parabacteroides</i> sp. Marseille-P3668 partial 16S rRNA gene, strain Marseille-P3668

TABLE 6-continued

Responder/non-responder bacteria identified by 16S sequencing OTUs.		
Family	Subject Seq-ID	Source
Porphyromonadaceae	gi 159159329 gb EU136685.1	<i>Parabacteroides merdae</i> strain JCM9497 16S ribosomal RNA gene, partial sequence
Porphyromonadaceae	gi 343200777 refl NR_041464.1	<i>Parabacteroides johnsonii</i> strain M-165 16S ribosomal RNA gene, partial sequence<> <i>Parabacteroides johnsonii</i> gene for 16S ribosomal RNA, partial sequence
Porphyromonadaceae	gi 89191778 dbj AB238929.1	<i>Parabacteroides merdae</i> gene for 16S ribosomal RNA, partial sequence, strain: JCM 13405
Bacteroidaceae	gi 1167601288 gb KX658684.1	<i>Bacteroides xylanisolvens</i> strain A3 16S ribosomal RNA gene, partial sequence
Bacteroidaceae	gi 1074125775 emb LT622246.1	<i>Bacteroides ovatus</i> V975 genome assembly, chromosome: 1
Bacteroidaceae	gi 1074125775 emb LT622246.1	<i>Bacteroides ovatus</i> V975 genome assembly, chromosome: 1
Bacteroidaceae	gi 988570982 gb KR364742.1	<i>Bacteroides</i> sp. JJM0207-12 16S ribosomal RNA gene, partial sequence
Bacteroidaceae	gi 745311129 gb KP202688.1	<i>Bacteroides</i> sp. G25 16S ribosomal RNA gene, partial sequence
Bacteroidaceae	gi 672228637 dbj AB908392.1	<i>Bacteroides xylanisolvens</i> gene for 16S ribosomal RNA, partial sequence, strain: EFEL001
Bacteroidaceae	gi 148791577 gb EF608211.1	<i>Bacteroides</i> sp. EBA5-17 16S ribosomal RNA gene, partial sequence
Bacteroidaceae	gi 343201773 refl NR_042499.1	<i>Bacteroides xylanisolvens</i> strain XB1A 16S ribosomal RNA gene, partial sequence<> <i>Bacteroides xylanisolvens</i> partial 16S rRNA gene, type strain XB1AT
Bacteroidaceae	gi 46370580 gb AY538687.1	Bacteroidaceae bacterium Smarlab 3301643 16S ribosomal RNA gene, partial sequence
Bacteroidaceae	gi 1173512652 gb CP015401.2	<i>Bacteroides caecimuris</i> strain I48, complete genome
Bacteroidaceae	gi 988570981 gb KR364741.1	<i>Bacteroides caecimuris</i> strain I48 16S ribosomal RNA gene, partial sequence
Bacteroidaceae	gi 1181557329 gb KY950632.1	<i>Bacteroides acidifaciens</i> strain V 16S ribosomal RNA gene, partial sequence
Bacteroidaceae	gi 1153695773 gb KY703634.1	<i>Anaerostipes</i> sp. strain KFT8 16S ribosomal RNA gene, partial sequence
Bacteroidaceae	gi 988570980 gb KR364740.1	<i>Bacteroides acidifaciens</i> strain JJM0207-2 16S ribosomal RNA gene, partial sequence
Bacteroidaceae	gi 728055098 gb KM396275.1	<i>Bacteroides ovatus</i> strain G19 16S ribosomal RNA gene, partial sequence
Bacteroidaceae	gi 723001884 dbj AB908394.1	<i>Bacteroides ovatus</i> gene for 16S ribosomal RNA, partial sequence, strain: EFEL003
Bacteroidaceae	gi 676402736 gb KM043747.1	Bacterium PIC8 16S ribosomal RNA gene, partial sequence
Bacteroidaceae	gi 159159338 gb EU136694.1	<i>Bacteroides acidifaciens</i> strain JCM10556 16S ribosomal RNA gene, partial sequence
Bacteroidaceae	gi 636560121 refl NR_116181.1	<i>Bacteroides ovatus</i> strain JCM5824 16S ribosomal RNA gene, partial sequence<> <i>Bacteroides ovatus</i> strain JCM5824 16S ribosomal RNA gene, partial sequence
Bacteroidaceae	gi 636559244 refl NR_115301.1	<i>Bacteroides ovatus</i> strain CIP 103756 16S ribosomal RNA gene, partial sequence<> <i>Bacteroides ovatus</i> 16S ribosomal RNA gene, partial sequence
Bacteroidaceae	gi 83627372 dbj AB222700.1	<i>Bacteroides finegoldii</i> gene for 16S rRNA, partial sequence, strain: JCM 13346
Ruminococcaceae	gi 34558694 gb AY305307.1	Butyrate-producing bacterium M21/2 16S ribosomal RNA gene, partial sequence
Ruminococcaceae	gi 1031487358 gb KX150462.1	<i>Faecalibacterium</i> CM04-06 16S ribosomal RNA gene, partial sequence
Ruminococcaceae	gi 1031486988 gb KX146426.1	<i>Faecalibacterium</i> AF52-21 16S ribosomal RNA gene, partial sequence
Ruminococcaceae	gi 685210693 gb KJ957877.1	<i>Faecalibacterium prausnitzii</i> strain 78_10294632078_070 16S ribosomal RNA gene, partial sequence

TABLE 6-continued

Responder/non-responder bacteria identified by 16S sequencing OTUs.		
Family	Subject Seq-ID	Source
Ruminococcaceae	gil685210692 gb KJ957876.1	<i>Faecalibacterium prausnitzii</i> strain 67_10294632067_075 16S ribosomal RNA gene, partial sequence
Ruminococcaceae	gil685210691 gb KJ957875.1	<i>Faecalibacterium prausnitzii</i> strain 63_10294632063_052 16S ribosomal RNA gene, partial sequence
Ruminococcaceae	gil685210690 gb KJ957874.1	<i>Faecalibacterium prausnitzii</i> strain 59_10294632059_060 16S ribosomal RNA gene, partial sequence
Ruminococcaceae	gil685210689 gb KJ957873.1	<i>Faecalibacterium prausnitzii</i> strain 56_10294632056_049 16S ribosomal RNA gene, partial sequence
Ruminococcaceae	gil685210687 gb KJ957871.1	<i>Faecalibacterium prausnitzii</i> strain 51_10294632051_059 16S ribosomal RNA gene, partial sequence
Ruminococcaceae	gil685210685 gb KJ957869.1	<i>Faecalibacterium prausnitzii</i> strain 45_10299630045_040 16S ribosomal RNA gene, partial sequence
Ruminococcaceae	gil685210684 gb KJ957868.1	<i>Faecalibacterium prausnitzii</i> strain 31_10294632031_020 16S ribosomal RNA gene, partial sequence
Ruminococcaceae	gil685210683 gb KJ957867.1	<i>Faecalibacterium prausnitzii</i> strain 29_10294632029_024 16S ribosomal RNA gene, partial sequence
Ruminococcaceae	gil685210682 gb KJ957866.1	<i>Faecalibacterium prausnitzii</i> strain 28_10294632028_026 16S ribosomal RNA gene, partial sequence
Ruminococcaceae	gil685210681 gb KJ957865.1	<i>Faecalibacterium prausnitzii</i> strain 27_10294632027_028 16S ribosomal RNA gene, partial sequence
Ruminococcaceae	gil685210680 gb KJ957864.1	<i>Faecalibacterium prausnitzii</i> strain 25_10294632025_032 16S ribosomal RNA gene, partial sequence
Ruminococcaceae	gil685210679 gb KJ957863.1	<i>Faecalibacterium prausnitzii</i> strain 24_10297420024_017 16S ribosomal RNA gene, partial sequence
Ruminococcaceae	gil685210678 gb KJ957862.1	<i>Faecalibacterium prausnitzii</i> strain 24_10294632024_017 16S ribosomal RNA gene, partial sequence
Ruminococcaceae	gil685210677 gb KJ957861.1	<i>Faecalibacterium prausnitzii</i> strain 23_10294632023_019 16S ribosomal RNA gene, partial sequence
Ruminococcaceae	gil685210676 gb KJ957860.1	<i>Faecalibacterium prausnitzii</i> strain 22_10294632022_021 16S ribosomal RNA gene, partial sequence
Ruminococcaceae	gil685210675 gb KJ957859.1	<i>Faecalibacterium prausnitzii</i> strain 20_10294632020_025 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gil823630656 emb LN850732.1	<i>Eubacterium</i> sp. SB2 partial 16S rRNA gene, strain SB2
Lachnospiraceae	gil676402749 gb KM043760.1	Bacterium P1B3 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gil343198924 ref NR_044048.1	<i>Coprococcus comes</i> strain ATCC 27758 16S ribosomal RNA gene, partial sequence<> <i>Coprococcus comes</i> strain ATCC 27758 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gil34558699 gb AY305312.1	Butyrate-producing bacterium SL7/1 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gil34558692 gb AY305305.1	Butyrate-producing bacterium A2-232 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gil1139937221 emb LT722679.1	<i>Lactonifactor</i> sp. Marseille-P3743 partial 16S rRNA gene, strain Marseille-P3743
Lachnospiraceae	gil1008904204 emb LT223592.1	<i>Lactonifactor longoviformis</i> partial 16S rRNA gene, strain Marseille-P2234
Lachnospiraceae	gil133779806 gb EF451053.1	<i>Clostridiaceae bacterium</i> END-2 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gil988571010 gb KR364770.1	<i>Muricomes intestini</i> strain 2PG-424-CC-1 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gil1192786034 dbj LC269264.1	<i>Ruminococcus gausvreauii</i> gene for 16S ribosomal RNA, partial sequence, strain: JCM 14987
Lachnospiraceae	gil343205824 ref NR_044265.1	<i>Ruminococcus gausvreauii</i> strain CCR1-16110 16S ribosomal RNA gene, partial

TABLE 6-continued

Responder/non-responder bacteria identified by 16S sequencing OTUs.		
Family	Subject Seq-ID	Source
Lachnospiraceae	gi 631251356 refl NR_112553.1	sequence<> <i>Ruminococcus gauvreaui</i> strain CCRI-16110 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gi 57283069 embl AJ518873.1	[<i>Clostridium</i>] <i>glycyrrhizinilyticum</i> strain ZM35 16S ribosomal RNA gene, complete sequences<> <i>Clostridium glycyrrhizinilyticum</i> gene for 16S ribosomal RNA
Lachnospiraceae	gi 73427036 gb DQ144122.1	Uncultured bacterium clone p-2205-s959-3 16S ribosomal RNA gene, partial sequence<> <i>Lachnospiraceae</i> sp. wal 14165 16S rRNA gene
Lachnospiraceae	gi 1126366538 dbj LC192831.1	Butyrate-producing bacterium PH05YB03 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gi 1004171141 gb KT889289.1	<i>Ruminococcus</i> sp. JCM 30896 gene for 16S ribosomal RNA, partial sequence, strain: EGH7<> <i>Ruminococcus</i> sp. JCM 30896 gene for 16S ribosomal RNA, partial sequence, strain: TSAH33
Lachnospiraceae	gi 657357060 dbj AB849410.1	Bacterium 14(2016) 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gi 33242862 gb AY269188.1	Lachnospiraceae bacterium CG22 gene for 16S ribosomal RNA, partial sequence
Lachnospiraceae	gi 90855248 embl AM039822.1	Human intestinal bacterium julong 601 16S ribosomal RNA gene, complete sequence
Lachnospiraceae	gi 55818570 gb AY804152.1	<i>Butyrivibrio fibrisolvens</i> 16S rRNA gene, strain Mz3
Enterobacteriaceae	gi 1151220730 gb CP019012.1	<i>Eubacterium rectale</i> strain S2Ss/2 16S ribosomal RNA gene, partial sequence
Enterobacteriaceae	gi 1151220730 gb CP019012.1	<i>Escherichia coli</i> strain Ecol_AZ161, complete genome
Enterobacteriaceae	gi 1151220730 gb CP019012.1	<i>Escherichia coli</i> strain Ecol_AZ161, complete genome
Enterobacteriaceae	gi 1160538609 gb CP011124.1	<i>Escherichia coli</i> strain Ecol_AZ161, complete genome
Enterobacteriaceae	gi 1154168274 gb KY711200.1	<i>Escherichia coli</i> strain USML2, complete genome
Enterobacteriaceae	gi 1154168267 gb KY711193.1	<i>Escherichia coli</i> strain AMuM12 16S ribosomal RNA gene, partial sequence
Enterobacteriaceae	gi 1039023268 gb KU744859.1	<i>Escherichia coli</i> strain AMuM2 16S ribosomal RNA gene, partial sequence
Enterobacteriaceae	gi 1015522073 gb KU923357.1	<i>Escherichia coli</i> strain SFSA62 16S ribosomal RNA gene, partial sequence
Enterobacteriaceae	gi 1015522072 gb KU923356.1	<i>Escherichia coli</i> strain USTRW17 16S ribosomal RNA gene, partial sequence
Enterobacteriaceae	gi 1015522068 gb KU923352.1	<i>Escherichia coli</i> strain USTRW16 16S ribosomal RNA gene, partial sequence
Enterobacteriaceae	gi 1154504417 gb CP019777.1	<i>Escherichia coli</i> strain USTRW12 16S ribosomal RNA gene, partial sequence
Enterobacteriaceae	gi 1154835240 embl LT795502.1	<i>Escherichia coli</i> NU14, complete genome
Enterobacteriaceae	gi 1150562860 gb KY678505.1	<i>Escherichia coli</i> strain KV7 genome assembly, chromosome: I
Enterobacteriaceae	gi 1150562859 gb KY678504.1	<i>Escherichia coli</i> strain WXL15 16S ribosomal RNA gene, partial sequence
Enterobacteriaceae	gi 1150562858 gb KY678503.1	<i>Escherichia coli</i> strain WXL13 16S ribosomal RNA gene, partial sequence
Enterobacteriaceae	gi 1150562857 gb KY678502.1	<i>Escherichia coli</i> strain WXL12 16S ribosomal RNA gene, partial sequence
Enterobacteriaceae	gi 1150562856 gb KY678501.1	<i>Escherichia coli</i> strain WXL11 16S ribosomal RNA gene, partial sequence
Enterobacteriaceae	gi 1150562855 gb KY678500.1	<i>Escherichia coli</i> strain WHD 16S ribosomal RNA gene, partial sequence
Enterobacteriaceae	gi 1150562854 gb KY678499.1	<i>Escherichia coli</i> strain XH2H 16S ribosomal RNA gene, partial sequence
Enterobacteriaceae	gi 1150562853 gb KY678498.1	<i>Escherichia coli</i> strain B22H 16S ribosomal RNA gene, partial sequence
Enterobacteriaceae	gi 1150562852 gb KY678497.1	<i>Escherichia coli</i> strain 2H 16S ribosomal RNA gene, partial sequence
Enterobacteriaceae	gi 1153112403 gb CP019953.1	<i>Escherichia coli</i> strain BDZH 16S ribosomal RNA gene, partial sequence
Porphyromonadaceae	gi 159159329 gb EU136685.1	<i>Escherichia coli</i> M8, complete genome
		<i>Parabacteroides merdae</i> strain JCM9497 16S ribosomal RNA gene, partial sequence

TABLE 6-continued

Responder/non-responder bacteria identified by 16S sequencing OTUs.		
Family	Subject Seq-ID	Source
Porphyromonadaceae	gi 89191778 dbj AB238929.1	<i>Parabacteroides merdae</i> gene for 16S ribosomal RNA, partial sequence, strain: JCM 13405
Porphyromonadaceae	gi 343200656 refl NR_041343.1	<i>Parabacteroides merdae</i> strain JCM 9497 16S ribosomal RNA gene, partial sequence<> <i>Parabacteroides merdae</i> gene for 16S ribosomal RNA, partial sequence, strain: JCM 9497
Porphyromonadaceae	gi 343200777 refl NR_041464.1	<i>Parabacteroides johnsonii</i> strain M-165 16S ribosomal RNA gene, partial sequence<> <i>Parabacteroides johnsonii</i> gene for 16S ribosomal RNA, partial sequence
Porphyromonadaceae	gi 1181557323 gb KY950626.1	<i>Bacteroides</i> sp. strain P 16S ribosomal RNA gene, partial sequence
Porphyromonadaceae	gi 816399474 gb GQ456205.2	<i>Parabacteroides goldsteinii</i> strain BS-C3-2 16S ribosomal RNA gene, partial sequence
Porphyromonadaceae	gi 159159377 gb EU136697.1	<i>Parabacteroides goldsteinii</i> strain JCM13446 16S ribosomal RNA gene, partial sequence
Porphyromonadaceae	gi 343202832 refl NR_043317.1	<i>Parabacteroides goldsteinii</i> strain WAL 12034 16S ribosomal RNA gene, partial sequence<> <i>Parabacteroides goldsteinii</i> strain WAL 12034 16S ribosomal RNA gene, partial sequence
Porphyromonadaceae	gi 1046811522 gb KX462878.1	<i>Parabacteroides</i> sp. strain B3181 16S ribosomal RNA gene, partial sequence
Porphyromonadaceae	gi 1142726810 emb LT725663.1	<i>Parabacteroides</i> sp. Marseille-P3668 partial 16S rRNA gene, strain Marseille-P3668
Porphyromonadaceae	gi 1024389103 emb LT558809.1	<i>Bacillus nealsonii</i> partial 16S rRNA gene, strain Marseille-P2085
Porphyromonadaceae	gi 1008904221 emb LT223609.1	<i>Parabacteroides faecis</i> partial 16S rRNA gene, strain Marseille-IHU_AA00074
Porphyromonadaceae	gi 1154838059 emb LT598573.4	<i>Parabacteroides</i> sp. Marseille-P3236 partial 16S rRNA gene, strain Marseille-P3136
Porphyromonadaceae	gi 1139937223 emb LT722681.1	<i>Parabacteroides</i> sp. Marseille-P3763 partial 16S rRNA gene, strain Marseille-P3763
Porphyromonadaceae	gi 10946530 gb AY008308.1	<i>Bacteroides</i> cf. <i>forsythus</i> oral clone BU063 16S ribosomal RNA gene, partial sequence
Porphyromonadaceae	gi 1061022501 gb CP017038.1	<i>Tannerella</i> sp. oral taxon BU063, complete genome
Porphyromonadaceae	gi 751868059 dbj LC021528.1	<i>Dysgonomonas alginatilytica</i> gene for 16S ribosomal RNA, partial sequence, strain: HUA-2
Porphyromonadaceae	gi 677007119 gb KJ888430.1	<i>Dysgonomonas</i> sp. DT183 16S ribosomal RNA gene, partial sequence
Porphyromonadaceae	gi 173915 gb M86695.1 BNRRR16S	<i>Bacteroides distasonis</i> 16S ribosomal RNA<> <i>Parabacteroides</i> sp. MC_17 partial 16S rRNA gene, strain DSM-20701, isolate MC_17
Porphyromonadaceae	gi 1153695770 gb KY703631.1	<i>Parabacteroides</i> sp. strain CT06 16S ribosomal RNA gene, partial sequence
Bifidobacteriaceae	gi 1153683744 gb KY705021.1	<i>Bifidobacterium longum</i> subsp. <i>suis</i> strain VB-5/9 16S ribosomal RNA gene, partial sequence
Bifidobacteriaceae	gi 1153683742 gb KY705019.1	<i>Bifidobacterium breve</i> strain VB-TA1 16S ribosomal RNA gene, partial sequence
Bifidobacteriaceae	gi 1153683740 gb KY705017.1	<i>Bifidobacterium adolescentis</i> strain VB-ES42 16S ribosomal RNA gene, partial sequence
Bifidobacteriaceae	gi 1141357803 gb KY448280.1	<i>Bifidobacterium longum</i> strain CFR815k 16S ribosomal RNA gene, partial sequence
Bifidobacteriaceae	gi 1141357802 gb KY448279.1	<i>Bifidobacterium longum</i> strain CFR56 16S ribosomal RNA gene, partial sequence
Bifidobacteriaceae	gi 1141357801 gb KY448278.1	<i>Bifidobacterium longum</i> strain CFR50 16S ribosomal RNA gene, partial sequence

TABLE 6-continued

Responder/non-responder bacteria identified by 16S sequencing OTUs.		
Family	Subject Seq-ID	Source
Bifidobacteriaceae	gi 1141357800 gb KY448277.1	<i>Bifidobacterium longum</i> strain CFR24 16S ribosomal RNA gene, partial sequence
Bifidobacteriaceae	gi 1141357799 gb KY448276.1	<i>Bifidobacterium longum</i> strain CFR 20 16S ribosomal RNA gene, partial sequence
Bifidobacteriaceae	gi 1141357789 gb KU297199.1	<i>Bifidobacterium longum</i> strain 815j 16S ribosomal RNA gene, partial sequence
Bifidobacteriaceae	gi 1141357788 gb KU297198.1	<i>Bifidobacterium breve</i> strain 142 16S ribosomal RNA gene, partial sequence
Bifidobacteriaceae	gi 1143275817 gb CP019596.1	<i>Bifidobacterium breve</i> strain LMC520, complete genome
Bifidobacteriaceae	gi 1137292025 gb KY523590.1	<i>Bifidobacterium adolescentis</i> strain S52 16S ribosomal RNA gene, partial sequence
Bifidobacteriaceae	gi 1137292022 gb KY523587.1	<i>Bifidobacterium adolescentis</i> strain S42 16S ribosomal RNA gene, partial sequence
Bifidobacteriaceae	gi 1137292017 gb KY523582.1	<i>Bifidobacterium</i> sp. strain S35 16S ribosomal RNA gene, partial sequence
Bifidobacteriaceae	gi 1137291996 gb KY523561.1	<i>Bifidobacterium longum</i> strain S53 16S ribosomal RNA gene, partial sequence
Bifidobacteriaceae	gi 1137291995 gb KY523560.1	<i>Bifidobacterium adolescentis</i> strain S50 16S ribosomal RNA gene, partial sequence
Bifidobacteriaceae	gi 1137291993 gb KY523558.1	<i>Bifidobacterium adolescentis</i> strain S47 16S ribosomal RNA gene, partial sequence
Bifidobacteriaceae	gi 1137291991 gb KY523556.1	<i>Bifidobacterium longum</i> strain S34 16S ribosomal RNA gene, partial sequence
Bifidobacteriaceae	gi 1137647753 ref NR_145535.1	<i>Bifidobacterium longum</i> subsp. <i>suillum</i> strain Su 851 16S ribosomal RNA, partial sequence
Bifidobacteriaceae	gi 1024389105 emb LT558811.1	<i>Bifidobacterium adolescentis</i> partial 16S rRNA gene, strain Marseille-P830
Bifidobacteriaceae	gi 1153683744 gb KY705021.1	<i>Bifidobacterium longum</i> subsp. <i>suis</i> strain VB-5/9 16S ribosomal RNA gene, partial sequence
Bifidobacteriaceae	gi 1153683742 gb KY705019.1	<i>Bifidobacterium breve</i> strain VB-TA1 16S ribosomal RNA gene, partial sequence
Bifidobacteriaceae	gi 1153683740 gb KY705017.1	<i>Bifidobacterium adolescentis</i> strain VB-ES42 16S ribosomal RNA gene, partial sequence
Bifidobacteriaceae	gi 1141357803 gb KY448280.1	<i>Bifidobacterium longum</i> strain CFR815k 16S ribosomal RNA gene, partial sequence
Bifidobacteriaceae	gi 1141357802 gb KY448279.1	<i>Bifidobacterium longum</i> strain CFR56 16S ribosomal RNA gene, partial sequence
Bifidobacteriaceae	gi 1141357801 gb KY448278.1	<i>Bifidobacterium longum</i> strain CFR50 16S ribosomal RNA gene, partial sequence
Bifidobacteriaceae	gi 1141357800 gb KY448277.1	<i>Bifidobacterium longum</i> strain CFR24 16S ribosomal RNA gene, partial sequence
Bifidobacteriaceae	gi 1141357799 gb KY448276.1	<i>Bifidobacterium longum</i> strain CFR 20 16S ribosomal RNA gene, partial sequence
Bifidobacteriaceae	gi 1141357789 gb KU297199.1	<i>Bifidobacterium longum</i> strain 815j 16S ribosomal RNA gene, partial sequence
Bifidobacteriaceae	gi 1141357788 gb KU297198.1	<i>Bifidobacterium breve</i> strain 142 16S ribosomal RNA gene, partial sequence
Bifidobacteriaceae	gi 1143275817 gb CP019596.1	<i>Bifidobacterium breve</i> strain LMC520, complete genome
Bifidobacteriaceae	gi 1137292025 gb KY523590.1	<i>Bifidobacterium adolescentis</i> strain S52 16S ribosomal RNA gene, partial sequence
Bifidobacteriaceae	gi 1137292022 gb KY523587.1	<i>Bifidobacterium adolescentis</i> strain S42 16S ribosomal RNA gene, partial sequence
Bifidobacteriaceae	gi 1137292017 gb KY523582.1	<i>Bifidobacterium</i> sp. strain S35 16S ribosomal RNA gene, partial sequence
Bifidobacteriaceae	gi 1137291996 gb KY523561.1	<i>Bifidobacterium longum</i> strain S53 16S ribosomal RNA gene, partial sequence

TABLE 6-continued

Responder/non-responder bacteria identified by 16S sequencing OTUs.		
Family	Subject Seq-ID	Source
Bifidobacteriaceae	gi 1137291995 gb KY523560.1	<i>Bifidobacterium adolescentis</i> strain S50 16S ribosomal RNA gene, partial sequence
Bifidobacteriaceae	gi 1137291993 gb KY523558.1	<i>Bifidobacterium adolescentis</i> strain S47 16S ribosomal RNA gene, partial sequence
Bifidobacteriaceae	gi 1137291991 gb KY523556.1	<i>Bifidobacterium longum</i> strain S34 16S ribosomal RNA gene, partial sequence
Bifidobacteriaceae	gi 1137647753 ref NR_145535.1	<i>Bifidobacterium longum</i> subsp. <i>suillum</i> strain Su 851 16S ribosomal RNA, partial sequence
Bifidobacteriaceae	gi 1024389105 emb LT558811.1	<i>Bifidobacterium adolescentis</i> partial 16S rRNA gene, strain Marseille-P830
Prevotellaceae	gi 82618889 gb DQ278861.1	<i>Prevotella</i> sp. 152R-1a 16S ribosomal RNA gene, partial sequence
Prevotellaceae	gi 343198858 ref NR_043894.1	<i>Prevotella timonensis</i> strain 4401737 16S ribosomal RNA gene, partial sequence<> <i>Prevotella timonensis</i> strain 4401737 16S ribosomal RNA gene, partial sequence
Prevotellaceae	gi 343200677 ref NR_041364.1	<i>Prevotella stercorea</i> strain CB35 16S ribosomal RNA gene, partial sequence<> <i>Prevotella stercorea</i> gene for 16S ribosomal RNA, partial sequence, strain: CB35
Prevotellaceae	gi 1139287453 emb LT699557.1	<i>Streptococcus anginosus</i> partial 16S rRNA gene, isolate R161T_24975
Prevotellaceae	gi 166063928 dbj AB298732.2	<i>Prevotellaceae bacterium</i> WR041 gene for 16S rRNA, partial sequence
Prevotellaceae	gi 343200237 ref NR_040924.1	<i>Prevotella paludivivens</i> strain KB7 16S ribosomal RNA gene, partial sequence<> <i>Prevotella paludivivens</i> gene for 16S ribosomal RNA, partial sequence, strain: KB7
Prevotellaceae	gi 343198484 ref NR_041907.1	<i>Prevotella marshii</i> strain E9.34 16S ribosomal RNA gene, partial sequence<> <i>Prevotella marshii</i> strain E9.34 16S ribosomal RNA gene, partial sequence
Prevotellaceae	gi 1139291348 emb LT677943.1	<i>Prevotella nigrescens</i> partial 16S rRNA gene, isolate 219N_3357
Prevotellaceae	gi 1139291337 emb LT677932.1	<i>Prevotella nigrescens</i> partial 16S rRNA gene, isolate 219N_3346
Prevotellaceae	gi 1139291306 emb LT677901.1	<i>Prevotella nigrescens</i> partial 16S rRNA gene, isolate 219N_3315
Prevotellaceae	gi 1139290143 emb LT676740.1	<i>Prevotella nigrescens</i> partial 16S rRNA gene, isolate 492N_2154
Prevotellaceae	gi 1139290140 emb LT676737.1	<i>Prevotella nigrescens</i> partial 16S rRNA gene, isolate 492N_2151
Prevotellaceae	gi 1139289788 emb LT676439.1	<i>Prevotella nigrescens</i> partial 16S rRNA gene, isolate W731N_1853
Prevotellaceae	gi 1139289722 emb LT676373.1	<i>Prevotella nigrescens</i> partial 16S rRNA gene, isolate W731N_1787
Prevotellaceae	gi 1139288883 emb LT675486.1	<i>Prevotella nigrescens</i> partial 16S rRNA gene, isolate W731T_900
Prevotellaceae	gi 1139288535 emb LT675139.1	<i>Prevotella nigrescens</i> partial 16S rRNA gene, isolate 256N_553
Prevotellaceae	gi 1139287425 emb LT699529.1	<i>Prevotella nigrescens</i> partial 16S rRNA gene, isolate R161T_24947
Prevotellaceae	gi 1139285456 emb LT699586.1	<i>Prevotella nigrescens</i> partial 16S rRNA gene, isolate R161T_25004
Prevotellaceae	gi 1139284226 emb LT698245.1	<i>Prevotella nigrescens</i> partial 16S rRNA gene, isolate W066N_23663
Prevotellaceae	gi 1139284222 emb LT698241.1	<i>Prevotella nigrescens</i> partial 16S rRNA gene, isolate W066N_23659
Veillonellaceae	gi 1148994964 gb CP019721.1	<i>Veillonella parvula</i> strain UTDB1-3, complete genome
Veillonellaceae	gi 1148994964 gb CP019721.1	<i>Veillonella parvula</i> strain UTDB1-3, complete genome
Veillonellaceae	gi 1148994964 gb CP019721.1	<i>Veillonella parvula</i> strain UTDB1-3, complete genome
Veillonellaceae	gi 1139270738 emb LT684451.1	<i>Veillonella dispar</i> partial 16S rRNA gene, isolate 189N_9866
Veillonellaceae	gi 17017249 gb AF439645.1	<i>Veillonella</i> sp. ADV 360.00 16S ribosomal RNA gene, partial sequence

TABLE 6-continued

Responder/non-responder bacteria identified by 16S sequencing OTUs.		
Family	Subject Seq-ID	Source
Veillonellaceae	gi 17017246 gb AF439642.1	<i>Veillonella</i> sp. ADV 360.00 16S ribosomal RNA gene, partial sequence
Veillonellaceae	gi 45861433 gb AY571668.1	<i>Veillonella</i> sp. ADV 269.01 16S ribosomal RNA gene, partial sequence
Veillonellaceae	gi 1175700537 gb CP020566.1	<i>Veillonella atypica</i> strain OK5, complete genome
Veillonellaceae	gi 1139289771 emb LT676422.1	<i>Veillonella dispar</i> partial 16S rRNA gene, isolate W731N_1836
Veillonellaceae	gi 1139281643 emb LT695664.1	<i>Veillonella dispar</i> partial 16S rRNA gene, isolate 468N_21082
Veillonellaceae	gi 1139281465 emb LT695486.1	<i>Veillonella dispar</i> partial 16S rRNA gene, isolate 39T_20904
Veillonellaceae	gi 1139276978 emb LT691051.1	<i>Veillonella dispar</i> partial 16S rRNA gene, isolate 210T_16467
Veillonellaceae	gi 1139272749 emb LT686946.1	<i>Veillonella dispar</i> partial 16S rRNA gene, isolate W153N_12361
Veillonellaceae	gi 1139271787 emb LT685808.1	<i>Veillonella dispar</i> partial 16S rRNA gene, isolate W297T_11223
Veillonellaceae	gi 1139269990 emb LT684009.1	<i>Veillonella dispar</i> partial 16S rRNA gene, isolate 377N_9424
Veillonellaceae	gi 1139269916 emb LT683633.1	<i>Veillonella dispar</i> partial 16S rRNA gene, isolate 482T_9048
Veillonellaceae	gi 1139269897 emb LT683614.1	<i>Veillonella dispar</i> partial 16S rRNA gene, isolate 482T_9029
Veillonellaceae	gi 1005392159 gb KU851244.1	<i>Veillonella atypica</i> strain TCD56.9 16S ribosomal RNA gene, partial sequence
Veillonellaceae	gi 343201163 ref NR_041879.1	<i>Veillonella dispar</i> strain ATCC 17748 16S ribosomal RNA gene, partial sequence<> <i>Veillonella dispar</i> 16S ribosomal RNA gene, partial sequence
Veillonellaceae	gi 1139289571 emb LT675824.1	<i>Veillonella dispar</i> partial 16S rRNA gene, isolate 467N_1238
Veillonellaceae	gi 1005392160 gb KU851245.1	<i>Veillonella atypica</i> strain TCD60.3 16S ribosomal RNA gene, partial sequence
Veillonellaceae	gi 1005392161 gb KU851246.1	<i>Veillonella atypica</i> strain R39.8 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gi 1158444432 emb LT799971.1	<i>Clostridium</i> sp. Marseille-P2378 partial 16S rRNA gene, strain Marseille-P2378
Lachnospiraceae	gi 1008904189 emb LT223578.1	<i>Ruminococcus</i> sp. Marseille-P328 partial 16S rRNA gene, strain Marseille-P328
Lachnospiraceae	gi 162949827 gb EU305624.1	Clostridiaceae bacterium K10 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gi 34558701 gb AY305314.1	Butyrate-producing bacterium SM4/1 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gi 34558696 gb AY305309.1	Butyrate-producing bacterium M62/1 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gi 1047487928 gb KX356509.1	Lachnospiraceae bacterium KNHs209 clone 2574497994 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gi 1047487919 gb KX356507.1	Lachnospiraceae bacterium KNHs209 clone 2574499676 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gi 1047487915 gb KX356506.1	Lachnospiraceae bacterium KNHs209 clone 2574499865 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gi 1154838044 emb LT631543.2	<i>Blautia</i> sp. Marseille-P3441 partial 16S rRNA gene, strain Marseille-P3441
Lachnospiraceae	gi 1147668874 gb KY621471.1	<i>Clostridium</i> sp. strain CS1GBYEI2 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gi 1047487923 gb KX356508.1	Lachnospiraceae bacterium KNHs209 clone 2574499031 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gi 1047487911 gb KX356505.1	Lachnospiraceae bacterium KNHs209 clone 2574501149 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gi 1023747088 gb KX009920.1	Lachnospiraceae bacterium DW22 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gi 1023747086 gb KX009918.1	Lachnospiraceae bacterium DW17 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gi 1008904263 emb LT223651.1	[<i>Clostridium</i>] <i>amygdalinum</i> partial 16S rRNA gene, strain Marseille-P2095
Lachnospiraceae	gi 1004171138 gb KT889286.1	Bacterium 11(2016) 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gi 1004171136 gb KT889284.1	Bacterium 9(2016) 16S ribosomal RNA gene, partial sequence

TABLE 6-continued

Responder/non-responder bacteria identified by 16S sequencing OTUs.		
Family	Subject Seq-ID	Source
Lachnospiraceae	gi 985742338 gb KT633847.1	[<i>Clostridium</i>] <i>celerecrescens</i> strain MCM B565 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gi 630257004 gb KJ722511.1	<i>Clostridium</i> sp. Nesulana3-1 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gi 630256999 gb KJ722506.1	<i>Desulfotomaculum</i> sp. Gec1-7ana4-1 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gi 34558709 gb AY305322.1	Butyrate-producing bacterium SR1/5 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gi 34558708 gb AY305321.1	Butyrate-producing bacterium SR1/1 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gi 73427042 gb DQ144128.1	Butyrate-producing bacterium PH07BW09 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gi 115607479 gb DQ986224.1	<i>Clostridiales</i> bacterium A2-162 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gi 1193829152 ref NR_147395.1	<i>Blautia marasmii</i> strain Marseille-P2377 16S ribosomal RNA, partial sequence
Lachnospiraceae	gi 1173534576 gb CP015405.2	<i>Blautia</i> sp. YL58, complete genome
Lachnospiraceae	gi 1153695771 gb KY703632.1	<i>Blautia</i> sp. strain KB1 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gi 104652739 gb KX594322.1	<i>Blautia coccoides</i> strain Ga-1 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gi 1023747089 gb KX009921.1	Lachnospiraceae bacterium DW28 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gi 988571034 gb KU196081.1	<i>Blautia coccoides</i> strain DSM 29138 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gi 988570987 gb KR364747.1	<i>Blautia</i> sp. YL58 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gi 816399476 gb GQ456208.2	<i>Blautia</i> sp. A-C6-0 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gi 676402738 gb KM043749.1	Bacterium P1G4 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gi 122725186 gb EF025906.1	<i>Clostridium coccoides</i> strain 8F 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gi 133779805 gb EF451052.1	<i>Ruminococcus</i> sp. END-1 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gi 62865582 gb AY937379.1	<i>Ruminococcus productus</i> strain SECO-Mt75m3 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gi 55975496 gb AY653234.1	<i>Ruminococcus</i> sp. MLG080-3 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gi 1079701574 emb LT623891.1	<i>Blautia</i> sp. Marseille-P3201T partial 16S rRNA gene, strain Marseille-P3201T
Lachnospiraceae	gi 1004171139 gb KT889287.1	Bacterium 12(2016) 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gi 988570986 gb KR364746.1	<i>Blautia caecimuris</i> strain SJ18 16S ribosomal RNA gene, partial sequence
Verrucomicrobiaceae	gi 1173526690 gb CP015409.2	<i>Akkermansia muciniphila</i> strain YL44, complete genome
Verrucomicrobiaceae	gi 988570971 gb KR364731.1	<i>Akkermansia muciniphila</i> strain YL44 16S ribosomal RNA gene, partial sequence
Verrucomicrobiaceae	gi 343202494 ref NR_042817.1	<i>Akkermansia muciniphila</i> strain Muc 16S ribosomal RNA gene, complete sequence
Verrucomicrobiaceae	gi 343200984 ref NR_041671.1	<i>Haloferula rosea</i> strain 06SJR1-1 16S ribosomal RNA gene, partial sequence
Verrucomicrobiaceae	gi 343200983 ref NR_041670.1	<i>Haloferula harenae</i> strain YM23-227 16S ribosomal RNA gene, partial sequence
Verrucomicrobiaceae	gi 343200986 ref NR_041673.1	<i>Haloferula helveola</i> strain 05IJR53-1 16S ribosomal RNA gene, partial sequence
Verrucomicrobiaceae	gi 152002650 dbj AB331894.1	<i>Luteolibacter algae</i> gene for 16S rRNA, partial sequence, strain: A5J-40
Verrucomicrobiaceae	gi 343200937 ref NR_041624.1	<i>Luteolibacter algae</i> strain A5J-41-2 16S ribosomal RNA gene, partial

TABLE 6-continued

Responder/non-responder bacteria identified by 16S sequencing OTUs.		
Family	Subject Seq-ID	Source
		sequence<> <i>Luteolibacter algae</i> gene for 16S rRNA, partial sequence, strain: A5J-41-2
Verrucomicrobiaceae	gi 163937810 dbj AB372857.1	<i>Haloferula sargassicola</i> gene for 16S rRNA, partial sequence, strain: MN1-1047
Verrucomicrobiaceae	gi 343200987 refl NR_041674.1	<i>Haloferula sargassicola</i> strain MN1-1037 16S ribosomal RNA gene, partial sequence<> <i>Haloferula sargassicola</i> gene for 16S rRNA, partial sequence, strain: MN1-1037
Verrucomicrobiaceae	gi 163929779 dbj AB373024.1	Verrucomicrobia bacterium MN 1-1006 gene for 16S rRNA, partial sequence
Verrucomicrobiaceae	gi 343200849 refl NR_041536.1	<i>Rubritalea squalenifaciens</i> strain HOact23 16S ribosomal RNA gene, partial sequence<> <i>Rubritalea squalenifaciens</i> gene for 16S rRNA, partial sequence
Verrucomicrobiaceae	gi 343200985 refl NR_041672.1	<i>Haloferula phyci</i> strain AK18-024 16S ribosomal RNA gene, partial sequence<> <i>Haloferula phyci</i> gene for 16S rRNA, partial sequence, strain: AK18-024
Verrucomicrobiaceae	gi 343200943 refl NR_041630.1	<i>Rubritalea sabuli</i> strain YM29-052 16S ribosomal RNA gene, partial sequence<> <i>Rubritalea sabuli</i> gene for 16S rRNA, partial sequence
Verrucomicrobiaceae	gi 343200938 refl NR_041625.1	<i>Luteolibacter pohmpiensis</i> strain A4T-83 16S ribosomal RNA gene, partial sequence<> <i>Luteolibacter pohmpiensis</i> gene for 16S rRNA, partial sequence, strain: A4T-83
Verrucomicrobiaceae	gi 760303835 gb KP030837.1	Verrucomicrobia bacterium PAORIC-16 16S ribosomal RNA gene, partial sequence
Verrucomicrobiaceae	gi 760303834 gb KP030836.1	Verrucomicrobia bacterium PAORIC-15 16S ribosomal RNA gene, partial sequence
Verrucomicrobiaceae	gi 152002643 dbj AB331887.1	<i>Persicirhabdus sediminis</i> gene for 16S rRNA, partial sequence, strain: YM21-151
Verrucomicrobiaceae	gi 343200933 refl NR_041620.1	<i>Persicirhabdus sediminis</i> strain YM20-087 16S ribosomal RNA gene, partial sequence<> <i>Persicirhabdus sediminis</i> gene for 16S rRNA, partial sequence, strain: YM20-087
Verrucomicrobiaceae	gi 164510703 emb AJ966882.1	<i>Prostheco bacter debontii</i> 16S rRNA gene (partial), 23 S rRNA gene (partial), tRNA-Ala gene, tRNA-Ile gene and ITS1, strain DSM 14044
	gi 165929482 emb AM932460.1	Alpha proteobacterium Jbg30 partial 16S rRNA gene, isolate Jbg30
	gi 74052580 gb DQ167235.1	<i>Brucella</i> sp. HJ114 16S ribosomal RNA gene, partial sequence
	gi 115529818 gb DQ869302.1	<i>Roseospira</i> sp. JL052 16S ribosomal RNA gene, partial sequence
	gi 1179766740 refl NR_146690.1	<i>Dongia soli</i> strain D78 16S ribosomal RNA, partial sequence
	gi 760303826 gb KP030828.1	Alpha proteobacterium SAORIC-614 16S ribosomal RNA gene, partial sequence
	gi 165929480 emb AM932458.1	Alpha proteobacterium Jbg28 partial 16S rRNA gene, isolate Jbg28
	gi 343205857 refl NR_044315.1	<i>Insolitispirillum peregrinum</i> subsp. <i>integrum</i> strain LMG 5407 16S ribosomal RNA gene, partial sequence<> <i>Aquaspirillum peregrinum</i> subsp. <i>integrum</i> strain LMG 5407 16S ribosomal RNA gene, partial sequence
	gi 343205856 refl NR_044314.1	<i>Insolitispirillum peregrinum</i> strain LMG 4340 16S ribosomal RNA gene, partial sequence<> <i>Aquaspirillum peregrinum</i> subsp. <i>peregrinum</i> strain LMG 4340 16S ribosomal RNA gene, partial sequence
	gi 111185844 emb AM180478.1	<i>Rhodospirillum</i> sp. C2T-PP-R1 partial 16S rRNA gene, isolate C2T-PP-R1

TABLE 6-continued

Responder/non-responder bacteria identified by 16S sequencing OTUs.		
Family	Subject Seq-ID	Source
	gi 729042038 emb LN650457.1	<i>Novispirillum itersonii</i> partial 16S rRNA gene, isolate TOSS-106
	gi 156968444 gb EF616604.1	Bacterium HTCC8037 16S ribosomal RNA gene, partial sequence
	gi 148509101 gb EF587969.1	Alpha proteobacterium UST061013-025 16S ribosomal RNA gene, partial sequence
	gi 343200914 ref NR_041601.1	<i>Tanticharoenia sakaeratensis</i> strain NBRC 103193 16S ribosomal RNA gene, partial sequences<> <i>Tanticharoenia sakaeratensis</i> gene for 16S ribosomal RNA, partial sequence, strain: NBRC 103193<> <i>Tanticharoenia sakaeratensis</i> gene for 16S ribosomal RNA, partial sequence, strain: NBRC 103194<> <i>Tanticharoenia sakaeratensis</i> gene for 16S ribosomal RNA, partial sequence, strain: NBRC 103195
	gi 157170576 emb AM411930.1	Alpha proteobacterium P-20 partial 16S rRNA gene, strain P-20
	gi 157170575 emb AM411929.1	Alpha proteobacterium P-4 partial 16S rRNA gene, strain P-4
	gi 37905460 gb AY225460.1	Acetobacteraceae bacterium SASB-15 16S ribosomal RNA gene, partial sequence
	gi 37905426 gb AY225458.1	Acetobacteraceae bacterium SASB-2 16S ribosomal RNA gene, partial sequence<>Acetobacteraceae bacterium SASB-4 16S ribosomal RNA gene, partial sequence
	gi 1018583283 gb KU865461.1	<i>Candidatus Neoehrlichia arcana</i> isolate HT136 16S ribosomal RNA gene, partial sequence
	gi 1015829900 gb KT946836.1	<i>Gluconobacter oxydans</i> strain G-1 16S ribosomal RNA gene, partial sequence
	gi 1005742402 gb KT283053.1	<i>Acetobacter pasteurianus</i> strain DY-5 16S ribosomal RNA gene, partial sequence
Erysipelotrichaceae	gi 988571035 gb KU196082.1	<i>Erysipelatoclostridium ramosum</i> strain DSM 29355 16S ribosomal RNA gene, partial sequence
Erysipelotrichaceae	gi 988570994 gb KR364754.1	<i>Erysipelatoclostridium ramosum</i> strain SRB509-5-F-B 16S ribosomal RNA gene, partial sequence
Erysipelotrichaceae	gi 631252045 ref NR_113243.1	<i>Erysipelatoclostridium ramosum</i> strain JCM 1298 16S ribosomal RNA gene, partial sequences <i>Clostridium ramosum</i> gene for 16S ribosomal RNA, partial sequence, strain: JCM 1298<> <i>Clostridium ramosum</i> gene for 16S ribosomal RNA, partial sequence, strain: JCM 5235<> <i>Clostridium ramosum</i> gene for 16S ribosomal RNA, partial sequence, strain: JCM 5234
Erysipelotrichaceae	gi 645322231 ref NR_119030.1	[<i>Clostridium</i>] <i>spiroforme</i> strain DSM 1552 16S ribosomal RNA gene, complete sequence
Erysipelotrichaceae	gi 631253195 ref NR_114393.1	[<i>Clostridium</i>] <i>spiroforme</i> strain JCM 1432 16S ribosomal RNA gene, partial sequence<> <i>Clostridium spiroforme</i> gene for 16S ribosomal RNA, partial sequence, strain: JCM 1432
Erysipelotrichaceae	gi 51512137 gb AY699288.1	<i>Clostridium ramosum</i> isolate M91 16S ribosomal RNA gene, partial sequence
Erysipelotrichaceae	gi 752910213 emb LN713275.1	Clostridiaceae bacterium GM1 partial 16S rRNA gene, strain GM1
Erysipelotrichaceae	gi 1187200865 gb KY992931.1	<i>Erysipelatoclostridium</i> sp. SNUG30099 16S ribosomal RNA gene, partial sequence
Erysipelotrichaceae	gi 1187200864 gb KY992930.1	<i>Erysipelatoclostridium</i> sp. SNUG30370 16S ribosomal RNA gene, partial sequence
Erysipelotrichaceae	gi 988571005 gb KR364765.1	<i>Longibaculum muris</i> strain MT10-315-CC-1.2-2 16S ribosomal RNA gene, partial sequence

TABLE 6-continued

Responder/non-responder bacteria identified by 16S sequencing OTUs.		
Family	Subject Seq-ID	Source
Erysipelotrichaceae	gi 647404815 emb LK021123.1	Bacterium LF-3 partial 16S rRNA gene, isolate LF-3
Erysipelotrichaceae	gi 117966651 dbj LC258136.1	<i>Sharpea azabuensis</i> gene for 16S ribosomal RNA, partial sequence, strain: JCM 14210
Erysipelotrichaceae	gi 121544027 gb AY265465.2	Bovine rumen bacterium niuD2 16S ribosomal RNA gene, complete sequence
Erysipelotrichaceae	gi 121544026 gb AY265464.2	Bovine rumen bacterium niuC23 16S ribosomal RNA gene, complete sequence
Erysipelotrichaceae	gi 121544025 gb AY263508.2	Bovine rumen bacterium niuO28 16S ribosomal RNA gene, complete sequence
Erysipelotrichaceae	gi 121544024 gb AY263507.2	Bovine rumen bacterium niuO25 16S ribosomal RNA gene, complete sequence
Erysipelotrichaceae	gi 121544023 gb AY263506.2	Bovine rumen bacterium niuO17 16S ribosomal RNA gene, complete sequence
Erysipelotrichaceae	gi 121544022 gb AY263505.2	Bovine rumen bacterium SNU-NiuO16 16S ribosomal RNA gene, complete sequence
Erysipelotrichaceae	gi 121544021 gb AY263503.2	Bovine rumen bacterium niuO6 16S ribosomal RNA gene, complete sequence
Erysipelotrichaceae	gi 121544020 gb AY263502.2	Bovine rumen bacterium niuD4 16S ribosomal RNA gene, complete sequence
Coriobacteriaceae	gi 117966652 dbj LC258146.1	<i>Collinsella aerofaciens</i> gene for 16S ribosomal RNA, partial sequence, strain: JCM 10808
Coriobacteriaceae	gi 1179666520 dbj LC258145.1	<i>Collinsella aerofaciens</i> gene for 16S ribosomal RNA, partial sequence, strain: JCM 10803
Coriobacteriaceae	gi 1179666519 dbj LC258144.1	<i>Collinsella aerofaciens</i> gene for 16S ribosomal RNA, partial sequence, strain: JCM 10800
Coriobacteriaceae	gi 1179666518 dbj LC258143.1	<i>Collinsella aerofaciens</i> gene for 16S ribosomal RNA, partial sequence, strain: JCM 10799
Coriobacteriaceae	gi 1179666517 dbj LC258142.1	<i>Collinsella aerofaciens</i> gene for 16S ribosomal RNA, partial sequence, strain: JCM 10798
Coriobacteriaceae	gi 1179666516 dbj LC258141.1	<i>Collinsella aerofaciens</i> gene for 16S ribosomal RNA, partial sequence, strain: JCM 10797
Coriobacteriaceae	gi 1179666515 dbj LC258140.1	<i>Collinsella aerofaciens</i> gene for 16S ribosomal RNA, partial sequence, strain: JCM 10795
Coriobacteriaceae	gi 1179666514 dbj LC258139.1	<i>Collinsella aerofaciens</i> gene for 16S ribosomal RNA, partial sequence, strain: JCM 10794
Coriobacteriaceae	gi 1179666513 dbj LC258138.1	<i>Collinsella aerofaciens</i> gene for 16S ribosomal RNA, partial sequence, strain: JCM 10793
Coriobacteriaceae	gi 1024389107 emb LT558813.1	<i>Collinsella aerofaciens</i> partial 16S rRNA gene, strain Marseille-P1069
Coriobacteriaceae	gi 1008904268 emb LT223656.1	<i>Collinsella aerofaciens</i> partial 16S rRNA gene, strain Marseille-P1069
Coriobacteriaceae	gi 745628439 gb KP233454.1	<i>Collinsella aerofaciens</i> strain D9-82 16S ribosomal RNA gene, partial sequence
Coriobacteriaceae	gi 745628433 gb KP233448.1	<i>Collinsella aerofaciens</i> strain D9-111 16S ribosomal RNA gene, partial sequence<> <i>Collinsella aerofaciens</i> strain D9-136 16S ribosomal RNA gene, partial sequence
Coriobacteriaceae	gi 745628432 gb KP233447.1	<i>Collinsella aerofaciens</i> strain D9-108 16S ribosomal RNA gene, partial sequence
Coriobacteriaceae	gi 745628431 gb KP233446.1	<i>Collinsella aerofaciens</i> strain D9-101 16S ribosomal RNA gene, partial sequence<> <i>Collinsella aerofaciens</i> strain D9-76 16S ribosomal RNA gene, partial sequence
Coriobacteriaceae	gi 745628430 gb KP233445.1	<i>Collinsella aerofaciens</i> strain D8-75 16S ribosomal RNA gene, partial sequence
Coriobacteriaceae	gi 745628429 gb KP233444.1	<i>Collinsella aerofaciens</i> strain D8-61 16S ribosomal RNA gene, partial sequence
Coriobacteriaceae	gi 745628428 gb KP233443.1	<i>Collinsella aerofaciens</i> strain D8-40 16S ribosomal RNA gene, partial sequence
Coriobacteriaceae	gi 745628427 gb KP233442.1	<i>Collinsella aerofaciens</i> strain D8-146 16S ribosomal RNA gene, partial sequence

TABLE 6-continued

Responder/non-responder bacteria identified by 16S sequencing OTUs.		
Family	Subject Seq-ID	Source
Coriobacteriaceae	gi 745628426 gb KP233441.1	<i>Collinsella aerofaciens</i> strain D8-124 16S ribosomal RNA gene, partial sequence
	gi 1023747096 gb KX009928.1	Lachnospiraceae bacterium DW60 16S ribosomal RNA gene, partial sequence
	gi 743404488 emb LN626356.1	<i>Herbinix</i> sp. RK1P partial 16S rRNA gene, isolate RK1P
	gi 743404487 emb LN626357.1	<i>Herbinix</i> sp. SD1I partial 16S rRNA gene, isolate SD1I
	gi 743404486 emb LN626358.1	<i>Herbinix</i> sp. SD1G partial 16S rRNA gene, isolate SD1G
	gi 743404485 emb LN626359.1	<i>Herbinix</i> sp. SD1D partial 16S rRNA gene, isolate SD1D
	gi 1040567056 ref NR_137405.1	<i>Anaerobium acetethylicum</i> strain GluBS11 16S ribosomal RNA, partial sequence<> <i>Anaerobium acetethylicum</i> strain GluBS11 16S ribosomal RNA gene, partial sequence
	gi 1024974678 ref NR_136763.1	<i>Herbinix hemicellulosilytica</i> strain T3/55 16S ribosomal RNA, partial sequence<> <i>Herbinix hemicellulosilytica</i> partial 16S rRNA gene, isolate T3/55
	gi 605097974 gb KJ016028.1	<i>Clostridium</i> sp. Pao32 16S ribosomal RNA gene, partial sequence
	gi 166063939 dbj AB298768.2	<i>Anaerotaenia torta</i> gene for 16S rRNA, partial sequence
	gi 343202586 ref NR_042953.1	<i>Anaerospobacter mobilis</i> strain IMSNU 40011 16S ribosomal RNA gene, partial sequence
	gi 775444676 emb LN794845.1	<i>Lachnotalea glycerini</i> partial 16S rRNA gene, type strain DL10T
	gi 664616078 emb LK391571.1	Lachnospiraceae bacterium V37_10_1 partial 16S rRNA gene, isolate V37_10_1
	gi 664616076 emb LK391569.1	Lachnospiraceae bacterium V37_06_2 partial 16S rRNA gene, isolate V37_06_2
	gi 664616071 emb LK391564.1	Lachnospiraceae bacterium V37_02_2 partial 16S rRNA gene, isolate V37_02_2
	gi 160426828 gb CP000885.1	<i>Clostridium phytofermentans</i> ISDg, complete genome
	gi 751868028 dbj LC020504.1	<i>Clostridium</i> sp. TB5 gene for 16S ribosomal RNA, partial sequence
	gi 664616057 emb LK391550.1	Lachnospiraceae bacterium S37_06_2 partial 16S rRNA gene, isolate S37_06_2
	gi 343198925 ref NR_044049.1	<i>Coprococcus eutactus</i> strain ATCC 27759 16S ribosomal RNA gene, partial sequence<> <i>Coprococcus eutactus</i> strain ATCC 27759 16S ribosomal RNA gene, partial sequence
	gi 2258201 gb U68616.1 UEU68616	Unidentified eubacterium from the Amazon 16S ribosomal RNA gene, partial sequence
gi 37993572 gb AY350746.1	Butyrate-producing bacterium ART55/1 16S ribosomal RNA gene, partial sequence	
Ruminococcaceae	gi 820661309 emb LN846907.1	<i>Clostridium</i> sp. GD3 partial 16S rRNA gene, strain GD3
Ruminococcaceae	gi 806475686 gb KP114242.1	<i>Intestinimonas</i> sp. FSAA-17 16S ribosomal RNA gene, partial sequence
Ruminococcaceae	gi 676402756 gb KM043767.1	Bacterium P1D11 16S ribosomal RNA gene, partial sequence
Ruminococcaceae	gi 1193829127 ref NR_147370.1	<i>Pseudoflavonifractor phocaensis</i> strain Marseille-P3064 16S ribosomal RNA, partial sequence
Ruminococcaceae	gi 1173509597 gb CP015406.2	<i>Flavonifractor plautii</i> strain YL31, complete genome
Ruminococcaceae	gi 1150560357 dbj LC175305.1	<i>Clostridium</i> sp. 19-20 gene for 16S ribosomal RNA, partial sequence, isolate: 19-20
Ruminococcaceae	gi 988571039 gb KU196086.1	<i>Flavonifractor plautii</i> strain DSM 29136 16S ribosomal RNA gene, partial sequence
Ruminococcaceae	gi 988571013 gb KR364773.1	<i>Flavonifractor plautii</i> strain YL31 16S ribosomal RNA gene, partial sequence
Ruminococcaceae	gi 988571012 gb KR364772.1	<i>Flavonifractor plautii</i> strain mOs-SRB-10A-2011 16S ribosomal RNA gene, partial sequence

TABLE 6-continued

Responder/non-responder bacteria identified by 16S sequencing OTUs.		
Family	Subject Seq-ID	Source
Ruminococcaceae	gi 343198636 ref NR_043142.1	<i>Flavonifractor plautii</i> strain Prevot S1 16S ribosomal RNA gene, partial sequence<> <i>Eubacterium plautii</i> strain CCUG 28093 16S ribosomal RNA gene, partial sequence<>Uncultured bacterium clone CFT19A5 16S ribosomal RNA gene, partial sequence
Ruminococcaceae	gi 52355230 gb AY730665.1	<i>Clostridium orbiscindens</i> strain NML 01-A-077 16S ribosomal RNA gene, partial sequence
Ruminococcaceae	gi 52355229 gb AY730664.1	<i>Clostridium orbiscindens</i> strain NML 00-A-095 16S ribosomal RNA gene, partial sequence
Ruminococcaceae	gi 52355228 gb AY730663.1	<i>Clostridium orbiscindens</i> strain NML 00-A-082 16S ribosomal RNA gene, partial sequence
Ruminococcaceae	gi 52355227 gb AY730662.1	<i>Clostridium orbiscindens</i> strain NML 98-A-029 16S ribosomal RNA gene, partial sequence
Ruminococcaceae	gi 676402757 gb KM043768.1	Bacterium P2C2 16S ribosomal RNA gene, partial sequence
Ruminococcaceae	gi 219846080 ref NR_025670.1	<i>Pseudoflavonifractor capillosus</i> strain ATCC 29799 16S ribosomal RNA gene, partial sequence<> <i>Bacteroides capillosus</i> 16S ribosomal RNA gene, partial sequence
Ruminococcaceae	gi 676402750 gb KM043761.1	Bacterium P1C2 16S ribosomal RNA gene, partial sequence
Ruminococcaceae	gi 50982374 gb AY654967.1	Unidentified bacterium clone CCCM81 16S ribosomal RNA gene, partial sequence
Ruminococcaceae	gi 1193829132 ref NR_147375.1	<i>Colidexibacter massiliensis</i> strain Marseille-P3083 16S ribosomal RNA, partial sequence
Ruminococcaceae	gi 676402755 gb KM043766.1	Bacterium P2G6 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gi 775444730 emb LN828944.1	Ruminococcaceae bacterium GD1 partial 16S rRNA gene, strain GD1
Lachnospiraceae	gi 676402759 gb KM043770.1	Bacterium P1A6 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gi 50982382 gb AY654975.1	Unidentified bacterium clone CDDN44 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gi 50982361 gb AY654954.1	Unidentified bacterium clone CCCM52 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gi 50982365 gb AY654958.1	Unidentified bacterium clone CCCM58 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gi 747220154 gb KC331157.2	Lachnospiraceae bacterium BTY6 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gi 50982356 gb AY654949.1	Unidentified bacterium clone CCCM15 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gi 1008904264 emb LT223652.1	[<i>Clostridium</i>] <i>lavalense</i> partial 16S rRNA gene, strain Marseille-P2117
Lachnospiraceae	gi 50982373 gb AY654966.1	Unidentified bacterium clone CCCM79 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gi 1023747088 gb KX009920.1	Lachnospiraceae bacterium DW22 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gi 631251356 ref NR_112553.1	[<i>Clostridium</i>] <i>glycyrrhizinilyticum</i> strain ZM35 16S ribosomal RNA gene, complete sequence<> <i>Clostridium glycyrrhizinilyticum</i> gene for 16S ribosomal RNA
Lachnospiraceae	gi 1193829163 ref NR_147406.1	<i>Mordavella massiliensis</i> strain Marseille-P3246 16S ribosomal RNA, partial sequence
Lachnospiraceae	gi 1173536882 gb CP015399.2	<i>Lachnoclostridium</i> sp. YL32, complete genome
Lachnospiraceae	gi 983963892 gb KU569989.1	<i>Clostridium</i> sp. V1 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gi 1008904250 emb LT223638.1	<i>Bacteroides xylanolyticus</i> partial 16S rRNA gene, strain Marseille-P2455

TABLE 6-continued

Responder/non-responder bacteria identified by 16S sequencing OTUs.		
Family	Subject Seq-ID	Source
Lachnospiraceae	gi 1005925626 emb LT161895.1	<i>Clostridium</i> sp. Marseille-P2415T partial 16S rRNA gene, type strain Marseille-P2415T
Lachnospiraceae	gi 988570995 gb KR364755.1	<i>Clostridium</i> sp. MT10-315-CC-82 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gi 988570990 gb KR364750.1	[<i>Clostridium</i>] <i>clostridioforme</i> strain YL32 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gi 985742343 gb KT633852.1	<i>Bacteroides xylanolyticus</i> strain MCM B570 16S ribosomal RNA gene, partial sequence
Lachnospiraceae	gi 985742341 gb KT633850.1	<i>Bacteroides xylanolyticus</i> strain MCM B568 16S ribosomal RNA gene, partial sequence
Enterobacteriaceae	gi 1158626387 gb KX929840.1	<i>Klebsiella pneumoniae</i> strain GPKP 16S ribosomal RNA gene, partial sequence
Enterobacteriaceae	gi 1158622117 gb KX377574.1	<i>Enterobacter</i> sp. strain YM29 16S ribosomal RNA gene, partial sequence
Enterobacteriaceae	gi 1158499421 gb KY750246.1	<i>Klebsiella</i> sp. strain QIUP 1 16S ribosomal RNA gene, partial sequence
Enterobacteriaceae	gi 1154259114 gb KY595448.1	<i>Enterobacter cloacae</i> strain S12 16S ribosomal RNA gene, partial sequence
Enterobacteriaceae	gi 1154259110 gb KY595444.1	<i>Enterobacter hormaechei</i> strain M55B 16S ribosomal RNA gene, partial sequence
Enterobacteriaceae	gi 1062337936 gb KX817276.1	<i>Enterobacter cloacae</i> strain AA4 16S ribosomal RNA gene, partial sequence
Enterobacteriaceae	gi 1062327603 gb KX817271.1	<i>Enterobacter</i> sp. strain AA25 16S ribosomal RNA gene, partial sequence
Enterobacteriaceae	gi 1062327580 gb KX817248.1	<i>Enterobacter</i> sp. strain AA13 16S ribosomal RNA gene, partial sequence
Enterobacteriaceae	gi 1039023274 gb KU744864.1	<i>Enterobacter</i> sp. SFS65 16S ribosomal RNA gene, partial sequence
Enterobacteriaceae	gi 1015522084 gb KU923368.1	<i>Enterobacter cloacae</i> strain UBGSS3 16S ribosomal RNA gene, partial sequence
Enterobacteriaceae	gi 1015522081 gb KU923365.1	<i>Enterobacter cancerogenus</i> strain TBGSS4 16S ribosomal RNA gene, partial sequence
Enterobacteriaceae	gi 1015522079 gb KU923363.1	<i>Bacillus andresenii</i> strain TBGSS2 16S ribosomal RNA gene, partial sequence
Enterobacteriaceae	gi 1015522063 gb KU923347.1	<i>Erwinia persicina</i> strain USTRW7 16S ribosomal RNA gene, partial sequence
Enterobacteriaceae	gi 1153684959 gb KY706100.1	<i>Klebsiella pneumoniae</i> strain NGR/A/2016 16S ribosomal RNA gene, partial sequence
Enterobacteriaceae	gi 1151099307 gb KY684230.1	<i>Enterobacter</i> sp. strain FYP1101 16S ribosomal RNA gene, partial sequence
Enterobacteriaceae	gi 1158824263 gb CP018785.1	<i>Enterobacter cloacae</i> strain AA4, complete genome
Enterobacteriaceae	gi 1151331582 gb KY476172.1	<i>Pantoeasp.</i> strain FA1-263 16S ribosomal RNA gene, partial sequence
Enterobacteriaceae	gi 1151331579 gb KY476169.1	<i>Enterobacter</i> sp. strain FA1-153 16S ribosomal RNA gene, partial sequence
Enterobacteriaceae	gi 1151124876 gb KX709881.1	<i>Lelliottia</i> sp. strain FOL02 16S ribosomal RNA gene, partial sequence
Enterobacteriaceae	gi 1150318051 gb KY673185.1	<i>Enterobacter</i> sp. strain MF90 16S ribosomal RNA gene, partial sequence
Lactobacillaceae	gi 770114072 gb KP178095.1	<i>Lactobacillus ruminis</i> strain 1313_A06 16S ribosomal RNA gene, partial sequence
Lactobacillaceae	gi 770114071 gb KP178094.1	<i>Lactobacillus ruminis</i> strain 1292_G05 16S ribosomal RNA gene, partial sequence
Lactobacillaceae	gi 756130194 gb KP159619.1	<i>Lactobacillus ruminis</i> strain CCFM8418 16S ribosomal RNA gene, partial sequence
Lactobacillaceae	gi 756130193 gb KP159618.1	<i>Lactobacillus ruminis</i> strain CCFM8417 16S ribosomal RNA gene, partial sequence
Lactobacillaceae	gi 723451374 gb KM269714.1	<i>Lactobacillus ruminis</i> strain 1291(LBF2)H02 16S ribosomal RNA gene, partial sequence
Lactobacillaceae	gi 343200924 ref NR_041611.1	<i>Lactobacillus ruminis</i> strain NBRC 102161 16S ribosomal RNA gene, partial sequence<> <i>Lactobacillus ruminis</i> gene

TABLE 6-continued

Responder/non-responder bacteria identified by 16S sequencing OTUs.		
Family	Subject Seq-ID	Source
Lactobacillaceae	gi 1008904203 emb LT223591.1	for 16S rRNA, partial sequence, strain: NBRC 102161
Lactobacillaceae	gi 1185965445 dbj LC259012.1	<i>Lactobacillus ruminis</i> partial 16S rRNA gene, strain Marseille-P908
Lactobacillaceae	gi 1062982723 gb KX826967.1	<i>Lactobacillus</i> sp. C-1 gene for 16S ribosomal RNA, partial sequence
Lactobacillaceae	gi 723451366 gb KM269706.1	<i>Lactobacillus ruminis</i> strain M1/34 16S ribosomal RNA gene, partial sequence
Lactobacillaceae	gi 937352297 gb KP317728.1	<i>Lactobacillus</i> sp. 1280(LBF2)G02 16S ribosomal RNA gene, partial sequence
Lactobacillaceae	gi 937352294 gb KP317725.1	<i>Lactobacillus salivarius</i> strain L64 16S ribosomal RNA gene, partial sequence
Lactobacillaceae	gi 937352288 gb KP317719.1	<i>Lactobacillus salivarius</i> strain L61 16S ribosomal RNA gene, partial sequence
Lactobacillaceae	gi 937352253 gb KP317685.1	<i>Lactobacillus salivarius</i> strain L52 16S ribosomal RNA gene, partial sequence
Lactobacillaceae	gi 937352252 gb KP317684.1	<i>Lactobacillus salivarius</i> strain L14 16S ribosomal RNA gene, partial sequence
Lactobacillaceae	gi 937352251 gb KP317683.1	<i>Lactobacillus salivarius</i> strain L13 16S ribosomal RNA gene, partial sequence
Lactobacillaceae	gi 937352249 gb KP317681.1	<i>Lactobacillus salivarius</i> strain L12 16S ribosomal RNA gene, partial sequence
Lactobacillaceae	gi 937352247 gb KP317679.1	<i>Lactobacillus salivarius</i> strain L8 16S ribosomal RNA gene, partial sequence
Lactobacillaceae	gi 937352246 gb KP317678.1	<i>Lactobacillus salivarius</i> strain L6 16S ribosomal RNA gene, partial sequence
Lactobacillaceae	gi 1024252056 gb KU163336.1	<i>Lactobacillus salivarius</i> strain L5 16S ribosomal RNA gene, partial sequence
Lactobacillaceae		<i>Lactobacillus</i> sp. YANG-11 16S ribosomal RNA gene, partial sequence

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<210> SEQ ID NO 32
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<210> SEQ ID NO 33

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<212> TYPE: DNA

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<210> SEQ ID NO 34

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<223> OTHER INFORMATION: Synthetic

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tgctgcctcc cgtaggag

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We claim:

1. A method of treating a subject suffering from cancer with an immune checkpoint inhibitor, the method comprising:

- (a) obtaining a stool sample from the subject;
- (b) characterizing the gut microflora of the subject by having the stool sample tested to determine levels of one or more beneficial bacteria selected from *Enterococcus faecium*, *Collinsella aerofaciens*, *Bifidobacterium adolescentis*, *Klebsiella pneumoniae*, *Veillonella parvula*, *Parabacteroides merdae*, *Lactobacillus* sp. and *Bifidobacterium longum*, and one or both non-beneficial bacteria selected from the species *Ruminococcus obeum* and *Roseburia intestinalis*;
- (c) calculating a ratio of beneficial bacteria to non-beneficial bacteria in the gut of the subject; and
- (d) administering to the subject (i) an immune checkpoint inhibitor intravenously to the subject if the ratio is above 1.5, or (ii) an immune checkpoint inhibitor intravenously and a bacterial formulation comprising one or more beneficial bacteria selected from *Enterococcus faecium*, *Collinsella aerofaciens*, *Bifidobacterium adolescentis*, *Klebsiella pneumoniae*, *Veillonella parvula*, *Parabacteroides merdae*, *Lactobacillus* sp. and *Bifidobacterium longum* orally or rectally to the subject if the ratio is below 1.5.

2. The method of claim 1, wherein at least 50% of the bacteria in the bacterial formulation are *Enterococcus faecium*, *Collinsella aerofaciens*, *Bifidobacterium adolescentis*, *Klebsiella pneumoniae*, *Veillonella parvula*, *Parabacteroides merdae*, *Lactobacillus* sp. and/or *Bifidobacterium longum*.

3. The method of claim 1, wherein at least 90% of the bacteria in the bacterial formulation are *Enterococcus faecium*, *Collinsella aerofaciens*, *Bifidobacterium adolescentis*, *Klebsiella pneumoniae*, *Veillonella parvula*, *Parabacteroides merdae*, *Lactobacillus* sp. and *Bifidobacterium longum*.

4. The method of claim 1, wherein the bacterial formulation comprises at least 5×10^6 CFU of bacteria.

5. The method of claim 1, wherein the bacterial formulation is administered to the subject in two or more doses.

6. The method of claim 5, wherein the administration of the two or more doses are separated by at least 1 week.

7. The method of claim 1, further comprising administering to the subject an antibiotic capable of killing the non-beneficial bacteria prior to the administration of the bacterial formulation.

8. The method of claim 7, wherein the antibiotic is administered to the subject at least 1 day before the bacterial formulation is administered to the subject.

9. The method of claim 1, wherein the immune checkpoint inhibitor is a protein or polypeptide that binds to an immune checkpoint protein.

10. The method of claim 9, wherein the immune checkpoint protein is CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3 or VISTA.

11. The method of claim 10, wherein the immune checkpoint protein is PD-1 or PD-L1.

12. The method of claim 1, wherein the immune checkpoint inhibitor is an antibody or antigen binding fragment thereof that binds to an immune checkpoint protein.

13. The method of claim 1, wherein the immune checkpoint inhibitor is nivolumab, pembrolizumab, pidilizumab, AMP-224, AMP-514, STI-A1110, TSR-042, RG-7446, BMS-936559, BMS-936558, MK-3475, CT 011, MPDL3280A, MEDI-4736, MSB-0020718C, AUR-012 and STI-A1010.

14. A method of treating a subject with cancer, wherein the subject has a ratio of beneficial bacteria to non-beneficial bacteria of less than 1.5, wherein the one or more beneficial bacteria are selected from *Enterococcus faecium*, *Collinsella aerofaciens*, *Bifidobacterium adolescentis*, *Klebsiella pneumoniae*, *Veillonella parvula*, *Parabacteroides merdae*, *Lactobacillus* sp. and *Bifidobacterium longum*, and wherein the non-beneficial bacteria are one or both of *Ruminococcus obeum* and *Roseburia intestinalis*, comprising intravenously administering to the subject a cancer immunotherapy comprising an immune checkpoint inhibitor and orally or rectally administering to the subject a bacterial formulation

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comprising one or more of *Enterococcus faecium*, *Collin-*
sella aerofaciens, *Bifidobacterium adolescentis*, *Klebsiella*
pneumoniae, *Veillonella parvula*, *Parabacteroides merdae*,
Lactobacillus sp. and/or *Bifidobacterium longum* bacteria.

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