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(54) Title: PROCESSED MICROBIAL EXTRACELLULAR VESICLES

(57) Abstract: Provided herein are methods and pharmaceutical compositions related to processed microbial extracellular vesicles (pmEVs) that can be useful as therapeutic agents.

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# Processed Microbial Extracellular Vesicles

## RELATED APPLICATIONS

[1] This application claims the benefit of U.S. Provisional Patent Application No. 62/860,029, filed June 11, 2019; U.S. Provisional Patent Application No. 62/860,049, filed June, 11, 2019; U.S. Provisional Patent Application No. 62/979,545, filed February 21, 2020; and U.S. Provisional Patent Application No. 62/991,767, filed March 19, 2020, the contents of each of which are hereby incorporated by reference in their entirety.

## SUMMARY

[2] As disclosed herein, certain types of microbial extracellular vesicles (mEVs), such as processed microbial extracellular vesicles (pmEVs) obtained from microbes (such as bacteria) have therapeutic effects and are useful for the treatment and/or prevention of disease and/or health disorders.

[3] In some embodiments, a pharmaceutical composition provided herein can contain mEVs (such as pmEVs) from one or more microbe source, e.g., one or more bacterial strain. In some embodiments, a pharmaceutical composition provided herein can contain mEVs from one microbe source, e.g., one bacterial strain. The bacterial strain used as a source of mEVs may be selected based on the properties of the bacteria (e.g., growth characteristics, yield, ability to modulate an immune response in an assay or a subject). A pharmaceutical composition comprising mEVs can contain pmEVs. The pharmaceutical composition can comprise a pharmaceutically acceptable excipient.

[4] In some embodiments, a pharmaceutical composition provided herein comprising mEVs (such as pmEVs) can be used for the treatment or prevention of a disease and/or a health disorder, e.g., in a subject (e.g., human).

[5] In some embodiments, a pharmaceutical composition provided herein comprising mEVs (such as pmEVs) can be prepared as powder (e.g., for resuspension) or as a solid dose form, such as a tablet, a minitablet, a capsule, a pill, or a powder; or a combination of these forms (e.g., minitablets comprised in a capsule). The solid dose form can comprise a coating (e.g., enteric coating).

[6] In some embodiments, a pharmaceutical composition provided herein can comprise lyophilized mEVs (such as pmEVs). The lyophilized mEVs (such as pmEVs) can be formulated

into a solid dose form, such as a tablet, a minitablet, a capsule, a pill, or a powder; or can be resuspended in a solution.

**[7]** In some embodiments, a pharmaceutical composition provided herein can comprise gamma irradiated mEVs (such as pmEVs). The gamma irradiated mEVs (such as pmEVs) can be formulated into a solid dose form, such as a tablet, a minitablet, a capsule, a pill, or a powder; or can be resuspended in a solution.

**[8]** In some embodiments, a pharmaceutical composition provided herein comprising mEVs (such as pmEVs) can be orally administered.

**[9]** In some embodiments, a pharmaceutical composition provided herein comprising mEVs (such as pmEVs) can be administered intravenously.

**[10]** In some embodiments, a pharmaceutical composition provided herein comprising mEVs (such as pmEVs) can be administered intratumorally or subtumorally, e.g., to a subject who has a tumor.

**[11]** In certain aspects, provided herein are pharmaceutical compositions comprising mEVs (such as pmEVs) useful for the treatment and/or prevention of a disease or a health disorder (e.g., adverse health disorders) (e.g., a cancer, an autoimmune disease, an inflammatory disease, a dysbiosis, or a metabolic disease), as well as methods of making and/or identifying such mEVs, and methods of using such pharmaceutical compositions (e.g., for the treatment of a cancer, an autoimmune disease, an inflammatory disease, a dysbiosis, or a metabolic disease, either alone or in combination with other therapeutics). In some embodiments, the pharmaceutical compositions comprise both mEVs and whole microbes from which they were obtained, such as bacteria, (e.g., live bacteria, killed bacteria, attenuated bacteria). In some embodiments, the pharmaceutical compositions comprise mEVs in the absence of microbes from which they were obtained, such as bacteria (e.g., over about 95% (or over about 99%) of the microbe-sourced content of the pharmaceutical composition comprises mEVs).

**[12]** In some embodiments, the pharmaceutical compositions comprise mEVs from one or more of the bacteria strains or species listed in Table 1, Table 2 and/or Table 3.

**[13]** In some embodiments, the pharmaceutical composition comprises isolated mEVs (e.g., from one or more strains of bacteria (e.g., bacteria of interest) (e.g., a therapeutically effective amount thereof). E.g., wherein at least 50%, at least 75%, at least 80%, at least 85%, at least

90%, at least 95%, or at least 99% of the content of the pharmaceutical composition is isolated mEV of bacteria (e.g., bacteria of interest).

**[14]** In some embodiments, the pharmaceutical composition comprises isolated mEVs (e.g., from one strain of bacteria (e.g., bacteria of interest) (e.g., a therapeutically effective amount thereof). E.g., wherein at least 50%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% of the content of the pharmaceutical composition is isolated mEV of bacteria (e.g., bacteria of interest).

**[15]** In some embodiments, the pharmaceutical composition comprises processed mEVs (pmEVs).

**[16]** In some embodiments, the pharmaceutical composition comprises pmEVs and the pmEVs are produced from bacteria that have been gamma irradiated, UV irradiated, heat inactivated, acid treated, or oxygen sparged.

**[17]** In some embodiments, the pharmaceutical composition comprises pmEVs and the pmEVs are produced from live bacteria.

**[18]** In some embodiments, the pharmaceutical composition comprises pmEVs and the pmEVs are produced from dead bacteria.

**[19]** In some embodiments, the pharmaceutical composition comprises pmEVs and the pmEVs are produced from non-replicating bacteria.

**[20]** In some embodiments, the pharmaceutical composition comprises mEVs and the mEVs are from one strain of bacteria.

**[21]** In some embodiments, the pharmaceutical composition comprises mEVs and the mEVs are from one strain of bacteria.

**[22]** In some embodiments, the mEVs are lyophilized (e.g., the lyophilized product further comprises a pharmaceutically acceptable excipient).

**[23]** In some embodiments, the mEVs are gamma irradiated.

**[24]** In some embodiments, the mEVs are UV irradiated.

**[25]** In some embodiments, the mEVs are heat inactivated (e.g., at 50°C for two hours or at 90°C for two hours).

**[26]** In some embodiments, the mEVs are acid treated.

**[27]** In some embodiments, the mEVs are oxygen sparged (e.g., at 0.1 vvm for two hours).

**[28]** In some embodiments, the mEVs are from Gram positive bacteria.

**[29]** In some embodiments, the mEVs are from Gram negative bacteria.

**[30]** In some embodiments, the Gram negative bacteria belong to class Negativicutes.

**[31]** In some embodiments, the mEVs are from aerobic bacteria.

**[32]** In some embodiments, the mEVs are from anaerobic bacteria.

**[33]** In some embodiments, the mEVs are from acidophile bacteria.

**[34]** In some embodiments, the mEVs are from alkaliphile bacteria.

**[35]** In some embodiments, the mEVs are from neutralophile bacteria.

**[36]** In some embodiments, the mEVs are from fastidious bacteria.

**[37]** In some embodiments, the mEVs are from nonfastidious bacteria.

**[38]** In some embodiments, the mEVs are from a bacterial strain listed in Table 1, Table 2, or Table 3.

**[39]** In some embodiments, the Gram negative bacteria belong to class *Negativicutes*.

**[40]** In some embodiments, the Gram negative bacteria belong to family *Veillonellaceae, Selenomonadaceae, Acidaminococcaceae*, or *Sporomusaceae*.

**[41]** In some embodiments, the mEVs are from bacteria of the genus *Megasphaera, Selenomonas, Propionospora,* or *Acidaminococcus*.

**[42]** In some embodiments, the mEVs are *Megasphaera sp., Selenomonas felix, Acidaminococcus intestine,* or *Propionospora sp.* bacteria.

**[43]** In some embodiments, the mEVs are from bacteria of the genus *Lactococcus, Prevotella, Bifidobacterium,* or *Veillonell*a.

**[44]** In some embodiments, the mEVs are from *Lactococcus lactis cremoris* bacteria.

**[45]** In some embodiments, the mEVs are from *Prevotella histicola* bacteria.

**[46]** In some embodiments, the mEVs are from *Bifidobacterium animalis* bacteria.

**[47]** In some embodiments, the mEVs are from *Veillonella parvula* bacteria.

**[48]** In some embodiments, the mEVs are from *Lactococcus lactis cremoris* bacteria. In some embodiments, the *Lactococcus lactis cremoris* bacteria are from a strain comprising at least 90% (or at least 97%) genomic, 16S and/or CRISPR sequence identity to the nucleotide sequence of the *Lactococcus lactis cremoris* Strain A (ATCC designation number PTA-125368). In some embodiments, the *Lactococcus* bacteria are from a strain comprising at least 99% genomic, 16S and/or CRISPR sequence identity to the nucleotide sequence of the *Lactococcus lactis cremoris* Strain A (ATCC designation number PTA-125368). In some embodiments, the *Lactococcus*

bacteria are from *Lactococcus lactis cremoris* Strain A (ATCC designation number PTA-125368).

**[49]** In some embodiments, the mEVs are from *Prevotella* bacteria. In some embodiments, the *Prevotella* bacteria are from a strain comprising at least 90% (or at least 97%) genomic, 16S and/or CRISPR sequence identity to the nucleotide sequence of the *Prevotella* Strain B 50329 (NRRL accession number B 50329). In some embodiments, the *Prevotella* bacteria are from a strain comprising at least 99% genomic, 16S and/or CRISPR sequence identity to the nucleotide sequence of the *Prevotella* Strain B 50329 (NRRL accession number B 50329). In some embodiments, the *Prevotella* bacteria are from *Prevotella* Strain B 50329 (NRRL accession number B 50329).

**[50]** In some embodiments, the mEVs are from *Bifidobacterium* bacteria. In some embodiments, the *Bifidobacterium* bacteria are from a strain comprising at least 90% (or at least 97%) genomic, 16S and/or CRISPR sequence identity to the nucleotide sequence of the *Bifidobacterium* bacteria deposited as ATCC designation number PTA-125097. In some embodiments, the *Bifidobacterium* bacteria are from a strain comprising at least 99% genomic, 16S and/or CRISPR sequence identity to the nucleotide sequence of the *Bifidobacterium* bacteria deposited as ATCC designation number PTA-125097. In some embodiments, the *Bifidobacterium* bacteria are from *Bifidobacterium* bacteria deposited as ATCC designation number PTA-125097.

**[51]** In some embodiments, the mEVs are from *Veillonella* bacteria. In some embodiments, the *Veillonella* bacteria are from a strain comprising at least 90% (or at least 97%) genomic, 16S and/or CRISPR sequence identity to the nucleotide sequence of the *Veillonell*a bacteria deposited as ATCC designation number PTA-125691. In some embodiments, the *Veillonella* bacteria are from a strain comprising at least 99% genomic, 16S and/or CRISPR sequence identity to the nucleotide sequence of the *Veillonella* bacteria deposited as ATCC designation number PTA-125691. In some embodiments, the *Veillonella* bacteria are from *Veillonella* bacteria deposited as ATCC designation number PTA-125691.

**[52]** In some embodiments, the mEVs are from *Ruminococcus gnavus* bacteria. In some embodiments, the *Ruminococcus gnavus* bacteria are from a strain comprising at least 90% (or at least 97%) genomic, 16S and/or CRISPR sequence identity to the nucleotide sequence of the *Ruminococcus gnavus* bacteria deposited as ATCC designation number PTA-126695. In some

embodiments, the *Ruminococcus gnavus* bacteria are from a strain comprising at least 99% genomic, 16S and/or CRISPR sequence identity to the nucleotide sequence of the *Ruminococcus gnavus* bacteria deposited as ATCC designation number PTA-126695. In some embodiments, the *Ruminococcus gnavus* bacteria are from *Ruminococcus gnavus* bacteria deposited as ATCC designation number PTA-126695.

**[53]** In some embodiments, the mEVs are from *Megasphaera sp.* bacteria. In some embodiments, the *Megasphaera sp.* bacteria are from a strain comprising at least 90% (or at least 97%) genomic, 16S and/or CRISPR sequence identity to the nucleotide sequence of the *Megasphaera sp.* bacteria deposited as ATCC designation number PTA-126770. In some embodiments, the *Megasphaera sp.*bacteria are from a strain comprising at least 99% genomic, 16S and/or CRISPR sequence identity to the nucleotide sequence of the *Megasphaera sp.*bacteria deposited as ATCC designation number PTA-126770. In some embodiments, the *Megasphaera sp.* bacteria are from *Megasphaera sp.* bacteria deposited as ATCC designation number PTA-126770.

**[54]** In some embodiments, the mEVs are from *Fournierella massiliensis* bacteria. In some embodiments, the *Fournierella massiliensis* bacteria are from a strain comprising at least 90% (or at least 97%) genomic, 16S and/or CRISPR sequence identity to the nucleotide sequence of the *Fournierella massiliensis* bacteria deposited as ATCC designation number PTA-126694. In some embodiments, the *Fournierella massiliensis* bacteria are from a strain comprising at least 99% genomic, 16S and/or CRISPR sequence identity to the nucleotide sequence of the *Fournierella massiliensis* bacteria deposited as ATCC designation number PTA-126694. In some embodiments, the *Fournierella massiliensis* bacteria are from *Fournierella massiliensis* bacteria deposited as ATCC designation number PTA-126694.

**[55]** In some embodiments, the mEVs are from *Harryflintia acetispora* bacteria. In some embodiments, the *Harryflintia acetispora* bacteria are from a strain comprising at least 90% (or at least 97%) genomic, 16S and/or CRISPR sequence identity to the nucleotide sequence of the *Harryflintia acetispora* bacteria deposited as ATCC designation number PTA-126696. In some embodiments, the *Harryflintia acetispora* bacteria are from a strain comprising at least 99% genomic, 16S and/or CRISPR sequence identity to the nucleotide sequence of the *Harryflintia acetispora* bacteria deposited as ATCC designation number PTA-126696. In some embodiments,

the *Harryflintia acetispora* bacteria are from *Harryflintia acetispora* bacteria deposited as ATCC designation number PTA-126696.

**[56]** In some embodiments, the mEVs are from bacteria of the genus *Akkermansia, Christensenella, Blautia, Enterococcus, Eubacterium, Roseburia, Bacteroides, Parabacteroides*, or *Erysipelatoclostridium*.

**[57]** In some embodiments, the mEVs are from *Blautia hydrogenotrophica, Blautia stercoris, Blautia wexlerae, Eubacterium faecium, Eubacterium contortum, Eubacterium rectale, Enterococcus faecalis, Enterococcus durans, Enterococcus villorum, Enterococcus gallinarum; Bifidobacterium lactis, Bifidobacterium bifidium, Bifidobacterium longum, Bifidobacterium animalis,* or *Bifidobacterium breve* bacteria.

**[58]** In some embodiments, the mEVs are from *BCG (bacillus Calmette-Guerin), Parabacteroides, Blautia, Veillonella, Lactobacillus salivarius, Agathobaculum, Ruminococcus gnavus, Paraclostridium benzoelyticum, Turicibacter sanguinus, Burkholderia, Klebsiella quasipneumoniae ssp similpneumoniae, Klebsiella oxytoca, Tyzzerela nexilis,* or *Neisseria* bacteria.

**[59]** In some embodiments, the mEVs are from *Blautia hydrogenotrophica* bacteria.

**[60]** In some embodiments, the mEVs are from *Blautia stercoris* bacteria.

**[61]** In some embodiments, the mEVs are from *Blautia wexlerae* bacteria.

**[62]** In some embodiments, the mEVs are from *Enterococcus gallinarum* bacteria.

**[63]** In some embodiments, the mEVs are from *Enterococcus faecium* bacteria.

**[64]** In some embodiments, the mEVs are from *Bifidobacterium bifidium* bacteria.

**[65]** In some embodiments, the mEVs are from *Bifidobacterium breve* bacteria.

**[66]** In some embodiments, the mEVs are from *Bifidobacterium longum* bacteria.

**[67]** In some embodiments, the mEVs are from *Roseburia hominis* bacteria.

**[68]** In some embodiments, the mEVs are from *Bacteroides thetaiotaomicron* bacteria.

**[69]** In some embodiments, the mEVs are from *Bacteroides coprocola* bacteria.

**[70]** In some embodiments, the mEVs are from *Erysipelatoclostridium ramosum* bacteria.

**[71]** In some embodiments, the mEVs are from *Megasphera massiliensis* bacteria.

**[72]** In some embodiments, the mEVs are from *Eubacterium* bacteria.

**[73]** In some embodiments, the mEVs are from *Parabacteroides distasonis* bacteria.

[74] In certain aspects, the mEVs (such as pmEVs) are obtained from bacteria that have been selected based on certain desirable properties, such as reduced toxicity and adverse effects (e.g., by removing or deleting lipopolysaccharide (LPS)), enhanced oral delivery (e.g., by improving acid resistance, muco-adherence and/or penetration and/or resistance to bile acids, resistance to anti-microbial peptides and/or antibody neutralization), target desired cell types (e.g., M-cells, goblet cells, enterocytes, dendritic cells, macrophages), improved bioavailability systemically or in an appropriate niche (e.g., mesenteric lymph nodes, Peyer's patches, lamina propria, tumor draining lymph nodes, and/or blood), enhanced immunomodulatory and/or therapeutic effect (e.g., either alone or in combination with another therapeutic agent), enhanced immune activation , and/or manufacturing attributes (e.g., growth characteristics, yield, greater stability, improved freeze-thaw tolerance, shorter generation times).

[75] In certain aspects, the mEVs are from engineered bacteria that are modified to enhance certain desirable properties. In some embodiments, the engineered bacteria are modified so that mEVs (such as pmEVs) produced therefrom will have reduced toxicity and adverse effects (e.g., by removing or deleting lipopolysaccharide (LPS)), enhanced oral delivery (e.g., by improving acid resistance, muco-adherence and/or penetration and/or resistance to bile acids, resistance to anti-microbial peptides and/or antibody neutralization), target desired cell types (e.g., M-cells, goblet cells, enterocytes, dendritic cells, macrophages), improved bioavailability systemically or in an appropriate niche (e.g., mesenteric lymph nodes, Peyer's patches, lamina propria, tumor draining lymph nodes, and/or blood), enhanced immunomodulatory and/or therapeutic effect (e.g., either alone or in combination with another therapeutic agent), enhanced immune activation, and/or improved manufacturing attributes (e.g., growth characteristics, yield, greater stability, improved freeze-thaw tolerance, shorter generation times). In some embodiments, provided herein are methods of making such mEVs (such as pmEVs).

[76] In certain aspects, provided herein are pharmaceutical compositions comprising mEVs (such as pmEVs) useful for the treatment and/or prevention of a disease or a health disorder (e.g., a cancer, an autoimmune disease, an inflammatory disease, or a metabolic disease), as well as methods of making and/or identifying such mEVs, and methods of using such pharmaceutical compositions (e.g., for the treatment of a cancer, an autoimmune disease, an inflammatory disease, or a metabolic disease), either alone or in combination with one or more other therapeutics.

**[77]** Pharmaceutical compositions containing mEVs (such pmEVs) can provide potency comparable to or greater than pharmaceutical compositions that contain the whole microbes from which the mEVs were obtained. For example, at the same dose of mEVs (e.g., based on particle count or protein content), a pharmaceutical composition containing mEVs can provide potency comparable to or greater than a comparable pharmaceutical composition that contains whole microbes of the same bacterial strain from which the mEVs were obtained. Such mEV containing pharmaceutical compositions can allow the administration of higher doses and elicit a comparable or greater (e.g., more effective) response than observed with a comparable pharmaceutical composition that contains whole microbes of the same bacterial strain from which the mEVs were obtained.

**[78]** As a further example, at the same dose (e.g., based on particle count or protein content), a pharmaceutical composition containing mEVs may contain less microbially-derived material (based on particle count or protein content), as compared to a pharmaceutical composition that contains the whole microbes of the same bacterial strain from which the mEVs were obtained, while providing an equivalent or greater therapeutic benefit to the subject receiving such pharmaceutical composition.

**[79]** As a further example, mEVs can be administered at doses e.g., of about $1x10^7$- about $1x10^{15}$ particles, e.g., as measured by NTA.

**[80]** As another example, mEVs can be administered at doses e.g., of about 5 mg to about 900 mg total protein, e.g., as measured by Bradford assay. As another example, mEVs can be administered at doses e.g., of about 5 mg to about 900 mg total protein, e.g., as measured by BCA assay.

**[81]** In certain embodiments, provided herein are methods of treating a subject who has cancer comprising administering to the subject a pharmaceutical composition described herein. In certain embodiments, provided herein are methods of treating a subject who has an immune disorder (e.g., an autoimmune disease, an inflammatory disease, an allergy) comprising administering to the subject a pharmaceutical composition described herein. In certain embodiments, provided herein are methods of treating a subject who has a metabolic disease comprising administering to the subject a pharmaceutical composition described herein. In certain embodiments, provided herein are methods of treating a subject who has a neurologic disease comprising administering to the subject a pharmaceutical composition described herein.

**[82]** In some embodiments, the method further comprises administering to the subject an antibiotic. In some embodiments, the method further comprises administering to the subject one or more other cancer therapies (e.g., surgical removal of a tumor, the administration of a chemotherapeutic agent, the administration of radiation therapy, and/or the administration of a cancer immunotherapy, such as an immune checkpoint inhibitor, a cancer-specific antibody, a cancer vaccine, a primed antigen presenting cell, a cancer-specific T cell, a cancer-specific chimeric antigen receptor (CAR) T cell, an immune activating protein, and/or an adjuvant). In some embodiments, the method further comprises the administration of another therapeutic bacterium and/or mEVs (such as pmEVs) from one or more other bacterial strains (e.g., therapeutic bacterium). In some embodiments, the method further comprises the administration of an immune suppressant and/or an anti-inflammatory agent. In some embodiments, the method further comprises the administration of a metabolic disease therapeutic agent.

**[83]** In certain aspects, provided herein is a pharmaceutical composition comprising mEVs (such as pmEVs) for use in the treatment and/or prevention of a disease (e.g., a cancer, an autoimmune disease, an inflammatory disease, a dysbiosis, or a metabolic disease) or a health disorder, either alone or in combination with one or more other therapeutic agent.

**[84]** In certain embodiments, provided herein is a pharmaceutical composition comprising mEVs (such as pmEVs) for use in treating and/or preventing a cancer in a subject (e.g., human). The pharmaceutical composition can be used either alone or in combination with one or more other therapeutic agent for the treatment of the cancer. In certain embodiments, provided herein is a pharmaceutical composition comprising mEVs (such as pmEVs) for use in treating and/or preventing an immune disorder (e.g., an autoimmune disease, an inflammatory disease, an allergy) in a subject (e.g., human). The pharmaceutical composition can be used either alone or in combination with one or more other therapeutic agent for the treatment of the immune disorder. In certain embodiments, provided herein is a pharmaceutical composition comprising mEVs (such as pmEVs) for use in treating and/or preventing a dysbiosis in a subject (e.g., human). The pharmaceutical composition can be used either alone or in combination with therapeutic agent for the treatment of the dysbiosis. In certain embodiments, provided herein is a pharmaceutical composition comprising mEVs (such as pmEVs) for use in treating and/or preventing a metabolic disease in a subject (e.g., human). The pharmaceutical composition can be used either alone or in combination with therapeutic agent for the treatment of the metabolic

disease. In certain embodiments, provided herein is a pharmaceutical composition comprising mEVs (such as pmEVs) for use in treating and/or preventing a neurologic disease in a subject (e.g., human). The pharmaceutical composition can be used either alone or in combination with one or more other therapeutic agent for treatment of the neurologic disorder.

**[85]** In some embodiments, the pharmaceutical composition comprising mEVs can be for use in combination with an antibiotic. In some embodiments, the pharmaceutical composition comprising mEVs can be for use in combination with one or more other cancer therapies (e.g., surgical removal of a tumor, the use of a chemotherapeutic agent, the use of radiation therapy, and/or the use of a cancer immunotherapy, such as an immune checkpoint inhibitor, a cancer-specific antibody, a cancer vaccine, a primed antigen presenting cell, a cancer-specific T cell, a cancer-specific chimeric antigen receptor (CAR) T cell, an immune activating protein, and/or an adjuvant). In some embodiments, the pharmaceutical composition comprising mEVs can be for use in combination with another therapeutic bacterium and/or mEVs obtained from one or more other bacterial strains (e.g., therapeutic bacterium). In some embodiments, the pharmaceutical composition comprising mEVs can be for use in combination with one or more immune suppressant(s) and/or an anti-inflammatory agent(s). In some embodiments, the pharmaceutical composition comprising mEVs can be for use in combination with one or more other metabolic disease therapeutic agents.

**[86]** In certain aspects, provided herein is use of a pharmaceutical composition comprising mEVs (such as pmEVs) for the preparation of a medicament for the treatment and/or prevention of a disease (e.g., a cancer, an autoimmune disease, an inflammatory disease, a dysbiosis, or a metabolic disease), either alone or in combination with another therapeutic agent. In some embodiments, the use is in combination with another therapeutic bacterium and/or mEVs obtained from one or more other bacterial strains (e.g., therapeutic bacterium).

**[87]** In certain embodiments, provided herein is use of a pharmaceutical composition comprising mEVs (such as pmEVs) for the preparation of a medicament for treating and/or preventing a cancer in a subject (e.g., human). The pharmaceutical composition can be for use either alone or in combination with another therapeutic agent for the cancer. In certain embodiments, provided herein is use of a pharmaceutical composition comprising mEVs (for the preparation of a medicament for treating and/or preventing an immune disorder (e.g., an autoimmune disease, an inflammatory disease, an allergy) in a subject (e.g., human). The

pharmaceutical composition can be for use either alone or in combination with another therapeutic agent for the immune disorder. In certain embodiments, provided herein is use of a pharmaceutical composition comprising mEVs (such as pmEVs) for the preparation of a medicament for treating and/or preventing a dysbiosis in a subject (e.g., human). The pharmaceutical composition can be for use either alone or in combination with another therapeutic agent for the dysbiosis. In certain embodiments, provided herein is use of a pharmaceutical composition comprising mEVs (such as pmEVs) for the preparation of a medicament for treating and/or preventing a metabolic disease in a subject (e.g., human). The pharmaceutical composition can be for use either alone or in combination with another therapeutic agent for the metabolic disease. In certain embodiments, provided herein is use of a pharmaceutical composition comprising mEVs (such as pmEVs) for the preparation of a medicament for treating and or preventing a neurologic disease in a subject (e.g., human). The pharmaceutical composition can be for use either alone or in combination with another therapeutic agent for the neurologic disorder.

**[88]** In some embodiments, the pharmaceutical composition comprising mEVs can be for use in combination with an antibiotic. In some embodiments, the pharmaceutical composition comprising mEVs can for use in combination with one or more other cancer therapies (e.g., surgical removal of a tumor, the use of a chemotherapeutic agent, the use of radiation therapy, and/or the use of a cancer immunotherapy, such as an immune checkpoint inhibitor, a cancer-specific antibody, a cancer vaccine, a primed antigen presenting cell, a cancer-specific T cell, a cancer-specific chimeric antigen receptor (CAR) T cell, an immune activating protein, and/or an adjuvant). In some embodiments, the pharmaceutical composition comprising mEVs can be for use in combination with another therapeutic bacterium and/or mEVs obtained from one or more other bacterial strains (e.g., therapeutic bacterium). In some embodiments, the pharmaceutical composition comprising mEVs can be for use in combination with one or more other immune suppressant(s) and/or an anti-inflammatory agent(s). In some embodiments, the pharmaceutical composition can be for use in combination with one or more other metabolic disease therapeutic agent(s).

**[89]** A pharmaceutical composition, e.g., as described herein, comprising mEVs (such as pmEVs) can provide a therapeutically effective amount of mEVs to a subject, e.g., a human.

**[90]** A pharmaceutical composition, e.g., as described herein, comprising mEVs (such as pmEVs) can provide a non-natural amount of the therapeutically effective components (e.g., present in the mEVs (such as pmEVs) to a subject, e.g., a human.

**[91]** A pharmaceutical composition, e.g., as described herein, comprising mEVs (such as pmEVs) can provide unnatural quantity of the therapeutically effective components (e.g., present in the mEVs (such as pmEVs) to a subject, e.g., a human.

**[92]** A pharmaceutical composition, e.g., as described herein, comprising mEVs (such as pmEVs) can bring about one or more changes to a subject, e.g., human, e.g., to treat or prevent a disease or a health disorder.

**[93]** A pharmaceutical composition, e.g., as described herein, comprising mEVs (such as pmEVs) has potential for significant utility, e.g., to affect a subject, e.g., a human, e.g., to treat or prevent a disease or a health disorder.

## BRIEF DESCRIPTION OF THE FIGURES

**[94]** **Figure 1** shows the efficacy of i.v. administered processed microbial extracellular vesicles (pmEVs) from *B. animalis ssp. lactis* compared to that of i.p. administered anti-PD-1 or vehicle in a mouse colorectal carcinoma model at day 11.

**[95]** **Figure 2** shows the efficacy of i.v. administered pmEVs from *Anaerostipes hadrus* compared to that of i.p. administered anti-PD-1 or vehicle in a mouse colorectal carcinoma model at day 11.

**[96]** **Figure 3** shows the efficacy of i.v. administered pmEVs from *S. pyogenes* compared to that of i.p. administered anti-PD-1 or vehicle in a mouse colorectal carcinoma model at day 11.

**[97]** **Figure 4** shows the efficacy of i.v. administered pmEVs from *P. benzoelyticum* compared to that of i.p. administered anti-PD-1 or vehicle in a mouse colorectal carcinoma model at day 11.

**[98]** **Figure 5** shows the efficacy of i.v. administered pmEVs from *Hungatella sp.* compared to that of i.p. administered anti-PD-1 or vehicle in a mouse colorectal carcinoma model at day 11.

**[99]** **Figure 6** shows the efficacy of i.v. administered pmEVs from *S. aureus* compared to that of i.p. administered anti-PD-1 or vehicle in a mouse colorectal carcinoma model at day 11.

**[100]** **Figure 7** shows the efficacy of i.v. administered pmEVs from *R. gnavus* compared to that of i.p. administered anti-PD-1 or vehicle in a mouse colorectal carcinoma model at day 11.

**[101]** **Figure 8** shows the efficacy of i.v. administered pmEVs from *B. animalis ssp. lactis* and *Megasphaera massiliensis* compared to that of i.p. administered anti-PD-1 or vehicle in a mouse colorectal carcinoma model at day 11.

**[102]** **Figure 9** shows the efficacy of i.v. administered pmEVs from *R. gnavus* compared to that of intraperitoneally (i.p.) administered anti-PD-1 or vehicle in a mouse colorectal carcinoma model at day 9.

**[103]** **Figure 10** shows the efficacy of i.v. administered pmEVs from *R. gnavus* compared to that of i.p. administered anti-PD-1 or vehicle in a mouse colorectal carcinoma model at day 11.

**[104]** **Figure 11** shows the efficacy of i.v. administered pmEVs from *B. animalis ssp. lactis* alone or in combination with anti-PD-1 compared to that of anti-PD-1 (alone) or vehicle in a mouse colorectal carcinoma model at day 9.

**[105]** **Figure 12** shows the efficacy of i.v. administered pmEVs from *B. animalis ssp. lactis* alone or in combination with anti-PD-1 compared to that of anti-PD-1 (alone) or vehicle in a mouse colorectal carcinoma model at day 11.

**[106]** **Figure 13** shows the efficacy of i.v. administered pmEVs from *P. distasonis* compared to that of i.p. administered anti-PD-1 or vehicle in a mouse colorectal carcinoma model at day 9.

**[107]** **Figure 14** shows the efficacy of i.v. administered pmEVs from *P. distasonis* compared to that of i.p. administered anti-PD-1 or vehicle in a mouse colorectal carcinoma model at day 11.

**[108]** **Figure 15** shows the efficacy of orally-gavaged pmEVs from *P. histicola* compared to dexamethasone. pmEVs from *P. histicola* were tested at low (6.0E+07), medium (6.0E+09), and high (6.0E+11) dosages.

**[109]** **Figure 16** shows the efficacy of i.v. administered smEVs from *V. parvula* compared to that of i.p. administered anti-PD-1 or vehicle in a mouse colorectal carcinoma model at day 11.

**[110]** **Figure 17** shows the efficacy of i.v. administered smEVs from *V. parvula* compared to that of i.p. administered anti-PD-1 or vehicle in a mouse colorectal carcinoma model at day 11. smEVs from *V. parvula* were tested at 2ug/dose, 5ug/dose, and 10ug/dose.

**[111]** **Figure 18** shows the efficacy of i.v. administered smEVs from *V. atypica* compared to that of i.p. administered anti-PD-1 or vehicle in a mouse colorectal carcinoma model at day 11. smEVs from *V. atypica* were tested at 2.0e+11PC, 7.0e+10PC, and 1.5e+10PC.

**[112]** **Figure 19** shows the efficacy of i.v. administered smEVs from *V. tobetsuensis* compared to that of i.p. administered anti-PD-1 or vehicle in a mouse colorectal carcinoma model at day 11. smEVs from *V. tobetsuensis* were tested at 2ug/dose, 5ug/dose, and 10ug/dose.

**[113]** **Figure 20** shows the efficacy of orally administered smEVs and lyophilized smEVs from *Prevotella histicola* at high (6.0 e+11 particle count), medium (6.0 e+9 particle count), and low (6.0 e+7 particle count) concentrations in reducing antigen-specific ear swelling (ear thickness) at 24 hours compared to vehicle (negative control) and dexamethasone (positive control) following antigen challenge in a KLH-based delayed type hypersensitivity model.

**[114]** **Figure 21** shows the efficacy (as determined by 24-hour ear measurements) of three doses (low, mid, and high) of pmEVs and lyophilized pmEVs from a *Prevotella histicola* (*P. histicola*) strain as compared to the efficacy of powder from the same *Prevotella histicola* strain in reducing ear thickness at a 24-hour time point in a DTH model. Dexamethasone was used as a positive control.

**[115]** **Figure 22** shows the efficacy (as determined by 24-hour ear measurements) of three doses (low, mid, and high) of smEVs from a *Veillonella parvula* (*V. parvula*) strain and of pmEVs and gamma irradiated (GI) pmEVs from the same *Veillonella parvula* strain as compared to the efficacy of gamma irradiated (GI) powder from the same *Veillonella parvula* strain in reducing ear thickness at a 24-hour time point in a DTH model. Dexamethasone was used as a positive control.

**[116]** **Figure 23** shows the efficacy (as determined by 24-hour ear measurements) of two doses (low and high) of smEVs from *Megasphaera Sp.* Strain A.

**[117]** **Figure 24** shows the efficacy (as determined by 24-hour ear measurements) of two doses (low and high) of smEVs from *Megasphaera Sp.* Strain B.

**[118]** **Figure 25** shows shows the efficacy (as determined by 24-hour ear measurements) of two doses (low and high) of smEVs from *Selenomonas felix.*

**[119]** **Figure 26** shows smEVs from *Megasphaera Sp.* Strain A induce cytokine production from PMA-differentiated U937 cells. U937 cells were treated with smEV at $1x10^6$-$1x10^9$ concentrations as well as TLR2 (FSL) and TLR4 (LPS) agonist controls for 24hrs and cytokine production was measured."Blank" indicates the medium control.

**[120]** **Figures 27A and 27B** show Day 22 Tumor Volume Summary (Figure 27A) and Tumor Volume Curves (Figure 27B) comparing *Megasphaera sp.* Strain A smEV (2e11) against a negative control (Vehicle PBS), and positive control (anti-PD-1).

**[121]** **Figures 28A and 28B** show Day 23 Tumor Volume Summary (Figure 28A) and Tumor Volume Curves (Figure 28B) comparing *Megasphaera sp.* Strain A smEV smEV*s* at 3 doses (2e11, 2e9, and 2e7) BID, as well as *Megasphaera sp.* smEVs (2e11) QD against a negative control (Vehicle PBS), and positive control (anti-PD-1).

**[122]** **Figure 29** shows tumor volumes after d10 tumors were dosed once daily for 14 days with pmEVs from *E. gallinarum* Strains A and B.

**[123]** **Figure 30** shows EVs from *Megasphaera Sp.* Strain A induce cytokine production from PMA-differentiated U937 cells. Cytokine release was measured by MSD ELISA. TLR2 (FSL) and TLR4 (LPS) agonists were used as controls. Blank indicates the media control.

**[124]** **Figure 31** shows EVs from *Megasphaera Sp.* Strain B induce cytokine production from PMA-differentiated U937 cells. Cytokine release was measured by MSD ELISA. TLR2 (FSL) and TLR4 (LPS) agonists were used as controls. Blank indicates the media control.

**[125]** **Figure 32** shows EVs from *Selenomonas felix* induce cytokine production from PMA-differentiated U937 cells. Cytokine release was measured by MSD ELISA. TLR2 (FSL) and TLR4 (LPS) agonists were used as controls. Blank indicates the media control.

**[126]** **Figure 33** shows EVs from *Acidaminococcus intestini* induce cytokine production from PMA-differentiated U937 cells. Cytokine release was measured by MSD ELISA. TLR2 (FSL) and TLR4 (LPS) agonists were used as controls. Blank indicates the media control.

**[127]** **Figure 34** shows EVs from *Propionospora sp.* induce cytokine production from PMA-differentiated U937 cells. Cytokine release was measured by MSD ELISA. TLR2 (FSL) and TLR4 (LPS) agonists were used as controls. Blank indicates the media control.

## DETAILED DESCRIPTION

Definitions

**[128]** "Adjuvant" or "Adjuvant therapy" broadly refers to an agent that affects an immunological or physiological response in a patient or subject (e.g., human). For example, an adjuvant might increase the presence of an antigen over time or to an area of interest like a tumor, help absorb an antigen presenting cell antigen, activate macrophages and lymphocytes and support the production of cytokines. By changing an immune response, an adjuvant might permit a smaller dose of an immune interacting agent to increase the effectiveness or safety of a particular dose of the immune interacting agent. For example, an adjuvant might prevent T cell exhaustion and thus increase the effectiveness or safety of a particular immune interacting agent.

**[129]** "Administration" broadly refers to a route of administration of a composition (e.g., a pharmaceutical composition) to a subject. Examples of routes of administration include oral administration, rectal administration, topical administration, inhalation (nasal) or injection. Administration by injection includes intravenous (IV), intramuscular (IM), intratumoral (IT) and subcutaneous (SC) administration. A pharmaceutical composition described herein can be administered in any form by any effective route, including but not limited to intratumoral, oral, parenteral, enteral, intravenous, intraperitoneal, topical, transdermal (e.g., using any standard patch), intradermal, ophthalmic, (intra)nasally, local, non-oral, such as aerosol, inhalation, subcutaneous, intramuscular, buccal, sublingual, (trans)rectal, vaginal, intra-arterial, and intrathecal, transmucosal (e.g., sublingual, lingual, (trans)buccal, (trans)urethral, vaginal (e.g., trans- and perivaginally), implanted, intravesical, intrapulmonary, intraduodenal, intragastrical, and intrabronchial. In preferred embodiments, a pharmaceutical composition described herein is administered orally, rectally, intratumorally, topically, intravesically, by injection into or adjacent to a draining lymph node, intravenously, by inhalation or aerosol, or subcutaneously. In another preferred embodiment, a pharmaceutical composition described herein is administered orally, intratumorally, or intravenously.

**[130]** As used herein, the term "antibody" may refer to both an intact antibody and an antigen binding fragment thereof. Intact antibodies are glycoproteins that include at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain includes a heavy chain variable region (abbreviated herein as $V_H$) and a heavy chain constant region. Each light chain includes a light chain variable region (abbreviated herein as $V_L$) and a

light chain constant region. The $V_H$ and $V_L$ regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each $V_H$ and $V_L$ is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The term "antibody" includes, for example, monoclonal antibodies, polyclonal antibodies, chimeric antibodies, humanized antibodies, human antibodies, multispecific antibodies (*e.g.,* bispecific antibodies), single-chain antibodies and antigen-binding antibody fragments.

**[131]** The terms "antigen binding fragment" and "antigen-binding portion" of an antibody, as used herein, refer to one or more fragments of an antibody that retain the ability to bind to an antigen. Examples of binding fragments encompassed within the term "antigen-binding fragment" of an antibody include Fab, Fab', $F(ab')_2$, Fv, scFv, disulfide linked Fv, Fd, diabodies, single-chain antibodies, NANOBODIES®, isolated CDRH3, and other antibody fragments that retain at least a portion of the variable region of an intact antibody. These antibody fragments can be obtained using conventional recombinant and/or enzymatic techniques and can be screened for antigen binding in the same manner as intact antibodies.

**[132]** "Cancer" broadly refers to an uncontrolled, abnormal growth of a host's own cells leading to invasion of surrounding tissue and potentially tissue distal to the initial site of abnormal cell growth in the host. Major classes include carcinomas which are cancers of the epithelial tissue (*e.g.*, skin, squamous cells); sarcomas which are cancers of the connective tissue (*e.g.*, bone, cartilage, fat, muscle, blood vessels, etc.); leukemias which are cancers of blood forming tissue (*e.g.*, bone marrow tissue); lymphomas and myelomas which are cancers of immune cells; and central nervous system cancers which include cancers from brain and spinal tissue. "Cancer(s) and" "neoplasm(s)"" are used herein interchangeably. As used herein, "cancer" refers to all types of cancer or neoplasm or malignant tumors including leukemias, carcinomas and sarcomas, whether new or recurring. Specific examples of cancers are: carcinomas, sarcomas, myelomas, leukemias, lymphomas and mixed type tumors. Non-limiting examples of cancers are new or recurring cancers of the brain, melanoma, bladder, breast, cervix, colon, head and neck, kidney, lung, non-small cell lung, mesothelioma, ovary, prostate, sarcoma,

stomach, uterus and medulloblastoma. In some embodiments, the cancer comprises a solid tumor. In some embodiments, the cancer comprises a metastasis.

**[133]** A "carbohydrate" refers to a sugar or polymer of sugars. The terms "saccharide," "polysaccharide," "carbohydrate," and "oligosaccharide" may be used interchangeably. Most carbohydrates are aldehydes or ketones with many hydroxyl groups, usually one on each carbon atom of the molecule. Carbohydrates generally have the molecular formula $C_nH_{2n}O_n$. A carbohydrate may be a monosaccharide, a disaccharide, trisaccharide, oligosaccharide, or polysaccharide. The most basic carbohydrate is a monosaccharide, such as glucose, galactose, mannose, ribose, arabinose, xylose, and fructose. Disaccharides are two joined monosaccharides. Exemplary disaccharides include sucrose, maltose, cellobiose, and lactose. Typically, an oligosaccharide includes between three and six monosaccharide units (e.g., raffinose, stachyose), and polysaccharides include six or more monosaccharide units. Exemplary polysaccharides include starch, glycogen, and cellulose. Carbohydrates may contain modified saccharide units such as 2'-deoxyribose wherein a hydroxyl group is removed, 2'-fluororibose wherein a hydroxyl group is replaced with a fluorine, or N-acetylglucosamine, a nitrogen-containing form of glucose (e.g., 2'-fluororibose, deoxyribose, and hexose). Carbohydrates may exist in many different forms, for example, conformers, cyclic forms, acyclic forms, stereoisomers, tautomers, anomers, and isomers.

**[134]** "Cellular augmentation" broadly refers to the influx of cells or expansion of cells in an environment that are not substantially present in the environment prior to administration of a composition and not present in the composition itself. Cells that augment the environment include immune cells, stromal cells, bacterial and fungal cells. Environments of particular interest are the microenvironments where cancer cells reside or locate. In some instances, the microenvironment is a tumor microenvironment or a tumor draining lymph node. In other instances, the microenvironment is a pre-cancerous tissue site or the site of local administration of a composition or a site where the composition will accumulate after remote administration.

**[135]** "Clade" refers to the OTUs or members of a phylogenetic tree that are downstream of a statistically valid node in a phylogenetic tree. The clade comprises a set of terminal leaves in the phylogenetic tree that is a distinct monophyletic evolutionary unit and that share some extent of sequence similarity.

**[136]** A “combination” of mEVs (such as smEVs) from two or more microbial strains includes the physical co-existence of the microbes from which the mEVs (such as smEVs) are obtained, either in the same material or product or in physically connected products, as well as the temporal co-administration or co-localization of the mEVs (such as smEVs) from the two strains.

**[137]** "Dysbiosis" refers to a state of the microbiota or microbiome of the gut or other body area, including, e.g., mucosal or skin surfaces (or any other microbiome niche) in which the normal diversity and/or function of the host gut microbiome ecological networks ( ”microbiome”) are disrupted. A state of dysbiosis may result in a diseased state, or it may be unhealthy under only certain conditions or only if present for a prolonged period. Dysbiosis may be due to a variety of factors, including, environmental factors, infectious agents , host genotype, host diet and/or stress. A dysbiosis may result in: a change (e.g., increase or decrease) in the prevalence of one or more bacteria types (e.g., anaerobic), species and/or strains, change (e.g., increase or decrease) in diversity of the host microbiome population composition; a change (e.g., increase or reduction) of one or more populations of symbiont organisms resulting in a reduction or loss of one or more beneficial effects; overgrowth of one or more populations of pathogens (e.g., pathogenic bacteria); and/or the presence of, and/or overgrowth of, symbiotic organisms that cause disease only when certain conditions are present.

**[138]** The term “decrease” or “deplete” means a change, such that the difference is, depending on circumstances, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 1/100, 1/1000, 1/10,000, 1/100,000, 1/1,000,000 or undetectable after treatment when compared to a pre-treatment state. Properties that may be decreased include the number of immune cells, bacterial cells, stromal cells, myeloid derived suppressor cells, fibroblasts, metabolites; the level of a cytokine; or another physical parameter (such as ear thickness (e.g., in a DTH animal model) or tumor size (e.g., in an animal tumor model)).

**[139]** The term “ecological consortium” is a group of bacteria which trades metabolites and positively co-regulates one another, in contrast to two bacteria which induce host synergy through activating complementary host pathways for improved efficacy.

**[140]** As used herein, “engineered bacteria” are any bacteria that have been genetically altered from their natural state by human activities, and the progeny of any such bacteria.

Engineered bacteria include, for example, the products of targeted genetic modification, the products of random mutagenesis screens and the products of directed evolution.

**[141]** The term "epitope" means a protein determinant capable of specific binding to an antibody or T cell receptor. Epitopes usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains. Certain epitopes can be defined by a particular sequence of amino acids to which an antibody is capable of binding.

**[142]** The term "gene" is used broadly to refer to any nucleic acid associated with a biological function. The term "gene" applies to a specific genomic sequence, as well as to a cDNA or an mRNA encoded by that genomic sequence.

**[143]** "Identity" as between nucleic acid sequences of two nucleic acid molecules can be determined as a percentage of identity using known computer algorithms such as the "FASTA" program, using for example, the default parameters as in Pearson *et al.* (1988) Proc. Natl. Acad. Sci. USA 85:2444 (other programs include the GCG program package (Devereux, J., *et al.*, Nucleic Acids Research 12(I):387 (1984)), BLASTP, BLASTN, FASTA Atschul, S. F., *et al.*, J Molec Biol 215:403 (1990); Guide to Huge Computers, Mrtin J. Bishop, ed., Academic Press, San Diego, 1994, and Carillo *et al.* (1988) SIAM J Applied Math 48:1073). For example, the BLAST function of the National Center for Biotechnology Information database can be used to determine identity. Other commercially or publicly available programs include, DNAStar "MegAlign" program (Madison, Wis.) and the University of Wisconsin Genetics Computer Group (UWG) "Gap" program (Madison Wis.)).

**[144]** As used herein, the term "immune disorder" refers to any disease, disorder or disease symptom caused by an activity of the immune system, including autoimmune diseases, inflammatory diseases and allergies. Immune disorders include, but are not limited to, autoimmune diseases (e.g., psoriasis, atopic dermatitis, lupus, scleroderma, hemolytic anemia, vasculitis, type one diabetes, Grave's disease, rheumatoid arthritis, multiple sclerosis, Goodpasture's syndrome, pernicious anemia and/or myopathy), inflammatory diseases (e.g., acne vulgaris, asthma, celiac disease, chronic prostatitis, glomerulonephritis, inflammatory bowel disease, pelvic inflammatory disease, reperfusion injury, rheumatoid arthritis, sarcoidosis, transplant rejection, vasculitis and/or interstitial cystitis), and/or an allergies (e.g., food allergies, drug allergies and/or environmental allergies).

**[145]** "Immunotherapy" is treatment that uses a subject's immune system to treat disease (e.g., immune disease, inflammatory disease, metabolic disease, cancer) and includes, for example, checkpoint inhibitors, cancer vaccines, cytokines, cell therapy, CAR-T cells, and dendritic cell therapy.

**[146]** The term "increase" means a change, such that the difference is, depending on circumstances, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 2-fold, 4-fold, 10-fold, 100-fold, 10^3 fold, 10^4 fold, 10^5 fold, 10^6 fold, and/or 10^7 fold greater after treatment when compared to a pre-treatment state. Properties that may be increased include the number of immune cells, bacterial cells, stromal cells, myeloid derived suppressor cells, fibroblasts, metabolites; the level of a cytokine; or another physical parameter (such as ear thickness (e.g., in a DTH animal model) or tumor size (e.g., in an animal tumor model).

**[147]** "Innate immune agonists" or "immuno-adjuvants" are small molecules, proteins, or other agents that specifically target innate immune receptors including Toll-Like Receptors (TLR), NOD receptors, RLRs, C-type lectin receptors, STING-cGAS Pathway components, inflammasome complexes. For example, LPS is a TLR-4 agonist that is bacterially derived or synthesized and aluminum can be used as an immune stimulating adjuvant. immuno-adjuvants are a specific class of broader adjuvant or adjuvant therapy. Examples of STING agonists include, but are not limited to, 2'3'- cGAMP, 3'3'-cGAMP, c-di-AMP, c-di-GMP, 2'2'-cGAMP, and 2'3'-cGAM(PS)2 (Rp/Sp) (Rp, Sp-isomers of the bis-phosphorothioate analog of 2'3'-cGAMP). Examples of TLR agonists include, but are not limited to, TLRl, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLRlO and TLRI l. Examples of NOD agonists include, but are not limited to, N-acetylmuramyl-L-alanyl-D-isoglutamine (muramyldipeptide (MDP)), gamma-D-glutamyl-meso-diaminopimelic acid (iE-DAP), and desmuramylpeptides (DMP).

**[148]** The "internal transcribed spacer" or " ITS" is a piece of non-functional RNA located between structural ribosomal RNAs (rRNA) on a common precursor transcript often used for identification of eukaryotic species in particular fungi. The rRNA of fungi that forms the core of the ribosome is transcribed as a signal gene and consists of the 8S, 5.8S and 28S regions with ITS4 and 5 between the 8S and 5.8S and 5.8S and 28S regions, respectively. These two intercistronic segments between the 18S and 5.8S and 5.8S and 28S regions are removed by splicing and contain significant variation between species for barcoding purposes as previously described (Schoch et al Nuclear ribosomal internal transcribed spacer (ITS) region as a universal

DNA barcode marker for Fungi. PNAS 109:6241-6246. 2012). 18S rDNA is traditionally used for phylogenetic reconstruction however the ITS can serve this function as it is generally highly conserved but contains hypervariable regions that harbor sufficient nucleotide diversity to differentiate genera and species of most fungus.

**[149]** The term "isolated" or "enriched" encompasses a microbe, an mEV (such as an smEV) or other entity or substance that has been (1) separated from at least some of the components with which it was associated when initially produced (whether in nature or in an experimental setting), and/or (2) produced, prepared, purified, and/or manufactured by the hand of man. Isolated microbes or mEVs may be separated from at least about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or more of the other components with which they were initially associated. In some embodiments, isolated microbes or mEVs are more than about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% pure, e.g., substantially free of other components. The terms "purify," "purifying" and "purified" refer to a microbe or mEV or other material that has been separated from at least some of the components with which it was associated either when initially produced or generated (*e.g.*, whether in nature or in an experimental setting), or during any time after its initial production. A microbe or a microbial population or mEV may be considered purified if it is isolated at or after production, such as from a material or environment containing the microbe or microbial population or mEV, and a purified microbe or microbial or mEV population may contain other materials up to about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or above about 90% and still be considered "isolated." In some embodiments, purified microbes or mEVs or microbial population are more than about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% pure. In the instance of microbial compositions provided herein, the one or more microbial types present in the composition can be independently purified from one or more other microbes produced and/or present in the material or environment containing the microbial type. Microbial compositions and the microbial components such as mEVs thereof are generally purified from residual habitat products.

**[150]** As used herein a "lipid" includes fats, oils, triglycerides, cholesterol, phospholipids, fatty acids in any form including free fatty acids. Fats, oils and fatty acids can be saturated, unsaturated (cis or trans) or partially unsaturated (cis or trans).

**[151]** The term "LPS mutant or lipopolysaccharide mutant" broadly refers to selected bacteria that comprises loss of LPS. Loss of LPS might be due to mutations or disruption to genes involved in lipid A biosynthesis, such as *lpxA*, *lpxC*, and *lpxD*. Bacteria comprising LPS mutants can be resistant to aminoglycosides and polymyxins (polymyxin B and colistin).

**[152]** "Metabolite" as used herein refers to any and all molecular compounds, compositions, molecules, ions, co-factors, catalysts or nutrients used as substrates in any cellular or microbial metabolic reaction or resulting as product compounds, compositions, molecules, ions, co-factors, catalysts or nutrients from any cellular or microbial metabolic reaction.

**[153]** "Microbe" refers to any natural or engineered organism characterized as a archaeaon, parasite, bacterium, fungus, microscopic alga, protozoan, and the stages of development or life cycle stages (*e.g.*, vegetative, spore (including sporulation, dormancy, and germination), latent, biofilm) associated with the organism. Examples of gut microbes include: *Actinomyces graevenitzii, Actinomyces odontolyticus, Akkermansia muciniphila, Bacteroides caccae, Bacteroides fragilis, Bacteroides putredinis, Bacteroides thetaiotaomicron, Bacteroides vultagus, Bifidobacterium adolescentis, Bifidobacterium bifidum, Bilophila wadsworthia, Blautia, Butyrivibrio, Campylobacter gracilis, Clostridia cluster III, Clostridia cluster IV, Clostridia cluster IX (Acidaminococcaceae group), Clostridia cluster XI, Clostridia cluster XIII (Peptostreptococcus group), Clostridia cluster XIV, Clostridia cluster XV, Collinsella aerofaciens, Coprococcus, Corynebacterium sunsvallense, Desulfomonas pigra, Dorea formicigenerans, Dorea longicatena, Escherichia coli, Eubacterium hadrum, Eubacterium rectale, Faecalibacteria prausnitzii, Gemella, Lactococcus, Lanchnospira, Mollicutes cluster XVI, Mollicutes cluster XVIII, Prevotella, Rothia mucilaginosa, Ruminococcus callidus, Ruminococcus gnavus, Ruminococcus torques,* and *Streptococcus.*

**[154]** "Microbial extracellular vesicles" (mEVs) can be obtained from microbes such as bacteria, archaea, fungi, microscopic algae, protozoans, and parasites. In some embodiments, the mEVs are obtained from bacteria. mEVs include secreted microbial extracellular vesicles (smEVs) and processed microbial extracellular vesicles (pmEVs). "Secreted microbial extracellular vesicles" (smEVs) are naturally-produced vesicles derived from microbes. smEVs

are comprised of microbial lipids and/or microbial proteins and/or microbial nucleic acids and/or microbial carbohydrate moieties, and are isolated from culture supernatant. The natural production of these vesicles can be artificially enhanced (e.g., increased) or decreased through manipulation of the environment in which the bacterial cells are being cultured (e.g., by media or temperature alterations). Further, smEV compositions may be modified to reduce, increase, add, or remove microbial components or foreign substances to alter efficacy, immune stimulation, stability, immune stimulatory capacity, stability, organ targeting (e.g., lymph node), absorption (e.g., gastrointestinal), and/or yield (e.g., thereby altering the efficacy). As used herein, the term "purified smEV composition" or "smEV composition" refers to a preparation of smEVs that have been separated from at least one associated substance found in a source material (e.g., separated from at least one other microbial component) or any material associated with the smEVs in any process used to produce the preparation. It can also refer to a composition that has been significantly enriched for specific components. "Processed microbial extracellular vesicles" (pmEVs) are a non-naturally-occurring collection of microbial membrane components that have been purified from artificially lysed microbes (e.g., bacteria) (e.g., microbial membrane components that have been separated from other, intracellular microbial cell components), and which may comprise particles of a varied or a selected size range, depending on the method of purification. A pool of pmEVs is obtained by chemically disrupting (e.g., by lysozyme and/or lysostaphin) and/or physically disrupting (e.g., by mechanical force) microbial cells and separating the microbial membrane components from the intracellular components through centrifugation and/or ultracentrifugation, or other methods. The resulting pmEV mixture contains an enrichment of the microbial membranes and the components thereof (e.g., peripherally associated or integral membrane proteins, lipids, glycans, polysaccharides, carbohydrates, other polymers), such that there is an increased concentration of microbial membrane components, and a decreased concentration (e.g., dilution) of intracellular contents, relative to whole microbes. For gram-positive bacteria, pmEVs may include cell or cytoplasmic membranes. For gram-negative bacteria, a pmEV may include inner and outer membranes. Gram-negative bacteria may belong to the class Negativicutes. pmEVs may be modified to increase purity, to adjust the size of particles in the composition, and/or modified to reduce, increase, add or remove, microbial components or foreign substances to alter efficacy, immune stimulation, stability, immune stimulatory capacity, stability, organ targeting (e.g., lymph node), absorption (e.g.,

gastrointestinal), and/or yield (e.g., thereby altering the efficacy). pmEVs can be modified by adding, removing, enriching for, or diluting specific components, including intracellular components from the same or other microbes. As used herein, the term "purified pmEV composition" or "pmEV composition" refers to a preparation of pmEVs that have been separated from at least one associated substance found in a source material (e.g., separated from at least one other microbial component) or any material associated with the pmEVs in any process used to produce the preparation. It can also refer to a composition that has been significantly enriched for specific components.

**[155]** "Microbiome" broadly refers to the microbes residing on or in body site of a subject or patient. Microbes in a microbiome may include bacteria, viruses, eukaryotic microorganisms, and/or viruses. Individual microbes in a microbiome may be metabolically active, dormant, latent, or exist as spores, may exist planktonically or in biofilms, or may be present in the microbiome in sustainable or transient manner. The microbiome may be a commensal or healthy-state microbiome or a disease-state microbiome. The microbiome may be native to the subject or patient, or components of the microbiome may be modulated, introduced, or depleted due to changes in health state (*e.g.*, precancerous or cancerous state) or treatment conditions (*e.g.*, antibiotic treatment, exposure to different microbes). In some aspects, the microbiome occurs at a mucosal surface. In some aspects, the microbiome is a gut microbiome. In some aspects, the microbiome is a tumor microbiome.

**[156]** A "microbiome profile" or a "microbiome signature" of a tissue or sample refers to an at least partial characterization of the bacterial makeup of a microbiome. In some embodiments, a microbiome profile indicates whether at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or more bacterial strains are present or absent in a microbiome. In some embodiments, a microbiome profile indicates whether at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or more cancer-associated bacterial strains are present in a sample. In some embodiments, the microbiome profile indicates the relative or absolute amount of each bacterial strain detected in the sample. In some embodiments, the microbiome profile is a cancer-associated microbiome profile. A cancer-associated microbiome profile is a microbiome profile that occurs with greater frequency in a subject who has cancer than in the general population. In some embodiments, the cancer-associated microbiome profile comprises a greater number of or amount of cancer-

associated bacteria than is normally present in a microbiome of an otherwise equivalent tissue or sample taken from an individual who does not have cancer.

**[157]** "Modified" in reference to a bacteria broadly refers to a bacteria that has undergone a change from its wild-type form. Bacterial modification can result from engineering bacteria. Examples of bacterial modifications include genetic modification, gene expression modification, phenotype modification, formulation modification, chemical modification, and dose or concentration. Examples of improved properties are described throughout this specification and include, e.g., attenuation, auxotrophy, homing, or antigenicity. Phenotype modification might include, by way of example, bacteria growth in media that modify the phenotype of a bacterium such that it increases or decreases virulence.

**[158]** An "oncobiome" as used herein comprises tumorigenic and/or cancer-associated microbiota, wherein the microbiota comprises one or more of a virus, a bacterium, a fungus, a protist, a parasite, or another microbe.

**[159]** "Oncotrophic" or "oncophilic" microbes and bacteria are microbes that are highly associated or present in a cancer microenvironment. They may be preferentially selected for within the environment, preferentially grow in a cancer microenvironment or hone to a said environment.

**[160]** "Operational taxonomic units" and "OTU(s)" refer to a terminal leaf in a phylogenetic tree and is defined by a nucleic acid sequence, e.g., the entire genome, or a specific genetic sequence, and all sequences that share sequence identity to this nucleic acid sequence at the level of species. In some embodiments the specific genetic sequence may be the 16S sequence or a portion of the 16S sequence. In other embodiments, the entire genomes of two entities are sequenced and compared. In another embodiment, select regions such as multilocus sequence tags (MLST), specific genes, or sets of genes may be genetically compared. For 16S, OTUs that share $\geq$ 97% average nucleotide identity across the entire 16S or some variable region of the 16S are considered the same OTU. See e.g., Claesson MJ, Wang Q, O'Sullivan O, Greene-Diniz R, Cole JR, Ross RP, and O'Toole PW. 2010. Comparison of two next-generation sequencing technologies for resolving highly complex microbiota composition using tandem variable 16S rRNA gene regions. Nucleic Acids Res 38: e200. Konstantinidis KT, Ramette A, and Tiedje JM. 2006. The bacterial species definition in the genomic era. Philos Trans R Soc Lond B Biol Sci 361: 1929–1940. For complete genomes, MLSTs, specific genes, other than

16S, or sets of genes OTUs that share ≥ 95% average nucleotide identity are considered the same OTU. See e.g., Achtman M, and Wagner M. 2008. Microbial diversity and the genetic nature of microbial species. Nat. Rev. Microbiol. 6: 431–440. Konstantinidis KT, Ramette A, and Tiedje JM. 2006. The bacterial species definition in the genomic era. Philos Trans R Soc Lond B Biol Sci 361: 1929–1940. OTUs are frequently defined by comparing sequences between organisms. Generally, sequences with less than 95% sequence identity are not considered to form part of the same OTU. OTUs may also be characterized by any combination of nucleotide markers or genes, in particular highly conserved genes (e.g., "house-keeping" genes), or a combination thereof. Operational Taxonomic Units (OTUs) with taxonomic assignments made to, e.g., genus, species, and phylogenetic clade are provided herein.

**[161]** As used herein, a gene is "overexpressed" in a bacteria if it is expressed at a higher level in an engineered bacteria under at least some conditions than it is expressed by a wild-type bacteria of the same species under the same conditions. Similarly, a gene is "underexpressed" in a bacteria if it is expressed at a lower level in an engineered bacteria under at least some conditions than it is expressed by a wild-type bacteria of the same species under the same conditions.

**[162]** The terms "polynucleotide", and "nucleic acid" are used interchangeably. They refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides, or analogs thereof. Polynucleotides may have any three-dimensional structure, and may perform any function. The following are non-limiting examples of polynucleotides: coding or non-coding regions of a gene or gene fragment, loci (locus) defined from linkage analysis, exons, introns, messenger RNA (mRNA), micro RNA (miRNA), silencing RNA (siRNA), transfer RNA, ribosomal RNA, ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes, and primers. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs. If present, modifications to the nucleotide structure may be imparted before or after assembly of the polymer. A polynucleotide may be further modified, such as by conjugation with a labeling component. In all nucleic acid sequences provided herein, U nucleotides are interchangeable with T nucleotides.

**[163]** As used herein, a substance is "pure" if it is substantially free of other components. The terms "purify," "purifying" and "purified" refer to an mEV (such as an smEV)

preparation or other material that has been separated from at least some of the components with which it was associated either when initially produced or generated (e.g., whether in nature or in an experimental setting), or during any time after its initial production. An mEV (such as an smEV) preparation or compositions may be considered purified if it is isolated at or after production, such as from one or more other bacterial components, and a purified microbe or microbial population may contain other materials up to about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or above about 90% and still be considered "purified." In some embodiments, purified mEVs (such as smEVs) are more than about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% pure. mEV (such as an smEV) compositions (or preparations) are, e.g., purified from residual habitat products.

**[164]** As used herein, the term "purified mEV composition" or "mEV composition" refers to a preparation that includes mEVs (such as smEVs) that have been separated from at least one associated substance found in a source material (*e.g.*, separated from at least one other bacterial component) or any material associated with the mEVs (such as smEVs) in any process used to produce the preparation. It also refers to a composition that has been significantly enriched or concentrated. In some embodiments, the mEVs (such as smEVs) are concentrated by 2 fold, 3-fold, 4-fold, 5-fold, 10-fold, 100-fold, 1000-fold, 10,000-fold or more than 10,000 fold.

**[165]** "Residual habitat products" refers to material derived from the habitat for microbiota within or on a subject. For example, fermentation cultures of microbes can contain contaminants, e.g., other microbe strains or forms (e.g., bacteria, virus, mycoplasm, and/or fungus). For example, microbes live in feces in the gastrointestinal tract, on the skin itself, in saliva, mucus of the respiratory tract, or secretions of the genitourinary tract (i.e., biological matter associated with the microbial community). Substantially free of residual habitat products means that the microbial composition no longer contains the biological matter associated with the microbial environment on or in the culture or human or animal subject and is 100% free, 99% free, 98% free, 97% free, 96% free, or 95% free of any contaminating biological matter associated with the microbial community. Residual habitat products can include abiotic materials (including undigested food) or it can include unwanted microorganisms. Substantially free of residual habitat products may also mean that the microbial composition contains no detectable cells from a culture contaminant or a human or animal and that only microbial cells are

detectable. In one embodiment, substantially free of residual habitat products may also mean that the microbial composition contains no detectable viral (including bacteria, viruses (e.g., phage)), fungal, mycoplasmal contaminants. In another embodiment, it means that fewer than $1x10^{-2}$%, $1x10^{-3}$%, $1x10^{-4}$%, $1x10^{-5}$%, $1x10^{-6}$%, $1x10^{-7}$%, $1x10^{-8}$% of the viable cells in the microbial composition are human or animal, as compared to microbial cells. There are multiple ways to accomplish this degree of purity, none of which are limiting. Thus, contamination may be reduced by isolating desired constituents through multiple steps of streaking to single colonies on solid media until replicate (such as, but not limited to, two) streaks from serial single colonies have shown only a single colony morphology. Alternatively, reduction of contamination can be accomplished by multiple rounds of serial dilutions to single desired cells (e.g., a dilution of $10^{-8}$ or $10^{-9}$), such as through multiple 10-fold serial dilutions. This can further be confirmed by showing that multiple isolated colonies have similar cell shapes and Gram staining behavior. Other methods for confirming adequate purity include genetic analysis (e.g., PCR, DNA sequencing), serology and antigen analysis, enzymatic and metabolic analysis, and methods using instrumentation such as flow cytometry with reagents that distinguish desired constituents from contaminants.

**[166]** As used herein, "specific binding" refers to the ability of an antibody to bind to a predetermined antigen or the ability of a polypeptide to bind to its predetermined binding partner. Typically, an antibody or polypeptide specifically binds to its predetermined antigen or binding partner with an affinity corresponding to a $K_D$ of about $10^{-7}$ M or less, and binds to the predetermined antigen/binding partner with an affinity (as expressed by $K_D$) that is at least 10 fold less, at least 100 fold less or at least 1000 fold less than its affinity for binding to a non-specific and unrelated antigen/binding partner (e.g., BSA, casein). Alternatively, specific binding applies more broadly to a two component system where one component is a protein, lipid, or carbohydrate or combination thereof and engages with the second component which is a protein, lipid, carbohydrate or combination thereof in a specific way.

**[167]** "Strain" refers to a member of a bacterial species with a genetic signature such that it may be differentiated from closely-related members of the same bacterial species. The genetic signature may be the absence of all or part of at least one gene, the absence of all or part of at least on regulatory region (e.g., a promoter, a terminator, a riboswitch, a ribosome binding site), the absence ("curing") of at least one native plasmid, the presence of at least one

recombinant gene, the presence of at least one mutated gene, the presence of at least one foreign gene (a gene derived from another species), the presence at least one mutated regulatory region (e.g., a promoter, a terminator, a riboswitch, a ribosome binding site), the presence of at least one non-native plasmid, the presence of at least one antibiotic resistance cassette, or a combination thereof. Genetic signatures between different strains may be identified by PCR amplification optionally followed by DNA sequencing of the genomic region(s) of interest or of the whole genome. In the case in which one strain (compared with another of the same species) has gained or lost antibiotic resistance or gained or lost a biosynthetic capability (such as an auxotrophic strain), strains may be differentiated by selection or counter-selection using an antibiotic or nutrient/metabolite, respectively.

**[168]** The terms "subject" or "patient" refers to any mammal. A subject or a patient described as "in need thereof" refers to one in need of a treatment (or prevention) for a disease. Mammals (i.e., mammalian animals) include humans, laboratory animals (e.g., primates, rats, mice), livestock (e.g., cows, sheep, goats, pigs), and household pets (e.g., dogs, cats, rodents). The subject may be a human. The subject may be a non-human mammal including but not limited to of a dog, a cat, a cow, a horse, a pig, a donkey, a goat, a camel, a mouse, a rat, a guinea pig, a sheep, a llama, a monkey, a gorilla or a chimpanzee. The subject may be healthy, or may be suffering from a cancer at any developmental stage, wherein any of the stages are either caused by or opportunistically supported of a cancer associated or causative pathogen, or may be at risk of developing a cancer, or transmitting to others a cancer associated or cancer causative pathogen. In some embodiments, a subject has lung cancer, bladder cancer, prostate cancer, plasmacytoma, colorectal cancer, rectal cancer, Merkel Cell carcinoma, salivary gland carcinoma, ovarian cancer, and/or melanoma. The subject may have a tumor. The subject may have a tumor that shows enhanced macropinocytosis with the underlying genomics of this process including Ras activation. In other embodiments, the subject has another cancer. In some embodiments, the subject has undergone a cancer therapy.

**[169]** As used herein, the term "treating" a disease in a subject or "treating" a subject having or suspected of having a disease refers to administering to the subject to a pharmaceutical treatment, *e.g.*, the administration of one or more agents, such that at least one symptom of the disease is decreased or prevented from worsening. Thus, in one embodiment, "treating" refers inter alia to delaying progression, expediting remission, inducing remission, augmenting

remission, speeding recovery, increasing efficacy of or decreasing resistance to alternative therapeutics, or a combination thereof. As used herein, the term "preventing" a disease in a subject refers to administering to the subject to a pharmaceutical treatment, e.g., the administration of one or more agents, such that onset of at least one symptom of the disease is delayed or prevented..

Bacteria

**[170]** In certain aspects, provided herein are pharmaceutical compositions that comprise mEVs (such as smEVs) obtained from bacteria.

**[171]** In some embodiments, the bacteria from which the mEVs (such as smEVs) are obtained are modified to reduce toxicity or other adverse effects, to enhance delivery) (e.g., oral delivery) of the mEVs (such as smEVs) (e.g., by improving acid resistance, muco-adherence and/or penetration and/or resistance to bile acids, digestive enzymes, resistance to anti-microbial peptides and/or antibody neutralization), to target desired cell types (e.g., M-cells, goblet cells, enterocytes, dendritic cells, macrophages), to enhance their immunomodulatory and/or therapeutic effect of the mEVs (such as smEVs) (e.g., either alone or in combination with another therapeutic agent), and/or to enhance immune activation or suppression by the mEVs (such as smEVs) (e.g., through modified production of polysaccharides, pili, fimbriae, adhesins). In some embodiments, the engineered bacteria described herein are modified to improve mEV (such as smEV) manufacturing (e.g., higher oxygen tolerance, stability, improved freeze-thaw tolerance, shorter generation times). For example, in some embodiments, the engineered bacteria described include bacteria harboring one or more genetic changes, such change being an insertion, deletion, translocation, or substitution, or any combination thereof, of one or more nucleotides contained on the bacterial chromosome or endogenous plasmid and/or one or more foreign plasmids, wherein the genetic change may results in the overexpression and/or underexpression of one or more genes. The engineered bacteria may be produced using any technique known in the art, including but not limited to site-directed mutagenesis, transposon mutagenesis, knock-outs, knock-ins, polymerase chain reaction mutagenesis, chemical mutagenesis, ultraviolet light mutagenesis, transformation (chemically or by electroporation), phage transduction, directed evolution, or any combination thereof.

**[172]** Examples of species and/or strains of bacteria that can be used as a source of mEVs (such as smEVs) described herein are provided in Table 1, Table 2, and/or Table 3 and elsewhere throughout the specification. In some embodiments, the bacterial strain is a bacterial strain having a genome that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8% or 99.9% sequence identity to a strain listed in Table 1, Table 2, and/or Table 3. In some embodiments, the mEVs are from an oncotrophic bacteria. In some embodiments, the mEVs are from an immunostimulatory bacteria. In some embodiments, the mEVs are from an immunosuppressive bacteria. In some embodiments, the mEVs are from an immunomodulatory bacteria. In certain embodiments, mEVs are generated from a combination of bacterial strains provided herein. In some embodiments, the combination is a combination of at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, 30, 35, 40, 45 or 50 bacterial strains. In some embodiments, the combination includes mEVs from bacterial strains listed in Table 1, Table 2, and/or Table 3 and/or bacterial strains having a genome that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8% or 99.9% sequence identity to a strain listed in Table 1, Table 2, and/or Table 3.

**[173]** In some embodiments, the mEVs are obtained from Gram negative bacteria.

**[174]** In some embodiments, the Gram negative bacteria belong to the class Negativicutes. The Negativicutes represent a unique class of microorganisms as they are the only diderm members of the Firmicutes phylum. These anaerobic organisms can be found in the environment and are normal commensals of the oral cavity and GI tract of humans. Because these organisms have an outer membrane, the yields of smEVs from this class were investigated. It was found that on a per cell basis these microbes produce a high number of vesicles (10-150 EVs/cell). The smEVs from these organisms are broadly stimulatory and highly potent in *in vitro* assays. Investigations into their therapeutic applications in several oncology and inflammation *in vivo* models have shown their therapeutic potential. The class Negativicutes includes the families *Veillonellaceae, Selenomonadaceae, Acidaminococcaceae*, and *Sporomusaceae*. The class Negativicutes includes the genera *Megasphaera, Selenomonas, Propionospora,* and *Acidaminococcus*. Exemplary Negativicutes species include, but are not limited to, *Megasphaera sp., Selenomonas felix, Acidaminococcus intestine,* and *Propionospora sp.*.

**[175]** In some embodiments, the mEVs are obtained from Gram positive bacteria.

**[176]** In some embodiments, the mEVs are obtained from aerobic bacteria.

**[177]** In some embodiments, the mEVs are obtained from anaerobic bacteria.

**[178]** In some embodiments, the mEVs are obtained from acidophile bacteria.

**[179]** In some embodiments, the mEVs are obtained from alkaliphile bacteria.

**[180]** In some embodiments, the mEVs are obtained from neutralophile bacteria.

**[181]** In some embodiments, the mEVs are obtained from fastidious bacteria.

**[182]** In some embodiments, the mEVs are obtained from nonfastidious bacteria.

**[183]** In some embodiments, bacteria from which mEVs are obtained are lyophilized.

**[184]** In some embodiments, bacteria from which mEVs are obtained are gamma irradiated (e.g., at 17.5 or 25 kGy).

**[185]** In some embodiments, bacteria from which mEVs are obtained are UV irradiated.

**[186]** In some embodiments, bacteria from which mEVs are obtained are heat inactivated (e.g., at 50°C for two hours or at 90°C for two hours).

**[187]** In some embodiments, bacteria from which mEVs are obtained are acid treated.

**[188]** In some embodiments, bacteria from which mEVs are obtained are oxygen sparged (e.g., at 0.1 vvm for two hours).

**[189]** In some embodiments, the mEVs are lyophilized.

**[190]** In some embodiments, the mEVs are gamma irradiated (e.g., at 17.5 or 25 kGy).

**[191]** In some embodiments, the mEVs are UV irradiated.

**[192]** In some embodiments, the mEVs are heat inactivated (e.g., at 50°C for two hours or at 90°C for two hours).

**[193]** In some embodiments, the mEVs are acid treated.

**[194]** In some embodiments, the mEVs are oxygen sparged (e.g., at 0.1 vvm for two hours).

**[195]** The phase of growth can affect the amount or properties of bacteria and/or smEVs produced by bacteria. For example, in the methods of smEVs preparation provided herein, smEVs can be isolated, e.g., from a culture, at the start of the log phase of growth, midway through the log phase, and/or once stationary phase growth has been reached.

**Table 1: Exemplary Bacterial Strains**

| OTU | Public DB Accession |
|---|---|

| | |
|---|---|
| *Abiotrophia defectiva* | ACIN02000016 |
| *Abiotrophia para_adiacens* | AB022027 |
| *Abiotrophia sp. oral clone P4PA_155 P1* | AY207063 |
| *Acetanaerobacterium elongatum* | NR_042930 |
| *Acetivibrio cellulolyticus* | NR_025917 |
| *Acetivibrio ethanolgignens* | FR749897 |
| *Acetobacter aceti* | NR_026121 |
| *Acetobacter fabarum* | NR_042678 |
| *Acetobacter lovaniensis* | NR_040832 |
| *Acetobacter malorum* | NR_025513 |
| *Acetobacter orientalis* | NR_028625 |
| *Acetobacter pasteurianus* | NR_026107 |
| *Acetobacter pomorum* | NR_042112 |
| *Acetobacter syzygii* | NR_040868 |
| *Acetobacter tropicalis* | NR_036881 |
| *Acetobacteraceae bacterium AT_5844* | AGEZ01000040 |
| *Acholeplasma laidlawii* | NR_074448 |
| *Achromobacter denitrificans* | NR_042021 |
| *Achromobacter piechaudii* | ADMS01000149 |
| *Achromobacter xylosoxidans* | ACRC01000072 |
| *Acidaminococcus fermentans* | CP001859 |
| *Acidaminococcus intestini* | CP003058 |
| *Acidaminococcus sp. D21* | ACGB01000071 |
| *Acidilobus saccharovorans* | AY350586 |
| *Acidithiobacillus ferrivorans* | NR_074660 |
| *Acidovorax sp. 98_63833* | AY258065 |
| *Acinetobacter baumannii* | ACYQ01000014 |
| *Acinetobacter calcoaceticus* | AM157426 |
| *Acinetobacter genomosp. C1* | AY278636 |

| | |
|---|---|
| *Acinetobacter haemolyticus* | ADMT01000017 |
| *Acinetobacter johnsonii* | ACPL01000162 |
| *Acinetobacter junii* | ACPM01000135 |
| *Acinetobacter lwoffii* | ACPN01000204 |
| *Acinetobacter parvus* | AIEB01000124 |
| *Acinetobacter radioresistens* | ACVR01000010 |
| *Acinetobacter schindleri* | NR_025412 |
| *Acinetobacter sp. 56A1* | GQ178049 |
| *Acinetobacter sp. CIP 101934* | JQ638573 |
| *Acinetobacter sp. CIP 102143* | JQ638578 |
| *Acinetobacter sp. CIP 53.82* | JQ638584 |
| *Acinetobacter sp. M16_22* | HM366447 |
| *Acinetobacter sp. RUH2624* | ACQF01000094 |
| *Acinetobacter sp. SH024* | ADCH01000068 |
| *Actinobacillus actinomycetemcomitans* | AY362885 |
| *Actinobacillus minor* | ACFT01000025 |
| *Actinobacillus pleuropneumoniae* | NR_074857 |
| *Actinobacillus succinogenes* | CP000746 |
| *Actinobacillus ureae* | AEVG01000167 |
| *Actinobaculum massiliae* | AF487679 |
| *Actinobaculum schaalii* | AY957507 |
| *Actinobaculum sp. BM#101342* | AY282578 |
| *Actinobaculum sp. P2P_19 P1* | AY207066 |
| *Actinomyces cardiffensis* | GU470888 |
| *Actinomyces europaeus* | NR_026363 |
| *Actinomyces funkei* | HQ906497 |
| *Actinomyces genomosp. C1* | AY278610 |
| *Actinomyces genomosp. C2* | AY278611 |
| *Actinomyces genomosp. P1 oral clone MB6_C03* | DQ003632 |

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| *Actinomyces georgiae* | GU561319 |
| *Actinomyces israelii* | AF479270 |
| *Actinomyces massiliensis* | AB545934 |
| *Actinomyces meyeri* | GU561321 |
| *Actinomyces naeslundii* | X81062 |
| *Actinomyces nasicola* | AJ508455 |
| *Actinomyces neuii* | X71862 |
| *Actinomyces odontolyticus* | ACYT01000123 |
| *Actinomyces oricola* | NR_025559 |
| *Actinomyces orihominis* | AJ575186 |
| *Actinomyces oris* | BABV01000070 |
| *Actinomyces sp. 7400942* | EU484334 |
| *Actinomyces sp. c109* | AB167239 |
| *Actinomyces sp. CCUG 37290* | AJ234058 |
| *Actinomyces sp. ChDC B197* | AF543275 |
| *Actinomyces sp. GEJ15* | GU561313 |
| *Actinomyces sp. HKU31* | HQ335393 |
| *Actinomyces sp. ICM34* | HQ616391 |
| *Actinomyces sp. ICM41* | HQ616392 |
| *Actinomyces sp. ICM47* | HQ616395 |
| *Actinomyces sp. ICM54* | HQ616398 |
| *Actinomyces sp. M2231_94_1* | AJ234063 |
| *Actinomyces sp. oral clone GU009* | AY349361 |
| *Actinomyces sp. oral clone GU067* | AY349362 |
| *Actinomyces sp. oral clone IO076* | AY349363 |
| *Actinomyces sp. oral clone IO077* | AY349364 |
| *Actinomyces sp. oral clone IP073* | AY349365 |
| *Actinomyces sp. oral clone IP081* | AY349366 |
| *Actinomyces sp. oral clone JA063* | AY349367 |

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| *Actinomyces sp. oral taxon 170* | AFBL01000010 |
| *Actinomyces sp. oral taxon 171* | AECW01000034 |
| *Actinomyces sp. oral taxon 178* | AEUH01000060 |
| *Actinomyces sp. oral taxon 180* | AEPP01000041 |
| *Actinomyces sp. oral taxon 848* | ACUY01000072 |
| *Actinomyces sp. oral taxon C55* | HM099646 |
| *Actinomyces sp. TeJ5* | GU561315 |
| *Actinomyces urogenitalis* | ACFH01000038 |
| *Actinomyces viscosus* | ACRE01000096 |
| *Adlercreutzia equolifaciens* | AB306661 |
| *Aerococcus sanguinicola* | AY837833 |
| *Aerococcus urinae* | CP002512 |
| *Aerococcus urinaeequi* | NR_043443 |
| *Aerococcus viridans* | ADNT01000041 |
| *Aeromicrobium marinum* | NR_025681 |
| *Aeromicrobium sp. JC14* | JF824798 |
| *Aeromonas allosaccharophila* | S39232 |
| *Aeromonas enteropelogenes* | X71121 |
| *Aeromonas hydrophila* | NC_008570 |
| *Aeromonas jandaei* | X60413 |
| *Aeromonas salmonicida* | NC_009348 |
| *Aeromonas trota* | X60415 |
| *Aeromonas veronii* | NR_044845 |
| *Afipia genomosp. 4* | EU117385 |
| *Aggregatibacter actinomycetemcomitans* | CP001733 |
| *Aggregatibacter aphrophilus* | CP001607 |
| *Aggregatibacter segnis* | AEPS01000017 |
| *Agrobacterium radiobacter* | CP000628 |
| *Agrobacterium tumefaciens* | AJ389893 |

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| *Agrococcus jenensis* | NR_026275 |
| *Akkermansia muciniphila* | CP001071 |
| *Alcaligenes faecalis* | AB680368 |
| *Alcaligenes sp. CO14* | DQ643040 |
| *Alcaligenes sp. S3* | HQ262549 |
| *Alicyclobacillus acidocaldarius* | NR_074721 |
| *Alicyclobacillus acidoterrestris* | NR_040844 |
| *Alicyclobacillus contaminans* | NR_041475 |
| *Alicyclobacillus cycloheptanicus* | NR_024754 |
| *Alicyclobacillus herbarius* | NR_024753 |
| *Alicyclobacillus pomorum* | NR_024801 |
| *Alicyclobacillus sp. CCUG 53762* | HE613268 |
| *Alistipes finegoldii* | NR_043064 |
| *Alistipes indistinctus* | AB490804 |
| *Alistipes onderdonkii* | NR_043318 |
| *Alistipes putredinis* | ABFK02000017 |
| *Alistipes shahii* | FP929032 |
| *Alistipes sp. HGB5* | AENZ01000082 |
| *Alistipes sp. JC50* | JF824804 |
| *Alistipes sp. RMA 9912* | GQ140629 |
| *Alkaliphilus metalliredigenes* | AY137848 |
| *Alkaliphilus oremlandii* | NR_043674 |
| *Alloscardovia omnicolens* | NR_042583 |
| *Alloscardovia sp. OB7196* | AB425070 |
| *Anaerobaculum hydrogeniformans* | ACJX02000009 |
| *Anaerobiospirillum succiniciproducens* | NR_026075 |
| *Anaerobiospirillum thomasii* | AJ420985 |
| *Anaerococcus hydrogenalis* | ABXA01000039 |
| *Anaerococcus lactolyticus* | ABYO01000217 |

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| *Anaerococcus octavius* | NR_026360 |
| *Anaerococcus prevotii* | CP001708 |
| *Anaerococcus sp. 8404299* | HM587318 |
| *Anaerococcus sp. 8405254* | HM587319 |
| *Anaerococcus sp. 9401487* | HM587322 |
| *Anaerococcus sp. 9403502* | HM587325 |
| *Anaerococcus sp. gpac104* | AM176528 |
| *Anaerococcus sp. gpac126* | AM176530 |
| *Anaerococcus sp. gpac155* | AM176536 |
| *Anaerococcus sp. gpac199* | AM176539 |
| *Anaerococcus sp. gpac215* | AM176540 |
| *Anaerococcus tetradius* | ACGC01000107 |
| *Anaerococcus vaginalis* | ACXU01000016 |
| *Anaerofustis stercorihominis* | ABIL02000005 |
| *Anaeroglobus geminatus* | AGCJ01000054 |
| *Anaerosporobacter mobilis* | NR_042953 |
| *Anaerostipes caccae* | ABAX03000023 |
| *Anaerostipes sp. 3_2_56FAA* | ACWB01000002 |
| *Anaerotruncus colihominis* | ABGD02000021 |
| *Anaplasma marginale* | ABOR01000019 |
| *Anaplasma phagocytophilum* | NC_007797 |
| *Aneurinibacillus aneurinilyticus* | AB101592 |
| *Aneurinibacillus danicus* | NR_028657 |
| *Aneurinibacillus migulanus* | NR_036799 |
| *Aneurinibacillus terranovensis* | NR_042271 |
| *Aneurinibacillus thermoaerophilus* | NR_029303 |
| *Anoxybacillus contaminans* | NR_029006 |
| *Anoxybacillus flavithermus* | NR_074667 |
| *Arcanobacterium haemolyticum* | NR_025347 |

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| *Arcanobacterium pyogenes* | GU585578 |
| *Arcobacter butzleri* | AEPT01000071 |
| *Arcobacter cryaerophilus* | NR_025905 |
| *Arthrobacter agilis* | NR_026198 |
| *Arthrobacter arilaitensis* | NR_074608 |
| *Arthrobacter bergerei* | NR_025612 |
| *Arthrobacter globiformis* | NR_026187 |
| *Arthrobacter nicotianae* | NR_026190 |
| *Atopobium minutum* | HM007583 |
| *Atopobium parvulum* | CP001721 |
| *Atopobium rimae* | ACFE01000007 |
| *Atopobium sp. BS2* | HQ616367 |
| *Atopobium sp. F0209* | EU592966 |
| *Atopobium sp. ICM42b10* | HQ616393 |
| *Atopobium sp. ICM57* | HQ616400 |
| *Atopobium vaginae* | AEDQ01000024 |
| *Aurantimonas coralicida* | AY065627 |
| *Aureimonas altamirensis* | FN658986 |
| *Auritibacter ignavus* | FN554542 |
| *Averyella dalhousiensis* | DQ481464 |
| *Bacillus aeolius* | NR_025557 |
| *Bacillus aerophilus* | NR_042339 |
| *Bacillus aestuarii* | GQ980243 |
| *Bacillus alcalophilus* | X76436 |
| *Bacillus amyloliquefaciens* | NR_075005 |
| *Bacillus anthracis* | AAEN01000020 |
| *Bacillus atrophaeus* | NR_075016 |
| *Bacillus badius* | NR_036893 |
| *Bacillus cereus* | ABDJ01000015 |

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| *Bacillus circulans* | AB271747 |
| *Bacillus clausii* | FN397477 |
| *Bacillus coagulans* | DQ297928 |
| *Bacillus firmus* | NR_025842 |
| *Bacillus flexus* | NR_024691 |
| *Bacillus fordii* | NR_025786 |
| *Bacillus gelatini* | NR_025595 |
| *Bacillus halmapalus* | NR_026144 |
| *Bacillus halodurans* | AY144582 |
| *Bacillus herbersteinensis* | NR_042286 |
| *Bacillus horti* | NR_036860 |
| *Bacillus idriensis* | NR_043268 |
| *Bacillus lentus* | NR_040792 |
| *Bacillus licheniformis* | NC_006270 |
| *Bacillus megaterium* | GU252124 |
| *Bacillus nealsonii* | NR_044546 |
| *Bacillus niabensis* | NR_043334 |
| *Bacillus niacini* | NR_024695 |
| *Bacillus pocheonensis* | NR_041377 |
| *Bacillus pumilus* | NR_074977 |
| *Bacillus safensis* | JQ624766 |
| *Bacillus simplex* | NR_042136 |
| *Bacillus sonorensis* | NR_025130 |
| *Bacillus sp. 10403023 MM10403188* | CAET01000089 |
| *Bacillus sp. 2_A_57_CT2* | ACWD01000095 |
| *Bacillus sp. 2008724126* | GU252108 |
| *Bacillus sp. 2008724139* | GU252111 |
| *Bacillus sp. 7_16AIA* | FN397518 |
| *Bacillus sp. 9_3AIA* | FN397519 |

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| *Bacillus sp. AP8* | JX101689 |
| *Bacillus sp. B27(2008)* | EU362173 |
| *Bacillus sp. BT1B_CT2* | ACWC01000034 |
| *Bacillus sp. GB1.1* | FJ897765 |
| *Bacillus sp. GB9* | FJ897766 |
| *Bacillus sp. HU19.1* | FJ897769 |
| *Bacillus sp. HU29* | FJ897771 |
| *Bacillus sp. HU33.1* | FJ897772 |
| *Bacillus sp. JC6* | JF824800 |
| *Bacillus sp. oral taxon F26* | HM099642 |
| *Bacillus sp. oral taxon F28* | HM099650 |
| *Bacillus sp. oral taxon F79* | HM099654 |
| *Bacillus sp. SRC_DSF1* | GU797283 |
| *Bacillus sp. SRC_DSF10* | GU797292 |
| *Bacillus sp. SRC_DSF2* | GU797284 |
| *Bacillus sp. SRC_DSF6* | GU797288 |
| *Bacillus sp. tc09* | HQ844242 |
| *Bacillus sp. zh168* | FJ851424 |
| *Bacillus sphaericus* | DQ286318 |
| *Bacillus sporothermodurans* | NR_026010 |
| *Bacillus subtilis* | EU627588 |
| *Bacillus thermoamylovorans* | NR_029151 |
| *Bacillus thuringiensis* | NC_008600 |
| *Bacillus weihenstephanensis* | NR_074926 |
| *Bacteroidales bacterium ph8* | JN837494 |
| *Bacteroidales genomosp. P1* | AY341819 |
| *Bacteroidales genomosp. P2 oral clone MB1_G13* | DQ003613 |

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| *Bacteroidales genomosp. P3 oral clone MB1_G34* | DQ003615 |
| *Bacteroidales genomosp. P4 oral clone MB2_G17* | DQ003617 |
| *Bacteroidales genomosp. P5 oral clone MB2_P04* | DQ003619 |
| *Bacteroidales genomosp. P6 oral clone MB3_C19* | DQ003634 |
| *Bacteroidales genomosp. P7 oral clone MB3_P19* | DQ003623 |
| *Bacteroidales genomosp. P8 oral clone MB4_G15* | DQ003626 |
| *Bacteroides acidifaciens* | NR_028607 |
| *Bacteroides barnesiae* | NR_041446 |
| *Bacteroides caccae* | EU136686 |
| *Bacteroides cellulosilyticus* | ACCH01000108 |
| *Bacteroides clarus* | AFBM01000011 |
| *Bacteroides coagulans* | AB547639 |
| *Bacteroides coprocola* | ABIY02000050 |
| *Bacteroides coprophilus* | ACBW01000012 |
| *Bacteroides dorei* | ABWZ01000093 |
| *Bacteroides eggerthii* | ACWG01000065 |
| *Bacteroides faecis* | GQ496624 |
| *Bacteroides finegoldii* | AB222699 |
| *Bacteroides fluxus* | AFBN01000029 |
| *Bacteroides fragilis* | AP006841 |
| *Bacteroides galacturonicus* | DQ497994 |
| *Bacteroides helcogenes* | CP002352 |
| *Bacteroides heparinolyticus* | JN867284 |
| *Bacteroides intestinalis* | ABJL02000006 |

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| *Bacteroides massiliensis* | AB200226 |
| *Bacteroides nordii* | NR_043017 |
| *Bacteroides oleiciplenus* | AB547644 |
| *Bacteroides ovatus* | ACWH01000036 |
| *Bacteroides pectinophilus* | ABVQ01000036 |
| *Bacteroides plebeius* | AB200218 |
| *Bacteroides pyogenes* | NR_041280 |
| *Bacteroides salanitronis* | CP002530 |
| *Bacteroides salyersiae* | EU136690 |
| *Bacteroides sp. 1_1_14* | ACRP01000155 |
| *Bacteroides sp. 1_1_30* | ADCL01000128 |
| *Bacteroides sp. 1_1_6* | ACIC01000215 |
| *Bacteroides sp. 2_1_22* | ACPQ01000117 |
| *Bacteroides sp. 2_1_56FAA* | ACWI01000065 |
| *Bacteroides sp. 2_2_4* | ABZZ01000168 |
| *Bacteroides sp. 20_3* | ACRQ01000064 |
| *Bacteroides sp. 3_1_19* | ADCJ01000062 |
| *Bacteroides sp. 3_1_23* | ACRS01000081 |
| *Bacteroides sp. 3_1_33FAA* | ACPS01000085 |
| *Bacteroides sp. 3_1_40A* | ACRT01000136 |
| *Bacteroides sp. 3_2_5* | ACIB01000079 |
| *Bacteroides sp. 315_5* | FJ848547 |
| *Bacteroides sp. 31SF15* | AJ583248 |
| *Bacteroides sp. 31SF18* | AJ583249 |
| *Bacteroides sp. 35AE31* | AJ583244 |
| *Bacteroides sp. 35AE37* | AJ583245 |
| *Bacteroides sp. 35BE34* | AJ583246 |
| *Bacteroides sp. 35BE35* | AJ583247 |
| *Bacteroides sp. 4_1_36* | ACTC01000133 |

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| *Bacteroides sp. 4_3_47FAA* | ACDR02000029 |
| *Bacteroides sp. 9_1_42FAA* | ACAA01000096 |
| *Bacteroides sp. AR20* | AF139524 |
| *Bacteroides sp. AR29* | AF139525 |
| *Bacteroides sp. B2* | EU722733 |
| *Bacteroides sp. D1* | ACAB02000030 |
| *Bacteroides sp. D2* | ACGA01000077 |
| *Bacteroides sp. D20* | ACPT01000052 |
| *Bacteroides sp. D22* | ADCK01000151 |
| *Bacteroides sp. F_4* | AB470322 |
| *Bacteroides sp. NB_8* | AB117565 |
| *Bacteroides sp. WH2* | AY895180 |
| *Bacteroides sp. XB12B* | AM230648 |
| *Bacteroides sp. XB44A* | AM230649 |
| *Bacteroides stercoris* | ABFZ02000022 |
| *Bacteroides thetaiotaomicron* | NR_074277 |
| *Bacteroides uniformis* | AB050110 |
| *Bacteroides ureolyticus* | GQ167666 |
| *Bacteroides vulgatus* | CP000139 |
| *Bacteroides xylanisolvens* | ADKP01000087 |
| *Bacteroidetes bacterium oral taxon D27* | HM099638 |
| *Bacteroidetes bacterium oral taxon F31* | HM099643 |
| *Bacteroidetes bacterium oral taxon F44* | HM099649 |
| *Barnesiella intestinihominis* | AB370251 |
| *Barnesiella viscericola* | NR_041508 |
| *Bartonella bacilliformis* | NC_008783 |
| *Bartonella grahamii* | CP001562 |
| *Bartonella henselae* | NC_005956 |
| *Bartonella quintana* | BX897700 |

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| *Bartonella tamiae* | EF672728 |
| *Bartonella washoensis* | FJ719017 |
| *Bdellovibrio sp. MPA* | AY294215 |
| *Bifidobacteriaceae genomosp. C1* | AY278612 |
| *Bifidobacterium adolescentis* | AAXD02000018 |
| *Bifidobacterium angulatum* | ABYS02000004 |
| *Bifidobacterium animalis* | CP001606 |
| *Bifidobacterium bifidum* | ABQP01000027 |
| *Bifidobacterium breve* | CP002743 |
| *Bifidobacterium catenulatum* | ABXY01000019 |
| *Bifidobacterium dentium* | CP001750 |
| *Bifidobacterium gallicum* | ABXB03000004 |
| *Bifidobacterium infantis* | AY151398 |
| *Bifidobacterium kashiwanohense* | AB491757 |
| *Bifidobacterium longum* | ABQQ01000041 |
| *Bifidobacterium pseudocatenulatum* | ABXX02000002 |
| *Bifidobacterium pseudolongum* | NR_043442 |
| *Bifidobacterium scardovii* | AJ307005 |
| *Bifidobacterium sp. HM2* | AB425276 |
| *Bifidobacterium sp. HMLN12* | JF519685 |
| *Bifidobacterium sp. M45* | HM626176 |
| *Bifidobacterium sp. MSX5B* | HQ616382 |
| *Bifidobacterium sp. TM_7* | AB218972 |
| *Bifidobacterium thermophilum* | DQ340557 |
| *Bifidobacterium urinalis* | AJ278695 |
| *Bilophila wadsworthia* | ADCP01000166 |
| *Bisgaard Taxon* | AY683487 |
| *Bisgaard Taxon* | AY683489 |
| *Bisgaard Taxon* | AY683491 |

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| *Bisgaard Taxon* | AY683492 |
| *Blastomonas natatoria* | NR_040824 |
| *Blautia coccoides* | AB571656 |
| *Blautia glucerasea* | AB588023 |
| *Blautia glucerasei* | AB439724 |
| *Blautia hansenii* | ABYU02000037 |
| *Blautia hydrogenotrophica* | ACBZ01000217 |
| *Blautia luti* | AB691576 |
| *Blautia producta* | AB600998 |
| *Blautia schinkii* | NR_026312 |
| *Blautia sp. M25* | HM626178 |
| *Blautia stercoris* | HM626177 |
| *Blautia wexlerae* | EF036467 |
| *Bordetella bronchiseptica* | NR_025949 |
| *Bordetella holmesii* | AB683187 |
| *Bordetella parapertussis* | NR_025950 |
| *Bordetella pertussis* | BX640418 |
| *Borrelia afzelii* | ABCU01000001 |
| *Borrelia burgdorferi* | ABGI01000001 |
| *Borrelia crocidurae* | DQ057990 |
| *Borrelia duttonii* | NC_011229 |
| *Borrelia garinii* | ABJV01000001 |
| *Borrelia hermsii* | AY597657 |
| *Borrelia hispanica* | DQ057988 |
| *Borrelia persica* | HM161645 |
| *Borrelia recurrentis* | AF107367 |
| *Borrelia sp. NE49* | AJ224142 |
| *Borrelia spielmanii* | ABKB01000002 |
| *Borrelia turicatae* | NC_008710 |

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| *Borrelia valaisiana* | ABCY01000002 |
| *Brachybacterium alimentarium* | NR_026269 |
| *Brachybacterium conglomeratum* | AB537169 |
| *Brachybacterium tyrofermentans* | NR_026272 |
| *Brachyspira aalborgi* | FM178386 |
| *Brachyspira pilosicoli* | NR_075069 |
| *Brachyspira sp. HIS3* | FM178387 |
| *Brachyspira sp. HIS4* | FM178388 |
| *Brachyspira sp. HIS5* | FM178389 |
| *Brevibacillus agri* | NR_040983 |
| *Brevibacillus brevis* | NR_041524 |
| *Brevibacillus centrosporus* | NR_043414 |
| *Brevibacillus choshinensis* | NR_040980 |
| *Brevibacillus invocatus* | NR_041836 |
| *Brevibacillus laterosporus* | NR_037005 |
| *Brevibacillus parabrevis* | NR_040981 |
| *Brevibacillus reuszeri* | NR_040982 |
| *Brevibacillus sp. phR* | JN837488 |
| *Brevibacillus thermoruber* | NR_026514 |
| *Brevibacterium aurantiacum* | NR_044854 |
| *Brevibacterium casei* | JF951998 |
| *Brevibacterium epidermidis* | NR_029262 |
| *Brevibacterium frigoritolerans* | NR_042639 |
| *Brevibacterium linens* | AJ315491 |
| *Brevibacterium mcbrellneri* | ADNU01000076 |
| *Brevibacterium paucivorans* | EU086796 |
| *Brevibacterium sanguinis* | NR_028016 |
| *Brevibacterium sp. H15* | AB177640 |
| *Brevibacterium sp. JC43* | JF824806 |

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| *Brevundimonas subvibrioides* | CP002102 |
| *Brucella abortus* | ACBJ01000075 |
| *Brucella canis* | NR_044652 |
| *Brucella ceti* | ACJD01000006 |
| *Brucella melitensis* | AE009462 |
| *Brucella microti* | NR_042549 |
| *Brucella ovis* | NC_009504 |
| *Brucella sp. 83_13* | ACBQ01000040 |
| *Brucella sp. BO1* | EU053207 |
| *Brucella suis* | ACBK01000034 |
| *Bryantella formatexigens* | ACCL02000018 |
| *Buchnera aphidicola* | NR_074609 |
| *Bulleidia extructa* | ADFR01000011 |
| *Burkholderia ambifaria* | AAUZ01000009 |
| *Burkholderia cenocepacia* | AAHI01000060 |
| *Burkholderia cepacia* | NR_041719 |
| *Burkholderia mallei* | CP000547 |
| *Burkholderia multivorans* | NC_010086 |
| *Burkholderia oklahomensis* | DQ108388 |
| *Burkholderia pseudomallei* | CP001408 |
| *Burkholderia rhizoxinica* | HQ005410 |
| *Burkholderia sp. 383* | CP000151 |
| *Burkholderia xenovorans* | U86373 |
| *Burkholderiales bacterium 1_1_47* | ADCQ01000066 |
| *Butyricicoccus pullicaecorum* | HH793440 |
| *Butyricimonas virosa* | AB443949 |
| *Butyrivibrio crossotus* | ABWN01000012 |
| *Butyrivibrio fibrisolvens* | U41172 |
| *Caldimonas manganoxidans* | NR_040787 |

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| *Caminicella sporogenes* | NR_025485 |
| *Campylobacter coli* | AAFL01000004 |
| *Campylobacter concisus* | CP000792 |
| *Campylobacter curvus* | NC_009715 |
| *Campylobacter fetus* | ACLG01001177 |
| *Campylobacter gracilis* | ACYG01000026 |
| *Campylobacter hominis* | NC_009714 |
| *Campylobacter jejuni* | AL139074 |
| *Campylobacter lari* | CP000932 |
| *Campylobacter rectus* | ACFU01000050 |
| *Campylobacter showae* | ACVQ01000030 |
| *Campylobacter sp. FOBRC14* | HQ616379 |
| *Campylobacter sp. FOBRC15* | HQ616380 |
| *Campylobacter sp. oral clone BB120* | AY005038 |
| *Campylobacter sputorum* | NR_044839 |
| *Campylobacter upsaliensis* | AEPU01000040 |
| *Candidatus Arthromitus sp. SFB_mouse_Yit* | NR_074460 |
| *Candidatus Sulcia muelleri* | CP002163 |
| *Capnocytophaga canimorsus* | CP002113 |
| *Capnocytophaga genomosp. C1* | AY278613 |
| *Capnocytophaga gingivalis* | ACLQ01000011 |
| *Capnocytophaga granulosa* | X97248 |
| *Capnocytophaga ochracea* | AEOH01000054 |
| *Capnocytophaga sp. GEJ8* | GU561335 |
| *Capnocytophaga sp. oral clone AH015* | AY005074 |
| *Capnocytophaga sp. oral clone ASCH05* | AY923149 |
| *Capnocytophaga sp. oral clone ID062* | AY349368 |
| *Capnocytophaga sp. oral strain A47ROY* | AY005077 |
| *Capnocytophaga sp. oral strain S3* | AY005073 |

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| *Capnocytophaga sp. oral taxon 338* | AEXX01000050 |
| *Capnocytophaga sp. S1b* | U42009 |
| *Capnocytophaga sputigena* | ABZV01000054 |
| *Cardiobacterium hominis* | ACKY01000036 |
| *Cardiobacterium valvarum* | NR_028847 |
| *Carnobacterium divergens* | NR_044706 |
| *Carnobacterium maltaromaticum* | NC_019425 |
| *Catabacter hongkongensis* | AB671763 |
| *Catenibacterium mitsuokai* | AB030224 |
| *Catonella genomosp. P1 oral clone MB5_P12* | DQ003629 |
| *Catonella morbi* | ACIL02000016 |
| *Catonella sp. oral clone FL037* | AY349369 |
| *Cedecea davisae* | AF493976 |
| *Cellulosimicrobium funkei* | AY501364 |
| *Cetobacterium somerae* | AJ438155 |
| *Chlamydia muridarum* | AE002160 |
| *Chlamydia psittaci* | NR_036864 |
| *Chlamydia trachomatis* | U68443 |
| *Chlamydiales bacterium NS11* | JN606074 |
| *Chlamydiales bacterium NS13* | JN606075 |
| *Chlamydiales bacterium NS16* | JN606076 |
| *Chlamydophila pecorum* | D88317 |
| *Chlamydophila pneumoniae* | NC_002179 |
| *Chlamydophila psittaci* | D85712 |
| *Chloroflexi genomosp. P1* | AY331414 |
| *Christensenella minuta* | AB490809 |
| *Chromobacterium violaceum* | NC_005085 |
| *Chryseobacterium anthropi* | AM982793 |
| *Chryseobacterium gleum* | ACKQ02000003 |

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| *Chryseobacterium hominis* | NR_042517 |
| *Citrobacter amalonaticus* | FR870441 |
| *Citrobacter braakii* | NR_028687 |
| *Citrobacter farmeri* | AF025371 |
| *Citrobacter freundii* | NR_028894 |
| *Citrobacter gillenii* | AF025367 |
| *Citrobacter koseri* | NC_009792 |
| *Citrobacter murliniae* | AF025369 |
| *Citrobacter rodentium* | NR_074903 |
| *Citrobacter sedlakii* | AF025364 |
| *Citrobacter sp. 30_2* | ACDJ01000053 |
| *Citrobacter sp. KMSI_3* | GQ468398 |
| *Citrobacter werkmanii* | AF025373 |
| *Citrobacter youngae* | ABWL02000011 |
| *Cloacibacillus evryensis* | GQ258966 |
| *Clostridiaceae bacterium END_2* | EF451053 |
| *Clostridiaceae bacterium JC13* | JF824807 |
| *Clostridiales bacterium 1_7_47FAA* | ABQR01000074 |
| *Clostridiales bacterium 9400853* | HM587320 |
| *Clostridiales bacterium 9403326* | HM587324 |
| *Clostridiales bacterium oral clone P4PA_66 P1* | AY207065 |
| *Clostridiales bacterium oral taxon 093* | GQ422712 |
| *Clostridiales bacterium oral taxon F32* | HM099644 |
| *Clostridiales bacterium ph2* | JN837487 |
| *Clostridiales bacterium SY8519* | AB477431 |
| *Clostridiales genomosp. BVAB3* | CP001850 |
| *Clostridiales sp. SM4_1* | FP929060 |
| *Clostridiales sp. SS3_4* | AY305316 |
| *Clostridiales sp. SSC_2* | FP929061 |

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| *Clostridium acetobutylicum* | NR_074511 |
| *Clostridium aerotolerans* | X76163 |
| *Clostridium aldenense* | NR_043680 |
| *Clostridium aldrichii* | NR_026099 |
| *Clostridium algidicarnis* | NR_041746 |
| *Clostridium algidixylanolyticum* | NR_028726 |
| *Clostridium aminovalericum* | NR_029245 |
| *Clostridium amygdalinum* | AY353957 |
| *Clostridium argentinense* | NR_029232 |
| *Clostridium asparagiforme* | ACCJ01000522 |
| *Clostridium baratii* | NR_029229 |
| *Clostridium bartlettii* | ABEZ02000012 |
| *Clostridium beijerinckii* | NR_074434 |
| *Clostridium bifermentans* | X73437 |
| *Clostridium bolteae* | ABCC02000039 |
| *Clostridium botulinum* | NC_010723 |
| *Clostridium butyricum* | ABDT01000017 |
| *Clostridium cadaveris* | AB542932 |
| *Clostridium carboxidivorans* | FR733710 |
| *Clostridium carnis* | NR_044716 |
| *Clostridium celatum* | X77844 |
| *Clostridium celerecrescens* | JQ246092 |
| *Clostridium cellulosi* | NR_044624 |
| *Clostridium chauvoei* | EU106372 |
| *Clostridium citroniae* | ADLJ01000059 |
| *Clostridium clariflavum* | NR_041235 |
| *Clostridium clostridiiformes* | M59089 |
| *Clostridium clostridioforme* | NR_044715 |
| *Clostridium coccoides* | EF025906 |

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| *Clostridium cochlearium* | NR_044717 |
| *Clostridium cocleatum* | NR_026495 |
| *Clostridium colicanis* | FJ957863 |
| *Clostridium colinum* | NR_026151 |
| *Clostridium difficile* | NC_013315 |
| *Clostridium disporicum* | NR_026491 |
| *Clostridium estertheticum* | NR_042153 |
| *Clostridium fallax* | NR_044714 |
| *Clostridium favososporum* | X76749 |
| *Clostridium felsineum* | AF270502 |
| *Clostridium frigidicarnis* | NR_024919 |
| *Clostridium gasigenes* | NR_024945 |
| *Clostridium ghonii* | AB542933 |
| *Clostridium glycolicum* | FJ384385 |
| *Clostridium glycyrrhizinilyticum* | AB233029 |
| *Clostridium haemolyticum* | NR_024749 |
| *Clostridium hathewayi* | AY552788 |
| *Clostridium hiranonis* | AB023970 |
| *Clostridium histolyticum* | HF558362 |
| *Clostridium hylemonae* | AB023973 |
| *Clostridium indolis* | AF028351 |
| *Clostridium innocuum* | M23732 |
| *Clostridium irregulare* | NR_029249 |
| *Clostridium isatidis* | NR_026347 |
| *Clostridium kluyveri* | NR_074165 |
| *Clostridium lactatifermentans* | NR_025651 |
| *Clostridium lavalense* | EF564277 |
| *Clostridium leptum* | AJ305238 |
| *Clostridium limosum* | FR870444 |

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| *Clostridium magnum* | X77835 |
| *Clostridium malenominatum* | FR749893 |
| *Clostridium mayombei* | FR733682 |
| *Clostridium methylpentosum* | ACEC01000059 |
| *Clostridium nexile* | X73443 |
| *Clostridium novyi* | NR_074343 |
| *Clostridium orbiscindens* | Y18187 |
| *Clostridium oroticum* | FR749922 |
| *Clostridium paraputrificum* | AB536771 |
| *Clostridium perfringens* | ABDW01000023 |
| *Clostridium phytofermentans* | NR_074652 |
| *Clostridium piliforme* | D14639 |
| *Clostridium putrefaciens* | NR_024995 |
| *Clostridium quinii* | NR_026149 |
| *Clostridium ramosum* | M23731 |
| *Clostridium rectum* | NR_029271 |
| *Clostridium saccharogumia* | DQ100445 |
| *Clostridium saccharolyticum* | CP002109 |
| *Clostridium sardiniense* | NR_041006 |
| *Clostridium sartagoforme* | NR_026490 |
| *Clostridium scindens* | AF262238 |
| *Clostridium septicum* | NR_026020 |
| *Clostridium sordellii* | AB448946 |
| *Clostridium sp. 7_2_43FAA* | ACDK01000101 |
| *Clostridium sp. D5* | ADBG01000142 |
| *Clostridium sp. HGF2* | AENW01000022 |
| *Clostridium sp. HPB_46* | AY862516 |
| *Clostridium sp. JC122* | CAEV01000127 |
| *Clostridium sp. L2_50* | AAYW02000018 |

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| *Clostridium sp. LMG 16094* | X95274 |
| *Clostridium sp. M62_1* | ACFX02000046 |
| *Clostridium sp. MLG055* | AF304435 |
| *Clostridium sp. MT4 E* | FJ159523 |
| *Clostridium sp. NMBHI_1* | JN093130 |
| *Clostridium sp. NML 04A032* | EU815224 |
| *Clostridium sp. SS2_1* | ABGC03000041 |
| *Clostridium sp. SY8519* | AP012212 |
| *Clostridium sp. TM_40* | AB249652 |
| *Clostridium sp. YIT 12069* | AB491207 |
| *Clostridium sp. YIT 12070* | AB491208 |
| *Clostridium sphenoides* | X73449 |
| *Clostridium spiroforme* | X73441 |
| *Clostridium sporogenes* | ABKW02000003 |
| *Clostridium sporosphaeroides* | NR_044835 |
| *Clostridium stercorarium* | NR_025100 |
| *Clostridium sticklandii* | L04167 |
| *Clostridium straminisolvens* | NR_024829 |
| *Clostridium subterminale* | NR_041795 |
| *Clostridium sulfidigenes* | NR_044161 |
| *Clostridium symbiosum* | ADLQ01000114 |
| *Clostridium tertium* | Y18174 |
| *Clostridium tetani* | NC_004557 |
| *Clostridium thermocellum* | NR_074629 |
| *Clostridium tyrobutyricum* | NR_044718 |
| *Clostridium viride* | NR_026204 |
| *Clostridium xylanolyticum* | NR_037068 |
| *Collinsella aerofaciens* | AAVN02000007 |
| *Collinsella intestinalis* | ABXH02000037 |

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| *Collinsella stercoris* | ABXJ01000150 |
| *Collinsella tanakaei* | AB490807 |
| *Comamonadaceae bacterium NML000135* | JN585335 |
| *Comamonadaceae bacterium NML790751* | JN585331 |
| *Comamonadaceae bacterium NML910035* | JN585332 |
| *Comamonadaceae bacterium NML910036* | JN585333 |
| *Comamonadaceae bacterium oral taxon F47* | HM099651 |
| *Comamonas sp. NSP5* | AB076850 |
| *Conchiformibius kuhniae* | NR_041821 |
| *Coprobacillus cateniformis* | AB030218 |
| *Coprobacillus sp. 29_1* | ADKX01000057 |
| *Coprobacillus sp. D7* | ACDT01000199 |
| *Coprococcus catus* | EU266552 |
| *Coprococcus comes* | ABVR01000038 |
| *Coprococcus eutactus* | EF031543 |
| *Coprococcus sp. ART55_1* | AY350746 |
| *Coriobacteriaceae bacterium BV3Ac1* | JN809768 |
| *Coriobacteriaceae bacterium JC110* | CAEM01000062 |
| *Coriobacteriaceae bacterium phI* | JN837493 |
| *Corynebacterium accolens* | ACGD01000048 |
| *Corynebacterium ammoniagenes* | ADNS01000011 |
| *Corynebacterium amycolatum* | ABZU01000033 |
| *Corynebacterium appendicis* | NR_028951 |
| *Corynebacterium argentoratense* | EF463055 |
| *Corynebacterium atypicum* | NR_025540 |
| *Corynebacterium aurimucosum* | ACLH01000041 |
| *Corynebacterium bovis* | AF537590 |
| *Corynebacterium canis* | GQ871934 |
| *Corynebacterium casei* | NR_025101 |

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| *Corynebacterium confusum* | Y15886 |
| *Corynebacterium coyleae* | X96497 |
| *Corynebacterium diphtheriae* | NC_002935 |
| *Corynebacterium durum* | Z97069 |
| *Corynebacterium efficiens* | ACLI01000121 |
| *Corynebacterium falsenii* | Y13024 |
| *Corynebacterium flavescens* | NR_037040 |
| *Corynebacterium genitalium* | ACLJ01000031 |
| *Corynebacterium glaucum* | NR_028971 |
| *Corynebacterium glucuronolyticum* | ABYP01000081 |
| *Corynebacterium glutamicum* | BA000036 |
| *Corynebacterium hansenii* | AM946639 |
| *Corynebacterium imitans* | AF537597 |
| *Corynebacterium jeikeium* | ACYW01000001 |
| *Corynebacterium kroppenstedtii* | NR_026380 |
| *Corynebacterium lipophiloflavum* | ACHJ01000075 |
| *Corynebacterium macginleyi* | AB359393 |
| *Corynebacterium mastitidis* | AB359395 |
| *Corynebacterium matruchotii* | ACSH02000003 |
| *Corynebacterium minutissimum* | X82064 |
| *Corynebacterium mucifaciens* | NR_026396 |
| *Corynebacterium propinquum* | NR_037038 |
| *Corynebacterium pseudodiphtheriticum* | X84258 |
| *Corynebacterium pseudogenitalium* | ABYQ01000237 |
| *Corynebacterium pseudotuberculosis* | NR_037070 |
| *Corynebacterium pyruviciproducens* | FJ185225 |
| *Corynebacterium renale* | NR_037069 |
| *Corynebacterium resistens* | ADGN01000058 |
| *Corynebacterium riegelii* | EU848548 |

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| *Corynebacterium simulans* | AF537604 |
| *Corynebacterium singulare* | NR_026394 |
| *Corynebacterium sp. 1 ex sheep* | Y13427 |
| *Corynebacterium sp. L_2012475* | HE575405 |
| *Corynebacterium sp. NML 93_0481* | GU238409 |
| *Corynebacterium sp. NML 97_0186* | GU238411 |
| *Corynebacterium sp. NML 99_0018* | GU238413 |
| *Corynebacterium striatum* | ACGE01000001 |
| *Corynebacterium sundsvallense* | Y09655 |
| *Corynebacterium tuberculostearicum* | ACVP01000009 |
| *Corynebacterium tuscaniae* | AY677186 |
| *Corynebacterium ulcerans* | NR_074467 |
| *Corynebacterium urealyticum* | X81913 |
| *Corynebacterium ureicelerivorans* | AM397636 |
| *Corynebacterium variabile* | NR_025314 |
| *Corynebacterium xerosis* | FN179330 |
| *Coxiella burnetii* | CP000890 |
| *Cronobacter malonaticus* | GU122174 |
| *Cronobacter sakazakii* | NC_009778 |
| *Cronobacter turicensis* | FN543093 |
| *Cryptobacterium curtum* | GQ422741 |
| *Cupriavidus metallidurans* | GU230889 |
| *Cytophaga xylanolytica* | FR733683 |
| *Deferribacteres sp. oral clone JV001* | AY349370 |
| *Deferribacteres sp. oral clone JV006* | AY349371 |
| *Deferribacteres sp. oral clone JV023* | AY349372 |
| *Deinococcus radiodurans* | AE000513 |
| *Deinococcus sp. R_43890* | FR682752 |
| *Delftia acidovorans* | CP000884 |

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| *Dermabacter hominis* | FJ263375 |
| *Dermacoccus sp. Ellin185* | AEIQ01000090 |
| *Desmospora activa* | AM940019 |
| *Desmospora sp. 8437* | AFHT01000143 |
| *Desulfitobacterium frappieri* | AJ276701 |
| *Desulfitobacterium hafniense* | NR_074996 |
| *Desulfobulbus sp. oral clone CH031* | AY005036 |
| *Desulfotomaculum nigrificans* | NR_044832 |
| *Desulfovibrio desulfuricans* | DQ092636 |
| *Desulfovibrio fairfieldensis* | U42221 |
| *Desulfovibrio piger* | AF192152 |
| *Desulfovibrio sp. 3_1_syn3* | ADDR01000239 |
| *Desulfovibrio vulgaris* | NR_074897 |
| *Dialister invisus* | ACIM02000001 |
| *Dialister micraerophilus* | AFBB01000028 |
| *Dialister microaerophilus* | AENT01000008 |
| *Dialister pneumosintes* | HM596297 |
| *Dialister propionicifaciens* | NR_043231 |
| *Dialister sp. oral taxon 502* | GQ422739 |
| *Dialister succinatiphilus* | AB370249 |
| *Dietzia natronolimnaea* | GQ870426 |
| *Dietzia sp. BBDP51* | DQ337512 |
| *Dietzia sp. CA149* | GQ870422 |
| *Dietzia timorensis* | GQ870424 |
| *Dorea formicigenerans* | AAXA02000006 |
| *Dorea longicatena* | AJ132842 |
| *Dysgonomonas gadei* | ADLV01000001 |
| *Dysgonomonas mossii* | ADLW01000023 |
| *Edwardsiella tarda* | CP002154 |

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| *Eggerthella lenta* | AF292375 |
| *Eggerthella sinensis* | AY321958 |
| *Eggerthella sp. 1_3_56FAA* | ACWN01000099 |
| *Eggerthella sp. HGA1* | AEXR01000021 |
| *Eggerthella sp. YY7918* | AP012211 |
| *Ehrlichia chaffeensis* | AAIF01000035 |
| *Eikenella corrodens* | ACEA01000028 |
| *Enhydrobacter aerosaccus* | ACYI01000081 |
| *Enterobacter aerogenes* | AJ251468 |
| *Enterobacter asburiae* | NR_024640 |
| *Enterobacter cancerogenus* | Z96078 |
| *Enterobacter cloacae* | FP929040 |
| *Enterobacter cowanii* | NR_025566 |
| *Enterobacter hormaechei* | AFHR01000079 |
| *Enterobacter sp. 247BMC* | HQ122932 |
| *Enterobacter sp. 638* | NR_074777 |
| *Enterobacter sp. JC163* | JN657217 |
| *Enterobacter sp. SCSS* | HM007811 |
| *Enterobacter sp. TSE38* | HM156134 |
| *Enterobacteriaceae bacterium 9_2_54FAA* | ADCU01000033 |
| *Enterobacteriaceae bacterium CF01Ent_1* | AJ489826 |
| *Enterobacteriaceae bacterium Smarlab 3302238* | AY538694 |
| *Enterococcus avium* | AF133535 |
| *Enterococcus caccae* | AY943820 |
| *Enterococcus casseliflavus* | AEWT01000047 |
| *Enterococcus durans* | AJ276354 |
| *Enterococcus faecalis* | AE016830 |
| *Enterococcus faecium* | AM157434 |
| *Enterococcus gallinarum* | AB269767 |

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| *Enterococcus gilvus* | AY033814 |
| *Enterococcus hawaiiensis* | AY321377 |
| *Enterococcus hirae* | AF061011 |
| *Enterococcus italicus* | AEPV01000109 |
| *Enterococcus mundtii* | NR_024906 |
| *Enterococcus raffinosus* | FN600541 |
| *Enterococcus sp. BV2CASA2* | JN809766 |
| *Enterococcus sp. CCRI_16620* | GU457263 |
| *Enterococcus sp. F95* | FJ463817 |
| *Enterococcus sp. RfL6* | AJ133478 |
| *Enterococcus thailandicus* | AY321376 |
| *Eremococcus coleocola* | AENN01000008 |
| *Erysipelothrix inopinata* | NR_025594 |
| *Erysipelothrix rhusiopathiae* | ACLK01000021 |
| *Erysipelothrix tonsillarum* | NR_040871 |
| *Erysipelotrichaceae bacterium 3_1_53* | ACTJ01000113 |
| *Erysipelotrichaceae bacterium 5_2_54FAA* | ACZW01000054 |
| *Escherichia albertii* | ABKX01000012 |
| *Escherichia coli* | NC_008563 |
| *Escherichia fergusonii* | CU928158 |
| *Escherichia hermannii* | HQ407266 |
| *Escherichia sp. 1_1_43* | ACID01000033 |
| *Escherichia sp. 4_1_40B* | ACDM02000056 |
| *Escherichia sp. B4* | EU722735 |
| *Escherichia vulneris* | NR_041927 |
| *Ethanoligenens harbinense* | AY675965 |
| *Eubacteriaceae bacterium P4P_50 P4* | AY207060 |
| *Eubacterium barkeri* | NR_044661 |
| *Eubacterium biforme* | ABYT01000002 |

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| *Eubacterium brachy* | U13038 |
| *Eubacterium budayi* | NR_024682 |
| *Eubacterium callanderi* | NR_026330 |
| *Eubacterium cellulosolvens* | AY178842 |
| *Eubacterium contortum* | FR749946 |
| *Eubacterium coprostanoligenes* | HM037995 |
| *Eubacterium cylindroides* | FP929041 |
| *Eubacterium desmolans* | NR_044644 |
| *Eubacterium dolichum* | L34682 |
| *Eubacterium eligens* | CP001104 |
| *Eubacterium fissicatena* | FR749935 |
| *Eubacterium hadrum* | FR749933 |
| *Eubacterium hallii* | L34621 |
| *Eubacterium infirmum* | U13039 |
| *Eubacterium limosum* | CP002273 |
| *Eubacterium moniliforme* | HF558373 |
| *Eubacterium multiforme* | NR_024683 |
| *Eubacterium nitritogenes* | NR_024684 |
| *Eubacterium nodatum* | U13041 |
| *Eubacterium ramulus* | AJ011522 |
| *Eubacterium rectale* | FP929042 |
| *Eubacterium ruminantium* | NR_024661 |
| *Eubacterium saburreum* | AB525414 |
| *Eubacterium saphenum* | NR_026031 |
| *Eubacterium siraeum* | ABCA03000054 |
| *Eubacterium sp. 3_1_31* | ACTL01000045 |
| *Eubacterium sp. AS15b* | HQ616364 |
| *Eubacterium sp. OBRC9* | HQ616354 |
| *Eubacterium sp. oral clone GI038* | AY349374 |

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| *Eubacterium sp. oral clone IR009* | AY349376 |
| *Eubacterium sp. oral clone JH012* | AY349373 |
| *Eubacterium sp. oral clone JI012* | AY349379 |
| *Eubacterium sp. oral clone JN088* | AY349377 |
| *Eubacterium sp. oral clone JS001* | AY349378 |
| *Eubacterium sp. oral clone OH3A* | AY947497 |
| *Eubacterium sp. WAL 14571* | FJ687606 |
| *Eubacterium tenue* | M59118 |
| *Eubacterium tortuosum* | NR_044648 |
| *Eubacterium ventriosum* | L34421 |
| *Eubacterium xylanophilum* | L34628 |
| *Eubacterium yurii* | AEES01000073 |
| *Ewingella americana* | JN175329 |
| *Exiguobacterium acetylicum* | FJ970034 |
| *Facklamia hominis* | Y10772 |
| *Faecalibacterium prausnitzii* | ACOP02000011 |
| *Filifactor alocis* | CP002390 |
| *Filifactor villosus* | NR_041928 |
| *Finegoldia magna* | ACHM02000001 |
| *Flavobacteriaceae genomosp. C1* | AY278614 |
| *Flavobacterium sp. NF2_1* | FJ195988 |
| *Flavonifractor plautii* | AY724678 |
| *Flexispira rappini* | AY126479 |
| *Flexistipes sinusarabici* | NR_074881 |
| *Francisella novicida* | ABSS01000002 |
| *Francisella philomiragia* | AY928394 |
| *Francisella tularensis* | ABAZ01000082 |
| *Fulvimonas sp. NML 060897* | EF589680 |
| *Fusobacterium canifelinum* | AY162222 |

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| *Fusobacterium genomosp. C1* | AY278616 |
| *Fusobacterium genomosp. C2* | AY278617 |
| *Fusobacterium gonidiaformans* | ACET01000043 |
| *Fusobacterium mortiferum* | ACDB02000034 |
| *Fusobacterium naviforme* | HQ223106 |
| *Fusobacterium necrogenes* | X55408 |
| *Fusobacterium necrophorum* | AM905356 |
| *Fusobacterium nucleatum* | ADVK01000034 |
| *Fusobacterium periodonticum* | ACJY01000002 |
| *Fusobacterium russii* | NR_044687 |
| *Fusobacterium sp. 1_1_41FAA* | ADGG01000053 |
| *Fusobacterium sp. 11_3_2* | ACUO01000052 |
| *Fusobacterium sp. 12_1B* | AGWJ01000070 |
| *Fusobacterium sp. 2_1_31* | ACDC02000018 |
| *Fusobacterium sp. 3_1_27* | ADGF01000045 |
| *Fusobacterium sp. 3_1_33* | ACQE01000178 |
| *Fusobacterium sp. 3_1_36A2* | ACPU01000044 |
| *Fusobacterium sp. 3_1_5R* | ACDD01000078 |
| *Fusobacterium sp. AC18* | HQ616357 |
| *Fusobacterium sp. ACB2* | HQ616358 |
| *Fusobacterium sp. AS2* | HQ616361 |
| *Fusobacterium sp. CM1* | HQ616371 |
| *Fusobacterium sp. CM21* | HQ616375 |
| *Fusobacterium sp. CM22* | HQ616376 |
| *Fusobacterium sp. D12* | ACDG02000036 |
| *Fusobacterium sp. oral clone ASCF06* | AY923141 |
| *Fusobacterium sp. oral clone ASCF11* | AY953256 |
| *Fusobacterium ulcerans* | ACDH01000090 |
| *Fusobacterium varium* | ACIE01000009 |

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| *Gardnerella vaginalis* | CP001849 |
| *Gemella haemolysans* | ACDZ02000012 |
| *Gemella morbillorum* | NR_025904 |
| *Gemella morbillorum* | ACRX01000010 |
| *Gemella sanguinis* | ACRY01000057 |
| *Gemella sp. oral clone ASCE02* | AY923133 |
| *Gemella sp. oral clone ASCF04* | AY923139 |
| *Gemella sp. oral clone ASCF12* | AY923143 |
| *Gemella sp. WAL 1945J* | EU427463 |
| *Gemmiger formicilis* | GU562446 |
| *Geobacillus kaustophilus* | NR_074989 |
| *Geobacillus sp. E263* | DQ647387 |
| *Geobacillus sp. WCH70* | CP001638 |
| *Geobacillus stearothermophilus* | NR_040794 |
| *Geobacillus thermocatenulatus* | NR_043020 |
| *Geobacillus thermodenitrificans* | NR_074976 |
| *Geobacillus thermoglucosidasius* | NR_043022 |
| *Geobacillus thermoleovorans* | NR_074931 |
| *Geobacter bemidjiensis* | CP001124 |
| *Gloeobacter violaceus* | NR_074282 |
| *Gluconacetobacter azotocaptans* | NR_028767 |
| *Gluconacetobacter diazotrophicus* | NR_074292 |
| *Gluconacetobacter entanii* | NR_028909 |
| *Gluconacetobacter europaeus* | NR_026513 |
| *Gluconacetobacter hansenii* | NR_026133 |
| *Gluconacetobacter johannae* | NR_024959 |
| *Gluconacetobacter oboediens* | NR_041295 |
| *Gluconacetobacter xylinus* | NR_074338 |
| *Gordonia bronchialis* | NR_027594 |

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| *Gordonia polyisoprenivorans* | DQ385609 |
| *Gordonia sp. KTR9* | DQ068383 |
| *Gordonia sputi* | FJ536304 |
| *Gordonia terrae* | GQ848239 |
| *Gordonibacter pamelaeae* | AM886059 |
| *Gordonibacter pamelaeae* | FP929047 |
| *Gracilibacter thermotolerans* | NR_043559 |
| *Gramella forsetii* | NR_074707 |
| *Granulicatella adiacens* | ACKZ01000002 |
| *Granulicatella elegans* | AB252689 |
| *Granulicatella paradiacens* | AY879298 |
| *Granulicatella sp. M658_99_3* | AJ271861 |
| *Granulicatella sp. oral clone ASC02* | AY923126 |
| *Granulicatella sp. oral clone ASCA05* | DQ341469 |
| *Granulicatella sp. oral clone ASCB09* | AY953251 |
| *Granulicatella sp. oral clone ASCG05* | AY923146 |
| *Grimontia hollisae* | ADAQ01000013 |
| *Haematobacter sp. BC14248* | GU396991 |
| *Haemophilus aegyptius* | AFBC01000053 |
| *Haemophilus ducreyi* | AE017143 |
| *Haemophilus genomosp. P2 oral clone MB3_C24* | DQ003621 |
| *Haemophilus genomosp. P3 oral clone MB3_C38* | DQ003635 |
| *Haemophilus haemolyticus* | JN175335 |
| *Haemophilus influenzae* | AADP01000001 |
| *Haemophilus parahaemolyticus* | GU561425 |
| *Haemophilus parainfluenzae* | AEWU01000024 |
| *Haemophilus paraphrophaemolyticus* | M75076 |

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| *Haemophilus parasuis* | GU226366 |
| *Haemophilus somnus* | NC_008309 |
| *Haemophilus sp. 70334* | HQ680854 |
| *Haemophilus sp. HK445* | FJ685624 |
| *Haemophilus sp. oral clone ASCA07* | AY923117 |
| *Haemophilus sp. oral clone ASCG06* | AY923147 |
| *Haemophilus sp. oral clone BJ021* | AY005034 |
| *Haemophilus sp. oral clone BJ095* | AY005033 |
| *Haemophilus sp. oral clone JM053* | AY349380 |
| *Haemophilus sp. oral taxon 851* | AGRK01000004 |
| *Haemophilus sputorum* | AFNK01000005 |
| *Hafnia alvei* | DQ412565 |
| *Halomonas elongata* | NR_074782 |
| *Halomonas johnsoniae* | FR775979 |
| *Halorubrum lipolyticum* | AB477978 |
| *Helicobacter bilis* | ACDN01000023 |
| *Helicobacter canadensis* | ABQS01000108 |
| *Helicobacter cinaedi* | ABQT01000054 |
| *Helicobacter pullorum* | ABQU01000097 |
| *Helicobacter pylori* | CP000012 |
| *Helicobacter sp. None* | U44756 |
| *Helicobacter winghamensis* | ACDO01000013 |
| *Heliobacterium modesticaldum* | NR_074517 |
| *Herbaspirillum seropedicae* | CP002039 |
| *Herbaspirillum sp. JC206* | JN657219 |
| *Histophilus somni* | AF549387 |
| *Holdemania filiformis* | Y11466 |
| *Hydrogenoanaerobacterium saccharovorans* | NR_044425 |
| *Hyperthermus butylicus* | CP000493 |

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| *Hyphomicrobium sulfonivorans* | AY468372 |
| *Hyphomonas neptunium* | NR_074092 |
| *Ignatzschineria indica* | HQ823562 |
| *Ignatzschineria sp. NML 95_0260* | HQ823559 |
| *Ignicoccus islandicus* | X99562 |
| *Inquilinus limosus* | NR_029046 |
| *Janibacter limosus* | NR_026362 |
| *Janibacter melonis* | EF063716 |
| *Janthinobacterium sp. SY12* | EF455530 |
| *Johnsonella ignava* | X87152 |
| *Jonquetella anthropi* | ACOO02000004 |
| *Kerstersia gyiorum* | NR_025669 |
| *Kingella denitrificans* | AEWV01000047 |
| *Kingella genomosp. P1 oral cone MB2_C20* | DQ003616 |
| *Kingella kingae* | AFHS01000073 |
| *Kingella oralis* | ACJW02000005 |
| *Kingella sp. oral clone ID059* | AY349381 |
| *Klebsiella oxytoca* | AY292871 |
| *Klebsiella pneumoniae* | CP000647 |
| *Klebsiella sp. AS10* | HQ616362 |
| *Klebsiella sp. Co9935* | DQ068764 |
| *Klebsiella sp. enrichment culture clone SRC_DSD25* | HM195210 |
| *Klebsiella sp. OBRC7* | HQ616353 |
| *Klebsiella sp. SP_BA* | FJ999767 |
| *Klebsiella sp. SRC_DSD1* | GU797254 |
| *Klebsiella sp. SRC_DSD11* | GU797263 |
| *Klebsiella sp. SRC_DSD12* | GU797264 |
| *Klebsiella sp. SRC_DSD15* | GU797267 |

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| *Klebsiella sp. SRC_DSD2* | GU797253 |
| *Klebsiella sp. SRC_DSD6* | GU797258 |
| *Klebsiella variicola* | CP001891 |
| *Kluyvera ascorbata* | NR_028677 |
| *Kluyvera cryocrescens* | NR_028803 |
| *Kocuria marina* | GQ260086 |
| *Kocuria palustris* | EU333884 |
| *Kocuria rhizophila* | AY030315 |
| *Kocuria rosea* | X87756 |
| *Kocuria varians* | AF542074 |
| *Lachnobacterium bovis* | GU324407 |
| *Lachnospira multipara* | FR733699 |
| *Lachnospira pectinoschiza* | L14675 |
| *Lachnospiraceae bacterium 1_1_57FAA* | ACTM01000065 |
| *Lachnospiraceae bacterium 1_4_56FAA* | ACTN01000028 |
| *Lachnospiraceae bacterium 2_1_46FAA* | ADLB01000035 |
| *Lachnospiraceae bacterium 2_1_58FAA* | ACTO01000052 |
| *Lachnospiraceae bacterium 3_1_57FAA_CT1* | ACTP01000124 |
| *Lachnospiraceae bacterium 4_1_37FAA* | ADCR01000030 |
| *Lachnospiraceae bacterium 5_1_57FAA* | ACTR01000020 |
| *Lachnospiraceae bacterium 5_1_63FAA* | ACTS01000081 |
| *Lachnospiraceae bacterium 6_1_63FAA* | ACTV01000014 |
| *Lachnospiraceae bacterium 8_1_57FAA* | ACWQ01000079 |
| *Lachnospiraceae bacterium 9_1_43BFAA* | ACTX01000023 |
| *Lachnospiraceae bacterium A4* | DQ789118 |
| *Lachnospiraceae bacterium DJF VP30* | EU728771 |
| *Lachnospiraceae bacterium ICM62* | HQ616401 |
| *Lachnospiraceae bacterium MSX33* | HQ616384 |
| *Lachnospiraceae bacterium oral taxon 107* | ADDS01000069 |

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| *Lachnospiraceae bacterium oral taxon F15* | HM099641 |
| *Lachnospiraceae genomosp. C1* | AY278618 |
| *Lactobacillus acidipiscis* | NR_024718 |
| *Lactobacillus acidophilus* | CP000033 |
| *Lactobacillus alimentarius* | NR_044701 |
| *Lactobacillus amylolyticus* | ADNY01000006 |
| *Lactobacillus amylovorus* | CP002338 |
| *Lactobacillus antri* | ACLL01000037 |
| *Lactobacillus brevis* | EU194349 |
| *Lactobacillus buchneri* | ACGH01000101 |
| *Lactobacillus casei* | CP000423 |
| *Lactobacillus catenaformis* | M23729 |
| *Lactobacillus coleohominis* | ACOH01000030 |
| *Lactobacillus coryniformis* | NR_044705 |
| *Lactobacillus crispatus* | ACOG01000151 |
| *Lactobacillus curvatus* | NR_042437 |
| *Lactobacillus delbrueckii* | CP002341 |
| *Lactobacillus dextrinicus* | NR_036861 |
| *Lactobacillus farciminis* | NR_044707 |
| *Lactobacillus fermentum* | CP002033 |
| *Lactobacillus gasseri* | ACOZ01000018 |
| *Lactobacillus gastricus* | AICN01000060 |
| *Lactobacillus genomosp. C1* | AY278619 |
| *Lactobacillus genomosp. C2* | AY278620 |
| *Lactobacillus helveticus* | ACLM01000202 |
| *Lactobacillus hilgardii* | ACGP01000200 |
| *Lactobacillus hominis* | FR681902 |
| *Lactobacillus iners* | AEKJ01000002 |
| *Lactobacillus jensenii* | ACQD01000066 |

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| *Lactobacillus johnsonii* | AE017198 |
| *Lactobacillus kalixensis* | NR_029083 |
| *Lactobacillus kefiranofaciens* | NR_042440 |
| *Lactobacillus kefiri* | NR_042230 |
| *Lactobacillus kimchii* | NR_025045 |
| *Lactobacillus leichmannii* | JX986966 |
| *Lactobacillus mucosae* | FR693800 |
| *Lactobacillus murinus* | NR_042231 |
| *Lactobacillus nodensis* | NR_041629 |
| *Lactobacillus oeni* | NR_043095 |
| *Lactobacillus oris* | AEKL01000077 |
| *Lactobacillus parabrevis* | NR_042456 |
| *Lactobacillus parabuchneri* | NR_041294 |
| *Lactobacillus paracasei* | ABQV01000067 |
| *Lactobacillus parakefiri* | NR_029039 |
| *Lactobacillus pentosus* | JN813103 |
| *Lactobacillus perolens* | NR_029360 |
| *Lactobacillus plantarum* | ACGZ02000033 |
| *Lactobacillus pontis* | HM218420 |
| *Lactobacillus reuteri* | ACGW02000012 |
| *Lactobacillus rhamnosus* | ABWJ01000068 |
| *Lactobacillus rogosae* | GU269544 |
| *Lactobacillus ruminis* | ACGS02000043 |
| *Lactobacillus sakei* | DQ989236 |
| *Lactobacillus salivarius* | AEBA01000145 |
| *Lactobacillus saniviri* | AB602569 |
| *Lactobacillus senioris* | AB602570 |
| *Lactobacillus sp. 66c* | FR681900 |
| *Lactobacillus sp. BT6* | HQ616370 |

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| *Lactobacillus sp. KLDS 1.0701* | EU600905 |
| *Lactobacillus sp. KLDS 1.0702* | EU600906 |
| *Lactobacillus sp. KLDS 1.0703* | EU600907 |
| *Lactobacillus sp. KLDS 1.0704* | EU600908 |
| *Lactobacillus sp. KLDS 1.0705* | EU600909 |
| *Lactobacillus sp. KLDS 1.0707* | EU600911 |
| *Lactobacillus sp. KLDS 1.0709* | EU600913 |
| *Lactobacillus sp. KLDS 1.0711* | EU600915 |
| *Lactobacillus sp. KLDS 1.0712* | EU600916 |
| *Lactobacillus sp. KLDS 1.0713* | EU600917 |
| *Lactobacillus sp. KLDS 1.0716* | EU600921 |
| *Lactobacillus sp. KLDS 1.0718* | EU600922 |
| *Lactobacillus sp. KLDS 1.0719* | EU600923 |
| *Lactobacillus sp. oral clone HT002* | AY349382 |
| *Lactobacillus sp. oral clone HT070* | AY349383 |
| *Lactobacillus sp. oral taxon 052* | GQ422710 |
| *Lactobacillus tucceti* | NR_042194 |
| *Lactobacillus ultunensis* | ACGU01000081 |
| *Lactobacillus vaginalis* | ACGV01000168 |
| *Lactobacillus vini* | NR_042196 |
| *Lactobacillus vitulinus* | NR_041305 |
| *Lactobacillus zeae* | NR_037122 |
| *Lactococcus garvieae* | AF061005 |
| *Lactococcus lactis* | CP002365 |
| *Lactococcus raffinolactis* | NR_044359 |
| *Lactonifactor longoviformis* | DQ100449 |
| *Laribacter hongkongensis* | CP001154 |
| *Lautropia mirabilis* | AEQP01000026 |
| *Lautropia sp. oral clone AP009* | AY005030 |

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|---|---|
| *Legionella hackeliae* | M36028 |
| *Legionella longbeachae* | M36029 |
| *Legionella pneumophila* | NC_002942 |
| *Legionella sp. D3923* | JN380999 |
| *Legionella sp. D4088* | JN381012 |
| *Legionella sp. H63* | JF831047 |
| *Legionella sp. NML 93L054* | GU062706 |
| *Legionella steelei* | HQ398202 |
| *Leminorella grimontii* | AJ233421 |
| *Leminorella richardii* | HF558368 |
| *Leptospira borgpetersenii* | NC_008508 |
| *Leptospira broomii* | NR_043200 |
| *Leptospira interrogans* | NC_005823 |
| *Leptospira licerasiae* | EF612284 |
| *Leptotrichia buccalis* | CP001685 |
| *Leptotrichia genomosp. C1* | AY278621 |
| *Leptotrichia goodfellowii* | ADAD01000110 |
| *Leptotrichia hofstadii* | ACVB02000032 |
| *Leptotrichia shahii* | AY029806 |
| *Leptotrichia sp. neutropenicPatient* | AF189244 |
| *Leptotrichia sp. oral clone GT018* | AY349384 |
| *Leptotrichia sp. oral clone GT020* | AY349385 |
| *Leptotrichia sp. oral clone HE012* | AY349386 |
| *Leptotrichia sp. oral clone IK040* | AY349387 |
| *Leptotrichia sp. oral clone P2PB_51 P1* | AY207053 |
| *Leptotrichia sp. oral taxon 223* | GU408547 |
| *Leuconostoc carnosum* | NR_040811 |
| *Leuconostoc citreum* | AM157444 |
| *Leuconostoc gasicomitatum* | FN822744 |

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| *Leuconostoc inhae* | NR_025204 |
| *Leuconostoc kimchii* | NR_075014 |
| *Leuconostoc lactis* | NR_040823 |
| *Leuconostoc mesenteroides* | ACKV01000113 |
| *Leuconostoc pseudomesenteroides* | NR_040814 |
| *Listeria grayi* | ACCR02000003 |
| *Listeria innocua* | JF967625 |
| *Listeria ivanovii* | X56151 |
| *Listeria monocytogenes* | CP002003 |
| *Listeria welshimeri* | AM263198 |
| *Luteococcus sanguinis* | NR_025507 |
| *Lutispora thermophila* | NR_041236 |
| *Lysinibacillus fusiformis* | FN397522 |
| *Lysinibacillus sphaericus* | NR_074883 |
| *Macrococcus caseolyticus* | NR_074941 |
| *Mannheimia haemolytica* | ACZX01000102 |
| *Marvinbryantia formatexigens* | AJ505973 |
| *Massilia sp. CCUG 43427A* | FR773700 |
| *Megamonas funiformis* | AB300988 |
| *Megamonas hypermegale* | AJ420107 |
| *Megasphaera elsdenii* | AY038996 |
| *Megasphaera genomosp. C1* | AY278622 |
| *Megasphaera genomosp. type_1* | ADGP01000010 |
| *Megasphaera micronuciformis* | AECS01000020 |
| *Megasphaera sp. BLPYG_07* | HM990964 |
| *Megasphaera sp. UPII 199_6* | AFIJ01000040 |
| *Metallosphaera sedula* | D26491 |
| *Methanobacterium formicicum* | NR_025028 |
| *Methanobrevibacter acididurans* | NR_028779 |

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| *Methanobrevibacter arboriphilus* | NR_042783 |
| *Methanobrevibacter curvatus* | NR_044796 |
| *Methanobrevibacter cuticularis* | NR_044776 |
| *Methanobrevibacter filiformis* | NR_044801 |
| *Methanobrevibacter gottschalkii* | NR_044789 |
| *Methanobrevibacter millerae* | NR_042785 |
| *Methanobrevibacter olleyae* | NR_043024 |
| *Methanobrevibacter oralis* | HE654003 |
| *Methanobrevibacter ruminantium* | NR_042784 |
| *Methanobrevibacter smithii* | ABYV02000002 |
| *Methanobrevibacter thaueri* | NR_044787 |
| *Methanobrevibacter woesei* | NR_044788 |
| *Methanobrevibacter wolinii* | NR_044790 |
| *Methanosphaera stadtmanae* | AY196684 |
| *Methylobacterium extorquens* | NC_010172 |
| *Methylobacterium podarium* | AY468363 |
| *Methylobacterium radiotolerans* | GU294320 |
| *Methylobacterium sp. 1sub* | AY468371 |
| *Methylobacterium sp. MM4* | AY468370 |
| *Methylocella silvestris* | NR_074237 |
| *Methylophilus sp. ECd5* | AY436794 |
| *Microbacterium chocolatum* | NR_037045 |
| *Microbacterium flavescens* | EU714363 |
| *Microbacterium gubbeenense* | NR_025098 |
| *Microbacterium lacticum* | EU714351 |
| *Microbacterium oleivorans* | EU714381 |
| *Microbacterium oxydans* | EU714348 |
| *Microbacterium paraoxydans* | AJ491806 |
| *Microbacterium phyllosphaerae* | EU714359 |

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| *Microbacterium schleiferi* | NR_044936 |
| *Microbacterium sp. 768* | EU714378 |
| *Microbacterium sp. oral strain C24KA* | AF287752 |
| *Microbacterium testaceum* | EU714365 |
| *Micrococcus antarcticus* | NR_025285 |
| *Micrococcus luteus* | NR_075062 |
| *Micrococcus lylae* | NR_026200 |
| *Micrococcus sp. 185* | EU714334 |
| *Microcystis aeruginosa* | NC_010296 |
| *Mitsuokella jalaludinii* | NR_028840 |
| *Mitsuokella multacida* | ABWK02000005 |
| *Mitsuokella sp. oral taxon 521* | GU413658 |
| *Mitsuokella sp. oral taxon G68* | GU432166 |
| *Mobiluncus curtisii* | AEPZ01000013 |
| *Mobiluncus mulieris* | ACKW01000035 |
| *Moellerella wisconsensis* | JN175344 |
| *Mogibacterium diversum* | NR_027191 |
| *Mogibacterium neglectum* | NR_027203 |
| *Mogibacterium pumilum* | NR_028608 |
| *Mogibacterium timidum* | Z36296 |
| *Mollicutes bacterium pACH93* | AY297808 |
| *Moorella thermoacetica* | NR_075001 |
| *Moraxella catarrhalis* | CP002005 |
| *Moraxella lincolnii* | FR822735 |
| *Moraxella osloensis* | JN175341 |
| *Moraxella sp. 16285* | JF682466 |
| *Moraxella sp. GM2* | JF837191 |
| *Morganella morganii* | AJ301681 |
| *Morganella sp. JB_T16* | AJ781005 |

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| *Morococcus cerebrosus* | JN175352 |
| *Moryella indoligenes* | AF527773 |
| *Mycobacterium abscessus* | AGQU01000002 |
| *Mycobacterium africanum* | AF480605 |
| *Mycobacterium alsiensis* | AJ938169 |
| *Mycobacterium avium* | CP000479 |
| *Mycobacterium chelonae* | AB548610 |
| *Mycobacterium colombiense* | AM062764 |
| *Mycobacterium elephantis* | AF385898 |
| *Mycobacterium gordonae* | GU142930 |
| *Mycobacterium intracellulare* | GQ153276 |
| *Mycobacterium kansasii* | AF480601 |
| *Mycobacterium lacus* | NR_025175 |
| *Mycobacterium leprae* | FM211192 |
| *Mycobacterium lepromatosis* | EU203590 |
| *Mycobacterium mageritense* | FR798914 |
| *Mycobacterium mantenii* | FJ042897 |
| *Mycobacterium marinum* | NC_010612 |
| *Mycobacterium microti* | NR_025234 |
| *Mycobacterium neoaurum* | AF268445 |
| *Mycobacterium parascrofulaceum* | ADNV01000350 |
| *Mycobacterium paraterrae* | EU919229 |
| *Mycobacterium phlei* | GU142920 |
| *Mycobacterium seoulense* | DQ536403 |
| *Mycobacterium smegmatis* | CP000480 |
| *Mycobacterium sp. 1761* | EU703150 |
| *Mycobacterium sp. 1776* | EU703152 |
| *Mycobacterium sp. 1781* | EU703147 |
| *Mycobacterium sp. 1791* | EU703148 |

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| *Mycobacterium sp. 1797* | EU703149 |
| *Mycobacterium sp. AQ1GA4* | HM210417 |
| *Mycobacterium sp. B10_07.09.0206* | HQ174245 |
| *Mycobacterium sp. GN_10546* | FJ497243 |
| *Mycobacterium sp. GN_10827* | FJ497247 |
| *Mycobacterium sp. GN_11124* | FJ652846 |
| *Mycobacterium sp. GN_9188* | FJ497240 |
| *Mycobacterium sp. GR_2007_210* | FJ555538 |
| *Mycobacterium sp. HE5* | AJ012738 |
| *Mycobacterium sp. NLA001000736* | HM627011 |
| *Mycobacterium sp. W* | DQ437715 |
| *Mycobacterium tuberculosis* | CP001658 |
| *Mycobacterium ulcerans* | AB548725 |
| *Mycobacterium vulneris* | EU834055 |
| *Mycoplasma agalactiae* | AF010477 |
| *Mycoplasma amphoriforme* | AY531656 |
| *Mycoplasma arthritidis* | NC_011025 |
| *Mycoplasma bovoculi* | NR_025987 |
| *Mycoplasma faucium* | NR_024983 |
| *Mycoplasma fermentans* | CP002458 |
| *Mycoplasma flocculare* | X62699 |
| *Mycoplasma genitalium* | L43967 |
| *Mycoplasma hominis* | AF443616 |
| *Mycoplasma orale* | AY796060 |
| *Mycoplasma ovipneumoniae* | NR_025989 |
| *Mycoplasma penetrans* | NC_004432 |
| *Mycoplasma pneumoniae* | NC_000912 |
| *Mycoplasma putrefaciens* | U26055 |
| *Mycoplasma salivarium* | M24661 |

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| *Mycoplasmataceae genomosp. P1 oral clone MB1_G23* | DQ003614 |
| *Myroides odoratimimus* | NR_042354 |
| *Myroides sp. MY15* | GU253339 |
| *Neisseria bacilliformis* | AFAY01000058 |
| *Neisseria cinerea* | ACDY01000037 |
| *Neisseria elongata* | ADBF01000003 |
| *Neisseria flavescens* | ACQV01000025 |
| *Neisseria genomosp. P2 oral clone MB5_P15* | DQ003630 |
| *Neisseria gonorrhoeae* | CP002440 |
| *Neisseria lactamica* | ACEQ01000095 |
| *Neisseria macacae* | AFQE01000146 |
| *Neisseria meningitidis* | NC_003112 |
| *Neisseria mucosa* | ACDX01000110 |
| *Neisseria pharyngis* | AJ239281 |
| *Neisseria polysaccharea* | ADBE01000137 |
| *Neisseria sicca* | ACKO02000016 |
| *Neisseria sp. KEM232* | GQ203291 |
| *Neisseria sp. oral clone AP132* | AY005027 |
| *Neisseria sp. oral clone JC012* | AY349388 |
| *Neisseria sp. oral strain B33KA* | AY005028 |
| *Neisseria sp. oral taxon 014* | ADEA01000039 |
| *Neisseria sp. SMC_A9199* | FJ763637 |
| *Neisseria sp. TM10_1* | DQ279352 |
| *Neisseria subflava* | ACEO01000067 |
| *Neorickettsia risticii* | CP001431 |
| *Neorickettsia sennetsu* | NC_007798 |
| *Nocardia brasiliensis* | AIHV01000038 |
| *Nocardia cyriacigeorgica* | HQ009486 |

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| *Nocardia farcinica* | NC_006361 |
| *Nocardia puris* | NR_028994 |
| *Nocardia sp. 01_Je_025* | GU574059 |
| *Nocardiopsis dassonvillei* | CP002041 |
| *Novosphingobium aromaticivorans* | AAAV03000008 |
| *Oceanobacillus caeni* | NR_041533 |
| *Oceanobacillus sp. Ndiop* | CAER01000083 |
| *Ochrobactrum anthropi* | NC_009667 |
| *Ochrobactrum intermedium* | ACQA01000001 |
| *Ochrobactrum pseudintermedium* | DQ365921 |
| *Odoribacter laneus* | AB490805 |
| *Odoribacter splanchnicus* | CP002544 |
| *Okadaella gastrococcus* | HQ699465 |
| *Oligella ureolytica* | NR_041998 |
| *Oligella urethralis* | NR_041753 |
| *Olsenella genomosp. C1* | AY278623 |
| *Olsenella profusa* | FN178466 |
| *Olsenella sp. F0004* | EU592964 |
| *Olsenella sp. oral taxon 809* | ACVE01000002 |
| *Olsenella uli* | CP002106 |
| *Opitutus terrae* | NR_074978 |
| *Oribacterium sinus* | ACKX01000142 |
| *Oribacterium sp. ACB1* | HM120210 |
| *Oribacterium sp. ACB7* | HM120211 |
| *Oribacterium sp. CM12* | HQ616374 |
| *Oribacterium sp. ICM51* | HQ616397 |
| *Oribacterium sp. OBRC12* | HQ616355 |
| *Oribacterium sp. oral taxon 078* | ACIQ02000009 |
| *Oribacterium sp. oral taxon 102* | GQ422713 |

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| *Oribacterium sp. oral taxon 108* | AFIH01000001 |
| *Orientia tsutsugamushi* | AP008981 |
| *Ornithinibacillus bavariensis* | NR_044923 |
| *Ornithinibacillus sp. 7_10AIA* | FN397526 |
| *Oscillibacter sp. G2* | HM626173 |
| *Oscillibacter valericigenes* | NR_074793 |
| *Oscillospira guilliermondii* | AB040495 |
| *Oxalobacter formigenes* | ACDQ01000020 |
| *Paenibacillus barcinonensis* | NR_042272 |
| *Paenibacillus barengoltzii* | NR_042756 |
| *Paenibacillus chibensis* | NR_040885 |
| *Paenibacillus cookii* | NR_025372 |
| *Paenibacillus durus* | NR_037017 |
| *Paenibacillus glucanolyticus* | D78470 |
| *Paenibacillus lactis* | NR_025739 |
| *Paenibacillus lautus* | NR_040882 |
| *Paenibacillus pabuli* | NR_040853 |
| *Paenibacillus polymyxa* | NR_037006 |
| *Paenibacillus popilliae* | NR_040888 |
| *Paenibacillus sp. CIP 101062* | HM212646 |
| *Paenibacillus sp. HGF5* | AEXS01000095 |
| *Paenibacillus sp. HGF7* | AFDH01000147 |
| *Paenibacillus sp. JC66* | JF824808 |
| *Paenibacillus sp. oral taxon F45* | HM099647 |
| *Paenibacillus sp. R_27413* | HE586333 |
| *Paenibacillus sp. R_27422* | HE586338 |
| *Paenibacillus timonensis* | NR_042844 |
| *Pantoea agglomerans* | AY335552 |
| *Pantoea ananatis* | CP001875 |

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| *Pantoea brenneri* | EU216735 |
| *Pantoea citrea* | EF688008 |
| *Pantoea conspicua* | EU216737 |
| *Pantoea septica* | EU216734 |
| *Papillibacter cinnamivorans* | NR_025025 |
| *Parabacteroides distasonis* | CP000140 |
| *Parabacteroides goldsteinii* | AY974070 |
| *Parabacteroides gordonii* | AB470344 |
| *Parabacteroides johnsonii* | ABYH01000014 |
| *Parabacteroides merdae* | EU136685 |
| *Parabacteroides sp. D13* | ACPW01000017 |
| *Parabacteroides sp. NS31_3* | JN029805 |
| *Parachlamydia sp. UWE25* | BX908798 |
| *Paracoccus denitrificans* | CP000490 |
| *Paracoccus marcusii* | NR_044922 |
| *Paraprevotella clara* | AFFY01000068 |
| *Paraprevotella xylaniphila* | AFBR01000011 |
| *Parascardovia denticolens* | ADEB01000020 |
| *Parasutterella excrementihominis* | AFBP01000029 |
| *Parasutterella secunda* | AB491209 |
| *Parvimonas micra* | AB729072 |
| *Parvimonas sp. oral taxon 110* | AFII01000002 |
| *Pasteurella bettyae* | L06088 |
| *Pasteurella dagmatis* | ACZR01000003 |
| *Pasteurella multocida* | NC_002663 |
| *Pediococcus acidilactici* | ACXB01000026 |
| *Pediococcus pentosaceus* | NR_075052 |
| *Peptococcus niger* | NR_029221 |
| *Peptococcus sp. oral clone JM048* | AY349389 |

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| *Peptococcus sp. oral taxon 167* | GQ422727 |
| *Peptoniphilus asaccharolyticus* | D14145 |
| *Peptoniphilus duerdenii* | EU526290 |
| *Peptoniphilus harei* | NR_026358 |
| *Peptoniphilus indolicus* | AY153431 |
| *Peptoniphilus ivorii* | Y07840 |
| *Peptoniphilus lacrimalis* | ADDO01000050 |
| *Peptoniphilus sp. gpac007* | AM176517 |
| *Peptoniphilus sp. gpac018A* | AM176519 |
| *Peptoniphilus sp. gpac077* | AM176527 |
| *Peptoniphilus sp. gpac148* | AM176535 |
| *Peptoniphilus sp. JC140* | JF824803 |
| *Peptoniphilus sp. oral taxon 386* | ADCS01000031 |
| *Peptoniphilus sp. oral taxon 836* | AEAA01000090 |
| *Peptostreptococcaceae bacterium ph1* | JN837495 |
| *Peptostreptococcus anaerobius* | AY326462 |
| *Peptostreptococcus micros* | AM176538 |
| *Peptostreptococcus sp. 9succ1* | X90471 |
| *Peptostreptococcus sp. oral clone AP24* | AB175072 |
| *Peptostreptococcus sp. oral clone FJ023* | AY349390 |
| *Peptostreptococcus sp. P4P_31 P3* | AY207059 |
| *Peptostreptococcus stomatis* | ADGQ01000048 |
| *Phascolarctobacterium faecium* | NR_026111 |
| *Phascolarctobacterium sp. YIT 12068* | AB490812 |
| *Phascolarctobacterium succinatutens* | AB490811 |
| *Phenylobacterium zucineum* | AY628697 |
| *Photorhabdus asymbiotica* | Z76752 |
| *Pigmentiphaga daeguensis* | JN585327 |
| *Planomicrobium koreense* | NR_025011 |

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| *Plesiomonas shigelloides* | X60418 |
| *Porphyromonadaceae bacterium NML 060648* | EF184292 |
| *Porphyromonas asaccharolytica* | AENO01000048 |
| *Porphyromonas endodontalis* | ACNN01000021 |
| *Porphyromonas gingivalis* | AE015924 |
| *Porphyromonas levii* | NR_025907 |
| *Porphyromonas macacae* | NR_025908 |
| *Porphyromonas somerae* | AB547667 |
| *Porphyromonas sp. oral clone BB134* | AY005068 |
| *Porphyromonas sp. oral clone F016* | AY005069 |
| *Porphyromonas sp. oral clone P2PB_52 P1* | AY207054 |
| *Porphyromonas sp. oral clone P4GB_100 P2* | AY207057 |
| *Porphyromonas sp. UQD 301* | EU012301 |
| *Porphyromonas uenonis* | ACLR01000152 |
| *Prevotella albensis* | NR_025300 |
| *Prevotella amnii* | AB547670 |
| *Prevotella bergensis* | ACKS01000100 |
| *Prevotella bivia* | ADFO01000096 |
| *Prevotella brevis* | NR_041954 |
| *Prevotella buccae* | ACRB01000001 |
| *Prevotella buccalis* | JN867261 |
| *Prevotella copri* | ACBX02000014 |
| *Prevotella corporis* | L16465 |
| *Prevotella dentalis* | AB547678 |
| *Prevotella denticola* | CP002589 |
| *Prevotella disiens* | AEDO01000026 |
| *Prevotella genomosp. C1* | AY278624 |
| *Prevotella genomosp. C2* | AY278625 |
| *Prevotella genomosp. P7 oral clone MB2_P31* | DQ003620 |

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| *Prevotella genomosp. P8 oral clone MB3_P13* | DQ003622 |
| *Prevotella genomosp. P9 oral clone MB7_G16* | DQ003633 |
| *Prevotella heparinolytica* | GQ422742 |
| *Prevotella histicola* | JN867315 |
| *Prevotella intermedia* | AF414829 |
| *Prevotella loescheii* | JN867231 |
| *Prevotella maculosa* | AGEK01000035 |
| *Prevotella marshii* | AEEI01000070 |
| *Prevotella melaninogenica* | CP002122 |
| *Prevotella micans* | AGWK01000061 |
| *Prevotella multiformis* | AEWX01000054 |
| *Prevotella multisaccharivorax* | AFJE01000016 |
| *Prevotella nanceiensis* | JN867228 |
| *Prevotella nigrescens* | AFPX01000069 |
| *Prevotella oralis* | AEPE01000021 |
| *Prevotella oris* | ADDV01000091 |
| *Prevotella oulorum* | L16472 |
| *Prevotella pallens* | AFPY01000135 |
| *Prevotella ruminicola* | CP002006 |
| *Prevotella salivae* | AB108826 |
| *Prevotella sp. BI_42* | AJ581354 |
| *Prevotella sp. CM38* | HQ610181 |
| *Prevotella sp. ICM1* | HQ616385 |
| *Prevotella sp. ICM55* | HQ616399 |
| *Prevotella sp. JCM 6330* | AB547699 |
| *Prevotella sp. oral clone AA020* | AY005057 |
| *Prevotella sp. oral clone ASCG10* | AY923148 |
| *Prevotella sp. oral clone ASCG12* | DQ272511 |
| *Prevotella sp. oral clone AU069* | AY005062 |

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| *Prevotella sp. oral clone CY006* | AY005063 |
| *Prevotella sp. oral clone DA058* | AY005065 |
| *Prevotella sp. oral clone FL019* | AY349392 |
| *Prevotella sp. oral clone FU048* | AY349393 |
| *Prevotella sp. oral clone FW035* | AY349394 |
| *Prevotella sp. oral clone GI030* | AY349395 |
| *Prevotella sp. oral clone GI032* | AY349396 |
| *Prevotella sp. oral clone GI059* | AY349397 |
| *Prevotella sp. oral clone GU027* | AY349398 |
| *Prevotella sp. oral clone HF050* | AY349399 |
| *Prevotella sp. oral clone ID019* | AY349400 |
| *Prevotella sp. oral clone IDR_CEC_0055* | AY550997 |
| *Prevotella sp. oral clone IK053* | AY349401 |
| *Prevotella sp. oral clone IK062* | AY349402 |
| *Prevotella sp. oral clone P4PB_83 P2* | AY207050 |
| *Prevotella sp. oral taxon 292* | GQ422735 |
| *Prevotella sp. oral taxon 299* | ACWZ01000026 |
| *Prevotella sp. oral taxon 300* | GU409549 |
| *Prevotella sp. oral taxon 302* | ACZK01000043 |
| *Prevotella sp. oral taxon 310* | GQ422737 |
| *Prevotella sp. oral taxon 317* | ACQH01000158 |
| *Prevotella sp. oral taxon 472* | ACZS01000106 |
| *Prevotella sp. oral taxon 781* | GQ422744 |
| *Prevotella sp. oral taxon 782* | GQ422745 |
| *Prevotella sp. oral taxon F68* | HM099652 |
| *Prevotella sp. oral taxon G60* | GU432133 |
| *Prevotella sp. oral taxon G70* | GU432179 |
| *Prevotella sp. oral taxon G71* | GU432180 |
| *Prevotella sp. SEQ053* | JN867222 |

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| *Prevotella sp. SEQ065* | JN867234 |
| *Prevotella sp. SEQ072* | JN867238 |
| *Prevotella sp. SEQ116* | JN867246 |
| *Prevotella sp. SG12* | GU561343 |
| *Prevotella sp. sp24* | AB003384 |
| *Prevotella sp. sp34* | AB003385 |
| *Prevotella stercorea* | AB244774 |
| *Prevotella tannerae* | ACIJ02000018 |
| *Prevotella timonensis* | ADEF01000012 |
| *Prevotella veroralis* | ACVA01000027 |
| *Prevotella jejuni*, *Prevotella aurantiaca*, *Prevotella baroniae*, *Prevotella colorans*, *Prevotella corporis*, *Prevotella dentasini*, *Prevotella enoeca*, *Prevotella falsenii*, *Prevotella fusca*, *Prevotella heparinolytica*, *Prevotella loescheii*, *Prevotella multisaccharivorax*, *Prevotella nanceiensis*, *Prevotella oryzae*, *Prevotella paludivivens*, *Prevotella pleuritidis*, *Prevotella ruminicola*, *Prevotella saccharolytica*, *Prevotella scopos*, *Prevotella shahii*, *Prevotella zoogleoformans* | |
| *Prevotellaceae bacterium P4P_62 P1* | AY207061 |
| *Prochlorococcus marinus* | CP000551 |
| *Propionibacteriaceae bacterium NML 02_0265* | EF599122 |
| *Propionibacterium acidipropionici* | NC_019395 |
| *Propionibacterium acnes* | ADJM01000010 |
| *Propionibacterium avidum* | AJ003055 |
| *Propionibacterium freudenreichii* | NR_036972 |
| *Propionibacterium granulosum* | FJ785716 |
| *Propionibacterium jensenii* | NR_042269 |

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| *Propionibacterium propionicum* | NR_025277 |
| *Propionibacterium sp. 434_HC2* | AFIL01000035 |
| *Propionibacterium sp. H456* | AB177643 |
| *Propionibacterium sp. LG* | AY354921 |
| *Propionibacterium sp. oral taxon 192* | GQ422728 |
| *Propionibacterium sp. S555a* | AB264622 |
| *Propionibacterium thoenii* | NR_042270 |
| *Proteus mirabilis* | ACLE01000013 |
| *Proteus penneri* | ABVP01000020 |
| *Proteus sp. HS7514* | DQ512963 |
| *Proteus vulgaris* | AJ233425 |
| *Providencia alcalifaciens* | ABXW01000071 |
| *Providencia rettgeri* | AM040492 |
| *Providencia rustigianii* | AM040489 |
| *Providencia stuartii* | AF008581 |
| *Pseudoclavibacter sp. Timone* | FJ375951 |
| *Pseudoflavonifractor capillosus* | AY136666 |
| *Pseudomonas aeruginosa* | AABQ07000001 |
| *Pseudomonas fluorescens* | AY622220 |
| *Pseudomonas gessardii* | FJ943496 |
| *Pseudomonas mendocina* | AAUL01000021 |
| *Pseudomonas monteilii* | NR_024910 |
| *Pseudomonas poae* | GU188951 |
| *Pseudomonas pseudoalcaligenes* | NR_037000 |
| *Pseudomonas putida* | AF094741 |
| *Pseudomonas sp. 2_1_26* | ACWU01000257 |
| *Pseudomonas sp. G1229* | DQ910482 |
| *Pseudomonas sp. NP522b* | EU723211 |
| *Pseudomonas stutzeri* | AM905854 |

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| *Pseudomonas tolaasii* | AF320988 |
| *Pseudomonas viridiflava* | NR_042764 |
| *Pseudoramibacter alactolyticus* | AB036759 |
| *Psychrobacter arcticus* | CP000082 |
| *Psychrobacter cibarius* | HQ698586 |
| *Psychrobacter cryohalolentis* | CP000323 |
| *Psychrobacter faecalis* | HQ698566 |
| *Psychrobacter nivimaris* | HQ698587 |
| *Psychrobacter pulmonis* | HQ698582 |
| *Psychrobacter sp. 13983* | HM212668 |
| *Pyramidobacter piscolens* | AY207056 |
| *Ralstonia pickettii* | NC_010682 |
| *Ralstonia sp. 5_7_47FAA* | ACUF01000076 |
| *Raoultella ornithinolytica* | AB364958 |
| *Raoultella planticola* | AF129443 |
| *Raoultella terrigena* | NR_037085 |
| *Rhodobacter sp. oral taxon C30* | HM099648 |
| *Rhodobacter sphaeroides* | CP000144 |
| *Rhodococcus corynebacterioides* | X80615 |
| *Rhodococcus equi* | ADNW01000058 |
| *Rhodococcus erythropolis* | ACNO01000030 |
| *Rhodococcus fascians* | NR_037021 |
| *Rhodopseudomonas palustris* | CP000301 |
| *Rickettsia akari* | CP000847 |
| *Rickettsia conorii* | AE008647 |
| *Rickettsia prowazekii* | M21789 |
| *Rickettsia rickettsii* | NC_010263 |
| *Rickettsia slovaca* | L36224 |
| *Rickettsia typhi* | AE017197 |

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| *Robinsoniella peoriensis* | AF445258 |
| *Roseburia cecicola* | GU233441 |
| *Roseburia faecalis* | AY804149 |
| *Roseburia faecis* | AY305310 |
| *Roseburia hominis* | AJ270482 |
| *Roseburia intestinalis* | FP929050 |
| *Roseburia inulinivorans* | AJ270473 |
| *Roseburia sp. 11SE37* | FM954975 |
| *Roseburia sp. 11SE38* | FM954976 |
| *Roseiflexus castenholzii* | CP000804 |
| *Roseomonas cervicalis* | ADVL01000363 |
| *Roseomonas mucosa* | NR_028857 |
| *Roseomonas sp. NML94_0193* | AF533357 |
| *Roseomonas sp. NML97_0121* | AF533359 |
| *Roseomonas sp. NML98_0009* | AF533358 |
| *Roseomonas sp. NML98_0157* | AF533360 |
| *Rothia aeria* | DQ673320 |
| *Rothia dentocariosa* | ADDW01000024 |
| *Rothia mucilaginosa* | ACVO01000020 |
| *Rothia nasimurium* | NR_025310 |
| *Rothia sp. oral taxon 188* | GU470892 |
| *Ruminobacter amylophilus* | NR_026450 |
| *Ruminococcaceae bacterium D16* | ADDX01000083 |
| *Ruminococcus albus* | AY445600 |
| *Ruminococcus bromii* | EU266549 |
| *Ruminococcus callidus* | NR_029160 |
| *Ruminococcus champanellensis* | FP929052 |
| *Ruminococcus flavefaciens* | NR_025931 |
| *Ruminococcus gnavus* | X94967 |

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| *Ruminococcus hansenii* | M59114 |
| *Ruminococcus lactaris* | ABOU02000049 |
| *Ruminococcus obeum* | AY169419 |
| *Ruminococcus sp. 18P13* | AJ515913 |
| *Ruminococcus sp. 5_1_39BFAA* | ACII01000172 |
| *Ruminococcus sp. 9SE51* | FM954974 |
| *Ruminococcus sp. ID8* | AY960564 |
| *Ruminococcus sp. K_1* | AB222208 |
| *Ruminococcus torques* | AAVP02000002 |
| *Saccharomonospora viridis* | X54286 |
| *Salmonella bongori* | NR_041699 |
| *Salmonella enterica* | NC_011149 |
| *Salmonella enterica* | NC_011205 |
| *Salmonella enterica* | DQ344532 |
| *Salmonella enterica* | ABEH02000004 |
| *Salmonella enterica* | ABAK02000001 |
| *Salmonella enterica* | NC_011080 |
| *Salmonella enterica* | EU118094 |
| *Salmonella enterica* | NC_011094 |
| *Salmonella enterica* | AE014613 |
| *Salmonella enterica* | ABFH02000001 |
| *Salmonella enterica* | ABEM01000001 |
| *Salmonella enterica* | ABAM02000001 |
| *Salmonella typhimurium* | DQ344533 |
| *Salmonella typhimurium* | AF170176 |
| *Sarcina ventriculi* | NR_026146 |
| *Scardovia inopinata* | AB029087 |
| *Scardovia wiggsiae* | AY278626 |
| *Segniliparus rotundus* | CP001958 |

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| *Segniliparus rugosus* | ACZI01000025 |
| *Selenomonas artemidis* | HM596274 |
| *Selenomonas dianae* | GQ422719 |
| *Selenomonas flueggei* | AF287803 |
| *Selenomonas genomosp. C1* | AY278627 |
| *Selenomonas genomosp. C2* | AY278628 |
| *Selenomonas genomosp. P5* | AY341820 |
| *Selenomonas genomosp. P6 oral clone MB3_C41* | DQ003636 |
| *Selenomonas genomosp. P7 oral clone MB5_C08* | DQ003627 |
| *Selenomonas genomosp. P8 oral clone MB5_P06* | DQ003628 |
| *Selenomonas infelix* | AF287802 |
| *Selenomonas noxia* | GU470909 |
| *Selenomonas ruminantium* | NR_075026 |
| *Selenomonas sp. FOBRC9* | HQ616378 |
| *Selenomonas sp. oral clone FT050* | AY349403 |
| *Selenomonas sp. oral clone GI064* | AY349404 |
| *Selenomonas sp. oral clone GT010* | AY349405 |
| *Selenomonas sp. oral clone HU051* | AY349406 |
| *Selenomonas sp. oral clone IK004* | AY349407 |
| *Selenomonas sp. oral clone IQ048* | AY349408 |
| *Selenomonas sp. oral clone JI021* | AY349409 |
| *Selenomonas sp. oral clone JS031* | AY349410 |
| *Selenomonas sp. oral clone OH4A* | AY947498 |
| *Selenomonas sp. oral clone P2PA_80 P4* | AY207052 |
| *Selenomonas sp. oral taxon 137* | AENV01000007 |
| *Selenomonas sp. oral taxon 149* | AEEJ01000007 |
| *Selenomonas sputigena* | ACKP02000033 |

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|---|---|
| *Serratia fonticola* | NR_025339 |
| *Serratia liquefaciens* | NR_042062 |
| *Serratia marcescens* | GU826157 |
| *Serratia odorifera* | ADBY01000001 |
| *Serratia proteamaculans* | AAUN01000015 |
| *Shewanella putrefaciens* | CP002457 |
| *Shigella boydii* | AAKA01000007 |
| *Shigella dysenteriae* | NC_007606 |
| *Shigella flexneri* | AE005674 |
| *Shigella sonnei* | NC_007384 |
| *Shuttleworthia satelles* | ACIP02000004 |
| *Shuttleworthia sp. MSX8B* | HQ616383 |
| *Shuttleworthia sp. oral taxon G69* | GU432167 |
| *Simonsiella muelleri* | ADCY01000105 |
| *Slackia equolifaciens* | EU377663 |
| *Slackia exigua* | ACUX01000029 |
| *Slackia faecicanis* | NR_042220 |
| *Slackia heliotrinireducens* | NR_074439 |
| *Slackia isoflavoniconvertens* | AB566418 |
| *Slackia piriformis* | AB490806 |
| *Slackia sp. NATTS* | AB505075 |
| *Solobacterium moorei* | AECQ01000039 |
| *Sphingobacterium faecium* | NR_025537 |
| *Sphingobacterium mizutaii* | JF708889 |
| *Sphingobacterium multivorum* | NR_040953 |
| *Sphingobacterium spiritivorum* | ACHA02000013 |
| *Sphingomonas echinoides* | NR_024700 |
| *Sphingomonas sp. oral clone FI012* | AY349411 |
| *Sphingomonas sp. oral clone FZ016* | AY349412 |

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|---|---|
| *Sphingomonas sp. oral taxon A09* | HM099639 |
| *Sphingomonas sp. oral taxon F71* | HM099645 |
| *Sphingopyxis alaskensis* | CP000356 |
| *Spiroplasma insolitum* | NR_025705 |
| *Sporobacter termitidis* | NR_044972 |
| *Sporolactobacillus inulinus* | NR_040962 |
| *Sporolactobacillus nakayamae* | NR_042247 |
| *Sporosarcina newyorkensis* | AFPZ01000142 |
| *Sporosarcina sp. 2681* | GU994081 |
| *Staphylococcaceae bacterium NML 92_0017* | AY841362 |
| *Staphylococcus aureus* | CP002643 |
| *Staphylococcus auricularis* | JQ624774 |
| *Staphylococcus capitis* | ACFR01000029 |
| *Staphylococcus caprae* | ACRH01000033 |
| *Staphylococcus carnosus* | NR_075003 |
| *Staphylococcus cohnii* | JN175375 |
| *Staphylococcus condimenti* | NR_029345 |
| *Staphylococcus epidermidis* | ACHE01000056 |
| *Staphylococcus equorum* | NR_027520 |
| *Staphylococcus fleurettii* | NR_041326 |
| *Staphylococcus haemolyticus* | NC_007168 |
| *Staphylococcus hominis* | AM157418 |
| *Staphylococcus lugdunensis* | AEQA01000024 |
| *Staphylococcus pasteuri* | FJ189773 |
| *Staphylococcus pseudintermedius* | CP002439 |
| *Staphylococcus saccharolyticus* | NR_029158 |
| *Staphylococcus saprophyticus* | NC_007350 |
| *Staphylococcus sciuri* | NR_025520 |
| *Staphylococcus sp. clone bottae7* | AF467424 |

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|---|---|
| *Staphylococcus sp. H292* | AB177642 |
| *Staphylococcus sp. H780* | AB177644 |
| *Staphylococcus succinus* | NR_028667 |
| *Staphylococcus vitulinus* | NR_024670 |
| *Staphylococcus warneri* | ACPZ01000009 |
| *Staphylococcus xylosus* | AY395016 |
| *Stenotrophomonas maltophilia* | AAVZ01000005 |
| *Stenotrophomonas sp. FG_6* | EF017810 |
| *Streptobacillus moniliformis* | NR_027615 |
| *Streptococcus agalactiae* | AAJO01000130 |
| *Streptococcus alactolyticus* | NR_041781 |
| *Streptococcus anginosus* | AECT01000011 |
| *Streptococcus australis* | AEQR01000024 |
| *Streptococcus bovis* | AEEL01000030 |
| *Streptococcus canis* | AJ413203 |
| *Streptococcus constellatus* | AY277942 |
| *Streptococcus cristatus* | AEVC01000028 |
| *Streptococcus downei* | AEKN01000002 |
| *Streptococcus dysgalactiae* | AP010935 |
| *Streptococcus equi* | CP001129 |
| *Streptococcus equinus* | AEVB01000043 |
| *Streptococcus gallolyticus* | FR824043 |
| *Streptococcus genomosp. C1* | AY278629 |
| *Streptococcus genomosp. C2* | AY278630 |
| *Streptococcus genomosp. C3* | AY278631 |
| *Streptococcus genomosp. C4* | AY278632 |
| *Streptococcus genomosp. C5* | AY278633 |
| *Streptococcus genomosp. C6* | AY278634 |
| *Streptococcus genomosp. C7* | AY278635 |

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|---|---|
| *Streptococcus genomosp. C8* | AY278609 |
| *Streptococcus gordonii* | NC_009785 |
| *Streptococcus infantarius* | ABJK02000017 |
| *Streptococcus infantis* | AFNN01000024 |
| *Streptococcus intermedius* | NR_028736 |
| *Streptococcus lutetiensis* | NR_037096 |
| *Streptococcus massiliensis* | AY769997 |
| *Streptococcus milleri* | X81023 |
| *Streptococcus mitis* | AM157420 |
| *Streptococcus mutans* | AP010655 |
| *Streptococcus oligofermentans* | AY099095 |
| *Streptococcus oralis* | ADMV01000001 |
| *Streptococcus parasanguinis* | AEKM01000012 |
| *Streptococcus pasteurianus* | AP012054 |
| *Streptococcus peroris* | AEVF01000016 |
| *Streptococcus pneumoniae* | AE008537 |
| *Streptococcus porcinus* | EF121439 |
| *Streptococcus pseudopneumoniae* | FJ827123 |
| *Streptococcus pseudoporcinus* | AENS01000003 |
| *Streptococcus pyogenes* | AE006496 |
| *Streptococcus ratti* | X58304 |
| *Streptococcus salivarius* | AGBV01000001 |
| *Streptococcus sanguinis* | NR_074974 |
| *Streptococcus sinensis* | AF432857 |
| *Streptococcus sp. 16362* | JN590019 |
| *Streptococcus sp. 2_1_36FAA* | ACOI01000028 |
| *Streptococcus sp. 2285_97* | AJ131965 |
| *Streptococcus sp. 69130* | X78825 |
| *Streptococcus sp. AC15* | HQ616356 |

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|---|---|
| *Streptococcus sp. ACS2* | HQ616360 |
| *Streptococcus sp. AS20* | HQ616366 |
| *Streptococcus sp. BS35a* | HQ616369 |
| *Streptococcus sp. C150* | ACRI01000045 |
| *Streptococcus sp. CM6* | HQ616372 |
| *Streptococcus sp. CM7* | HQ616373 |
| *Streptococcus sp. ICM10* | HQ616389 |
| *Streptococcus sp. ICM12* | HQ616390 |
| *Streptococcus sp. ICM2* | HQ616386 |
| *Streptococcus sp. ICM4* | HQ616387 |
| *Streptococcus sp. ICM45* | HQ616394 |
| *Streptococcus sp. M143* | ACRK01000025 |
| *Streptococcus sp. M334* | ACRL01000052 |
| *Streptococcus sp. OBRC6* | HQ616352 |
| *Streptococcus sp. oral clone ASB02* | AY923121 |
| *Streptococcus sp. oral clone ASCA03* | DQ272504 |
| *Streptococcus sp. oral clone ASCA04* | AY923116 |
| *Streptococcus sp. oral clone ASCA09* | AY923119 |
| *Streptococcus sp. oral clone ASCB04* | AY923123 |
| *Streptococcus sp. oral clone ASCB06* | AY923124 |
| *Streptococcus sp. oral clone ASCC04* | AY923127 |
| *Streptococcus sp. oral clone ASCC05* | AY923128 |
| *Streptococcus sp. oral clone ASCC12* | DQ272507 |
| *Streptococcus sp. oral clone ASCD01* | AY923129 |
| *Streptococcus sp. oral clone ASCD09* | AY923130 |
| *Streptococcus sp. oral clone ASCD10* | DQ272509 |
| *Streptococcus sp. oral clone ASCE03* | AY923134 |
| *Streptococcus sp. oral clone ASCE04* | AY953253 |
| *Streptococcus sp. oral clone ASCE05* | DQ272510 |

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|---|---|
| *Streptococcus sp. oral clone ASCE06* | AY923135 |
| *Streptococcus sp. oral clone ASCE09* | AY923136 |
| *Streptococcus sp. oral clone ASCE10* | AY923137 |
| *Streptococcus sp. oral clone ASCE12* | AY923138 |
| *Streptococcus sp. oral clone ASCF05* | AY923140 |
| *Streptococcus sp. oral clone ASCF07* | AY953255 |
| *Streptococcus sp. oral clone ASCF09* | AY923142 |
| *Streptococcus sp. oral clone ASCG04* | AY923145 |
| *Streptococcus sp. oral clone BW009* | AY005042 |
| *Streptococcus sp. oral clone CH016* | AY005044 |
| *Streptococcus sp. oral clone GK051* | AY349413 |
| *Streptococcus sp. oral clone GM006* | AY349414 |
| *Streptococcus sp. oral clone P2PA_41 P2* | AY207051 |
| *Streptococcus sp. oral clone P4PA_30 P4* | AY207064 |
| *Streptococcus sp. oral taxon 071* | AEEP01000019 |
| *Streptococcus sp. oral taxon G59* | GU432132 |
| *Streptococcus sp. oral taxon G62* | GU432146 |
| *Streptococcus sp. oral taxon G63* | GU432150 |
| *Streptococcus sp. SHV515* | Y07601 |
| *Streptococcus suis* | FM252032 |
| *Streptococcus thermophilus* | CP000419 |
| *Streptococcus uberis* | HQ391900 |
| *Streptococcus urinalis* | DQ303194 |
| *Streptococcus vestibularis* | AEKO01000008 |
| *Streptococcus viridans* | AF076036 |
| *Streptomyces albus* | AJ697941 |
| *Streptomyces griseus* | NR_074787 |
| *Streptomyces sp. 1 AIP_2009* | FJ176782 |
| *Streptomyces sp. SD 511* | EU544231 |

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|---|---|
| *Streptomyces sp. SD 524* | EU544234 |
| *Streptomyces sp. SD 528* | EU544233 |
| *Streptomyces sp. SD 534* | EU544232 |
| *Streptomyces thermoviolaceus* | NR_027616 |
| *Subdoligranulum variabile* | AJ518869 |
| *Succinatimonas hippei* | AEVO01000027 |
| *Sutterella morbirenis* | AJ832129 |
| *Sutterella parvirubra* | AB300989 |
| *Sutterella sanguinus* | AJ748647 |
| *Sutterella sp. YIT 12072* | AB491210 |
| *Sutterella stercoricanis* | NR_025600 |
| *Sutterella wadsworthensis* | ADMF01000048 |
| *Synergistes genomosp. C1* | AY278615 |
| *Synergistes sp. RMA 14551* | DQ412722 |
| *Synergistetes bacterium ADV897* | GQ258968 |
| *Synergistetes bacterium LBVCM1157* | GQ258969 |
| *Synergistetes bacterium oral taxon 362* | GU410752 |
| *Synergistetes bacterium oral taxon D48* | GU430992 |
| *Syntrophococcus sucromutans* | NR_036869 |
| *Syntrophomonadaceae genomosp. P1* | AY341821 |
| *Tannerella forsythia* | CP003191 |
| *Tannerella sp. 6_1_58FAA_CT1* | ACWX01000068 |
| *Tatlockia micdadei* | M36032 |
| *Tatumella ptyseos* | NR_025342 |
| *Tessaracoccus sp. oral taxon F04* | HM099640 |
| *Tetragenococcus halophilus* | NR_075020 |
| *Tetragenococcus koreensis* | NR_043113 |
| *Thermoanaerobacter pseudethanolicus* | CP000924 |
| *Thermobifida fusca* | NC_007333 |

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|---|---|
| *Thermofilum pendens* | X14835 |
| *Thermus aquaticus* | NR_025900 |
| *Tissierella praeacuta* | NR_044860 |
| *Trabulsiella guamensis* | AY373830 |
| *Treponema denticola* | ADEC01000002 |
| *Treponema genomosp. P1* | AY341822 |
| *Treponema genomosp. P4 oral clone MB2_G19* | DQ003618 |
| *Treponema genomosp. P5 oral clone MB3_P23* | DQ003624 |
| *Treponema genomosp. P6 oral clone MB4_G11* | DQ003625 |
| *Treponema lecithinolyticum* | NR_026247 |
| *Treponema pallidum* | CP001752 |
| *Treponema parvum* | AF302937 |
| *Treponema phagedenis* | AEFH01000172 |
| *Treponema putidum* | AJ543428 |
| *Treponema refringens* | AF426101 |
| *Treponema socranskii* | NR_024868 |
| *Treponema sp. 6:H:D15A_4* | AY005083 |
| *Treponema sp. clone DDKL_4* | Y08894 |
| *Treponema sp. oral clone JU025* | AY349417 |
| *Treponema sp. oral clone JU031* | AY349416 |
| *Treponema sp. oral clone P2PB_53 P3* | AY207055 |
| *Treponema sp. oral taxon 228* | GU408580 |
| *Treponema sp. oral taxon 230* | GU408603 |
| *Treponema sp. oral taxon 231* | GU408631 |
| *Treponema sp. oral taxon 232* | GU408646 |
| *Treponema sp. oral taxon 235* | GU408673 |
| *Treponema sp. oral taxon 239* | GU408738 |
| *Treponema sp. oral taxon 247* | GU408748 |
| *Treponema sp. oral taxon 250* | GU408776 |

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|---|---|
| *Treponema sp. oral taxon 251* | GU408781 |
| *Treponema sp. oral taxon 254* | GU408803 |
| *Treponema sp. oral taxon 265* | GU408850 |
| *Treponema sp. oral taxon 270* | GQ422733 |
| *Treponema sp. oral taxon 271* | GU408871 |
| *Treponema sp. oral taxon 508* | GU413616 |
| *Treponema sp. oral taxon 518* | GU413640 |
| *Treponema sp. oral taxon G85* | GU432215 |
| *Treponema sp. ovine footrot* | AJ010951 |
| *Treponema vincentii* | ACYH01000036 |
| *Tropheryma whipplei* | BX251412 |
| *Trueperella pyogenes* | NR_044858 |
| *Tsukamurella paurometabola* | X80628 |
| *Tsukamurella tyrosinosolvens* | AB478958 |
| *Turicibacter sanguinis* | AF349724 |
| *Ureaplasma parvum* | AE002127 |
| *Ureaplasma urealyticum* | AAYN01000002 |
| *Ureibacillus composti* | NR_043746 |
| *Ureibacillus suwonensis* | NR_043232 |
| *Ureibacillus terrenus* | NR_025394 |
| *Ureibacillus thermophilus* | NR_043747 |
| *Ureibacillus thermosphaericus* | NR_040961 |
| *Vagococcus fluvialis* | NR_026489 |
| *Veillonella atypica* | AEDS01000059 |
| *Veillonella dispar* | ACIK02000021 |
| *Veillonella genomosp. P1 oral clone MB5_P17* | DQ003631 |
| *Veillonella montpellierensis* | AF473836 |
| *Veillonella parvula* | ADFU01000009 |
| *Veillonella sp. 3_1_44* | ADCV01000019 |

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|---|---|
| *Veillonella sp. 6_1_27* | ADCW01000016 |
| *Veillonella sp. ACP1* | HQ616359 |
| *Veillonella sp. AS16* | HQ616365 |
| *Veillonella sp. BS32b* | HQ616368 |
| *Veillonella sp. ICM51a* | HQ616396 |
| *Veillonella sp. MSA12* | HQ616381 |
| *Veillonella sp. NVG 100cf* | EF108443 |
| *Veillonella sp. OK11* | JN695650 |
| *Veillonella sp. oral clone ASCA08* | AY923118 |
| *Veillonella sp. oral clone ASCB03* | AY923122 |
| *Veillonella sp. oral clone ASCG01* | AY923144 |
| *Veillonella sp. oral clone ASCG02* | AY953257 |
| *Veillonella sp. oral clone OH1A* | AY947495 |
| *Veillonella sp. oral taxon 158* | AENU01000007 |
| *Veillonellaceae bacterium oral taxon 131* | GU402916 |
| *Veillonellaceae bacterium oral taxon 155* | GU470897 |
| *Vibrio cholerae* | AAUR01000095 |
| *Vibrio fluvialis* | X76335 |
| *Vibrio furnissii* | CP002377 |
| *Vibrio mimicus* | ADAF01000001 |
| *Vibrio parahaemolyticus* | AAWQ01000116 |
| *Vibrio sp. RC341* | ACZT01000024 |
| *Vibrio vulnificus* | AE016796 |
| *Victivallaceae bacterium NML 080035* | FJ394915 |
| *Victivallis vadensis* | ABDE02000010 |
| *Virgibacillus proomii* | NR_025308 |
| *Weissella beninensis* | EU439435 |
| *Weissella cibaria* | NR_036924 |
| *Weissella confusa* | NR_040816 |

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|---|---|
| *Weissella hellenica* | AB680902 |
| *Weissella kandleri* | NR_044659 |
| *Weissella koreensis* | NR_075058 |
| *Weissella paramesenteroides* | ACKU01000017 |
| *Weissella sp. KLDS 7.0701* | EU600924 |
| *Wolinella succinogenes* | BX571657 |
| *Xanthomonadaceae bacterium NML 03_0222* | EU313791 |
| *Xanthomonas campestris* | EF101975 |
| *Xanthomonas sp. kmd_489* | EU723184 |
| *Xenophilus aerolatus* | JN585329 |
| *Yersinia aldovae* | AJ871363 |
| *Yersinia aleksiciae* | AJ627597 |
| *Yersinia bercovieri* | AF366377 |
| *Yersinia enterocolitica* | FR729477 |
| *Yersinia frederiksenii* | AF366379 |
| *Yersinia intermedia* | AF366380 |
| *Yersinia kristensenii* | ACCA01000078 |
| *Yersinia mollaretii* | NR_027546 |
| *Yersinia pestis* | AE013632 |
| *Yersinia pseudotuberculosis* | NC_009708 |
| *Yersinia rohdei* | ACCD01000071 |
| *Yokenella regensburgei* | AB273739 |
| *Zimmermannella bifida* | AB012592 |
| *Zymomonas mobilis* | NR_074274 |

**Table 2: Exemplary Oncophilic Bacteria**

| Genera | Species | Tumor Association |
|---|---|---|
| *Mycoplasma* | *hyorhinis* | Gastric Carcinoma |

| | | |
|---|---|---|
| *Propionibacterium* | *Acnes* | Prostate Cancer |
| *Mycoplasma* | *genitalium* | Prostate Cancer |
| *Methylophilus* | *sp.* | Prostate Cancer |
| *Chlamydia* | *trachomatis* | Prostate Cancer |
| *Helicobacter* | *pylori* | Gastric MALT |
| *Listeria* | *welshimeri* | Renal Cancer |
| *Streptococcus* | *pneumoniae* | Lymphoma and Leukemia |
| *Haemophilus* | *influenzae* | Lymphoma and Leukemia |
| *Staphylococcus* | *aureus* | Breast Cancer |
| *Listeria* | *monocytogenes* | Breast Cancer |
| *Methylobacterium* | *radiotolerans* | Breast Cancer |
| *Shingomonas* | *yanoikuyae* | breast Cancer |
| *Fusobacterium* | *sp* | Larynx cancer |
| *Provetelis* | *sp* | Larynx cancer |
| *streptococcus* | *pneumoniae* | Larynx cancer |
| *Gemella* | *sp* | Larynx cancer |
| *Bordetella* | *Pertussis* | Larynx cancer |
| *Corumebacterium* | *tuberculosterarícum* | Oral squamous cell carcinoma |
| *Micrococcus* | *luteus* | Oral squamous cell carcinoma |
| *Prevotella* | *melaninogenica* | Oral squamous cell carcinoma |
| *Exiguobacterium* | *oxidotolerans* | Oral squamous cell carcinoma |
| *Fusobacterium* | *naviforme* | Oral squamous cell carcinoma |
| *Veillonella* | *parvula* | Oral squamous cell carcinoma |
| *Streptococcus* | *salivarius* | Oral squamous cell carcinoma |
| *Streptococcus* | *mitis/oralis* | Oral squamous cell carcinoma |

| | | |
|---|---|---|
| *veillonella* | *dispar* | Oral squamous cell carcinoma |
| *Peptostreptococcus* | *stomatis* | Oral squamous cell carcinoma |
| *Streptococcus* | *gordonii* | Oral squamous cell carcinoma |
| *Gemella* | *Haemolysans* | Oral squamous cell carcinoma |
| *Gemella* | *morbillorum* | Oral squamous cell carcinoma |
| *Johnsonella* | *ignava* | Oral squamous cell carcinoma |
| *Streptococcus* | *parasanguins* | Oral squamous cell carcinoma |
| *Granulicatella* | *adiacens* | Oral squamous cell carcinoma |
| *Mycobacteria* | *marinum* | lung infection |
| *Campylobacter* | *concisus* | Barrett's Esophagus |
| *Campylobacter* | *rectus* | Barrett's Esophagus |
| *Oribacterium* | *sp* | Esophageal adenocarcinoma |
| *Catonella* | *sp* | Esophageal adenocarcinoma |
| *Peptostreptococcus* | *sp* | Esophageal adenocarcinoma |
| *Eubacterium* | *sp* | Esophageal adenocarcinoma |
| *Dialister* | *sp* | Esophageal adenocarcinoma |
| *Veillonella* | *sp* | Esophageal adenocarcinoma |
| *Anaeroglobus* | *sp* | Esophageal adenocarcinoma |
| *Megasphaera* | *sp* | Esophageal adenocarcinoma |
| *Atoppbium* | *sp* | Esophageal adenocarcinoma |
| *Solobacterium* | *sp* | Esophageal adenocarcinoma |
| *Rothia* | *sp* | Esophageal adenocarcinoma |
| *Actinomyces* | *sp* | Esophageal adenocarcinoma |
| *Fusobacterium* | *sp* | Esophageal adenocarcinoma |
| *Sneathia* | *sp* | Esophageal adenocarcinoma |

| | | |
|---|---|---|
| *Leptotrichia* | *sp* | Esophageal adenocarcinoma |
| *Capnocytophaga* | *sp* | Esophageal adenocarcinoma |
| *Prevotella* | *sp* | Esophageal adenocarcinoma |
| *Porphyromonas* | *sp* | Esophageal adenocarcinoma |
| *Campylobacter* | *sp* | Esophageal adenocarcinoma |
| *Haemophilus* | *sp* | Esophageal adenocarcinoma |
| *Neisseria* | *sp* | Esophageal adenocarcinoma |
| *TM7* | *sp* | Esophageal adenocarcinoma |
| *Granulicatella* | *sp* | Esophageal adenocarcinoma |
| *Variovorax* | *sp* | Psuedomyxoma Peritonei |
| *Escherichia* | *Shigella* | Psuedomyxoma Peritonei |
| *Pseudomonas* | *sp* | Psuedomyxoma Peritonei |
| *Tessaracoccus* | *sp* | Psuedomyxoma Peritonei |
| *Acinetobacter* | *sp* | Psuedomyxoma Peritonei |
| *Helicobacter* | *hepaticus* | Breast cancer |
| *Chlamydia* | *psittaci* | MALT lymphoma |
| *Borrelia* | *burgdorferi* | B cell lymphoma skin |
| *Escherichia* | *Coli NC101* | Colorectal Cancer |
| *Salmonella* | *typhimurium* | Tool |
| *Eterococcus* | *faecalis* | blood |
| *Streptococcus* | *mitis* | blood |
| *Streptococcus* | *sanguis* | blood |
| *Streptococcus* | *anginosus* | blood |
| *Streptococcus* | *salvarius* | blood |
| *Staphylococcus* | *epidermidis* | blood |

| | | |
|---|---|---|
| *Streptococcus* | *gallolyticus* | Colorectal Cancer |
| *Campylobacter* | *showae CC57C* | Colorectal Cancer |
| *Leptotrichia* | *sp* | Colorectal Cancer |

**[196]** In certain embodiments, the mEVs (such as smEVs) described herein are obtained from obligate anaerobic bacteria. Examples of obligate anaerobic bacteria include gram-negative rods (including the genera of *Bacteroides*, *Prevotella*, *Porphyromonas*, *Fusobacterium*, *Bilophila* and *Sutterella spp.*), gram-positive cocci (primarily *Peptostreptococcus spp.*), gram-positive spore-forming (*Clostridium spp.*), non-spore-forming bacilli (*Actinomyces*, *Propionibacterium*, *Eubacterium*, *Lactobacillus* and *Bifidobacterium spp.*), and gram-negative cocci (mainly *Veillonella spp.*). In some embodiments, the obligate anaerobic bacteria are of a genus selected from the group consisting of Agathobaculum, Atopobium, Blautia, Burkholderia, Dielma, Longicatena, Paraclostridium, Turicibacter, and Tyzzerella.

**[197]** In some embodiments, the mEVs (such as smEVs) described herein are obtained from bacterium of a genus selected from the group consisting of *Escherichia, Klebsiella, Lactobacillus, Shigella,* and *Staphylococcus.*

**[198]** In some embodiments, the mEVs (such as smEVs) described herein are obtained from a species selected from the group consisting of *Blautia massiliensis*, *Paraclostridium benzoelyticum*, *Dielma fastidiosa*, *Longicatena caecimuris*, *Lactococcus lactis cremoris*, *Tyzzerella nexilis*, *Hungatella effluvia*, *Klebsiella quasipneumoniae subsp. Similipneumoniae, Klebsiella oxytoca,* and *Veillonella tobetsuensis*.

**[199]** In some embodiments, the mEVs (such as smEVs) described herein are obtained from a *Prevotella* bacteria selected from the group consisting of *Prevotella albensis, Prevotella amnii, Prevotella bergensis, Prevotella bivia, Prevotella brevis, Prevotella bryantii, Prevotella buccae, Prevotella buccalis, Prevotella copri, Prevotella dentalis, Prevotella denticola, Prevotella disiens, Prevotella histicola, Prevotella intermedia, Prevotella maculosa, Prevotella marshii, Prevotella melaninogenica, Prevotella micans, Prevotella multiformis, Prevotella nigrescens, Prevotella oralis, Prevotella oris, Prevotella oulorum, Prevotella pallens, Prevotella salivae, Prevotella stercorea, Prevotella tannerae, Prevotella timonensis, Prevotella jejuni, Prevotella aurantiaca, Prevotella baroniae, Prevotella colorans, Prevotella corporis, Prevotella*

*dentasini, Prevotella enoeca, Prevotella falsenii, Prevotella fusca, Prevotella heparinolytica, Prevotella loescheii, Prevotella multisaccharivorax, Prevotella nanceiensis, Prevotella oryzae, Prevotella paludivivens, Prevotella pleuritidis, Prevotella ruminicola, Prevotella saccharolytica, Prevotella scopos, Prevotella shahii, Prevotella zoogleoformans,* and *Prevotella veroralis.*

**[200]** In some embodiments, the mEVs (such as smEVs) described herein are obtained from a strain of bacteria comprising a genomic sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity (*e.g.,* at least 99.5% sequence identity, at least 99.6% sequence identity, at least 99.7% sequence identity, at least 99.8% sequence identity, at least 99.9% sequence identity) to the genomic sequence of the strain of bacteria deposited with the ATCC Deposit number as provided in Table 3. In some embodiments, the mEVs (such as smEVs) described herein are obtained from a strain of bacteria comprising a 16S sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity (e.g., at least 99.5% sequence identity, at least 99.6% sequence identity, at least 99.7% sequence identity, at least 99.8% sequence identity, at least 99.9% sequence identity) to the 16S sequence as provided in Table 3.

**Table 3 Exemplary Bacterial Strains**

| SEQ ID No. | Strain | Deposit Number | 16S Sequence |
|---|---|---|---|
| | Parabacteroides goldsteinii Strain A | | |
| | Bifidobacterium animalis ssp. lactis Strain A | PTA-125097 | |
| | Bifidobacterium animalis ssp. lactis Strain B | | |
| | Bifidobacterium animalis ssp. lactis Strain C | | |
| | Blautia Massiliensis Strain A | PTA-125134 | |
| | Prevotella Strain B | NRRL accession Number B 50329 | |
| | Prevotella Histicola Strain A | | |

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|---|---|---|---|
| | Prevotella melanogenica Strain A | | |
| | Blautia Strain A | PTA-125346 | |
| | Lactococcus lactis cremoris Strain A | PTA-125368 | |
| | Lactococcus lactis cremoris Strain B | | |
| | Ruminococcus gnavus strain | PTA-125706 | |
| | Tyzzerella nexilis strain | PTA-125707 | |
| | Clostridium symbiosum S10-19 | | >S10-19-contig<br>CAGCGACGCCGCGTGAGTGAAGAAGTATTTC<br>GGTATGTAAAGCTCTATCAGCAGGGAAGAAA<br>ATGACGGTACCTGACTAAGAAGCCCCGGCTA<br>ACTACGTGCCAGCAGCCGCGGTAATACGTAG<br>GGGGCAAGCGTTATCCGGATTTACTGGGTGTA<br>AAGGGAGCGTAGACGGTAAAGCAAGTCTGAA<br>GTGAAAGCCCGCGGCTCAACTGCGGGACTGC<br>TTTGGAAACTGTTTAACTGGAGTGTCGGAGAG<br>GTAAGTGGAATTCCTAGTGTAGCGGTGAAAT<br>GCGTAGATATTAGGAGGAACACCAGTGGCGA<br>AGGCGACTTACTGGACGATAACTGACGTTGA<br>GGCTCGAAAGCGTGGGGAGCAAACAGGATTA<br>GATACCCTGGTAGTCCACGCCGTAAACGATG<br>AATACTAGGTGTTGGGGAGCAAAGCTCTTCG<br>GTGCCGTCGCAAACGCAGTAAGTATTCCACCT<br>GGGGAGTACGTTCGCAAGAATGAAACTCAAA<br>GGAATTGACGGGGACCCGCACAAGCGGTGGA<br>GCATGTGGTTTAATTCGAAGCAACGCGAAGA<br>ACCTTACCAGGTCTTGACATCGATCCGACGGG<br>GGAGTAACGTCCCCTTCCCTTCGGGGCGGAG<br>AAGACAGGTGGTGCATGGTTGTCGTCAGCTC<br>GTGTCGTGAGATGTTGGGTTAAGTCCCGCAAC<br>GAGCGCAACCCTTATTCTAAGTAGCCAGCGGT<br>TCGGCCGGGAACTCTTGGGAGACTGCCAGGG<br>ATAACCTGGAGGAAGGTGGGGATGACGTCAA<br>ATCATCATGCCCCTTATGATCTGGGCTACACA<br>CGTGCTACAATGGCGTAAACAAAGAGAAGCA<br>AGACCGCGAGGTGGAGCAAATCTCAAAAATA<br>ACGTCTCAGTTCGGACTGCAGGCTGCAACTCG<br>CCTGCACGAAGCTGGAATCGCTAGTAATCGC<br>GAATCAGAATGTCGCGGTGAATACGTTCCCG<br>GGTCTTGTACACACCGCCCGTCACACCATGGG<br>AGTCAGTAACGCCCGAAGTCAGTGACCCAAC<br>CGCAAGG |
| | Clostridium symbiosum S6-202 | | >S6-202-contig<br>GATGCAGCGACGCCGCGTGAGTGAAGAAGTA<br>TTTCGGTATGTAAAGCTCTATCAGCAGGGAAG<br>AAAATGACGGTACCTGACTAAGAAGCCCCGG |

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| | | | CTAACTACGTGCCAGCAGCCGCGGTAATACG<br>TAGGGGGCAAGCGTTATCCGGATTTACTGGGT<br>GTAAAGGGAGCGTAGACGGTAAAGCAAGTCT<br>GAAGTGAAAGCCCGCGGCTCAACTGCGGGAC<br>TGCTTTGGAAACTGTTTAACTGGAGTGTCGGA<br>GAGGTAAGTGGAATTCCTAGTGTAGCGGTGA<br>AATGCGTAGATATTAGGAGGAACACCAGTGG<br>CGAAGGCGACTTACTGGACGATAACTGACGT<br>TGAGGCTCGAAAGCGTGGGGAGCAAACAGGA<br>TTAGATACCCTGGTAGTCCACGCCGTAAACGA<br>TGAATACTAGGTGTTGGGGAGCAAAGCTCTTC<br>GGTGCCGTCGCAAACGCAGTAAGTATTCCAC<br>CTGGGGAGTACGTTCGCAAGAATGAAACTCA<br>AAGGAATTGACGGGGACCCGCACAAGCGGTG<br>GAGCATGTGGTTTAATTCGAAGCAACGCGAA<br>GAACCTTACCAGGTCTTGACATCGATCCGACG<br>GGGGAGTAACGTCCCCTTCCCTTCGGGGCGG<br>AGAAGACAGGTGGTGCATGGTTGTCGTCAGC<br>TCGTGTCGTGAGATGTTGGGTTAAGTCCCGCA<br>ACGAGCGCAACCCTTATTCTAAGTAGCCAGC<br>GGTTCGGCCGGGAACTCTTGGGAGACTGCCA<br>GGGATAACCTGGAGGAAGGTGGGGATGACGT<br>CAAATCATCATGCCCCTTATGATCTGGGCTAC<br>ACACGTGCTACAATGGCGTAAACAAAGAGAA<br>GCAAGACCGCGAGGTGGAGCAAATCTCAAAA<br>ATAACGTCTCAGTTCGGACTGCAGGCTGCAAC<br>TCGCCTGCACGAAGCTGGAATCGCTAGTAATC<br>GCGAATCAGAATGTCGCGGTGAATACGTTCC<br>CGGGTCTTGTACACACCGCCCGTCACACCATG<br>GGAGTCAGTAACGCCCGAAGTCAGTGACCCA<br>ACCGCAAGGAGGG |
| | Clostridium symbiosum S10-257 | | >consensus sequence<br>TGACTAAGAAGCCCCGGCTAACTACGTGCCA<br>GCAGCCGCGGTAATACGTAGGGGGCAAGCGT<br>TATCCGGATTTACTGGGTGTAAAGGGAGCGT<br>AGACGGTAAAGCAAGTCTGAAGTGAAAGCCC<br>GCGGCTCAACTGCGGGACTGCTTTGGAAACT<br>GTTTAACTGGAGTGTCGGAGAGGTAAGTGGA<br>ATTCCTAGTGTAGCGGTGAAATGCGTAGATAT<br>TAGGAGGAACACCAGTGGCGAAGGCGACTTA<br>CTGGACGATAACTGACGTTGAGGCTCGAAAG<br>CGTGGGGAGCAAACAGGATTAGATACCCTGG<br>TAGTCCACGCCGTAAACGATGAATACTAGGT<br>GTTGGGGAGCAAAGCTCTTCGGTGCCGTCGC<br>AAACGCAGTAAGTATTCCACCTGGGGAGTAC<br>GTTCGCAAGAATGAAACTCAAAGGAATTGAC<br>GGGGACCCGCACAAGCGGTGGAGCATGTGGT<br>TTAATTCGAAGCAACGCGAAGAACCTTACCA<br>GGTCTTGACATCGATCCGACGGGGGAGTAAC<br>GTCCCCTTCCCTTCGGGGCGGAGAAGACAGG<br>TGGTGCATGGTTGTCGTCAGCTCGTGTCGTGA<br>GATGTTGGGTTAAGTCCCGCAACGAGCGCAA<br>CCCTTATTCTAAGTAGCCAGCGGTTCGGCCGG<br>GAACTCTTGGGAGACTGCCAGGGATAACCTG<br>GAGGAAGGTGGGGATGACGTCAAATCATCAT |

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| | | | GCCCCTTATGATCTGGGCTACACACGTGCTAC<br>AATGGCGTAAACAAAGAGAAGCAAGACCGCG<br>AGGTGGAGCAAATCTCAAAAATAACGTCTCA<br>GTTCGGACTGCAGGCTGCAACTCGCCTGCACG<br>AAGCTGGAATCGCTAGTAATCGCGAATCAGA<br>ATGTCGC GGTGAATACGTTCCC |
| | Clostridium symbiosum S10-552 | | >10-552 consensus<br>sequenceCGTATTCACCGCGACATTCTGATTCGC<br>GATTACTAGCGATTCCAGCTTCGTGCAGGCGA<br>GTTGCAGCCTGCAGTCCGAACTGAGACGTTAT<br>TTTTGAGATTTGCTCCACCTCGCGGTCTTGCTT<br>CTCTTTGTTTACGCCATTGTAGCACGTGTGTA<br>GCCCAGATCATAAGGGGCATGATGATTTGAC<br>GTCATCCCCACCTTCCTCCAGGTTATCCCTGG<br>CAGTCTCCCAAGAGTTCCCGGCCGAACCGCTG<br>GCTACTTAGAATAAGGGTTGCGCTCGTTGCGG<br>GACTTAACCCAACATCTCACGACACGAGCTG<br>ACGACAACCATGCACCACCTGTCTTCTCCGCC<br>CCGAAGGGAAGGGGACGTTACTCCCCCGTCG<br>GATCGATGTCAAGACCTGGTAAGGTTCTTCGC<br>GTTGCTTCGAATTAAACCACATGCTCCACCGC<br>TTGTGCGGGTCCCCGTCAATTCCTTTGAGTTT<br>CATTCTTGCGAACGTACTCCCCAGGTGGAATA<br>CTTACTGCGTTTGCGACGGCACCGAAGAGCTT<br>TGCTCCCCAACACCTAGTATTCATCGTTTACG<br>GCGTGGACTACCAGGGTATCTAATCCTGTTTG<br>CTCCCCACGCTTTCGAGCCTCAACGTCAGTTA<br>TCGTCCAGTAAGTCGCCTTCGCCACTGGTGTT<br>CCTCCTAATATCTACGCATTTCACCGCTACAC<br>TAGGAATTCCACTTACCTCTCCGACACTCCAG<br>TTAAACAGTTTCCAAAGCAGTCCCGCAGTTGA<br>GCCGCGGGCTTTCACTTCAGACTTGCTTTACC<br>GTCTACGCTCCCTTTACACCCAGTAAATCCGG<br>ATAACGCTTGCCCCCTACGTATTACCGCGGCT<br>GCTGGCACGTAGTTAGCCGGGGCTTCTTAGT |
| | Clostridium symbiosum S10-551 | | >10-511_consensus_sequence 2 reads from 10-511<br>ACTAAGAAGCCCCGGCTAACTACGTGCCAGC<br>AGCCGCGGTAATACGTAGGGGGCAAGCGTTA<br>TCCGGATTTACTGGGTGTAAAGGGAGCGTAG<br>ACGGTAAAGCAAGTCTGAAGTGAAAGCCCGC<br>GGCTCAACTGCGGGACTGCTTTGGAAACTGTT<br>TAACTGGAGTGTCGGAGAGGTAAGTGGAATT<br>CCTAGTGTAGCGGTGAAATGCGTAGATATTA<br>GGAGGAACACCAGTGGCGAAGGCGACTTACT<br>GGACGATAACTGACGTTGAGGCTCGAAAGCG<br>TGGGGAGCAAACAGGATTAGATACCCTGGTA<br>GTCCACGCCGTAAACGATGAATACTAGGTGTT<br>GGGGAGCAAAGCTCTTCGGTGCCGTCGCAAA<br>CGCAGTAAGTATTCCACCTGGGGAGTACGTTC<br>GCAAGAATGAAACTCAAAGGAATTGACGGGG<br>ACCCGCACAAGCGGTGGAGCATGTGGTTTAA<br>TTCGAAGCAACGCGAAGAACCTTACCAGGTC<br>TTGACATCGATCCGACGGGGGAGTAACGTCC<br>CCTTCCCTTCGGGGCGGAGAAGACAGGTGGT |

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| | | | GCATGGTTGTCGTCAGCTCGTGTCGTGAGATG<br>TTGGGTTAAGTCCCGCAACGAGCGCAACCCTT<br>ATTCTAAGTAGCCAGCGGTTCGGCCGGGAAC<br>TCTTGGGAGACTGCCAGGGATAACCTGGAGG<br>AAGGTGGGGATGACGTCAAATCATCATGCCC<br>CTTATGATCTGGGCTACACACGTGCTACAATG<br>GCGTAAACAAAGAGAAGCAAGACCGCGAGGT<br>GGAGCAAATCTCAAAAATAACGTCTCAGTTC<br>GGACTGCAGGCTGCAACTCGCCTGCACGAAG<br>CTGGAATCGCTAGTAATCGCGAATCAGAATG<br>TCGCGGTGAATACGTTCCC |
| | Clostridium symbiosum S10-530 | | >10-<br>530GAAAATGACGGTACCTGACTAAGAAGCCC<br>CGGCTAACTACGTGCCAGCAGCCGCGGTAAT<br>ACGTAGGGGGCAAGCGTTATCCGGATTTACT<br>GGGTGTAAAGGGAGCGTAGACGGTAAAGCAA<br>GTCTGAAGTGAAAGCCCGCGGCTCAACTGCG<br>GGACTGCTTTGGAAACTGTTTAACTGGAGTGT<br>CGGAGAGGTAAGTGGAATTCCTAGTGTAGCG<br>GTGAAATGCGTAGATATTAGGAGGAACACCA<br>GTGGCGAAGGCGACTTACTGGACGATAACTG<br>ACGTTGAGGCTCGAAAGCGTGGGGAGCAAAC<br>AGGATTAGATACCCTGGTAGTCCACGCCGTA<br>AACGATGAATACTAGGTGTTGGGGAGCAAAG<br>CTCTTCGGTGCCGTCGCAAACGCAGTAAGTAT<br>TCCACCTGGGGAGTACGTTCGCAAGAATGAA<br>ACTCAAAGGAATTGACGGGGACCCGCACAAG<br>CGGTGGAGCATGTGGTTTAATTCGAAGCAAC<br>GCGAAGAACCTTACCAGGTCTTGACATCGATC<br>CGACGGGGGAGTAACGTCCCCTTCCCTTCGGG<br>GCGGA |
| | Clostridium symbiosum S10-533 | | >10-533_consensus_sequence 2 reads from 10-<br>533GAACGTATTCACCGCGACATTCTGATTCGC<br>GATTACTAGCGATTCCAGCTTCGTGCAGGCGA<br>GTTGCAGCCTGCAGTCCGAACTGAGACGTTAT<br>TTTTGAGATTTGCTCCACCTCGCGGTCTTGCTT<br>CTCTTTGTTTACGCCATTGTAGCACGTGTGTA<br>GCCCAGATCATAAGGGGCATGATGATTTGAC<br>GTCATCCCCACCTTCCTCCAGGTTATCCCTGG<br>CAGTCTCCCAAGAGTTCCCGGCCGAACCGCTG<br>GCTACTTAGAATAAGGGTTGCGCTCGTTGCGG<br>GACTTAACCCAACATCTCACGACACGAGCTG<br>ACGACAACCATGCACCACCTGTCTTCTCCGCC<br>CCGAAGGGAAGGGGACGTTACTCCCCCGTCG<br>GATCGATGTCAAGACCTGGTAAGGTTCTTCGC<br>GTTGCTTCGAATTAAACCACATGCTCCACCGC<br>TTGTGCGGGTCCCCGTCAATTCCTTTGAGTTT<br>CATTCTTGCGAACGTACTCCCCAGGTGGAATA<br>CTTACTGCGTTTGCGACGGCACCGAAGAGCTT<br>TGCTCCCCAACACCTAGTATTCATCGTTTACG<br>GCGTGGACTACCAGGGTATCTAATCCTGTTTG<br>CTCCCCACGCTTTCGAGCCTCAACGTCAGTTA<br>TCGTCCAGTAAGTCGCCTTCGCCACTGGTGTT<br>CCTCCTAATATCTACGCATTTCACCGCTACAC |

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| | | | TAGGAATTCCACTTACCTCTCCGACACTCCAG<br>TTAAACAGTTTCCAAAGCAGTCCCGCAGTTGA<br>GCCGCGGGCTTTCACTTCAGACTTGCTTTACC<br>GTCTACGCTCCCTTTACACCCAGTAAATCCGG<br>ATAACGCTTGCCCCCTACGTATTACCGCGGCT<br>GCTGGCACGTAGTTAGCCGGGGCTTCTTAG |
| | Clostridium symbiosum S10-537 | | >10-537_consensus_sequence 2 reads from 10-537ACTAAGAAGCCCCGGCTAACTACGTGCCA<br>GCAGCCGCGGTAATACGTAGGGGGCAAGCGT<br>TATCCGGATTTACTGGGTGTAAAGGGAGCGT<br>AGACGGTAAAGCAAGTCTGAAGTGAAAGCCC<br>GCGGCTCAACTGCGGGACTGCTTTGGAAACT<br>GTTTAACTGGAGTGTCGGAGAGGTAAGTGGA<br>ATTCCTAGTGTAGCGGTGAAATGCGTAGATAT<br>TAGGAGGAACACCAGTGGCGAAGGCGACTTA<br>CTGGACGATAACTGACGTTGAGGCTCGAAAG<br>CGTGGGGAGCAAACAGGATTAGATACCCTGG<br>TAGTCCACGCCGTAAACGATGAATACTAGGT<br>GTTGGGGAGCAAAGCTCTTCGGTGCCGTCGC<br>AAACGCAGTAAGTATTCCACCTGGGGAGTAC<br>GTTCGCAAGAATGAAACTCAAAGGAATTGAC<br>GGGGACCCGCACAAGCGGTGGAGCATGTGGT<br>TTAATTCGAAGCAACGCGAAGAACCTTACCA<br>GGTCTTGACATCGATCCGACGGGGGAGTAAC<br>GTCCCCTTCCCTTCGGGGCGGAGAAGACAGG<br>TGGTGCATGGTTGTCGTCAGCTCGTGTCGTGA<br>GATGTTGGGTTAAGTCCCGCAACGAGCGCAA<br>CCCTTATTCTAAGTAGCCAGCGGTTCGGCCGG<br>GAACTCTTGGGAGACTGCCAGGGATAACCTG<br>GAGGAAGGTGGGGATGACGTCAAATCATCAT<br>GCCCCTTATGATCTGGGCTACACACGTGCTAC<br>AATGGCGTAAACAAAGAGAAGCAAGACCGCG<br>AGGTGGAGCAAATCTCAAAAATAACGTCTCA<br>GTTCGGACTGCAGGCTGCAACTCGCCTGCACG<br>AAGCTGGAATCGCTAGTAATCGCGAATCAGA<br>ATGTCGCGGTGAATACGTT |
| | Clostridium symbiosum S10-544 | | >10-544ATGACGGTACCTGACTAAGAAGCCCCGGC<br>TAACTACGTGCCAGCAGCCGCGGTAATACGT<br>AGGGGGCAAGCGTTATCCGGATTTACTGGGT<br>GTAAAGGGAGCGTAGACGGTAAAGCAAGTCT<br>GAAGTGAAAGCCCGCGGCTCAACTGCGGGAC<br>TGCTTTGGAAACTGTTTAACTGGAGTGTCGGA<br>GAGGTAAGTGGAATTCCTAGTGTAGCGGTGA<br>AATGCGTAGATATTAGGAGGAACACCAGTGG<br>CGAAGGCGACTTACTGGACGATAACTGACGT<br>TGAGGCTCGAAAGCGTGGGGAGCAAACAGGA<br>TTAGATACCCTGGTAGTCCACGCCGTAAACGA<br>TGAATACTAGGTGTTGGGGAGCAAAGCTCTTC<br>GGTGCCGTCGCAAACGCAGTAAGTATTCCAC<br>CTGGGGAGTACGTTCGCAAGAATGAAACTCA<br>AAGGAATTGACGGGGACCCGCACAAGCGGTG<br>GAGCATGTGGTTTAATTCGAAGCAACGCGAA<br>GAACCTTACCAGGTCTTGACATCGATCCGACG |

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| | | | GGGGAGTAACGTCCCCTTCCCTTCGGGGCGG<br>AGAAGACAGGTGGTGCATGGTTGTCGTCAGC<br>TCGTGTCGTGAGATGTTGGGTTAAGTCCCGCA<br>ACGAGCGCAACCCTTATTCTAAGTAGCCAGC<br>GGTTCGGCCGGGAACTCTTGGGAGACTGCCA<br>GGGATAACCTG |
| | Clostridium symbiosum S10-547 | | >10-<br>547GGGAAGAAAATGACGGTACCTGACTAAGA<br>AGCCCCGGCTAACTACGTGCCAGCAGCCGCG<br>GTAATACGTAGGGGGCAAGCGTTATCCGGAT<br>TTACTGGGTGTAAAGGGAGCGTAGACGGTAA<br>AGCAAGTCTGAAGTGAAAGCCCGCGGCTCAA<br>CTGCGGGACTGCTTTGGAAACTGTTTAACTGG<br>AGTGTCGGAGAGGTAAGTGGAATTCCTAGTG<br>TAGCGGTGAAATGCGTAGATATTAGGAGGAA<br>CACCAGTGGCGAAGGCGACTTACTGGACGAT<br>AACTGACGTTGAGGCTCGAAAGCGTGGGGAG<br>CAAACAGGATTAGATACCCTGGTAGTCCACG<br>CCGTAAACGATGAATACTAGGTGTTGGGGAG<br>CAAAGCTCTTCGGTGCCGTCGCAAACGCAGT<br>AAGTATTCCACCTGGGGAGTACGTTCGCAAG<br>AATGAAACTCAAAGGAATTGACGGGGACCCG<br>CACAAGCGGTGGAGCATGTGGTTTAATTCGA<br>AGCAACGCGAAGAACCTTACCAGGTCTTGAC<br>ATCGATCCGACGGGGGAGTAACGTCCCCTTCC<br>CTTCGGGGCGGAGAAGACAGGTGGTGCATGG<br>TTGTCGTCAGCTCGTGTCGTGAGATGTTGGGT<br>TAAGTCCCGCAACGAGCGCAACCCTTATTCTA<br>AGTAGCCAGCGGTTCGGCCGGGAACTC |
| | Clostridium symbiosum S10-548 | | >10-548_consensus_sequence 2 reads from 10-<br>548AAGAAGCCCCGGCTAACTACGTGCCAGCA<br>GCCGCGGTAATACGTAGGGGGCAAGCGTTAT<br>CCGGATTTACTGGGTGTAAAGGGAGCGTAGA<br>CGGTAAAGCAAGTCTGAAGTGAAAGCCCGCG<br>GCTCAACTGCGGGACTGCTTTGGAAACTGTTT<br>AACTGGAGTGTCGGAGAGGTAAGTGGAATTC<br>CTAGTGTAGCGGTGAAATGCGTAGATATTAG<br>GAGGAACACCAGTGGCGAAGGCGACTTACTG<br>GACGATAACTGACGTTGAGGCTCGAAAGCGT<br>GGGGAGCAAACAGGATTAGATACCCTGGTAG<br>TCCACGCCGTAAACGATGAATACTAGGTGTTG<br>GGGAGCAAAGCTCTTCGGTGCCGTCGCAAAC<br>GCAGTAAGTATTCCACCTGGGGAGTACGTTCG<br>CAAGAATGAAACTCAAAGGAATTGACGGGGA<br>CCCGCACAAGCGGTGGAGCATGTGGTTTAATT<br>CGAAGCAACGCGAAGAACCTTACCAGGTCTT<br>GACATCGATCCGACGGGGGAGTAACGTCCCC<br>TTCCCTTCGGGGCGGAGAAGACAGGTGGTGC<br>ATGGTTGTCGTCAGCTCGTGTCGTGAGATGTT<br>GGGTTAAGTCCCGCAACGAGCGCAACCCTTA<br>TTCTAAGTAGCCAGCGGTTCGGCCGGGAACTC<br>TTGGGAGACTGCCAGGGATAACCTGGAGGAA<br>GGTGGGGATGACGTCAAATCATCATGCCCCTT<br>ATGATCTGGGCTACACACGTGCTACAATGGC |

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| | | | GTAAACAAAGAGAAGCAAGACCGCGAGGTG<br>GAGCAAATCTCAAAAATAACGTCTCAGTTCG<br>GACTGCAGGCTGCAACTCGCCTGCACGAAGC<br>TGGAATCGCTAGTAATCGCGAATCAGAATGT<br>CGCGGTGAATACGTT |
| | Clostridium sp. S7-203 | | >S7-203-357F<br>TGATGCAGCGACGCCGCGTGAGTGAAGAAGT<br>ATTTCGGTATGTAAAGCTCTATCAGCAGGGAA<br>GAAAATGACGGTACCTGACTAAGAAGCCCCG<br>GCTAACTACGTGCCAGCAGCCGCGGTAATAC<br>GTAGGGGGCAAGCGTTATCCGGATTTACTGG<br>GTGTAAAGGGAGCGTAGACGGTAAAGCAAGT<br>CTGAAGTGAAAGCCCGCGGCTCAACTGCGGG<br>ACTGCTTTGGAAACTGTTTAACTGGAGTGTCG<br>GAGAGGTAAGTGGAATTCCTAGTGTAGCGGT<br>GAAATGCGTAGATATTAGGAGGAACACCAGT<br>GGCGAAGGCGACTTACTGGACGATAACTGAC<br>GTTGAGGCTCGAAAGCGTGGGGAGCAAACAG<br>GATTAGATACCCTGGTAGTCCACGCCGTAAAC<br>GATGAATACTAGGTGTTGGGGAGCAAAGCTC<br>TTCGGTGCCGTCGCAAACGCAGTAAGTATTCC<br>ACCTGGGGAGTACGTTCGCAAGAATGAAACT<br>CAAAGGAATTGACGGGGACCCGCACAAGCGG<br>TGGAGCATGTGGTTTAATTCGAAGCAACGCG<br>AAGAACCTTACCAGGTCTTGACATCGATCCGA<br>CGGGGGAGTAACGTCCCCTTCCCTTCGGGGCG<br>GAGAAGACAGGTGGTGCATGGTTGTCGTCAG<br>CTCGTGTCGTGAGATGTTGGGTTAAGTCCCGC<br>AACGAGCGCAACCCTTATTCTAAGTAGCCAG<br>CGGTTCGGCCGGGAACTCTTGGGAGACTGCC<br>AGGGATAACCTGGAGGAAGGTGGGGATGACG<br>TCAAATCATCATGCCCCT |
| | Clostridium sp. 36A7-1014 | | GCCGCGTGAGTGAAGAAGTATTTCGGTATGT<br>AAAGCTCTATCAGCAGGGAAGAAAATGACGG<br>TACCTGACTAAGAAGCCCCGGCTAACTACGT<br>GCCAGCAGCCGCGGTAATACGTAGGGGGCAA<br>GCGTTATCCGGATTTACTGGGTGTAAAGGGA<br>GCGTAGACGGTAAAGCAAGTCTGAAGTGAAA<br>GCCCGCGGCTCAACTGCGGGACTGCTTTGGA<br>AACTGTTTAACTGGAGTGTCGGAGAGGTAAG<br>TGGAATTCCTAGTGTAGCGGTGAAATGCGTA<br>GATATTAGGAGGAACACCAGTGGCGAAGGCG<br>ACTTACTGGACGATAACTGACGTTGAGGCTCG<br>AAAGCGTGGGGAGCAAACAGGATTAGATACC<br>CTGGTAGTCCACGCCGTAAACGATGAATACT<br>AGGTGTTGGGGAGCAAAGCTCTTCGGTGCCG<br>TCGCAAACGCAGTAAGTATTCCACCTGGGGA<br>GTACGTTCGCAAGAATGAAACTCAAAGGAAT<br>TGACGGGGACCCGCACAAGCGGTGGAGCATG<br>TGGTTTAATTCGAAGCAACGCGAAGAACCTT<br>ACCAGGTCTTGACATCGATCCGACGGGGGAG<br>TAACGTCCCCTTCCCTTCGGGGCGGAGAAGAC<br>AGGTGGTGCATGGTTGTCGTCAGCTCGTGTCG |

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| | | | TGAGATGTTGGGTTAAGTCCCGCAACGAGCG<br>CAACCCTTATTCTAAGTAGCCAGCGGTTC |
| | Clostridium sp. S4-31 | | >4-31-contig<br>GCCTGATGCAGCGACGCCGCGTGAGTGAAGA<br>AGTATTTCGGTATGTAAAGCTCTATCAGCAGG<br>GAAGAAAATGACGGTACCTGACTAAGAAGCC<br>CCGGCTAACTACGTGCCAGCAGCCGCGGTAA<br>TACGTAGGGGGCAAGCGTTATCCGGATTTACT<br>GGGTGTAAAGGGAGCGTAGACGGTAAAGCAA<br>GTCTGAAGTGAAAGCCCGCGGCTCAACTGCG<br>GGACTGCTTTGGAAACTGTTTAACTGGAGTGT<br>CGGAGAGGTAAGTGGAATTCCTAGTGTAGCG<br>GTGAAATGCGTAGATATTAGGAGGAACACCA<br>GTGGCGAAGGCGACTTACTGGACGATAACTG<br>ACGTTGAGGCTCGAAAGCGTGGGGAGCAAAC<br>AGGATTAGATACCCTGGTAGTCCACGCCGTA<br>AACGATGAATACTAGGTGTTGGGGAGCAAAG<br>CTCTTCGGTGCCGTCGCAAACGCAGTAAGTAT<br>TCCACCTGGGGAGTACGTTCGCAAGAATGAA<br>ACTCAAAGGAATTGACGGGGACCCGCACAAG<br>CGGTGGAGCATGTGGTTTAATTCGAAGCAAC<br>GCGAAGAACCTTACCAGGTCTTGACATCGATC<br>CGACGGGGGAGTAACGTCCCCTTCCCTTCGGG<br>GCGGAGAAGACAGGTGGTGCATGGTTGTCGT<br>CAGCTCGTGTCGTGAGATGTTGGGTTAAGTCC<br>CGCAACGAGCGCAACCCTTATTCTAAGTAGCC<br>AGCGGTTCGGCCGGGAACTCTTGGGAGACTG<br>CCAGGGATAACCTGGAGGAAGGTGGGGATGA<br>CGTCAAATCATCATGCCCCTTATGATCTGGGC<br>TACACACGTGCTACAATGGCGTAAACAAAGA<br>GAAGCAAGACCGCGAGGTGGAGCAAATCTCA<br>AAAATAACGTCTCAGTTCGGACTGCAGGCTG<br>CAACTCGCCTGCACGAAGCTGGAATCGCTAG<br>TAATCGCGAATCAGAATGTCGCGGTGAATAC<br>GTTCCCGGGTCTTGTACACACCGCCCGTCACA<br>CCATGGGAGTCAGTAACGCCCGAAGTCAGTG<br>ACCCAACCGCAAGGAGGGAGCTG |
| | Clostridium sp. S210-133 | | >210-133-Contig<br>TTCGGTATGTAAAGCTCTATCAGCAGGGAAG<br>AAAATGACGGTACCTGACTAAGAAGCCCCGG<br>CTAACTACGTGCCAGCAGCCGCGGTAATACG<br>TAGGGGGCAAGCGTTATCCGGATTTACTGGGT<br>GTAAAGGGAGCGTAGACGGTAAAGCAAGTCT<br>GAAGTGAAAGCCCGCGGCTCAACTGCGGGAC<br>TGCTTTGGAAACTGTTTAACTGGAGTGTCGGA<br>GAGGTAAGTGGAATTCCTAGTGTAGCGGTGA<br>AATGCGTAGATATTAGGAGGAACACCAGTGG<br>CGAAGGCGACTTACTGGACGATAACTGACGT<br>TGAGGCTCGAAAGCGTGGGGAGCAAACAGGA<br>TTAGATACCCTGGTAGTCCACGCCGTAAACGA<br>TGAATACTAGGTGTTGGGGAGCAAAGCTCTTC<br>GGTGCCGTCGCAAACGCAGTAAGTATTCCAC<br>CTGGGGAGTACGTTCGCAAGAATGAAACTCA<br>AAGGAATTGACGGGGACCCGCACAAGCGGTG |

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| | | | GAGCATGTGGTTTAATTCGAAGCAACGCGAA<br>GAACCTTACCAGGTCTTGACATCGATCCGACG<br>GGGGAGTAACGTCCCCTTCCCTTCGGGGCGG<br>AGAAGACAGGTGGTGCATGGTTGTCGTCAGC<br>TCGTGTCGTGAGATGTTGGGTTAAGTCCCGCA<br>ACGAGCGCAACCCTTATTCTAAGTAGCCAGC<br>GGTTCGGCCGGGAACTCTTGGGAGACTGCCA<br>GGGATAACCTGGAGGAAGGTGGGGGATGACG<br>TCAAATCATCATGCCCCTTATGATCTGGGCTA<br>CACACGTGCTACAATGGCGTAAACAAAGAGA<br>AGCAAGACCGCGAGGTGGAGCAAATCTCAAA<br>AATAACGTCTCAGTTCGGACTGCAGGCTGCA<br>ACTCGCCTGCACGAAGCTGGAATCGCTAGTA<br>ATCGCGAATCAGAATGTCGCGGTGAATACGT<br>TCCCGGGTCTTGTACACACCGCCCGTCACACC<br>ATGGGAGTCAGTAACGCCCGAAGTCAGTGAC<br>CCA |
| | Clostridium symbiosum S10-534 | | >10-534_consensus_sequence 2 reads from 10-534ACTAAGAAGCCCCGGCTAACTACGTGCCA<br>GCAGCCGCGGTAATACGTAGGGGGCAAGCGT<br>TATCCGGATTTACTGGGTGTAAAGGGAGCGT<br>AGACGGTAAAGCAAGTCTGAAGTGAAAGCCC<br>GCGGCTCAACTGCGGGACTGCTTTGGAAACT<br>GTTTAACTGGAGTGTCGGAGAGGTAAAGTGG<br>AATTCCTAGTGTAGCGGTGAAATGCGTAGAT<br>ATTAGGAGGAACACCAGTGGCGAAGGCGACT<br>TACTGGACGATAACTGACGTTGAGGCTCGAA<br>AGCGTGGGGAGCAAACAGGATTAGATACCCT<br>GGTAGTCCACGCCGTAAACGATGAATACTAG<br>GTGTTGGGGAGCAAAGCTCTTCGGTGCCGTCG<br>CAAACGCAGTAAGTATTCCACCTGGGGAGTA<br>CGTTCGCAAGAATGAAACTCAAAGGAATTGA<br>CGGGGACCCGCACAAGCGGTGGAGCATGTGG<br>TTTAATTCGAAGCAACGCGAAGAACCTTACC<br>AGGTCTTGACATCGATCCGACGGGGGAGTAA<br>CGTCCCCTTCCCTTCGGGGCGGAGAAGACAG<br>GTGGTGCATGGTTGTCGTCAGCTCGTGTCGTG<br>AGATGTTGGGTTAAGTCCCGCAACGAGCGCA<br>ACCCTTATTCTAAGTAGCCAGCGGTTCGGCCG<br>GGAACTCTTGGGAGACTGCCAGGGATAACCT<br>GGAGGAAGGTGGGGATGACGTCAAATCATCA<br>TGCCCCTTATGATCTGGGCTACACACGTGCTA<br>CAATGGCGTAAACAAAGAGAAGCAAGACCGC<br>GAGGTGGAGCAAATCTCAAAAATAACGTCTC<br>AGTTCGGACTGCAGGCTGCAACTCGCCTGCAC<br>GAAGCTGGAATCGCTAGTAATCGCGAATCAG<br>AATGTCGCGGTGAATACGTTCC |
| | Clostridium sp. S4-44 | | >4-44-contig<br>CTGATGCAGCGACGCCGCGTGAGTGAAGAAG<br>TAGTTTCGGTATGTAAAGCTCTATCAGCAGGG<br>AAGAAAATGACGGTACCTGACTAAGAAGCCC<br>CGGCTAACTACGTGCCAGCAGCCGCGGTAAT<br>ACGTAGGGGGCAAGCGTTATCCGGATTTACT<br>GGGTGTAAAGGGAGCGTAGACGGTAAAGCAA |

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| | | | GTCTGAAGTGAAAGCCCGCGGCTCAACTGCG<br>GGACTGCTTTGGAAACTGTTTAACTGGAGTGT<br>CGGAGAGGTAAGTGGAATTCCTAGTGTAGCG<br>GTGAAATGCGTAGATATTAGGAGGAACACCA<br>GTGGCGAAGGCGACTTACTGGACGATAACTG<br>ACGTTGAGGCTCGAAAGCGTGGGGAGCAAAC<br>AGGATTAGATACCCTGGTAGTCCACGCCGTA<br>AACGATGAATACTAGGTGTTGGGGAGCAAAG<br>CTCTTCGGTGCCGTCGCAAACGCAGTAAGTAT<br>TCCACCTGGGGAGTACGTTCGCAAGAATGAA<br>ACTCAAAGGAATTGACGGGGACCCGCACAAG<br>CGGTGGAGCATGTGGTTTAATTCGAAGCAAC<br>GCGAAGAACCTTACCAGGTCTTGACATCGATC<br>CGACGGGGGAGTAACGTCCCCTTCCCTTCGGG<br>GCGGAGAAGACAGGTGGTGCATGGTTGTCGT<br>CAGCTCGTGTCGTGAGATGTTGGGTTAAGTCC<br>CGCAACGAGCGCAACCCTTATTCTAAGTAGCC<br>AGCGGTTCGGCCGGGAACTCTTGGGAGACTG<br>CCAGGGATAACCTGGAGGAAGGTGGGGGATG<br>ACGTCAAATCATCATGCCCCTTATGATCTGGG<br>CTACACACGTGCTACAATGGCGTAAACAAAG<br>AGAAGCAAGACCGCGAGGTGGAGCAAATCTC<br>AAAAATAACGTCTCAGTTCGGACTGCAGGCT<br>GCAACTCGCCTGCACGAAGCTGGAATCGCTA<br>GTAATCGCGAATCAGAATGTCGCGGTGAATA<br>CGTTCCCGGGTCTTGTACACACCGCCCGTCAC<br>ACCATGGGAGTCAGTAACGCCCGAAGTCAGT<br>GACCCAACCGCAAGGAGGGAGCTGCCGA |
| | Hungatella hathewayi or [Clostridium] hathewayi 34D2-1004 | | GAAGTATTTCGGTATGTAAAGCTCTATCAGCA<br>GGGAAGAAAATGACGGTACCTGACTAAGAAG<br>CCCCGGCTAACTACGTGCCAGCAGCCGCGGT<br>AATACGTAGGGGGCAAGCGTTATCCGGATTT<br>ACTGGGTGTAAAGGGAGCGTAGACGGTTTAG<br>CAAGTCTGAAGTGAAAGCCCGGGGCTCAACC<br>CCGGTACTGCTTTGGAAACTGTTAGACTTGAG<br>TGCAGGAGAGGTAAGTGGAATTCCTAGTGTA<br>GCGGTGAAATGCGTAGATATTAGGAGGAACA<br>CCAGTGGCGAAGGCGGCTTACTGGACTGTAA<br>CTGACGTTGAGGCTCGAAAGCGTGGGGAGCA<br>AACAGGATTAGATACCCTGGTAGTCCACGCC<br>GTAAACGATGAATACTAGGTGTCGGGGGGCA<br>AAGCCCTTCGGTGCCGCCGCAAACGCAATAA<br>GTATTCCACCTGGGGAGTACGTTCGCAAGAAT<br>GAAACTCAAAGGAATTGACGGGGACCCGCAC<br>AAGCGGTGGAGCATGTGGTTTAATTCGAAGC<br>AACGCGAAGAACCTTACCAAGTCTTGACATC |
| | Hungatella hathewayi or [Clostridium] hathewayi 34H6-1004 | | TTCGGTATGTAAAGCTCTATCAGCAGGGAAG<br>AAAATGACGGTACCTGACTAAGAAGCCCCGG<br>CTAACTACGTGCCAGCAGCCGCGGTAATACG<br>TAGGGGGCAAGCGTTATCCGGATTTACTGGGT<br>GTAAAGGGAGCGTAGACGGTTTAGCAAGTCT<br>GAAGTGAAAGCCCGGGGCTCAACCCCGGTAC<br>TGCTTTGGAAACTGTTAGACTTGAGTGCAGGA<br>GAGGTAAGTGGAATTCCTAGTGTAGCGGTGA |

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| | | | AATGCGTAGATATTAGGAGGAACACCAGTGG<br>CGAAGGCGGCTTACTGGACTGTAACTGACGTT<br>GAGGCTCGAAAGCGTGGGGAGCAAACAGGAT<br>TAGATACCCTGGTAGTCCACGCCGTAAACGAT<br>GAATACTAGGTGTCGGGGGGCAAAGCCCTTC<br>GGTGCCGCCGCAAACGCAATAAGTATTCCAC<br>CTGGGGAGTACGTTCGCAAGAATGAAACTCA<br>AAGGAATTGACGGGGACCCGCACAAGCGGTG<br>GAGCATGTGGTTTAATTCGAAGCAACGCGAA<br>GAACCTTACCAAGTCTTGACATCCCA |
| | Hungatella effluvia 36B10-1014 | | GCCGCGTGAGTGAAGAAGTATTTCGGTATGT<br>AAAGCTCTATCAGCAGGGAAGAAAATGACGG<br>TACCTGACTAAGAAGCCCCGGCTAACTACGT<br>GCCAGCAGCCGCGGTAATACGTAGGGGGCAA<br>GCGTTATCCGGATTTACTGGGTGTAAAGGGA<br>GCGTAGACGGTTAAGCAAGTCTGAAGTGAAA<br>GCCCGGGGCTCAACCCCGGTACTGCTTTGGAA<br>ACTGTTTGACTTGAGTGCAGGAGAGGTAAGT<br>GGAATTCCTAGTGTAGCGGTGAAATGCGTAG<br>ATATTAGGAGGAACACCAGTGGCGAAGGCGG<br>CTTACTGGACTGTAACTGACGTTGAGGCTCGA<br>AAGCGTGGGGAGCAAACAGGATTAGATACCC<br>TGGTAGTCCACGCCGTAAACGATGAATACTA<br>GGTGTCGGGGGACAAAGTCCTTCGGTGCCGC<br>CGCTAACGCAATAAGTATTCCACCTGGGGAG<br>TACGTTCGCAAGAATGAAACTCAAAGGAATT<br>GACGGGGACCCGCACAAGCGGTGGAGCATGT<br>GGTTTAATTCGAAGCAACGCGAAGAACCTTA<br>CCAAGTCTTGACATCCCATTGAAAATCATTTA<br>ACCG |
| | Hungatella effluvia 36C4-1014 | | GCCGCGTGAGTGAAGAAGTATTTCGGTATGT<br>AAAGCTCTATCAGCAGGGAAGAAAATGACGG<br>TACCTGACTAAGAAGCCCCGGCTAACTACGT<br>GCCAGCAGCCGCGGTAATACGTAGGGGGCAA<br>GCGTTATCCGGATTTACTGGGTGTAAAGGGA<br>GCGTAGACGGTTAAGCAAGTCTGAAGTGAAA<br>GCCCGGGGCTCAACCCCGGTACTGCTTTGGAA<br>ACTGTTTGACTTGAGTGCAGGAGAGGTAAGT<br>GGAATTCCTAGTGTAGCGGTGAAATGCGTAG<br>ATATTAGGAGGAACACCAGTGGCGAAGGCGG<br>CTTACTGGACTGTAACTGACGTTGAGGCTCGA<br>AAGCGTGGGGAGCAAACAGGATTAGATACCC<br>TGGTAGTCCACGCCGTAAACGATGAATACTA<br>GGTGTCGGGGGACAAAGTCCTTCGGTGCCGC<br>CGCTAACGCAATAAGTATTCCACCTGGGGAG<br>TACGTTCGCAAGAATGAAACTCAAAGGAATT<br>GACGGGGACCCGCACAAGCGGTGGAGCATGT<br>GGTTTAATTCGAAGCAACGCGAAGAACCTTA<br>CCAAGTCTTGACATCCCATTGAAAA |
| | Hungatella effluvii 36F7- 1014 | | GCCGCGTGAGTGAAGAAGTATTTCGGTATGT<br>AAAGCTCTATCAGCAGGGAAGAAAATGACGG<br>TACCTGACTAAGAAGCCCCGGCTAACTACGT<br>GCCAGCAGCCGCGGTAATACGTAGGGGGCAA |

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| | | | GCGTTATCCGGATTTACTGGGTGTAAAGGGA<br>GCGTAGACGGTTAAGCAAGTCTGAAGTGAAA<br>GCCCGGGGCTCAACCCCGGTACTGCTTTGGAA<br>ACTGTTTGACTTGAGTGCAGGAGAGGTAAGT<br>GGAATTCCTAGTGTAGCGGTGAAATGCGTAG<br>ATATTAGGAGGAACACCAGTGGCGAAGGCGG<br>CTTACTGGACTGTAACTGACGTTGAGGCTCGA<br>AAGCGTGGGGAGCAAACAGGATTAGATACCC<br>TGGTAGTCCACGCCGTAAACGATGAATACTA<br>GGTGTCGGGGGACAAAGTCCTTCGGTGCCGC<br>CGCTAACGCAATAAGTATTCCACCTGGGGAG<br>TACGTTCGCAAGAATGAAACTCAAAGGAATT<br>GACGGGGACCCGCACAAGCGGTGGAGCATGT<br>GGTTTAATTCGAAGCAACGCGAAGAACCTTA<br>CCAAGTCTTGACATCCCATTGAA |
| | Lachnospiraceae sp or [Clostridium] Citroniae 39A7-1014 | | GACGGTACCTGACTAAGAAGCCCCGGCTAAC<br>TACGTGCCAGCAGCCGCGGTAATACGTAGGG<br>GGCAAGCGTTATCCGGATTTACTGGGTGTAAA<br>GGGAGCGTAGACGGCGAAGCAAGTCTGGAGT<br>GAAAACCCAGGGCTCAACCCTGGGACTGCTT<br>TGGAAACTGTTTTGCTAGAGTGTCGGAGAGGT<br>AAGTGGAATTCCTAGTGTAGCGGTGAAATGC<br>GTAGATATTAGGAGGAACACCAGTGGCGAAG<br>GCGGCTTACTGGACGATAACTGACGTTGAGG<br>CTCGAAAGCGTGGGGAGCAAACAGGATTAGA<br>TACCCTGGTAGTCCACGCCGTAAACGATGAAT<br>GCTAGGTGTTGGGGGG |
| | Lachnospiraceae sp or [Clostridium] citroniae 39A8-1014 | | GACGGTACCTGACTAAGAAGCCCCGGCTAAC<br>TACGTGCCAGCAGCCGCGGTAATACGTAGGG<br>GGCAAGCGTTATCCGGATTTACTGGGTGTAAA<br>GGGAGCGTAGACGGCGAAGCAAGTCTGGAGT<br>GAAAACCCAGGGCTCAACCCTGGGACTGCTT<br>TGGAAACTGTTTTGCTAGAGTGTCGGAGAGGT<br>AAGTGGAATTCCTAGTGTAGCGGTGAAATGC<br>GTAGATATTAGGAGGAACACCAGTGGCGAAG<br>GCGGCTTACTGGACGATAACTGACGTTGAGG<br>CTCGAAAGCGTGGGGAGCAAACAGGATTAGA<br>TACCCTGGTAGTCCACGCCGTAAACGATGAAT<br>GCTAGGTGTTGGGGGG |
| | Lachnospiraceae sp or [Clostridium] citroniae 36A6-1014 | | GCCGCGTGAGTGAAGAAGTATTTCGGTATGT<br>AAAGCTCTATCAGCAGGGAAGAAACTGACGG<br>TACCTGACTAAGAAGCCCCGGCTAACTACGT<br>GCCAGCAGCCGCGGTAATACGTAGGGGGCAA<br>GCGTTATCCGGATTTACTGGGTGTAAAGGGA<br>GCGTAGACGGCGAAGCAAGTCTGGAGTGAAA<br>ACCCAGGGCTCAACCCTGGGACTGCTTTGGA<br>AACTGTTTTGCTAGAGTGTCGGAGAGGTAAGT<br>GGAATTCCTAGTGTAGCGGTGAAATGCGTAG<br>ATATTAGGAGGAACACCAGTGGCGAAGGCGG<br>CTTACTGGACGATAACTGACGTTGAGGCTCGA<br>AAGCGTGGGGAGCAAACAGGATTAGATACCC |

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| | | | TGGTAGTCCACGCCGTAAACGATGAATGCTA<br>GGTGTTGGGGGGCAAAGCCCTTC |
| | Lachnospiraceae sp or [Clostridium] sp 36C9-1014 | | GAAGTATTTCGGTATGTAAACTTCTATCAGCA<br>GGGAAGAAAATGACGGTACCTGACTAAGAAG<br>CCCCGGCTAACTACGTGCCAGCAGCCGCGGT<br>AATACGTAGGGGGCAAGCGTTATCCGGATTT<br>ACTGGGTGTAAAGGGAGCGTAGACGGCAGTG<br>CAAGTCTGAAGTGAAAGCCCGGGGCTCAACC<br>CCGGGACTGCTTTGGAAACTGTGCAGCTAGA<br>GTGTCGGAGAGGCAAGCGGAATTCCTAGTGT<br>AGCGGTGAAATGCGTAGATATTAGGAGGAAC<br>ACCAGTGGCGAAGGCGGCTTGCTGGACGATG<br>ACTGACGTTGAGGCTCGAAAGCGTGGGGAGC<br>AAACAGGATTAGATACCCTGGTAGTCCACGC<br>CGTAAACGATGACTACTAGGTGTCGGGGAGC<br>AAAGCTCTTCGGTGCCGCAGCCAACGCAATA<br>AGTAGTCCACCTGGGGAGTACGTTCGCAAGA<br>ATGAAACTCAAAGGAATTGACGGGGACCCGC<br>ACAAGCGGTGGAGCATGTGGTTTAATTCGAA<br>GCAACGCGAAGAACCTTACCTGCTCTTGACAT<br>CCCTCTGACCG |
| | [Clostridium] bolteae S10-21 | | >S10-121-contig<br>GATGCAGCGACGCCGCGTGAGTGAAGAAGTA<br>TTTCGGTATGTAAAGCTCTATCAGCAGGGAAG<br>AAAATGACGGTACCTGACTAAGAAGCCCCGG<br>CTAACTACGTGCCAGCAGCCGCGGTAATACG<br>TAGGGGGCAAGCGTTATCCGGATTTACTGGGT<br>GTAAAGGGAGCGTAGACGGCGAAGCAAGTCT<br>GAAGTGAAAACCCAGGGCTCAACCCTGGGAC<br>TGCTTTGGAAACTGTTTTGCTAGAGTGTCGGA<br>GAGGTAAGTGGAATTCCTAGTGTAGCGGTGA<br>AATGCGTAGATATTAGGAGGAACACCAGTGG<br>CGAAGGCGGCTTACTGGACGATAACTGACGT<br>TGAGGCTCGAAAGCGTGGGGAGCAAACAGGA<br>TTAGATACCCTGGTAGTCCACGCCGTAAACGA<br>TGAATGCTAGGTGTTGGGGGGCAAAGCCCTT<br>CGGTGCCGTCGCAAACGCAGTAAGCATTCCA<br>CCTGGGGAGTACGTTCGCAAGAATGAAACTC<br>AAAGGAATTGACGGGGACCCGCACAAGCGGT<br>GGAGCATGTGGTTTAATTCGAAGCAACGCGA<br>AGAACCTTACCAAGTCTTGACATCCTCTTGAC<br>CGGCGTGTAACGGCGCCTTCCCTTCGGGGCAG<br>GAGAGACAGGTGGTGCATGGTTGTCGTCAGC<br>TCGTGTCGTGAGATGTTGGGTTAAGTCCCGCA<br>ACGAGCGCAACCCTTATCCTTAGTAGCCAGCA<br>GGTAAAGCTGGGCACTCTAGGGAGACTGCCA<br>GGGATAACCTGGAGGAAGGTGGGGATGACGT<br>CAAATCATCATGCCCCTTATGATTTGGGCTAC<br>ACACGTGCTACAATGGCGTAAACAAAGGGAA<br>GCAAGACAGTGATGTGGAGCAAATCCCAAAA<br>ATAACGTCCCAGTTCGGACTGTAGTCTGCAAC<br>CCGACTACACGAAGCTGGAATCGCTAGTAAT<br>CGCGAATCAGAATGTCGCGGTGAATACGTTC<br>CCGGGTCTTGTACACACCGCCCGTCACACCAT |

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| | | | GGGAGTCAGCAACGCCCGAAGTCAGTGACCC<br>AACTCGCAAGAGAGGG |
| | Ruminococcus gnavus Strain A | PTA-126695 | CCTTAGCGGTTGGGTCACTGACTTCGGGCGTT<br>ACTGACTCCCATGGTGTGACGGGCGGTGTGTA<br>CAAGACCCGGGAACGTATTCACCGCGACATT<br>CTGATTCGCGATTACTAGCGATTCCAGCTTCA<br>TGTAGTCGAGTTGCAGACTACAATCCGAACTG<br>AGACGTTATTTTTGGGATTTGCTCCCCCTCGC<br>GGGCTCGCTTCCCTTTGTTTACGCCATTGTAG<br>CACGTGTGTAGCCCTGGTCATAAGGGGCATG<br>ATGATTTGACGTCATCCCCACCTTCCTCCAGG<br>TTATCCCTGGCAGTCTCTCTAGAGTGCCCATC<br>CTAAATGCTGGCTACTAAAGATAGGGGTTGC<br>GCTCGTTGCGGGACTTAACCCAACATCTCACG<br>ACACGAGCTGACGACAACCATGCACCACCTG<br>TCTCCTCTGTCCCGAAGGAAAGCTCCGATTAA<br>AGAGCGGTCAGAGGGATGTCAAGACCAGGTA<br>AGGTTCTTCGCGTTGCTTCGAATTAAACCACA<br>TGCTCCACCGCTTGTGCGGGTCCCCGTCAATT<br>CCTTTGAGTTTCATTCTTGCGAACGTACTCCC<br>CAGGTGGAATACTTATTGCGTTTGCTGCGGCA<br>CCGAATGGCTTTGCCACCCGACACCTAGTATT<br>CATCGTTTACGGCGTGGACTACCAGGGTATCT<br>AATCCTGTTTGCTCCCCACGCTTTCGAGCCTC<br>AACGTCAGTCATCGTCCAGAAAGCCGCCTTCG<br>CCACTGGTGTTCCTCCTAATATCTACGCATTT<br>CACCGCTACACTAGGAATTCCGCTTTCCTCTC<br>CGACACTCTAGCCTGACAGTTCCAAATGCAGT |
| | Tyzzerella nexilis Strain A | | > T. nexilis S10-231 consensus sequence<br>GGCTAAATACGTGCCAGCAGCCGCGGTAATA<br>CGTATGGTGCAAGCGTTATCCGGATTTACTGG<br>GTGTAAAGGGAGCGTAGACGGTTGTGTAAGT<br>CTGATGTGAAAGCCCGGGGCTCAACCCCGGG<br>ACTGCATTGGAAACTATGTAACTAGAGTGTCG<br>GAGAGGTAAGCGGAATTCCTAGTGTAGCGGT<br>GAAATGCGTAGATATTAGGAGGAACACCAGT<br>GGCGAAGGCGGCTTACTGGACGATCACTGAC<br>GTTGAGGCTCGAAAGCGTGGGGAGCAAACAG<br>GATTAGATACCCTGGTAGTCCACGCCGTAAAC<br>GATGACTACTAGGTGTCGGGGAGCAAAGCTC<br>TTCGGTGCCGCAGCAAACGCAATAAGTAGTC<br>CACCTGGGGAGTACGTTCGCAAGAATGAAAC<br>TCAAAGGAATTGACGGGGACCCGCACAAGCG<br>GTGGAGCATGTGGTTTAATTCGAAGCAACGC<br>GAAGAACCTTACCTGGTCTTGACATCCCTCTG<br>ACCGCTCTTTAATCGGAGTTTTCCTTCGGGAC<br>AGAGGAGACAGGTGGTGCATGGTTGTCGTCA<br>GCTCGTGTCGTGAGATGTTGGGTTAAGTCCCG<br>CAACGAGCGCAACCCCTATCTTCAGTAGCCA<br>GCATTTAAGGTGGGCACTCTGGAGAGACTGC<br>CAGGGATAACCTGGAGGAAGGTGGGGATGAC<br>GTCAAATCATCATGCCCCTTATGACCAGGGCT<br>ACACACGTGCTACAATGGCGTAAACAAAGGG |

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| | | | AAGCGAACCTGTGAGGGGAAGCAAATCTCAA<br>AAATAACGTCTCAGTTCGGATTGTAGTCTGCA<br>ACTCGACTACATGAAGCTGGAATCGCTAGTA<br>ATCGCGAATCAGCATGTCGCGGTGAATACGTT<br>CCCGGGTCTTGTACACACCGCCCGTC |
| | Veillonella tobetsuensis | | >S11-19-357F<br>AGCAACGCCGCGTGAGTGATGACGGCCTTCG<br>GGTTGTAAAGCTCTGTTAATCGGGACGAAAG<br>GCCTTCTTGCGAATAGTTAGAAGGATTGACGG<br>TACCGGAATAGAAAGCCACGGCTAACTACGT<br>GCCAGCAGCCGCGGTAATACGTAGGTGGCAA<br>GCGTTGTCCGGAATTATTGGGCGTAAAGCGC<br>GCGCAGGCGGATCGGTCAGTCTGTCTTAAAA<br>GTTCGGGGCTTAACCCCGTGAGGGGATGGAA<br>ACTGCTGATCTAGAGTATCGGAGAGGAAAGT<br>GGAATTCCTAGTGTAGCGGTGAAATGCGTAG<br>ATATTAGGAAGAACACCAGTGGCGAAGGCGA<br>CTTTCTGGACGAAAACTGACGCTGAGGCGCG<br>AAAGCCAGGGGAGCGAACGGGATTAGATACC<br>CCGGTAGTCCTGGCCGTAAACGATGGGTACT<br>AGGTGTAGGAGGTATCGACCCCTTCTGTGCCG<br>GAGTTAACGCAATAAGTACCCCGCCTGGGGA<br>GTACGACCGCAAGGTTGAAACTCAAAGGAAT<br>TGACGGGGGCCCGCACAAGCGGTGGAGTATG<br>TGGTTTAATTCGACGCAACGCGAAGAACCTTA<br>CCAGGTCTTGACATTGATGGACAGAACTAGA<br>GATAGTTCCTCTTCTTCGGAAGCCAGAAAACA<br>GGTGGTGCACGGTTGTCGTCAGCTCGTGTCGT<br>GAGATGTTGGGTTAAGTCCCGCAACGAGCGC<br>AACCCCTATCTTATGTTGCCAGCACTTCGGGT<br>GGGAACTCAT |
| | Veillonella parvula | | >S14-201 Contig<br>GAGTGATGACGGCCTTCGGGTTGTAAAGCTCT<br>GTTAATCGGGACGAAAGGCCTTCTTGCGAAT<br>AGTGAGAAGGATTGACGGTACCGGAATAGAA<br>AGCCACGGCTAACTACGTGCCAGCAGCCGCG<br>GTAATACGTAGGTGGCAAGCGTTGTCCGGAA<br>TTATTGGGCGTAAAGCGCGCGCAGGCGGATA<br>GGTCAGTCTGTCTTAAAAGTTCGGGGCTTAAC<br>CCCGTGATGGGATGGAAACTGCCAATCTAGA<br>GTATCGGAGAGGAAAGTGGAATTCCTAGTGT<br>AGCGGTGAAATGCGTAGATATTAGGAAGAAC<br>ACCAGTGGCGAAGGCGACTTTCTGGACGAAA<br>ACTGACGCTGAGGCGCGAAAGCCAGGGGAGC<br>GAACGGGATTAGATACCCCGGTAGTCCTGGC<br>CGTAAACGATGGGTACTAGGTGTAGGAGGTA<br>TCGACCCCTTCTGTGCCGGAGTTAACGCAATA<br>AGTACCCCGCCTGGGGAGTACGACCGCAAGG<br>TTGAAACTCAAAGGAATTGACGGGGGCCCGC<br>ACAAGCGGTGGAGTATGTGGTTTAATTCGAC<br>GCAACGCGAAGAACCTTACCAGGTCTTGACA<br>TTGATGGACAGAACCAGAGATGGTTCCTCTTC<br>TTCGGAAGCCAGAAAACAGGTGGTGCACGGT |

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| | | | TGTCGTCAGCTCGTGTCGTGAGATGTTGGGTT<br>AAGTCCCGCAACGAGCGCAACCCCTATCTTAT<br>GTTGCCAGCACTTTGGGTGGGGACTCATGAG<br>AGACTGCCGCAGACAATGCGGAGGAAGGCGG<br>GGATGACGTCAAATCATCATGCCCCTTATGAC<br>CTGGGCTACACACGTACTACAATGGGAGTTA<br>ATAGACGGAAGCGAGATCGCGAGATGGAGCA<br>AACCCGAGAAACACTCTCTCAGTTCGGATCGT<br>AGGCTGCAACTCGCCTACGTGAAGTCGGAAT<br>CGCTAGTAATCGCAGGTCAGCATACTGCGGT<br>GAATACGTTCCCGGGCCTTGTACACACCGCCC<br>GTCACACCACGAAAGTCGGAAGTGCCCAAAG<br>CCGGTGGGGTAACCTTC |
| | Veillonella parvula | | >S14-205 Contig<br>GAGTGATGACGGCCTTCGGGTTGTAAAGCTCT<br>GTTAATCGGGACGAAAGGCCTTCTTGCGAAT<br>AGTGAGAAGGATTGACGGTACCGGAATAGAA<br>AGCCACGGCTAACTACGTGCCAGCAGCCGCG<br>GTAATACGTAGGTGGCAAGCGTTGTCCGGAA<br>TTATTGGGCGTAAAGCGCGCGCAGGCGGATA<br>GGTCAGTCTGTCTTAAAAGTTCGGGGCTTAAC<br>CCCGTGATGGGATGGAAACTGCCAATCTAGA<br>GTATCGGAGAGGAAAGTGGAATTCCTAGTGT<br>AGCGGTGAAATGCGTAGATATTAGGAAGAAC<br>ACCAGTGGCGAAGGCGACTTTCTGGACGAAA<br>ACTGACGCTGAGGCGCGAAAGCCAGGGGAGC<br>GAACGGGATTAGATACCCCGGTAGTCCTGGC<br>CGTAAACGATGGGTACTAGGTGTAGGAGGTA<br>TCGACCCCTTCTGTGCCGGAGTTAACGCAATA<br>AGTACCCCGCCTGGGGAGTACGACCGCAAGG<br>TTGAAACTCAAAGGAATTGACGGGGGCCCGC<br>ACAAGCGGTGGAGTATGTGGTTTAATTCGAC<br>GCAACGCGAAGAACCTTACCAGGTCTTGACA<br>TTGATGGACAGAACCAGAGATGGTTCCTCTTC<br>TTCGGAAGCCAGAAAACAGGTGGTGCACGGT<br>TGTCGTCAGCTCGTGTCGTGAGATGTTGGGTT<br>AAGTCCCGCAACGAGCGCAACCCCTATCTTAT<br>GTTGCCAGCACTTTGGGTGGGGACTCATGAG<br>AGACTGCCGCAGACAATGCGGAGGAAGGCGG<br>GGATGACGTCAAATCATCATGCCCCTTATGAC<br>CTGGGCTACACACGTACTACAATGGGAGTTA<br>ATAGACGGAAGCGAGATCGCGAGATGGAGCA<br>AACCCGAGAAACACTCTCTCAGTTCGGATCGT<br>AGGCTGCAACTCGCCTACGTGAAGTCGGAAT<br>CGCTAGTAATCGCAGGTCAGCATACTGCGGT<br>GAATACGTTCCCGGGCCTTGTACACACCGCCC<br>GTCACACCACGAAAGTCGGAAGTGCCCAAAG<br>CCGGTG |
| | Veillonella atypica Strain A | PTA-125709 | |
| | Veillonella atypica Strain B | PTA-125711 | |

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| | Veillonella dispar | | |
| | Veillonella parvula Strain A | PTA-125691 | |
| | Veillonella parvula Strain B | PTA-125711 | |
| | Veillonella tobetsuensis Strain A | PTA-125708 | |
| | Veillonella tobetsuensis Strain B | | |
| | Lactobacillus salivarius Strain A | | ATGGAGCAACGCCGCGTGAGTGAAGAAGGTC<br>TTCGGATCGTAAAACTCTGTTGTTAGAGAAGA<br>ACACGAGTGAGAGTAACTGTTCATTCGATGA<br>CGGTATCTAACCAGCAAGTCACGGCTAACTA<br>CGTGCCAGCAGCCGCGGTAATACGTAGGTGG<br>CAAGCGTTGTCCGGATTTATTGGGCGTAAAGG<br>GAACGCAGGCGGTCTTTTAAGTCTGATGTGAA<br>AGCCTTCGGCTTAACCGGAGTAGTGCATTGGA<br>AACTGGAAGACTTGAGTGCAGAAGAGGAGAG<br>TGGAACTCCATGTGTAGCGGTGAAATGCGTA<br>GATATATGGAAGAACACCAGTGGCGAAAGCG<br>GCTCTCTGGTCTGTAACTGACGCTGAGGTTCG<br>AAAGCGTGGGTAGCAAACAGGATTAGATACC<br>CTGGTAGTCCACGCCGTAAACGATGAATGCT<br>AGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCG<br>CAGCTAACGCAATAAGCATTCCGCCTGGGGA<br>GTACGACCGCAAGGTTGAAACTCAAAGGAAT<br>TGACGGGGGCCCGCACAAGCGGTGGAGCATG<br>TGGTTTAATTCGAAGCAACGCGAAGAACCTT<br>ACCAGGTCTTGACATCCTTTGACCACCTAAGA<br>GATTAGGCTTTCCCTTCGGGGACAAAGTGACA<br>GGTGGTGCATGGCTGTCGTCAGCTCGTGTCGT<br>GAGATGTTGGGTTAAGTCCCGCAACGAGCGC<br>AACCCTTGTTGTCAGTTGCCAGCATTAAGTTG<br>GGCACTCTGGCGAGACTGCCGGTGACAAACC<br>GGAGGAAGGTGGGGACGACGTCAAGTCATCA<br>TGCCCCTTATGACCTGGGCTACACACGTGCTA<br>CAATGGACGGTACAACGAGTCGCGAGACCGC<br>GAGGTTTAGCTAATCTCTTAAAGCCGTTCTCA<br>GTTCGGATTGTAGGCTGCAACTCGCCTACATG<br>AAGTCGGAATCGCTAGTAATCGCGAATCAGC<br>ATGTCGCGGTGAATACGTTCCCGGGCCTTGTA<br>CACACCGCCCGTCACACCATGAGAGTTTGTAA<br>CACCCAAAGCCGGTGGGGTAACCGCAAGGAG<br>CCAGCCG |
| | Agathobaculum Strain A | | CCGCGTGATTGAAGAAGGCCTNTCGGGTTGT<br>AAAGATCTTTAATTCGGGACGAAAAATGACG<br>GTACCGAAAGAATAAGCTCCGGCTAACTACG<br>TGCCAGCAGCCGCGGTAATACGTAGGGAGCA<br>AGCGTTATCCGGATTTACTGGGTGTAAAGGGC<br>GCGCAGGCGGGCTGGCAAGTTGGAAGTGAAA |

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| | | | TCTAGGGGCTTAACCCCTAAACTGCTTTCAAA<br>ACTGCTGGTCTTGAGTGATGGAGAGGCAGGC<br>GGAATTCCGTGTGTAGCGGTGAAATGCGTAG<br>ATATACGGAGGAACACCAGTGGCGAAGGCGG<br>CCTGCTGGACATTAACTGACGCTGAGGCGCG<br>AAAGCGTGGGGAGCAAACAGGATTAGATACC<br>CTGGTAGTCCACGCCGTAAACGATGGATACT<br>AGGTGTGGGAGGTATTGACCCCTTCCGTGCCG<br>CAGTTAACACAATAAGTATCCCACCTGGGGA<br>GTACGGCCGCAAGGTTGAAACTCAAAGGAAT<br>TGACGGGGGCCCGCACAAGCAGTGGAGTATG<br>TGGTTTAATTCGAAGCAACGCGAAGAACCTT<br>ACCAGGCCTTGACATCCCGATGACCGGTCTAG<br>AGATAGACCTTCTCTTCGGAGCATCGGTGACA<br>GGTGGTGCATGGTTGTCGTCAGCTCGTGTCGT<br>GAGATGTTGGGTTAAGTCCCGCAACGAGCGC<br>AACCCTTACGGTTAGTTGATACGCAAGATCAC<br>TCTAGCCGGACTGCCGTTGACAAAACGGAGG<br>AAGGTGGGGACGACGTCAAATCATCATGCCC<br>CTTATGGCCTGGGCTACACACGTACTACAATG<br>GCAGTCATACAGAGGGAAGCAAAGCTGTGAG<br>GCGGAGCAAATCCCTAAAAGCTGTCCCAGTT<br>CAGATTGCAGGCTGCAACCCGCCTGCATGAA<br>GTCGGAATTGCTAGTAATCGCGGATCAGCAT<br>GCCGCGGTGAATACGTTCCCGGGCCTTGTACA<br>CACCGCCCGTCACACCATGAGAGCCGTCAAT<br>ACCCGAAGTCCGTAGCCTAACCGCAAG |
| | Paraclostridium benzoelyticum Strain A | | GAATTACTGGGCGTAAAGGGTGCGTAGGTGG<br>TTTTTTAAGTCAGAAGTGAAAGGCTACGGCTC<br>AACCGTAGTAAGCTTTTGAAACTAGAGAACTT<br>GAGTGCAGGAGAGGAGAGTAGAATTCCTAGT<br>GTAGCGGTGAAATGCGTAGATATTAGGAGGA<br>ATACCAGTAGCGAAGGCGGCTCTCTGGACTG<br>TAACTGACACTGAGGCACGAAAGCGTGGGGA<br>GCAAACAGGATTAGATACCCTGGTAGTCCAC<br>GCCGTAAACGATGAGTACTAGGTGTCGGGGG<br>TTACCCCCCTCGGTGCCGCAGCTAACGCATTA<br>AGTACTCCGCCTGGGAAGTACGCTCGCAAGA<br>GTGAAACTCAAAGGAATTGACGGGGACCCGC<br>ACAAGTAGCGGAGCATGTGGTTTAATTCGAA<br>GCAACGCGAAGAACCTTACCTAAGCTTGACA<br>TCCCACTGACCTCTCCCTAATCGGAGATTTCC<br>CTTCGGGGACAGTGGTGACAGGTGGTGCATG<br>GTTGTCGTCAGCTCGTGTCGTGAGATGTTGGG<br>TTAAGTCCCGCAACGAGCGCAACCCTTGCCTT<br>TAGTTGCCAGCATTAAGTTGGGCACTCTAGAG<br>GGACTGCCGAGGATAACTCGGAGGAAGGTGG<br>GGATGACGTCAAATCATCATGCCCCTTATGCT<br>TAGGGCTACACACGTGCTACAATGGGTGGTA<br>CAGAGGGTTGCCAAGCCGCGAGGTGGAGCTA<br>ATCCCTTAAAGCCATTCTCAGTTCGGATTGTA<br>GGCTGAAACTCGCCTACATGAAGCTGGAGTT<br>ACTAGTAATCGCAGATCAGAATGCTGCGGTG<br>AATGCGTTCCCGGGTCTTGTACACACCGCCCG |

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| | | | TCACACCATGGAAGTTGGGGGCGCCCGAAGC CGGTTAGCTAACCTTTTAGGAAGCGGCCGT |
| | Turicibacter sanguinis Strain A | | ATGGCTAGAGTGTGACGGTACCTTATGAGAA AGCCACGGCTAACTACGTGCCAGCAGCCGCG GTAATACGTAGGTGGCGAGCGTTATCCGGAA TTATTGGGCGTAAAGAGCGCGCAGGTGGTTG ATTAAGTCTGATGTGAAAGCCCACGGCTTAAC CGTGGAGGGTCATTGGAAACTGGTCAACTTG AGTGCAGAAGAGGGAAGTGGAATTCCATGTG TAGCGGTGAAATGCGTAGAGATATGGAGGAA CACCAGTGGCGAAGGCGGCTTCCTGGTCTGTA ACTGACACTGAGGCGCGAAAGCGTGGGGAGC AAACAGGATTAGATACCCTGGTAGTCCACGC CGTAAACGATGAGTGCTAAGTGTTGGGGGTC GAACCTCAGTGCTGAAGTTAACGCATTAAGC ACTCCGCCTGGGGAGTACGGTCGCAAGACTG AAACTCAAAGGAATTGACGGGGACCCGCACA AGCGGTGGAGCATGTGGTTTAATTCGAAGCA ACGCGAAGAACCTTACCAGGTCTTGACATAC CAGTGACCGTCCTAGAGATAGGATTTTCCCT TCGGGGACAATGGATACAGGTGGTGCATGGT TGTCGTCAGCTCGTGTCGTGAGATGTTGGGTT AAGTCCCGCAACGAGCGCAACCCCTGTCGTT AGTTGCCAGCATTCAGTTGGGGACTCTAACGA GACTGCCAGTGACAAACTGGAGGAAGGTGGG GATGACGTCAAATCATCATGCCCCTTATGACC TGGGCTACACACGTGCTACAATGGTTGGTACA AAGAGAAGCGAAGCGGTGACGTGGAGCAAA CCTCATAAAGCCAATCTCAGTTCGGATTGTAG GCTGCAACTCGCCTACATGAAGTTGGAATCGC TAGTAATCGCGAATCAGCATGTCGCGGTGAA TACGTT |
| | Burkholderia pseudomallei | | |
| | Klebsiella quasipneumoniae subsp. similipneumoniae | | |
| | Klebsiella oxytoca Strain A | | |
| | Megasphaera Sp. Strain A | PTA-126770 | TATCAATTCGAGTGGCAAACGGGTGA GTAACGCGTAAGCAACCTGCCCTTCA GATGGGGACAACAGCTGGAAACGGCT GCTAATACCGAATACGTTCTTTCCGCC GCATGACGGGATGAAGAAAGGGAGG CCTTCGGGCTTTCGCTGGAGGAGGGG CTTGCGTCTGATTAGCTAGTTGGAGG GGTAACGGCCCACCAAGGCGACGATC AGTAGCCGGTCTGAGAGGATGAACGG |

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| | | | CCACATTGGGACTGAGACACGGCCCA<br>GACTCCTACGGGAGGCAGCAGTGGGG<br>AATCTTCCGCAATGGACGAAAGTCTG<br>ACGGAGCAACGCCGCGTGAACGATGA<br>CGGCCTTCGGGTTGTAAAGTTCTGTTA<br>TATGGGACGAACAGGATAGCGGTCAA<br>TACCCGTTATCCCTGACGGTACCGTAA<br>GAGAAAGCCACGGCTAACTACGTGCC<br>AGCAGCCGCGGTAATACGTAGGTGGC<br>AAGCGTTGTCCGGAATTATTGGGCGT<br>AAAGGGCGCGCAGGCGGCATCGCAA<br>GTCGGTCTTAAAAGTGCGGGGCTTAA<br>CCCCGTGAGGGGACCGAAACTGTGAA<br>GCTCGAGTGTCGGAGAGGAAAGCGGA<br>ATTCCTAGTGTAGCGGTGAAATGCGT<br>AGATATTAGGAGGAACACCAGTGGCG<br>AAAGCGGCTTTCTGGACGACAACTGA<br>CGCTGAGGCGCGAAAGCCAGGGGAG<br>CAAACGGGATTAGATACCCCGGTAGT<br>CCTGGCCGTAAACGATGGATACTAGG<br>TGTAGGAGGTATCGACTCCTTCTGTGC<br>CGGAGTTAACGCAATAAGTATCCCGC<br>CTGGGGAGTACGGCCGCAAGGCTGAA<br>ACTCAAAGGAATTGACGGGGGCCCGC<br>ACAAGCGGTGGAGTATGTGGTTTAAT<br>TCGACGCAACGCGAAGAACCTTACCA<br>AGCCTTGACATTGATTGCTACGGAAA<br>GAGATTTCCGGTTCTTCTTCGGAAGAC<br>AAGAAAACAGGTGGTGCACGGCTGTC<br>GTCAGCTCGTGTCGTGAGATGTTGGG<br>TTAAGTCCCGCAACGAGCGCAACCCC<br>TATCTTCTGTTGCCAGCACTAAGGGTG<br>GGGACTCAGAAGAGACTGCCGCAGAC<br>AATGCGGAGGAAGGCGGGGATGACG<br>TCAAGTCATCATGCCCCTTATGGCTTG<br>GGCTACACACGTACTACAATGGCTCT<br>TAATAGAGGGAAGCGAAGGAGCGAT<br>CCGGAGCAAACCCCAAAAACAGAGTC<br>CCAGTTCGGATTGCAGGCTGCAACTC<br>GCCTGCATGAAGCAGGAATCGCTAGT<br>AATCGCAGGTCAGCATACTGCGGTGA<br>ATACGTTCCCGGGCCTTGTACACACC<br>GCCCGTCACACCACGAAAGTCATTCA<br>CACCCGAAGCCGGTGAGGCAACCGCA<br>AGGAACCAGCCGTCGAAGGTGGGGGC<br>GATGATTGGGGTGAAGTCGTAACAAG |

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| | | | GTAGCCGTATCGGAAGGTGCGGCTGG<br>ATCACCTCCTTT |
| | Megasphaera Sp.<br>Strain B | | ATGGAGAGTTTGATCCTGGCTCAGGA<br>CGAACGCTGGCGGCGTGCTTAACACA<br>TGCAAGTCGAACGAGAAGAGATGAG<br>AAGCTTGCTTCTTATCAATTCGAGTGG<br>CAAACGGGTGAGTAACGCGTAAGCAA<br>CCTGCCCTTCAGATGGGGACAACAGC<br>TGGAAACGGCTGCTAATACCGAATAC<br>GTTCTTTCCGCCGCATGACGGGATGA<br>AGAAAGGGAGGCCTTCGGGCTTTCGC<br>TGGAGGAGGGGCTTGCGTCTGATTAG<br>CTAGTTGGAGGGGTAACGGCCCACCA<br>AGGCGACGATCAGTAGCCGGTCTGAG<br>AGGATGAACGGCCACATTGGGACTGA<br>GACACGGCCCAGACTCCTACGGGAGG<br>CAGCAGTGGGGAATCTTCCGCAATGG<br>ACGAAAGTCTGACGGAGCAACGCCGC<br>GTGAACGATGACGGCCTTCGGGTTGT<br>AAAGTTCTGTTATATGGGACGAACAG<br>GATAGCGGTCAATACCCGTTATCCCT<br>GACGGTACCGTAAGAGAAAGCCACGG<br>CTAACTACGTGCCAGCAGCCGCGGTA<br>ATACGTAGGTGGCAAGCGTTGTCCGG<br>AATTATTGGGCGTAAAGGGCGCGCAG<br>GCGGCATCGCAAGTCGGTCTTAAAAG<br>TGCGGGGCTTAACCCCGTGAGGGGAC<br>CGAAACTGTGAAGCTCGAGTGTCGGA<br>GAGGAAAGCGGAATTCCTAGTGTAGC<br>GGTGAAATGCGTAGATATTAGGAGGA<br>ACACCAGTGGCGAAAGCGGCTTTCTG<br>GACGACAACTGACGCTGAGGCGCGAA<br>AGCCAGGGGAGCAAACGGGATTAGAT<br>ACCCCGGTAGTCCTGGCCGTAAACGA<br>TGGATACTAGGTGTAGGAGGTATCGA<br>CTCCTTCTGTGCCGGAGTTAACGCAAT<br>AAGTATCCCGCCTGGGGAGTACGGCC<br>GCAAGGCTGAAACTCAAAGGAATTGA<br>CGGGGGCCCGCACAAGCGGTGGAGTA<br>TGTGGTTTAATTCGACGCAACGCGAA<br>GAACCTTACCAAGCCTTGACATTGATT<br>GCTACGGAAAGAGATTTCCGGTTCTT<br>CTTCGGAAGACAAGAAAACAGGTGGT<br>GCACGGCTGTCGTCAGCTCGTGTCGT<br>GAGATGTTGGGTTAAGTCCCGCAACG |

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| | | | AGCGCAACCCCTATCTTCTGTTGCCAG<br>CACTAAGGGTGGGGACTCAGAAGAGA<br>CTGCCGCAGACAATGCGGAGGAAGGC<br>GGGGATGACGTCAAGTCATCATGCCC<br>CTTATGGCTTGGGCTACACACGTACTA<br>CAATGGCTCTTAATAGAGGGAAGCGA<br>AGGAGCGATCCGGAGCAAACCCCAAA<br>AACAGAGTCCCAGTTCGGATTGCAGG<br>CTGCAACTCGCCTGCATGAAGCAGGA<br>ATCGCTAGTAATCGCAGGTCAGCATA<br>CTGCGGTGAATACGTTCCCGGGCCTT<br>GTACACACCGCCCGTCACACCACGAA<br>AGTCATTCACACCCGAAGCCGGTGAG<br>GCAACCGCAAGGAACCAGCCGTCGAA<br>GGTGGGGGCGATGATTGGGGTGAAGT<br>CGTAACAAGGTAGCCGTATCGGAAGG<br>TGCGGCTGGATCACCTCCTTT |
| | Selenomonas felix | | GTTGGTGAGGTAACGGCTCACCAAGG<br>CGACGATCAGTAGCCGGTCTGAGAGG<br>ATGAACGGCCACATTGGGACTGAGAC<br>ACGGCCCAGACTCCTACGGGAGGCAG<br>CAGTGGGGAATCTTCCGCAATGGGCG<br>CAAGCCTGACGGAGCAACGCCGCGTG<br>AGTGAAGAAGGTCTTCGGATCGTAAA<br>GCTCTGTTGACGGGGACGAACGTGCG<br>GAGTGCGAATAGCGCTTTGTAATGAC<br>GGTACCTGTCGAGGAAGCCACGGCTA<br>ACTACGTGCCAGCAGCCGCGGTAATA<br>CGTAGGTGGCGAGCGTTGTCCGGAAT<br>CATTGGGCGTAAAGGGAGCGCAGGCG<br>GGCCGGTAAGTCTTACTTAAAAGTGC<br>GGGGCTCAACCCCGTGATGGGAGAGA<br>AACTATCGGTCTTGAGTACAGGAGAG<br>GAAAGCGGAATTCCCAGTGTAGCGGT<br>GAAATGCGTAGATATTGGGAAGAACA<br>CCAGTGGCGAAGGCGGCTTTCTGGAC<br>TGCAACTGACGCTGAGGCTCGAAAGC<br>CAGGGGAGCGAACGGGATTAGATACC<br>CCGGTAGTCCTGGCCGTAAACGATGG<br>ATACTAGGTGTGGGAGGTATCGACCC<br>CTACCGTGCCGGAGTTAACGCAATAA<br>GTATCCCGCCTGGGGAGTACGGCCGC<br>AAGGCTGAAACTCAAAGGAATTGACG<br>GGGACCCGCACAAGCGGTGGAGTATG<br>TGGTTTAATTCGAAGCAACGCGAAGA |

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| | | | ACCTTACCAGGCCTTGACATTGACTG<br>AAAGCACTAGAGATAGTGCCCTCTCT<br>TCGGAGACAGGAAAACAGGTGGTGCA<br>TGGCTGTCGTCAGCTCGTGTCGTGAG<br>ATGTTGGGTTAAGTCCCGCAACGAGC<br>GCAACCCCTGTTCTTTGTTGCCATCAG<br>GTAAAGCTGGGCACTCAAAGGAGACT<br>GCCGCGGAGAACGCGGAGGAAGGCG<br>GGGATGACGTCAAGTCATCATGCCCC<br>TTATGGCCTGGGCTACACACGTACTA<br>CAATGGAACGGACAGAGAGCAGCGA<br>ACCCGCGAGGGCAAGCGAACCTCAAA<br>AACCGTTTCCCAGTTCGGATTGCAGG<br>CTGCAACCCGCCTGCATGAAGTCGGA<br>ATCGCTAGTAATCGCAGGTCAGCATA<br>CTGCGGTGAATACGTTCCCGGGTCTTG<br>TACACACCGCCCGTCACACCACGGAA<br>GTCATTCACACCCGAAGCCGGCGCAG<br>CCGTCTAAGGTGGGGAAGGTGACTGG<br>GGTGAAGTCGTAACAAGGTAGCCGTA<br>TCGGAAGGTGCGGCTGGATCACCTCC<br>TTT |
| | Enterococcus gallinarum Strain A | | CTGACCGAGCACGCCGCGTGAGTGAA<br>GAAGGTTTTCGGATCGTAAAACTCTG<br>TTGTTAGAGAAGAACAAGGATGAGAG<br>TAAAACGTTCATCCCTTGACGGTATCT<br>AACCAGAAAGCCACGGCTAACTACGT<br>GCCAGCAGCCGCGGTAATACGTAGGT<br>GGCAAGCGTTGTCCGGATTTATTGGG<br>CGTAAAGCGAGCGCAGGCGGTTTCTT<br>AAGTCTGATGTGAAAGCCCCCGGCTC<br>AACCGGGGAGGGTCATTGGAAACTGG<br>GAGACTTGAGTGCAGAAGAGGAGAGT<br>GGAATTCCATGTGTAGCGGTGAAATG<br>CGTAGATATATGGAGGAACACCAGTG<br>GCGAAGGCGGCTCTCTGGTCTGTAAC<br>TGACGCTGAGGCTCGAAAGCGTGGGG<br>AGCGAACAGGATTAGATACCCTGGTA<br>GTCCACGCCGTAAACGATGAGTGCTA<br>AGTGTTGGAGGGTTTCCGCCCTTCAGT<br>GCTGCAGCAAACGCATTAAGCACTCC<br>GCCTGGGGAGTACGACCGCAAGGTTG<br>AAACTCAAAGGAATTGACGGGGGCCC<br>GCACAAGCGGTGGAGCATGTGGTTTA<br>ATTCGAAGCAACGCGAAGAACCTTAC |

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| | | | CAGGTCTTGACATCCTTTGACCACTCT<br>AGAGATAGAGCTTCCCCTTCGGGGGC<br>AAAGTGACAGGTGGTGCATGGTTGTC<br>GTCAGCTCGTGTCGTGAGATGTTGGG<br>TTAAGTCCCGCAACGAGCGCAACCCT<br>TATTGTTAGTTGCCATCATTTAGTTGG<br>GCACTCTAGCGAGACTGCCGGTGACA<br>AACCGGAGGAAGGTGGGGATGACGTC<br>AAATCATCATGCCCCTTATGACCTGG<br>GCTACACACGTGCTACAATGGGAAGT<br>ACAACGAGTTGCGAAGTCGCGAGGCT<br>AAGCTAATCTCTTAAAGCTTCTCTCAG<br>TTCGGATTGTAGGCTGCAACTCGCCTA<br>CATGAAGCCGGAATCGCTAGTAATCG<br>CGGATCAGCACGCCGCGGTGAATACG<br>TTCCCGGGCCTTGTACACACCGCCCGT<br>CACACCACGAGAGTTTGTAACACCCG<br>AAGTCGGTGAGGTAACCTTT |
| | Enterococcus Gallinarum Strain B | | CGCGTGAGTGAAGAAGGTTTTCGGAT<br>CGTAAAACTCTGTTGTTAGAGAAGAA<br>CAAGGATGAGAGTAGAACGTTCATCC<br>CTTGACGGTATCTAACCAGAAAGCCA<br>CGGCTAACTACGTGCCAGCAGCCGCG<br>GTAATACGTAGGTGGCAAGCGTTGTC<br>CGGATTTATTGGGCGTAAAGCGAGCG<br>CAGGCGGTTTCTTAAGTCTGATGTGA<br>AAGCCCCCGGCTCAACCGGGGAGGGT<br>CATTGGAAACTGGGAGACTTGAGTGC<br>AGAAGAGGAGAGTGGAATTCCATGTG<br>TAGCGGTGAAATGCGTAGATATATGG<br>AGGAACACCAGTGGCGAAGGCGGCTC<br>TCTGGTCTGTAACTGACGCTGAGGCTC<br>GAAAGCGTGGGGAGCGAACAGGATT<br>AGATACCCTGGTAGTCCACGCCGTAA<br>ACGATGAGTGCTAAGTGTTGGAGGGT<br>TTCCGCCCTTCAGTGCTGCAGCAAAC<br>GCATTAAGCACTCCGCCTGGGGAGTA<br>CGACCGCAAGGTTGAAACTCAAAGGA<br>ATTGACGGGGGCCCGCACAAGCGGTG<br>GAGCATGTGGTTTAATTCGAAGCAAC<br>GCGAAGAACCTTACCAGGTCTTGACA<br>TCCTTTGACCACTCTAGAGATAGAGCT<br>TCCCCTTCGGGGGCAAAGTGACAGGT<br>GGTGCATGGTTGTCGTCAGCTCGTGTC<br>GTGAGATGTTGGGTTAAGTCCCGCAA |

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| | | | CGAGCGCAACCCTTATTGTTAGTTGCC ATCATTTAGTTGGGCACTCTAGCGAG ACTGCCGGTGACAAACCGGAGGAAGG TGGGGATGACGTCAAATCATCATGCC CCTTATGACCTGGGCTACACACGTGCT ACAATGGGAAGTACAACGAGTTGCGA AGTCGCGAGGCTAAGCTAATCTCTTA AAGCTTCTCTCAGTTCGGATTGTAGGC TGCAACTCGCCTACATGAAGCCGGAA TCGCTAGTAATCGCGGATCAGCACGC CGCGGTGAATACGTTCCCGGGCCTTG TACACACCGCCCGTCACACCACGAGA GTTTGTAACACCCGAAGTCGGTGAGG TAACCTTTTNGGAGCCAGCCGC |
| Fournierella massiliensis | PTA-126694 | | Fournierella massiliensis |
| Harryflintia acetispora | PTA-126696 | | Harryflintia acetispora |

**[201]** In some embodiments, the mEVs from one or more of the following bacteria:

- *Akkermansia, Christensenella, Blautia, Enterococcus, Eubacterium, Roseburia, Bacteroides, Parabacteroides, or Erysipelatoclostridium*
- *Blautia hydrogenotrophica, Blautia stercoris, Blautia wexlerae, Eubacterium faecium, Eubacterium contortum, Eubacterium rectale, Enterococcus faecalis, Enterococcus durans, Enterococcus villorum, Enterococcus gallinarum; Bifidobacterium lactis, Bifidobacterium bifidium, Bifidobacterium longum, Bifidobacterium animalis, or Bifidobacterium breve*
- *BCG, Parabacteroides, Blautia, Veillonella, Lactobacillus salivarius, Agathobaculum, Ruminococcus gnavus, Paraclostridium benzoelyticum, Turicibacter sanguinus, Burkholderia, Klebsiella quasipneumoniae ssp similpneumoniae, Klebsiella oxytoca, Tyzzerela nexilis, or Neisseria*
- *Blautia hydrogenotrophica*
- *Blautia stercoris*

- *Blautia wexlerae*
- *Enterococcus gallinarum*
- *Enterococcus faecium*
- *Bifidobacterium bifidium*
- *Bifidobacterium breve*
- *Bifidobacterium longum*
- *Roseburia hominis*
- *Bacteroides thetaiotaomicron*
- *Bacteroides coprocola*
- *Erysipelatoclostridium ramosum*
- *Megasphera, including Megasphera massiliensis*
- *Parabacteroides distasonis*
- *Eubacterium contortum*
- *Eubacterium hallii*
- *Intestimonas butyriciproducens*
- *Streptococcus australis*
- *Eubacterium eligens*
- *Faecalibacterium prausnitzii*
- *Anaerostipes caccae*
- *Erysipelotrichaceae*
- *Rikenellaceae*
- *Lactococcus, Prevotella, Bifidobacterium, Veillonella*
- *Lactococcus lactis cremoris*
- *Prevotella histicola*
- *Bifidobacterium animalis lactis*
- *Veillonella parvula*

**[202]** In some embodiments, the mEVs are from *Lactococcus lactis cremoris* bacteria, e.g., from a strain comprising at least 90% or at least 99% genomic, 16S and/or CRISPR sequence identity to the nucleotide sequence of the *Lactococcus lactis cremoris* Strain A (ATCC designation number PTA-125368). In some embodiments, the mEVs are from *Lactococcus*

bacteria, e.g., from *Lactococcus lactis cremoris* Strain A (ATCC designation number PTA-125368).

**[203]** In some embodiments, the mEVs are from *Prevotella* bacteria, e.g., from a strain comprising at least 90% or at least 99% genomic, 16S and/or CRISPR sequence identity to the nucleotide sequence of the *Prevotella* Strain B 50329 (NRRL accession number B 50329). In some embodiments, the mEVs are from *Prevotella* bacteria, e.g., from *Prevotella* Strain B 50329 (NRRL accession number B 50329).

**[204]** In some embodiments, the mEVs are from *Bifidobacterium* bacteria, e.g., from a strain comprising at least 90% or at least 99% genomic, 16S and/or CRISPR sequence identity to the nucleotide sequence of the *Bifidobacterium* bacteria deposited as ATCC designation number PTA-125097. In some embodiments, the mEVs are from *Bifidobacterium* bacteria, e.g., from *Bifidobacterium* bacteria deposited as ATCC designation number PTA-125097.

**[205]** In some embodiments, the mEVs are from *Veillonella* bacteria, e.g., from a strain comprising at least 90% or at least 99% genomic, 16S and/or CRISPR sequence identity to the nucleotide sequence of the *Veillonella* bacteria deposited as ATCC designation number PTA-125691. In some embodiments, the mEVs are from *Veillonella* bacteria, e.g., from *Veillonella* bacteria deposited as ATCC designation number PTA-125691.

Modified mEVs

**[206]** In some aspects, the mEVs (such as smEVs) described herein are modified such that they comprise, are linked to, and/or are bound by a therapeutic moiety.

**[207]** In some embodiments, the therapeutic moiety is a cancer-specific moiety. In some embodiments, the cancer-specific moiety has binding specificity for a cancer cell (e.g., has binding specificity for a cancer-specific antigen). In some embodiments, the cancer-specific moiety comprises an antibody or antigen binding fragment thereof. In some embodiments, the cancer-specific moiety comprises a T cell receptor or a chimeric antigen receptor (CAR). In some embodiments, the cancer-specific moiety comprises a ligand for a receptor expressed on the surface of a cancer cell or a receptor-binding fragment thereof. In some embodiments, the cancer-specific moiety is a bipartite fusion protein that has two parts: a first part that binds to and/or is linked to the bacterium and a second part that is capable of binding to a cancer cell (e.g., by having binding specificity for a cancer-specific antigen). In some embodiments, the first

part is a fragment of or a full-length peptidoglycan recognition protein, such as PGRP. In some embodiments the first part has binding specificity for the mEV (e.g., by having binding specificity for a bacterial antigen). In some embodiments, the first and/or second part comprises an antibody or antigen binding fragment thereof. In some embodiments, the first and/or second part comprises a T cell receptor or a chimeric antigen receptor (CAR). In some embodiments, the first and/or second part comprises a ligand for a receptor expressed on the surface of a cancer cell or a receptor-binding fragment thereof. In certain embodiments, co-administration of the cancer-specific moiety with the mEVs (either in combination or in separate administrations) increases the targeting of the mEVs to the cancer cells.

**[208]** In some embodiments, the mEVs described herein are modified such that they comprise, are linked to, and/or are bound by a magnetic and/or paramagnetic moiety (e.g., a magnetic bead). In some embodiments, the magnetic and/or paramagnetic moiety is comprised by and/or directly linked to the bacteria. In some embodiments, the magnetic and/or paramagnetic moiety is linked to and/or a part of an mEV-binding moiety that that binds to the mEV. In some embodiments, the mEV-binding moiety is a fragment of or a full-length peptidoglycan recognition protein, such as PGRP. In some embodiments the mEV-binding moiety has binding specificity for the mEV (e.g., by having binding specificity for a bacterial antigen). In some embodiments, the mEV-binding moiety comprises an antibody or antigen binding fragment thereof. In some embodiments, the mEV-binding moiety comprises a T cell receptor or a chimeric antigen receptor (CAR). In some embodiments, the mEV-binding moiety comprises a ligand for a receptor expressed on the surface of a cancer cell or a receptor-binding fragment thereof. In certain embodiments, co-administration of the magnetic and/or paramagnetic moiety with the mEVs (either together or in separate administrations) can be used to increase the targeting of the mEVs (e.g., to cancer cells and/or a part of a subject where cancer cells are present.

Production of Secreted Microbial Extracellular Vesicles (smEVs)

**[209]** In certain aspects, the smEVs described herein can be prepared using any method known in the art.

**[210]** In some embodiments, the smEVs are prepared without an smEV purification step. For example, in some embodiments, bacteria described herein are killed using a method

that leaves the smEVs intact and the resulting bacterial components, including the smEVs, are used in the methods and compositions described herein. In some embodiments, the bacteria are killed using an antibiotic (e.g., using an antibiotic described herein). In some embodiments, the bacteria are killed using UV irradiation. In some embodiments, the bacteria are heat-killed.

**[211]** In some embodiments, the smEVs described herein are purified from one or more other bacterial components. Methods for purifying smEVs from bacteria are known in the art. In some embodiments, smEVs are prepared from bacterial cultures using methods described in S. Bin Park, et al. PLoS ONE. 6(3):e17629 (2011) or G. Norheim, et al. PLoS ONE. 10(9): e0134353 (2015) or Jeppesen, et al. Cell 177:428 (2019), each of which is hereby incorporated by reference in its entirety. In some embodiments, the bacteria are cultured to high optical density and then centrifuged to pellet bacteria (e.g., at 10,000 x g for 30 min at 4°C, at 15,500 x g for 15 min at 4°C). In some embodiments, the culture supernatants are then passed through filters to exclude intact bacterial cells (e.g., a 0.22 μm filter). In some embodiments, the supernatants are then subjected to tangential flow filtration, during which the supernatant is concentrated, species smaller than 100 kDa are removed, and the media is partially exchanged with PBS. In some embodiments, filtered supernatants are centrifuged to pellet bacterial smEVs (e.g., at 100,000-150,000 x g for 1-3 hours at 4°C, at 200,000 x g for 1-3 hours at 4°C). In some embodiments, the smEVs are further purified by resuspending the resulting smEV pellets (e.g., in PBS), and applying the resuspended smEVs to an Optiprep (iodixanol) gradient or gradient (e.g., a 30-60% discontinuous gradient, a 0-45% discontinuous gradient), followed by centrifugation (e.g., at 200,000 x g for 4-20 hours at 4°C). smEV bands can be collected, diluted with PBS, and centrifuged to pellet the smEVs (e.g., at 150,000 x g for 3 hours at 4°C, at 200,000 x g for 1 hour at 4°C). The purified smEVs can be stored, for example, at -80°C or -20°C until use. In some embodiments, the smEVs are further purified by treatment with DNase and/or proteinase K.

**[212]** For example, in some embodiments, cultures of bacteria can be centrifuged at 11,000 x g for 20-40 min at 4°C to pellet bacteria. Culture supernatants may be passed through a 0.22 μm filter to exclude intact bacterial cells. Filtered supernatants may then be concentrated using methods that may include, but are not limited to, ammonium sulfate precipitation, ultracentrifugation, or filtration. For example, for ammonium sulfate precipitation, 1.5-3 M ammonium sulfate can be added to filtered supernatant slowly, while stirring at 4°C.

Precipitations can be incubated at 4°C for 8-48 hours and then centrifuged at 11,000 x g for 20-40 min at 4°C. The resulting pellets contain bacteria smEVs and other debris. Using ultracentrifugation, filtered supernatants can be centrifuged at 100,000-200,000 x g for 1-16 hours at 4°C. The pellet of this centrifugation contains bacteria smEVs and other debris such as large protein complexes. In some embodiments, using a filtration technique, such as through the use of an Amicon Ultra spin filter or by tangential flow filtration, supernatants can be filtered so as to retain species of molecular weight > 50 or 100 kDa.

**[213]** Alternatively, smEVs can be obtained from bacteria cultures continuously during growth, or at selected time points during growth, for example, by connecting a bioreactor to an alternating tangential flow (ATF) system (e.g., XCell ATF from Repligen). The ATF system retains intact cells (>0.22 um) in the bioreactor, and allows smaller components (e.g., smEVs, free proteins) to pass through a filter for collection. For example, the system may be configured so that the <0.22 um filtrate is then passed through a second filter of 100 kDa, allowing species such as smEVs between 0.22 um and 100 kDa to be collected, and species smaller than 100 kDa to be pumped back into the bioreactor. Alternatively, the system may be configured to allow for medium in the bioreactor to be replenished and/or modified during growth of the culture. smEVs collected by this method may be further purified and/or concentrated by ultracentrifugation or filtration as described above for filtered supernatants.

**[214]** smEVs obtained by methods provided herein may be further purified by size-based column chromatography, by affinity chromatography, by ion-exchange chromatography, and by gradient ultracentrifugation, using methods that may include, but are not limited to, use of a sucrose gradient or Optiprep gradient. Briefly, using a sucrose gradient method, if ammonium sulfate precipitation or ultracentrifugation were used to concentrate the filtered supernatants, pellets are resuspended in 60% sucrose, 30 mM Tris, pH 8.0. If filtration was used to concentrate the filtered supernatant, the concentrate is buffer exchanged into 60% sucrose, 30 mM Tris, pH 8.0, using an Amicon Ultra column. Samples are applied to a 35-60% discontinuous sucrose gradient and centrifuged at 200,000 x g for 3-24 hours at 4°C. Briefly, using an Optiprep gradient method, if ammonium sulfate precipitation or ultracentrifugation were used to concentrate the filtered supernatants, pellets are resuspended in PBS and 3 volumes of 60% Optiprep are added to the sample. In some embodiments, if filtration was used to concentrate the filtered supernatant, the concentrate is diluted using 60% Optiprep to a final concentration of

35% Optiprep. Samples are applied to a 0-45% discontinuous Optiprep gradient and centrifuged at 200,000 x g for 3-24 hours at 4°C, e.g., 4-24 hours at 4°C.

**[215]** In some embodiments, to confirm sterility and isolation of the smEV preparations, smEVs are serially diluted onto agar medium used for routine culture of the bacteria being tested, and incubated using routine conditions. Non-sterile preparations are passed through a 0.22 um filter to exclude intact cells. To further increase purity, isolated smEVs may be DNase or proteinase K treated.

**[216]** In some embodiments, for preparation of smEVs used for in vivo injections, purified smEVs are processed as described previously (G. Norheim, et al. PLoS ONE. 10(9): e0134353 (2015)). Briefly, after sucrose gradient centrifugation, bands containing smEVs are resuspended to a final concentration of 50 μg/mL in a solution containing 3% sucrose or other solution suitable for in vivo injection known to one skilled in the art. This solution may also contain adjuvant, for example aluminum hydroxide at a concentration of 0-0.5% (w/v). In some embodiments, for preparation of smEVs used for in vivo injections, smEVs in PBS are sterile-filtered to < 0.22 um.

**[217]** In certain embodiments, to make samples compatible with further testing (e.g., to remove sucrose prior to TEM imaging or in vitro assays), samples are buffer exchanged into PBS or 30 mM Tris, pH 8.0 using filtration (e.g., Amicon Ultra columns), dialysis, or ultracentrifugation (200,000 x g, ≥ 3 hours, 4°C) and resuspension.

**[218]** In some embodiments, the sterility of the smEV preparations can be confirmed by plating a portion of the smEVs onto agar medium used for standard culture of the bacteria used in the generation of the smEVs and incubating using standard conditions.

**[219]** In some embodiments, select smEVs are isolated and enriched by chromatography and binding surface moieties on smEVs. In other embodiments, select smEVs are isolated and/or enriched by fluorescent cell sorting by methods using affinity reagents, chemical dyes, recombinant proteins or other methods known to one skilled in the art.

**[220]** The smEVs can be analyzed, e.g., as described in Jeppesen, et al. Cell 177:428 (2019).

**[221]** In some embodiments, smEVs are lyophilized.

**[222]** In some embodiments, smEVs are gamma irradiated (e.g., at 17.5 or 25 kGy).

**[223]** In some embodiments, smEVs are UV irradiated.

**[224]** In some embodiments, smEVs are heat inactivated (e.g., at 50°C for two hours or at 90°C for two hours).

**[225]** In some embodiments, smEVs s are acid treated.

**[226]** In some embodiments, smEVs are oxygen sparged (e.g., at 0.1 vvm for two hours).

**[227]** The phase of growth can affect the amount or properties of bacteria and/or smEVs produced by bacteria. For example, in the methods of smEV preparation provided herein, smEVs can be isolated, e.g., from a culture, at the start of the log phase of growth, midway through the log phase, and/or once stationary phase growth has been reached.

**[228]** The growth environment (e.g., culture conditions) can affect the amount of smEVs produced by bacteria. For example, the yield of smEVs can be increased by an smEV inducer, as provided in Table 4.

**Table 4: Culture Techniques to Increase smEV Production**

| smEV inducement | smEV inducer | Acts on |
|---|---|---|
| Temperature | | |
| | Heat<br>RT to 37 °C temp change<br>37 to 40 °C temp change | stress response<br>simulates infection<br>febrile infection |
| ROS | | |
| | Plumbagin<br>Cumene hydroperoxide<br>Hydrogen Peroxide | oxidative stress response<br>oxidative stress response<br>oxidative stress response |
| Antibiotics | | |
| | Ciprofloxacin<br>Gentamycin<br>Polymyxin B<br>D-cylcloserine | bacterial SOS response<br>protein synthesis<br>outer membrane<br>cell wall |
| Osmolyte | | |
| | NaCl | osmotic stress |
| Metal Ion Stress | | |
| | Iron Chelation<br>EDTA<br>Low Hemin | iron levels<br>removes divalent cations<br>iron levels |
| Media additives or removal | | |

| | | |
|---|---|---|
| | Lactate | growth |
| | Amino acid deprivation | stress |
| | Hexadecane | stress |
| | Glucose | growth |
| | Sodium bicarbonate | ToxT induction |
| | PQS | vesiculator (from bacteria) |
| | Diamines+ DFMO | membrane anchoring (negativicutes only) |
| | High nutrients | enhanced growth |
| | Low nutrients | |
| Other mechanisms | | |
| | Oxygen | oxygen stress in anaerobe |
| | No Cysteine | oxygen stress in anaerobe |
| | Inducing biofilm or floculation | |
| | Diauxic Growth | |
| | Phage | |
| | Urea | |

**[229]** In the methods of smEVs preparation provided herein, the method can optionally include exposing a culture of bacteria to an smEV inducer prior to isolating smEVs from the bacterial culture. The culture of bacteria can be exposed to an smEV inducer at the start of the log phase of growth, midway through the log phase, and/or once stationary phase growth has been reached.

Pharmaceutical Compositions

**[230]** In certain embodiments, provided herein are pharmaceutical compositions comprising mEVs (such as smEVs) (e.g., an mEV composition (e.g., an smEV composition)). In some embodiments, the mEV composition comprises mEVs (such as smEVs) and/or a combination of mEVs (such as smEVs) described herein and a pharmaceutically acceptable carrier. In some embodiments, the smEV composition comprises smEVs and/or a combination of smEVs described herein and a pharmaceutically acceptable carrier.

**[231]** In some embodiments, the pharmaceutical compositions comprise mEVs (such as smEVs) substantially or entirely free of whole bacteria (e.g., live bacteria, killed bacteria, attenuated bacteria). In some embodiments, the pharmaceutical compositions comprise both

mEVs and whole bacteria (e.g., live bacteria, killed bacteria, attenuated bacteria). In some embodiments, the pharmaceutical compositions comprise mEVs from one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of the bacteria strains or species listed in Table 1, Table 2, and/or Table 3. In some embodiments, the pharmaceutical compositions comprise mEVs from one of the bacteria strains or species listed in Table 1, Table 2, and/or Table 3. In some embodiments, the pharmaceutical composition comprises lyophilized mEVs (such as smEVs). In some embodiments, the pharmaceutical composition comprises gamma irradiated mEVs (such as smEVs). The mEVs (such as smEVs) can be gamma irradiated after the mEVs are isolated (e.g., prepared).

**[232]** In some embodiments, to quantify the numbers of mEVs (such as smEVs) and/or bacteria present in a bacterial sample, electron microscopy (e.g., EM of ultrathin frozen sections) can be used to visualize the mEVs (such as smEVs) and/or bacteria and count their relative numbers. Alternatively, nanoparticle tracking analysis (NTA), Coulter counting, or dynamic light scattering (DLS) or a combination of these techniques can be used. NTA and the Coulter counter count particles and show their sizes. DLS gives the size distribution of particles, but not the concentration. Bacteria frequently have diameters of 1-2 um (microns). The full range is 0.2-20 um. Combined results from Coulter counting and NTA can reveal the numbers of bacteria and/or mEVs (such as smEVs) in a given sample. Coulter counting reveals the numbers of particles with diameters of 0.7-10 um. For most bacterial and/or mEV (such as smEV) samples, the Coulter counter alone can reveal the number of bacteria and/or mEVs (such as smEVs) in a sample. For NTA, a Nanosight instrument can be obtained from Malvern Pananlytical. For example, the NS300 can visualize and measure particles in suspension in the size range 10-2000nm. NTA allows for counting of the numbers of particles that are, for example, 50-1000 nm in diameter. DLS reveals the distribution of particles of different diameters within an approximate range of 1 nm – 3 um.

**[233]** mEVs can be characterized by analytical methods known in the art (e.g., Jeppesen, et al. Cell 177:428 (2019)).

**[234]** In some embodiments, the mEVs may be quantified based on particle count. For example, total protein content of an mEV preparation can be measured using NTA.

**[235]** In some embodiments, the mEVs may be quantified based on the amount of protein, lipid, or carbohydrate. For example, total protein content of an mEV preparation can be measured using the Bradford assay.

**[236]** In some embodiments, the mEVs are isolated away from one or more other bacterial components of the source bacteria. In some embodiments, the pharmaceutical composition further comprises other bacterial components.

**[237]** In certain embodiments, the mEV preparation obtained from the source bacteria may be fractionated into subpopulations based on the physical properties (e.g., sized, density, protein content, binding affinity) of the subpopulations. One or more of the mEV subpopulations can then be incorporated into the pharmaceutical compositions of the invention.

**[238]** In certain aspects, provided herein are pharmaceutical compositions comprising mEVs (such as smEVs) useful for the treatment and/or prevention of disease (e.g., a cancer, an autoimmune disease, an inflammatory disease, or a metabolic disease), as well as methods of making and/or identifying such mEVs, and methods of using such pharmaceutical compositions (e.g., for the treatment of a cancer, an autoimmune disease, an inflammatory disease, or a metabolic disease, either alone or in combination with other therapeutics). In some embodiments, the pharmaceutical compositions comprise both mEVs (such as smEVs), and whole bacteria (e.g., live bacteria, killed bacteria, attenuated bacteria). In some embodiments, the pharmaceutical compositions comprise mEVs (such as smEVs) in the absence of bacteria. In some embodiments, the pharmaceutical compositions comprise mEVs (such as smEVs) and/or bacteria from one or more of the bacteria strains or species listed in Table 1, Table 2, and/or Table 3. In some embodiments, the pharmaceutical compositions comprise mEVs (such as smEVs) and/or bacteria from one of the bacteria strains or species listed in Table 1, Table 2, and/or Table 3.

**[239]** In certain aspects, provided are pharmaceutical compositions for administration to a subject (e.g., human subject). In some embodiments, the pharmaceutical compositions are combined with additional active and/or inactive materials in order to produce a final product, which may be in single dosage unit or in a multi-dose format. In some embodiments, the pharmaceutical composition is combined with an adjuvant such as an immuno-adjuvant (e.g., a STING agonist, a TLR agonist, or a NOD agonist).

**[240]** In some embodiments, the pharmaceutical composition comprises at least one carbohydrate.

**[241]** In some embodiments, the pharmaceutical composition comprises at least one lipid. In some embodiments the lipid comprises at least one fatty acid selected from lauric acid (12:0), myristic acid (14:0), palmitic acid (16:0), palmitoleic acid (16:1), margaric acid (17:0), heptadecenoic acid (17:1), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), linolenic acid (18:3), octadecatetraenoic acid (18:4), arachidic acid (20:0), eicosenoic acid (20:1), eicosadienoic acid (20:2), eicosatetraenoic acid (20:4), eicosapentaenoic acid (20:5) (EPA), docosanoic acid (22:0), docosenoic acid (22:1), docosapentaenoic acid (22:5), docosahexaenoic acid (22:6) (DHA), and tetracosanoic acid (24:0).

**[242]** In some embodiments, the pharmaceutical composition comprises at least one supplemental mineral or mineral source. Examples of minerals include, without limitation: chloride, sodium, calcium, iron, chromium, copper, iodine, zinc, magnesium, manganese, molybdenum, phosphorus, potassium, and selenium. Suitable forms of any of the foregoing minerals include soluble mineral salts, slightly soluble mineral salts, insoluble mineral salts, chelated minerals, mineral complexes, non-reactive minerals such as carbonyl minerals, and reduced minerals, and combinations thereof.

**[243]** In some embodiments, the pharmaceutical composition comprises at least one supplemental vitamin. The at least one vitamin can be fat-soluble or water soluble vitamins. Suitable vitamins include but are not limited to vitamin C, vitamin A, vitamin E, vitamin B12, vitamin K, riboflavin, niacin, vitamin D, vitamin B6, folic acid, pyridoxine, thiamine, pantothenic acid, and biotin. Suitable forms of any of the foregoing are salts of the vitamin, derivatives of the vitamin, compounds having the same or similar activity of the vitamin, and metabolites of the vitamin.

**[244]** In some embodiments, the pharmaceutical composition comprises an excipient. Non-limiting examples of suitable excipients include a buffering agent, a preservative, a stabilizer, a binder, a compaction agent, a lubricant, a dispersion enhancer, a disintegration agent, a flavoring agent, a sweetener, and a coloring agent.

**[245]** In some embodiments, the excipient is a buffering agent. Non-limiting examples of suitable buffering agents include sodium citrate, magnesium carbonate, magnesium bicarbonate, calcium carbonate, and calcium bicarbonate.

**[246]** In some embodiments, the excipient comprises a preservative. Non-limiting examples of suitable preservatives include antioxidants, such as alpha-tocopherol and ascorbate, and antimicrobials, such as parabens, chlorobutanol, and phenol.

**[247]** In some embodiments, the pharmaceutical composition comprises a binder as an excipient. Non-limiting examples of suitable binders include starches, pregelatinized starches, gelatin, polyvinylpyrolidone, cellulose, methylcellulose, sodium carboxymethylcellulose, ethylcellulose, polyacrylamides, polyvinyloxoazolidone, polyvinylalcohols, $C_{12}$-$C_{18}$ fatty acid alcohol, polyethylene glycol, polyols, saccharides, oligosaccharides, and combinations thereof.

**[248]** In some embodiments, the pharmaceutical composition comprises a lubricant as an excipient. Non-limiting examples of suitable lubricants include magnesium stearate, calcium stearate, zinc stearate, hydrogenated vegetable oils, sterotex, polyoxyethylene monostearate, talc, polyethyleneglycol, sodium benzoate, sodium lauryl sulfate, magnesium lauryl sulfate, and light mineral oil.

**[249]** In some embodiments, the pharmaceutical composition comprises a dispersion enhancer as an excipient. Non-limiting examples of suitable dispersants include starch, alginic acid, polyvinylpyrrolidones, guar gum, kaolin, bentonite, purified wood cellulose, sodium starch glycolate, isoamorphous silicate, and microcrystalline cellulose as high HLB emulsifier surfactants.

**[250]** In some embodiments, the pharmaceutical composition comprises a disintegrant as an excipient. In some embodiments the disintegrant is a non-effervescent disintegrant. Non-limiting examples of suitable non-effervescent disintegrants include starches such as corn starch, potato starch, pregelatinized and modified starches thereof, sweeteners, clays, such as bentonite, micro-crystalline cellulose, alginates, sodium starch glycolate, gums such as agar, guar, locust bean, karaya, pectin, and tragacanth. In some embodiments the disintegrant is an effervescent disintegrant. Non-limiting examples of suitable effervescent disintegrants include sodium bicarbonate in combination with citric acid, and sodium bicarbonate in combination with tartaric acid.

**[251]** In some embodiments, the pharmaceutical composition is a food product (*e.g.*, a food or beverage) such as a health food or beverage, a food or beverage for infants, a food or beverage for pregnant women, athletes, senior citizens or other specified group, a functional food, a beverage, a food or beverage for specified health use, a dietary supplement, a food or

beverage for patients, or an animal feed. Specific examples of the foods and beverages include various beverages such as juices, refreshing beverages, tea beverages, drink preparations, jelly beverages, and functional beverages; alcoholic beverages such as beers; carbohydrate-containing foods such as rice food products, noodles, breads, and pastas; paste products such as fish hams, sausages, paste products of seafood; retort pouch products such as curries, food dressed with a thick starchy sauces, and Chinese soups; soups; dairy products such as milk, dairy beverages, ice creams, cheeses, and yogurts; fermented products such as fermented soybean pastes, yogurts, fermented beverages, and pickles; bean products; various confectionery products, including biscuits, cookies, and the like, candies, chewing gums, gummies, cold desserts including jellies, cream caramels, and frozen desserts; instant foods such as instant soups and instant soy-bean soups; microwavable foods; and the like. Further, the examples also include health foods and beverages prepared in the forms of powders, granules, tablets, capsules, liquids, pastes, and jellies.

**[252]** In some embodiments, the pharmaceutical composition is a food product for animals, including humans. The animals, other than humans, are not particularly limited, and the composition can be used for various livestock, poultry, pets, experimental animals, and the like. Specific examples of the animals include pigs, cattle, horses, sheep, goats, chickens, wild ducks, ostriches, domestic ducks, dogs, cats, rabbits, hamsters, mice, rats, monkeys, and the like, but the animals are not limited thereto.

Dose Forms

**[253]** A pharmaceutical composition comprising mEVs (such as smEVs) can be formulated as a solid dose form, e.g., for oral administration. The solid dose form can comprise one or more excipients, e.g., pharmaceutically acceptable excipients. The mEVs in the solid dose form can be isolated mEVs. Optionally, the mEVs in the solid dose form can be lyophilized. Optionally, the mEVs in the solid dose form are gamma irradiated. The solid dose form can comprise a tablet, a minitablet, a capsule, a pill, or a powder; or a combination of these forms (e.g., minitablets comprised in a capsule).

**[254]** The solid dose form can comprise a tablet (e.g., > 4mm).

**[255]** The solid dose form can comprise a mini tablet (e.g., 1-4 mm sized minitablet, e.g., a 2mm minitablet or a 3mm minitablet).

**[256]** The solid dose form can comprise a capsule, e.g., a size 00, size 0, size 1, size 2, size 3, size 4, or size 5 capsule; e.g., a size 0 capsule.

**[257]** The solid dose form can comprise a coating. The solid dose form can comprise a single layer coating, e.g., enteric coating, e.g., a Eudragit-based coating, e.g., EUDRAGIT L30 D-55, triethylcitrate, and talc. The solid dose form can comprise two layers of coating. For example, an inner coating can comprise, e.g., EUDRAGIT L30 D-55, triethylcitrate, talc, citric acid anhydrous, and sodium hydroxide, and an outer coating can comprise, e.g., EUDRAGIT L30 D-55, triethylcitrate, and talc. EUDRAGIT is the brand name for a diverse range of polymethacrylate-based copolymers. It includes anionic, cationic, and neutral copolymers based on methacrylic acid and methacrylic/acrylic esters or their derivatives. Eudragits are amorphous polymers having glass transition temperatures between 9 to > 150°C. Eudragits are non-biodegradable, nonabsorbable, and nontoxic. Anionic Eudragit L dissolves at pH > 6 and is used for enteric coating, while Eudragit S, soluble at pH > 7 is used for colon targeting. Eudragit RL and RS, having quaternary ammonium groups, are water insoluble, but swellable/permeable polymers which are suitable for the sustained release film coating applications. Cationic Eudragit E, insoluble at pH ≥ 5, can prevent drug release in saliva.

**[258]** The solid dose form (e.g., a capsule) can comprise a single layer coating, e.g., a non-enteric coating such as HPMC (hydroxyl propyl methyl cellulose) or gelatin.

**[259]** A pharmaceutical composition comprising mEVs (such as smEVs) can be formulated as a suspension, e.g., for oral administration or for injection. Administration by injection includes intravenous (IV), intramuscular (IM), intratumoral (IT) and subcutaneous (SC) administration. For a suspension, mEVs can be in a buffer, e.g., a pharmaceutically acceptable buffer, e.g., saline or PBS. The suspension can comprise one or more excipients, e.g., pharmaceutically acceptable excipients. The suspension can comprise, e.g., sucrose or glucose. The mEVs in the suspension can be isolated mEVs. Optionally, the mEVs in the suspension can be lyophilized. Optionally, the mEVs in the suspension can be gamma irradiated.

Dosage

**[260]** For oral administration to a human subject, the dose of mEVs (such as smEVs) can be, e.g., about $2x10^6$- about $2x10^{16}$ particles. The dose can be, e.g., about $1x10^7$- about $1x10^{15}$, about $1x10^8$- about $1x10^{14}$, about $1x10^9$- about $1x10^{13}$, about $1x10^{10}$- about $1x10^{14}$, or

about $1x10^8$- about $1x10^{12}$ particles. The dose can be, e.g., about $2x10^6$, about $2x10^7$, about $2x10^8$, about $2x10^9$, about $1x10^{10}$, about $2x10^{10}$, about $2x10^{11}$, about $2x10^{12}$, about $2x10^{13}$, about $2x10^{14}$, or about $1x10^{15}$ particles. The dose can be, e.g., about $2x10^{14}$ particles. The dose can be, e.g., about $2x10^{12}$ particles. The dose can be, e.g., about $2x10^{10}$ particles. The dose can be, e.g., about $1x10^{10}$ particles. Particle count can be determined, e.g., by NTA.

**[261]** For oral administration to a human subject, the dose of mEVs (such as smEVs) can be, e.g., based on total protein. The dose can be, e.g., about 5 mg to about 900 mg total protein. The dose can be, e.g., about 20 mg to about 800 mg, about 50 mg to about 700 mg, about 75 mg to about 600 mg, about 100 mg to about 500 mg, about 250 mg to about 750 mg, or about 200 mg to about 500 mg total protein. The dose can be, e.g., about 10 mg, about 25 mg, about 50 mg, about 75 mg, about 100 mg, about 150 mg, about 200 mg, about 250 mg, about 300 mg, about 400 mg, about 500 mg, about 600 mg, or about 750 mg total protein. Total protein can be determined, e.g., by Bradford assay.

**[262]** For administration by injection (e.g., intravenous administration) to a human subject, the dose of mEVs (such as smEVs) can be, e.g., about $1x10^6$- about $1x10^{16}$ particles. The dose can be, e.g., about $1x10^7$- about $1x10^{15}$, about $1x10^8$- about $1x10^{14}$, about $1x10^9$- about $1x10^{13}$, about $1x10^{10}$- about $1x10^{14}$, or about $1x10^8$- about $1x10^{12}$ particles. The dose can be, e.g., about $2x10^6$, about $2x10^7$, about $2x10^8$, about $2x10^9$, about $1x10^{10}$, about $2x10^{10}$, about $2x10^{11}$, about $2x10^{12}$, about $2x10^{13}$, about $2x10^{14}$, or about $1x10^{15}$ particles. The dose can be, e.g., about $1x10^{15}$ particles. The dose can be, e.g., about $2x10^{14}$ particles. The dose can be, e.g., about $2x10^{13}$ particles. Particle count can be determined, e.g., by NTA.

**[263]** For administration by injection (e.g., intravenous administration), the dose of mEVs (such as smEVs) can be, e.g., about 5 mg to about 900 mg total protein. The dose can be, e.g., about 20 mg to about 800 mg, about 50 mg to about 700 mg, about 75 mg to about 600 mg, about 100 mg to about 500 mg, about 250 mg to about 750 mg, or about 200 mg to about 500 mg total protein. The dose can be, e.g., about 10 mg, about 25 mg, about 50 mg, about 75 mg, about 100 mg, about 150 mg, about 200 mg, about 250 mg, about 300 mg, about 400 mg, about 500 mg, about 600 mg, or about 750 mg total protein. The dose can be, e.g., about 700 mg total protein. The dose can be, e.g., about 350 mg total protein. The dose can be, e.g., about 175 mg total protein. Total protein can be determined, e.g., by Bradford assay.

Gamma-irradiation

**[264]** Powders (e.g., of mEVs (such as smEVs)) can be gamma-irradiated at 17.5 kGy radiation unit at ambient temperature.

**[265]** Frozen biomasses (e.g., of mEVs (such as smEVs)) can be gamma-irradiated at 25 kGy radiation unit in the presence of dry ice.

Additional Therapeutic Agents

**[266]** In certain aspects, the methods provided herein include the administration to a subject of a pharmaceutical composition described herein either alone or in combination with an additional therapeutic agent. In some embodiments, the additional therapeutic agent is an immunosuppressant, an anti-inflammatory agent, a steroid, and/or a cancer therapeutic.

**[267]** In some embodiments, the pharmaceutical composition comprising mEVs (such as smEVs) is administered to the subject before the additional therapeutic agent is administered (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24 hours before or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 days before). In some embodiments , the pharmaceutical composition comprising mEVs (such as smEVs) is administered to the subject after the additional therapeutic agent is administered (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24 hours after or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 days after). In some embodiments, the pharmaceutical composition comprising mEVs (such as smEVs) and the additional therapeutic agent are administered to the subject simultaneously or nearly simultaneously (e.g., administrations occur within an hour of each other).

**[268]** In some embodiments, an antibiotic is administered to the subject before the pharmaceutical composition comprising mEVs (such as smEVs) is administered to the subject (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24 hours before or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 days before). In some embodiments, an antibiotic is administered to the subject after pharmaceutical composition comprising mEVs (such as smEVs) is administered to the subject (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24 hours before or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16,

17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 days after). In some embodiments, the pharmaceutical composition comprising mEVs (such as smEVs) and the antibiotic are administered to the subject simultaneously or nearly simultaneously (e.g., administrations occur within an hour of each other).

**[269]** In some embodiments, the additional therapeutic agent is a cancer therapeutic. In some embodiments, the cancer therapeutic is a chemotherapeutic agent. Examples of such chemotherapeutic agents include, but are not limited to, alkylating agents such as thiotepa and cyclosphosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethiylenethiophosphoramide and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including the synthetic analogue topotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimnustine; antibiotics such as the enediyne antibiotics (e.g., calicheamicin, especially calicheamicin gammalI and calicheamicin omegal1; dynemicin, including dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromophores, aclacinomysins, actinomycin, authrarnycin, azaserine, bleomycins, cactinomycin, carabicin, caminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine

analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK polysaccharide complex); razoxane; rhizoxin; sizofuran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, *e.g.*, paclitaxel and doxetaxel; chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum coordination complexes such as cisplatin, oxaliplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; vinorelbine; novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; irinotecan (*e.g.*, CPT-11); topoisomerase inhibitor RFS 2000; difluoromethylomithine (DMFO); retinoids such as retinoic acid; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

**[270]** In some embodiments, the cancer therapeutic is a cancer immunotherapy agent. Immunotherapy refers to a treatment that uses a subject's immune system to treat cancer, *e.g.*, checkpoint inhibitors, cancer vaccines, cytokines, cell therapy, CAR-T cells, and dendritic cell therapy. Non-limiting examples of immunotherapies are checkpoint inhibitors include Nivolumab (BMS, anti-PD-1), Pembrolizumab (Merck, anti-PD-1), Ipilimumab (BMS, anti-CTLA-4), MEDI4736 (AstraZeneca, anti-PD-L1), and MPDL3280A (Roche, anti-PD-L1). Other immunotherapies may be tumor vaccines, such as Gardail, Cervarix, BCG, sipulencel-T, Gp100:209-217, AGS-003, DCVax-L, Algenpantucel-L, Tergenpantucel-L, TG4010, ProstAtak, Prostvac-V/R-TRICOM, Rindopepimul, E75 peptide acetate, IMA901, POL-103A, Belagenpumatucel-L, GSK1572932A, MDX-1279, GV1001, and Tecemotide. The

immunotherapy agent may be administered via injection (e.g., intravenously, intratumorally, subcutaneously, or into lymph nodes), but may also be administered orally, topically, or via aerosol. Immunotherapies may comprise adjuvants such as cytokines.

**[271]** In some embodiments, the immunotherapy agent is an immune checkpoint inhibitor. Immune checkpoint inhibition broadly refers to inhibiting the checkpoints 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 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, MEDI-4736, MSB-0010718C (avelumab), AUR-012 and STI-A1010.

**[272]** In some embodiments, the methods provided herein include the administration of a pharmaceutical composition described herein in combination with one or more additional therapeutic agents. In some embodiments, the methods disclosed herein include the administration of two immunotherapy agents (e.g., immune checkpoint inhibitor). For example, the methods provided herein include the administration of a pharmaceutical composition described herein in combination with a PD-1 inhibitor (such as pemrolizumab or nivolumab or pidilizumab) or a CLTA-4 inhibitor (such as ipilimumab) or a PD-L1 inhibitor (such as avelumab).

**[273]** In some embodiments, the immunotherapy agent is an antibody or antigen binding fragment thereof that, for example, binds to a cancer-associated antigen. Examples of cancer-associated antigens include, but are not limited to, adipophilin, AIM-2, ALDH1A1, alpha-actinin-4, alpha-fetoprotein ("AFP"), ARTC1, B-RAF, BAGE-1, BCLX (L), BCR-ABL fusion protein b3a2, beta-catenin, BING-4, CA-125, CALCA, carcinoembryonic antigen ("CEA"), CASP-5, CASP-8, CD274, CD45, Cdc27, CDK12, CDK4, CDKN2A, CEA, CLPP, COA-1, CPSF, CSNK1A1, CTAG1, CTAG2, cyclin D1, Cyclin-A1, dek-can fusion protein, DKK1, EFTUD2, Elongation factor 2, ENAH (hMena), Ep-CAM, EpCAM, EphA3, epithelial tumor antigen ("ETA"), ETV6-AML1 fusion protein, EZH2, FGF5, FLT3-ITD, FN1, G250/MN/CAIX, GAGE-1,2,8, GAGE-3,4,5,6,7, GAS7, glypican-3, GnTV, gp100/Pmel17, GPNMB, HAUS3, Hepsin, HER-2/neu, HERV-K-MEL, HLA-A11, HLA-A2, HLA-DOB, hsp70-2, IDO1, IGF2B3,

IL13Ralpha2, Intestinal carboxyl esterase, K-ras, Kallikrein 4, KIF20A, KK-LC-1, KKLC1, KM-HN-1, KMHN1 also known as CCDC110, LAGE-1, LDLR-fucosyltransferaseAS fusion protein, Lengsin, M-CSF, MAGE-A1, MAGE-A10, MAGE-A12, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A6, MAGE-A9, MAGE-C1, MAGE-C2, malic enzyme, mammaglobin-A, MART2, MATN, MC1R, MCSP, mdm-2, ME1, Melan-A/MART-1, Meloe, Midkine, MMP-2, MMP-7, MUC1, MUC5AC, mucin, MUM-1, MUM-2, MUM-3, Myosin, Myosin class I, N-raw, NA88-A, neo-PAP, NFYC, NY-BR-1, NY-ESO-1/LAGE-2, OA1, OGT, OS-9, P polypeptide, p53, PAP, PAX5, PBF, pml-RARalpha fusion protein, polymorphic epithelial mucin ("PEM"), PPP1R3B, PRAME, PRDX5, PSA, PSMA, PTPRK, RAB38/NY-MEL-1, RAGE-1, RBAF600, RGS5, RhoC, RNF43, RU2AS, SAGE, secernin 1, SIRT2, SNRPD1, SOX10, Sp17, SPA17, SSX-2, SSX-4, STEAP1, survivin, SYT-SSX1 or -SSX2 fusion protein, TAG-1, TAG-2, Telomerase, TGF-betaRII, TPBG, TRAG-3, Triosephosphate isomerase, TRP-1/gp75, TRP-2, TRP2-INT2, tyrosinase, tyrosinase ("TYR"), VEGF, WT1, XAGE-1b/GAGED2a. In some embodiments, the antigen is a neo-antigen.

**[274]** In some embodiments, the immunotherapy agent is a cancer vaccine and/or a component of a cancer vaccine (*e.g.,* an antigenic peptide and/or protein). The cancer vaccine can be a protein vaccine, a nucleic acid vaccine or a combination thereof. For example, in some embodiments, the cancer vaccine comprises a polypeptide comprising an epitope of a cancer-associated antigen. In some embodiments, the cancer vaccine comprises a nucleic acid (*e.g.,* DNA or RNA, such as mRNA) that encodes an epitope of a cancer-associated antigen. Examples of cancer-associated antigens include, but are not limited to, adipophilin, AIM-2, ALDH1A1, alpha-actinin-4, alpha-fetoprotein ("AFP"), ARTC1, B-RAF, BAGE-1, BCLX (L), BCR-ABL fusion protein b3a2, beta-catenin, BING-4, CA-125, CALCA, carcinoembryonic antigen ("CEA"), CASP-5, CASP-8, CD274, CD45, Cdc27, CDK12, CDK4, CDKN2A, CEA, CLPP, COA-1, CPSF, CSNK1A1, CTAG1, CTAG2, cyclin D1, Cyclin-A1, dek-can fusion protein, DKK1, EFTUD2, Elongation factor 2, ENAH (hMena), Ep-CAM, EpCAM, EphA3, epithelial tumor antigen ("ETA"), ETV6-AML1 fusion protein, EZH2, FGF5, FLT3-ITD, FN1, G250/MN/CAIX, GAGE-1,2,8, GAGE-3,4,5,6,7, GAS7, glypican-3, GnTV, gp100/Pmel17, GPNMB, HAUS3, Hepsin, HER-2/neu, HERV-K-MEL, HLA-A11, HLA-A2, HLA-DOB, hsp70-2, IDO1, IGF2B3, IL13Ralpha2, Intestinal carboxyl esterase, K-ras, Kallikrein 4, KIF20A, KK-LC-1, KKLC1, KM-HN-1, KMHN1 also known as CCDC110, LAGE-1, LDLR-

fucosyltransferaseAS fusion protein, Lengsin, M-CSF, MAGE-A1, MAGE-A10, MAGE-A12, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A6, MAGE-A9, MAGE-C1, MAGE-C2, malic enzyme, mammaglobin-A, MART2, MATN, MC1R, MCSP, mdm-2, ME1, Melan-A/MART-1, Meloe, Midkine, MMP-2, MMP-7, MUC1, MUC5AC, mucin, MUM-1, MUM-2, MUM-3, Myosin, Myosin class I, N-raw, NA88-A, neo-PAP, NFYC, NY-BR-1, NY-ESO-1/LAGE-2, OA1, OGT, OS-9, P polypeptide, p53, PAP, PAX5, PBF, pml-RARalpha fusion protein, polymorphic epithelial mucin ("PEM"), PPP1R3B, PRAME, PRDX5, PSA, PSMA, PTPRK, RAB38/NY-MEL-1, RAGE-1, RBAF600, RGS5, RhoC, RNF43, RU2AS, SAGE, secernin 1, SIRT2, SNRPD1, SOX10, Sp17, SPA17, SSX-2, SSX-4, STEAP1, survivin, SYT-SSX1 or -SSX2 fusion protein, TAG-1, TAG-2, Telomerase, TGF-betaRII, TPBG, TRAG-3, Triosephosphate isomerase, TRP-1/gp75, TRP-2, TRP2-INT2, tyrosinase, tyrosinase ("TYR"), VEGF, WT1, XAGE-1b/GAGED2a. In some embodiments, the antigen is a neo-antigen. In some embodiments, the cancer vaccine is administered with an adjuvant. Examples of adjuvants include, but are not limited to, an immune modulatory protein, Adjuvant 65, α-GalCer, aluminum phosphate, aluminum hydroxide, calcium phosphate, β-Glucan Peptide, CpG ODN DNA, GPI-0100, lipid A, lipopolysaccharide, Lipovant, Montanide, N-acetyl-muramyl-L-alanyl-D-isoglutamine, Pam3CSK4, quil A , cholera toxin (CT) and heat-labile toxin from enterotoxigenic *Escherichia coli* (LT) including derivatives of these (CTB, mmCT, CTA1-DD, LTB, LTK63, LTR72, dmLT) and trehalose dimycolate.

**[275]** In some embodiments, the immunotherapy agent is an immune modulating protein to the subject. In some embodiments, the immune modulatory protein is a cytokine or chemokine. Examples of immune modulating proteins include, but are not limited to, B lymphocyte chemoattractant ("BLC"), C-C motif chemokine 11 ("Eotaxin-1"), Eosinophil chemotactic protein 2 ("Eotaxin-2"), Granulocyte colony-stimulating factor ("G-CSF"), Granulocyte macrophage colony-stimulating factor ("GM-CSF"), 1-309, Intercellular Adhesion Molecule 1 ("ICAM-1"), Interferon alpha ("IFN-alpha"), Interferon beta ("IFN-beta") Interferon gamma ("IFN-gamma"), Interlukin-1 alpha ("IL-1 alpha"), Interlukin-1 beta ("IL-1 beta"), Interleukin 1 receptor antagonist ("IL-1 ra"), Interleukin-2 ("IL-2"), Interleukin-4 ("IL-4"), Interleukin-5 ("IL-5"), Interleukin-6 ("IL-6"), Interleukin-6 soluble receptor ("IL-6 sR"), Interleukin-7 ("IL-7"), Interleukin-8 ("IL-8"), Interleukin- 10 ("IL-10"), Interleukin- 11 ("IL-11"), Subunit beta of Interleukin- 12 ("IL-12 p40" or "IL-12 p70"), Interleukin-13 ("IL-13"),

Interleukin-15 ("IL-15"), Interleukin-16 ("IL-16"), Interleukin-17A-F ("IL-17A-F"), Interleukin-18 ("IL-18"), Interleukin-21 ("IL-21"), Interleukin-22 ("IL-22"), Interleukin-23 ("IL-23"), Interleukin-33 ("IL-33"), Chemokine (C-C motif) Ligand 2 ("MCP-1"), Macrophage colony-stimulating factor ("M-CSF"), Monokine induced by gamma interferon ("MIG"), Chemokine (C-C motif) ligand 2 ("MIP-1 alpha"), Chemokine (C-C motif) ligand 4 ("MIP-1 beta"), Macrophage inflammatory protein- 1 -delta ("MIP-1 delta"), Platelet-derived growth factor subunit B ("PDGF-BB"), Chemokine (C-C motif) ligand 5, Regulated on Activation, Normal T cell Expressed and Secreted ("RANTES"), TIMP metallopeptidase inhibitor 1 ("TIMP-1"), TIMP metallopeptidase inhibitor 2 ("TIMP-2"), Tumor necrosis factor, lymphotoxin-alpha ("TNF alpha"), Tumor necrosis factor, lymphotoxin-beta ("TNF beta"), Soluble TNF receptor type 1 ("sTNFRI"), sTNFRIIAR, Brain-derived neurotrophic factor ("BDNF"), Basic fibroblast growth factor ("bFGF"), Bone morphogenetic protein 4 ("BMP-4"), Bone morphogenetic protein 5 ("BMP-5"), Bone morphogenetic protein 7 ("BMP-7"), Nerve growth factor ("b-NGF"), Epidermal growth factor ("EGF"), Epidermal growth factor receptor ("EGFR"), Endocrine-gland-derived vascular endothelial growth factor ("EG-VEGF"), Fibroblast growth factor 4 ("FGF-4"), Keratinocyte growth factor ("FGF-7"), Growth differentiation factor 15 ("GDF-15"), Glial cell-derived neurotrophic factor ("GDNF"), Growth Hormone, Heparin-binding EGF-like growth factor ("HB-EGF"), Hepatocyte growth factor ("HGF"), Insulin-like growth factor binding protein 1 ("IGFBP-1"), Insulin-like growth factor binding protein 2 ("IGFBP-2"), Insulin-like growth factor binding protein 3 (" IGFBP-3"), Insulin-like growth factor binding protein 4 ("IGFBP-4"), Insulin-like growth factor binding protein 6 ("IGFBP-6"), Insulin-like growth factor 1 ("IGF-1"), Insulin, Macrophage colony-stimulating factor ("M-CSF R"), Nerve growth factor receptor ("NGF R"), Neurotrophin-3 ("NT-3"), Neurotrophin-4 ("NT-4"), Osteoclastogenesis inhibitory factor ("Osteoprotegerin"), Platelet-derived growth factor receptors ("PDGF-AA"), Phosphatidylinositol-glycan biosynthesis ("PIGF"), Skp, Cullin, F-box containing comples ("SCF"), Stem cell factor receptor ("SCF R"), Transforming growth factor alpha ("TGFalpha"), Transforming growth factor beta-1 ("TGF beta 1"), Transforming growth factor beta-3 ("TGF beta 3"), Vascular endothelial growth factor ("VEGF"), Vascular endothelial growth factor receptor 2 ("VEGFR2"), Vascular endothelial growth factor receptor 3 ("VEGFR3"), VEGF-D 6Ckine, Tyrosine-protein kinase receptor UFO ("Axl"), Betacellulin ("BTC"), Mucosae-associated epithelial chemokine ("CCL28"), Chemokine (C-C motif) ligand

27 ("CTACK"), Chemokine (C-X-C motif) ligand 16 ("CXCL16"), C-X-C motif chemokine 5 ("ENA-78"), Chemokine (C-C motif) ligand 26 ("Eotaxin-3"), Granulocyte chemotactic protein 2 ("GCP-2"), GRO, Chemokine (C-C motif) ligand 14 ("HCC-l"), Chemokine (C-C motif) ligand 16 ("HCC-4"), Interleukin-9 ("IL-9"), Interleukin-17 F ("IL-17F"), Interleukin- 18-binding protein ("IL-18 BPa"), Interleukin-28 A ("IL-28A"), Interleukin 29 ("IL-29"), Interleukin 31 ("IL-31"), C-X-C motif chemokine 10 ("IP-10"), Chemokine receptor CXCR3 ("I-TAC"), Leukemia inhibitory factor ("LIF"), Light, Chemokine (C motif) ligand ("Lymphotactin"), Monocyte chemoattractant protein 2 ("MCP-2"), Monocyte chemoattractant protein 3 ("MCP-3"), Monocyte chemoattractant protein 4 ("MCP-4"), Macrophage-derived chemokine ("MDC"), Macrophage migration inhibitory factor ("MIF"), Chemokine (C-C motif) ligand 20 ("MIP-3 alpha"), C-C motif chemokine 19 ("MIP-3 beta"), Chemokine (C-C motif) ligand 23 ("MPIF-1"), Macrophage stimulating protein alpha chain ("MSPalpha"), Nucleosome assembly protein 1-like 4 ("NAP-2"), Secreted phosphoprotein 1 ("Osteopontin"), Pulmonary and activation-regulated cytokine ("PARC"), Platelet factor 4 ("PF4"), Stroma cell-derived factor- 1 alpha ("SDF-1 alpha"), Chemokine (C-C motif) ligand 17 ("TARC"), Thymus-expressed chemokine ("TECK"), Thymic stromal lymphopoietin ("TSLP 4- IBB"), CD 166 antigen ("ALCAM"), Cluster of Differentiation 80 ("B7-1"), Tumor necrosis factor receptor superfamily member 17 ("BCMA"), Cluster of Differentiation 14 ("CD14"), Cluster of Differentiation 30 ("CD30"), Cluster of Differentiation 40 ("CD40 Ligand"), Carcinoembryonic antigen-related cell adhesion molecule 1 (biliary glycoprotein) ("CEACAM-1"), Death Receptor 6 ("DR6"), Deoxythymidine kinase ("Dtk"), Type 1 membrane glycoprotein ("Endoglin"), Receptor tyrosine-protein kinase erbB-3 ("ErbB3"), Endothelial-leukocyte adhesion molecule 1 ("E-Selectin"), Apoptosis antigen 1 ("Fas"), Fms-like tyrosine kinase 3 ("Flt-3L"), Tumor necrosis factor receptor superfamily member 1 ("GITR"), Tumor necrosis factor receptor superfamily member 14 ("HVEM"), Intercellular adhesion molecule 3 ("ICAM-3"), IL-1 R4, IL-1 RI, IL-10 Rbeta, IL-17R, IL-2Rgamma, IL-21R, Lysosome membrane protein 2 ("LIMPII"), Neutrophil gelatinase-associated lipocalin ("Lipocalin-2"), CD62L ("L-Selectin"), Lymphatic endothelium ("LYVE-1"), MHC class I polypeptide-related sequence A ("MICA"), MHC class I polypeptide-related sequence B ("MICB"), NRGl-betal, Beta-type platelet-derived growth factor receptor ("PDGF Rbeta"), Platelet endothelial cell adhesion molecule ("PECAM-1"), RAGE, Hepatitis A virus cellular receptor 1 ("TIM-1"), Tumor necrosis factor receptor superfamily member IOC ("TRAIL R3"),

Trappin protein transglutaminase binding domain ("Trappin-2"), Urokinase receptor ("uPAR"), Vascular cell adhesion protein 1 ("VCAM-1"), XEDARActivin A, Agouti-related protein ("AgRP"), Ribonuclease 5 ("Angiogenin"), Angiopoietin 1, Angiostatin, Catheprin S, CD40, Cryptic family protein IB ("Cripto-1"), DAN, Dickkopf-related protein 1 ("DKK-1"), E-Cadherin, Epithelial cell adhesion molecule ("EpCAM"), Fas Ligand (FasL or CD95L), Fcg RIIB/C, Follistatin, Galectin-7, Intercellular adhesion molecule 2 ("ICAM-2"), IL-13 Rl, IL-13R2, IL-17B, IL-2 Ra, IL-2 Rb, IL-23, LAP, Neuronal cell adhesion molecule ("NrCAM"), Plasminogen activator inhibitor- 1 ("PAI-1"), Platelet derived growth factor receptors ("PDGF-AB"), Resistin, stromal cell-derived factor 1 ("SDF-1 beta"), sgpl30, Secreted frizzled-related protein 2 ("ShhN"), Sialic acid-binding immunoglobulin-type lectins ("Siglec-5"), ST2, Transforming growth factor-beta 2 ("TGF beta 2"), Tie-2, Thrombopoietin ("TPO"), Tumor necrosis factor receptor superfamily member 10D ("TRAIL R4"), Triggering receptor expressed on myeloid cells 1 ("TREM-1"), Vascular endothelial growth factor C ("VEGF-C"), VEGFRlAdiponectin, Adipsin ("AND"), Alpha-fetoprotein ("AFP"), Angiopoietin-like 4 ("ANGPTL4"), Beta-2-microglobulin ("B2M"), Basal cell adhesion molecule ("BCAM"), Carbohydrate antigen 125 ("CA125"), Cancer Antigen 15-3 ("CA15-3"), Carcinoembryonic antigen ("CEA"), cAMP receptor protein ("CRP"), Human Epidermal Growth Factor Receptor 2 ("ErbB2"), Follistatin, Follicle-stimulating hormone ("FSH"), Chemokine (C-X-C motif) ligand 1 ("GRO alpha"), human chorionic gonadotropin ("beta HCG"), Insulin-like growth factor 1 receptor ("IGF-1 sR"), IL-1 sRII, IL-3, IL-18 Rb, IL-21, Leptin, Matrix metalloproteinase-1 ("MMP-1"), Matrix metalloproteinase-2 ("MMP-2"), Matrix metalloproteinase-3 ("MMP-3"), Matrix metalloproteinase-8 ("MMP-8"), Matrix metalloproteinase-9 ("MMP-9"), Matrix metalloproteinase-10 ("MMP-10"), Matrix metalloproteinase-13 ("MMP-13"), Neural Cell Adhesion Molecule ("NCAM-1"), Entactin ("Nidogen-1"), Neuron specific enolase ("NSE"), Oncostatin M ("OSM"), Procalcitonin, Prolactin, Prostate specific antigen ("PSA"), Sialic acid-binding Ig-like lectin 9 ("Siglec-9"), ADAM 17 endopeptidase ("TACE"), Thyroglobulin, Metalloproteinase inhibitor 4 ("TIMP-4"), TSH2B4, Disintegrin and metalloproteinase domain-containing protein 9 ("ADAM-9"), Angiopoietin 2, Tumor necrosis factor ligand superfamily member 13/ Acidic leucine-rich nuclear phosphoprotein 32 family member B ("APRIL"), Bone morphogenetic protein 2 ("BMP-2"), Bone morphogenetic protein 9 ("BMP-9"), Complement component 5a ("C5a"), Cathepsin L, CD200, CD97, Chemerin, Tumor necrosis factor receptor

superfamily member 6B ("DcR3"), Fatty acid-binding protein 2 ("FABP2"), Fibroblast activation protein, alpha ("FAP"), Fibroblast growth factor 19 ("FGF-19"), Galectin-3, Hepatocyte growth factor receptor ("HGF R"), IFN-gammalpha/beta R2, Insulin-like growth factor 2 ("IGF-2"), Insulin-like growth factor 2 receptor ("IGF-2 R"), Interleukin-1 receptor 6 ("IL-1R6"), Interleukin 24 ("IL-24"), Interleukin 33 ("IL-33", Kallikrein 14, Asparaginyl endopeptidase ("Legumain"), Oxidized low-density lipoprotein receptor 1 ("LOX-1"), Mannose-binding lectin ("MBL"), Neprilysin ("NEP"), Notch homolog 1, translocation-associated (Drosophila) ("Notch-1"), Nephroblastoma overexpressed ("NOV"), Osteoactivin, Programmed cell death protein 1 ("PD-1"), N-acetylmuramoyl-L-alanine amidase ("PGRP-5"), Serpin A4, Secreted frizzled related protein 3 ("sFRP-3"), Thrombomodulin, Tolllike receptor 2 ("TLR2"), Tumor necrosis factor receptor superfamily member 10A ("TRAIL Rl"), Transferrin ("TRF"), WIF-lACE-2, Albumin, AMICA, Angiopoietin 4, B-cell activating factor ("BAFF"), Carbohydrate antigen 19-9 ("CA19-9"), CD 163 , Clusterin, CRT AM, Chemokine (C-X-C motif) ligand 14 ("CXCL14"), Cystatin C, Decorin ("DCN"), Dickkopf-related protein 3 ("Dkk-3"), Delta-like protein 1 ("DLL1"), Fetuin A, Heparin-binding growth factor 1 ("aFGF"), Folate receptor alpha ("FOLR1"), Furin, GPCR-associated sorting protein 1 ("GASP-1"), GPCR-associated sorting protein 2 ("GASP-2"), Granulocyte colony-stimulating factor receptor ("GCSF R"), Serine protease hepsin ("HAI-2"), Interleukin-17B Receptor ("IL-17B R"), Interleukin 27 ("IL-27"), Lymphocyte-activation gene 3 ("LAG-3"), Apolipoprotein A-V ("LDL R"), Pepsinogen I, Retinol binding protein 4 ("RBP4"), SOST, Heparan sulfate proteoglycan ("Syndecan-1"), Tumor necrosis factor receptor superfamily member 13B ("TACI"), Tissue factor pathway inhibitor ("TFPI"), TSP-1, Tumor necrosis factor receptor superfamily, member 10b ("TRAIL R2"), TRANCE, Troponin I, Urokinase Plasminogen Activator ("uPA"), Cadherin 5, type 2 or VE-cadherin (vascular endothelial) also known as CD144 ("VE-Cadherin"), WNTl-inducible-signaling pathway protein 1 ("WISP-1"), and Receptor Activator of Nuclear Factor κ B ("RANK").

**[276]** In some embodiments, the cancer therapeutic is an anti-cancer compound. Exemplary anti-cancer compounds include, but are not limited to, Alemtuzumab (Campath®), Alitretinoin (Panretin®), Anastrozole (Arimidex®), Bevacizumab (Avastin®), Bexarotene (Targretin®), Bortezomib (Velcade®), Bosutinib (Bosulif®), Brentuximab vedotin (Adcetris®), Cabozantinib (Cometriq™), Carfilzomib (Kyprolis™), Cetuximab (Erbitux®), Crizotinib

(Xalkori®), Dasatinib (Sprycel®), Denileukin diftitox (Ontak®), Erlotinib hydrochloride (Tarceva®), Everolimus (Afinitor®), Exemestane (Aromasin®), Fulvestrant (Faslodex®), Gefitinib (Iressa®), Ibritumomab tiuxetan (Zevalin®), Imatinib mesylate (Gleevec®), Ipilimumab (Yervoy™), Lapatinib ditosylate (Tykerb®), Letrozole (Femara®), Nilotinib (Tasigna®), Ofatumumab (Arzerra®), Panitumumab (Vectibix®), Pazopanib hydrochloride (Votrient®), Pertuzumab (Perjeta™), Pralatrexate (Folotyn®), Regorafenib (Stivarga®), Rituximab (Rituxan®), Romidepsin (Istodax®), Sorafenib tosylate (Nexavar®), Sunitinib malate (Sutent®), Tamoxifen, Temsirolimus (Torisel®), Toremifene (Fareston®), Tositumomab and 131I-tositumomab (Bexxar®), Trastuzumab (Herceptin®), Tretinoin (Vesanoid®), Vandetanib (Caprelsa®), Vemurafenib (Zelboraf®), Vorinostat (Zolinza®), and Ziv-aflibercept (Zaltrap®).

**[277]** Exemplary anti-cancer compounds that modify the function of proteins that regulate gene expression and other cellular functions (*e.g.*, HDAC inhibitors, retinoid receptor ligants) are Vorinostat (Zolinza®), Bexarotene (Targretin®) and Romidepsin (Istodax®), Alitretinoin (Panretin®), and Tretinoin (Vesanoid®).

**[278]** Exemplary anti-cancer compounds that induce apoptosis (*e.g.*, proteasome inhibitors, antifolates) are Bortezomib (Velcade®), Carfilzomib (Kyprolis™), and Pralatrexate (Folotyn®).

**[279]** Exemplary anti-cancer compounds that increase anti-tumor immune response (*e.g.*, anti CD20, anti CD52; anti-cytotoxic T-lymphocyte-associated antigen-4) are Rituximab (Rituxan®), Alemtuzumab (Campath®), Ofatumumab (Arzerra®), and Ipilimumab (Yervoy™).

**[280]** Exemplary anti-cancer compounds that deliver toxic agents to cancer cells (*e.g.*, anti-CD20-radionuclide fusions; IL-2-diphtheria toxin fusions; anti-CD30- monomethylauristatin E (MMAE)-fusions) are Tositumomab and 131I-tositumomab (Bexxar®)and Ibritumomab tiuxetan (Zevalin®), Denileukin diftitox (Ontak®), and Brentuximab vedotin (Adcetris®).

**[281]** Other exemplary anti-cancer compounds are small molecule inhibitors and conjugates thereof of, *e.g.*, Janus kinase, ALK, Bcl-2, PARP, PI3K, VEGF receptor, Braf, MEK, CDK, and HSP90.

**[282]** Exemplary platinum-based anti-cancer compounds include, for example, cisplatin, carboplatin, oxaliplatin, satraplatin, picoplatin, Nedaplatin, Triplatin, and Lipoplatin. Other metal-based drugs suitable for treatment include, but are not limited to ruthenium-based compounds, ferrocene derivatives, titanium-based compounds, and gallium-based compounds.

**[283]** In some embodiments, the cancer therapeutic is a radioactive moiety that comprises a radionuclide. Exemplary radionuclides include, but are not limited to Cr-51, Cs-131, Ce-134, Se-75, Ru-97, I-125, Eu-149, Os-189m, Sb-119, I-123, Ho-161, Sb-117, Ce-139, In-111, Rh-103m, Ga-67, Tl-201, Pd-103, Au-195, Hg-197, Sr-87m, Pt-191, P-33, Er-169, Ru-103, Yb-169, Au-199, Sn-121, Tm-167, Yb-175, In-113m, Sn-113, Lu-177, Rh-105, Sn-117m, Cu-67, Sc-47, Pt-195m, Ce-141, I-131, Tb-161, As-77, Pt-197, Sm-153, Gd-159, Tm-173, Pr-143, Au-198, Tm-170, Re-186, Ag-111, Pd-109, Ga-73, Dy-165, Pm-149, Sn-123, Sr-89, Ho-166, P-32, Re-188, Pr-142, Ir-194, In-114m/In-114, and Y-90.

**[284]** In some embodiments, the cancer therapeutic is an antibiotic. For example, if the presence of a cancer-associated bacteria and/or a cancer-associated microbiome profile is detected according to the methods provided herein, antibiotics can be administered to eliminate the cancer-associated bacteria from the subject. "Antibiotics" broadly refers to compounds capable of inhibiting or preventing a bacterial infection. Antibiotics can be classified in a number of ways, including their use for specific infections, their mechanism of action, their bioavailability, or their spectrum of target microbe (e.g., Gram-negative vs. Gram-positive bacteria, aerobic vs. anaerobic bacteria, etc.) and these may be used to kill specific bacteria in specific areas of the host ("niches") (Leekha, et al 2011. General Principles of Antimicrobial Therapy. Mayo Clin Proc. 86(2): 156-167). In certain embodiments, antibiotics can be used to selectively target bacteria of a specific niche. In some embodiments, antibiotics known to treat a particular infection that includes a cancer niche may be used to target cancer-associated microbes, including cancer-associated bacteria in that niche. In other embodiments, antibiotics are administered after the pharmaceutical composition comprising mEVs (such as smEVs). In some embodiments, antibiotics are administered before pharmaceutical composition comprising mEVs (such as smEVs).

**[285]** In some aspects, antibiotics can be selected based on their bactericidal or bacteriostatic properties. Bactericidal antibiotics include mechanisms of action that disrupt the cell wall (e.g., β-lactams), the cell membrane (e.g., daptomycin), or bacterial DNA (e.g., fluoroquinolones). Bacteriostatic agents inhibit bacterial replication and include sulfonamides, tetracyclines, and macrolides, and act by inhibiting protein synthesis. Furthermore, while some drugs can be bactericidal in certain organisms and bacteriostatic in others, knowing the target organism allows one skilled in the art to select an antibiotic with the appropriate properties. In

certain treatment conditions, bacteriostatic antibiotics inhibit the activity of bactericidal antibiotics. Thus, in certain embodiments, bactericidal and bacteriostatic antibiotics are not combined.

**[286]** Antibiotics include, but are not limited to aminoglycosides, ansamycins, carbacephems, carbapenems, cephalosporins, glycopeptides, lincosamides, lipopeptides, macrolides, monobactams, nitrofurans, oxazolidonones, penicillins, polypeptide antibiotics, quinolones, fluoroquinolone, sulfonamides, tetracyclines, and anti-mycobacterial compounds, and combinations thereof.

**[287]** Aminoglycosides include, but are not limited to Amikacin, Gentamicin, Kanamycin, Neomycin, Netilmicin, Tobramycin, Paromomycin, and Spectinomycin. Aminoglycosides are effective, e.g., against Gram-negative bacteria, such as Escherichia coli, Klebsiella, Pseudomonas aeruginosa, and Francisella tularensis, and against certain aerobic bacteria but less effective against obligate/facultative anaerobes. Aminoglycosides are believed to bind to the bacterial 30S or 50S ribosomal subunit thereby inhibiting bacterial protein synthesis.

**[288]** Ansamycins include, but are not limited to, Geldanamycin, Herbimycin, Rifamycin, and Streptovaricin. Geldanamycin and Herbimycin are believed to inhibit or alter the function of Heat Shock Protein 90.

**[289]** Carbacephems include, but are not limited to, Loracarbef. Carbacephems are believed to inhibit bacterial cell wall synthesis.

**[290]** Carbapenems include, but are not limited to, Ertapenem, Doripenem, Imipenem/Cilastatin, and Meropenem. Carbapenems are bactericidal for both Gram-positive and Gram-negative bacteria as broad-spectrum antibiotics. Carbapenems are believed to inhibit bacterial cell wall synthesis.

**[291]** Cephalosporins include, but are not limited to, Cefadroxil, Cefazolin, Cefalotin, Cefalothin, Cefalexin, Cefaclor, Cefamandole, Cefoxitin, Cefprozil, Cefuroxime, Cefixime, Cefdinir, Cefditoren, Cefoperazone, Cefotaxime, Cefpodoxime, Ceftazidime, Ceftibuten, Ceftizoxime, Ceftriaxone, Cefepime, Ceftaroline fosamil,and Ceftobiprole. Selected Cephalosporins are effective, e.g., against Gram-negative bacteria and against Gram-positive bacteria, including *Pseudomonas*, certain Cephalosporins are effective against methicillin-

resistant *Staphylococcus aureus* (MRSA). Cephalosporins are believed to inhibit bacterial cell wall synthesis by disrupting synthesis of the peptidoglycan layer of bacterial cell walls.

**[292]** Glycopeptides include, but are not limited to, Teicoplanin, Vancomycin, and Telavancin. Glycopeptides are effective, e.g., against aerobic and anaerobic Gram-positive bacteria including MRSA and *Clostridium difficile*. Glycopeptides are believed to inhibit bacterial cell wall synthesis by disrupting synthesis of the peptidoglycan layer of bacterial cell walls.

**[293]** Lincosamides include, but are not limited to, Clindamycin and Lincomycin. Lincosamides are effective, e.g., against anaerobic bacteria, as well as Staphylococcus, and Streptococcus. Lincosamides are believed to bind to the bacterial 50S ribosomal subunit thereby inhibiting bacterial protein synthesis.

**[294]** Lipopeptides include, but are not limited to, Daptomycin. Lipopeptides are effective, e.g., against Gram-positive bacteria. Lipopeptides are believed to bind to the bacterial membrane and cause rapid depolarization.

**[295]** Macrolides include, but are not limited to, Azithromycin, Clarithromycin, Dirithromycin, Erythromycin, Roxithromycin, Troleandomycin, Telithromycin, and Spiramycin. Macrolides are effective, e.g., against Streptococcus and Mycoplasma. Macrolides are believed to bind to the bacterial or 50S ribosomal subunit, thereby inhibiting bacterial protein synthesis.

**[296]** Monobactams include, but are not limited to, Aztreonam. Monobactams are effective, *e.g.*, against Gram-negative bacteria. Monobactams are believed to inhibit bacterial cell wall synthesis by disrupting synthesis of the peptidoglycan layer of bacterial cell walls.

**[297]** Nitrofurans include, but are not limited to, Furazolidone and Nitrofurantoin.

**[298]** Oxazolidonones include, but are not limited to, Linezolid, Posizolid, Radezolid, and Torezolid. Oxazolidonones are believed to be protein synthesis inhibitors.

**[299]** Penicillins include, but are not limited to, Amoxicillin, Ampicillin, Azlocillin, Carbenicillin, Cloxacillin, Dicloxacillin, Flucloxacillin, Mezlocillin, Methicillin, Nafcillin, Oxacillin, Penicillin G, Penicillin V, Piperacillin, Temocillin and Ticarcillin. Penicillins are effective, *e.g.*, against Gram-positive bacteria, facultative anaerobes, *e.g.*, Streptococcus, Borrelia, and Treponema. Penicillins are believed to inhibit bacterial cell wall synthesis by disrupting synthesis of the peptidoglycan layer of bacterial cell walls.

**[300]** Penicillin combinations include, but are not limited to, Amoxicillin/clavulanate, Ampicillin/sulbactam, Piperacillin/tazobactam, and Ticarcillin/clavulanate.

**[301]** Polypeptide antibiotics include, but are not limited to, Bacitracin, Colistin, and Polymyxin B and E. Polypeptide Antibiotics are effective, e.g., against Gram-negative bacteria. Certain polypeptide antibiotics are believed to inhibit isoprenyl pyrophosphate involved in synthesis of the peptidoglycan layer of bacterial cell walls, while others destabilize the bacterial outer membrane by displacing bacterial counter-ions.

**[302]** Quinolones and Fluoroquinolone include, but are not limited to, Ciprofloxacin, Enoxacin, Gatifloxacin, Gemifloxacin, Levofloxacin, Lomefloxacin, Moxifloxacin, Nalidixic acid, Norfloxacin, Ofloxacin, Trovafloxacin, Grepafloxacin, Sparfloxacin, and Temafloxacin. Quinolones/Fluoroquinolone are effective, e.g., against *Streptococcus* and *Neisseria*. Quinolones/Fluoroquinolone are believed to inhibit the bacterial DNA gyrase or topoisomerase IV, thereby inhibiting DNA replication and transcription.

**[303]** Sulfonamides include, but are not limited to, Mafenide, Sulfacetamide, Sulfadiazine, Silver sulfadiazine, Sulfadimethoxine, Sulfamethizole, Sulfamethoxazole, Sulfanilimide, Sulfasalazine, Sulfisoxazole, Trimethoprim-Sulfamethoxazole (Co-trimoxazole), and Sulfonamidochrysoidine. Sulfonamides are believed to inhibit folate synthesis by competitive inhibition of dihydropteroate synthetase, thereby inhibiting nucleic acid synthesis.

**[304]** Tetracyclines include, but are not limited to, Demeclocycline, Doxycycline, Minocycline, Oxytetracycline, and Tetracycline. Tetracyclines are effective, e.g., against Gram-negative bacteria. Tetracyclines are believed to bind to the bacterial 30S ribosomal subunit thereby inhibiting bacterial protein synthesis.

**[305]** Anti-mycobacterial compounds include, but are not limited to, Clofazimine, Dapsone, Capreomycin, Cycloserine, Ethambutol, Ethionamide, Isoniazid, Pyrazinamide, Rifampicin, Rifabutin, Rifapentine, and Streptomycin.

**[306]** Suitable antibiotics also include arsphenamine, chloramphenicol, fosfomycin, fusidic acid, metronidazole, mupirocin, platensimycin, quinupristin/dalfopristin, tigecycline, tinidazole, trimethoprim amoxicillin/clavulanate, ampicillin/sulbactam, amphomycin ristocetin, azithromycin, bacitracin, buforin II, carbomycin, cecropin Pl, clarithromycin, erythromycins, furazolidone, fusidic acid, Na fusidate, gramicidin, imipenem, indolicidin, josamycin, magainan II, metronidazole, nitroimidazoles, mikamycin, mutacin B-Ny266, mutacin B-JHl 140, mutacin

J-T8, nisin, nisin A, novobiocin, oleandomycin, ostreogrycin, piperacillin/tazobactam, pristinamycin, ramoplanin, ranalexin, reuterin, rifaximin, rosamicin, rosaramicin, spectinomycin, spiramycin, staphylomycin, streptogramin, streptogramin A, synergistin, taurolidine, teicoplanin, telithromycin, ticarcillin/clavulanic acid, triacetyloleandomycin, tylosin, tyrocidin, tyrothricin, vancomycin, vemamycin, and virginiamycin.

**[307]** In some embodiments, the additional therapeutic agent is an immunosuppressive agent, a DMARD, a pain-control drug, a steroid, a non-steroidal antiinflammatory drug (NSAID), or a cytokine antagonist, and combinations thereof. Representative agents include, but are not limited to, cyclosporin, retinoids, corticosteroids, propionic acid derivative, acetic acid derivative, enolic acid derivatives, fenamic acid derivatives, Cox-2 inhibitors, lumiracoxib, ibuprophen, cholin magnesium salicylate, fenoprofen, salsalate, difunisal, tolmetin, ketoprofen, flurbiprofen, oxaprozin, indomethacin, sulindac, etodolac, ketorolac, nabumetone, naproxen, valdecoxib, etoricoxib, MK0966; rofecoxib, acetominophen, Celecoxib, Diclofenac, tramadol, piroxicam, meloxicam, tenoxicam, droxicam, lornoxicam, isoxicam, mefanamic acid, meclofenamic acid, flufenamic acid, tolfenamic, valdecoxib, parecoxib, etodolac, indomethacin, aspirin, ibuprophen, firocoxib, methotrexate (MTX), antimalarial drugs (e.g., hydroxychloroquine and chloroquine), sulfasalazine, Leflunomide, azathioprine, cyclosporin, gold salts, minocycline, cyclophosphamide, D-penicillamine, minocycline, auranofin, tacrolimus, myocrisin, chlorambucil, TNF alpha antagonists (e.g., TNF alpha antagonists or TNF alpha receptor antagonists), e.g., ADALIMUMAB (Humira®), ETANERCEPT (Enbrel®), INFLIXIMAB (Remicade®; TA-650), CERTOLIZUMAB PEGOL (Cimzia®; CDP870), GOLIMUMAB (Simpom®; CNTO 148), ANAKINRA (Kineret®), RITUXIMAB (Rituxan®; MabThera®), ABATACEPT (Orencia®), TOCILIZUMAB (RoActemra /Actemra®), integrin antagonists (TYSABRI® (natalizumab)), IL-1 antagonists (ACZ885 (Ilaris)), Anakinra (Kineret®)), CD4 antagonists, IL-23 antagonists, IL-20 antagonists, IL-6 antagonists, BLyS antagonists (e.g., Atacicept, Benlysta®/ LymphoStat-B® (belimumab)), p38 Inhibitors, CD20 antagonists (Ocrelizumab, Ofatumumab (Arzerra®)), interferon gamma antagonists (Fontolizumab), prednisolone, Prednisone, dexamethasone, Cortisol, cortisone, hydrocortisone, methylprednisolone, betamethasone, triamcinolone, beclometasome, fludrocortisone, deoxycorticosterone, aldosterone, Doxycycline, vancomycin, pioglitazone, SBI-087, SCIO-469, Cura-100, Oncoxin + Viusid, TwHF, Methoxsalen, Vitamin D - ergocalciferol, Milnacipran,

Paclitaxel, rosig tazone, Tacrolimus (Prograf®), RADOOl, rapamune, rapamycin, fostamatinib, Fentanyl, XOMA 052, Fostamatinib disodium,rosightazone, Curcumin (Longvida™), Rosuvastatin, Maraviroc, ramipnl, Milnacipran, Cobiprostone, somatropin, tgAAC94 gene therapy vector, MK0359, GW856553, esomeprazole, everolimus, trastuzumab, JAKl and JAK2 inhibitors, pan JAK inhibitors, e.g., tetracyclic pyridone 6 (P6), 325, PF-956980, denosumab, IL-6 antagonists, CD20 antagonistis, CTLA4 antagonists, IL-8 antagonists, IL-21 antagonists, IL-22 antagonist, integrin antagonists (Tysarbri® (natalizumab)), VGEF antagnosits, CXCL antagonists, MMP antagonists, defensin antagonists, IL-1 antagonists (including IL-1 beta antagonsits), and IL-23 antagonists (e.g., receptor decoys, antagonistic antibodies, etc.).

**[308]** In some embodiments, the additional therapeutic agent is an immunosuppressive agent. Examples of immunosuppressive agents include, but are not limited to, corticosteroids, mesalazine, mesalamine, sulfasalazine, sulfasalazine derivatives, immunosuppressive drugs, cyclosporin A, mercaptopurine, azathiopurine, prednisone, methotrexate, antihistamines, glucocorticoids, epinephrine, theophylline, cromolyn sodium, anti-leukotrienes, anti-cholinergic drugs for rhinitis, TLR antagonists, inflammasome inhibitors, anti-cholinergic decongestants, mast-cell stabilizers, monoclonal anti-IgE antibodies, vaccines (e.g., vaccines used for vaccination where the amount of an allergen is gradually increased), cytokine inhibitors, such as anti-IL-6 antibodies, TNF inhibitors such as infliximab, adalimumab, certolizumab pegol, golimumab, or etanercept, iand combinations thereof.

Administration

**[309]** In certain aspects, provided herein is a method of delivering a pharmaceutical composition described herein (e.g., a pharmaceutical composition comprising mEVs (such as smEVs) to a subject. In some embodiments of the methods provided herein, the pharmaceutical composition is administered in conjunction with the administration of an additional therapeutic agent. In some embodiments, the pharmaceutical composition comprises mEVs (such as smEVs) co-formulated with the additional therapeutic agent. In some embodiments, the pharmaceutical composition comprising mEVs (such as smEVs) is co-administered with the additional therapeutic agent. In some embodiments, the additional therapeutic agent is administered to the subject before administration of the pharmaceutical composition that comprises mEVs (such as smEVs) (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50 or 55 minutes

before, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22 or 23 hours before, or about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or 14 days before). In some embodiments, the additional therapeutic agent is administered to the subject after administration of the pharmaceutical composition that comprises mEVs (such as smEVs) (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50 or 55 minutes after, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22 or 23 hours after, or about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or 14 days after). In some embodiments, the same mode of delivery is used to deliver both the pharmaceutical composition that comprises mEVs (such as smEVs) and the additional therapeutic agent. In some embodiments, different modes of delivery are used to administer the pharmaceutical composition that comprises mEVs (such as smEVs) and the additional therapeutic agent. For example, in some embodiments the pharmaceutical composition that comprises mEVs (such as smEVs) is administered orally while the additional therapeutic agent is administered via injection (e.g., an intravenous, intramuscular and/or intratumoral injection).

**[310]** In some embodiments, the pharmaceutical composition described herein is administered once a day. In some embodiments, the pharmaceutical composition described herein is administered twice a day. In some embodiments, the pharmaceutical composition described herein is formulated for a daily dose. In some embodiments, the pharmaceutical composition described herein is formulated for twice a day dose, wherein each dose is half of the daily dose.

**[311]** In certain embodiments, the pharmaceutical compositions and dosage forms described herein can be administered in conjunction with any other conventional anti-cancer treatment, such as, for example, radiation therapy and surgical resection of the tumor. These treatments may be applied as necessary and/or as indicated and may occur before, concurrent with or after administration of the pharmaceutical composition that comprises mEVs (such as smEVs) or dosage forms described herein.

**[312]** The dosage regimen can be any of a variety of methods and amounts, and can be determined by one skilled in the art according to known clinical factors. As is known in the medical arts, dosages for any one patient can depend on many factors, including the subject's species, size, body surface area, age, sex, immunocompetence, and general health, the particular microorganism to be administered, duration and route of administration, the kind and stage of the disease, for example, tumor size, and other compounds such as drugs being administered

concurrently or near-concurrently. In addition to the above factors, such levels can be affected by the infectivity of the microorganism, and the nature of the microorganism, as can be determined by one skilled in the art. In the present methods, appropriate minimum dosage levels of microorganisms can be levels sufficient for the microorganism to survive, grow and replicate. The dose of a pharmaceutical composition that comprises mEVs (such as smEVs) described herein may be appropriately set or adjusted in accordance with the dosage form, the route of administration, the degree or stage of a target disease, and the like. For example, the general effective dose of the agents may range between 0.01 mg/kg body weight/day and 1000 mg/kg body weight/day, between 0.1 mg/kg body weight/day and 1000 mg/kg body weight/day, 0.5 mg/kg body weight/day and 500 mg/kg body weight/day, 1 mg/kg body weight/day and 100 mg/kg body weight/day, or between 5 mg/kg body weight/day and 50 mg/kg body weight/day. The effective dose may be 0.01, 0.05, 0.1, 0.5, 1, 2, 3, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 500, or 1000 mg/kg body weight/day or more, but the dose is not limited thereto.

**[313]** In some embodiments, the dose administered to a subject is sufficient to prevent disease (e.g., autoimmune disease, inflammatory disease, metabolic disease, or cancer), delay its onset, or slow or stop its progression, or relieve one or more symptoms of the disease. One skilled in the art will recognize that dosage will depend upon a variety of factors including the strength of the particular agent (e.g., therapeutic agent) employed, as well as the age, species, condition, and body weight of the subject. The size of the dose will also be determined by the route, timing, and frequency of administration as well as the existence, nature, and extent of any adverse side-effects that might accompany the administration of a particular therapeutic agent and the desired physiological effect.

**[314]** Suitable doses and dosage regimens can be determined by conventional range-finding techniques known to those of ordinary skill in the art. Generally, treatment is initiated with smaller dosages, which are less than the optimum dose of the compound. Thereafter, the dosage is increased by small increments until the optimum effect under the circumstances is reached. An effective dosage and treatment protocol can be determined by routine and conventional means, starting *e.g.,* with a low dose in laboratory animals and then increasing the dosage while monitoring the effects, and systematically varying the dosage regimen as well. Animal studies are commonly used to determine the maximal tolerable dose ("MTD") of

bioactive agent per kilogram weight. Those skilled in the art regularly extrapolate doses for efficacy, while avoiding toxicity, in other species, including humans.

**[315]** In accordance with the above, in therapeutic applications, the dosages of the therapeutic agents used in accordance with the invention vary depending on the active agent, the age, weight, and clinical condition of the recipient patient, and the experience and judgment of the clinician or practitioner administering the therapy, among other factors affecting the selected dosage. For example, for cancer treatment, the dose should be sufficient to result in slowing, and preferably regressing, the growth of a tumor and most preferably causing complete regression of the cancer, or reduction in the size or number of metastases As another example, the dose should be sufficient to result in slowing of progression of the disease for which the subject is being treated, and preferably amelioration of one or more symptoms of the disease for which the subject is being treated.

**[316]** Separate administrations can include any number of two or more administrations, including two, three, four, five or six administrations. One skilled in the art can readily determine the number of administrations to perform or the desirability of performing one or more additional administrations according to methods known in the art for monitoring therapeutic methods and other monitoring methods provided herein. Accordingly, the methods provided herein include methods of providing to the subject one or more administrations of a pharmaceutical composition, where the number of administrations can be determined by monitoring the subject, and, based on the results of the monitoring, determining whether or not to provide one or more additional administrations. Deciding on whether or not to provide one or more additional administrations can be based on a variety of monitoring results.

**[317]** The time period between administrations can be any of a variety of time periods. The time period between administrations can be a function of any of a variety of factors, including monitoring steps, as described in relation to the number of administrations, the time period for a subject to mount an immune response. In one example, the time period can be a function of the time period for a subject to mount an immune response; for example, the time period can be more than the time period for a subject to mount an immune response, such as more than about one week, more than about ten days, more than about two weeks, or more than about a month; in another example, the time period can be less than the time period for a subject

to mount an immune response, such as less than about one week, less than about ten days, less than about two weeks, or less than about a month.

**[318]** In some embodiments, the delivery of an additional therapeutic agent in combination with the pharmaceutical composition described herein reduces the adverse effects and/or improves the efficacy of the additional therapeutic agent.

**[319]** The effective dose of an additional therapeutic agent described herein is the amount of the additional therapeutic agent that is effective to achieve the desired therapeutic response for a particular subject, composition, and mode of administration, with the least toxicity to the subject. The effective dosage level can be identified using the methods described herein and will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions or agents administered, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the subject being treated, and like factors well known in the medical arts. In general, an effective dose of an additional therapeutic agent will be the amount of the additional therapeutic agent which is the lowest dose effective to produce a therapeutic effect. Such an effective dose will generally depend upon the factors described above.

**[320]** The toxicity of an additional therapeutic agent is the level of adverse effects experienced by the subject during and following treatment. Adverse events associated with additional therapy toxicity can include, but are not limited to, abdominal pain, acid indigestion, acid reflux, allergic reactions, alopecia, anaphylasix, anemia, anxiety, lack of appetite, arthralgias, asthenia, ataxia, azotemia, loss of balance, bone pain, bleeding, blood clots, low blood pressure, elevated blood pressure, difficulty breathing, bronchitis, bruising, low white blood cell count, low red blood cell count, low platelet count, cardiotoxicity, cystitis, hemorrhagic cystitis, arrhythmias, heart valve disease, cardiomyopathy, coronary artery disease, cataracts, central neurotoxicity, cognitive impairment, confusion, conjunctivitis, constipation, coughing, cramping, cystitis, deep vein thrombosis, dehydration, depression, diarrhea, dizziness, dry mouth, dry skin, dyspepsia, dyspnea, edema, electrolyte imbalance, esophagitis, fatigue, loss of fertility, fever, flatulence, flushing, gastric reflux, gastroesophageal reflux disease, genital pain, granulocytopenia, gynecomastia, glaucoma, hair loss, hand-foot syndrome, headache,

hearing loss, heart failure, heart palpitations, heartburn, hematoma, hemorrhagic cystitis, hepatotoxicity, hyperamylasemia, hypercalcemia, hyperchloremia, hyperglycemia, hyperkalemia, hyperlipasemia, hypermagnesemia, hypernatremia, hyperphosphatemia, hyperpigmentation, hypertriglyceridemia, hyperuricemia, hypoalbuminemia, hypocalcemia, hypochloremia, hypoglycemia, hypokalemia, hypomagnesemia, hyponatremia, hypophosphatemia, impotence, infection, injection site reactions, insomnia, iron deficiency, itching, joint pain, kidney failure, leukopenia, liver dysfunction, memory loss, menopause, mouth sores, mucositis, muscle pain, myalgias, myelosuppression, myocarditis, neutropenic fever, nausea, nephrotoxicity, neutropenia, nosebleeds, numbness, ototoxicity, pain, palmar-plantar erythrodysesthesia, pancytopenia, pericarditis, peripheral neuropathy, pharyngitis, photophobia, photosensitivity, pneumonia, pneumonitis, proteinuria, pulmonary embolus, pulmonary fibrosis, pulmonary toxicity, rash, rapid heart beat, rectal bleeding, restlessness, rhinitis, seizures, shortness of breath, sinusitis, thrombocytopenia, tinnitus, urinary tract infection, vaginal bleeding, vaginal dryness, vertigo, water retention, weakness, weight loss, weight gain, and xerostomia. In general, toxicity is acceptable if the benefits to the subject achieved through the therapy outweigh the adverse events experienced by the subject due to the therapy.

Immune disorders

**[321]** In some embodiments, the methods and pharmaceutical compositions described herein relate to the treatment or prevention of a disease or disorder associated a pathological immune response, such as an autoimmune disease, an allergic reaction and/or an inflammatory disease. In some embodiments, the disease or disorder is an inflammatory bowel disease (e.g., Crohn's disease or ulcerative colitis). In some embodiments, the disease or disorder is psoriasis. In some embodiments, the disease or disorder is atopic dermatitis.

**[322]** The methods described herein can be used to treat any subject in need thereof. As used herein, a "subject in need thereof" includes any subject that has a disease or disorder associated with a pathological immune response (e.g., an inflammatory bowel disease), as well as any subject with an increased likelihood of acquiring a such a disease or disorder.

**[323]** The pharmaceutical compositions described herein can be used, for example, as a pharmaceutical composition for preventing or treating (reducing, partially or completely, the

adverse effects of) an autoimmune disease, such as chronic inflammatory bowel disease, systemic lupus erythematosus, psoriasis, muckle-wells syndrome, rheumatoid arthritis, multiple sclerosis, or Hashimoto's disease; an allergic disease, such as a food allergy, pollenosis, or asthma; an infectious disease, such as an infection with Clostridium difficile; an inflammatory disease such as a TNF-mediated inflammatory disease (e.g., an inflammatory disease of the gastrointestinal tract, such as pouchitis, a cardiovascular inflammatory condition, such as atherosclerosis, or an inflammatory lung disease, such as chronic obstructive pulmonary disease); a pharmaceutical composition for suppressing rejection in organ transplantation or other situations in which tissue rejection might occur; a supplement, food, or beverage for improving immune functions; or a reagent for suppressing the proliferation or function of immune cells.

**[324]** In some embodiments, the methods provided herein are useful for the treatment of inflammation. In certain embodiments, the inflammation of any tissue and organs of the body, including musculoskeletal inflammation, vascular inflammation, neural inflammation, digestive system inflammation, ocular inflammation, inflammation of the reproductive system, and other inflammation, as discussed below.

**[325]** Immune disorders of the musculoskeletal system include, but are not limited, to those conditions affecting skeletal joints, including joints of the hand, wrist, elbow, shoulder, jaw, spine, neck, hip, knew, ankle, and foot, and conditions affecting tissues connecting muscles to bones such as tendons. Examples of such immune disorders, which may be treated with the methods and compositions described herein include, but are not limited to, arthritis (including, for example, osteoarthritis, rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, acute and chronic infectious arthritis, arthritis associated with gout and pseudogout, and juvenile idiopathic arthritis), tendonitis, synovitis, tenosynovitis, bursitis, fibrositis (fibromyalgia), epicondylitis, myositis, and osteitis (including, for example, Paget's disease, osteitis pubis, and osteitis fibrosa cystic).

**[326]** Ocular immune disorders refers to a immune disorder that affects any structure of the eye, including the eye lids. Examples of ocular immune disorders which may be treated with the methods and compositions described herein include, but are not limited to, blepharitis, blepharochalasis, conjunctivitis, dacryoadenitis, keratitis, keratoconjunctivitis sicca (dry eye), scleritis, trichiasis, and uveitis

[327] Examples of nervous system immune disorders which may be treated with the methods and compositions described herein include, but are not limited to, encephalitis, Guillain-Barre syndrome, meningitis, neuromyotonia, narcolepsy, multiple sclerosis, myelitis and schizophrenia. Examples of inflammation of the vasculature or lymphatic system which may be treated with the methods and compositions described herein include, but are not limited to, arthrosclerosis, arthritis, phlebitis, vasculitis, and lymphangitis.

[328] Examples of digestive system immune disorders which may be treated with the methods and pharmaceutical compositions described herein include, but are not limited to, cholangitis, cholecystitis, enteritis, enterocolitis, gastritis, gastroenteritis, inflammatory bowel disease, ileitis, and proctitis. Inflammatory bowel diseases include, for example, certain art-recognized forms of a group of related conditions. Several major forms of inflammatory bowel diseases are known, with Crohn's disease (regional bowel disease, *e.g.*, inactive and active forms) and ulcerative colitis (*e.g.*, inactive and active forms) the most common of these disorders. In addition, the inflammatory bowel disease encompasses irritable bowel syndrome, microscopic colitis, lymphocytic-plasmocytic enteritis, coeliac disease, collagenous colitis, lymphocytic colitis and eosinophilic enterocolitis. Other less common forms of IBD include indeterminate colitis, pseudomembranous colitis (necrotizing colitis), ischemic inflammatory bowel disease, Behcet's disease, sarcoidosis, scleroderma, IBD-associated dysplasia, dysplasia associated masses or lesions, and primary sclerosing cholangitis.

[329] Examples of reproductive system immune disorders which may be treated with the methods and pharmaceutical compositions described herein include, but are not limited to, cervicitis, chorioamnionitis, endometritis, epididymitis, omphalitis, oophoritis, orchitis, salpingitis, tubo-ovarian abscess, urethritis, vaginitis, vulvitis, and vulvodynia.

[330] The methods and pharmaceutical compositions described herein may be used to treat autoimmune conditions having an inflammatory component. Such conditions include, but are not limited to, acute disseminated alopecia universalise, Behcet's disease, Chagas' disease, chronic fatigue syndrome, dysautonomia, encephalomyelitis, ankylosing spondylitis, aplastic anemia, hidradenitis suppurativa, autoimmune hepatitis, autoimmune oophoritis, celiac disease, Crohn's disease, diabetes mellitus type 1, giant cell arteritis, goodpasture's syndrome, Grave's disease, Guillain-Barre syndrome, Hashimoto's disease, Henoch-Schonlein purpura, Kawasaki's disease, lupus erythematosus, microscopic colitis, microscopic polyarteritis, mixed connective

tissue disease, Muckle-Wells syndrome, multiple sclerosis, myasthenia gravis, opsoclonus myoclonus syndrome, optic neuritis, ord's thyroiditis, pemphigus, polyarteritis nodosa, polymyalgia, rheumatoid arthritis, Reiter's syndrome, Sjogren's syndrome, temporal arteritis, Wegener's granulomatosis, warm autoimmune haemolytic anemia, interstitial cystitis, Lyme disease, morphea, psoriasis, sarcoidosis, scleroderma, ulcerative colitis, and vitiligo.

**[331]** The methods and pharmaceutical compositions described herein may be used to treat T-cell mediated hypersensitivity diseases having an inflammatory component. Such conditions include, but are not limited to, contact hypersensitivity, contact dermatitis (including that due to poison ivy), uticaria, skin allergies, respiratory allergies (hay fever, allergic rhinitis, house dustmite allergy) and gluten-sensitive enteropathy (Celiac disease).

**[332]** Other immune disorders which may be treated with the methods and pharmaceutical compositions include, for example, appendicitis, dermatitis, dermatomyositis, endocarditis, fibrositis, gingivitis, glossitis, hepatitis, hidradenitis suppurativa, iritis, laryngitis, mastitis, myocarditis, nephritis, otitis, pancreatitis, parotitis, percarditis, peritonoitis, pharyngitis, pleuritis, pneumonitis, prostatistis, pyelonephritis, and stomatisi, transplant rejection (involving organs such as kidney, liver, heart, lung, pancreas (e.g., islet cells), bone marrow, cornea, small bowel, skin allografts, skin homografts, and heart valve xengrafts, sewrum sickness, and graft vs host disease), acute pancreatitis, chronic pancreatitis, acute respiratory distress syndrome, Sexary's syndrome, congenital adrenal hyperplasis, nonsuppurative thyroiditis, hypercalcemia associated with cancer, pemphigus, bullous dermatitis herpetiformis, severe erythema multiforme, exfoliative dermatitis, seborrheic dermatitis, seasonal or perennial allergic rhinitis, bronchial asthma, contact dermatitis, atopic dermatitis, drug hypersensistivity reactions, allergic conjunctivitis, keratitis, herpes zoster ophthalmicus, iritis and oiridocyclitis, chorioretinitis, optic neuritis, symptomatic sarcoidosis, fulminating or disseminated pulmonary tuberculosis chemotherapy, idiopathic thrombocytopenic purpura in adults, secondary thrombocytopenia in adults, acquired (autoimmune) haemolytic anemia, leukaemia and lymphomas in adults, acute leukaemia of childhood, regional enteritis, autoimmune vasculitis, multiple sclerosis, chronic obstructive pulmonary disease, solid organ transplant rejection, sepsis. Preferred treatments include treatment of transplant rejection, rheumatoid arthritis, psoriatic arthritis, multiple sclerosis, Type 1 diabetes, asthma, inflammatory bowel disease, systemic lupus erythematosus,

psoriasis, chronic obstructive pulmonary disease, and inflammation accompanying infectious conditions (e.g., sepsis).

Metabolic disorders

**[333]** In some embodiments, the methods and pharmaceutical compositions described herein relate to the treatment or prevention of a metabolic disease or disorder a, such as type II diabetes, impaired glucose tolerance, insulin resistance, obesity, hyperglycemia, hyperinsulinemia, fatty liver, non-alcoholic steatohepatitis, hypercholesterolemia, hypertension, hyperlipoproteinemia, hyperlipidemia, hypertriglylceridemia, ketoacidosis, hypoglycemia, thrombotic disorders, dyslipidemia, non-alcoholic fatty liver disease (NAFLD), Nonalcoholic Steatohepatitis (NASH) or a related disease. In some embodiments, the related disease is cardiovascular disease, atherosclerosis, kidney disease, nephropathy, diabetic neuropathy, diabetic retinopathy, sexual dysfunction, dermatopathy, dyspepsia, or edema. In some embodiments, the methods and pharmaceutical compositions described herein relate to the treatment of Nonalcoholic Fatty Liver Disease (NAFLD) and Nonalcoholic Steatohepatitis (NASH).

**[334]** The methods described herein can be used to treat any subject in need thereof. As used herein, a "subject in need thereof" includes any subject that has a metabolic disease or disorder, as well as any subject with an increased likelihood of acquiring a such a disease or disorder.

**[335]** The pharmaceutical compositions described herein can be used, for example, for preventing or treating (reducing, partially or completely, the adverse effects of) a metabolic disease, such as type II diabetes, impaired glucose tolerance, insulin resistance, obesity, hyperglycemia, hyperinsulinemia, fatty liver, non-alcoholic steatohepatitis, hypercholesterolemia, hypertension, hyperlipoproteinemia, hyperlipidemia, hypertriglylceridemia, ketoacidosis, hypoglycemia, thrombotic disorders, dyslipidemia, non-alcoholic fatty liver disease (NAFLD), Nonalcoholic Steatohepatitis (NASH), or a related disease. In some embodiments, the related disease is cardiovascular disease, atherosclerosis, kidney disease, nephropathy, diabetic neuropathy, diabetic retinopathy, sexual dysfunction, dermatopathy, dyspepsia, or edema.

Cancer

**[336]** In some embodiments, the methods and pharmaceutical compositions described herein relate to the treatment of cancer. In some embodiments, any cancer can be treated using the methods described herein. Examples of cancers that may treated by methods and pharmaceutical compositions described herein include, but are not limited to, cancer cells from the bladder, blood, bone, bone marrow, brain, breast, colon, esophagus, gastrointestine, 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; branchiolo-alveolar 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; endometroid 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; extra-mammary 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.

**[337]** In some embodiments, the methods and pharmaceutical compositions provided herein relate to the treatment of a leukemia. The term "leukemia" includes 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.

**[338]** In some embodiments, the methods and pharmaceutical compositions provided herein relate to the treatment 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 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, cerebriform 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, epiennoid 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, mucoepidermoid carcinoma, carcinoma mucosum, mucous carcinoma, carcinoma myxomatodes, naspharyngeal 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, and carcinoma scroti.

**[339]** In some embodiments, the methods and pharmaceutical compositions provided herein relate to the treatment 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.

**[340]** Additional exemplary neoplasias that can be treated using the methods and pharmaceutical compositions 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, plasmacytoma, colorectal cancer, rectal cancer, and adrenal cortical cancer.

**[341]** In some embodiments, the cancer treated 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.

**[342]** In some embodiments, the cancer comprises breast cancer (e.g., triple negative breast cancer).

**[343]** In some embodiments, the cancer comprises colorectal cancer (e.g., microsatellite stable (MSS) colorectal cancer).

**[344]** In some embodiments, the cancer comprises renal cell carcinoma.

**[345]** In some embodiments, the cancer comprises lung cancer (e.g., non small cell lung cancer).

**[346]** In some embodiments, the cancer comprises bladder cancer.

**[347]** In some embodiments, the cancer comprises gastroesophageal cancer.

**[348]** Particular categories of tumors that can be treated using methods and pharmaceutical compositions 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, leimyosarcoma, rhabdotheliosarcoma, 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, plasmacytoma, colorectal cancer, and rectal cancer.

**[349]** Cancers treated in certain embodiments also include precancerous lesions, e.g., actinic keratosis (solar keratosis), moles (dysplastic nevi), acitinic chelitis (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.

**[350]** Cancers 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.

Other Diseases and Disorders

**[351]** In some embodiments, the methods and pharmaceutical compositions described herein relate to the treatment of liver diseases. Such diseases include, but are not limited to, Alagille Syndrome, Alcohol-Related Liver Disease, Alpha-1 Antitrypsin Deficiency, Autoimmune Hepatitis, Benign Liver Tumors, Biliary Atresia, Cirrhosis, Galactosemia, Gilbert Syndrome, Hemochromatosis, Hepatitis A, Hepatitis B, Hepatitis C, Hepatic Encephalopathy, Intrahepatic Cholestasis of Pregnancy (ICP), Lysosomal Acid Lipase Deficiency (LAL-D), Liver Cysts, Liver Cancer, Newborn Jaundice, Primary Biliary Cholangitis (PBC), Primary Sclerosing Cholangitis (PSC), Reye Syndrome, Type I Glycogen Storage Disease, and Wilson Disease.

**[352]** The methods and pharmaceutical compositions described herein may be used to treat neurodegenerative and neurological diseases. In certain embodiments, the neurodegenerative and/or neurological disease is Parkinson’s disease, Alzheimer’s disease, prion disease, Huntington’s disease, motor neuron diseases (MND), spinocerebellar ataxia, spinal muscular atrophy, dystonia, idiopathicintracranial hypertension, epilepsy, nervous system

disease, central nervous system disease, movement disorders, multiple sclerosis, encephalopathy, peripheral neuropathy or post-operative cognitive dysfunction.

Dysbiosis

**[353]** The gut microbiome (also called the "gut microbiota") can have a significant impact on an individual's health through microbial activity and influence (local and/or distal) on immune and other cells of the host (Walker, W.A., Dysbiosis. The Microbiota in Gastrointestinal Pathophysiology. Chapter 25. 2017; Weiss and Thierry, Mechanisms and consequences of intestinal dysbiosis. *Cellular and Molecular Life Sciences*. (2017) 74(16):2959-2977. Zurich Open Repository and Archive, doi: https://doi.org/10.1007/s00018-017-2509-x)).

**[354]** A healthy host-gut microbiome homeostasis is sometimes referred to as a "eubiosis" or "normobiosis," whereas a detrimental change in the host microbiome composition and/or its diversity can lead to an unhealthy imbalance in the microbiome, or a "dysbiosis" (Hooks and O'Malley. *Dysbiosis and its discontents*. American Society for Microbiology. Oct 2017. Vol. 8. Issue 5. mBio 8:e01492-17. https://doi.org/10.1128/mBio.01492-17). Dysbiosis, and associated local or distal host inflammatory or immune effects, may occur where microbiome homeostasis is lost or diminished, resulting in: increased susceptibility to pathogens; altered host bacterial metabolic activity; induction of host proinflammatory activity and/or reduction of host anti-inflammatory activity. Such effects are mediated in part by interactions between host immune cells (e.g., T cells, dendritic cells, mast cells, NK cells, intestinal epithelial lymphocytes (IEC), macrophages and phagocytes) and cytokines, and other substances released by such cells and other host cells.

**[355]** A dysbiosis may occur within the gastrointestinal tract (a "gastrointestinal dysbiosis" or "gut dysbiosis") or may occur outside the lumen of the gastrointestinal tract (a "distal dysbiosis"). Gastrointestinal dysbiosis is often associated with a reduction in integrity of the intestinal epithelial barrier, reduced tight junction integrity and increased intestinal permeability. Citi, S. Intestinal Barriers protect against disease, *Science* 359:1098-99 (2018); Srinivasan et al., TEER measurement techniques for in vitro barrier model systems. *J. Lab. Autom*. 20:107-126 (2015). A gastrointestinal dysbiosis can have physiological and immune effects within and outside the gastrointestinal tract.

**[356]** The presence of a dysbiosis can be associated with a wide variety of diseases and conditions including: infection, cancer, autoimmune disorders (e.g., systemic lupus erythematosus (SLE)) or inflammatory disorders (e.g., functional gastrointestinal disorders such as inflammatory bowel disease (IBD), ulcerative colitis, and Crohn's disease), neuroinflammatory diseases (e.g., multiple sclerosis), transplant disorders (e.g., graft-versus-host disease), fatty liver disease, type I diabetes, rheumatoid arthritis, Sjögren's syndrome, celiac disease, cystic fibrosis, chronic obstructive pulmonary disorder (COPD), and other diseases and conditions associated with immune dysfunction. Lynch et al., The Human Microbiome in Health and Disease, N. *Engl. J. Med* .375:2369-79 (2016), Carding et al., Dysbiosis of the gut microbiota in disease. *Microb. Ecol. Health Dis.* (2015); 26: 10: 3402/mehd.v26.2619; Levy et al, Dysbiosis and the Immune System, *Nature Reviews Immunology* 17:219 (April 2017)

**[357]** In certain embodiments, exemplary pharmaceutical compositions disclosed herein can treat a dysbiosis and its effects by modifying the immune activity present at the site of dysbiosis. As described herein, such compositions can modify a dysbiosis via effects on host immune cells, resulting in, e.g., an increase in secretion of anti-inflammatory cytokines and/or a decrease in secretion of pro-inflammatory cytokines, reducing inflammation in the subject recipient or via changes in metabolite production.

**[358]** Exemplary pharmaceutical compositions disclosed herein that are useful for treatment of disorders associated with a dysbiosis contain one or more types of mEVs (microbial extracellular vesicles) derived from immunomodulatory bacteria (e.g., anti-inflammatory bacteria). Such compositions are capable of affecting the recipient host's immune function, in the gastrointestinal tract, and/or a systemic effect at distal sites outside the subject's gastrointestinal tract.

**[359]** Exemplary pharmaceutical compositions disclosed herein that are useful for treatment of disorders associated with a dysbiosis contain a population of immunomodulatory bacteria of a single bacterial species (e.g., a single strain) (e.g., anti-inflammatory bacteria) and/or a population of mEVs derived from immunomodulatory bacteria of a single bacterial species (e.g., a single strain) (e.g., anti-inflammatory bacteria). Such compositions are capable of affecting the recipient host's immune function, in the gastrointestinal tract, and /or a systemic effect at distal sites outside the subject's gastrointestinal tract.

**[360]** In one embodiment, pharmaceutical compositions containing an isolated population of mEVs derived from immunomodulatory bacteria (e.g., anti-inflammatory bacterial cells) are administered (e.g., orally) to a mammalian recipient in an amount effective to treat a dysbiosis and one or more of its effects in the recipient. The dysbiosis may be a gastrointestinal tract dysbiosis or a distal dysbiosis.

**[361]** In another embodiment, pharmaceutical compositions of the instant invention can treat a gastrointestinal dysbiosis and one or more of its effects on host immune cells, resulting in an increase in secretion of anti-inflammatory cytokines and/or a decrease in secretion of pro-inflammatory cytokines, reducing inflammation in the subject recipient.

**[362]** In another embodiment, the pharmaceutical compositions can treat a gastrointestinal dysbiosis and one or more of its effects by modulating the recipient immune response via cellular and cytokine modulation to reduce gut permeability by increasing the integrity of the intestinal epithelial barrier.

**[363]** In another embodiment, the pharmaceutical compositions can treat a distal dysbiosis and one or more of its effects by modulating the recipient immune response at the site of dysbiosis via modulation of host immune cells.

**[364]** Other exemplary pharmaceutical compositions are useful for treatment of disorders associated with a dysbiosis, which compositions contain one or more types of bacteria or mEVs capable of altering the relative proportions of host immune cell subpopulations, e.g., subpopulations of T cells, immune lymphoid cells, dendritic cells, NK cells and other immune cells, or the function thereof, in the recipient.

**[365]** Other exemplary pharmaceutical compositions are useful for treatment of disorders associated with a dysbiosis, which compositions contain a population of mEVs of a single immunomodulatory bacterial (e.g., anti-inflammatory bacterial cells) species (e.g., a single strain) capable of altering the relative proportions of immune cell subpopulations, e.g., T cell subpopulations, immune lymphoid cells, NK cells and other immune cells, or the function thereof, in the recipient subject.

**[366]** In one embodiment, the invention provides methods of treating a gastrointestinal dysbiosis and one or more of its effects by orally administering to a subject in need thereof a pharmaceutical composition which alters the microbiome population existing at the site of the dysbiosis. The pharmaceutical composition can contain one or more types of mEVs from

immunomodulatory bacteria or a population of mEVs of a single immunomodulatory bacterial species (e.g., anti-inflammatory bacterial cells) (e.g., a single strain).

**[367]** In one embodiment, the invention provides methods of treating a distal dysbiosis and one or more of its effects by orally administering to a subject in need thereof a pharmaceutical composition which alters the subject's immune response outside the gastrointestinal tract. The pharmaceutical composition can contain one or more types of mEVs from immunomodulatory bacteria (e.g., anti-inflammatory bacterial cells) or a population of mEVs of a single immunomodulatory bacterial (e.g., anti-inflammatory bacterial cells) species (e.g., a single strain).

**[368]** In exemplary embodiments, pharmaceutical compositions useful for treatment of disorders associated with a dysbiosis stimulate secretion of one or more anti-inflammatory cytokines by host immune cells. Anti-inflammatory cytokines include, but are not limited to, IL-10, IL-13, IL-9, IL-4, IL-5, TGFβ, and combinations thereof. In other exemplary embodiments, pharmaceutical compositions useful for treatment of disorders associated with a dysbiosis that decrease (e.g., inhibit) secretion of one or more pro-inflammatory cytokines by host immune cells. Pro-inflammatory cytokines include, but are not limited to, IFNγ, IL-12p70, IL-1α, IL-6, IL-8, MCP1, MIP1α, MIP1β, TNFα, and combinations thereof. Other exemplary cytokines are known in the art and are described herein.

**[369]** In another aspect, the invention provides a method of treating or preventing a disorder associated with a dysbiosis in a subject in need thereof, comprising administering (e.g., orally administering) to the subject a therapeutic composition in the form of a probiotic or medical food comprising bacteria or mEVs in an amount sufficient to alter the microbiome at a site of the dysbiosis, such that the disorder associated with the dysbiosis is treated.

**[370]** In another embodiment, a therapeutic composition of the instant invention in the form of a probiotic or medical food may be used to prevent or delay the onset of a dysbiosis in a subject at risk for developing a dysbiosis.

Methods of Making Enhanced Bacteria

**[371]** In certain aspects, provided herein are methods of making engineered bacteria for the production of the mEVs (such as smEVs) described herein. In some embodiments, the engineered bacteria are modified to enhance certain desirable properties. For example, in some

embodiments, the engineered bacteria are modified to enhance the immunomodulatory and/or therapeutic effect of the mEVs (such as smEVs) (e.g., either alone or in combination with another therapeutic agent), to reduce toxicity and/or to improve bacterial and/or mEV (such as smEV) manufacturing (e.g., higher oxygen tolerance, improved freeze-thaw tolerance, shorter generation times). The engineered bacteria may be produced using any technique known in the art, including but not limited to site-directed mutagenesis, transposon mutagenesis, knock-outs, knock-ins, polymerase chain reaction mutagenesis, chemical mutagenesis, ultraviolet light mutagenesis, transformation (chemically or by electroporation), phage transduction, directed evolution, CRISPR/Cas9, or any combination thereof.

**[372]** In some embodiments of the methods provided herein, the bacterium is modified by directed evolution. In some embodiments, the directed evolution comprises exposure of the bacterium to an environmental condition and selection of bacterium with improved survival and/or growth under the environmental condition. In some embodiments, the method comprises a screen of mutagenized bacteria using an assay that identifies enhanced bacterium. In some embodiments, the method further comprises mutagenizing the bacteria (e.g., by exposure to chemical mutagens and/or UV radiation) or exposing them to a therapeutic agent (e.g., antibiotic) followed by an assay to detect bacteria having the desired phenotype (e.g., an *in vivo* assay, an *ex vivo* assay, or an *in vitro* assay).

## EXAMPLES

### Example 1: Purification and preparation of membranes from bacteria to obtain processed microbial extracellular vesicles (pmEVs)

*Purification*

**[373]** Processed microbial extracellular vesicles (pmEVs) are purified and prepared from bacterial cultures (e.g., bacteria listed in Table 1, Table 2, and/or Table 3) using methods known to those skilled in the art (Thein et al, 2010. Efficient subfractionation of gram-negative bacteria for proteomics studies. J. Proteome Res. 2010 Dec 3; 9(12): 6135-47. Doi: 10.1021/pr1002438. Epub 2010 Oct. 28; Sandrini et al. 2014. Fractionation by Ultracentrifugation of Gram negative cytoplasmic and membrane proteins. Bio-Protocol. Vol. 4 (21) Doi: 10.21769/BioProtoc.1287).

**[374]** Alternatively, pmEVs are purified by methods adapted from Thein et al. For example, bacterial cultures are centrifuged at 10,000-15,500 x g for 10-30 minutes at room temperature or at 4°C. Supernatants are discarded and cell pellets are frozen at -80°C. Cell pellets are thawed on ice and resuspended in 100 mM Tris-HCl, pH 7.5, and may be supplemented with 1 mg/mL DNase I and/or 100mM NaCl. Thawed cells are incubated in 500ug/ml lysozyme, 40ug/ml lyostaphin, and/or 1 mg/ml DNaseI for 40 minutes to facilitate cell lysis. Additional enzymes may be used to facilitate the lysing process (e.g., EDTA (5mM), PMSF (Sigma Aldrich), and/or benzamidine (Sigma Aldrich). Cells are then lysed using an Emulsiflex C-3 (Avestin, Inc.) under conditions recommended by the manufacturer. Alternatively, pellets may be frozen at -80°C and thawed again prior to lysis. Debris and unlysed cells are pelleted by centrifugation at 10,000-12,500 x g for 15 minutes at 4°C. Supernatants are then centrifuged at 120,000 x g for 1 hour at 4°C. Pellets are resuspended in ice-cold 100 mM sodium carbonate, pH 11, incubated with agitation for 1 hour at 4°C. Alternatively, pellets are centrifuged at 120,000 x g for 1 hour at 4°C in sodium carbonate immediately following resuspension. Pellets are resuspended in 100mM Tris-HCl, pH 7.5 supplemented with 100mM NaCl re-centrifuged at 120,000 x g for 20 minutes at 4°C, and then resuspended in 100mM Tris-HCl, pH 7.5 supplemented with up to or around 100mM NaCl or in PBS. Samples are stored at -20°C. To protect the pmEV preparation during the freeze/thaw steps, 250mM sucrose and up to 500mM NaCl may be added to the final preparation to stabilize the vesicles in the pmEV preparation.

**[375]** Alternatively, pmEVs are obtained by methods adapted from Sandrini et al, 2014. After, bacterial cultures are centrifuged at 10,000-15,500 x g for 10-15 minutes at room temperature or at 4°C, cell pellets are frozen at -80°C and supernatants are discarded. Then, cell pellets are thawed on ice and resuspended in 10 mM Tris-HCl, pH 8.0, 1 mM EDTA supplemented with 0.1 mg/mL lysozyme. Samples are then incubated with mixing at room temperature or at 37°C for 30 min. In an optional step, samples are re-frozen at -80°C and thawed again on ice. DNase I is added to a final concentration of 1.6 mg/mL and MgCl2 to a final concentration of 100mM. Samples are sonicated using a QSonica Q500 sonicator with 7 cycles of 30 sec on and 30 sec off. Debris and unlysed cells are pelleted by centrifugation at 10,000 x g for 15 min. at 4°C. Supernatants are then centrifuged at 110,000 x g for 15 minutes at 4°C. Pellets are resuspended in 10 mM Tris-HCl, pH 8.0 and incubated 30-60 minutes with mixing at

room temperature. Samples are centrifuged at 110,000 x g for 15 minutes at 4°C. Pellets are resuspended in PBS and stored at -20°C.

**[376]** Optionally, pmEVs can be separated from other bacterial components and debris using methods known in the art. Size-exclusion chromatography or fast protein liquid chromatography (FPLC) may be used for pmEV purification. Additional separation methods that could be used include field flow fractionation, microfluidic filtering, contact-free sorting, and/or immunoaffinity enrichment chromatography. Alternatively, high resolution density gradient fractionation could be used to separate pmEV particles based on density.

*Preparation*

**[377]** Bacterial cultures are centrifuged at 10,000-15,500 x g for 10-30 minutes at room temperature or at 4°C. Supernatants are discarded and cell pellets are frozen at -80°C. Cell pellets are thawed on ice and resuspended in 100mM Tris-HCl, pH 7.5, 100mM NaCl, 500ug/ml lysozyme and/or 40ug/ml Lysostaphin to facilitate cell lysis; up to 0.5 mg/ml DNaseI to reduce genomic DNA size, and EDTA (5mM), PMSF (1mM, Sigma Aldrich), and Benzamidine (1mM, Sigma Aldrich) to inhibit proteases. Cells are then lysed using an Emulsiflex C-3 (Avestin, Inc.) under conditions recommended by the manufacturer. Alternatively, pellets may be frozen at -80°C and thawed again prior to lysis. Debris and unlysed are pelleted by centrifugation at 10,000-12,500 x g at for 15 minutes at 4°C. Supernatants are subjected to size exclusion chromatography (Sepharose 4 FF, GE Healthcare) using an FPLC instrument (AKTA Pure 150, GE Healthcare) with PBS and running buffer supplemented with up to 0.3M NaCl. Pure pmEVs are collected in the column void volume, concentrated and stored at -20°C. Concentration may be performed by a number of methods. For example, ultra-centrifugation may be used (140l x g, 1 hour, 4°C, followed by resuspension in small volume of PBS). To protect the pmEV preparation during the freeze-thaw steps, 250mM sucrose and up to 500mM NaCl may be added to the final preparation to stabilize the vesicles in the pmEV preparation. Additional separation methods that could be used include field flow fractionation, microfluidic filtering, contact-free sorting, and/or immunoaffinity enrichment chromatography. Other techniques that may be employed using methods known in the arts include Whipped Film Evaporation, Molecular Distillation, Short Pass Distillation, and/or Tangential Flow Filtration.

**[378]** In some instances, pmEVs are weighed and are administered at varying doses (in ug/ml). Optionally, pmEVs are assessed for particle count and size distribution using

Nanoparticle Tracking Analysis (NTA), using methods known in the art. For example, a Malvern NS300 instrument may be used according to manufacturer's instructions or as described by Bachurski et al. 2019. Journal of Extracellular Vesicles. Vol. 8(1). Alternatively, for the pmEVs, total protein may be measured using Bio-rad assays (Cat# 5000205) performed per manufacturer's instructions and administered at varying doses based on protein content/dose.

**[379]** For all of the studies described below, the pmEVs may be irradiated, heated, and/or lyophilized prior to administration (as described in Example 49).

**Example 2: A colorectal carcinoma model**

**[380]** To study the efficacy of pmEVs in a tumor model, one of many cancer cell lines may be used according to rodent tumor models known in the art.

**[381]** For example, female 6-8 week old Balb/c mice are obtained from Taconic (Germantown, NY) or other vendor. 100,000 CT-26 colorectal tumor cells (ATCC CRL-2638) are resuspended in sterile PBS and inoculated in the presence of 50% Matrigel. CT-26 tumor cells are subcutaneously injected into one hind flank of each mouse. When tumor volumes reach an average of $100mm^3$ (approximately 10-12 days following tumor cell inoculation), animals are distributed into various treatment groups (e.g., Vehicle; *Veillonella* pmEVs, *Bifidobacteria* pmEVs, with or without anti-PD-1 antibody). Antibodies are administered intraperitoneally (i.p.) at 200 µg/mouse (100 µl final volume) every four days, starting on day 1, for a total of 3 times (Q4Dx3), and pmEVs are administered orally or intravenously and at varied doses and varied times. For example, pmEVs (5 µg) are intravenously (i.v.) injected every third day, starting on day 1 for a total of 4 times (Q3Dx4) and mice are assessed for tumor growth.

**[382]** Alternatively, when tumor volumes reach an average of $100mm^3$ (approximately 10-12 days following tumor cell inoculation), animals are distributed into the following groups: 1) Vehicle; 2) *Neisseria Meningitidis* pmEVs isolated from the Bexsero® vaccine; and 3) anti-PD-1 antibody. Antibodies are administered intraperitoneally (i.p.) at 200ug/mouse (100ul final volume) every four days, starting on day 1, and *Neisseria Meningitidis* pmEVs are administered intraperitoneally (i.p.) daily, starting on day 1 until the conclusion of the study.

**[383]** When tumor volumes reached an average of $100mm^3$ (approximately 10-12 days following tumor cell inoculation), animals were distributed into the following groups: 1) Vehicle; 2) anti-PD-1 antibody; 3) pmEV *B. animalis ssp. lactis* (7.0 e+10 particle count); 4)

pmEV *Anaerostipes hadrus* (7.0 e+10 particle count); 5) pmEV *S. pyogenes* (3.0 e+10 particle count); 6) pmEV *P. benzoelyticum* (3.0 e+10 particle count); 7) pmEV *Hungatella sp.* (7.0 e+10 particle count); 8) pmEV *S. aureus* (7.0 e+10 particle count); and 9) pmEV *R. gnavus* (7.0 e+10 particle count). Antibodies were administered intraperitoneally (i.p.) at 200 µg/mouse (100 µl final volume) every four days, starting on day 1, and pmEVs were intravenously (i.v.) injected daily, starting on day 1 until the conclusion of the study and tumors measured for growth. At day 11, all of the pmEV groups exhibited tumor growth inhibition (**Figures 1-7**). The pmEV *B. animalis ssp. lactis* (**Figure 1**), pmEV *Anaerostipes hadrus* (**Figure 2**), pmEV *S. pyogenes* (**Figure 3**), pmEV *P. benzoelyticum* (**Figure 4**), and pmEV *Hungatella sp.* (**Figure 5**) groups all showed tumor growth inhibition comparable to the anti-PD-1 group, while the pmEV *S. aureus* and pmEV *R. gnavus* groups showed tumor growth inhibition better than that seen in the anti-PD-1 group (**Figures 6 and 7**). In a similar dose-response study, the highest dose of pmEV *B. animalis lactis* demonstrated the greatest efficacy, although pmEV *Megasphaera massiliensis* showed significant efficacy at a lower dose (**Figure 8**). Welch's test is performed for treatment versus vehicle.

**[384]** Yet another study demonstrated significant efficacy of pmEVs earlier than on day 11. The pmEV *R. gnavus* 7.0E+10 (**Figures 9 and 10**), pmEV *B. animalis ssp. lactis* 2.0E+11 (**Figures 11 and 12**), and pmEV *P. distasonis* groups 7.0E+10 (**Figures 13 and 14**) all showed efficacy as early as day 9.

**Example 3: Administering pmEV compositions to treat mouse tumor models**

**[385]** As described in Example 2, a mouse model of cancer is generated by subcutaneously injecting a tumor cell line or patient-derived tumor sample and allowing it to engraft into healthy mice. The methods provided herein may be performed using one of several different tumor cell lines including, but not limited to: B16-F10 or B16-F10-SIY cells as an orthotopic model of melanoma, Panc02 cells as an orthotopic model of pancreatic cancer (Maletzki et al., 2008, Gut 57:483-491), LLC1 cells as an orthotopic model of lung cancer, and RM-1 as an orthotopic model of prostate cancer. As an example, but without limitation, methods for studying the efficacy of pmEVs in the B16-F10 model are provided in depth herein.

**[386]** A syngeneic mouse model of spontaneous melanoma with a very high metastatic frequency is used to test the ability of bacteria to reduce tumor growth and the spread of

metastases. The pmEVs chosen for this assay are compositions that may display enhanced activation of immune cell subsets and stimulate enhanced killing of tumor cells *in vitro*. The mouse melanoma cell line B16-F10 is obtained from ATCC. The cells are cultured in vitro as a monolayer in RPMI medium, supplemented with 10% heat-inactivated fetal bovine serum and 1% penicillin/streptomycin at 37□ in an atmosphere of 5% CO2 in air. The exponentially growing tumor cells are harvested by trypsinization, washed three times with cold 1x PBS, and a suspension of 5E6 cells/ml is prepared for administration. Female C57BL/6 mice are used for this experiment. The mice are 6-8 weeks old and weigh approximately 16-20 g. For tumor development, each mouse is injected SC into the flank with 100 µl of the B16-F10 cell suspension. The mice are anesthetized by ketamine and xylazine prior to the cell transplantation. The animals used in the experiment may be started on an antibiotic treatment via instillation of a cocktail of kanamycin (0.4 mg/ml), gentamicin, (0.035 mg/ml), colistin (850 U/ml), metronidazole (0.215 mg/ml) and vancomycin (0.045 mg/ml) in the drinking water from day 2 to 5 and an intraperitoneal injection of clindamycin (10 mg/kg) on day 7 after tumor injection.

**[387]** The size of the primary flank tumor is measured with a caliper every 2-3 days and the tumor volume is calculated using the following formula: tumor volume = the tumor width × tumor length × 0.5. After the primary tumor reaches approximately 100 mm3, the animals are sorted into several groups based on their body weight. The mice are then randomly taken from each group and assigned to a treatment group. pmEV compositions are prepared as previously described. The mice are orally inoculated by gavage with approximately 7.0e+09 to 3.0e+12 pmEV particles. Alternatively, pmEVs are administered intravenously. Mice receive pmEVs daily, weekly, bi-weekly, monthly, bi-monthly, or on any other dosing schedule throughout the treatment period. Mice may be IV injected with pmEVs in the tail vein, or directly injected into the tumor. Mice can be injected with pmEVs, with or without live bacteria, with or without inactivated/weakened or killed bacteria. Mice can be injected or orally gavaged weekly or once a month. Mice may receive combinations of purified pmEVs and live bacteria to maximize tumor-killing potential. All mice are housed under specific pathogen-free conditions following approved protocols. Tumor size, mouse weight, and body temperature are monitored every 3-4 days and the mice are humanely sacrificed 6 weeks after the B16-F10 mouse melanoma cell injection or when the volume of the primary tumor reaches 1000 mm3. Blood draws are taken

weekly and a full necropsy under sterile conditions is performed at the termination of the protocol.

**[388]** Cancer cells can be easily visualized in the mouse B16-F10 melanoma model due to their melanin production. Following standard protocols, tissue samples from lymph nodes and organs from the neck and chest region are collected and the presence of micro- and macro-metastases is analyzed using the following classification rule. An organ is classified as positive for metastasis if at least two micro-metastatic and one macro-metastatic lesion per lymph node or organ are found. Micro-metastases are detected by staining the paraffin-embedded lymphoid tissue sections with hematoxylin-eosin following standard protocols known to one skilled in the art. The total number of metastases is correlated to the volume of the primary tumor and it is found that the tumor volume correlates significantly with tumor growth time and the number of macro- and micro-metastases in lymph nodes and visceral organs and also with the sum of all observed metastases. Twenty-five different metastatic sites are identified as previously described (Bobek V., et al., Syngeneic lymph-node-targeting model of green fluorescent protein-expressing Lewis lung carcinoma, Clin. Exp. Metastasis, 2004;21(8):705-8).

**[389]** The tumor tissue samples are further analyzed for tumor infiltrating lymphocytes. The CD8+ cytotoxic T cells can be isolated by FACS and can then be further analyzed using customized p/MHC class I microarrays to reveal their antigen specificity (see e.g., Deviren G., et al., Detection of antigen-specific T cells on p/MHC microarrays, J. Mol. Recognit., 2007 Jan-Feb;20(1):32-8). CD4+ T cells can be analyzed using customized p/MHC class II microarrays.

**[390]** At various timepoints, mice are sacrificed and tumors, lymph nodes, or other tissues may be removed for *ex vivo* flow cytometric analysis using methods known in the art. For example, tumors are dissociated using a Miltenyi tumor dissociation enzyme cocktail according to the manufacturer's instructions. Tumor weights are recorded and tumors are chopped then placed in 15ml tubes containing the enzyme cocktail and placed on ice. Samples are then placed on a gentle shaker at 37°C for 45 minutes and quenched with up to 15ml complete RPMI. Each cell suspension is strained through a 70μm filter into a 50ml falcon tube and centrifuged at 1000 rpm for 10 minutes. Cells are resuspended in FACS buffer and washed to remove remaining debris. If necessary, samples are strained again through a second 70μm filter into a new tube. Cells are stained for analysis by flow cytometry using techniques known in the art. Staining antibodies can include anti-CD11c (dendritic cells), anti-CD80, anti-CD86, anti-CD40, anti-

MHCII, anti-CD8a, anti-CD4, and anti-CD103. Other markers that may be analyzed include pan-immune cell marker CD45, T cell markers (CD3, CD4, CD8, CD25, Foxp3, T-bet, Gata3, Ror□t, Granzyme B, CD69, PD-1, CTLA-4), and macrophage/myeloid markers (CD11b, MHCII, CD206, CD40, CSF1R, PD-L1, Gr-1). In addition to immunophenotyping, serum cytokines can be analyzed including, but not limited to, TNFa, IL-17, IL-13, IL-12p70, IL12p40, IL-10, IL-6, IL-5, IL-4, IL-2, IL-1b, IFNy, GM-CSF, G-CSF, M-CSF, MIG, IP10, MIP1b, RANTES, and MCP-1. Cytokine analysis may be carried out immune cells obtained from lymph nodes or other tissue, and/or on purified CD45+ tumor-infiltrated immune cells obtained ex vivo. Finally, immunohistochemistry is carried out on tumor sections to measure T cells, macrophages, dendritic cells, and checkpoint molecule protein expression.

**[391]** The same experiment is also performed with a mouse model of multiple pulmonary melanoma metastases. The mouse melanoma cell line B16-BL6 is obtained from ATCC and the cells are cultured in vitro as described above. Female C57BL/6 mice are used for this experiment. The mice are 6-8 weeks old and weigh approximately 16-20 g. For tumor development, each mouse is injected into the tail vein with 100 μl of a 2E6 cells/ml suspension of B16-BL6 cells. The tumor cells that engraft upon IV injection end up in the lungs.

**[392]** The mice are humanely killed after 9 days. The lungs are weighed and analyzed for the presence of pulmonary nodules on the lung surface. The extracted lungs are bleached with Fekete's solution, which does not bleach the tumor nodules because of the melanin in the B16 cells though a small fraction of the nodules is amelanotic (i.e. white). The number of tumor nodules is carefully counted to determine the tumor burden in the mice. Typically, 200-250 pulmonary nodules are found on the lungs of the control group mice (i.e. PBS gavage).

**[393]** The percentage tumor burden is calculated for the three treatment groups. Percentage tumor burden is defined as the mean number of pulmonary nodules on the lung surfaces of mice that belong to a treatment group divided by the mean number of pulmonary nodules on the lung surfaces of the control group mice.

**[394]** The tumor biopsies and blood samples are submitted for metabolic analysis via LCMS techniques or other methods known in the art. Differential levels of amino acids, sugars, lactate, among other metabolites, between test groups demonstrate the ability of the microbial composition to disrupt the tumor metabolic state.

*RNA Seq to Determine Mechanism of Action*

**[395]** Dendritic cells are purified from tumors, Peyers patches, and mesenteric lymph nodes. RNAseq analysis is carried out and analyzed according to standard techniques known to one skilled in the art (Z. Hou. *Scientific Reports*. 5(9570):doi:10.1038/srep09570 (2015)). In the analysis, specific attention is placed on innate inflammatory pathway genes including TLRs, CLRs, NLRs, and STING, cytokines, chemokines, antigen processing and presentation pathways, cross presentation, and T cell co-stimulation.

**[396]** Rather than being sacrificed, some mice may be rechallenged with tumor cell injection into the contralateral flank (or other area) to determine the impact of the immune system's memory response on tumor growth.

**Example 4: Administering pmEVs to treat mouse tumor models in combination with PD-1 or PD-L1 inhibition**

**[397]** To determine the efficacy of pmEVs in tumor mouse models, in combination with PD-1 or PD-L1 inhibition, a mouse tumor model may be used as described above.

**[398]** pmEVs are tested for their efficacy in the mouse tumor model, either alone or in combination with whole bacterial cells and with or without anti-PD-1 or anti-PD-L1. pmEVs, bacterial cells, and/or anti-PD-1 or anti-PD-L1 are administered at varied time points and at varied doses. For example, on day 10 after tumor injection, or after the tumor volume reaches $100mm^3$, the mice are treated with pmEVs alone or in combination with anti-PD-1 or anti-PD-L1.

**[399]** Mice may be administered pmEVs orally, intravenously, or intratumorally. For example, some mice are intravenously injected with anywhere between 7.0e+09 to 3.0e+12 pmEV particles. While some mice receive pmEVs through i.v. injection, other mice may receive pmEVs through intraperitoneal (i.p.) injection, subcutaneous (s.c.) injection, nasal route administration, oral gavage, or other means of administration. Some mice may receive pmEVs every day (e.g., starting on day 1), while others may receive pmEVs at alternative intervals (e.g., every other day, or once every three days). Groups of mice may be administered a pharmaceutical composition of the invention comprising a mixture of pmEVs and bacterial cells. For example, the composition may comprise pmEV particles and whole bacteria in a ratio from 1:1 (pmEVs: bacterial cells) to $1\text{-}1x10^{12}$:1 (pmEVs: bacterial cells).

**[400]** Alternatively, some groups of mice may receive between $1x10^4$ and $5x10^9$ bacterial cells in an administration separate from, or comingled with, the pmEV administration. As with the pmEVs, bacterial cell administration may be varied by route of administration, dose, and schedule. The bacterial cells may be live, dead, or weakened. The bacterial cells may be harvested fresh (or frozen) and administered, or they may be irradiated or heat-killed prior to administration with the pmEVs. Some groups of mice are also injected with effective doses of checkpoint inhibitor. For example, mice receive 100 μg anti-PD-L1 mAB (clone 10f.9g2, BioXCell) or another anti-PD-1 or anti-PD-L1 mAB in 100 μl PBS, and some mice receive vehicle and/or other appropriate control (e.g., control antibody). Mice are injected with mABs 3, 6, and 9 days after the initial injection. To assess whether checkpoint inhibition and pmEV immunotherapy have an additive anti-tumor effect, control mice receiving anti-PD-1 or anti-PD-L1 mABs are included to the standard control panel. Primary (tumor size) and secondary (tumor infiltrating lymphocytes and cytokine analysis) endpoints are assessed, and some groups of mice may be rechallenged with a subsequent tumor cell inoculation to assess the effect of treatment on memory response.

**Example 5: pmEVs in a mouse model of delayed-type hypersensitivity (DTH)**

**[401]** Delayed-type hypersensitivity (DTH) is an animal model of atopic dermatitis (or allergic contact dermatitis), as reviewed by Petersen et al. (In vivo pharmacological disease models for psoriasis and atopic dermatitis in drug discovery. Basic & Clinical Pharm & Toxicology. 2006. 99(2): 104-115; see also Irving C. Allen (ed.) Mouse Models of Innate Immunity: Methods and Protocols, Methods in Molecular Biology, 2013. vol. 1031, DOI 10.1007/978-1-62703-481-4_13). Several variations of the DTH model have been used and are well known in the art (Irving C. Allen (ed.). *Mouse Models of Innate Immunity: Methods and Protocols*, Methods in Molecular Biology. Vol. 1031, DOI 10.1007/978-1-62703-481-4_13, Springer Science + Business Media, LLC 2013).

**[402]** DTH can be induced in a variety of mouse and rat strains using various haptens or antigens, for example an antigen emulsified with an adjuvant. DTH is characterized by sensitization as well as an antigen-specific T cell-mediated reaction that results in erythema, edema, and cellular infiltration – especially infiltration of antigen presenting cells (APCs), eosinophils, activated CD4+ T cells, and cytokine-expressing Th2 cells.

**[403]** Generally, mice are primed with an antigen administered in the context of an adjuvant (e.g., Complete Freund's Adjuvant) in order to induce a secondary (or memory) immune response measured by swelling and antigen-specific antibody titer.

**[404]** Dexamethasone, a corticosteroid, is a known anti-inflammatory that ameliorates DTH reactions in mice and serves as a positive control for suppressing inflammation in this model (Taube and Carlsten, Action of dexamethasone in the suppression of delayed-type hypersensitivity in reconstituted SCID mice. Inflamm Res. 2000. 49(10): 548-52). For the positive control group, a stock solution of 17 mg/mL of Dexamethasone is prepared on Day 0 by diluting 6.8 mg Dexamethasone in 400 µL 96% ethanol. For each day of dosing, a working solution is prepared by diluting the stock solution 100x in sterile PBS to obtain a final concentration of 0.17 mg/mL in a septum vial for intraperitoneal dosing. Dexamethasone-treated mice receive 100 µL Dexamethasone i.p. (5 mL/kg of a 0.17 mg/mL solution). Frozen sucrose serves as the negative control (vehicle). In the study described below, vehicle, Dexamethasone (positive control) and pmEVs were dosed daily.

**[405]** pmEVs are tested for their efficacy in the mouse model of DTH, either alone or in combination with whole bacterial cells, with or without the addition of other anti-inflammatory treatments. For example, 6-8 week old C57Bl/6 mice are obtained from Taconic (Germantown, NY), or other vendor. Groups of mice are administered four subcutaneous (s.c.) injections at four sites on the back (upper and lower) of antigen (e.g., Ovalbumin (OVA) or Keyhole Limpet Hemocyanin (KLH)) in an effective dose (e.g., 50ul total volume per site). For a DTH response, animals are injected intradermally (i.d.) in the ears under ketamine/xylazine anesthesia (approximately 50mg/kg and 5 mg/kg, respectively). Some mice serve as control animals. Some groups of mice are challenged with 10ul per ear (vehicle control (0.01% DMSO in saline) in the left ear and antigen (21.2 ug (12nmol) in the right ear) on day 8. To measure ear inflammation, the ear thickness of manually restrained animals is measured using a Mitutoyo micrometer. The ear thickness is measured before intradermal challenge as the baseline level for each individual animal. Subsequently, the ear thickness is measured two times after intradermal challenge, at approximately 24 hours and 48 hours (i.e., days 9 and 10).

**[406]** Treatment with pmEVs is initiated at some point, either around the time of priming or around the time of DTH challenge. For example, pmEVs may be administered at the same time as the subcutaneous injections (day 0), or they may be administered prior to, or upon,

intradermal injection. pmEVs are administered at varied doses and at defined intervals. For example, some mice are intravenously injected with pmEVs at 10, 15, or 20 ug/mouse. Other mice may receive 25, 50, or 100 mg of pmEVs per mouse. Alternatively, some mice receive between 7.0e+09 to 3.0e+12 pmEV particles per dose. While some mice receive pmEVs through i.v. injection, other mice may receive pmEVs through intraperitoneal (i.p.) injection, subcutaneous (s.c.) injection, nasal route administration, oral gavage, topical administration, intradermal (i.d.) injection, or other means of administration. Some mice may receive pmEVs every day (e.g., starting on day 0), while others may receive pmEVs at alternative intervals (e.g., every other day, or once every three days). Groups of mice may be administered a pharmaceutical composition of the invention comprising a mixture of pmEVs and bacterial cells. For example, the composition may comprise pmEV particles and whole bacteria in a ratio from 1:1 (pmEVs: bacterial cells) to 1-1x$10^{12}$:1 (pmEVs: bacterial cells).

**[407]** Alternatively, some groups of mice may receive between 1x$10^{4}$ and 5x$10^{9}$ bacterial cells in an administration separate from, or comingled with, the pmEV administration. As with the pmEVs, bacterial cell administration may be varied by route of administration, dose, and schedule. The bacterial cells may be live, dead, or weakened. The bacterial cells may be harvested fresh (or frozen) and administered, or they may be irradiated or heat-killed prior to administration with the pmEVs.

**[408]** For the pmEVs, total protein is measured using Bio-rad assays (Cat# 5000205) performed per manufacturer's instructions.

**[409]** An emulsion of Keyhole Limpet Hemocyanin (KLH) and Complete Freund's Adjuvant (CFA) was prepared freshly on the day of immunization (day 0). To this end, 8 mg of KLH powder is weighed and is thoroughly re-suspended in 16 mL saline. An emulsion was prepared by mixing the KLH/saline with an equal volume of CFA solution (*e.g.*, 10 mL KLH/saline + 10 mL CFA solution) using syringes and a luer lock connector. KLH and CFA were mixed vigorously for several minutes to form a white-colored emulsion to obtain maximum stability. A drop test was performed to check if a homogenous emulsion was obtained.

**[410]** On day 0, C57Bl/6J female mice, approximately 7 weeks old, were primed with KLH antigen in CFA by subcutaneous immunization (4 sites, 50 μL per site). Orally-gavaged *P. histicola* pmEVs were tested at low (6.0E+07), medium (6.0E+09), and high (6.0E+11) dosages.

**[411]** On day 8, mice were challenged intradermally (i.d.) with 10 μg KLH in saline (in a volume of 10 μL) in the left ear. Ear pinna thickness was measured at 24 hours following antigen challenge (**Figure 15**). As determined by ear thickness, *P. histicola* pmEVs were efficacious at suppressing inflammation.

**[412]** For future inflammation studies, some groups of mice may be treated with anti-inflammatory agent(s) (e.g., anti-CD154, blockade of members of the TNF family, or other treatment), and/or an appropriate control (e.g., vehicle or control antibody) at various timepoints and at effective doses.

**[413]** At various timepoints, serum samples may be taken. Other groups of mice may be sacrificed and lymph nodes, spleen, mesenteric lymph nodes (MLN), the small intestine, colon, and other tissues may be removed for histology studies, ex vivo histological, cytokine and/or flow cytometric analysis using methods known in the art. Some mice are exsanguinated from the orbital plexus under O2/CO2 anesthesia and ELISA assays performed.

**[414]** Tissues may be dissociated using dissociation enzymes according to the manufacturer's instructions. Cells are stained for analysis by flow cytometry using techniques known in the art. Staining antibodies can include anti-CD11c (dendritic cells), anti-CD80, anti-CD86, anti-CD40, anti-MHCII, anti-CD8a, anti-CD4, and anti-CD103. Other markers that may be analyzed include pan-immune cell marker CD45, T cell markers (CD3, CD4, CD8, CD25, Foxp3, T-bet, Gata3, Rory-gamma-t, Granzyme B, CD69, PD-1, CTLA-4), and macrophage/myeloid markers (CD11b, MHCII, CD206, CD40, CSF1R, PD-L1, Gr-1, F4/80). In addition to immunophenotyping, serum cytokines can be analyzed including, but not limited to, TNFa, IL-17, IL-13, IL-12p70, IL12p40, IL-10, IL-6, IL-5, IL-4, IL-2, IL-1b, IFNy, GM-CSF, G-CSF, M-CSF, MIG, IP10, MIP1b, RANTES, and MCP-1. Cytokine analysis may be carried out on immune cells obtained from lymph nodes or other tissue, and/or on purified CD45+ infiltrated immune cells obtained ex vivo. Finally, immunohistochemistry is carried out on various tissue sections to measure T cells, macrophages, dendritic cells, and checkpoint molecule protein expression.

**[415]** Ears may be removed from the sacrificed animals and placed in cold EDTA-free protease inhibitor cocktail (Roche). Ears are homogenized using bead disruption and supernatants analyzed for various cytokines by Luminex kit (EMD Millipore) as per manufacturer's instructions. In addition, cervical lymph nodes are dissociated through a cell

strainer, washed, and stained for FoxP3 (PE-FJK-16s) and CD25 (FITC-PC61.5) using methods known in the art.

**[416]** In order to examine the impact and longevity of DTH protection, rather than being sacrificed, some mice may be rechallenged with the challenging antigen at a later time and mice analyzed for susceptibility to DTH and severity of response.

**Example 6: pmEVs in a mouse model of Experimental Autoimmune Encephalomyelitis (EAE)**

**[417]** EAE is a well-studied animal model of multiple sclerosis, as reviewed by Constantinescu et al., (Experimental autoimmune encephalomyelitis (EAE) as a model for multiple sclerosis (MS). Br J Pharmacol. 2011 Oct; 164(4): 1079-1106). It can be induced in a variety of mouse and rat strains using different myelin-associated peptides, by the adoptive transfer of activated encephalitogenic T cells, or the use of TCR transgenic mice susceptible to EAE, as discussed in Mangalam et al., (Two discreet subsets of CD8+ T cells modulate $PLP_{91-110}$ induced experimental autoimmune encephalomyelitis in HLA-DR3 transgenic mice. J Autoimmun. 2012 Jun; 38(4): 344-353).

**[418]** pmEVs are tested for their efficacy in the rodent model of EAE, either alone or in combination with whole bacterial cells, with or without the addition of other anti-inflammatory treatments. Additionally, pmEVs may be administered orally or via intravenous administration. For example, female 6-8 week old C57Bl/6 mice are obtained from Taconic (Germantown, NY). Groups of mice are administered two subcutaneous (s.c.) injections at two sites on the back (upper and lower) of 0.1 ml myelin oligodentrocyte glycoprotein 35-55 (MOG35-55; 100ug per injection; 200ug per mouse (total 0.2ml per mouse)), emulsified in Complete Freund's Adjuvant (CFA; 2-5mg killed mycobacterium tuberculosis H37Ra/ml emulsion). Approximately 1-2 hours after the above, mice are intraperitoneally (i.p.) injected with 200ng Pertussis toxin (PTx) in 0.1ml PBS (2ug/ml). An additional IP injection of PTx is administered on day 2. Alternatively, an appropriate amount of an alternative myelin peptide (e.g., proteolipid protein (PLP)) is used to induce EAE. Some animals serve as naïve controls. EAE severity is assessed and a disability score is assigned daily beginning on day 4 according to methods known in the art (Mangalam et al. 2012).

**[419]** Treatment with pmEVs is initiated at some point, either around the time of immunization or following EAE immunization. For example, pmEVs may be administered at the same time as immunization (day 1), or they may be administered upon the first signs of disability (e.g., limp tail), or during severe EAE. pmEVs are administered at varied doses and at defined intervals. For example, some mice are intravenously injected with pmEVs at 10, 15, or 20 ug/mouse. Other mice may receive 25, 50, or 100 mg of pmEVs per mouse. Alternatively, some mice receive between 7.0e+09 to 3.0e+12 pmEV particles per dose. While some mice receive pmEVs through i.v. injection, other mice may receive pmEVs through intraperitoneal (i.p.) injection, subcutaneous (s.c.) injection, nasal route administration, oral gavage, or other means of administration. Some mice may receive pmEVs every day (e.g., starting on day 1), while others may receive pmEVs at alternative intervals (e.g., every other day, or once every three days). Groups of mice may be administered a pharmaceutical composition of the invention comprising a mixture of pmEVs and bacterial cells. For example, the composition may comprise pmEV particles and whole bacteria in a ratio from 1:1 (pmEVs: bacterial cells) to 1-1x$10^{12}$:1 (pmEVs: bacterial cells).

**[420]** Alternatively, some groups of mice may receive between 1x$10^{4}$ and 5x$10^{9}$ bacterial cells in an administration separate from, or comingled with, the pmEV administration. As with the pmEVs, bacterial cell administration may be varied by route of administration, dose, and schedule. The bacterial cells may be live, dead, or weakened. The bacterial cells may be harvested fresh (or frozen) and administered, or they may be irradiated or heat-killed prior to administration with the pmEVs.

**[421]** Some groups of mice may be treated with additional anti-inflammatory agent(s) or EAE therapeutic(s) (e.g., anti-CD154, blockade of members of the TNF family, Vitamin D, steroids, anti-inflammatory agents, or other treatment(s)), and/or an appropriate control (e.g., vehicle or control antibody) at various time points and at effective doses.

**[422]** In addition, some mice are treated with antibiotics prior to treatment. For example, vancomycin (0.5g/L), ampicillin (1.0g/L), gentamicin (1.0g/L) and amphotericin B (0.2g/L) are added to the drinking water, and antibiotic treatment is halted at the time of treatment or a few days prior to treatment. Some immunized mice are treated without receiving antibiotics.

**[423]** At various timepoints, mice are sacrificed and sites of inflammation (e.g., brain and spinal cord), lymph nodes, or other tissues may be removed for ex vivo histological, cytokine and/or flow cytometric analysis using methods known in the art. For example, tissues are dissociated using dissociation enzymes according to the manufacturer's instructions. Cells are stained for analysis by flow cytometry using techniques known in the art. Staining antibodies can include anti-CD11c (dendritic cells), anti-CD80, anti-CD86, anti-CD40, anti-MHCII, anti-CD8a, anti-CD4, and anti-CD103. Other markers that may be analyzed include pan-immune cell marker CD45, T cell markers (CD3, CD4, CD8, CD25, Foxp3, T-bet, Gata3, Roryt, Granzyme B, CD69, PD-1, CTLA-4), and macrophage/myeloid markers (CD11b, MHCII, CD206, CD40, CSF1R, PD-L1, Gr-1, F4/80). In addition to immunophenotyping, serum cytokines can be analyzed including, but not limited to, TNFa, IL-17, IL-13, IL-12p70, IL12p40, IL-10, IL-6, IL-5, IL-4, IL-2, IL-1b, IFNy, GM-CSF, G-CSF, M-CSF, MIG, IP10, MIP1b, RANTES, and MCP-1. Cytokine analysis may be carried out on immune cells obtained from lymph nodes or other tissue, and/or on purified CD45+ central nervous system (CNS)-infiltrated immune cells obtained ex vivo. Finally, immunohistochemistry is carried out on various tissue sections to measure T cells, macrophages, dendritic cells, and checkpoint molecule protein expression.

**[424]** In order to examine the impact and longevity of disease protection, rather than being sacrificed, some mice may be rechallenged with a disease trigger (e.g., activated encephalitogenic T cells or re-injection of EAE-inducing peptides). Mice are analyzed for susceptibility to disease and EAE severity following rechallenge.

**Example 7: pmEVs in a mouse model of collagen-induced arthritis (CIA)**

**[425]** Collagen-induced arthritis (CIA) is an animal model commonly used to study rheumatoid arthritis (RA), as described by Caplazi et al. (Mouse models of rheumatoid arthritis. Veterinary Pathology. Sept. 1, 2015. 52(5): 819-826) (see also Brand et al. Collagen-induced arthritis. Nature Protocols. 2007. 2: 1269-1275; Pietrosimone et al. Collagen-induced arthritis: a model for murine autoimmune arthritis. Bio Protoc. 2015 Oct. 20; 5(20): e1626).

**[426]** Among other versions of the CIA rodent model, one model involves immunizing HLA-DQ8 Tg mice with chick type II collagen as described by Taneja et al. (J. Immunology. 2007. 56: 69-78; see also Taneja et al. J. Immunology 2008. 181: 2869-2877; and Taneja et al. Arthritis Rheum., 2007. 56: 69-78). Purification of chick CII has been described by Taneja et al.

(Arthritis Rheum., 2007. 56: 69-78). Mice are monitored for CIA disease onset and progression following immunization, and severity of disease is evaluated and "graded" as described by Wooley, J. Exp. Med. 1981. 154: 688-700.

**[427]** Mice are immunized for CIA induction and separated into various treatment groups. pmEVs are tested for their efficacy in CIA, either alone or in combination with whole bacterial cells, with or without the addition of other anti-inflammatory treatments.

**[428]** Treatment with pmEVs is initiated either around the time of immunization with collagen or post-immunization. For example, in some groups, pmEVs may be administered at the same time as immunization (day 1), or pmEVs may be administered upon first signs of disease, or upon the onset of severe symptoms. pmEVs are administered at varied doses and at defined intervals. For example, some mice are intravenously injected with pmEVs at 10, 15, or 20 ug/mouse. Other mice may receive 25, 50, or 100 mg of pmEVs per mouse. Alternatively, some mice receive between 7.0e+09 to 3.0e+12 pmEV particles per dose. While some mice receive pmEVs through oral gavage or i.v. injection, while other groups of mice may receive pmEVs through intraperitoneal (i.p.) injection, subcutaneous (s.c.) injection, nasal route administration, or other means of administration. Some mice may receive pmEVs every day (e.g., starting on day 1), while others may receive pmEVs at alternative intervals (e.g., every other day, or once every three days). Groups of mice may be administered a pharmaceutical composition of the invention comprising a mixture of pmEVs and bacterial cells. For example, the composition may comprise pmEV particles and whole bacteria in a ratio from 1:1 (pmEVs: bacterial cells) to 1-$1x10^{12}$:1 (pmEVs: bacterial cells).

**[429]** Alternatively, some groups of mice may receive between $1x10^4$ and $5x10^9$ bacterial cells in an administration separate from, or comingled with, the pmEV administration. As with the pmEVs, bacterial cell administration may be varied by route of administration, dose, and schedule. The bacterial cells may be live, dead, or weakened. The bacterial cells may be harvested fresh (or frozen) and administered, or they may be irradiated or heat-killed prior to administration with the pmEVs.

**[430]** Some groups of mice may be treated with additional anti-inflammatory agent(s) or CIA therapeutic(s) (e.g., anti-CD154, blockade of members of the TNF family, Vitamin D, steroid(s), anti-inflammatory agent(s), and/or other treatment), and/or an appropriate control (e.g., vehicle or control antibody) at various timepoints and at effective doses.

**[431]** In addition, some mice are treated with antibiotics prior to treatment. For example, vancomycin (0.5g/L), ampicillin (1.0g/L), gentamicin (1.0g/L) and amphotericin B (0.2g/L) are added to the drinking water, and antibiotic treatment is halted at the time of treatment or a few days prior to treatment. Some immunized mice are treated without receiving antibiotics.

**[432]** At various timepoints, serum samples are obtained to assess levels of anti-chick and anti-mouse CII IgG antibodies using a standard ELISA (Batsalova et al. Comparative analysis of collagen type II-specific immune responses during development of collagen-induced arthritis in two B10 mouse strains. Arthritis Res Ther. 2012. 14(6): R237). Also, some mice are sacrificed and sites of inflammation (e.g., synovium), lymph nodes, or other tissues may be removed for ex vivo histological, cytokine and/or flow cytometric analysis using methods known in the art. The synovium and synovial fluid are analyzed for plasma cell infiltration and the presence of antibodies using techniques known in the art. In addition, tissues are dissociated using dissociation enzymes according to the manufacturer's instructions to examine the profiles of the cellular infiltrates. Cells are stained for analysis by flow cytometry using techniques known in the art. Staining antibodies can include anti-CD11c (dendritic cells), anti-CD80, anti-CD86, anti-CD40, anti-MHCII, anti-CD8a, anti-CD4, and anti-CD103. Other markers that may be analyzed include pan-immune cell marker CD45, T cell markers (CD3, CD4, CD8, CD25, Foxp3, T-bet, Gata3, Roryt, Granzyme B, CD69, PD-1, CTLA-4), and macrophage/myeloid markers (CD11b, MHCII, CD206, CD40, CSF1R, PD-L1, Gr-1, F4/80). In addition to immunophenotyping, serum cytokines can be analyzed including, but not limited to, TNFa, IL-17, IL-13, IL-12p70, IL12p40, IL-10, IL-6, IL-5, IL-4, IL-2, IL-1b, IFNy, GM-CSF, G-CSF, M-CSF, MIG, IP10, MIP1b, RANTES, and MCP-1. Cytokine analysis may be carried out on immune cells obtained from lymph nodes or other tissue, and/or on purified CD45+ synovium-infiltrated immune cells obtained ex vivo. Finally, immunohistochemistry is carried out on various tissue sections to measure T cells, macrophages, dendritic cells, and checkpoint molecule protein expression.

**[433]** In order to examine the impact and longevity of disease protection, rather than being sacrificed, some mice may be rechallenged with a disease trigger (e.g., activated re-injection with CIA-inducing peptides). Mice are analyzed for susceptibility to disease and CIA severity following rechallenge.

**Example 8: pmEVs in a mouse model of colitis**

**[434]** Dextran sulfate sodium (DSS)-induced colitis is a well-studied animal model of colitis, as reviewed by Randhawa et al. (A review on chemical-induced inflammatory bowel disease models in rodents. Korean J Physiol Pharmacol. 2014. 18(4): 279-288; see also Chassaing et al. Dextran sulfate sodium (DSS)-induced colitis in mice. Curr Protoc Immunol. 2014 Feb 4; 104: Unit 15.25).

**[435]** pmEVs are tested for their efficacy in a mouse model of DSS-induced colitis, either alone or in combination with whole bacterial cells, with or without the addition of other anti-inflammatory agents.

**[436]** Groups of mice are treated with DSS to induce colitis as known in the art (Randhawa et al. 2014; Chassaing et al. 2014; see also Kim et al. Investigating intestinal inflammation in DSS-induced model of IBD. J Vis Exp. 2012. 60: 3678). For example, male 6-8 week old C57Bl/6 mice are obtained from Charles River Labs, Taconic, or other vendor. Colitis is induced by adding 3% DSS (MP Biomedicals, Cat. #0260110) to the drinking water. Some mice do not receive DSS in the drinking water and serve as naïve controls. Some mice receive water for five (5) days. Some mice may receive DSS for a shorter duration or longer than five (5) days. Mice are monitored and scored using a disability activity index known in the art based on weight loss (e.g., no weight loss (score 0); 1-5% weight loss (score 1); 5-10% weight loss (score 2)); stool consistency (e.g., normal (score 0); loose stool (score 2); diarrhea (score 4)); and bleeding (e.g., no blood (score 0), hemoccult positive (score 1); hemoccult positive and visual pellet bleeding (score 2); blood around anus, gross bleeding (score 4).

**[437]** Treatment with pmEVs is initiated at some point, either on day 1 of DSS administration, or sometime thereafter. For example, pmEVs may be administered at the same time as DSS initiation (day 1), or they may be administered upon the first signs of disease (e.g., weight loss or diarrhea), or during the stages of severe colitis. Mice are observed daily for weight, morbidity, survival, presence of diarrhea and/or bloody stool.

**[438]** pmEVs are administered at various doses and at defined intervals. For example, some mice receive between 7.0e+09 and 3.0e+12 pmEV particles. While some mice receive pmEVs through oral gavage or i.v. injection, while other groups of mice may receive pmEVs through intraperitoneal (i.p.) injection, subcutaneous (s.c.) injection, nasal route administration,

or other means of administration. Some mice may receive pmEVs every day (e.g., starting on day 1), while others may receive pmEVs at alternative intervals (e.g., every other day, or once every three days). Groups of mice may be administered a pharmaceutical composition of the invention comprising a mixture of pmEVs and bacterial cells. For example, the composition may comprise pmEV particles and whole bacteria in a ratio from 1:1 (pmEVs: bacterial cells) to 1-$1x10^{12}$:1 (pmEVs: bacterial cells).

**[439]** Alternatively, some groups of mice may receive between $1x10^4$ and $5x10^9$ bacterial cells in an administration separate from, or comingled with, the pmEV administration. As with the pmEVs, bacterial cell administration may be varied by route of administration, dose, and schedule. The bacterial cells may be live, dead, or weakened. The bacterial cells may be harvested fresh (or frozen) and administered, or they may be irradiated or heat-killed prior to administration with the pmEVs.

**[440]** Some groups of mice may be treated with additional anti-inflammatory agent(s) (e.g., anti-CD154, blockade of members of the TNF family, or other treatment), and/or an appropriate control (e.g., vehicle or control antibody) at various timepoints and at effective doses.

**[441]** In addition, some mice are treated with antibiotics prior to treatment. For example, vancomycin (0.5g/L), ampicillin (1.0g/L), gentamicin (1.0g/L) and amphotericin B (0.2g/L) are added to the drinking water, and antibiotic treatment is halted at the time of treatment or a few days prior to treatment. Some mice receive DSS without receiving antibiotics beforehand.

**[442]** At various timepoints, mice undergo video endoscopy using a small animal endoscope (Karl Storz Endoskipe, Germany) under isoflurane anesthesia. Still images and video are recorded to evaluate the extent of colitis and the response to treatment. Colitis is scored using criteria known in the art. Fecal material is collected for study.

**[443]** At various timepoints, mice are sacrificed and the colon, small intestine, spleen, and lymph nodes (e.g., mesenteric lymph nodes) are collected. Additionally, blood is collected into serum separation tubes. Tissue damage is assessed through histological studies that evaluate, but are not limited to, crypt architecture, degree of inflammatory cell infiltration, and goblet cell depletion.

**[444]** The gastrointestinal (GI) tract, lymph nodes, and/or other tissues may be removed for ex vivo histological, cytokine and/or flow cytometric analysis using methods known in the art. For example, tissues are harvested and may be dissociated using dissociation enzymes according to the manufacturer's instructions. Cells are stained for analysis by flow cytometry using techniques known in the art. Staining antibodies can include anti-CD11c (dendritic cells), anti-CD80, anti-CD86, anti-CD40, anti-MHCII, anti-CD8a, anti-CD4, and anti-CD103. Other markers that may be analyzed include pan-immune cell marker CD45, T cell markers (CD3, CD4, CD8, CD25, Foxp3, T-bet, Gata3, Roryt, Granzyme B, CD69, PD-1, CTLA-4), and macrophage/myeloid markers (CD11b, MHCII, CD206, CD40, CSF1R, PD-L1, Gr-1, F4/80). In addition to immunophenotyping, serum cytokines can be analyzed including, but not limited to, TNFa, IL-17, IL-13, IL-12p70, IL12p40, IL-10, IL-6, IL-5, IL-4, IL-2, IL-1b, IFNy, GM-CSF, G-CSF, M-CSF, MIG, IP10, MIP1b, RANTES, and MCP-1. Cytokine analysis may be carried out on immune cells obtained from lymph nodes or other tissue, and/or on purified CD45+ GI tract-infiltrated immune cells obtained ex vivo. Finally, immunohistochemistry is carried out on various tissue sections to measure T cells, macrophages, dendritic cells, and checkpoint molecule protein expression.

**[445]** In order to examine the impact and longevity of disease protection, rather than being sacrificed, some mice may be rechallenged with a disease trigger. Mice are analyzed for susceptibility to colitis severity following rechallenge.

**Example 9: pmEVs in a mouse model of Type 1 Diabetes (T1D)**

**[446]** Type 1 diabetes (T1D) is an autoimmune disease in which the immune system targets the islets of Langerhans of the pancreas, thereby destroying the body's ability to produce insulin.

**[447]** There are various models of animal models of T1D, as reviewed by Belle et al. (Mouse models for type 1 diabetes. Drug Discov Today Dis Models. 2009; 6(2): 41-45; see also Aileen JF King. The use of animal models in diabetes research. Br J Pharmacol. 2012 Jun; 166(3): 877-894. There are models for chemically-induced T1D, pathogen-induced T1D, as well as models in which the mice spontaneously develop T1D.

**[448]** pmEVs are tested for their efficacy in a mouse model of T1D, either alone or in combination with whole bacterial cells, with or without the addition of other anti-inflammatory treatments.

**[449]** Depending on the method of T1D induction and/or whether T1D development is spontaneous, treatment with pmEVs is initiated at some point, either around the time of induction or following induction, or prior to the onset (or upon the onset) of spontaneously-occurring T1D. pmEVs are administered at varied doses and at defined intervals. For example, some mice are intravenously injected with pmEVs at 10, 15, or 20 ug/mouse. Other mice may receive 25, 50, or 100 mg of pmEVs per mouse. Alternatively, some mice receive between 7.0e+09 to 3.0e+12 pmEV particles per dose. While some mice receive pmEVs through oral gavage or i.v. injection, while other groups of mice may receive pmEVs through intraperitoneal (i.p.) injection, subcutaneous (s.c.) injection, nasal route administration, or other means of administration. Some mice may receive pmEVs every day, while others may receive pmEVs at alternative intervals (e.g., every other day, or once every three days). Groups of mice may be administered a pharmaceutical composition of the invention comprising a mixture of pmEVs and bacterial cells. For example, the composition may comprise pmEV particles and whole bacteria in a ratio from 1:1 (pmEVs: bacterial cells) to 1-1x$10^{12}$:1 (pmEVs: bacterial cells).

**[450]** Alternatively, some groups of mice may receive between 1x$10^{4}$ and 5x$10^{9}$ bacterial cells in an administration separate from, or comingled with, the pmEV administration. As with the pmEVs, bacterial cell administration may be varied by route of administration, dose, and schedule. The bacterial cells may be live, dead, or weakened. The bacterial cells may be harvested fresh (or frozen) and administered, or they may be irradiated or heat-killed prior to administration with the pmEVs.

**[451]** Some groups of mice may be treated with additional treatments and/or an appropriate control (e.g., vehicle or control antibody) at various timepoints and at effective doses.

**[452]** In addition, some mice are treated with antibiotics prior to treatment. For example, vancomycin (0.5g/L), ampicillin (1.0g/L), gentamicin (1.0g/L) and amphotericin B (0.2g/L) are added to the drinking water, and antibiotic treatment is halted at the time of treatment or a few days prior to treatment. Some immunized mice are treated without receiving antibiotics.

[453] Blood glucose is monitored biweekly prior to the start of the experiment. At various timepoints thereafter, nonfasting blood glucose is measured. At various timepoints, mice are sacrificed and site the pancreas, lymph nodes, or other tissues may be removed for ex vivo histological, cytokine and/or flow cytometric analysis using methods known in the art. For example, tissues are dissociated using dissociation enzymes according to the manufacturer's instructions. Cells are stained for analysis by flow cytometry using techniques known in the art. Staining antibodies can include anti-CD11c (dendritic cells), anti-CD80, anti-CD86, anti-CD40, anti-MHCII, anti-CD8a, anti-CD4, and anti-CD103. Other markers that may be analyzed include pan-immune cell marker CD45, T cell markers (CD3, CD4, CD8, CD25, Foxp3, T-bet, Gata3, Roryt, Granzyme B, CD69, PD-1, CTLA-4), and macrophage/myeloid markers (CD11b, MHCII, CD206, CD40, CSF1R, PD-L1, Gr-1, F4/80). In addition to immunophenotyping, serum cytokines can be analyzed including, but not limited to, TNFa, IL-17, IL-13, IL-12p70, IL12p40, IL-10, IL-6, IL-5, IL-4, IL-2, IL-1b, IFNy, GM-CSF, G-CSF, M-CSF, MIG, IP10, MIP1b, RANTES, and MCP-1. Cytokine analysis may be carried out on immune cells obtained from lymph nodes or other tissue, and/or on purified tissue-infiltrating immune cells obtained ex vivo. Finally, immunohistochemistry is carried out on various tissue sections to measure T cells, macrophages, dendritic cells, and checkpoint molecule protein expression. Antibody production may also be assessed by ELISA.

[454] In order to examine the impact and longevity of disease protection, rather than being sacrificed, some mice may be rechallenged with a disease trigger, or assessed for susceptibility to relapse. Mice are analyzed for susceptibility to diabetes onset and severity following rechallenge (or spontaneously-occurring relapse).

**Example 10: pmEVs in a mouse model of Primary Sclerosing Cholangitis (PSC)**

[455] Primary Sclerosing Cholangitis (PSC) is a chronic liver disease that slowly damages the bile ducts and leads to end-stage cirrhosis. It is associated with inflammatory bowel disease (IBD).

[456] There are various animal models for PSC, as reviewed by Fickert et al. (Characterization of animal models for primary sclerosing cholangitis (PSC). J Hepatol. 2014 Jun. 60(6): 1290-1303; see also Pollheimer and Fickert. Animal models in primary biliary cirrhosis and primary sclerosing cholangitis. Clin Rev Allergy Immunol. 2015 Jun. 48(2-3): 207-

17). Induction of disease in PSC models includes chemical induction (e.g., 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC)-induced cholangitis), pathogen-induced (e.g., Cryptosporidium parvum), experimental biliary obstruction (e.g., common bile duct ligation (CBDL)), and transgenic mouse model of antigen-driven biliary injury (e.g., Ova-Bil transgenic mice). For example, bile duct ligation is performed as described by Georgiev et al. (Characterization of time-related changes after experimental bile duct ligation. Br J Surg. 2008. 95(5): 646-56), or disease is induced by DCC exposure as described by Fickert et al. (A new xenobiotic-induced mouse model of sclerosing cholangitis and biliary fibrosis. Am J Path. Vol 171(2): 525-536.

**[457]** pmEVs are tested for their efficacy in a mouse model of PSC, either alone or in combination with whole bacterial cells, with or without the addition of some other therapeutic agent.

*DCC-induced Cholangitis*

**[458]** For example, 6-8 week old C57bl/6 mice are obtained from Taconic or other vendor. Mice are fed a 0.1% DCC-supplemented diet for various durations. Some groups receive DCC-supplement food for 1 week, others for 4 weeks, others for 8 weeks. Some groups of mice may receive a DCC-supplemented diet for a length of time and then be allowed to recover, thereafter receiving a normal diet. These mice may be studied for their ability to recover from disease and/or their susceptibility to relapse upon subsequent exposure to DCC. Treatment with pmEVs is initiated at some point, either around the time of DCC-feeding or subsequent to initial exposure to DCC. For example, pmEVs may be administered on day 1, or they may be administered sometime thereafter. pmEVs are administered at varied doses and at defined intervals. For example, some mice are intravenously injected with pmEVs at 10, 15, or 20 ug/mouse. Alternatively, some mice may receive between 7.0e+09 and 3.0e+12 pmEV particles. While some mice receive pmEVs through oral gavage or i.v. injection, while other groups of mice may receive pmEVs through intraperitoneal (i.p.) injection, subcutaneous (s.c.) injection, nasal route administration, or other means of administration. Some mice may receive pmEVs every day (e.g., starting on day 1), while others may receive pmEVs at alternative intervals (e.g., every other day, or once every three days). Groups of mice may be administered a pharmaceutical composition of the invention comprising a mixture of pmEVs and bacterial cells. For example, the composition may comprise pmEV particles and whole bacteria in a ratio from 1:1 (pmEVs: bacterial cells) to 1-1x$10^{12}$:1 (pmEVs: bacterial cells).

**[459]** Alternatively, some groups of mice may receive between $1x10^4$ and $5x10^9$ bacterial cells in an administration separate from, or comingled with, the pmEV administration. As with the pmEVs, bacterial cell administration may be varied by route of administration, dose, and schedule. The bacterial cells may be live, dead, or weakened. The bacterial cells may be harvested fresh (or frozen) and administered, or they may be irradiated or heat-killed prior to administration with the pmEVs.

**[460]** Some groups of mice may be treated with additional agents and/or an appropriate control (e.g., vehicle or antibody) at various timepoints and at effective doses.

**[461]** In addition, some mice are treated with antibiotics prior to treatment. For example, vancomycin (0.5g/L), ampicillin (1.0g/L), gentamicin (1.0g/L) and amphotericin B (0.2g/L) are added to the drinking water, and antibiotic treatment is halted at the time of treatment or a few days prior to treatment. Some immunized mice are treated without receiving antibiotics. At various timepoints, serum samples are analyzed for ALT, AP, bilirubin, and serum bile acid (BA) levels.

**[462]** At various timepoints, mice are sacrificed, body and liver weight are recorded, and sites of inflammation (e.g., liver, small and large intestine, spleen), lymph nodes, or other tissues may be removed for *ex vivo* histolomorphological characterization, cytokine and/or flow cytometric analysis using methods known in the art (see Fickert et al. Characterization of animal models for primary sclerosing cholangitis (PSC)). J Hepatol. 2014. 60(6): 1290-1303). For example, bile ducts are stained for expression of ICAM-1, VCAM-1, MadCAM-1. Some tissues are stained for histological examination, while others are dissociated using dissociation enzymes according to the manufacturer's instructions. Cells are stained for analysis by flow cytometry using techniques known in the art. Staining antibodies can include anti-CD11c (dendritic cells), anti-CD80, anti-CD86, anti-CD40, anti-MHCII, anti-CD8a, anti-CD4, and anti-CD103. Other markers that may be analyzed include pan-immune cell marker CD45, T cell markers (CD3, CD4, CD8, CD25, Foxp3, T-bet, Gata3, Roryt, Granzyme B, CD69, PD-1, CTLA-4), and macrophage/myeloid markers (CD11b, MHCII, CD206, CD40, CSF1R, PD-L1, Gr-1, F4/80), as well as adhesion molecule expression (ICAM-1, VCAM-1, MadCAM-1). In addition to immunophenotyping, serum cytokines can be analyzed including, but not limited to, TNFa, IL-17, IL-13, IL-12p70, IL12p40, IL-10, IL-6, IL-5, IL-4, IL-2, IL-1b, IFNy, GM-CSF, G-CSF, M-CSF, MIG, IP10, MIP1b, RANTES, and MCP-1. Cytokine analysis may be carried out on

immune cells obtained from lymph nodes or other tissue, and/or on purified CD45+ bile duct-infiltrated immune cells obtained ex vivo.

**[463]** Liver tissue is prepared for histological analysis, for example, using Sirius-red staining followed by quantification of the fibrotic area. At the end of the treatment, blood is collected for plasma analysis of liver enzymes, for example, AST or ALT, and to determine Bilirubin levels. The hepatic content of Hydroxyproline can be measured using established protocols. Hepatic gene expression analysis of inflammation and fibrosis markers may be performed by qRT-PCR using validated primers. These markers may include, but are not limited to, MCP-1, alpha-SMA, Coll1a1, and TIMP. Metabolite measurements may be performed in plasma, tissue and fecal samples using established metabolomics methods. Finally, immunohistochemistry is carried out on liver sections to measure neutrophils, T cells, macrophages, dendritic cells, or other immune cell infiltrates.

**[464]** In order to examine the impact and longevity of disease protection, rather than being sacrificed, some mice may be rechallenged with DCC at a later time. Mice are analyzed for susceptibility to cholangitis and cholangitis severity following rechallenge.

*BDL-induced Cholangitis*

**[465]** Alternatively, pmEVs are tested for their efficacy in BDL-induced cholangitis. For example, 6-8 week old C57Bl/6J mice are obtained from Taconic or other vendor. After an acclimation period the mice are subjected to a surgical procedure to perform a bile duct ligation (BDL). Some control animals receive a sham surgery. The BDL procedure leads to liver injury, inflammation and fibrosis within 7-21 days.

**[466]** Treatment with pmEVs is initiated at some point, either around the time of surgery or some time following the surgery. pmEVs are administered at varied doses and at defined intervals. For example, some mice are intravenously injected with pmEVs at 10, 15, or 20 ug/mouse. Other mice may receive 25, 50, or 100 mg of pmEVs per mouse. Alternatively, some mice receive between 7.0e+09 to 3.0e+12 pmEV particles per dose. While some mice receive pmEVs through oral gavage or i.v. injection, while other groups of mice may receive pmEVs through intraperitoneal (i.p.) injection, subcutaneous (s.c.) injection, nasal route administration, or other means of administration. Some mice receive pmEVs every day (e.g., starting on day 1), while others may receive pmEVs at alternative intervals (e.g., every other day,

or once every three days). Groups of mice may be administered a pharmaceutical composition of the invention comprising a mixture of pmEVs and bacterial cells. For example, the composition may comprise pmEV particles and whole bacteria in a ratio from 1:1 (pmEVs: bacterial cells) to 1-1x$10^{12}$:1 (pmEVs: bacterial cells).

**[467]** Alternatively, some groups of mice may receive between 1x$10^4$ and 5x$10^9$ bacterial cells in an administration separate from, or comingled with, the pmEV administration. As with the pmEVs, bacterial cell administration may be varied by route of administration, dose, and schedule. The bacterial cells may be live, dead, or weakened. The bacterial cells may be harvested fresh (or frozen) and administered, or they may be irradiated or heat-killed prior to administration with the pmEVs.

**[468]** Some groups of mice may be treated with additional agents and/or an appropriate control (e.g., vehicle or antibody) at various timepoints and at effective doses.

**[469]** In addition, some mice are treated with antibiotics prior to treatment. For example, vancomycin (0.5g/L), ampicillin (1.0g/L), gentamicin (1.0g/L) and amphotericin B (0.2g/L) are added to the drinking water, and antibiotic treatment is halted at the time of treatment or a few days prior to treatment. Some immunized mice are treated without receiving antibiotics. At various timepoints, serum samples are analyzed for ALT, AP, bilirubin, and serum bile acid (BA) levels.

**[470]** At various timepoints, mice are sacrificed, body and liver weight are recorded, and sites of inflammation (e.g., liver, small and large intestine, spleen), lymph nodes, or other tissues may be removed for ex vivo histolomorphological characterization, cytokine and/or flow cytometric analysis using methods known in the art (see Fickert et al. Characterization of animal models for primary sclerosing cholangitis (PSC)). J Hepatol. 2014. 60(6): 1290-1303). For example, bile ducts are stained for expression of ICAM-1, VCAM-1, MadCAM-1. Some tissues are stained for histological examination, while others are dissociated using dissociation enzymes according to the manufacturer's instructions. Cells are stained for analysis by flow cytometry using techniques known in the art. Staining antibodies can include anti-CD11c (dendritic cells), anti-CD80, anti-CD86, anti-CD40, anti-MHCII, anti-CD8a, anti-CD4, and anti-CD103. Other markers that may be analyzed include pan-immune cell marker CD45, T cell markers (CD3, CD4, CD8, CD25, Foxp3, T-bet, Gata3, Roryt, Granzyme B, CD69, PD-1, CTLA-4), and macrophage/myeloid markers (CD11b, MHCII, CD206, CD40, CSF1R, PD-L1, Gr-1, F4/80), as

well as adhesion molecule expression (ICAM-1, VCAM-1, MadCAM-1). In addition to immunophenotyping, serum cytokines can be analyzed including, but not limited to, TNFa, IL-17, IL-13, IL-12p70, IL12p40, IL-10, IL-6, IL-5, IL-4, IL-2, IL-1b, IFNy, GM-CSF, G-CSF, M-CSF, MIG, IP10, MIP1b, RANTES, and MCP-1. Cytokine analysis may be carried out on immune cells obtained from lymph nodes or other tissue, and/or on purified CD45+ bile duct-infiltrated immune cells obtained ex vivo.

**[471]** Liver tissue is prepared for histological analysis, for example, using Sirius-red staining followed by quantification of the fibrotic area. At the end of the treatment, blood is collected for plasma analysis of liver enzymes, for example, AST or ALT, and to determine Bilirubin levels. The hepatic content of Hydroxyproline can be measured using established protocols. Hepatic gene expression analysis of inflammation and fibrosis markers may be performed by qRT-PCR using validated primers. These markers may include, but are not limited to, MCP-1, alpha-SMA, Coll1a1, and TIMP. Metabolite measurements may be performed in plasma, tissue and fecal samples using established metabolomics methods. Finally, immunohistochemistry is carried out on liver sections to measure neutrophils, T cells, macrophages, dendritic cells, or other immune cell infiltrates.

**[472]** In order to examine the impact and longevity of disease protection, rather than being sacrificed, some mice may be analyzed for recovery.

**Example 11: pmEVs in a mouse model of Nonalcoholic Steatohepatitis (NASH)**

**[473]** Nonalcoholic Steatohepatitis (NASH) is a severe form of Nonalcoholic Fatty Liver Disease (NAFLD), where buildup of hepatic fat (steatosis) and inflammation lead to liver injury and hepatocyte cell death (ballooning).

**[474]** There are various animal models of NASH, as reviewed by Ibrahim et al. (Animal models of nonalcoholic steatohepatitis: Eat, Delete, and Inflame. Dig Dis Sci. 2016 May. 61(5): 1325-1336; see also Lau et al. Animal models of non-alcoholic fatty liver disease: current perspectives and recent advances 2017 Jan. 241(1): 36-44).

**[475]** pmEVs are tested for their efficacy in a mouse model of NASH, either alone or in combination with whole bacterial cells, with or without the addition of another therapeutic agent. For example, 8-10 week old C57Bl/6J mice, obtained from Taconic (Germantown, NY), or other

vendor, are placed on a methionine choline deficient (MCD) diet for a period of 4-8 weeks during which NASH features develop, including steatosis, inflammation, ballooning and fibrosis.

**[476]** *P. histicola* pmEVs are tested for their efficacy in a mouse model of NASH, either alone or in combination with each other, in varying proportions, with or without the addition of another therapeutic agent. For example, 8 week old C57Bl/6J mice, obtained from Charles River (France), or other vendor, are acclimated for a period of 5 days, randomized intro groups of 10 mice based on body weight, and placed on a methionine choline deficient (MCD) diet for example A02082002B from Research Diets (USA), for a period of 4 weeks during which NASH features developed, including steatosis, inflammation, ballooning and fibrosis. Control chow mice are fed a normal chow diet, for example RM1 (E) 801492 from SDS Diets (UK). Control chow, MCD diet, and water are provided *ad libitum*.

**[477]** An NAS scoring system adapted from Kleiner et al. (Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology. 2005 Jun. 41(6): 1313-1321) is used to determine the degree of steatosis (scored 0-3), lobular inflammation (scored 0-3), hepatocyte ballooning (scored 0-3), and fibrosis (scored 0-4). An individual mouse NAS score may be calculated by summing the score for steatosis, inflammation, ballooning, and fibrosis (scored 0-13). In addition, the levels of plasma AST and ALT are determined using a Pentra 400 instrument from Horiba (USA), according to manufacturer's instructions. The levels of hepatic total cholesterol, triglycerides, fatty acids, alanine aminotransferase, and aspartate aminotransferase are also determined using methods known in the art.

**[478]** In other studies, hepatic gene expression analysis of inflammation, fibrosis, steatosis, ER stress, or oxidative stress markers may be performed by qRT-PCR using validated primers. These markers may include, but are not limited to, IL-1β, TNF-α, MCP-1, α-SMA, Coll1a1, CHOP, and NRF2.

**[479]** In other studies, hepatic gene expression analysis of inflammation, fibrosis, steatosis, ER stress, or oxidative stress markers may be performed by qRT-PCR using validated primers. These markers may include, but are not limited to, IL-1β, TNF-α, MCP-1, α-SMA, Coll1a1, CHOP, and NRF2.

**[480]** Treatment with pmEVs is initiated at some point, either at the beginning of the diet, or at some point following diet initiation (for example, one week after). For example, pmEVs may be administered starting in the same day as the initiation of the MCD diet. pmEVs

are administered at varied doses and at defined intervals. For example, some mice are intravenously injected with pmEVs at 10, 15, or 20 ug/mouse. Other mice may receive 25, 50, or 100 mg of pmEVs per mouse. Alternatively, some mice receive between 7.0e+09 to 3.0e+12 pmEV particles per dose. While some mice receive pmEVs through oral gavage or i.v. injection, while other groups of mice may receive pmEVs through intraperitoneal (i.p.) injection, subcutaneous (s.c.) injection, nasal route administration, or other means of administration. Some mice may receive pmEVs every day (e.g., starting on day 1), while others may receive pmEVs at alternative intervals (e.g., every other day, or once every three days). Groups of mice may be administered a pharmaceutical composition of the invention comprising a mixture of pmEVs and bacterial cells. For example, the composition may comprise pmEV particles and whole bacteria in a ratio from 1:1 (pmEVs: bacterial cells) to 1-1x$10^{12}$:1 (pmEVs: bacterial cells).

**[481]** Alternatively, some groups of mice may receive between 1x$10^4$ and 5x$10^9$ bacterial cells in an administration separate from, or comingled with, the pmEV administration. As with the pmEVs, bacterial cell administration may be varied by route of administration, dose, and schedule. The bacterial cells may be live, dead, or weakened. The bacterial cells may be harvested fresh (or frozen) and administered, or they may be irradiated or heat-killed prior to administration with the pmEVs.

**[482]** Some groups of mice may be treated with additional NASH therapeutic(s) (e.g., FXR agonists, PPAR agonists, CCR2/5 antagonists or other treatment) and/or appropriate control at various timepoints and effective doses.

**[483]** At various timepoints and/or at the end of the treatment, mice are sacrificed and liver, intestine, blood, feces, or other tissues may be removed for ex vivo histological, biochemical, molecular or cytokine and/or flow cytometry analysis using methods known in the art. For example, liver tissues are weighed and prepared for histological analysis, which may comprise staining with H&E, Sirius Red, and determination of NASH activity score (NAS). At various timepoints, blood is collected for plasma analysis of liver enzymes, for example, AST or ALT, using standards assays. In addition, the hepatic content of cholesterol, triglycerides, or fatty acid acids can be measured using established protocols. Hepatic gene expression analysis of inflammation, fibrosis, steatosis, ER stress, or oxidative stress markers may be performed by qRT-PCR using validated primers. These markers may include, but are not limited to, IL-6, MCP-1, alpha-SMA, Coll1a1, CHOP, and NRF2. Metabolite measurements may be performed in

plasma, tissue and fecal samples using established biochemical and mass-spectrometry-based metabolomics methods. Serum cytokines can be analyzed including, but not limited to, TNFa, IL-17, IL-13, IL-12p70, IL12p40, IL-10, IL-6, IL-5, IL-4, IL-2, IL-1b, IFNy, GM-CSF, G-CSF, M-CSF, MIG, IP10, MIP1b, RANTES, and MCP-1. Cytokine analysis may be carried out on immune cells obtained from lymph nodes or other tissue, and/or on purified CD45+ bile duct-infiltrated immune cells obtained ex vivo. Finally, immunohistochemistry is carried out on liver or intestine sections to measure neutrophils, T cells, macrophages, dendritic cells, or other immune cell infiltrates.

**[484]** In order to examine the impact and longevity of disease protection, rather than being sacrificed, some mice may be analyzed for recovery.

**Example 12: pmEVs in a mouse model of psoriasis**

**[485]** Psoriasis is a T-cell-mediated chronic inflammatory skin disease. So-called "plaque-type" psoriasis is the most common form of psoriasis and is typified by dry scales, red plaques, and thickening of the skin due to infiltration of immune cells into the dermis and epidermis. Several animal models have contributed to the understanding of this disease, as reviewed by Gudjonsson et al. (Mouse models of psoriasis. J Invest Derm. 2007. 127: 1292-1308; see also van der Fits et al. Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis. J. Immunol. 2009 May 1. 182(9): 5836-45).

**[486]** Psoriasis can be induced in a variety of mouse models, including those that use transgenic, knockout, or xenograft models, as well as topical application of imiquimod (IMQ), a TLR7/8 ligand.

**[487]** pmEVs are tested for their efficacy in the mouse model of psoriasis, either alone or in combination with whole bacterial cells, with or without the addition of other anti-inflammatory treatments. For example, 6-8 week old C57Bl/6 or Balb/c mice are obtained from Taconic (Germantown, NY), or other vendor. Mice are shaved on the back and the right ear. Groups of mice receive a daily topical dose of 62.5 mg of commercially available IMQ cream (5%) (Aldara; 3M Pharmaceuticals). The dose is applied to the shaved areas for 5 or 6 consecutive days. At regular intervals, mice are scored for erythema, scaling, and thickening on a scale from 0 to 4, as described by van der Fits et al. (2009). Mice are monitored for ear thickness using a Mitutoyo micrometer.

**[488]** Treatment with pmEVs is initiated at some point, either around the time of the first application of IMQ, or something thereafter. For example, pmEVs may be administered at the same time as the subcutaneous injections (day 0), or they may be administered prior to, or upon, application. pmEVs are administered at varied doses and at defined intervals. For example, some mice are intravenously injected with pmEVs at 10, 15, or 20 ug/mouse. Other mice may receive 25, 50, or 100 mg of pmEVs per mouse. Alternatively, some mice receive between 7.0e+09 to 3.0e+12 pmEV particles per dose. While some mice receive pmEVs through oral gavage or i.v. injection, while other groups of mice may receive pmEVs through intraperitoneal (i.p.) injection, subcutaneous (s.c.) injection, nasal route administration, or other means of administration. Some mice may receive pmEVs every day (e.g., starting on day 0), while others may receive pmEVs at alternative intervals (e.g., every other day, or once every three days). Groups of mice may be administered a pharmaceutical composition of the invention comprising a mixture of pmEVs and bacterial cells. For example, the composition may comprise pmEV particles and whole bacteria in a ratio from 1:1 (pmEVs: bacterial cells) to $1\text{-}1x10^{12}$:1 (pmEVs: bacterial cells).

**[489]** Alternatively, some groups of mice may receive between $1x10^4$ and $5x10^9$ bacterial cells in an administration separate from, or comingled with, the pmEV administration. As with the pmEVs, bacterial cell administration may be varied by route of administration, dose, and schedule. The bacterial cells may be live, dead, or weakened. The bacterial cells may be harvested fresh (or frozen) and administered, or they may be irradiated or heat-killed prior to administration with the pmEVs.

**[490]** Some groups of mice may be treated with anti-inflammatory agent(s) (e.g., anti-CD154, blockade of members of the TNF family, or other treatment), and/or an appropriate control (e.g., vehicle or control antibody) at various timepoints and at effective doses.

**[491]** In addition, some mice are treated with antibiotics prior to treatment. For example, vancomycin (0.5g/L), ampicillin (1.0g/L), gentamicin (1.0g/L) and amphotericin B (0.2g/L) are added to the drinking water, and antibiotic treatment is halted at the time of treatment or a few days prior to treatment. Some immunized mice are treated without receiving antibiotics.

**[492]** At various timepoints, samples from back and ear skin are taken for cryosection staining analysis using methods known in the art. Other groups of mice are sacrificed and lymph

nodes, spleen, mesenteric lymph nodes (MLN), the small intestine, colon, and other tissues may be removed for histology studies, ex vivo histological, cytokine and/or flow cytometric analysis using methods known in the art. Some tissues may be dissociated using dissociation enzymes according to the manufacturer's instructions. Cryosection samples, tissue samples, or cells obtained ex vivo are stained for analysis by flow cytometry using techniques known in the art. Staining antibodies can include anti-CD11c (dendritic cells), anti-CD80, anti-CD86, anti-CD40, anti-MHCII, anti-CD8a, anti-CD4, and anti-CD103. Other markers that may be analyzed include pan-immune cell marker CD45, T cell markers (CD3, CD4, CD8, CD25, Foxp3, T-bet, Gata3, Roryt, Granzyme B, CD69, PD-1, CTLA-4), and macrophage/myeloid markers (CD11b, MHCII, CD206, CD40, CSF1R, PD-L1, Gr-1, F4/80). In addition to immunophenotyping, serum cytokines can be analyzed including, but not limited to, TNFa, IL-17, IL-13, IL-12p70, IL12p40, IL-10, IL-6, IL-5, IL-4, IL-2, IL-1b, IFNy, GM-CSF, G-CSF, M-CSF, MIG, IP10, MIP1b, RANTES, and MCP-1. Cytokine analysis may be carried out on immune cells obtained from lymph nodes or other tissue, and/or on purified CD45+ skin-infiltrated immune cells obtained ex vivo. Finally, immunohistochemistry is carried out on various tissue sections to measure T cells, macrophages, dendritic cells, and checkpoint molecule protein expression.

**[493]** In order to examine the impact and longevity of psoriasis protection, rather than being sacrificed, some mice may be studied to assess recovery, or they may be rechallenged with IMQ. The groups of rechallenged mice are analyzed for susceptibility to psoriasis and severity of response.

**Example 13: pmEVs in a mouse model of obesity (DIO)**

**[494]** There are various animal models of DIO, as reviewed by Tschop et al. (A guide to analysis of mouse energy metabolism. Nat. Methods. 2012; 9(1):57-63) and Ayala et al. (Standard operating procedures for describing and performing metabolic tests of glucose homeostasis in mice. Disease Models and Mechanisms. 2010; 3:525-534) and provided by Physiogenex.

**[495]** pmEVs are tested for their efficacy in a mouse model of DIO, either alone or in combination with other whole bacterial cells (live, killed, irradiated, and/or inactivated, etc) with or without the addition of other anti-inflammatory treatments.

**[496]** Depending on the method of DIO induction and/or whether DIO development is spontaneous, treatment with pmEVs is initiated at some point, either around the time of induction or following induction, or prior to the onset (or upon the onset) of spontaneously-occurring T1D. pmEVs are administered at varied doses and at defined intervals. For example, some mice are intravenously injected with pmEVs at 10, 15, or 20 ug/mouse. Other mice may receive 25, 50, or 100 mg of pmEVs per mouse. Alternatively, some mice receive between 7.0e+09 to 3.0e+12 pmEV particles per dose. While some mice receive pmEVs through i.v. injection, other mice may receive pmEVs through intraperitoneal (i.p.) injection, subcutaneous (s.c.) injection, nasal route administration, oral gavage, or other means of administration. Some mice may receive pmEVs every day, while others may receive pmEVs at alternative intervals (*e.g.,* every other day, or once every three days). Groups of mice may be administered a pharmaceutical composition of the invention comprising a mixture of pmEVs and bacterial cells. For example, the composition may comprise pmEV particles and whole bacteria in a ratio from 1:1 (pmEVs: bacterial cells) to 1-1x$10^{12}$:1 (pmEVs: bacterial cells).

**[497]** Alternatively, some groups of mice may receive between 1x$10^{4}$ and 5x$10^{9}$ bacterial cells in an administration separate from, or comingled with, the pmEV administration. As with the pmEVs, bacterial cell administration may be varied by route of administration, dose, and schedule. The bacterial cells may be live, dead, or weakened. The bacterial cells may be harvested fresh (or frozen) and administered, or they may be irradiated or heat-killed prior to administration with the pmEVs.

**[498]** Some groups of mice may be treated with additional treatments and/or an appropriate control (*e.g.,* vehicle or control antibody) at various timepoints and at effective doses.

**[499]** In addition, some mice are treated with antibiotics prior to treatment. For example, vancomycin (0.5g/L), ampicillin (1.0g/L), gentamicin (1.0g/L) and amphotericin B (0.2g/L) are added to the drinking water, and antibiotic treatment is halted at the time of treatment or a few days prior to treatment. Some immunized mice are treated without receiving antibiotics.

**[500]** Blood glucose is monitored biweekly prior to the start of the experiment. At various timepoints thereafter, nonfasting blood glucose is measured. At various timepoints, mice are sacrificed and site the pancreas, lymph nodes, or other tissues may be removed for ex vivo

histological, cytokine and/or flow cytometric analysis using methods known in the art. For example, tissues are dissociated using dissociation enzymes according to the manufacturer's instructions. Cells are stained for analysis by flow cytometry using techniques known in the art. Staining antibodies can include anti-CD11c (dendritic cells), anti-CD80, anti-CD86, anti-CD40, anti-MHCII, anti-CD8a, anti-CD4, and anti-CD103. Other markers that may be analyzed include pan-immune cell marker CD45, T cell markers (CD3, CD4, CD8, CD25, Foxp3, T-bet, Gata3, Roryt, Granzyme B, CD69, PD-1, CTLA-4), and macrophage/myeloid markers (CD11b, MHCII, CD206, CD40, CSF1R, PD-L1, Gr-1, F4/80). In addition to immunophenotyping, serum cytokines can be analyzed including, but not limited to, TNFa, IL-17, IL-13, IL-12p70, IL12p40, IL-10, IL-6, IL-5, IL-4, IL-2, IL-1b, IFNy, GM-CSF, G-CSF, M-CSF, MIG, IP10, MIP1b, RANTES, and MCP-1. Cytokine analysis may be carried out on immune cells obtained from lymph nodes or other tissue, and/or on purified tissue-infiltrating immune cells obtained ex vivo. Finally, immunohistochemistry is carried out on various tissue sections to measure T cells, macrophages, dendritic cells, and checkpoint molecule protein expression. Antibody production may also be assessed by ELISA.

**[501]** In order to examine the impact and longevity of disease protection, rather than being sacrificed, some mice may be rechallenged with a disease trigger, or assessed for susceptibility to relapse. Mice are analyzed for susceptibility to diabetes onset and severity following rechallenge (or spontaneously-occurring relapse).

**Example 14: Labeling bacterial pmEVs**

**[502]** pmEVs may be labeled in order to track their biodistribution *in vivo* and to quantify and track cellular localization in various preparations and in assays conducted with mammalian cells. For example, pmEVs may be radio-labeled, incubated with dyes, fluorescently labeled, luminescently labeled, or labeled with conjugates containing metals and isotopes of metals.

**[503]** For example, pmEVs may be incubated with dyes conjugated to functional groups such as NHS-ester, click-chemistry groups, streptavidin or biotin. The labeling reaction may occur at a variety of temperatures for minutes or hours, and with or without agitation or rotation. The reaction may then be stopped by adding a reagent such as bovine serum albumin (BSA), or similar agent, depending on the protocol, and free or unbound dye molecule removed by ultra-

centrifugation, filtration, centrifugal filtration, column affinity purification or dialysis. Additional washing steps involving wash buffers and vortexing or agitation may be employed to ensure complete removal of free dyes molecules such as described in Su Chul Jang et al, Small. 11, No.4, 456-461(2017).

**[504]** Optionally, pmEVs may be concentrated to 5.0 E12 particle/ml (300ug) and diluted up to 1.8mo using 2X concentrated PBS buffer pH 8.2 and pelleted by centrifugation at 165,000 x g at 4 C using a benchtop ultracentrifuge. The pellet is resuspended in 300ul 2X PBS pH 8.2 and an NHS-ester fluorescent dye is added at a final concentration of 0.2mM from a 10mM dye stock (dissolved in DMSO). The sample is gently agitated at 24°C for 1.5 hours, and then incubated overnight at 4°C. Free non-reacted dye is removed by 2 repeated steps of dilution/pelleting as described above, using 1X PBS buffer, and resuspending in 300ul final volume.

**[505]** Fluorescently labeled pmEVs are detected in cells or organs, or in *in vitro* and/or *ex vivo* samples by confocal microscopy, nanoparticle tracking analysis, flow cytometry, fluorescence activated cell sorting (FACs) or fluorescent imaging system such as the Odyssey CLx LICOR (see e.g., Wiklander et al. 2015. J. Extracellular Vesicles. 4:10.3402/jev.v4.26316). Additionally, fluorescently labeled pmEVs are detected in whole animals and/or dissected organs and tissues using an instrument such as the IVIS spectrum CT (Perkin Elmer) or Pearl Imager, as in H-I. Choi, et al. *Experimental & Molecular Medicine*. 49: e330 (2017).

**[506]** pmEVs may be labeled with conjugates containing metals and isotopes of metals using the protocols described above. Metal-conjugated pmEVs may be administered *in vivo* to animals. Cells may then be harvested from organs at various time-points, and analyzed *ex vivo*. Alternatively, cells derived from animals, humans, or immortalized cell lines may be treated with metal-labelled pmEVs *in vitro* and cells subsequently labelled with metal-conjugated antibodies and phenotyped using a Cytometry by Time of Flight (CyTOF) instrument such as the Helios CyTOF (Fluidigm) or imaged and analyzed using and Imaging Mass Cytometry instrument such as the Hyperion Imaging System (Fluidigm). Additionally, pmEVs may be labelled with a radioisotope to track the pmEVs biodistribution (see, *e.g.*, Miller et al., Nanoscale. 2014 May 7;6(9):4928-35).

**Example 15: Transmission electron microscopy to visualize bacterial pmEVs**

**[507]** pmEVs are prepared from bacteria batch cultures. Transmission electron microscopy (TEM) may be used to visualize purified bacterial pmEVs (S. Bin Park, et al. PLoS ONE. 6(3):e17629 (2011). pmEVs are mounted onto 300- or 400-mesh-size carbon-coated copper grids (Electron Microscopy Sciences, USA) for 2 minutes and washed with deionized water. pmEVs are negatively stained using 2% (w/v) uranyl acetate for 20 sec – 1 min. Copper grids are washed with sterile water and dried. Images are acquired using a transmission electron microscope with 100-120 kV acceleration voltage. Stained pmEVs appear between 20-600 nm in diameter and are electron dense. 10-50 fields on each grid are screened.

**Example 16: Profiling pmEV composition and content**

**[508]** pmEVs may be characterized by any one of various methods including, but not limited to, NanoSight characterization, SDS-PAGE gel electrophoresis, Western blot, ELISA, liquid chromatography-mass spectrometry and mass spectrometry, dynamic light scattering, lipid levels, total protein, lipid to protein ratios, nucleic acid analysis and/or zeta potential.

*NanoSight Characterization of pmEVs*

**[509]** Nanoparticle tracking analysis (NTA) is used to characterize the size distribution of purified bacterial pmEVs. Purified pmEV preparations are run on a NanoSight machine (Malvern Instruments) to assess pmEV size and concentration.

*SDS-PAGE Gel Electrophoresis*

**[510]** To identify the protein components of purified pmEVs, samples are run on a gel, for example a Bolt Bis-Tris Plus 4-12% gel (Thermo-Fisher Scientific), using standard techniques. Samples are boiled in 1x SDS sample buffer for 10 minutes, cooled to 4°C, and then centrifuged at 16,000 x g for 1 min. Samples are then run on a SDS-PAGE gel and stained using one of several standard techniques (e.g., Silver staining, Coomassie Blue, Gel Code Blue ) for visualization of bands.

*Western blot analysis*

**[511]** To identify and quantify specific protein components of purified pmEVs, pmEV proteins are separated by SDS-PAGE as described above and subjected to Western blot analysis (Cvjetkovic et al., *Sci. Rep.* **6**, 36338 (2016)) and are quantified via ELISA.

*pmEV proteomics and Liquid Chromatography-Mass Spectrometry (LC-MS/MS) and Mass Spectrometry (MS)*

**[512]** Proteins present in pmEVs are identified and quantified by Mass Spectrometry techniques. pmEV proteins may be prepared for LC-MS/MS using standard techniques including protein reduction using dithiotreitol solution (DTT) and protein digestion using enzymes such as LysC and trypsin as described in Erickson et al, 2017 (Molecular Cell, VOLUME 65, ISSUE 2, P361-370, JANUARY 19, 2017). Alternatively, peptides are prepared as described by Liu et al. 2010 (JOURNAL OF BACTERIOLOGY, June 2010, p. 2852–2860 Vol. 192, No. 11), Kieselbach and Oscarsson 2017 (Data Brief. 2017 Feb; 10: 426–431.), Vildhede et al, 2018 (Drug Metabolism and Disposition February 8, 2018). Following digestion, peptide preparations are run directly on liquid chromatography and mass spectrometry devices for protein identification within a single sample. For relative quantitation of proteins between samples, peptide digests from different samples are labeled with isobaric tags using the iTRAQ Reagent-8plex Multiplex Kit (Applied Biosystems, Foster City, CA) or TMT 10plex and 11plex Label Reagents (Thermo Fischer Scientific, San Jose, CA, USA). Each peptide digest is labeled with a different isobaric tag and then the labeled digests are combined into one sample mixtur. The combined peptide mixture is analyzed by LC-MS/MS for both identification and quantification. A database search is performed using the LC-MS/MS data to identify the labeled peptides and the corresponding proteins. In the case of isobaric labeling, the fragmentation of the attached tag generates a low molecular mass reporter ion that is used to obtain a relative quantitation of the peptides and proteins present in each pmEV.

**[513]** Additionally, metabolic content is ascertained using liquid chromatography techniques combined with mass spectrometry. A variety of techniques exist to determine metabolomic content of various samples and are known to one skilled in the art involving solvent extraction, chromatographic separation and a variety of ionization techniques coupled to mass determination (Roberts et al 2012 Targeted Metabolomics. Curr Protoc Mol Biol. 30: 1-24; Dettmer et al 2007, Mass spectrometry-based metabolomics. Mass Spectrom Rev. 26(1):51-78). As a non-limiting example, a LC-MS system includes a 4000 QTRAP triple quadrupole mass spectrometer (AB SCIEX) combined with 1100 Series pump (Agilent) and an HTS PAL autosampler (Leap Technologies). Media samples or other complex metabolic mixtures (~10 µL) are extracted using nine volumes of 74.9:24.9:0.2 (v/v/v) acetonitrile/methanol/formic acid

containing stable isotope-labeled internal standards (valine-d8, Isotec; and phenylalanine-d8, Cambridge Isotope Laboratories). Standards may be adjusted or modified depending on the metabolites of interest. The samples are centrifuged (10 minutes, 9,000g, 4 °C), and the supernatants (10 µL) are submitted to LCMS by injecting the solution onto the HILIC column (150 × 2.1 mm, 3 µm particle size). The column is eluted by flowing a 5% mobile phase [10mM ammonium formate, 0.1% formic acid in water] for 1 minute at a rate of 250uL/minute followed by a linear gradient over 10 minutes to a solution of 40% mobile phase [acetonitrile with 0.1% formic acid]. The ion spray voltage is set to 4.5 kV and the source temperature is 450 °C.

**[514]** The data are analyzed using commercially available software like Multiquant 1.2 from AB SCIEX for mass spectrum peak integration. Peaks of interest should be manually curated and compared to standards to confirm the identity of the peak. Quantitation with appropriate standards is performed to determine the number of metabolites present in the initial media, after bacterial conditioning and after tumor cell growth. A non-targeted metabolomics approach may also be used using metabolite databases, such as but not limited to the NIST database, for peak identification.

*Dynamic light scattering (DLS)*

**[515]** DLS measurements, including the distribution of particles of different sizes in different pmEV preparations are taken using instruments such as the DynaPro NanoStar (Wyatt Technology) and the Zetasizer Nano ZS (Malvern Instruments).

*Lipid Levels*

**[516]** Lipid levels are quantified using FM4-64 (Life Technologies), by methods similar to those described by A.J. McBroom *et al. J Bacteriol* 188:5385-5392. and A. Frias, *et al. Microb Ecol.* 59:476-486 (2010). Samples are incubated with FM4-64 (3.3 µg/mL in PBS for 10 minutes at 37ºC in the dark). After excitation at 515 nm, emission at 635 nm is measured using a Spectramax M5 plate reader (Molecular Devices). Absolute concentrations are determined by comparison of unknown samples to standards (such as palmitoyloleoylphosphatidylglycerol (POPG) vesicles) of known concentrations. Lipidomics can be used to identify the lipids present in the pmEVs.

*Total Protein*

**[517]** Protein levels are quantified by standard assays such as the Bradford and BCA assays. The Bradford assays are run using Quick Start Bradford 1x Dye Reagent (Bio-Rad),

according to manufacturer's protocols. BCA assays are run using the Pierce BCA Protein Assay Kit (Thermo-Fisher Scientific). Absolute concentrations are determined by comparison to a standard curve generated from BSA of known concentrations. Alternatively, protein concentration can be calculated using the Beer-Lambert equation using the sample absorbance at 280nm (A280) as measured on a Nanodrop spectrophotometer (Thermo-Fisher Scientific).In addition, proteomics may be used to identify proteins in the sample.

*Lipid:Protein Ratios*

**[518]** Lipid:protein ratios are generated by dividing lipid concentrations by protein concentrations. These provide a measure of the purity of vesicles as compared to free protein in each preparation.

*Nucleic acid analysis*

**[519]** Nucleic acids are extracted from pmEVs and quantified using a Qubit fluorometer. Size distribution is assessed using a BioAnalyzer and the material is sequenced.

*Zeta Potential*

**[520]** The zeta potential of different preparations are measured using instruments such as the Zetasizer ZS (Malvern Instruments).

**Example 17: In vitro screening of pmEVs for enhanced activation of dendritic cells**

**[521]** *In vitro* immune responses are thought to simulate mechanisms by which immune responses are induced *in vivo*, e.g., as in response to a cancer microenvironment. Briefly, PBMCs are isolated from heparinized venous blood from healthy donors by gradient centrifugation using Lymphoprep (Nycomed, Oslo, Norway), or from mouse spleens or bone marrow using the magnetic bead-based Human Blood Dendritic cell isolation kit (Miltenyi Biotech, Cambridge, MA). Using anti-human CD14 mAb, the monocytes are purified by Moflo and cultured in cRPMI at a cell density of 5e5 cells/ml in a 96-well plate (Costar Corp) for 7 days at 37°C. For maturation of dendritic cells, the culture is stimulated with 0.2 ng/mL IL-4 and 1000 U/ml GM-CSF at 37°C for one week. Alternatively, maturation is achieved through incubation with recombinant GM-CSF for a week, or using other methods known in the art. Mouse DCs can be harvested directly from spleens using bead enrichment or differentiated from hematopoietic stem cells. Briefly, bone marrow may be obtained from the femurs of mice. Cells are recovered and red blood cells lysed. Stem cells are cultured in cell culture medium in 20ng/ml mouse GMCSF

for 4 days. Additional medium containing 20ng/ml mouse GM-CSF is added. On day 6 the medium and non-adherent cells are removed and replaced with fresh cell culture medium containing 20ng/ml GMCSF. A final addition of cell culture medium with 20ng/ml GM-CSF is added on day 7. On day10, non-adherent cells are harvested and seeded into cell culture plates overnight and stimulated as required. Dendritic cells are then treated with various doses of pmEVs with or without antibiotics. For example, 25-75 ug/mL pmEVs for 24 hours with antibiotics. pmEV compositions tested may include pmEVs from a single bacterial species or strain, or a mixture of pmEVs from one or more genus, 1 or more species, or 1 or more strains (e.g., one or more strains within one species). PBS is included as a negative control and LPS, anti-CD40 antibodies, from *Bifidobacterium* spp. are used as positive controls. Following incubation, DCs are stained with anti CD11b, CD11c, CD103, CD8a, CD40, CD80, CD83, CD86, MHCI and MHCII, and analyzed by flow cytometry. DCs that are significantly increased in CD40, CD80, CD83, and CD86 as compared to negative controls are considered to be activated by the associated bacterial pmEV composition. These experiments are repeated three times at minimum.

**[522]** To screen for the ability of pmEV-activated epithelial cells to stimulate DCs, the above protocol is followed with the addition of a 24-hour epithelial cell pmEV co-culture prior to incubation with DCs. Epithelial cells are washed after incubation with pmEVs and are then co-cultured with DCs in an absence of pmEVs for 24 hours before being processed as above. Epithelial cell lines may include Int407, HEL293, HT29, T84 and CACO2.

**[523]** As an additional measure of DC activation, 100 µl of culture supernatant is removed from wells following 24-hour incubation of DCs with pmEVs or pmEV-treated epithelial cells and is analyzed for secreted cytokines, chemokines, and growth factors using the multiplexed Luminex Magpix. Kit (EMD Millipore, Darmstadt, Germany). Briefly, the wells are pre-wet with buffer, and 25 µl of 1x antibody-coated magnetic beads are added and 2x 200 µl of wash buffer are performed in every well using the magnet. 50 µl of Incubation buffer, 50 µl of diluent and 50 µl of samples are added and mixed via shaking for 2hrs at room temperature in the dark. The beads are then washed twice with 200 µl wash buffer. 100 µl of 1X biotinylated detector antibody is added and the suspension is incubated for 1 hour with shaking in the dark. Two, 200 µl washes are then performed with wash buffer. 100 µl of 1x SAV-RPE reagent is added to each well and is incubated for 30 min at RT in the dark. Three 200 µl washes are

performed and 125 µl of wash buffer is added with 2-3 min shaking occurs. The wells are then submitted for analysis in the Luminex xMAP system.

**[524]** Standards allow for careful quantitation of the cytokines including GM-CSF, IFN-g, IFN-a, IFN-B, IL-1a, IL-1B, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, IL-12 (p40/p70), IL-17A, IL-17F, IL-21, IL-22 IL-23, IL-25, IP-10, KC, MCP-1, MIG, MIP1a, TNFa, and VEGF. These cytokines are assessed in samples of both mouse and human origin. Increases in these cytokines in the bacterial treated samples indicate enhanced production of proteins and cytokines from the host. Other variations on this assay examining specific cell types ability to release cytokines are assessed by acquiring these cells through sorting methods and are recognized by one of ordinary skill in the art. Furthermore, cytokine mRNA is also assessed to address cytokine release in response to an pmEV composition.

**[525]** This DC stimulation protocol may be repeated using combinations of purified pmEVs and live bacterial strains to maximize immune stimulation potential.

**Example 18: In vitro screening of pmEVs for enhanced activation of CD8+ T cell killing when incubated with tumor cells**

**[526]** *In vitro* methods for screening pmEVs that can activate CD8+ T cell killing of tumor cells are described. Briefly, DCs are isolated from human PBMCs or mouse spleens, using techniques known in the art, and incubated *in vitro* with single-strain pmEVs, mixtures of pmEVs, and/or appropriate controls. In addition, CD8+ T cells are obtained from human PBMCs or mouse spleens using techniques known in the art, for example the magnetic bead-based Mouse CD8a+ T Cell Isolation Kit and the magnetic bead-based Human CD8+ T Cell Isolation Kit (both from Miltenyi Biotech, Cambridge, MA). After incubation of DCs with pmEVs for some time (e.g., for 24-hours), or incubation of DCs with pmEV-stimulated epithelial cells, pmEVs are removed from the cell culture with PBS washes and 100ul of fresh media with antibiotics is added to each well, and 200,000 T cells are added to each experimental well in the 96-well plate. Anti-CD3 antibody is added at a final concentration of 2ug/ml. Co-cultures are then allowed to incubate at 37˚C for 96 hours under normal oxygen conditions.

**[527]** For example, approximately 72 hours into the coculture incubation, tumor cells are plated for use in the assay using techniques known in the art. For example, 50,000 tumor cells/well are plated per well in new 96-well plates. Mouse tumor cell lines used may include

B16.F10, SIY+ B16.F10, and others. Human tumor cell lines are HLA-matched to donor, and can include PANC-1, UNKPC960/961, UNKC, and HELA cell lines. After completion of the 96-hour co-culture, 100 µl of the CD8+ T cell and DC mixture is transferred to wells containing tumor cells. Plates are incubated for 24 hours at 37°C under normal oxygen conditions. Staurospaurine may be used as negative control to account for cell death.

**[528]** Following this incubation, flow cytometry is used to measure tumor cell death and characterize immune cell phenotype. Briefly, tumor cells are stained with viability dye. FACS analysis is used to gate specifically on tumor cells and measure the percentage of dead (killed) tumor cells. Data are also displayed as the absolute number of dead tumor cells per well. Cytotoxic CD8+ T cell phenotype may be characterized by the following methods: a) concentration of supernatant granzyme B, IFNy and TNFa in the culture supernatant as described below, b) CD8+ T cell surface expression of activation markers such as DC69, CD25, CD154, PD-1, gamma/delta TCR, Foxp3, T-bet, granzyme B, c) intracellular cytokine staining of IFNy, granzyme B, TNFa in CD8+ T cells. CD4+ T cell phenotype may also be assessed by intracellular cytokine staining in addition to supernatant cytokine concentration including INFy, TNFa, IL-12, IL-4, IL-5, IL-17, IL-10, chemokines etc.

**[529]** As an additional measure of CD8+ T cell activation, 100 µl of culture supernatant is removed from wells following the 96-hour incubation of T cells with DCs and is analyzed for secreted cytokines, chemokines, and growth factors using the multiplexed Luminex Magpix. Kit (EMD Millipore, Darmstadt, Germany). Briefly, the wells are pre-wet with buffer, and 25 µl of 1x antibody-coated magnetic beads are added and 2x 200 µl of wash buffer are performed in every well using the magnet. 50 µl of Incubation buffer, 50 µl of diluent and 50 µl of samples are added and mixed via shaking for 2hrs at room temperature in the dark. The beads are then washed twice with 200 µl wash buffer. 100 µl of 1X biotinylated detector antibody is added and the suspension is incubated for 1 hour with shaking in the dark. Two, 200 µl washes are then performed with wash buffer. 100 µl of 1x SAV-RPE reagent is added to each well and is incubated for 30 min at RT in the dark. Three 200 µl washes are performed and 125 µl of wash buffer is added with 2-3 min shaking occurs. The wells are then submitted for analysis in the Luminex xMAP system.

**[530]** Standards allow for careful quantitation of the cytokines including GM-CSF, IFN-g, IFN-a, IFN-B IL-1a, IL-1B, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, IL-12 (p40/p70), IL-17,

IL-23, IP-10, KC, MCP-1, MIG, MIP1a, TNFa, and VEGF. These cytokines are assessed in samples of both mouse and human origin. Increases in these cytokines in the bacterial treated samples indicate enhanced production of proteins and cytokines from the host. Other variations on this assay examining specific cell types ability to release cytokines are assessed by acquiring these cells through sorting methods and are recognized by one of ordinary skill in the art. Furthermore, cytokine mRNA is also assessed to address cytokine release in response to an pmEV composition. These changes in the cells of the host stimulate an immune response similarly to in vivo response in a cancer microenvironment.

**[531]** This CD8+ T cell stimulation protocol may be repeated using combinations of purified pmEVs and live bacterial strains to maximize immune stimulation potential.

**Example 19: In vitro screening of pmEVs for enhanced tumor cell killing by PBMCs**

**[532]** Various methods may be used to screen pmEVs for the ability to stimulate PBMCs, which in turn activate CD8+ T cells to kill tumor cells. For example, PBMCs are isolated from heparinized venous blood from healthy human donors by ficoll-paque gradient centrifugation for mouse or human blood, or with Lympholyte Cell Separation Media (Cedarlane Labs, Ontario, Canada) from mouse blood. PBMCs are incubated with single-strain pmEVs, mixtures of pmEVs, and appropriate controls. In addition, CD8+ T cells are obtained from human PBMCs or mouse spleens. After the 24-hour incubation of PBMCs with pmEVs, pmEVs are removed from the cells using PBS washes. 100ul of fresh media with antibiotics is added to each well. An appropriate number of T cells (e.g., 200,000 T cells) are added to each experimental well in the 96-well plate. Anti-CD3 antibody is added at a final concentration of 2ug/ml. Co-cultures are then allowed to incubate at 37°C for 96 hours under normal oxygen conditions.

**[533]** For example, 72 hours into the coculture incubation, 50,000 tumor cells/well are plated per well in new 96-well plates. Mouse tumor cell lines used include B16.F10, SIY+ B16.F10, and others. Human tumor cell lines are HLA-matched to donor, and can include PANC-1, UNKPC960/961, UNKC, and HELA cell lines. After completion of the 96-hour co-culture, 100 µl of the CD8+ T cell and PBMC mixture is transferred to wells containing tumor cells. Plates are incubated for 24 hours at 37°C under normal oxygen conditions. Staurospaurine is used as negative control to account for cell death.

**[534]** Following this incubation, flow cytometry is used to measure tumor cell death and characterize immune cell phenotype. Briefly, tumor cells are stained with viability dye. FACS analysis is used to gate specifically on tumor cells and measure the percentage of dead (killed) tumor cells. Data are also displayed as the absolute number of dead tumor cells per well. Cytotoxic CD8+ T cell phenotype may be characterized by the following methods: a) concentration of supernatant granzyme B, IFNy and TNFa in the culture supernatant as described below, b) CD8+ T cell surface expression of activation markers such as DC69, CD25, CD154, PD-1, gamma/delta TCR, Foxp3, T-bet, granzyme B, c) intracellular cytokine staining of IFNy, granzyme B, TNFa in CD8+ T cells. CD4+ T cell phenotype may also be assessed by intracellular cytokine staining in addition to supernatant cytokine concentration including INFy, TNFa, IL-12, IL-4, IL-5, IL-17, IL-10, chemokines etc.

**[535]** As an additional measure of CD8+ T cell activation, 100 µl of culture supernatant is removed from wells following the 96-hour incubation of T cells with DCs and is analyzed for secreted cytokines, chemokines, and growth factors using the multiplexed Luminex Magpix. Kit (EMD Millipore, Darmstadt, Germany). Briefly, the wells are pre-wet with buffer, and 25 µl of 1x antibody-coated magnetic beads are added and 2x 200 µl of wash buffer are performed in every well using the magnet. 50 µl of Incubation buffer, 50 µl of diluent and 50 µl of samples are added and mixed via shaking for 2hrs at room temperature in the dark. The beads are then washed twice with 200 µl wash buffer. 100 µl of 1X biotinylated detector antibody is added and the suspension is incubated for 1 hour with shaking in the dark. Two, 200 µl washes are then performed with wash buffer. 100 µl of 1x SAV-RPE reagent is added to each well and is incubated for 30 min at RT in the dark. Three 200 µl washes are performed and 125 µl of wash buffer is added with 2-3 min shaking occurs. The wells are then submitted for analysis in the Luminex xMAP system.

**[536]** Standards allow for careful quantitation of the cytokines including GM-CSF, IFN-g, IFN-a, IFN-B IL-1a, IL-1B, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, IL-12 (p40/p70), IL-17, IL-23, IP-10, KC, MCP-1, MIG, MIP1a, TNFa, and VEGF. These cytokines are assessed in samples of both mouse and human origin. Increases in these cytokines in the bacterial treated samples indicate enhanced production of proteins and cytokines from the host. Other variations on this assay examining specific cell types ability to release cytokines are assessed by acquiring these cells through sorting methods and are recognized by one of ordinary skill in the art.

Furthermore, cytokine mRNA is also assessed to address cytokine release in response to an pmEV composition. These changes in the cells of the host stimulate an immune response similarly to in vivo response in a cancer microenvironment.

**[537]** This PBMC stimulation protocol may be repeated using combinations of purified pmEVs with or without combinations of live, dead, or inactivated/weakened bacterial strains to maximize immune stimulation potential.

**Example 20: In vitro detection of pmEVs in antigen-presenting cells**

**[538]** Dendritic cells in the lamina propria constantly sample live bacteria, dead bacteria, and microbial products in the gut lumen by extending their dendrites across the gut epithelium, which is one way that pmEVs produced by bacteria in the intestinal lumen may directly stimulate dendritic cells. The following methods represent a way to assess the differential uptake of pmEVs by antigen-presenting cells. Optionally, these methods may be applied to assess immunomodulatory behavior of pmEVs administered to a patient.

**[539]** Dendritic cells (DCs) are isolated from human or mouse bone marrow, blood, or spleens according to standard methods or kit protocols (e.g., Inaba K, Swiggard WJ, Steinman RM, Romani N, Schuler G, 2001. Isolation of dendritic cells. Current Protocols in Immunology. Chapter 3:Unit3.7).

**[540]** To evaluate pmEV entrance into and/or presence in DCs, 250,000 DCs are seeded on a round cover slip in complete RPMI-1640 medium and are then incubated with pmEVs from single bacterial strains or combinations pmEVs at various ratios. Purified pmEVs may be labeled with fluorochromes or fluorescent proteins. After incubation for various timepoints (e.g., 1 hour, 2 hours), the cells are washed twice with ice-cold PBS and detached from the plate using trypsin. Cells are either allowed to remain intact or are lysed. Samples are then processed for flow cytometry. Total internalized pmEVs are quantified from lysed samples, and percentage of cells that uptake pmEVs is measured by counting fluorescent cells. The methods described above may also be performed in substantially the same manner using macrophages or epithelial cell lines (obtained from the ATCC) in place of DCs.

**Example 21: In vitro screening of pmEVs with an enhanced ability to activate NK cell killing when incubated with target cells**

**[541]** To demonstrate the ability of the selected pmEV compositions to elicit potent NK cell cytotoxicity to tumor cells, the following *in vitro* assay is used. Briefly, mononuclear cells from heparinized blood are obtained from healthy human donors. Optionally, an expansion step to increase the numbers of NK cells is performed as previously described (e.g., see Somanschi et al., *J Vis Exp.* 2011;(48):2540). The cells may be adjusted to a concentration of ,cells/ml in RPMI-1640 medium containing 5% human serum. The PMNC cells are then labeled with appropriate antibodies and NK cells are isolated through FACS as CD3-/CD56+ cells and are ready for the subsequent cytotoxicity assay. Alternatively, NK cells are isolated using the autoMACs instrument and NK cell isolation kit following manufacturer's instructions (Miltenyl Biotec).

**[542]** NK cells are counted and plated in a 96 well format with 20,000 or more cells per well, and incubated with single-strain pmEVs, with or without addition of antigen presenting cells (e.g., monocytes derived from the same donor), pmEVs from mixtures of bacterial strains, and appropriate controls. After 5-24 hours incubation of NK cells with pmEVs, pmEVs are removed from cells with PBS washes, NK cells are resuspended in10 mL fresh media with antibiotics and are added to 96-well plates containing 20,000 target tumor cells/well. Mouse tumor cell lines used include B16.F10, SIY+ B16.F10, and others. Human tumor cell lines are HLA-matched to donor, and can include PANC-1, UNKPC960/961, UNKC, and HELA cell lines. Plates are incubated for 2-24 hours at 37°C under normal oxygen conditions. Staurospaurine is used as negative control to account for cell death.

**[543]** Following this incubation, flow cytometry is used to measure tumor cell death using methods known in the art. Briefly, tumor cells are stained with viability dye. FACS analysis is used to gate specifically on tumor cells and measure the percentage of dead (killed) tumor cells. Data are also displayed as the absolute number of dead tumor cells per well.

**[544]** This NK cell stimulation protocol may be repeated using combinations of purified pmEVs and live bacterial strains to maximize immune stimulation potential.

**Example 22: Using in vitro immune activation assays to predict in vivo cancer immunotherapy efficacy of pmEV compositions**

**[545]** *In vitro* immune activation assays identify pmEVs that are able to stimulate dendritic cells, which in turn activate CD8+ T cell killing. Therefore, the *in vitro* assays described above are used as a predictive screen of a large number of candidate pmEVs for potential immunotherapy activity. pmEVs that display enhanced stimulation of dendritic cells, enhanced stimulation of CD8+ T cell killing, enhanced stimulation of PBMC killing, and/or enhanced stimulation of NK cell killing, are preferentially chosen for *in vivo* cancer immunotherapy efficacy studies.

**Example 23: Determining the biodistribution of pmEVs when delivered orally to mice**

**[546]** Wild-type mice (*e.g.,* C57BL/6 or BALB/c) are orally inoculated with the pmEV composition of interest to determine the *in vivo* biodistibution profile of purified pmEVs. pmEVs are labeled to aide in downstream analyses. Alternatively, tumor-bearing mice or mice with some immune disorder (e.g., systemic lupus erythematosus, experimental autoimmune encephalomyelitis, NASH) may be studied for *in vivo* distribution of pmEVs over a given time-course.

**[547]** Mice can receive a single dose of the pmEV (e.g., 25-100 µg) or several doses over a defined time course (25-100 µg). Alternatively, pmEVs dosages may be administered based on particle count (e.g., 7e+08 to 6e+11 particles). Mice are housed under specific pathogen-free conditions following approved protocols. Alternatively, mice may be bred and maintained under sterile, germ-free conditions. Blood, stool, and other tissue samples can be taken at appropriate time points.

**[548]** The mice are humanely sacrificed at various time points (*i.e.,* hours to days) post administration of the pmEV compositions, and a full necropsy under sterile conditions is performed. Following standard protocols, lymph nodes, adrenal glands, liver, colon, small intestine, cecum, stomach, spleen, kidneys, bladder, pancreas, heart, skin, lungs, brain, and other tissue of interest are harvested and are used directly or snap frozen for further testing. The tissue samples are dissected and homogenized to prepare single-cell suspensions following standard protocols known to one skilled in the art. The number of pmEVs present in the sample is then quantified through flow cytometry. Quantification may also proceed with use of fluorescence

microscopy after appropriate processing of whole mouse tissue (Vankelecom H., Fixation and paraffin-embedding of mouse tissues for GFP visualization, *Cold Spring Harb. Protoc.*, 2009). Alternatively, the animals may be analyzed using live-imaging according to the pmEV labeling technique.

**[549]** Biodistribution may be performed in mouse models of cancer such as but not limited to CT-26 and B16 (see, e.g., Kim et al., Nature Communications vol. 8, no. 626 (2017)) or autoimmunity such as but not limited to EAE and DTH (see, e.g., Turjeman et al., PLoS One 10(7): e0130442 (20105).

**Example 24: Purification and preparation of secreted microbial extracellular vesicles (smEVs) from bacteria**

*Purification*

**[550]** Secreted microbial extracellular vesicles (smEVs) are purified and prepared from bacterial cultures (e.g., bacteria from Table 1, Table 2, and/or Table 3) using methods known to those skilled in the art (S. Bin Park, et al. PLoS ONE. 6(3):e17629 (2011)).

**[551]** For example, bacterial cultures are centrifuged at 10,000-15,500 x g for 10-40 min at 4°C or room temperature to pellet bacteria. Culture supernatants are then filtered to include material $\leq$ 0.22 μm (for example, via a 0.22 μm or 0.45 μm filter) and to exclude intact bacterial cells. Filtered supernatants are concentrated using methods that may include, but are not limited to, ammonium sulfate precipitation, ultracentrifugation, or filtration. Briefly, for ammonium sulfate precipitation, 1.5-3 M ammonium sulfate is added to filtered supernatant slowly, while stirring at 4°C. Precipitations are incubated at 4°C for 8-48 hours and then centrifuged at 11,000 x g for 20-40 min at 4°C. The pellets contain smEVs and other debris. Briefly, using ultracentrifugation, filtered supernatants are centrifuged at 100,000-200,000 x g for 1-16 hours at 4°C. The pellet of this centrifugation contains smEVs and other debris. Briefly, using a filtration technique, using an Amicon Ultra spin filter or by tangential flow filtration, supernatants are filtered so as to retain species of molecular weight > 50, 100, 300, or 500 kDa.

**[552]** Alternatively, smEVs are obtained from bacterial cultures continuously during growth, or at selected time points during growth, by connecting a bioreactor to an alternating tangential flow (ATF) system (e.g., XCell ATF from Repligen) according to manufacturer's instructions. The ATF system retains intact cells (> 0.22 um) in the bioreactor, and allows

smaller components (e.g., smEVs, free proteins) to pass through a filter for collection. For example, the system may be configured so that the < 0.22 um filtrate is then passed through a second filter of 100 kDa, allowing species such as smEVs between 0.22 um and 100 kDa to be collected, and species smaller than 100 kDa to be pumped back into the bioreactor. Alternatively, the system may be configured to allow for medium in the bioreactor to be replenished and/or modified during growth of the culture. smEVs collected by this method may be further purified and/or concentrated by ultracentrifugation or filtration as described above for filtered supernatants.

**[553]** smEVs obtained by methods described above may be further purified by gradient ultracentrifugation, using methods that may include, but are not limited to, use of a sucrose gradient or Optiprep gradient. Briefly, using a sucrose gradient method, if ammonium sulfate precipitation or ultracentrifugation were used to concentrate the filtered supernatants, pellets are resuspended in 60% sucrose, 30 mM Tris, pH 8.0. If filtration was used to concentrate the filtered supernatant, the concentrate is buffer exchanged into 60% sucrose, 30 mM Tris, pH 8.0, using an Amicon Ultra column. Samples are applied to a 35-60% discontinuous sucrose gradient and centrifuged at 200,000 x g for 3-24 hours at 4°C. Briefly, using an Optiprep gradient method, if ammonium sulfate precipitation or ultracentrifugation were used to concentrate the filtered supernatants, pellets are resuspended in 45% Optiprep in PBS. If filtration was used to concentrate the filtered supernatant, the concentrate is diluted using 60% Optiprep to a final concentration of 45% Optiprep. Samples are applied to a 0-45% discontinuous sucrose gradient and centrifuged at 200,000 x g for 3-24 hours at 4°C. Alternatively, high resolution density gradient fractionation could be used to separate smEVs based on density.

*Preparation*

**[554]** To confirm sterility and isolation of the smEV preparations, smEVs are serially diluted onto agar medium used for routine culture of the bacteria being tested and incubated using routine conditions. Non-sterile preparations are passed through a 0.22 um filter to exclude intact cells. To further increase purity, isolated smEVs may be DNase or proteinase K treated.

**[555]** Alternatively, for preparation of smEVs used for *in vivo* injections, purified smEVs are processed as described previously (G. Norheim, et al. *PLoS ONE.* 10(9): e0134353 (2015)). Briefly, after sucrose gradient centrifugation, bands containing smEVs are resuspended to a final concentration of 50 μg/mL in a solution containing 3% sucrose or other solution

suitable for *in vivo* injection known to one skilled in the art. This solution may also contain adjuvant, for example aluminum hydroxide at a concentration of 0-0.5% (w/v).

**[556]** To make samples compatible with further testing (e.g., to remove sucrose prior to TEM imaging or *in vitro* assays), samples are buffer exchanged into PBS or 30 mM Tris, pH 8.0 using filtration (e.g., Amicon Ultra columns), dialysis, or ultracentrifugation (following 15-fold or greater dilution in PBS, 200,000 x g, 1-3 hours, 4°C) and resuspension in PBS.

**[557]** For all of these studies, smEVs may be heated, irradiated, and/or lyophilized prior to administration (as described in Example 49).

**Example 25: Manipulating bacteria through stress to produce various amounts of smEVs and/or to vary content of smEVs**

**[558]** Stress, and in particular envelope stress, has been shown to increase production of smEVs by some bacterial strains (I. MacDonald, M. Kuehn. *J Bacteriol* 195(13): doi: 10/1128/JB.02267-12). In order to vary production of smEVs by bacteria, bacteria are stressed using various methods.

**[559]** Bacteria may be subjected to single stressors or stressors in combination. The effects of different stressors on different bacteria is determined empirically by varying the stress condition and determining the IC50 value (the conditions required to inhibit cell growth by 50%). smEV purification, quantification, and characterization occurs. smEV production is quantified (1) in complex samples of bacteria and smEVs by nanoparticle tracking analysis (NTA) or transmission electron microscopy (TEM); or (2) following smEV purification by NTA, lipid quantification, or protein quantification. smEV content is assessed following purification by methods described above.

**[560]** *Antibiotic Stress*

**[561]** Bacteria are cultivated under standard growth conditions with the addition of sublethal concentrations of antibiotics. This may include 0.1-1 μg/mL chloramphenicol, or 0.1-0.3 μg/mL gentamicin, or similar concentrations of other antibiotics (e.g., ampicillin, polymyxin B). Host antimicrobial products such as lysozyme, defensins, and Reg proteins may be used in place of antibiotics. Bacterially-produced antimicrobial peptides, including bacteriocins and microcins may also be used.

**[562]** *Temperature Stress*

**[563]** Bacteria are cultivated under standard growth conditions, but at higher or lower temperatures than are typical for their growth. Alternatively, bacteria are grown under standard conditions, and then subjected to cold shock or heat shock by incubation for a short period of time at low or high temperatures respectively. For example, bacteria grown at 37°C are incubated for 1 hour at 4°C-18°C for cold shock or 42°C-50°C for heat shock.

**[564]** *Starvation and nutrient limitation*

**[565]** To induce nutritional stress, bacteria are cultivated under conditions where one or more nutrients are limited. Bacteria may be subjected to nutritional stress throughout growth or shifted from a rich medium to a poor medium. Some examples of media components that are limited are carbon, nitrogen, iron, and sulfur. An example medium is M9 minimal medium (Sigma-Aldrich), which contains low glucose as the sole carbon source. Particularly for *Prevotella spp.*, iron availability is varied by altering the concentration of hemin in media and/or by varying the type of porphyrin or other iron carrier present in the media, as cells grown in low hemin conditions were found to produce greater numbers of smEVs (S. Stubbs et al. *Letters in Applied Microbiology.* 29:31-36 (1999). Media components are also manipulated by the addition of chelators such as EDTA and deferoxamine.

**[566]** *Saturation*

**[567]** Bacteria are grown to saturation and incubated past the saturation point for various periods of time. Alternatively, conditioned media is used to mimic saturating environments during exponential growth. Conditioned media is prepared by removing intact cells from saturated cultures by centrifugation and filtration, and conditioned media may be further treated to concentrate or remove specific components.

**[568]** *Salt Stress*

**[569]** Bacteria are cultivated in or exposed for brief periods to medium containing NaCl, bile salts, or other salts.

**[570]** *UV Stress*

**[571]** UV stress is achieved by cultivating bacteria under a UV lamp or by exposing bacteria to UV using an instrument such as a Stratalinker (Agilent). UV may be administered throughout the entire cultivation period, in short bursts, or for a single defined period following growth.

**[572]** *Reactive Oxygen Stress*

**[573]** Bacteria are cultivated in the presence of sublethal concentrations of hydrogen peroxide (250-1,000 μM) to induce stress in the form of reactive oxygen species. Anaerobic bacteria are cultivated in or exposed to concentrations of oxygen that are toxic to them.

**[574]** *Detergent stress*

**[575]** Bacteria are cultivated in or exposed to detergent, such as sodium dodecyl sulfate (SDS) or deoxycholate.

**[576]** *pH stress*

**[577]** Bacteria are cultivated in or exposed for limited times to media of different pH.

**Example 26: Preparation of smEV-free bacteria**

**[578]** Bacterial samples containing minimal amounts of smEVs are prepared. smEV production is quantified (1) in complex samples of bacteria and extracellular components by NTA or TEM; or (2) following smEV purification from bacterial samples, by NTA, lipid quantification, or protein quantification.

**[579]** a. Centrifugation and washing: Bacterial cultures are centrifuged at 11,000 x g to separate intact cells from supernatant (including free proteins and vesicles). The pellet is washed with buffer, such as PBS, and stored in a stable way (e.g., mixed with glycerol, flash frozen, and stored at -80°C).

**[580]** b. ATF: Bacteria and smEVs are separated by connection of a bioreactor to an ATF system. smEV-free bacteria are retained within the bioreactor, and may be further separated from residual smEVs by centrifugation and washing, as described above.

**[581]** c. Bacteria are grown under conditions that are found to limit production of smEVs. Conditions that may be varied.

**Example 27: A colorectal carcinoma model**

**[582]** To study the efficacy of smEVs in a tumor model, one of many cancer cell lines may be used according to rodent tumor models known in the art. smEVs may be generated from any one of several bacterial species, for instance *Veillonella parvula* or *V. atypica*.

**[583]** For example, female 6-8 week old Balb/c mice are obtained from Taconic (Germantown, NY) or other vendor. 100,000 CT-26 colorectal tumor cells (ATCC CRL-2638) are resuspended in sterile PBS and inoculated in the presence of 50% Matrigel. CT-26 tumor

cells are subcutaneously injected into one hind flank of each mouse. When tumor volumes reach an average of 100mm$^3$ (approximately 10-12 days following tumor cell inoculation), animals are distributed into various treatment groups (e.g., Vehicle; *Veillonella* smEVs, *Bifidobacteria* smEVs, with or without anti-PD-1 antibody). Antibodies are administered intraperitoneally (i.p.) at 200 µg/mouse (100 µl final volume) every four days, starting on day 1, for a total of 3 times (Q4Dx3), and smEVs are administered orally or intravenously and at varied doses and varied times. For example, smEVs (5 µg) are intravenously (i.v.) injected every third day, starting on day 1 for a total of 4 times (Q3Dx4) and mice are assessed for tumor growth. Some mice may be intravenously injected with smEVs at 10, 15, or 20 ug smEVs/mouse. Other mice may receive 25, 50, or 100 mg of smEVs per mouse. Alternatively, some mice receive between 7.0e+09 to 3.0e+12 smEV particles per dose.

**[584]** Alternatively, when tumor volumes reach an average of 100mm$^3$ (approximately 10-12 days following tumor cell inoculation), animals are distributed into the following groups: 1) Vehicle; 2) *Neisseria Meningitidis* smEVs isolated from the Bexsero® vaccine; and 3) anti-PD-1 antibody. Antibodies are administered intraperitoneally (i.p.) at 200ug/mouse (100ul final volume) every four days, starting on day 1, and *Neisseria Meningitidis* smEVs are administered intraperitoneally (i.p.) daily, starting on day 1 until the conclusion of the study.

**[585]** When tumor volumes reached an average of 100mm$^3$ (approximately 10-12 days following tumor cell inoculation), animals were distributed into the following groups: 1) Vehicle; 2) anti-PD-1 antibody; and 3) smEV *V. parvula* (7.0 e+10 particle count). Antibodies were administered intraperitoneally (i.p.) at 200 µg/mouse (100 µl final volume) every four days, starting on day 1, and smEVs were intravenously (i.v.) injected daily, starting on day 1 until the conclusion of the study and tumors measured for growth. At day 11, the smEV *V. parvula* group exhibited tumor growth inhibition that was significantly better than that seen in the anti-PD-1 group (**Figure 16**). Welch's test is performed for treatment vs. vehicle. In a study looking at dose-response of smEVs purified from *V.* parvula and *V. atypica*, the highest dose of smEVs demonstrated the greatest efficacy (**Figures 17 and 18**), although in a study with smEVs from *V. tobetsuensis*, higher doses do not necessarily correspond to greater efficacy (**Figure 19**).

**Example 28: Administering smEV compositions to treat mouse tumor models**

**[586]** As described in Example 27 a mouse model of cancer is generated by subcutaneously injecting a tumor cell line or patient-derived tumor sample and allowing it to engraft into healthy mice. The methods provided herein may be performed using one of several different tumor cell lines including, but not limited to: B16-F10 or B16-F10-SIY cells as an orthotopic model of melanoma, Panc02 cells as an orthotopic model of pancreatic cancer (Maletzki et al., 2008, Gut 57:483-491), LLC1 cells as an orthotopic model of lung cancer, and RM-1 as an orthotopic model of prostate cancer. As an example, but without limitation, methods for studying the efficacy of smEVs in the B16-F10 model are provided in depth herein.

**[587]** A syngeneic mouse model of spontaneous melanoma with a very high metastatic frequency is used to test the ability of bacteria to reduce tumor growth and the spread of metastases. The smEVs chosen for this assay are compositions that may display enhanced activation of immune cell subsets and stimulate enhanced killing of tumor cells *in vitro*. The mouse melanoma cell line B16-F10 is obtained from ATCC. The cells are cultured in vitro as a monolayer in RPMI medium, supplemented with 10% heat-inactivated fetal bovine serum and 1% penicillin/streptomycin at 37□ in an atmosphere of 5% CO2 in air. The exponentially growing tumor cells are harvested by trypsinization, washed three times with cold 1x PBS, and a suspension of 5E6 cells/ml is prepared for administration. Female C57BL/6 mice are used for this experiment. The mice are 6-8 weeks old and weigh approximately 16-20 g. For tumor development, each mouse is injected SC into the flank with 100 µl of the B16-F10 cell suspension. The mice are anesthetized by ketamine and xylazine prior to the cell transplantation. The animals used in the experiment may be started on an antibiotic treatment via instillation of a cocktail of kanamycin (0.4 mg/ml), gentamicin, (0.035 mg/ml), colistin (850 U/ml), metronidazole (0.215 mg/ml) and vancomycin (0.045 mg/ml) in the drinking water from day 2 to 5 and an intraperitoneal injection of clindamycin (10 mg/kg) on day 7 after tumor injection.

**[588]** The size of the primary flank tumor is measured with a caliper every 2-3 days and the tumor volume is calculated using the following formula: tumor volume = the tumor width × tumor length × 0.5. After the primary tumor reaches approximately 100 mm3, the animals are sorted into several groups based on their body weight. The mice are then randomly taken from each group and assigned to a treatment group. smEV compositions are prepared as previously described. The mice are orally inoculated by gavage with approximately 7.0e+09 to 3.0e+12

smEV particles. Alternatively, smEVs are administered intravenously. Mice receive smEVs daily, weekly, bi-weekly, monthly, bi-monthly, or on any other dosing schedule throughout the treatment period. Mice may be IV injected with smEVs in the tail vein, or directly injected into the tumor. Mice can be injected with smEVs, with or without live bacteria, and/or smEVs with or without inactivated/weakened or killed bacteria. Mice can be injected or orally gavaged weekly or once a month. Mice may receive combinations of purified smEVs and live bacteria to maximize tumor-killing potential. All mice are housed under specific pathogen-free conditions following approved protocols. Tumor size, mouse weight, and body temperature are monitored every 3-4 days and the mice are humanely sacrificed 6 weeks after the B16-F10 mouse melanoma cell injection or when the volume of the primary tumor reaches 1000 mm3. Blood draws are taken weekly and a full necropsy under sterile conditions is performed at the termination of the protocol.

**[589]** Cancer cells can be easily visualized in the mouse B16-F10 melanoma model due to their melanin production. Following standard protocols, tissue samples from lymph nodes and organs from the neck and chest region are collected and the presence of micro- and macro-metastases is analyzed using the following classification rule. An organ is classified as positive for metastasis if at least two micro-metastatic and one macro-metastatic lesion per lymph node or organ are found. Micro-metastases are detected by staining the paraffin-embedded lymphoid tissue sections with hematoxylin-eosin following standard protocols known to one skilled in the art. The total number of metastases is correlated to the volume of the primary tumor and it is found that the tumor volume correlates significantly with tumor growth time and the number of macro- and micro-metastases in lymph nodes and visceral organs and also with the sum of all observed metastases. Twenty-five different metastatic sites are identified as previously described (Bobek V., et al., Syngeneic lymph-node-targeting model of green fluorescent protein-expressing Lewis lung carcinoma, Clin. Exp. Metastasis, 2004;21(8):705-8).

**[590]** The tumor tissue samples are further analyzed for tumor infiltrating lymphocytes. The CD8+ cytotoxic T cells can be isolated by FACS and can then be further analyzed using customized p/MHC class I microarrays to reveal their antigen specificity (see e.g., Deviren G., et al., Detection of antigen-specific T cells on p/MHC microarrays, J. Mol. Recognit., 2007 Jan-Feb;20(1):32-8). CD4+ T cells can be analyzed using customized p/MHC class II microarrays.

**[591]** At various timepoints, mice are sacrificed and tumors, lymph nodes, or other tissues may be removed for *ex vivo* flow cytometric analysis using methods known in the art. For example, tumors are dissociated using a Miltenyi tumor dissociation enzyme cocktail according to the manufacturer's instructions. Tumor weights are recorded and tumors are chopped then placed in 15ml tubes containing the enzyme cocktail and placed on ice. Samples are then placed on a gentle shaker at 37°C for 45 minutes and quenched with up to 15ml complete RPMI. Each cell suspension is strained through a 70μm filter into a 50ml falcon tube and centrifuged at 1000 rpm for 10 minutes. Cells are resuspended in FACS buffer and washed to remove remaining debris. If necessary, samples are strained again through a second 70μm filter into a new tube. Cells are stained for analysis by flow cytometry using techniques known in the art. Staining antibodies can include anti-CD11c (dendritic cells), anti-CD80, anti-CD86, anti-CD40, anti-MHCII, anti-CD8a, anti-CD4, and anti-CD103. Other markers that may be analyzed include pan-immune cell marker CD45, T cell markers (CD3, CD4, CD8, CD25, Foxp3, T-bet, Gata3, Ror□t, Granzyme B, CD69, PD-1, CTLA-4), and macrophage/myeloid markers (CD11b, MHCII, CD206, CD40, CSF1R, PD-L1, Gr-1). In addition to immunophenotyping, serum cytokines can be analyzed including, but not limited to, TNFa, IL-17, IL-13, IL-12p70, IL12p40, IL-10, IL-6, IL-5, IL-4, IL-2, IL-1b, IFNy, GM-CSF, G-CSF, M-CSF, MIG, IP10, MIP1b, RANTES, and MCP-1. Cytokine analysis may be carried out immune cells obtained from lymph nodes or other tissue, and/or on purified CD45+ tumor-infiltrated immune cells obtained ex vivo. Finally, immunohistochemistry is carried out on tumor sections to measure T cells, macrophages, dendritic cells, and checkpoint molecule protein expression.

**[592]** The same experiment is also performed with a mouse model of multiple pulmonary melanoma metastases. The mouse melanoma cell line B16-BL6 is obtained from ATCC and the cells are cultured in vitro as described above. Female C57BL/6 mice are used for this experiment. The mice are 6-8 weeks old and weigh approximately 16-20 g. For tumor development, each mouse is injected into the tail vein with 100 μl of a 2E6 cells/ml suspension of B16-BL6 cells. The tumor cells that engraft upon IV injection end up in the lungs.

**[593]** The mice are humanely killed after 9 days. The lungs are weighed and analyzed for the presence of pulmonary nodules on the lung surface. The extracted lungs are bleached with Fekete's solution, which does not bleach the tumor nodules because of the melanin in the B16 cells though a small fraction of the nodules is amelanotic (i.e. white). The number of tumor

nodules is carefully counted to determine the tumor burden in the mice. Typically, 200-250 pulmonary nodules are found on the lungs of the control group mice (i.e. PBS gavage).

**[594]** The percentage tumor burden is calculated for the various treatment groups. Percentage tumor burden is defined as the mean number of pulmonary nodules on the lung surfaces of mice that belong to a treatment group divided by the mean number of pulmonary nodules on the lung surfaces of the control group mice.

**[595]** The tumor biopsies and blood samples are submitted for metabolic analysis via LCMS techniques or other methods known in the art. Differential levels of amino acids, sugars, lactate, among other metabolites, between test groups demonstrate the ability of the microbial composition to disrupt the tumor metabolic state.

*RNA Seq to Determine Mechanism of Action*

**[596]** Dendritic cells are purified from tumors, Peyers patches, and mesenteric lymph nodes. RNAseq analysis is carried out and analyzed according to standard techniques known to one skilled in the art (Z. Hou. *Scientific Reports*. 5(9570):doi:10.1038/srep09570 (2015)). In the analysis, specific attention is placed on innate inflammatory pathway genes including TLRs, CLRs, NLRs, and STING, cytokines, chemokines, antigen processing and presentation pathways, cross presentation, and T cell co-stimulation.

**[597]** Rather than being sacrificed, some mice may be rechallenged with tumor cell injection into the contralateral flank (or other area) to determine the impact of the immune system's memory response on tumor growth.

## Example 29: Administering smEVs to treat mouse tumor models in combination with PD-1 or PD-L1 inhibition

**[598]** To determine the efficacy of smEVs in tumor mouse models in combination with PD-1 or PD-L1 inhibition, a mouse tumor model may be used as described above.

**[599]** smEVs are tested for their efficacy in the mouse tumor model, either alone or in combination with whole bacterial cells and with or without anti-PD-1 or anti-PD-L1. smEVs, bacterial cells, and/or anti-PD-1 or anti-PD-L1 are administered at varied time points and at varied doses. For example, on day 10 after tumor injection, or after the tumor volume reaches $100mm^3$, the mice are treated with smEVs alone or in combination with anti-PD-1 or anti-PD-L1.

**[600]** Mice may be administered smEVs orally, intravenously, or intratumorally. For example, some mice are intravenously injected with anywhere between 7.0e+09 to 3.0e+12 smEV particles. While some mice receive smEVs through i.v. injection, other mice may receive smEVs through intraperitoneal (i.p.) injection, subcutaneous (s.c.) injection, nasal route administration, oral gavage, or other means of administration. Some mice may receive smEVs every day (e.g., starting on day 1), while others may receive smEVs at alternative intervals (e.g., every other day, or once every three days). Groups of mice may be administered a pharmaceutical composition of the invention comprising a mixture of smEVs and bacterial cells. For example, the composition may comprise smEV particles and whole bacteria in a ratio from 1:1 (smEVs: bacterial cells) to 1-1x$10^{12}$:1 (smEVs: bacterial cells).

**[601]** Alternatively, some groups of mice may receive between 1x$10^{4}$ and 5x$10^{9}$ bacterial cells in an administration separate from, or comingled with, the smEV administration. As with the smEVs, bacterial cell administration may be varied by route of administration, dose, and schedule. The bacterial cells may be live, dead, or weakened. The bacterial cells may be harvested fresh (or frozen) and administered, or they may be irradiated or heat-killed prior to administration with the smEVs.

**[602]** Some groups of mice are also injected with effective doses of checkpoint inhibitor. For example, mice receive 100 μg anti-PD-L1 mAB (clone 10f.9g2, BioXCell) or another anti-PD-1 or anti-PD-L1 mAB in 100 μl PBS, and some mice receive vehicle and/or other appropriate control (e.g., control antibody). Mice are injected with mABs 3, 6, and 9 days after the initial injection. To assess whether checkpoint inhibition and smEV immunotherapy have an additive anti-tumor effect, control mice receiving anti-PD-1 or anti-PD-L1 mABs are included to the standard control panel. Primary (tumor size) and secondary (tumor infiltrating lymphocytes and cytokine analysis) endpoints are assessed, and some groups of mice may be rechallenged with a subsequent tumor cell inoculation to assess the effect of treatment on memory response.

**Example 30: smEVs in a mouse model of delayed-type hypersensitivity (DTH)**

**[603]** Delayed-type hypersensitivity (DTH) is an animal model of atopic dermatitis (or allergic contact dermatitis), as reviewed by Petersen et al. (In vivo pharmacological disease models for psoriasis and atopic dermatitis in drug discovery. Basic & Clinical Pharm &

Toxicology. 2006. 99(2): 104-115; see also Irving C. Allen (ed.) Mouse Models of Innate Immunity: Methods and Protocols, Methods in Molecular Biology, 2013. vol. 1031, DOI 10.1007/978-1-62703-481-4_13). Several variations of the DTH model have been used and are well known in the art (Irving C. Allen (ed.). *Mouse Models of Innate Immunity: Methods and Protocols*, Methods in Molecular Biology. Vol. 1031, DOI 10.1007/978-1-62703-481-4_13, Springer Science + Business Media, LLC 2013).

**[604]** DTH can be induced in a variety of mouse and rat strains using various haptens or antigens, for example an antigen emulsified with an adjuvant. DTH is characterized by sensitization as well as an antigen-specific T cell-mediated reaction that results in erythema, edema, and cellular infiltration – especially infiltration of antigen presenting cells (APCs), eosinophils, activated CD4+ T cells, and cytokine-expressing Th2 cells.

**[605]** Generally, mice are primed with an antigen administered in the context of an adjuvant (e.g., Complete Freund's Adjuvant) in order to induce a secondary (or memory) immune response measured by swelling and antigen-specific antibody titer.

**[606]** Dexamethasone, a corticosteroid, is a known anti-inflammatory that ameliorates DTH reactions in mice and serves as a positive control for suppressing inflammation in this model (Taube and Carlsten, Action of dexamethasone in the suppression of delayed-type hypersensitivity in reconstituted SCID mice. Inflamm Res. 2000. 49(10): 548-52). For the positive control group, a stock solution of 17 mg/mL of Dexamethasone is prepared on Day 0 by diluting 6.8 mg Dexamethasone in 400 µL 96% ethanol. For each day of dosing, a working solution is prepared by diluting the stock solution 100x in sterile PBS to obtain a final concentration of 0.17 mg/mL in a septum vial for intraperitoneal dosing. Dexamethasone-treated mice receive 100 µL Dexamethasone i.p. (5 mL/kg of a 0.17 mg/mL solution). Frozen sucrose serves as the negative control (vehicle). In the study described below, vehicle, Dexamethasone (positive control) and smEVs were dosed daily.

**[607]** smEVs are tested for their efficacy in the mouse model of DTH, either alone or in combination with whole bacterial cells, with or without the addition of other anti-inflammatory treatments. For example, 6-8 week old C57Bl/6 mice are obtained from Taconic (Germantown, NY), or other vendor. Groups of mice are administered four subcutaneous (s.c.) injections at four sites on the back (upper and lower) of antigen (e.g., Ovalbumin (OVA) or Keyhole Limpet Hemocyanin (KLH)) in an effective dose (e.g., 50ul total volume per site). For a DTH response,

animals are injected intradermally (i.d.) in the ears under ketamine/xylazine anesthesia (approximately 50mg/kg and 5 mg/kg, respectively). Some mice serve as control animals. Some groups of mice are challenged with 10ul per ear (vehicle control (0.01% DMSO in saline) in the left ear and antigen (21.2 ug (12nmol) in the right ear) on day 8. To measure ear inflammation, the ear thickness of manually restrained animals is measured using a Mitutoyo micrometer. The ear thickness is measured before intradermal challenge as the baseline level for each individual animal. Subsequently, the ear thickness is measured two times after intradermal challenge, at approximately 24 hours and 48 hours (i.e., days 9 and 10).

**[608]** Treatment with smEVs is initiated at some point, either around the time of priming or around the time of DTH challenge. For example, smEVs may be administered at the same time as the subcutaneous injections (day 0), or they may be administered prior to, or upon, intradermal injection. smEVs are administered at varied doses and at defined intervals. For example, some mice are intravenously injected with smEVs at 10, 15, or 20 ug/mouse. Other mice may receive 25, 50, or 100 mg of smEVs per mouse. Other mice may receive 25, 50, or 100 mg of smEVs per mouse. Alternatively, some mice receive between 7.0e+09 to 3.0e+12 smEV particles per dose.

**[609]** While some mice receive smEVs through i.v. injection, other mice may receive smEVs through intraperitoneal (i.p.) injection, subcutaneous (s.c.) injection, nasal route administration, oral gavage, topical administration, intradermal (i.d.) injection, or other means of administration. Some mice may receive smEVs every day (e.g., starting on day 0), while others may receive smEVs at alternative intervals (e.g., every other day, or once every three days). Groups of mice may be administered a pharmaceutical composition of the invention comprising a mixture of smEVs and bacterial cells. For example, the composition may comprise smEV particles and whole bacteria in a ratio from 1:1 (smEVs: bacterial cells) to 1-1x$10^{12}$:1 (smEVs: bacterial cells).

**[610]** Alternatively, some groups of mice may receive between 1x$10^{4}$ and 5x$10^{9}$ bacterial cells in an administration separate from, or comingled with, the smEV administration. As with the smEVs, bacterial cell administration may be varied by route of administration, dose, and schedule. The bacterial cells may be live, dead, or weakened. The bacterial cells may be harvested fresh (or frozen) and administered, or they may be irradiated or heat-killed prior to administration with the smEVs.

**[611]** For the smEVs, total protein is measured using Bio-rad assays (Cat# 5000205) performed per manufacturer's instructions.

**[612]** An emulsion of Keyhole Limpet Hemocyanin (KLH) and Complete Freund's Adjuvant (CFA) was prepared freshly on the day of immunization (day 0). To this end, 8 mg of KLH powder is weighed and is thoroughly re-suspended in 16 mL saline. An emulsion was prepared by mixing the KLH/saline with an equal volume of CFA solution (*e.g.*, 10 mL KLH/saline + 10 mL CFA solution) using syringes and a luer lock connector. KLH and CFA were mixed vigorously for several minutes to form a white-colored emulsion to obtain maximum stability. A drop test was performed to check if a homogenous emulsion was obtained.

**[613]** On day 0, C57Bl/6J female mice, approximately 7 weeks old, were primed with KLH antigen in CFA by subcutaneous immunization (4 sites, 50 μL per site). *P. histicola* smEVs and lyophilized *P. histicola* smEVs were tested by oral gavage at low (6.0E+07), medium (6.0E+09), and high (6.0E+11) dosages.

**[614]** On day 8, mice were challenged intradermally (i.d.) with 10 μg KLH in saline (in a volume of 10 μL) in the left ear. Ear pinna thickness was measured at 24 hours following antigen challenge (**Figure 20**). As determined by ear thickness, *P. histicola* smEVs were efficacious at suppressing inflammation in both their non-lyophilized and lyophilized forms.

**[615]** For future inflammation studies, some groups of mice may be treated with anti-inflammatory agent(s) (e.g., anti-CD154, blockade of members of the TNF family, or other treatment), and/or an appropriate control (e.g., vehicle or control antibody) at various timepoints and at effective doses.

**[616]** At various timepoints, serum samples may be taken. Other groups of mice may be sacrificed and lymph nodes, spleen, mesenteric lymph nodes (MLN), the small intestine, colon, and other tissues may be removed for histology studies, ex vivo histological, cytokine and/or flow cytometric analysis using methods known in the art. Some mice are exsanguinated from the orbital plexus under O2/CO2 anesthesia and ELISA assays performed.

**[617]** Tissues may be dissociated using dissociation enzymes according to the manufacturer's instructions. Cells are stained for analysis by flow cytometry using techniques known in the art. Staining antibodies can include anti-CD11c (dendritic cells), anti-CD80, anti-CD86, anti-CD40, anti-MHCII, anti-CD8a, anti-CD4, and anti-CD103. Other markers that may be analyzed include pan-immune cell marker CD45, T cell markers (CD3, CD4, CD8, CD25,

Foxp3, T-bet, Gata3, Rory-gamma-t, Granzyme B, CD69, PD-1, CTLA-4), and macrophage/myeloid markers (CD11b, MHCII, CD206, CD40, CSF1R, PD-L1, Gr-1, F4/80). In addition to immunophenotyping, serum cytokines can be analyzed including, but not limited to, TNFa, IL-17, IL-13, IL-12p70, IL12p40, IL-10, IL-6, IL-5, IL-4, IL-2, IL-1b, IFNy, GM-CSF, G-CSF, M-CSF, MIG, IP10, MIP1b, RANTES, and MCP-1. Cytokine analysis may be carried out on immune cells obtained from lymph nodes or other tissue, and/or on purified CD45+ infiltrated immune cells obtained ex vivo. Finally, immunohistochemistry is carried out on various tissue sections to measure T cells, macrophages, dendritic cells, and checkpoint molecule protein expression.

**[618]** Ears may be removed from the sacrificed animals and placed in cold EDTA-free protease inhibitor cocktail (Roche). Ears are homogenized using bead disruption and supernatants analyzed for various cytokines by Luminex kit (EMD Millipore) as per manufacturer's instructions. In addition, cervical lymph nodes are dissociated through a cell strainer, washed, and stained for FoxP3 (PE-FJK-16s) and CD25 (FITC-PC61.5) using methods known in the art.

**[619]** In order to examine the impact and longevity of DTH protection, rather than being sacrificed, some mice may be rechallenged with the challenging antigen at a later time and mice analyzed for susceptibility to DTH and severity of response.

**Example 31: smEVs in a mouse model of Experimental Autoimmune Encephalomyelitis (EAE)**

**[620]** EAE is a well-studied animal model of multiple sclerosis, as reviewed by Constantinescu et al., (Experimental autoimmune encephalomyelitis (EAE) as a model for multiple sclerosis (MS). Br J Pharmacol. 2011 Oct; 164(4): 1079-1106). It can be induced in a variety of mouse and rat strains using different myelin-associated peptides, by the adoptive transfer of activated encephalitogenic T cells, or the use of TCR transgenic mice susceptible to EAE, as discussed in Mangalam et al., (Two discreet subsets of CD8+ T cells modulate $PLP_{91-110}$ induced experimental autoimmune encephalomyelitis in HLA-DR3 transgenic mice. J Autoimmun. 2012 Jun; 38(4): 344-353).

**[621]** smEVs are tested for their efficacy in the rodent model of EAE, either alone or in combination with whole bacterial cells, with or without the addition of other anti-inflammatory

treatments. Additionally, smEVs may be administered orally or via intravenous administration. For example, female 6-8 week old C57Bl/6 mice are obtained from Taconic (Germantown, NY). Groups of mice are administered two subcutaneous (s.c.) injections at two sites on the back (upper and lower) of 0.1 ml myelin oligodentrocyte glycoprotein 35-55 (MOG35-55; 100ug per injection; 200ug per mouse (total 0.2ml per mouse)), emulsified in Complete Freund's Adjuvant (CFA; 2-5mg killed mycobacterium tuberculosis H37Ra/ml emulsion). Approximately 1-2 hours after the above, mice are intraperitoneally (i.p.) injected with 200ng Pertussis toxin (PTx) in 0.1ml PBS (2ug/ml). An additional IP injection of PTx is administered on day 2. Alternatively, an appropriate amount of an alternative myelin peptide (e.g., proteolipid protein (PLP)) is used to induce EAE. Some animals serve as naïve controls. EAE severity is assessed and a disability score is assigned daily beginning on day 4 according to methods known in the art (Mangalam et al. 2012).

**[622]** Treatment with smEVs is initiated at some point, either around the time of immunization or following EAE immunization. For example, smEVs may be administered at the same time as immunization (day 1), or they may be administered upon the first signs of disability (e.g., limp tail), or during severe EAE. smEVs are administered at varied doses and at defined intervals. For example, some mice are intravenously injected with smEVs at 10, 15, or 20 ug/mouse. Other mice may receive 25, 50, or 100 mg of smEVs per mouse. Alternatively, some mice receive between 7.0e+09 to 3.0e+12 smEV particles per dose. While some mice receive smEVs through i.v. injection, other mice may receive smEVs through intraperitoneal (i.p.) injection, subcutaneous (s.c.) injection, nasal route administration, oral gavage, or other means of administration. Some mice may receive smEVs every day (e.g., starting on day 1), while others may receive smEVs at alternative intervals (e.g., every other day, or once every three days). Groups of mice may be administered a pharmaceutical composition of the invention comprising a mixture of smEVs and bacterial cells. For example, the composition may comprise smEV particles and whole bacteria in a ratio from 1:1 (smEVs: bacterial cells) to $1\text{-}1x10^{12}$:1 (smEVs: bacterial cells).

**[623]** Alternatively, some groups of mice may receive between $1x10^4$ and $5x10^9$ bacterial cells in an administration separate from, or comingled with, the smEV administration. As with the smEVs, bacterial cell administration may be varied by route of administration, dose, and schedule. The bacterial cells may be live, dead, or weakened. The bacterial cells may be

harvested fresh (or frozen) and administered, or they may be irradiated or heat-killed prior to administration with the smEVs.

**[624]** Some groups of mice may be treated with additional anti-inflammatory agent(s) or EAE therapeutic(s) (e.g., anti-CD154, blockade of members of the TNF family, Vitamin D, steroids, anti-inflammatory agents, or other treatment(s)), and/or an appropriate control (e.g., vehicle or control antibody) at various time points and at effective doses.

**[625]** In addition, some mice are treated with antibiotics prior to treatment. For example, vancomycin (0.5g/L), ampicillin (1.0g/L), gentamicin (1.0g/L) and amphotericin B (0.2g/L) are added to the drinking water, and antibiotic treatment is halted at the time of treatment or a few days prior to treatment. Some immunized mice are treated without receiving antibiotics.

**[626]** At various timepoints, mice are sacrificed and sites of inflammation (e.g., brain and spinal cord), lymph nodes, or other tissues may be removed for ex vivo histological, cytokine and/or flow cytometric analysis using methods known in the art. For example, tissues are dissociated using dissociation enzymes according to the manufacturer's instructions. Cells are stained for analysis by flow cytometry using techniques known in the art. Staining antibodies can include anti-CD11c (dendritic cells), anti-CD80, anti-CD86, anti-CD40, anti-MHCII, anti-CD8a, anti-CD4, and anti-CD103. Other markers that may be analyzed include pan-immune cell marker CD45, T cell markers (CD3, CD4, CD8, CD25, Foxp3, T-bet, Gata3, Roryt, Granzyme B, CD69, PD-1, CTLA-4), and macrophage/myeloid markers (CD11b, MHCII, CD206, CD40, CSF1R, PD-L1, Gr-1, F4/80). In addition to immunophenotyping, serum cytokines can be analyzed including, but not limited to, TNFa, IL-17, IL-13, IL-12p70, IL12p40, IL-10, IL-6, IL-5, IL-4, IL-2, IL-1b, IFNy, GM-CSF, G-CSF, M-CSF, MIG, IP10, MIP1b, RANTES, and MCP-1. Cytokine analysis may be carried out on immune cells obtained from lymph nodes or other tissue, and/or on purified CD45+ central nervous system (CNS)-infiltrated immune cells obtained ex vivo. Finally, immunohistochemistry is carried out on various tissue sections to measure T cells, macrophages, dendritic cells, and checkpoint molecule protein expression.

**[627]** In order to examine the impact and longevity of disease protection, rather than being sacrificed, some mice may be rechallenged with a disease trigger (e.g., activated encephalitogenic T cells or re-injection of EAE-inducing peptides). Mice are analyzed for susceptibility to disease and EAE severity following rechallenge.

**Example 32: smEVs in a mouse model of collagen-induced arthritis (CIA)**

**[628]** Collagen-induced arthritis (CIA) is an animal model commonly used to study rheumatoid arthritis (RA), as described by Caplazi et al. (Mouse models of rheumatoid arthritis. Veterinary Pathology. Sept. 1, 2015. 52(5): 819-826) (see also Brand et al. Collagen-induced arthritis. Nature Protocols. 2007. 2: 1269-1275; Pietrosimone et al. Collagen-induced arthritis: a model for murine autoimmune arthritis. Bio Protoc. 2015 Oct. 20; 5(20): e1626).

**[629]** Among other versions of the CIA rodent model, one model involves immunizing HLA-DQ8 Tg mice with chick type II collagen as described by Taneja et al. (J. Immunology. 2007. 56: 69-78; see also Taneja et al. J. Immunology 2008. 181: 2869-2877; and Taneja et al. Arthritis Rheum., 2007. 56: 69-78). Purification of chick CII has been described by Taneja et al. (Arthritis Rheum., 2007. 56: 69-78). Mice are monitored for CIA disease onset and progression following immunization, and severity of disease is evaluated and "graded" as described by Wooley, J. Exp. Med. 1981. 154: 688-700.

**[630]** Mice are immunized for CIA induction and separated into various treatment groups. smEVs are tested for their efficacy in CIA, either alone or in combination with whole bacterial cells, with or without the addition of other anti-inflammatory treatments.

**[631]** Treatment with smEVs is initiated either around the time of immunization with collagen or post-immunization. For example, in some groups, smEVs may be administered at the same time as immunization (day 1), or smEVs may be administered upon first signs of disease, or upon the onset of severe symptoms. smEVs are administered at varied doses and at defined intervals. For example, some mice are intravenously injected with smEVs at 10, 15, or 20 ug/mouse. Other mice may receive 25, 50, or 100 mg of smEVs per mouse. Alternatively, some mice receive between 7.0e+09 to 3.0e+12 smEV particles per dose. While some mice receive smEVs through oral gavage or i.v. injection, while other groups of mice may receive smEVs through intraperitoneal (i.p.) injection, subcutaneous (s.c.) injection, nasal route administration, or other means of administration. Some mice may receive smEVs every day (e.g., starting on day 1), while others may receive smEVs at alternative intervals (e.g., every other day, or once every three days). Groups of mice may be administered a pharmaceutical composition of the invention comprising a mixture of smEVs and bacterial cells. For example, the composition may comprise

smEV particles and whole bacteria in a ratio from 1:1 (smEVs: bacterial cells) to 1-1x$10^{12}$:1 (smEVs: bacterial cells).

**[632]** Alternatively, some groups of mice may receive between 1x$10^4$ and 5x$10^9$ bacterial cells in an administration separate from, or comingled with, the smEV administration. As with the smEVs, bacterial cell administration may be varied by route of administration, dose, and schedule. The bacterial cells may be live, dead, or weakened. The bacterial cells may be harvested fresh (or frozen) and administered, or they may be irradiated or heat-killed prior to administration with the smEVs.

**[633]** Some groups of mice may be treated with additional anti-inflammatory agent(s) or CIA therapeutic(s) (e.g., anti-CD154, blockade of members of the TNF family, Vitamin D, steroid(s), anti-inflammatory agent(s), and/or other treatment), and/or an appropriate control (e.g., vehicle or control antibody) at various timepoints and at effective doses.

**[634]** In addition, some mice are treated with antibiotics prior to treatment. For example, vancomycin (0.5g/L), ampicillin (1.0g/L), gentamicin (1.0g/L) and amphotericin B (0.2g/L) are added to the drinking water, and antibiotic treatment is halted at the time of treatment or a few days prior to treatment. Some immunized mice are treated without receiving antibiotics.

**[635]** At various timepoints, serum samples are obtained to assess levels of anti-chick and anti-mouse CII IgG antibodies using a standard ELISA (Batsalova et al. Comparative analysis of collagen type II-specific immune responses during development of collagen-induced arthritis in two B10 mouse strains. Arthritis Res Ther. 2012. 14(6): R237). Also, some mice are sacrificed and sites of inflammation (e.g., synovium), lymph nodes, or other tissues may be removed for ex vivo histological, cytokine and/or flow cytometric analysis using methods known in the art. The synovium and synovial fluid are analyzed for plasma cell infiltration and the presence of antibodies using techniques known in the art. In addition, tissues are dissociated using dissociation enzymes according to the manufacturer's instructions to examine the profiles of the cellular infiltrates. Cells are stained for analysis by flow cytometry using techniques known in the art. Staining antibodies can include anti-CD11c (dendritic cells), anti-CD80, anti-CD86, anti-CD40, anti-MHCII, anti-CD8a, anti-CD4, and anti-CD103. Other markers that may be analyzed include pan-immune cell marker CD45, T cell markers (CD3, CD4, CD8, CD25, Foxp3, T-bet, Gata3, Roryt, Granzyme B, CD69, PD-1, CTLA-4), and macrophage/myeloid

markers (CD11b, MHCII, CD206, CD40, CSF1R, PD-L1, Gr-1, F4/80). In addition to immunophenotyping, serum cytokines can be analyzed including, but not limited to, TNFa, IL-17, IL-13, IL-12p70, IL12p40, IL-10, IL-6, IL-5, IL-4, IL-2, IL-1b, IFNy, GM-CSF, G-CSF, M-CSF, MIG, IP10, MIP1b, RANTES, and MCP-1. Cytokine analysis may be carried out on immune cells obtained from lymph nodes or other tissue, and/or on purified CD45+ synovium-infiltrated immune cells obtained ex vivo. Finally, immunohistochemistry is carried out on various tissue sections to measure T cells, macrophages, dendritic cells, and checkpoint molecule protein expression.

**[636]** In order to examine the impact and longevity of disease protection, rather than being sacrificed, some mice may be rechallenged with a disease trigger (e.g., activated re-injection with CIA-inducing peptides). Mice are analyzed for susceptibility to disease and CIA severity following rechallenge.

**Example 33: smEVs in a mouse model of colitis**

**[637]** Dextran sulfate sodium (DSS)-induced colitis is a well-studied animal model of colitis, as reviewed by Randhawa et al. (A review on chemical-induced inflammatory bowel disease models in rodents. Korean J Physiol Pharmacol. 2014. 18(4): 279-288; see also Chassaing et al. Dextran sulfate sodium (DSS)-induced colitis in mice. Curr Protoc Immunol. 2014 Feb 4; 104: Unit 15.25).

**[638]** smEVs are tested for their efficacy in a mouse model of DSS-induced colitis, either alone or in combination with whole bacterial cells, with or without the addition of other anti-inflammatory agents.

**[639]** Groups of mice are treated with DSS to induce colitis as known in the art (Randhawa et al. 2014; Chassaing et al. 2014; see also Kim et al. Investigating intestinal inflammation in DSS-induced model of IBD. J Vis Exp. 2012. 60: 3678). For example, male 6-8 week old C57Bl/6 mice are obtained from Charles River Labs, Taconic, or other vendor. Colitis is induced by adding 3% DSS (pmEV Biomedicals, Cat. #0260110) to the drinking water. Some mice do not receive DSS in the drinking water and serve as naïve controls. Some mice receive water for five (5) days. Some mice may receive DSS for a shorter duration or longer than five (5) days. Mice are monitored and scored using a disability activity index known in the art based on weight loss (e.g., no weight loss (score 0); 1-5% weight loss (score 1); 5-10% weight loss (score

2)); stool consistency (e.g., normal (score 0); loose stool (score 2); diarrhea (score 4)); and bleeding (e.g., no blood (score 0), hemoccult positive (score 1); hemoccult positive and visual pellet bleeding (score 2); blood around anus, gross bleeding (score 4).

**[640]** Treatment with smEVs is initiated at some point, either on day 1 of DSS administration, or sometime thereafter. For example, smEVs may be administered at the same time as DSS initiation (day 1), or they may be administered upon the first signs of disease (e.g., weight loss or diarrhea), or during the stages of severe colitis. Mice are observed daily for weight, morbidity, survival, presence of diarrhea and/or bloody stool.

**[641]** smEVs are administered at various doses and at defined intervals. For example, some mice receive between 7.0e+09 and 3.0e+12 smEV particles. While some mice receive smEVs through oral gavage or i.v. injection, while other groups of mice may receive smEVs through intraperitoneal (i.p.) injection, subcutaneous (s.c.) injection, nasal route administration, or other means of administration. Some mice may receive smEVs every day (e.g., starting on day 1), while others may receive smEVs at alternative intervals (e.g., every other day, or once every three days). Groups of mice may be administered a pharmaceutical composition of the invention comprising a mixture of smEVs and bacterial cells. For example, the composition may comprise smEV particles and whole bacteria in a ratio from 1:1 (smEVs: bacterial cells) to $1\text{-}1x10^{12}$:1 (smEVs: bacterial cells).

**[642]** Alternatively, some groups of mice may receive between $1x10^4$ and $5x10^9$ bacterial cells in an administration separate from, or comingled with, the smEV administration. As with the smEVs, bacterial cell administration may be varied by route of administration, dose, and schedule. The bacterial cells may be live, dead, or weakened. The bacterial cells may be harvested fresh (or frozen) and administered, or they may be irradiated or heat-killed prior to administration with the smEVs.

**[643]** Some groups of mice may be treated with additional anti-inflammatory agent(s) (e.g., anti-CD154, blockade of members of the TNF family, or other treatment), and/or an appropriate control (e.g., vehicle or control antibody) at various timepoints and at effective doses.

**[644]** In addition, some mice are treated with antibiotics prior to treatment. For example, vancomycin (0.5g/L), ampicillin (1.0g/L), gentamicin (1.0g/L) and amphotericin B (0.2g/L) are added to the drinking water, and antibiotic treatment is halted at the time of

treatment or a few days prior to treatment. Some mice receive DSS without receiving antibiotics beforehand.

**[645]** At various timepoints, mice undergo video endoscopy using a small animal endoscope (Karl Storz Endoskipe, Germany) under isoflurane anesthesia. Still images and video are recorded to evaluate the extent of colitis and the response to treatment. Colitis is scored using criteria known in the art. Fecal material is collected for study.

**[646]** At various timepoints, mice are sacrificed and the colon, small intestine, spleen, and lymph nodes (e.g., mesenteric lymph nodes) are collected. Additionally, blood is collected into serum separation tubes. Tissue damage is assessed through histological studies that evaluate, but are not limited to, crypt architecture, degree of inflammatory cell infiltration, and goblet cell depletion.

**[647]** The gastrointestinal (GI) tract, lymph nodes, and/or other tissues may be removed for ex vivo histological, cytokine and/or flow cytometric analysis using methods known in the art. For example, tissues are harvested and may be dissociated using dissociation enzymes according to the manufacturer's instructions. Cells are stained for analysis by flow cytometry using techniques known in the art. Staining antibodies can include anti-CD11c (dendritic cells), anti-CD80, anti-CD86, anti-CD40, anti-MHCII, anti-CD8a, anti-CD4, and anti-CD103. Other markers that may be analyzed include pan-immune cell marker CD45, T cell markers (CD3, CD4, CD8, CD25, Foxp3, T-bet, Gata3, Roryt, Granzyme B, CD69, PD-1, CTLA-4), and macrophage/myeloid markers (CD11b, MHCII, CD206, CD40, CSF1R, PD-L1, Gr-1, F4/80). In addition to immunophenotyping, serum cytokines can be analyzed including, but not limited to, TNFa, IL-17, IL-13, IL-12p70, IL12p40, IL-10, IL-6, IL-5, IL-4, IL-2, IL-1b, IFNy, GM-CSF, G-CSF, M-CSF, MIG, IP10, MIP1b, RANTES, and MCP-1. Cytokine analysis may be carried out on immune cells obtained from lymph nodes or other tissue, and/or on purified CD45+ GI tract-infiltrated immune cells obtained ex vivo. Finally, immunohistochemistry is carried out on various tissue sections to measure T cells, macrophages, dendritic cells, and checkpoint molecule protein expression.

**[648]** In order to examine the impact and longevity of disease protection, rather than being sacrificed, some mice may be rechallenged with a disease trigger. Mice are analyzed for susceptibility to colitis severity following rechallenge.

**Example 34: smEVs in a mouse model of Type 1 Diabetes (T1D)**

**[649]** Type 1 diabetes (T1D) is an autoimmune disease in which the immune system targets the islets of Langerhans of the pancreas, thereby destroying the body's ability to produce insulin.

**[650]** There are various models of animal models of T1D, as reviewed by Belle et al. (Mouse models for type 1 diabetes. Drug Discov Today Dis Models. 2009; 6(2): 41-45; see also Aileen JF King. The use of animal models in diabetes research. Br J Pharmacol. 2012 Jun; 166(3): 877-894. There are models for chemically-induced T1D, pathogen-induced T1D, as well as models in which the mice spontaneously develop T1D.

**[651]** smEVs are tested for their efficacy in a mouse model of T1D, either alone or in combination with whole bacterial cells, with or without the addition of other anti-inflammatory treatments.

**[652]** Depending on the method of T1D induction and/or whether T1D development is spontaneous, treatment with smEVs is initiated at some point, either around the time of induction or following induction, or prior to the onset (or upon the onset) of spontaneously-occurring T1D. smEVs are administered at varied doses and at defined intervals. For example, some mice are intravenously injected with smEVs at 10, 15, or 20 ug/mouse. Other mice may receive 25, 50, or 100 mg of smEVs per mouse. Alternatively, some mice receive between 7.0e+09 to 3.0e+12 smEV particles per dose. While some mice receive smEVs through oral gavage or i.v. injection, while other groups of mice may receive smEVs through intraperitoneal (i.p.) injection, subcutaneous (s.c.) injection, nasal route administration, or other means of administration. Some mice may receive smEVs every day, while others may receive smEVs at alternative intervals (e.g., every other day, or once every three days). Groups of mice may be administered a pharmaceutical composition of the invention comprising a mixture of smEVs and bacterial cells. For example, the composition may comprise smEV particles and whole bacteria in a ratio from 1:1 (smEVs: bacterial cells) to $1\text{-}1x10^{12}$:1 (smEVs: bacterial cells).

**[653]** Alternatively, some groups of mice may receive between $1x10^4$ and $5x10^9$ bacterial cells in an administration separate from, or comingled with, the smEV administration. As with the smEVs, bacterial cell administration may be varied by route of administration, dose, and schedule. The bacterial cells may be live, dead, or weakened. The bacterial cells may be

harvested fresh (or frozen) and administered, or they may be irradiated or heat-killed prior to administration with the smEVs.

**[654]** Some groups of mice may be treated with additional treatments and/or an appropriate control (e.g., vehicle or control antibody) at various timepoints and at effective doses.

**[655]** In addition, some mice are treated with antibiotics prior to treatment. For example, vancomycin (0.5g/L), ampicillin (1.0g/L), gentamicin (1.0g/L) and amphotericin B (0.2g/L) are added to the drinking water, and antibiotic treatment is halted at the time of treatment or a few days prior to treatment. Some immunized mice are treated without receiving antibiotics.

**[656]** Blood glucose is monitored biweekly prior to the start of the experiment. At various timepoints thereafter, nonfasting blood glucose is measured. At various timepoints, mice are sacrificed and site the pancreas, lymph nodes, or other tissues may be removed for ex vivo histological, cytokine and/or flow cytometric analysis using methods known in the art. For example, tissues are dissociated using dissociation enzymes according to the manufacturer's instructions. Cells are stained for analysis by flow cytometry using techniques known in the art. Staining antibodies can include anti-CD11c (dendritic cells), anti-CD80, anti-CD86, anti-CD40, anti-MHCII, anti-CD8a, anti-CD4, and anti-CD103. Other markers that may be analyzed include pan-immune cell marker CD45, T cell markers (CD3, CD4, CD8, CD25, Foxp3, T-bet, Gata3, Roryt, Granzyme B, CD69, PD-1, CTLA-4), and macrophage/myeloid markers (CD11b, MHCII, CD206, CD40, CSF1R, PD-L1, Gr-1, F4/80). In addition to immunophenotyping, serum cytokines can be analyzed including, but not limited to, TNFa, IL-17, IL-13, IL-12p70, IL12p40, IL-10, IL-6, IL-5, IL-4, IL-2, IL-1b, IFNy, GM-CSF, G-CSF, M-CSF, MIG, IP10, MIP1b, RANTES, and MCP-1. Cytokine analysis may be carried out on immune cells obtained from lymph nodes or other tissue, and/or on purified tissue-infiltrating immune cells obtained ex vivo. Finally, immunohistochemistry is carried out on various tissue sections to measure T cells, macrophages, dendritic cells, and checkpoint molecule protein expression. Antibody production may also be assessed by ELISA.

**[657]** In order to examine the impact and longevity of disease protection, rather than being sacrificed, some mice may be rechallenged with a disease trigger, or assessed for

susceptibility to relapse. Mice are analyzed for susceptibility to diabetes onset and severity following rechallenge (or spontaneously-occurring relapse).

**Example 35: smEVs in a mouse model of Primary Sclerosing Cholangitis (PSC)**

**[658]** Primary Sclerosing Cholangitis (PSC) is a chronic liver disease that slowly damages the bile ducts and leads to end-stage cirrhosis. It is associated with inflammatory bowel disease (IBD).

**[659]** There are various animal models for PSC, as reviewed by Fickert et al. (Characterization of animal models for primary sclerosing cholangitis (PSC). J Hepatol. 2014 Jun. 60(6): 1290-1303; see also Pollheimer and Fickert. Animal models in primary biliary cirrhosis and primary sclerosing cholangitis. Clin Rev Allergy Immunol. 2015 Jun. 48(2-3): 207-17). Induction of disease in PSC models includes chemical induction (e.g., 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC)-induced cholangitis), pathogen-induced (e.g., Cryptosporidium parvum), experimental biliary obstruction (e.g., common bile duct ligation (CBDL)), and transgenic mouse model of antigen-driven biliary injury (e.g., Ova-Bil transgenic mice). For example, bile duct ligation is performed as described by Georgiev et al. (Characterization of time-related changes after experimental bile duct ligation. Br J Surg. 2008. 95(5): 646-56), or disease is induced by DCC exposure as described by Fickert et al. (A new xenobiotic-induced mouse model of sclerosing cholangitis and biliary fibrosis. Am J Path. Vol 171(2): 525-536.

**[660]** smEVs are tested for their efficacy in a mouse model of PSC, either alone or in combination with whole bacterial cells, with or without the addition of some other therapeutic agent.

*DCC-induced Cholangitis*

**[661]** For example, 6-8 week old C57bl/6 mice are obtained from Taconic or other vendor. Mice are fed a 0.1% DCC-supplemented diet for various durations. Some groups receive DCC-supplement food for 1 week, others for 4 weeks, others for 8 weeks. Some groups of mice may receive a DCC-supplemented diet for a length of time and then be allowed to recover, thereafter receiving a normal diet. These mice may be studied for their ability to recover from disease and/or their susceptibility to relapse upon subsequent exposure to DCC. Treatment with smEVs is initiated at some point, either around the time of DCC-feeding or subsequent to initial exposure to DCC. For example, smEVs may be administered on day 1, or they may be

administered sometime thereafter. smEVs are administered at varied doses and at defined intervals. For example, some mice are intravenously injected with smEVs at 10, 15, or 20 ug/mouse. Alternatively, some mice may receive between 7.0e+09 and 3.0e+12 smEV particles. While some mice receive smEVs through oral gavage or i.v. injection, while other groups of mice may receive smEVs through intraperitoneal (i.p.) injection, subcutaneous (s.c.) injection, nasal route administration, or other means of administration. Some mice may receive smEVs every day (e.g., starting on day 1), while others may receive smEVs at alternative intervals (e.g., every other day, or once every three days). Groups of mice may be administered a pharmaceutical composition of the invention comprising a mixture of smEVs and bacterial cells. For example, the composition may comprise smEV particles and whole bacteria in a ratio from 1:1 (smEVs: bacterial cells) to 1-1x$10^{12}$:1 (smEVs: bacterial cells).

**[662]** Alternatively, some groups of mice may receive between 1x$10^{4}$ and 5x$10^{9}$ bacterial cells in an administration separate from, or comingled with, the smEV administration. As with the smEVs, bacterial cell administration may be varied by route of administration, dose, and schedule. The bacterial cells may be live, dead, or weakened. The bacterial cells may be harvested fresh (or frozen) and administered, or they may be irradiated or heat-killed prior to administration with the smEVs.

**[663]** Some groups of mice may be treated with additional agents and/or an appropriate control (e.g., vehicle or antibody) at various timepoints and at effective doses.

**[664]** In addition, some mice are treated with antibiotics prior to treatment. For example, vancomycin (0.5g/L), ampicillin (1.0g/L), gentamicin (1.0g/L) and amphotericin B (0.2g/L) are added to the drinking water, and antibiotic treatment is halted at the time of treatment or a few days prior to treatment. Some immunized mice are treated without receiving antibiotics. At various timepoints, serum samples are analyzed for ALT, AP, bilirubin, and serum bile acid (BA) levels.

**[665]** At various timepoints, mice are sacrificed, body and liver weight are recorded, and sites of inflammation (e.g., liver, small and large intestine, spleen), lymph nodes, or other tissues may be removed for ex vivo histolomorphological characterization, cytokine and/or flow cytometric analysis using methods known in the art (see Fickert et al. Characterization of animal models for primary sclerosing cholangitis (PSC)). J Hepatol. 2014. 60(6): 1290-1303). For example, bile ducts are stained for expression of ICAM-1, VCAM-1, MadCAM-1. Some tissues

are stained for histological examination, while others are dissociated using dissociation enzymes according to the manufacturer's instructions. Cells are stained for analysis by flow cytometry using techniques known in the art. Staining antibodies can include anti-CD11c (dendritic cells), anti-CD80, anti-CD86, anti-CD40, anti-MHCII, anti-CD8a, anti-CD4, and anti-CD103. Other markers that may be analyzed include pan-immune cell marker CD45, T cell markers (CD3, CD4, CD8, CD25, Foxp3, T-bet, Gata3, Roryt, Granzyme B, CD69, PD-1, CTLA-4), and macrophage/myeloid markers (CD11b, MHCII, CD206, CD40, CSF1R, PD-L1, Gr-1, F4/80), as well as adhesion molecule expression (ICAM-1, VCAM-1, MadCAM-1). In addition to immunophenotyping, serum cytokines can be analyzed including, but not limited to, TNFa, IL-17, IL-13, IL-12p70, IL12p40, IL-10, IL-6, IL-5, IL-4, IL-2, IL-1b, IFNy, GM-CSF, G-CSF, M-CSF, MIG, IP10, MIP1b, RANTES, and MCP-1. Cytokine analysis may be carried out on immune cells obtained from lymph nodes or other tissue, and/or on purified CD45+ bile duct-infiltrated immune cells obtained ex vivo.

**[666]** Liver tissue is prepared for histological analysis, for example, using Sirius-red staining followed by quantification of the fibrotic area. At the end of the treatment, blood is collected for plasma analysis of liver enzymes, for example, AST or ALT, and to determine Bilirubin levels. The hepatic content of Hydroxyproline can be measured using established protocols. Hepatic gene expression analysis of inflammation and fibrosis markers may be performed by qRT-PCR using validated primers. These markers may include, but are not limited to, MCP-1, alpha-SMA, Coll1a1, and TIMP-. Metabolite measurements may be performed in plasma, tissue and fecal samples using established metabolomics methods. Finally, immunohistochemistry is carried out on liver sections to measure neutrophils, T cells, macrophages, dendritic cells, or other immune cell infiltrates.

**[667]** In order to examine the impact and longevity of disease protection, rather than being sacrificed, some mice may be rechallenged with DCC at a later time. Mice are analyzed for susceptibility to cholangitis and cholangitis severity following rechallenge.

*BDL-induced Cholangitis*

**[668]** Alternatively, smEVs are tested for their efficacy in BDL-induced cholangitis. For example, 6-8 week old C57Bl/6J mice are obtained from Taconic or other vendor. After an acclimation period the mice are subjected to a surgical procedure to perform a bile duct ligation

(BDL). Some control animals receive a sham surgery. The BDL procedure leads to liver injury, inflammation and fibrosis within 7-21 days.

**[669]** Treatment with smEVs is initiated at some point, either around the time of surgery or some time following the surgery. smEVs are administered at varied doses and at defined intervals. For example, some mice are intravenously injected with smEVs at 10, 15, or 20 ug/mouse. Other mice may receive 25, 50, or 100 mg of smEVs per mouse. Alternatively, some mice receive between 7.0e+09 to 3.0e+12 smEV particles per dose. While some mice receive smEVs through oral gavage or i.v. injection, while other groups of mice may receive smEVs through intraperitoneal (i.p.) injection, subcutaneous (s.c.) injection, nasal route administration, or other means of administration. Some mice receive smEVs every day (e.g., starting on day 1), while others may receive smEVs at alternative intervals (e.g., every other day, or once every three days). Groups of mice may be administered a pharmaceutical composition of the invention comprising a mixture of smEVs and bacterial cells. For example, the composition may comprise smEV particles and whole bacteria in a ratio from 1:1 (smEVs: bacterial cells) to $1\text{-}1x10^{12}$:1 (smEVs: bacterial cells).

**[670]** Alternatively, some groups of mice may receive between $1x10^4$ and $5x10^9$ bacterial cells in an administration separate from, or comingled with, the smEV administration. As with the smEVs, bacterial cell administration may be varied by route of administration, dose, and schedule. The bacterial cells may be live, dead, or weakened. The bacterial cells may be harvested fresh (or frozen) and administered, or they may be irradiated or heat-killed prior to administration with the smEVs.

**[671]** Some groups of mice may be treated with additional agents and/or an appropriate control (e.g., vehicle or antibody) at various timepoints and at effective doses.

**[672]** In addition, some mice are treated with antibiotics prior to treatment. For example, vancomycin (0.5g/L), ampicillin (1.0g/L), gentamicin (1.0g/L) and amphotericin B (0.2g/L) are added to the drinking water, and antibiotic treatment is halted at the time of treatment or a few days prior to treatment. Some immunized mice are treated without receiving antibiotics. At various timepoints, serum samples are analyzed for ALT, AP, bilirubin, and serum bile acid (BA) levels.

**[673]** At various timepoints, mice are sacrificed, body and liver weight are recorded, and sites of inflammation (e.g., liver, small and large intestine, spleen), lymph nodes, or other

tissues may be removed for ex vivo histolomorphological characterization, cytokine and/or flow cytometric analysis using methods known in the art (see Fickert et al. Characterization of animal models for primary sclerosing cholangitis (PSC)). J Hepatol. 2014. 60(6): 1290-1303). For example, bile ducts are stained for expression of ICAM-1, VCAM-1, MadCAM-1. Some tissues are stained for histological examination, while others are dissociated using dissociation enzymes according to the manufacturer's instructions. Cells are stained for analysis by flow cytometry using techniques known in the art. Staining antibodies can include anti-CD11c (dendritic cells), anti-CD80, anti-CD86, anti-CD40, anti-MHCII, anti-CD8a, anti-CD4, and anti-CD103. Other markers that may be analyzed include pan-immune cell marker CD45, T cell markers (CD3, CD4, CD8, CD25, Foxp3, T-bet, Gata3, Roryt, Granzyme B, CD69, PD-1, CTLA-4), and macrophage/myeloid markers (CD11b, MHCII, CD206, CD40, CSF1R, PD-L1, Gr-1, F4/80), as well as adhesion molecule expression (ICAM-1, VCAM-1, MadCAM-1). In addition to immunophenotyping, serum cytokines can be analyzed including, but not limited to, TNFa, IL-17, IL-13, IL-12p70, IL12p40, IL-10, IL-6, IL-5, IL-4, IL-2, IL-1b, IFNy, GM-CSF, G-CSF, M-CSF, MIG, IP10, MIP1b, RANTES, and MCP-1. Cytokine analysis may be carried out on immune cells obtained from lymph nodes or other tissue, and/or on purified CD45+ bile duct-infiltrated immune cells obtained ex vivo.

**[674]** Liver tissue is prepared for histological analysis, for example, using Sirius-red staining followed by quantification of the fibrotic area. At the end of the treatment, blood is collected for plasma analysis of liver enzymes, for example, AST or ALT, and to determine Bilirubin levels. The hepatic content of Hydroxyproline can be measured using established protocols. Hepatic gene expression analysis of inflammation and fibrosis markers may be performed by qRT-PCR using validated primers. These markers may include, but are not limited to, MCP-1, alpha-SMA, Coll1a1, and TIMP. Metabolite measurements may be performed in plasma, tissue and fecal samples using established metabolomics methods. Finally, immunohistochemistry is carried out on liver sections to measure neutrophils, T cells, macrophages, dendritic cells, or other immune cell infiltrates.

**[675]** In order to examine the impact and longevity of disease protection, rather than being sacrificed, some mice may be analyzed for recovery.

**Example 36: smEVs in a mouse model of Nonalcoholic Steatohepatitis (NASH)**

**[676]** Nonalcoholic Steatohepatitis (NASH) is a severe form of Nonalcoholic Fatty Liver Disease (NAFLD), where buildup of hepatic fat (steatosis) and inflammation lead to liver injury and hepatocyte cell death (ballooning).

**[677]** There are various animal models of NASH, as reviewed by Ibrahim et al. (Animal models of nonalcoholic steatohepatitis: Eat, Delete, and Inflame. Dig Dis Sci. 2016 May. 61(5): 1325-1336; see also Lau et al. Animal models of non-alcoholic fatty liver disease: current perspectives and recent advances 2017 Jan. 241(1): 36-44).

**[678]** smEVs are tested for their efficacy in a mouse model of NASH, either alone or in combination with whole bacterial cells, with or without the addition of another therapeutic agent. For example, 8-10 week old C57Bl/6J mice, obtained from Taconic (Germantown, NY), or other vendor, are placed on a methionine choline deficient (MCD) diet for a period of 4-8 weeks during which NASH features develop, including steatosis, inflammation, ballooning and fibrosis.

**[679]** *P. histicola*-derived smEVs are tested for their efficacy in a mouse model of NASH, either alone or in combination with each other, in varying proportions, with or without the addition of another therapeutic agent. For example, 8 week old C57Bl/6J mice, obtained from Charles River (France), or other vendor, are acclimated for a period of 5 days, randomized intro groups of 10 mice based on body weight, and placed on a methionine choline deficient (MCD) diet for example A02082002B from Research Diets (USA), for a period of 4 weeks during which NASH features developed, including steatosis, inflammation, ballooning and fibrosis. Control chow mice are fed a normal chow diet, for example RM1 (E) 801492 from SDS Diets (UK). Control chow, MCD diet, and water are provided *ad libitum*.

**[680]** An NAS scoring system adapted from Kleiner et al. (Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology. 2005 Jun. 41(6): 1313-1321) is used to determine the degree of steatosis (scored 0-3), lobular inflammation (scored 0-3), hepatocyte ballooning (scored 0-3), and fibrosis (scored 0-4). An individual mouse NAS score may be calculated by summing the score for steatosis, inflammation, ballooning, and fibrosis (scored 0-13). In addition, the levels of plasma AST and ALT are determined using a Pentra 400 instrument from Horiba (USA), according to manufacturer's instructions. The levels of hepatic total cholesterol, triglycerides, fatty acids, alanine aminotransferase, and aspartate aminotransferase are also determined using methods known in the art.

**[681]** In other studies, hepatic gene expression analysis of inflammation, fibrosis, steatosis, ER stress, or oxidative stress markers may be performed by qRT-PCR using validated primers. These markers may include, but are not limited to, IL-1β, TNF-α, MCP-1, α-SMA, Coll1a1, CHOP, and NRF2.

**[682]** Treatment with smEVs is initiated at some point, either at the beginning of the diet, or at some point following diet initiation (for example, one week after). For example, smEVs may be administered starting in the same day as the initiation of the MCD diet. smEVs are administered at varied doses and at defined intervals. For example, some mice are intravenously injected with smEVs at 10, 15, or 20 ug/mouse. Other mice may receive 25, 50, or 100 mg of smEVs per mouse. Alternatively, some mice receive between 7.0e+09 to 3.0e+12 smEV particles per dose. While some mice receive smEVs through oral gavage or i.v. injection, while other groups of mice may receive smEVs through intraperitoneal (i.p.) injection, subcutaneous (s.c.) injection, nasal route administration, or other means of administration. Some mice may receive smEVs every day (e.g., starting on day 1), while others may receive smEVs at alternative intervals (e.g., every other day, or once every three days). Groups of mice may be administered a pharmaceutical composition of the invention comprising a mixture of smEVs and bacterial cells. For example, the composition may comprise smEV particles and whole bacteria in a ratio from 1:1 (smEVs: bacterial cells) to 1-1x$10^{12}$:1 (smEVs: bacterial cells).

**[683]** Alternatively, some groups of mice may receive between 1x$10^{4}$ and 5x$10^{9}$ bacterial cells in an administration separate from, or comingled with, the smEV administration. As with the smEVs, bacterial cell administration may be varied by route of administration, dose, and schedule. The bacterial cells may be live, dead, or weakened. The bacterial cells may be harvested fresh (or frozen) and administered, or they may be irradiated or heat-killed prior to administration with the smEVs.

**[684]** Some groups of mice may be treated with additional NASH therapeutic(s) (e.g., FXR agonists, PPAR agonists, CCR2/5 antagonists or other treatment) and/or appropriate control at various timepoints and effective doses.

**[685]** At various timepoints and/or at the end of the treatment, mice are sacrificed and liver, intestine, blood, feces, or other tissues may be removed for ex vivo histological, biochemical, molecular or cytokine and/or flow cytometry analysis using methods known in the art. For example, liver tissues are weighed and prepared for histological analysis, which may

comprise staining with H&E, Sirius Red, and determination of NASH activity score (NAS). At various timepoints, blood is collected for plasma analysis of liver enzymes, for example, AST or ALT, using standards assays. In addition, the hepatic content of cholesterol, triglycerides, or fatty acid acids can be measured using established protocols. Hepatic gene expression analysis of inflammation, fibrosis, steatosis, ER stress, or oxidative stress markers may be performed by qRT-PCR using validated primers. These markers may include, but are not limited to, IL-6, MCP-1, alpha-SMA, Coll1a1, CHOP, and NRF2. Metabolite measurements may be performed in plasma, tissue and fecal samples using established biochemical and mass-spectrometry-based metabolomics methods. Serum cytokines can be analyzed including, but not limited to, TNFa, IL-17, IL-13, IL-12p70, IL12p40, IL-10, IL-6, IL-5, IL-4, IL-2, IL-1b, IFNy, GM-CSF, G-CSF, M-CSF, MIG, IP10, MIP1b, RANTES, and MCP-1. Cytokine analysis may be carried out on immune cells obtained from lymph nodes or other tissue, and/or on purified CD45+ bile duct-infiltrated immune cells obtained ex vivo. Finally, immunohistochemistry is carried out on liver or intestine sections to measure neutrophils, T cells, macrophages, dendritic cells, or other immune cell infiltrates.

**[686]** In order to examine the impact and longevity of disease protection, rather than being sacrificed, some mice may be analyzed for recovery.

**Example 37: smEVs in a mouse model of psoriasis**

**[687]** Psoriasis is a T-cell-mediated chronic inflammatory skin disease. So-called "plaque-type" psoriasis is the most common form of psoriasis and is typified by dry scales, red plaques, and thickening of the skin due to infiltration of immune cells into the dermis and epidermis. Several animal models have contributed to the understanding of this disease, as reviewed by Gudjonsson et al. (Mouse models of psoriasis. J Invest Derm. 2007. 127: 1292-1308; see also van der Fits et al. Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis. J. Immunol. 2009 May 1. 182(9): 5836-45).

**[688]** Psoriasis can be induced in a variety of mouse models, including those that use transgenic, knockout, or xenograft models, as well as topical application of imiquimod (IMQ), a TLR7/8 ligand.

**[689]** smEVs are tested for their efficacy in the mouse model of psoriasis, either alone or in combination with whole bacterial cells, with or without the addition of other anti-

inflammatory treatments. For example, 6-8 week old C57Bl/6 or Balb/c mice are obtained from Taconic (Germantown, NY), or other vendor. Mice are shaved on the back and the right ear. Groups of mice receive a daily topical dose of 62.5 mg of commercially available IMQ cream (5%) (Aldara; 3M Pharmaceuticals). The dose is applied to the shaved areas for 5 or 6 consecutive days. At regular intervals, mice are scored for erythema, scaling, and thickening on a scale from 0 to 4, as described by van der Fits et al. (2009). Mice are monitored for ear thickness using a Mitutoyo micrometer.

**[690]** Treatment with smEVs is initiated at some point, either around the time of the first application of IMQ, or something thereafter. For example, smEVs may be administered at the same time as the subcutaneous injections (day 0), or they may be administered prior to, or upon, application. smEVs are administered at varied doses and at defined intervals. For example, some mice are intravenously injected with smEVs at 10, 15, or 20 ug/mouse. Other mice may receive 25, 50, or 100 mg of smEVs per mouse. Alternatively, some mice receive between 7.0e+09 to 3.0e+12 smEV particles per dose. While some mice receive smEVs through oral gavage or i.v. injection, while other groups of mice may receive smEVs through intraperitoneal (i.p.) injection, subcutaneous (s.c.) injection, nasal route administration, or other means of administration. Some mice may receive smEVs every day (e.g., starting on day 0), while others may receive smEVs at alternative intervals (e.g., every other day, or once every three days). Groups of mice may be administered a pharmaceutical composition of the invention comprising a mixture of smEVs and bacterial cells. For example, the composition may comprise smEV particles and whole bacteria in a ratio from 1:1 (smEVs: bacterial cells) to $1\text{-}1x10^{12}$:1 (smEVs: bacterial cells).

**[691]** Alternatively, some groups of mice may receive between $1x10^4$ and $5x10^9$ bacterial cells in an administration separate from, or comingled with, the smEV administration. As with the smEVs, bacterial cell administration may be varied by route of administration, dose, and schedule. The bacterial cells may be live, dead, or weakened. The bacterial cells may be harvested fresh (or frozen) and administered, or they may be irradiated or heat-killed prior to administration with the smEVs.

**[692]** Some groups of mice may be treated with anti-inflammatory agent(s) (e.g., anti-CD154, blockade of members of the TNF family, or other treatment), and/or an appropriate control (e.g., vehicle or control antibody) at various timepoints and at effective doses.

**[693]** In addition, some mice are treated with antibiotics prior to treatment. For example, vancomycin (0.5g/L), ampicillin (1.0g/L), gentamicin (1.0g/L) and amphotericin B (0.2g/L) are added to the drinking water, and antibiotic treatment is halted at the time of treatment or a few days prior to treatment. Some immunized mice are treated without receiving antibiotics.

**[694]** At various timepoints, samples from back and ear skin are taken for cryosection staining analysis using methods known in the art. Other groups of mice are sacrificed and lymph nodes, spleen, mesenteric lymph nodes (MLN), the small intestine, colon, and other tissues may be removed for histology studies, ex vivo histological, cytokine and/or flow cytometric analysis using methods known in the art. Some tissues may be dissociated using dissociation enzymes according to the manufacturer's instructions. Cryosection samples, tissue samples, or cells obtained ex vivo are stained for analysis by flow cytometry using techniques known in the art. Staining antibodies can include anti-CD11c (dendritic cells), anti-CD80, anti-CD86, anti-CD40, anti-MHCII, anti-CD8a, anti-CD4, and anti-CD103. Other markers that may be analyzed include pan-immune cell marker CD45, T cell markers (CD3, CD4, CD8, CD25, Foxp3, T-bet, Gata3, Roryt, Granzyme B, CD69, PD-1, CTLA-4), and macrophage/myeloid markers (CD11b, MHCII, CD206, CD40, CSF1R, PD-L1, Gr-1, F4/80). In addition to immunophenotyping, serum cytokines can be analyzed including, but not limited to, TNFa, IL-17, IL-13, IL-12p70, IL12p40, IL-10, IL-6, IL-5, IL-4, IL-2, IL-1b, IFNy, GM-CSF, G-CSF, M-CSF, MIG, IP10, MIP1b, RANTES, and MCP-1. Cytokine analysis may be carried out on immune cells obtained from lymph nodes or other tissue, and/or on purified CD45+ skin-infiltrated immune cells obtained ex vivo. Finally, immunohistochemistry is carried out on various tissue sections to measure T cells, macrophages, dendritic cells, and checkpoint molecule protein expression.

**[695]** In order to examine the impact and longevity of psoriasis protection, rather than being sacrificed, some mice may be studied to assess recovery, or they may be rechallenged with IMQ. The groups of rechallenged mice are analyzed for susceptibility to psoriasis and severity of response.

**Example 38: smEVs in a mouse model of obesity (DIO)**

**[696]** There are various animal models of DIO, as reviewed by Tschop et al. (A guide to analysis of mouse energy metabolism. Nat. Methods. 2012; 9(1):57-63) and Ayala et al.

(Standard operating procedures for describing and performing metabolic tests of glucose homeostasis in mice. Disease Models and Mechanisms. 2010; 3:525-534) and provided by Physiogenex.

**[697]** smEVs are tested for their efficacy in a mouse model of DIO, either alone or in combination with other whole bacterial cells (live, killed, irradiated, and/or inactivated, etc) with or without the addition of other anti-inflammatory treatments.

**[698]** Depending on the method of DIO induction and/or whether DIO development is spontaneous, treatment with smEVs is initiated at some point, either around the time of induction or following induction, or prior to the onset (or upon the onset) of spontaneously-occurring T1D. smEVs are administered at varied doses and at defined intervals. For example, some mice are intravenously injected with smEVs at 10, 15, or 20 ug/mouse. Other mice may receive 25, 50, or 100 mg of smEVs per mouse. Alternatively, some mice receive between 7.0e+09 to 3.0e+12 smEV particles per dose. While some mice receive smEVs through i.v. injection, other mice may receive smEVs through intraperitoneal (i.p.) injection, subcutaneous (s.c.) injection, nasal route administration, oral gavage, or other means of administration. Some mice may receive smEVs every day, while others may receive smEVs at alternative intervals (*e.g.,* every other day, or once every three days). Groups of mice may be administered a pharmaceutical composition of the invention comprising a mixture of smEVs and bacterial cells. For example, the composition may comprise smEV particles and whole bacteria in a ratio from 1:1 (smEVs: bacterial cells) to 1-$1x10^{12}$:1 (smEVs: bacterial cells).

**[699]** Alternatively, some groups of mice may receive between $1x10^4$ and $5x10^9$ bacterial cells in an administration separate from, or comingled with, the smEV administration. As with the smEVs, bacterial cell administration may be varied by route of administration, dose, and schedule. The bacterial cells may be live, dead, or weakened. The bacterial cells may be harvested fresh (or frozen) and administered, or they may be irradiated or heat-killed prior to administration with the smEVs.

**[700]** Some groups of mice may be treated with additional treatments and/or an appropriate control (*e.g.,* vehicle or control antibody) at various timepoints and at effective doses.

**[701]** In addition, some mice are treated with antibiotics prior to treatment. For example, vancomycin (0.5g/L), ampicillin (1.0g/L), gentamicin (1.0g/L) and amphotericin B

(0.2g/L) are added to the drinking water, and antibiotic treatment is halted at the time of treatment or a few days prior to treatment. Some immunized mice are treated without receiving antibiotics.

**[702]** Blood glucose is monitored biweekly prior to the start of the experiment. At various timepoints thereafter, nonfasting blood glucose is measured. At various timepoints, mice are sacrificed and site the pancreas, lymph nodes, or other tissues may be removed for ex vivo histological, cytokine and/or flow cytometric analysis using methods known in the art. For example, tissues are dissociated using dissociation enzymes according to the manufacturer's instructions. Cells are stained for analysis by flow cytometry using techniques known in the art. Staining antibodies can include anti-CD11c (dendritic cells), anti-CD80, anti-CD86, anti-CD40, anti-MHCII, anti-CD8a, anti-CD4, and anti-CD103. Other markers that may be analyzed include pan-immune cell marker CD45, T cell markers (CD3, CD4, CD8, CD25, Foxp3, T-bet, Gata3, Roryt, Granzyme B, CD69, PD-1, CTLA-4), and macrophage/myeloid markers (CD11b, MHCII, CD206, CD40, CSF1R, PD-L1, Gr-1, F4/80). In addition to immunophenotyping, serum cytokines can be analyzed including, but not limited to, TNFa, IL-17, IL-13, IL-12p70, IL12p40, IL-10, IL-6, IL-5, IL-4, IL-2, IL-1b, IFNy, GM-CSF, G-CSF, M-CSF, MIG, IP10, MIP1b, RANTES, and MCP-1. Cytokine analysis may be carried out on immune cells obtained from lymph nodes or other tissue, and/or on purified tissue-infiltrating immune cells obtained ex vivo. Finally, immunohistochemistry is carried out on various tissue sections to measure T cells, macrophages, dendritic cells, and checkpoint molecule protein expression. Antibody production may also be assessed by ELISA.

**[703]** In order to examine the impact and longevity of disease protection, rather than being sacrificed, some mice may be rechallenged with a disease trigger, or assessed for susceptibility to relapse. Mice are analyzed for susceptibility to diabetes onset and severity following rechallenge (or spontaneously-occurring relapse).

**Example 39: Labeling bacterial smEVs**

**[704]** smEVs may be labeled in order to track their biodistribution *in vivo* and to quantify and track cellular localization in various preparations and in assays conducted with mammalian cells. For example, smEVs may be radio-labeled, incubated with dyes, fluorescently

labeled, luminescently labeled, or labeled with conjugates containing metals and isotopes of metals.

**[705]** For example, smEVs may be incubated with dyes conjugated to functional groups such as NHS-ester, click-chemistry groups, streptavidin or biotin. The labeling reaction may occur at a variety of temperatures for minutes or hours, and with or without agitation or rotation. The reaction may then be stopped by adding a reagent such as bovine serum albumin (BSA), or similar agent, depending on the protocol, and free or unbound dye molecule removed by ultra-centrifugation, filtration, centrifugal filtration, column affinity purification or dialysis. Additional washing steps involving wash buffers and vortexing or agitation may be employed to ensure complete removal of free dyes molecules such as described in Su Chul Jang et al, Small. 11, No.4, 456-461(2017).

**[706]** Fluorescently labeled smEVs are detected in cells or organs, or in *in vitro* and/or *ex vivo* samples by confocal microscopy, nanoparticle tracking analysis, flow cytometry, fluorescence activated cell sorting (FACs) or fluorescent imaging system such as the Odyssey CLx LICOR (see e.g., Wiklander et al. 2015. J. Extracellular Vesicles. 4:10.3402/jev.v4.26316). Additionally, fluorescently labeled smEVs are detected in whole animals and/or dissected organs and tissues using an instrument such as the IVIS spectrum CT (Perkin Elmer) or Pearl Imager, as in H-I. Choi, et al. *Experimental & Molecular Medicine*. 49: e330 (2017).

**[707]** smEVs may be labeled with conjugates containing metals and isotopes of metals using the protocols described above. Metal-conjugated smEVs may be administered *in vivo* to animals. Cells may then be harvested from organs at various time-points, and analyzed *ex vivo*. Alternatively, cells derived from animals, humans, or immortalized cell lines may be treated with metal-labelled smEVs *in vitro* and cells subsequently labelled with metal-conjugated antibodies and phenotyped using a Cytometry by Time of Flight (CyTOF) instrument such as the Helios CyTOF (Fluidigm) or imaged and analyzed using and Imaging Mass Cytometry instrument such as the Hyperion Imaging System (Fluidigm). Additionally, smEVs may be labelled with a radioisotope to track the smEVs biodistribution (see, *e.g.*, Miller et al., Nanoscale. 2014 May 7;6(9):4928-35).

**Example 40: Transmission electron microscopy to visualize purified bacterial smEVs**

**[708]** smEVs are purified from bacteria batch cultures. Transmission electron microscopy (TEM) may be used to visualize purified bacterial smEVs (S. Bin Park, et al. PLoS ONE. 6(3):e17629 (2011). smEVs are mounted onto 300- or 400-mesh-size carbon-coated copper grids (Electron Microscopy Sciences, USA) for 2 minutes and washed with deionized water. smEVs are negatively stained using 2% (w/v) uranyl acetate for 20 sec – 1 min. Copper grids are washed with sterile water and dried. Images are acquired using a transmission electron microscope with 100-120 kV acceleration voltage. Stained smEVs appear between 20-600 nm in diameter and are electron dense. 10-50 fields on each grid are screened.

**Example 41: Profiling smEV composition and content**

**[709]** smEVs may be characterized by any one of various methods including, but not limited to, NanoSight characterization, SDS-PAGE gel electrophoresis, Western blot, ELISA, liquid chromatography-mass spectrometry and mass spectrometry, dynamic light scattering, lipid levels, total protein, lipid to protein ratios, nucleic acid analysis and/or zeta potential.

*NanoSight Characterization of smEVs*

**[710]** Nanoparticle tracking analysis (NTA) is used to characterize the size distribution of purified smEVs. Purified smEV preparations are run on a NanoSight machine (Malvern Instruments) to assess smEV size and concentration.

*SDS-PAGE Gel Electrophoresis*

**[711]** To identify the protein components of purified smEVs, samples are run on a gel, for example a Bolt Bis-Tris Plus 4-12% gel (Thermo-Fisher Scientific), using standard techniques. Samples are boiled in 1x SDS sample buffer for 10 minutes, cooled to 4°C, and then centrifuged at 16,000 x g for 1 min. Samples are then run on a SDS-PAGE gel and stained using one of several standard techniques (e.g., Silver staining, Coomassie Blue, Gel Code Blue) for visualization of bands.

*Western blot analysis*

**[712]** To identify and quantify specific protein components of purified smEVs, smEV proteins are separated by SDS-PAGE as described above and subjected to Western blot analysis (Cvjetkovic et al., *Sci. Rep.* **6**, 36338 (2016)) and are quantified via ELISA.

*smEV proteomics and Liquid Chromatography-Mass Spectrometry (LC-MS/MS) and Mass Spectrometry (MS)*

**[713]** Proteins present in smEVs are identified and quantified by Mass Spectrometry techniques. smEV proteins may be prepared for LC-MS/MS using standard techniques including protein reduction using dithiotreitol solution (DTT) and protein digestion using enzymes such as LysC and trypsin as described in Erickson et al, 2017 (Molecular Cell, VOLUME 65, ISSUE 2, P361-370, JANUARY 19, 2017). Alternatively, peptides are prepared as described by Liu et al. 2010 (JOURNAL OF BACTERIOLOGY, June 2010, p. 2852–2860 Vol. 192, No. 11), Kieselbach and Oscarsson 2017 (Data Brief. 2017 Feb; 10: 426–431.), Vildhede et al, 2018 (Drug Metabolism and Disposition February 8, 2018). Following digestion, peptide preparations are run directly on liquid chromatography and mass spectrometry devices for protein identification within a single sample. For relative quantitation of proteins between samples, peptide digests from different samples are labeled with isobaric tags using the iTRAQ Reagent-8plex Multiplex Kit (Applied Biosystems, Foster City, CA) or TMT 10plex and 11plex Label Reagents (Thermo Fischer Scientific, San Jose, CA, USA). Each peptide digest is labeled with a different isobaric tag and then the labeled digests are combined into one sample mixtur. The combined peptide mixture is analyzed by LC-MS/MS for both identification and quantification. A database search is performed using the LC-MS/MS data to identify the labeled peptides and the corresponding proteins. In the case of isobaric labeling, the fragmentation of the attached tag generates a low molecular mass reporter ion that is used to obtain a relative quantitation of the peptides and proteins present in each smEV.

**[714]** Additionally, metabolic content is ascertained using liquid chromatography techniques combined with mass spectrometry. A variety of techniques exist to determine metabolomic content of various samples and are known to one skilled in the art involving solvent extraction, chromatographic separation and a variety of ionization techniques coupled to mass determination (Roberts et al 2012 Targeted Metabolomics. Curr Protoc Mol Biol. 30: 1-24; Dettmer et al 2007, Mass spectrometry-based metabolomics. Mass Spectrom Rev. 26(1):51-78). As a non-limiting example, a LC-MS system includes a 4000 QTRAP triple quadrupole mass spectrometer (AB SCIEX) combined with 1100 Series pump (Agilent) and an HTS PAL autosampler (Leap Technologies). Media samples or other complex metabolic mixtures (~10 µL) are extracted using nine volumes of 74.9:24.9:0.2 (v/v/v) acetonitrile/methanol/formic acid

containing stable isotope-labeled internal standards (valine-d8, Isotec; and phenylalanine-d8, Cambridge Isotope Laboratories). Standards may be adjusted or modified depending on the metabolites of interest. The samples are centrifuged (10 minutes, 9,000g, 4 °C), and the supernatants (10 µL) are submitted to LCMS by injecting the solution onto the HILIC column (150 × 2.1 mm, 3 µm particle size). The column is eluted by flowing a 5% mobile phase [10mM ammonium formate, 0.1% formic acid in water] for 1 minute at a rate of 250uL/minute followed by a linear gradient over 10 minutes to a solution of 40% mobile phase [acetonitrile with 0.1% formic acid]. The ion spray voltage is set to 4.5 kV and the source temperature is 450 °C.

**[715]** The data are analyzed using commercially available software like Multiquant 1.2 from AB SCIEX for mass spectrum peak integration. Peaks of interest should be manually curated and compared to standards to confirm the identity of the peak. Quantitation with appropriate standards is performed to determine the number of metabolites present in the initial media, after bacterial conditioning and after tumor cell growth. A non-targeted metabolomics approach may also be used using metabolite databases, such as but not limited to the NIST database, for peak identification.

**[716]** *Dynamic light scattering (DLS)*

**[717]** DLS measurements, including the distribution of particles of different sizes in different smEV preparations are taken using instruments such as the DynaPro NanoStar (Wyatt Technology) and the Zetasizer Nano ZS (Malvern Instruments).

*Lipid Levels*

**[718]** Lipid levels are quantified using FM4-64 (Life Technologies), by methods similar to those described by A.J. McBroom *et al. J Bacteriol* 188:5385-5392. and A. Frias, *et al. Microb Ecol.* 59:476-486 (2010). Samples are incubated with FM4-64 (3.3 µg/mL in PBS for 10 minutes at 37ºC in the dark). After excitation at 515 nm, emission at 635 nm is measured using a Spectramax M5 plate reader (Molecular Devices). Absolute concentrations are determined by comparison of unknown samples to standards (such as palmitoyloleoylphosphatidylglycerol (POPG) vesicles) of known concentrations. Lipidomics can be used to identify the lipids present in the smEVs.

*Total Protein*

**[719]** Protein levels are quantified by standard assays such as the Bradford and BCA assays. The Bradford assays are run using Quick Start Bradford 1x Dye Reagent (Bio-Rad),

according to manufacturer's protocols. BCA assays are run using the Pierce BCA Protein Assay Kit (Thermo-Fisher Scientific). Absolute concentrations are determined by comparison to a standard curve generated from BSA of known concentrations. Alternatively, protein concentration can be calculated using the Beer-Lambert equation using the sample absorbance at 280nm (A280) as measured on a Nanodrop spectrophotometer (Thermo-Fisher Scientific).In addition, proteomics may be used to identify proteins in the sample.

*Lipid:Protein Ratios*

**[720]** Lipid:protein ratios are generated by dividing lipid concentrations by protein concentrations. These provide a measure of the purity of vesicles as compared to free protein in each preparation.

*Nucleic acid analysis*

**[721]** Nucleic acids are extracted from smEVs and quantified using a Qubit fluorometer. Size distribution is assessed using a BioAnalyzer and the material is sequenced.

*Zeta Potential*

**[722]** The zeta potential of different preparations are measured using instruments such as the Zetasizer ZS (Malvern Instruments).

**Example 42: In vitro screening of smEVs for enhanced activation of dendritic cells**

**[723]** *In vitro* immune responses are thought to simulate mechanisms by which immune responses are induced *in vivo*, e.g., as in response to a cancer microenvironment. Briefly, PBMCs are isolated from heparinized venous blood from healthy donors by gradient centrifugation using Lymphoprep (Nycomed, Oslo, Norway), or from mouse spleens or bone marrow using the magnetic bead-based Human Blood Dendritic cell isolation kit (Miltenyi Biotech, Cambridge, MA). Using anti-human CD14 mAb, the monocytes are purified by Moflo and cultured in cRPMI at a cell density of 5e5 cells/ml in a 96-well plate (Costar Corp) for 7 days at 37°C. For maturation of dendritic cells, the culture is stimulated with 0.2 ng/mL IL-4 and 1000 U/ml GM-CSF at 37°C for one week. Alternatively, maturation is achieved through incubation with recombinant GM-CSF for a week, or using other methods known in the art. Mouse DCs can be harvested directly from spleens using bead enrichment or differentiated from hematopoietic stem cells. Briefly, bone marrow may be obtained from the femurs of mice. Cells are recovered and red blood cells lysed. Stem cells are cultured in cell culture medium in 20ng/ml mouse GMCSF

for 4 days. Additional medium containing 20ng/ml mouse GM-CSF is added. On day 6 the medium and non-adherent cells are removed and replaced with fresh cell culture medium containing 20ng/ml GMCSF. A final addition of cell culture medium with 20ng/ml GM-CSF is added on day 7. On day10, non-adherent cells are harvested and seeded into cell culture plates overnight and stimulated as required. Dendritic cells are then treated with various doses of smEVs with or without antibiotics. For example, 25-75 ug/mL smEVs for 24 hours with antibiotics. smEV compositions tested may include smEVs from a single bacterial species or strain, or a mixture of smEVs from one or more genus, 1 or more species, or 1 or more strains (e.g., one or more strains within one species). PBS is included as a negative control and LPS, anti-CD40 antibodies, and/or smEVs from *Bifidobacterium* spp. are used as positive controls. Following incubation, DCs are stained with anti CD11b, CD11c, CD103, CD8a, CD40, CD80, CD83, CD86, MHCI and MHCII, and analyzed by flow cytometry. DCs that are significantly increased in CD40, CD80, CD83, and CD86 as compared to negative controls are considered to be activated by the associated bacterial smEV composition. These experiments are repeated three times at minimum.

**[724]** To screen for the ability of smEV-activated epithelial cells to stimulate DCs, the above protocol is followed with the addition of a 24-hour epithelial cell smEV co-culture prior to incubation with DCs. Epithelial cells are washed after incubation with smEVs and are then co-cultured with DCs in an absence of smEVs for 24 hours before being processed as above. Epithelial cell lines may include Int407, HEL293, HT29, T84 and CACO2.

**[725]** As an additional measure of DC activation, 100 µl of culture supernatant is removed from wells following 24-hour incubation of DCs with smEVs or smEV-treated epithelial cells and is analyzed for secreted cytokines, chemokines, and growth factors using the multiplexed Luminex Magpix. Kit (EMD Millipore, Darmstadt, Germany). Briefly, the wells are pre-wet with buffer, and 25 µl of 1x antibody-coated magnetic beads are added and 2x 200 µl of wash buffer are performed in every well using the magnet. 50 µl of Incubation buffer, 50 µl of diluent and 50 µl of samples are added and mixed via shaking for 2hrs at room temperature in the dark. The beads are then washed twice with 200 µl wash buffer. 100 µl of 1X biotinylated detector antibody is added and the suspension is incubated for 1 hour with shaking in the dark. Two, 200 µl washes are then performed with wash buffer. 100 µl of 1x SAV-RPE reagent is added to each well and is incubated for 30 min at RT in the dark. Three 200 µl washes are

performed and 125 µl of wash buffer is added with 2-3 min shaking occurs. The wells are then submitted for analysis in the Luminex xMAP system.

**[726]** Standards allow for careful quantitation of the cytokines including GM-CSF, IFN-g, IFN-a, IFN-B, IL-1a, IL-1B, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, IL-12 (p40/p70), IL-17A, IL-17F, IL-21, IL-22 IL-23, IL-25, IP-10, KC, MCP-1, MIG, MIP1a, TNFa, and VEGF. These cytokines are assessed in samples of both mouse and human origin. Increases in these cytokines in the bacterial treated samples indicate enhanced production of proteins and cytokines from the host. Other variations on this assay examining specific cell types ability to release cytokines are assessed by acquiring these cells through sorting methods and are recognized by one of ordinary skill in the art. Furthermore, cytokine mRNA is also assessed to address cytokine release in response to an smEV composition.

**[727]** This DC stimulation protocol may be repeated using combinations of purified smEVs and live bacterial strains to maximize immune stimulation potential.

**Example 43: In vitro screening of smEVs for enhanced activation of CD8+ T cell killing when incubated with tumor cells**

**[728]** *In vitro* methods for screening smEVs that can activate CD8+ T cell killing of tumor cells are described. Briefly, DCs are isolated from human PBMCs or mouse spleens, using techniques known in the art, and incubated *in vitro* with single-strain smEVs, mixtures of smEVs, and/or appropriate controls. In addition, CD8+ T cells are obtained from human PBMCs or mouse spleens using techniques known in the art, for example the magnetic bead-based Mouse CD8a+ T Cell Isolation Kit and the magnetic bead-based Human CD8+ T Cell Isolation Kit (both from Miltenyi Biotech, Cambridge, MA). After incubation of DCs with smEVs for some time (e.g., for 24-hours), or incubation of DCs with smEV-stimulated epithelial cells, smEVs are removed from the cell culture with PBS washes and 100ul of fresh media with antibiotics is added to each well, and 200,000 T cells are added to each experimental well in the 96-well plate. Anti-CD3 antibody is added at a final concentration of 2ug/ml. Co-cultures are then allowed to incubate at 37˚C for 96 hours under normal oxygen conditions.

**[729]** For example, approximately 72 hours into the coculture incubation, tumor cells are plated for use in the assay using techniques known in the art. For example, 50,000 tumor cells/well are plated per well in new 96-well plates. Mouse tumor cell lines used may include

B16.F10, SIY+ B16.F10, and others. Human tumor cell lines are HLA-matched to donor, and can include PANC-1, UNKPC960/961, UNKC, and HELA cell lines. After completion of the 96-hour co-culture, 100 µl of the CD8+ T cell and DC mixture is transferred to wells containing tumor cells. Plates are incubated for 24 hours at 37°C under normal oxygen conditions. Staurospaurine may be used as negative control to account for cell death.

**[730]** Following this incubation, flow cytometry is used to measure tumor cell death and characterize immune cell phenotype. Briefly, tumor cells are stained with viability dye. FACS analysis is used to gate specifically on tumor cells and measure the percentage of dead (killed) tumor cells. Data are also displayed as the absolute number of dead tumor cells per well. Cytotoxic CD8+ T cell phenotype may be characterized by the following methods: a) concentration of supernatant granzyme B, IFNy and TNFa in the culture supernatant as described below, b) CD8+ T cell surface expression of activation markers such as DC69, CD25, CD154, PD-1, gamma/delta TCR, Foxp3, T-bet, granzyme B, c) intracellular cytokine staining of IFNy, granzyme B, TNFa in CD8+ T cells. CD4+ T cell phenotype may also be assessed by intracellular cytokine staining in addition to supernatant cytokine concentration including INFy, TNFa, IL-12, IL-4, IL-5, IL-17, IL-10, chemokines etc.

**[731]** As an additional measure of CD8+ T cell activation, 100 µl of culture supernatant is removed from wells following the 96-hour incubation of T cells with DCs and is analyzed for secreted cytokines, chemokines, and growth factors using the multiplexed Luminex Magpix. Kit (EMD Millipore, Darmstadt, Germany). Briefly, the wells are pre-wet with buffer, and 25 µl of 1x antibody-coated magnetic beads are added and 2x 200 µl of wash buffer are performed in every well using the magnet. 50 µl of Incubation buffer, 50 µl of diluent and 50 µl of samples are added and mixed via shaking for 2hrs at room temperature in the dark. The beads are then washed twice with 200 µl wash buffer. 100 µl of 1X biotinylated detector antibody is added and the suspension is incubated for 1 hour with shaking in the dark. Two, 200 µl washes are then performed with wash buffer. 100 µl of 1x SAV-RPE reagent is added to each well and is incubated for 30 min at RT in the dark. Three 200 µl washes are performed and 125 µl of wash buffer is added with 2-3 min shaking occurs. The wells are then submitted for analysis in the Luminex xMAP system.

**[732]** Standards allow for careful quantitation of the cytokines including GM-CSF, IFN-g, IFN-a, IFN-B IL-1a, IL-1B, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, IL-12 (p40/p70), IL-17,

IL-23, IP-10, KC, MCP-1, MIG, MIP1a, TNFa, and VEGF. These cytokines are assessed in samples of both mouse and human origin. Increases in these cytokines in the bacterial treated samples indicate enhanced production of proteins and cytokines from the host. Other variations on this assay examining specific cell types ability to release cytokines are assessed by acquiring these cells through sorting methods and are recognized by one of ordinary skill in the art. Furthermore, cytokine mRNA is also assessed to address cytokine release in response to an smEV composition. These changes in the cells of the host stimulate an immune response similarly to in vivo response in a cancer microenvironment.

**[733]** This CD8+ T cell stimulation protocol may be repeated using combinations of purified smEVs and live bacterial strains to maximize immune stimulation potential.

**Example 44: In vitro screening of smEVs for enhanced tumor cell killing by PBMCs**

**[734]** Various methods may be used to screen smEVs for the ability to stimulate PBMCs, which in turn activate CD8+ T cells to kill tumor cells. For example, PBMCs are isolated from heparinized venous blood from healthy human donors by ficoll-paque gradient centrifugation for mouse or human blood, or with Lympholyte Cell Separation Media (Cedarlane Labs, Ontario, Canada) from mouse blood. PBMCs are incubated with single-strain smEVs, mixtures of smEVs, and appropriate controls. In addition, CD8+ T cells are obtained from human PBMCs or mouse spleens. After the 24-hour incubation of PBMCs with smEVs, smEVs are removed from the cells using PBS washes. 100ul of fresh media with antibiotics is added to each well. An appropriate number of T cells (e.g., 200,000 T cells) are added to each experimental well in the 96-well plate. Anti-CD3 antibody is added at a final concentration of 2ug/ml. Co-cultures are then allowed to incubate at 37°C for 96 hours under normal oxygen conditions.

**[735]** For example, 72 hours into the coculture incubation, 50,000 tumor cells/well are plated per well in new 96-well plates. Mouse tumor cell lines used include B16.F10, SIY+ B16.F10, and others. Human tumor cell lines are HLA-matched to donor, and can include PANC-1, UNKPC960/961, UNKC, and HELA cell lines. After completion of the 96-hour co-culture, 100 µl of the CD8+ T cell and PBMC mixture is transferred to wells containing tumor cells. Plates are incubated for 24 hours at 37°C under normal oxygen conditions. Staurospaurine is used as negative control to account for cell death.

[736] Following this incubation, flow cytometry is used to measure tumor cell death and characterize immune cell phenotype. Briefly, tumor cells are stained with viability dye. FACS analysis is used to gate specifically on tumor cells and measure the percentage of dead (killed) tumor cells. Data are also displayed as the absolute number of dead tumor cells per well. Cytotoxic CD8+ T cell phenotype may be characterized by the following methods: a) concentration of supernatant granzyme B, IFNy and TNFa in the culture supernatant as described below, b) CD8+ T cell surface expression of activation markers such as DC69, CD25, CD154, PD-1, gamma/delta TCR, Foxp3, T-bet, granzyme B, c) intracellular cytokine staining of IFNy, granzyme B, TNFa in CD8+ T cells. CD4+ T cell phenotype may also be assessed by intracellular cytokine staining in addition to supernatant cytokine concentration including INFy, TNFa, IL-12, IL-4, IL-5, IL-17, IL-10, chemokines etc.

[737] As an additional measure of CD8+ T cell activation, 100 µl of culture supernatant is removed from wells following the 96-hour incubation of T cells with DCs and is analyzed for secreted cytokines, chemokines, and growth factors using the multiplexed Luminex Magpix. Kit (EMD Millipore, Darmstadt, Germany). Briefly, the wells are pre-wet with buffer, and 25 µl of 1x antibody-coated magnetic beads are added and 2x 200 µl of wash buffer are performed in every well using the magnet. 50 µl of Incubation buffer, 50 µl of diluent and 50 µl of samples are added and mixed via shaking for 2hrs at room temperature in the dark. The beads are then washed twice with 200 µl wash buffer. 100 µl of 1X biotinylated detector antibody is added and the suspension is incubated for 1 hour with shaking in the dark. Two, 200 µl washes are then performed with wash buffer. 100 µl of 1x SAV-RPE reagent is added to each well and is incubated for 30 min at RT in the dark. Three 200 µl washes are performed and 125 µl of wash buffer is added with 2-3 min shaking occurs. The wells are then submitted for analysis in the Luminex xMAP system.

[738] Standards allow for careful quantitation of the cytokines including GM-CSF, IFN-g, IFN-a, IFN-B IL-1a, IL-1B, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, IL-12 (p40/p70), IL-17, IL-23, IP-10, KC, MCP-1, MIG, MIP1a, TNFa, and VEGF. These cytokines are assessed in samples of both mouse and human origin. Increases in these cytokines in the bacterial treated samples indicate enhanced production of proteins and cytokines from the host. Other variations on this assay examining specific cell types ability to release cytokines are assessed by acquiring these cells through sorting methods and are recognized by one of ordinary skill in the art.

Furthermore, cytokine mRNA is also assessed to address cytokine release in response to an smEV composition. These changes in the cells of the host stimulate an immune response similarly to in vivo response in a cancer microenvironment.

**[739]** This PBMC stimulation protocol may be repeated using combinations of purified smEVs with or without combinations of live, dead, or inactivated/weakened bacterial strains to maximize immune stimulation potential.

**Example 45: In vitro detection of smEVs in antigen-presenting cells**

**[740]** Dendritic cells in the lamina propria constantly sample live bacteria, dead bacteria, and microbial products in the gut lumen by extending their dendrites across the gut epithelium, which is one way that smEVs produced by bacteria in the intestinal lumen may directly stimulate dendritic cells. The following methods represent a way to assess the differential uptake of smEVs by antigen-presenting cells. Optionally, these methods may be applied to assess immunomodulatory behavior of smEVs administered to a patient.

**[741]** Dendritic cells (DCs) are isolated from human or mouse bone marrow, blood, or spleens according to standard methods or kit protocols (e.g., Inaba K, Swiggard WJ, Steinman RM, Romani N, Schuler G, 2001. Isolation of dendritic cells. Current Protocols in Immunology. Chapter 3:Unit3.7).

**[742]** To evaluate smEV entrance into and/or presence in DCs, 250,000 DCs are seeded on a round cover slip in complete RPMI-1640 medium and are then incubated with smEVs from single bacterial strains or combinations smEVs at various ratios. Purified smEVs may be labeled with fluorochromes or fluorescent proteins. After incubation for various timepoints (e.g., 1 hour, 2 hours), the cells are washed twice with ice-cold PBS and detached from the plate using trypsin. Cells are either allowed to remain intact or are lysed. Samples are then processed for flow cytometry. Total internalized smEVs are quantified from lysed samples, and percentage of cells that uptake smEVs is measured by counting fluorescent cells. The methods described above may also be performed in substantially the same manner using macrophages or epithelial cell lines (obtained from the ATCC) in place of DCs.

**Example 46: In vitro screening of smEVs with an enhanced ability to activate NK cell killing when incubated with target cells**

**[743]** To demonstrate the ability of the selected smEV compositions to elicit potent NK cell cytotoxicity to tumor cells, the following *in vitro* assay is used. Briefly, mononuclear cells from heparinized blood are obtained from healthy human donors. Optionally, an expansion step to increase the numbers of NK cells is performed as previously described (e.g., see Somanschi et al., *J Vis Exp.* 2011;(48):2540). The cells may be adjusted to a concentration of ,cells/ml in RPMI-1640 medium containing 5% human serum. The PMNC cells are then labeled with appropriate antibodies and NK cells are isolated through FACS as CD3-/CD56+ cells and are ready for the subsequent cytotoxicity assay. Alternatively, NK cells are isolated using the autoMACs instrument and NK cell isolation kit following manufacturer's instructions (Miltenyl Biotec).

**[744]** NK cells are counted and plated in a 96 well format with 20,000 or more cells per well, and incubated with single-strain smEVs, with or without addition of antigen presenting cells (e.g., monocytes derived from the same donor), smEVs from mixtures of bacterial strains, and appropriate controls. After 5-24 hours incubation of NK cells with smEVs, smEVs are removed from cells with PBS washes, NK cells are resuspended in10 mL fresh media with antibiotics and are added to 96-well plates containing 20,000 target tumor cells/well. Mouse tumor cell lines used include B16.F10, SIY+ B16.F10, and others. Human tumor cell lines are HLA-matched to donor, and can include PANC-1, UNKPC960/961, UNKC, and HELA cell lines. Plates are incubated for 2-24 hours at 37°C under normal oxygen conditions. Staurospaurine is used as negative control to account for cell death.

**[745]** Following this incubation, flow cytometry is used to measure tumor cell death using methods known in the art. Briefly, tumor cells are stained with viability dye. FACS analysis is used to gate specifically on tumor cells and measure the percentage of dead (killed) tumor cells. Data are also displayed as the absolute number of dead tumor cells per well.

**[746]** This NK cell stimulation protocol may be repeated using combinations of purified smEVs and live bacterial strains to maximize immune stimulation potential.

**Example 47: Using in vitro immune activation assays to predict in vivo cancer immunotherapy efficacy of smEV compositions**

**[747]** *In vitro* immune activation assays identify smEVs that are able to stimulate dendritic cells, which in turn activate CD8+ T cell killing. Therefore, the *in vitro* assays described above are used as a predictive screen of a large number of candidate smEVs for potential immunotherapy activity. smEVs that display enhanced stimulation of dendritic cells, enhanced stimulation of CD8+ T cell killing, enhanced stimulation of PBMC killing, and/or enhanced stimulation of NK cell killing, are preferentially chosen for *in vivo* cancer immunotherapy efficacy studies.

**Example 48: Determining the biodistribution of smEVs when delivered orally to mice**

**[748]** Wild-type mice (*e.g.,* C57BL/6 or BALB/c) are orally inoculated with the smEV composition of interest to determine the *in vivo* biodistibution profile of purified smEVs. smEVs are labeled to aide in downstream analyses. Alternatively, tumor-bearing mice or mice with some immune disorder (e.g., systemic lupus erythematosus, experimental autoimmune encephalomyelitis, NASH) may be studied for *in vivo* distribution of smEVs over a given time-course.

**[749]** Mice can receive a single dose of the smEV (e.g., 25-100 μg) or several doses over a defined time course (25-100 μg). Alternatively, smEVs dosages may be administered based on particle count (e.g., 7e+08 to 6e+11 particles). Mice are housed under specific pathogen-free conditions following approved protocols. Alternatively, mice may be bred and maintained under sterile, germ-free conditions. Blood, stool, and other tissue samples can be taken at appropriate time points.

**[750]** The mice are humanely sacrificed at various time points (*i.e.,* hours to days) post administration of the smEV compositions, and a full necropsy under sterile conditions is performed. Following standard protocols, lymph nodes, adrenal glands, liver, colon, small intestine, cecum, stomach, spleen, kidneys, bladder, pancreas, heart, skin, lungs, brain, and other tissue of interest are harvested and are used directly or snap frozen for further testing. The tissue samples are dissected and homogenized to prepare single-cell suspensions following standard protocols known to one skilled in the art. The number of smEVs present in the sample is then quantified through flow cytometry. Quantification may also proceed with use of fluorescence

microscopy after appropriate processing of whole mouse tissue (Vankelecom H., Fixation and paraffin-embedding of mouse tissues for GFP visualization, *Cold Spring Harb. Protoc.*, 2009). Alternatively, the animals may be analyzed using live-imaging according to the smEV labeling technique.

**[751]** Biodistribution may be performed in mouse models of cancer such as but not limited to CT-26 and B16 (see, e.g., Kim et al., Nature Communications vol. 8, no. 626 (2017)) or autoimmunity such as but not limited to EAE and DTH (see, e.g., Turjeman et al., PLoS One 10(7): e0130442 (20105).

**Example 49: Manufacturing conditions**

**[752]** Enriched media is used to grow and prepare the bacteria for *in vitro* and *in vivo* use and, ultimately, for pmEV and smEV preparations. For example, media may contain sugar, yeast extracts, plant-based peptones, buffers, salts, trace elements, surfactants, anti-foaming agents, and vitamins. Composition of complex components such as yeast extracts and peptones may be undefined or partially defined (including approximate concentrations of amino acids, sugars etc.). Microbial metabolism may be dependent on the availability of resources such as carbon and nitrogen. Various sugars or other carbon sources may be tested. Alternatively, media may be prepared and the selected bacterium grown as shown by Saarela *et al., J. Applied Microbiology*. 2005. 99: 1330-1339, which is hereby incorporated by reference. Influence of fermentation time, cryoprotectant and neutralization of cell concentrate on freeze-drying survival, storage stability, and acid and bile exposure of the selected bacterium produced without milk-based ingredients.

**[753]** At large scale, the media is sterilized. Sterilization may be accomplished by Ultra High Temperature (UHT) processing. The UHT processing is performed at very high temperature for short periods of time. The UHT range may be from 135-180°C. For example, the medium may be sterilized from between 10 to 30 seconds at 135°C.

**[754]** Inoculum can be prepared in flasks or in smaller bioreactors and growth is monitored. For example, the inoculum size may be between approximately 0.5 and 3% of the total bioreactor volume. Depending on the application and need for material, bioreactor volume can be at least 2L, 10L, 80L, 100L, 250L, 1000L, 2500L, 5000L, 10,000L.

[755] Before the inoculation, the bioreactor is prepared with medium at desired pH, temperature, and oxygen concentration. The initial pH of the culture medium may be different that the process set-point. pH stress may be detrimental at low cell centration; the initial pH could be between pH 7.5 and the process set-point. For example, pH may be set between 4.5 and 8.0. During the fermentation, the pH can be controlled through the use of sodium hydroxide, potassium hydroxide, or ammonium hydroxide. The temperature may be controlled from $25^0$C to $45^0$C, for example at $37^0$C. Anaerobic conditions are created by reducing the level of oxygen in the culture broth from around 8mg/L to 0mg/L. For example, nitrogen or gas mixtures (N2, CO2, and H2) may be used in order to establish anaerobic conditions. Alternatively, no gases are used and anaerobic conditions are established by cells consuming remaining oxygen from the medium. Depending on strain and inoculum size, the bioreactor fermentation time can vary. For example, fermentation time can vary from approximately 5 hours to 48 hours.

[756] Reviving microbes from a frozen state may require special considerations. Production medium may stress cells after a thaw; a specific thaw medium may be required to consistently start a seed train from thawed material. The kinetics of transfer or passage of seed material to fresh medium, for the purposes of increasing the seed volume or maintaining the microbial growth state, may be influenced by the current state of the microbes (ex. exponential growth, stationary growth, unstressed, stressed).

[757] Inoculation of the production fermenter(s) can impact growth kinetics and cellular activity. The initial state of the bioreactor system must be optimized to facilitate successful and consistent production. The fraction of seed culture to total medium (*e.g.,* a percentage) has a dramatic impact on growth kinetics. The range may be 1-5% of the fermenter's working volume. The initial pH of the culture medium may be different from the process set-point. pH stress may be detrimental at low cell concentration; the initial pH may be between pH 7.5 and the process set-point. Agitation and gas flow into the system during inoculation may be different from the process set-points. Physical and chemical stresses due to both conditions may be detrimental at low cell concentration.

[758] Process conditions and control settings may influence the kinetics of microbial growth and cellular activity. Shifts in process conditions may change membrane composition, production of metabolites, growth rate, cellular stress, etc. Optimal temperature range for growth may vary with strain. The range may be 20-40 °C. Optimal pH for cell growth and performance

of downstream activity may vary with strain. The range may be pH 5-8. Gasses dissolved in the medium may be used by cells for metabolism. Adjusting concentrations of O2, CO2, and $N_2$ throughout the process may be required. Availability of nutrients may shift cellular growth. Microbes may have alternate kinetics when excess nutrients are available.

**[759]** The state of microbes at the end of a fermentation and during harvesting may impact cell survival and activity. Microbes may be preconditioned shortly before harvest to better prepare them for the physical and chemical stresses involved in separation and downstream processing. A change in temperature (often reducing to 20-5 °C) may reduce cellular metabolism, slowing growth (and/or death) and physiological change when removed from the fermenter. Effectiveness of centrifugal concentration may be influenced by culture pH. Raising pH by 1-2 points can improve effectiveness of concentration but can also be detrimental to cells. Microbes may be stressed shortly before harvest by increasing the concentration of salts and/or sugars in the medium. Cells stressed in this way may better survive freezing and lyophilization during downstream.

**[760]** Separation methods and technology may impact how efficiently microbes are separated from the culture medium. Solids may be removed using centrifugation techniques. Effectiveness of centrifugal concentration can be influenced by culture pH or by the use of flocculating agents. Raising pH by 1-2 points may improve effectiveness of concentration but can also be detrimental to cells. Microbes may be stressed shortly before harvest by increasing the concentration of salts and/or sugars in the medium. Cells stressed in this way may better survive freezing and lyophilization during downstream. Additionally, Microbes may also be separated via filtration. Filtration is superior to centrifugation techniques for purification if the cells require excessive g-minutes to successfully centrifuge. Excipients can be added before after separation. Excipients can be added for cryo protection or for protection during lyophilization. Excipients can include, but are not limited to, sucrose, trehalose, or lactose, and these may be alternatively mixed with buffer and anti-oxidants. Prior to lyophilization, droplets of cell pellets mixed with excipients are submerged in liquid nitrogen.

**[761]** Harvesting can be performed by continuous centrifugation. Product may be resuspended with various excipients to a desired final concentration. Excipients can be added for cryo protection or for protection during lyophilization. Excipients can include, but are not limited to, sucrose, trehalose, or lactose, and these may be alternatively mixed with buffer and anti-

oxidants. Prior to lyophilization, droplets of cell pellets mixed with excipients are submerged in liquid nitrogen.

**[762]** Lyophilization of material, including live bacteria, vesicles, or other bacterial derivative includes a freezing, primary drying, and secondary drying phase. Lyophilization begins with freezing. The product material may or may not be mixed with a lyoprotectant or stabilizer prior to the freezing stage. A product may be frozen prior to the loading of the lyophilizer, or under controlled conditions on the shelf of the lyophilizer. During the next phase, the primary drying phase, ice is removed via sublimation. Here, a vacuum is generated and an appropriate amount of heat is supplied to the material. The ice will sublime while keeping the product temperature below freezing, and below the material's critical temperature ($T_c$). The temperature of the shelf on which the material is loaded and the chamber vacuum can be manipulated to achieve the desired product temperature. During the secondary drying phase, product-bound water molecules are removed. Here, the temperature is generally raised higher than in the primary drying phase to break any physico-chemical interactions that have formed between the water molecules and the product material. After the freeze-drying process is complete, the chamber may be filled with an inert gas, such as nitrogen. The product may be sealed within the freeze dryer under dry conditions, in a glass vial or other similar container, preventing exposure to atmospheric water and contaminates.

**Example 50: Oral *Prevotella histicola* and *Veillonella parvula* smEVs and pmEVs: DTH studies**

**[763]** I. Female 5 week old C57BL/6 mice were purchased from Taconic Biosciences and acclimated at a vivarium for one week. Mice were primed with an emulsion of KLH and CFA (1:1) by subcutaneous immunization on day 0. Mice were orally gavaged daily with pmEVs or powder of whole microbe of the indicated strain or dosed intraperitoneally with dexamethasone at 1 mg/kg from days 1-8. After dosing on day 8, mice were anaesthetized with isoflurane, left ears were measured for baseline measurements with Fowler calipers and the mice were challenged intradermally with KLH in saline (10 µl) in the left ear and ear thickness measurements were taken at 24 hours.

**[764]** The 24 hour ear measurement results are shown in **Figure 21**. The efficacy of *P. histicola* pmEVs at three doses (high: 6.0E+11, mid: 6.0E+09 and low: 6.0E+07) was tested in

comparison to lyophilized *P. histicola* pmEVs at the same doses and to 10 mg of powder (with total cell count 3.13E+09). The results show that the high dose of pmEVs displayed comparable efficacy to the 10 mg dose of powder. The efficacy of *P. histicola* pmEVs is not affected by lyophilization.

**[765]** II. Female 5 week old C57BL/6 mice were purchased from Taconic Biosciences and acclimated at a vivarium for one week. Mice were primed with an emulsion of KLH and CFA (1:1) by subcutaneous immunization on day 0. Mice were orally gavaged daily with smEVs, pmEVs, gamma irradiated (GI) pmEVs, or gamma irradiated (GI) powder (of whole microbe) of the indicated strain or dosed intraperitoneally with dexamethasone at 1 mg/kg from days 1-8. After dosing on day 8, mice were anaesthetized with isoflurane, left ears were measured for baseline measurements with Fowler calipers and the mice were challenged intradermally with KLH in saline (10 µl) in the left ear and ear thickness measurements were taken at 24 hours.

**[766]** The 24 hour ear measurement results are shown in **Figure 22**. The efficacy of *V. parvula* smEVs, pmEVs and gamma-irradiated (GI) pmEVs were tested head-to-head at three doses (high: 3.0E+11, mid: 3.0E+09 and low: 3.0E+07). There was not a significant difference between the highest dose of each group. *V. parvula* pmEVs, both gamma-irradiated and non-gamma-irradiated, are just as efficacious as smEVs.

## Example 51: smEV and pmEV preparation

**[767]** For the studies described in Example 50, the smEVs and pmEVs were prepared as follows.

**[768]** *smEVs:* Downstream processing of smEVs began immediately following harvest of the bioreactor. Centrifugation at 20,000 g was used to remove the cells from the broth. The resulting supernatant was clarified using 0.22 µm filter. The smEVs were concentrated and washed using tangential flow filtration (TFF) with flat sheet cassettes ultrafiltration (UF) membranes with 100 kDa molecular weight cutoff (MWCO). Diafiltration (DF) was used to washout small molecules and small proteins using 5 volumes of phosphate buffer solution (PBS). The retentate from TFF was spun down in an ultracentrifuge at 200,000 g for 1 hour to form a pellet rich in smEVs called a high-speed pellet (HSP). The pellet was resuspended with minimal PBS and a gradient was prepared with optiprep™ density gradient medium and ultracentrifuged

at 200,000 g for 16 hours. Of the resulting fractions, 2 middle bands contained smEVs. The fractions were washed with 15 fold PBS and the smEVs spun down at 200,000 g for 1 hr to create the fractionated HSP or fHSP. It was subsequently resuspended with minimal PBS, pooled, and analyzed for particles per mL and protein content. Dosing was prepared from the particle / mL count to achieve desired concentration. The smEVs were characterized using a NanoSight NS300 by Malvern Panalytical in scatter mode using the 532 nm laser.

**[769]** *Prevotella histicola pmEVs:*

**[770]** Cell pellets were removed from freezer and placed on ice. Pellet weights were noted.

**[771]** Cold 100 mM Tris-HCl pH 7.5 was added to the frozen pellets and the pellets were thawed rotating at 4°C.

**[772]** 10mg/ml DNase stock was added to the thawed pellets to a final concentration of 1mg/mL.

**[773]** The pellets were incubated on the inverter for 40 min at RT (room temperature).

**[774]** The sample was filtered in a 70um cell strainer before running through the Emulsiflex.

**[775]** The samples were lysed using the Emulsiflex with 8 discrete cycles at 22,000psi.

**[776]** To remove the cellular debris from the lysed sample, the sample was centrifuged at 12,500 x g, 15 min, 4°C.

**[777]** The sample was centrifuged two additional times at 12,500 x g, 15 min, 4°C, each time moving the supernatant to a fresh tube.

**[778]** To pellet the membrane proteins, the sample was centrifuged at 120,000 x g, 1 hr, 4°C.

**[779]** The pellet was resuspended in 10 mL ice-cold 0.1 M sodium carbonate pH 11. The sample was incubated on the inverter at 4°C for 1 hour.

**[780]** The sample was centrifuged at 120,000 x g, 1 hr, 4°C.

**[781]** 10 mL 100 mM Tris-HCl pH 7.5 was added to pellet and incubate O/N (overnight) at 4°C.

**[782]** The pellet was resuspended and the sample was centrifuged at 120,000xg for 1 hour at 4°C.

**[783]** The supernatant was discarded and the pellet was resuspended in a minimal volume of PBS.

**[784]** *Veillonella parvula pmEVs:*

**[785]** The *V. parvula* pmEVs used in the studies in Example 50 came from three different isolations (isolations 1, 2 and 3). There were small variations in protocol.

**[786]** Cell pellets were removed from freezer and place on ice. Pellet weights were noted.

**[787]** Cold MP Buffer (100 mM Tris-HCl pH 7.5) was added to the frozen pellets and the pellets were thawed rotating at RT.

**[788]** 10mg/ml DNase stock was added to the thawed pellets from isolations 1 and 2 to a final concentration of 1mg/mL and incubate. The pellets were incubated an additional 40' on the inverter.

**[789]** The samples were lysed using the Emulsiflex with 8 discrete cycles at 20,000-30,000 psi.

**[790]** For isolations 1 and 2, the samples were filtered in a 70um cell strainer before running through the Emulsiflex to remove clumps.

**[791]** For isolation 3, 1mM PMSF (Phenylmethylsulfonyl fluoride, Sigma) and 1mM Benzamidine (Sigma) were added immediately prior to passage through the Emulsiflex and the sample was first cycled through the Emulsiflex continuously for 1.5 minutes at 15,000 psi to break up large clumps.

**[792]** To remove the cellular debris from the cell lysate, the samples were centrifuged at 12,500 x g, 15 min, 4ºC.

**[793]** The supernatant from isolation 3 was centrifuged one additional time while the supernatants from isolations 1 and 2 were cycled two additional times at 12,500 x g, 15 min, 4°C. After each centrifugation the supernatant was moved to a fresh tube.

**[794]** The final supernatant was centrifuged 120,000 x g, 1 hr, 4ºC.

**[795]** The membrane pellet was resuspended in 10 mL ice-cold 0.1 M sodium carbonate pH 11. For isolations 1 and 2, the samples were incubated in sodium carbonate for 1 hour prior to high speed spin.

**[796]** The samples were spun at 120,000 x g, 1 hr, 4°C.

**[797]** 10 mL 100 mM Tris-HCl pH 7.5 was added to the pellet and the pellet was resuspended.

**[798]** The sample was centrifuged at 120,000xg for 1 hour at 4°C.

**[799]** The supernatant was discarded and the pellets were in a minimal volume of in PBS (isolations 1 and 2) or PBS containing 250mM sucrose (isolation 3).

**[800]** Dosing pmEVs was based on particle counts, as assessed by Nanoparticle Tracking Analysis (NTA) using a NanoSight NS300 (Malvern Panalytical) according to manufacturer instructions. Counts for each sample were based on at least three videos of 30 sec duration each, counting 40-140 particles per frame.

**[801]** *Gamma irradiation:* For gamma irradiation, *V. parvula* pmEVs were prepared in frozen form and gamma irradiated on dry ice at 25kGy radiation dose*; V. parvula* whole microbe lyophilized powder was gamma irradiated at ambient temperature at 17.5kGy radiation dose.

**[802]** *Lyophilization:* Samples were placed in lyophilization equipment and frozen at -45 °C. The lyophilization cycle included a hold step at -45 °C for 10 min. The vacuum began and was set to 100 mTorr and the sample was held at -45 °C for another 10 min. Primary drying began with a temperature ramp to -25 °C over 300 minutes and it was held at this temperature for 4630 min. Secondary drying started with a temperature ramp to 20 °C over 200 min while the vacuum was decreased to 20 mTorr. It was held at this temperature and pressure for 1200 min. The final step increased the temperature from 20 to 25 °C where it remained at a vacuum of 20 mTorr for 10 min.

## Example 52: smEV Isolation and Enumeration

**[803]** The equipment used in smEV isolation includes a Sorvall RC-5C centrifuge with SLA-3000 rotor; an Optima XE-90 Ultracentrifuge by Beckman-Coulter 45Ti rotor; a Sorvall wX+ Ultra Series Centrifuge by Thermo Scientific; and a Fiberlite F37L-8x100 rotor.

*Microbial Supernatant Collection and Filtration*

**[804]** Microbes must be pelleted and filtered away from supernatant in order to recover smEVs and not microbes.

**[805]** Pellet microbial culture is generated by using a Sorvall RC-5C centrifuge with the SLA-3000 rotor and centrifuge culture for a minimum of 15min at a minimum of 7,000rpm. And then decanting the supernatant into new and sterile container.

**[806]** The supernatant is filtered through a 0.2um filter. For supernatants with poor filterability (less than 300ml of supernatant pass through filter) a 0.45um capsule filter is attached ahead of the 0.2um vacuum filter. The filtered supernatant is stored atat 4°C. The filtered supernatant can then be concentrated using TFF.

*Isolation of smEVs using Ultracentrifugation*

**[807]** Concentrated supernatant is centrifuged in the ultracentrifuge to pellet smEVs and isolate the smEVs from smaller biomolecules. The speed is for 200,000g, time for 1 hour, and temperature at 4°C. When rotor has stopped, tubes are removed from the ultracentrifuge and the supernatant is gently poured off. More supernatant is added the tubes are centrifuged again. After all concentrated supernatant has been centrifuged, the pellets generated are referred to as 'crude' smEV pellets. Sterile 1xPBS is added to pellets, which are placed in a container. The container is placed on a shaker set at speed 70, in a 4°C fridge overnight or longer. The smEV pellets are resuspended with additional sterile 1xPBS. The resuspended crude EV samples are stored at 4°C or at -80°C.

*smEV Purification using Density Gradients*

**[808]** Density gradients are used for smEV purification. During ultracentrifugation, particles in the sample will move, and separate, within the graded density medium based on their 'buoyant' densities. In this way smEVs are separated from other particles, such as sugars, lipids, or other proteins, in the sample.

**[809]** For smEV purification, four different percentages of the density medium (60% Optiprep) are used, a 45% layer, a 35% layer, a 25%, and a 15% layer. This will create the graded layers. A 0% layer is added at the top consisting of sterile 1xPBS. The 45% gradient layer should contain the crude smEV sample. 5ml of sample is added to 15ml of Optiprep. If crude smEV sample is less than 5ml, bring up to volume using sterile 1xPBS.

**[810]** Using a serological pipette, the 45% gradient mixture is pipetted up and down to mix. The sample is then pipetted into a labeled clean and sterile ultracentrifuge tube. Next, a

10ml serological pipette is used to slowly add 13ml of 35% gradient mixture. Next 13ml of the 25% gradient mixture is added, followed by 13ml of the 15% mixture and finally 6ml of sterile 1xPBS. The ultracentrifuge tubes are balanced with sterile 1xPBS. The gradients are carefully placed in a rotor and the ultracentrifuge is set for for 200,000g and 4°C. The gradients are centrifuged for a minimum of 16 hours.

**[811]** A clean pipette is used to remove fraction(s) of interest, which are added to 15ml conical tube. These 'purified' smEV samples are kept at 4°C.

**[812]** In order to clean and remove residual optiprep from smEVs, 10x volume of PBS are added to purified smEVs. The ultracentrifuge is set for 200,000g and 4°C. Centrifuge and spun for 1 hour. The tubes are carefully removed from ultracentrifuge and the supernatant decanted. The purified EVs are washed until all sample has been pelleted. 1xPBS is added to th purified pellets, which are placed in a container.The container is placed on a shaker set at speed 70 in a 4°C fridge overnight or longer. The 'purified' smEV pellets are resuspended with additional sterile 1xPBS. The resuspended purified smEV samples are stored at 4°C or at -80°C .

**Example 53: KLH DTH Study**

**[813]** Female 5 week old C57BL/6 mice were purchased from Taconic Biosciences and acclimated at a vivarium for one week. Mice were primed with an emulsion of KLH and CFA (1:1) by subcutaneous immunization on day 0. Mice were orally gavaged daily with smEVs or dosed intraperitoneally with dexamethasone at 1 mg/kg from days 1-8. After dosing on day 8, mice were anaesthetized with isoflurane, left ears were measured for baseline measurements with Fowler calipers and the mice were challenged intradermally with KLH in saline (10 µl) in the left ear and ear thickness measurements were taken at 24 hours. Dose was determined by particle count by NTA.

**[814]** The 24 hour ear measurement results are shown in **Figure 23**. smEVs made from *Megasphaera Sp.* Strain A were compared at two doses, 2E+11 and 2E+07 (based on particles per dose). The smEVs were efficacious, showing decreased ear inflammation 24 hours after challenge.

**[815]** The 24 hour ear measurement results are shown in **Figure 24**. smEVs made from *Megasphaera Sp.* Strain B were compared at two doses, 2E+11 and 2E+07 (based on particles

per dose). The smEVs were efficacious, showing decreased ear inflammation 24 hours after challenge.

**[816]** The 24 hour ear measurement results are shown in **Figure 25**. smEVs made from Selenomonas felix were compared at two doses, 2E+11 and 2E+07 (based on particles per dose). The smEVs were efficacious, showing decreased ear inflammation 24 hours after challenge.

**Example 54: smEV and Gamma-Irradiated whole bacterium U937 Testing Protocol**

**[817]** Cell line preparation: The U937 Monocyte cell line (ATCC) was propagated in RPMI medium with added FBS HEPES, sodium pyruvate, and antibiotic. at 37°C with 5% $CO_2$. Cells were enumerated using a cellometer with live/dead staining to determine viability. Next, Cells were diluted to a concentration of $5x10^5$ cells per ml in RPMI medium with 20nM phorbol-12-myristate-13-acetate (PMA) to differentiate the monocytes into macrophage-like cells. Next, 200 microliters of cell suspension was added to each well of a 96-well plate and incubated 37°C with 5% $CO_2$ for 72hrs. The adherent, differentiated cells were washed and incubated in fresh medium without PMA for 24hrs before experimentation.

**[818]** Experimental Setup: smEVs were diluted to the appropriate concentration in RPMI medium without antibiotics (typically $1x10^5$-$1x10^{10}$). Treatment-free and TLR 2 and 4 agonist control samples were also prepared. The 96-well plate containing the differentiated U937 cells was washed with fresh medium without antibiotics, to remove residual antibiotics. Next, the suspension of smEVs was added to the washed plate. The plate was incubated for 24hrs at 37°C with 5% $CO_2$.

**[819]** Experimental Endpoints: After 24hrs of coincubation, the supernatants were removed from the U937 cells into a separate 96-well plate. The cells were observed for any obvious lysis (plaques) in the wells. Two treatment-free wells did not have the supernatants removed and Lysis buffer was added to the wells and incubated at 37°C for 30 minutes to lyse cells (maximum lysis control). 50 microliters of each supernatant or maximum lysis control was added to a new 96-well plate and cell lysis was determined (CytoTox 96® Non-Radioactive Cytotoxicity Assay, Promega) per manufacturer's instructions. Cytokines were measured from the supernatants using U-plex MSD plates (Meso Scale Discovery) per manufacturer's instructions.

**[820]** Results are shown in **Figure 26**. smEVs from *Megasphaera Sp.* Strain A induce cytokine production from PMA-differentiated U937 cells. U937 cells were treated with smEV at $1x10^6$-$1x10^9$ concentrations as well as TLR2 (FSL) and TLR4 (LPS) agonist controls for 24hrs and cytokine production was measured."Blank" indicates the medium control.

**Example 55: Oral Delivery of *Megasphaera sp.* smEVs in CT26 Tumor Studies, First Representative Oncology Study**

**[821]** Female 8 week old BALB/c mice were acquired from Taconic Biosciences and allowed to acclimate at a vivarium for 3 weeks. On Day 0, mice were anesthetized with isoflurane, and inoculated subcutaneously on the left flank with 1.0e5 CT-26 cells (0.1mL) prepared in PBS and Corning (GFR) Phenol Red-Free Matrigel (1:1). Mice were allowed to rest for 9 days post CT-26 inoculation to allow formation of palpable tumors. On Day 9, tumors were measured using a sliding digital caliper to collect length and width in measurements (in millimeters) to calculate estimated tumor volume ((L x W x W)/2)=TVmm3)). Mice were randomized into different treatment groups with a total of 9 or 10 mice per group. Randomization was done to balance all treatment groups, allowing each group to begin treatment with a similar average tumor volume and standard deviation. Dosing began on Day 10, and ended on Day 22 for 13 consecutive days of dosing. Mice were orally dosed BID with *Megasphaera sp.* Strain AsmEVs, or Q4D intraperitoneally with 200ug anti-mouse PD-1 antibody. Body weight and tumor measurements were collected on a MWF (Monday-Wednesday-Friday) schedule. Dose of smEVs was determined by particle count by NTA. Two mice from the *Megasphaera sp.* smEV group were censored out of the study due to mortality caused by dosing injury.

**[822]** Results are shown in **Figures 27A and 27B.** The Day 22 Tumor Volume Summary compares *Megasphaera sp.* smEV (2e11) against a negative control (Vehicle PBS), and positive control (anti-PD-1). *Megasphaera sp.* smEV (2e11) compared to Vehicle PBS showed statistically significant efficacy and is not significantly different than anti-PD-1. The Tumor Volume Curves show similar growth trends *Megasphaera sp.* smEVs and anti-PD-1, along with sustained efficacy after 13 days of treatment.

**Example 56: Oral Delivery of *Megasphaera sp.* smEVs in CT26 Tumor Studies, Second Representative Oncology Study**

**[823]** Female 8 week old BALB/c mice were acquired from Taconic Biosciences and allowed to acclimate at a vivarium for 1 week. On Day 0, mice were anesthetized with isoflurane, and inoculated subcutaneously on the left flank with 1.0e5 CT-26 cells (0.1mL) prepared in PBS and Corning (GFR) Phenol Red-Free Matrigel (1:1). Mice were allowed to rest for 9 days post CT-26 inoculation to allow formation of palpable tumors. On Day 9, tumors were measured using a sliding digital caliper to collect length and width in measurements (in millimeters) to calculate estimated tumor volume ((L x W x W)/2)=TVmm3)). Mice were randomized into different treatment groups with a total of 9 mice per group. Randomization was done to balance all treatment groups, allowing each group to begin treatment with a similar average tumor volume and standard deviation. Dosing began on Day 10, and ended on Day 23 for 14 consecutive days of dosing. Mice were orally dosed BID and QD with *Megasphaera sp.* Strain A smEVs, or Q4D intraperitoneally with 200ug anti-mouse PD-1 antibody. Body weight and tumor measurements were collected on a MWF schedule. Dose of smEVs was determined by particle count by NTA.

**[824]** Results are shown in **Figures 28A** and **28B.** The Day 23 Tumor Volume Summary compares *Megasphaera sp.* smEVs at 3 doses (2e11, 2e9, and 2e7) BID, as well as *Megasphaera sp.* smEVs (2e11) QD against a negative control (Vehicle PBS), and positive control (anti-PD-1). All *Megasphaera sp.* smEV treatment groups compared to Vehicle PBS show statistically significant efficacy compared to Vehicle (PBS). All Megasphaera sp. smEV doses tested are not significantly different than anti-PD-1. The Tumor Growth Curve shows sustained efficacy of *Megasphaera sp.* smEV treatment groups over 14 days of treatment similar to anti-PD-1.

## Example 57: Isolation of pmEVs from Enterococcus gallinarum strains

**[825]** pmEVs from both Enterococcus gallinarum strains were prepared as follows: Cold MP Buffer (50 mM Tris-HCl pH 7.5 with 100 mM NaCl) was added to frozen cell pellets and pellets were thawed rotating at RT (room temperature) or 4°C. Cells were lysed on the Emulsiflex. The samples were lysd on the Emulsiflex with 4 discrete passes at 24,000 psi. Immediately prior to lysis a proteinase inhibitors, phenylmethylsulfonyl fluoride (PMSF) and benzamidine were added to the sample to a final concentration of 1mM each. Debris and unlysed cells were pelleted: 6,000 x g, 30 min, 40C.

**[826]** pmEVs were purified by FPLC from Low Speed Supernatant (LSS) Setup: A large column (GE XK 26/70) packed with Captocore 700 was used for pmEV purification: 70%

EtOH for sterilization; 0.1X PBS for running buffer; Milli-Q water for washing; 20% EtOH w/ 0.1 M NaOH for cleaning and storage. Benzonase was added to LSS sample and incubate at RT for 30 minutes while rotating (Final concentration of 100 U/ml Benzonase and 1 mM MgCl).LSS from bacterial lysis was kept on ice and at 4C until ready to load into the Superloop.

**[827]** FPLC purification was run: Flow rate was set to 5 ml/min and set delta column pressure to 0.25 psi. Throughout the purification process, the UV absorbance, pressure, and flow rate were monitored. Run was started and sample (Superloop) was manually loaded. When the sample became visible on the chromatogram (~50mAU), the fraction collector was engaged. The entire sample peak was collected.

**[828]** Final pmEV sample was concentrated: Final pmEV fractions were added to clean ultracentrifuge tubes and balance. Tubes were spun at 120,000 x g for 1 hour at 40C. Supernatant was discarded and pellets were resuspended in a minimal volume of sterile PBS.

**Example 58: In vivo data generated with pmEVs**

**[829]** Female 8 week old BALB/c mice were allowed to acclimate at a vivarium for 1 week. On Day 0, mice were anesthetized with isoflurane, and inoculated subcutaneously on the left flank with 1 x $10^5$ CT-26 cells (0.1mL) prepared in PBS and Corning (GFR) Phenol Red-Free Matrigel (1:1). Mice were allowed to rest for 9 days post CT-26 inoculation to allow formation of palpable tumors. On Day 9, tumors were measured using a sliding digital caliper to collect length and width in measurements (in millimeters) to calculate estimated tumor volume ((L x W x W)/2)=TVmm3)). Mice were randomized into different treatment groups with a total of (9) mice per group. Randomization was done to balance all treatment groups, allowing begin each group to begin treatment with a similar average tumor volume and standard deviation. Dosing began on Day 10, and ended on Day 23 for 14 consecutive days of dosing. Mice were orally dosed once daily with the Enterococcus gallinarum pmEVs, or Q4D intraperitoneally with 200 μg anti-mouse PD-1. Body weight and tumor measurements were collected on a MWF schedule.

**[830]** pmEVs were prepared from two strains of Enterococcus gallinarum. One strain was obtained from a JAX mouse; one strain was obtained from a human source. The dose particle count for the pmEVs was 2 x $10^{11}$. The dose was determined as particle count by NTA.

**[831]** **Figure 29** shows tumor volumes after d10 tumors were dosed once daily for 14 days with pmEVs from *E. gallinarum* Strain A.

## Example 59: Negativicutes U937 Results

**[832]** To demonstrate the therapeutic utility of the Negativicutes as a class, representatives from each family in Table 5 were selected and EVs were harvested from culture supernatants. The EVs were added to PMA-differentiated U937 cells and incubated for 24hrs. Cytokine release was measured by MSD ELISA.

**[833]** The results are shown in **Figures 30-34**. The broad robust stimulation exhibited by each strain's EVs follows a similar profile between strains. TLR2 (FSL) and TLR4 (LPS) agonists were used as controls. Blank indicates the media control.

**Table 5**

| **Strain Name** | **Family within Negativicutes Class** |
|---|---|
| *Megasphaera sp.* Strain A | Veillonellaceae |
| *Megasphaera sp.* Strain B | Veillonellaceae |
| *Selenomonas felix* | Selenomonadaceae |
| *Acidaminococcus intestini* | Acidaminococcaceae |
| *Propionospora sp.* | Sporomusaceae |

## Incorporation by Reference

**[834]** All publications patent applications mentioned herein are hereby incorporated by reference in their entirety as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference. In case of conflict, the present application, including any definitions herein, will control.

**Equivalents**

**[835]** Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

What is claimed is:

1. A pharmaceutical composition comprising isolated processed microbial extracellular vesicles (pmEVs).

2. The pharmaceutical composition of claim 1, wherein at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% of the microbial-derived content of the pharmaceutical composition is pmEVs.

3. The pharmaceutical composition of claim 1 or claim 2 for use in the treatment of a disease via immune suppression.

4. The pharmaceutical composition of claim 1 or claim 2 for use in the treatment of a disease via immune activation.

5. The pharmaceutical composition of claim 1 or claim 2 for use in the treatment of a disease via activation or enhancement of one or more immune responses in the subject.

6. The pharmaceutical composition of claim 1 or claim 2 for use in the treatment of a disease via promotion of immune suppression in the subject.

7. The pharmaceutical composition of any one of claims 2 to 6, wherein the disease is a cancer, an autoimmune disease, an inflammatory disease, or a metabolic disease.

8. The pharmaceutical composition of any one of claims 1 to 7, comprising a therapeutically effective amount of the pmEVs.

9. The pharmaceutical composition of any one of claims 1 to 8, wherein the composition activates innate antigen presenting cells.

10. The pharmaceutical composition of any one of claims 1 to 9, wherein the composition has one or more beneficial immune effects outside the gastrointestinal tract when orally administered.

11. The pharmaceutical composition of any one of claims 1 to 10, wherein the composition modulates immune effects outside the gastrointestinal tract in the subject when orally administered.

12. The pharmaceutical composition of any one of claims 1 to 11, wherein the composition comprises pmEVs from one strain of bacteria.

13. The pharmaceutical composition of any one of claims 1 to 12, wherein the pmEVs are lyophilized (e.g., the lyophilized product further comprises a pharmaceutically acceptable excipient).

14. The pharmaceutical composition of any one of claims 1 to 13, wherein the pmEVs are gamma irradiated.

15. The pharmaceutical composition of any one of claims 1 to 14, wherein the pmEVs are UV irradiated.

16. The pharmaceutical composition of any one of claims 1 to 15, wherein the pmEVs are heat inactivated.

17. The pharmaceutical composition of claim 16, wherein the pmEVs are heat inactivated at about 50°C for two hours or at about 90°C for two hours.

18. The pharmaceutical composition of any one of claims 1 to 17, wherein the pmEVs are acid treated.

19. The pharmaceutical composition of any one of claims 1 to 18, wherein the pmEVs are oxygen sparge.

20. The pharmaceutical composition of claim 19, wherein the pmEVs are ozygen sparged at about 0.1 vvm for at least two hours.

21. The pharmaceutical composition of any one of claims 1 to 20, wherein the dose of pmEVs is about $2x10^6$ to about $2x10^{16}$ particles.

22. The pharmaceutical composition of any one of claims 1 to 21, wherein the dose of pmEVs is about 5 mg to about 900 mg total protein.

23. The pharmaceutical composition of any one of claims 1 to 22, wherein the pharmaceutical composition is a solid dose form.

24. The pharmaceutical composition of claim 23, wherein the solid dose form comprises a tablet, a minitablet, a capsule, a pill, or a powder, or a combination of the foregoing.

25. The pharmaceutical composition of claim 23 or 24, wherein the solid dose form further comprises a pharmaceutically acceptable excipient.

26. The pharmaceutical composition of any one of claims 23 to 25, wherein the solid dose form comprises an enteric coating.

27. The pharmaceutical composition of any one of claims 23 to 26, wherein the solid dose form is formulated for oral administration.

28. The pharmaceutical composition of any one of claims 1 to 22, wherein the pharmaceutical composition is in the form of a suspension.

29. The pharmaceutical composition of claim 28, wherein the suspension is formulated for oral administration.

30. The pharmaceutical composition of claim 29, wherein the suspension comprises PBS, and optionally, sucrose or glucose.

31. The pharmaceutical composition of claim 28, wherein the suspension is formulated for intravenous, intraperitoneal, or intratumoral administration.

32. The pharmaceutical composition of claim 31, wherein the suspension comprises PBS.

33. The pharmaceutical composition of any one of claims 28 to 32, wherein the suspension further comprises a pharmaceutically acceptable excipient or a buffer.

34. The pharmaceutical composition of any one of claims 1 to 33, wherein the pmEvs are from Gram positive bacteria.

35. The pharmaceutical composition of any one of claims 1 to 33, wherein the pmEvs are from Gram negative bacteria.

36. The pharmaceutical composition of claim 35, wherein the Gram negative bacteria belongs to the class Negativicutes.

37. The pharmaceutical composition of any one of claims 1 to 36, wherein the pmEVs are from aerobic bacteria, anaerobic bacteria, acidophile bacteria, alkaliniphile bacteria, neutralophile bacteria, fastidious bacteria, nonfastidiouius bacteria, or a combination thereof.

38. The pharmaceutical composition of any one of claims 1 to 37, wherein the pmEVs are from one or more bacterial strain listed in Table 1, Table 2 or Table 3.

39. The pharmaceutical composition of any one of claims 1 to 38, wherein the composition further comprises one or more additional therapeutic agents.

40. Use of a pharmaceutical composition of any one of claims 1 to 39 for the preparation of a medicament for the treatment of a disease.

41. The use of claim 49, wherein the disease is a cancer, an autoimmune disease, an inflammatory disease, a dysbiosis, and/or a metabolic disease.

42. A method of treating a subject comprising administering to the subject a pharmaceutical composition of any one of claims 1 to 41.

43. The method of claim 42, wherein the pmEVs are from bacteria that have been gamma irradiated, UV irradiated, heat inactivated, acid treated, oxygen sparged, or a combination thereof.

44. The method of claim 42, wherein the pmEVs are from live bacteria.

45. The method of any one of claims 42 to 44, wherein the composition activates or enhances of one or more immune responses in the subject.

46. The method of claim 45, wherein the one or more immune responses comprises a systemic immune response.

47. The method of any one of claims 42 to 44, wherein the composition suppresses an immune response in the subject.

48. The method of any one of claims 42 to 44, wherein the composition promotes immune activation in the subject.

49. The method of any one of claims 42 to 48, wherein the pharmaceutical composition comprising the pmEVs has comparable potency or increased potency compared to a pharmaceutical composition that contains whole microbes from the same bacterial strain from which the pmEVs were produced).

50. The method of any one of claims 42 to 48, wherein the pharmaceutical composition comprising the pmEVs has more therapeutically active microbial material compared to a pharmaceutical composition that contains whole microbesfrom which the pmEVs were obtained.

51. The method of any one of claims 42 to 50, wherein the subject is in need of treatment for a cancer.

52. The method of any one of claims 42 to 50, wherein the subject is in need of treatment for an autoimmune disease and/or an inflammatory disease.

53. The method of any one of claims 42 to 50, wherein the subject is in need of treatment for a dysbiosis.

54. The method of any one of claims 42 to 50, wherein the subject is in need of treatment for a metabolic disease.

55. The method of any one of claims 42 to 50, wherein the pharmaceutical composition is administered in combination with an additional therapeutic agent.

56. The method of any one of claims 42 to 55, wherein the composition comprises pmEVs from one strain of bacteria.

57. The method of any one of claims 42 to 56, wherein the pmEVs are lyophilized.

58. The method of any one of claims 42 to 57, wherein the pharmaceutical composition is orally administered.

59. The method of any one of claims 42 to 57, wherein the pharmaceutical composition is administered intravenously.

60. The method of any one of claims 42 to 57, wherein the pharmaceutical composition is administered intratumorally.

61. The method of any one of claims 42 to 57, wherein the pharmaceutical composition is administered subtumorally.

62. The method of any one of claims 42 to 57, wherein the pharmaceutical composition is administered by injection.

63. A method for preparing a pharmaceutical composition comprising pmEVs in a suspension, the method comprising: combining pmEVs with a pharmaceutically acceptable buffer, thereby preparing the pharmaceutical composition.

64. The method of claim 63, wherein the pharmaceutically acceptable buffer comprises PBS.

65. The method of claim 63 or 64, wherein the suspension further comprises sucrose or glucose.

66. The method of any one of claims 63 to 65, wherein the pmEVs comprise about $2x10^6$ to about $2x10^{16}$ particles of pmEVs.

67. The method of any one of claims 63 to 66, wherein the pmEVs comprise about 5 mg to about 900 mg total protein.

68. A pharmaceutical composition prepared by the method of any one of claims 62 to 67.

69. A method for preparing a solid dose form of pharmaceutical composition comprising pmEVs (e.g., a therapeutically effective amount thereof) in a solid dose form, the method comprising:

a) combining pmEVs with a pharmaceutically acceptable excipient; and

b) compressing the combined pmEVs and pharmaceutically acceptable excipient; thereby preparing a solid dose form of a pharmaceutical composition.

70. The method of claim 69, further comprising enterically coating the solid dose form.

71. The method of claim 69 or 70, wherein the solid dose form comprises a tablet or a minitablet.

72. The method of any one of claims 69 to 71, wherein the composition comprises pmEVs from one strain of bacteria.

73. The method of any one of claims 69 to 72, wherein the pmEVs are lyophilized.

74. The method of any one of claims 69 to 73, wherein the pmEVs comprise about $2x10^6$ to about $2x10^{16}$ particles.

75. The method of any one of claims 69 to 74, wherein the pmEVs comprise about 5 mg to about 900 mg total protein.

76. A pharmaceutical composition prepared by the method of any one of claims 69 to 75.

Figure 1

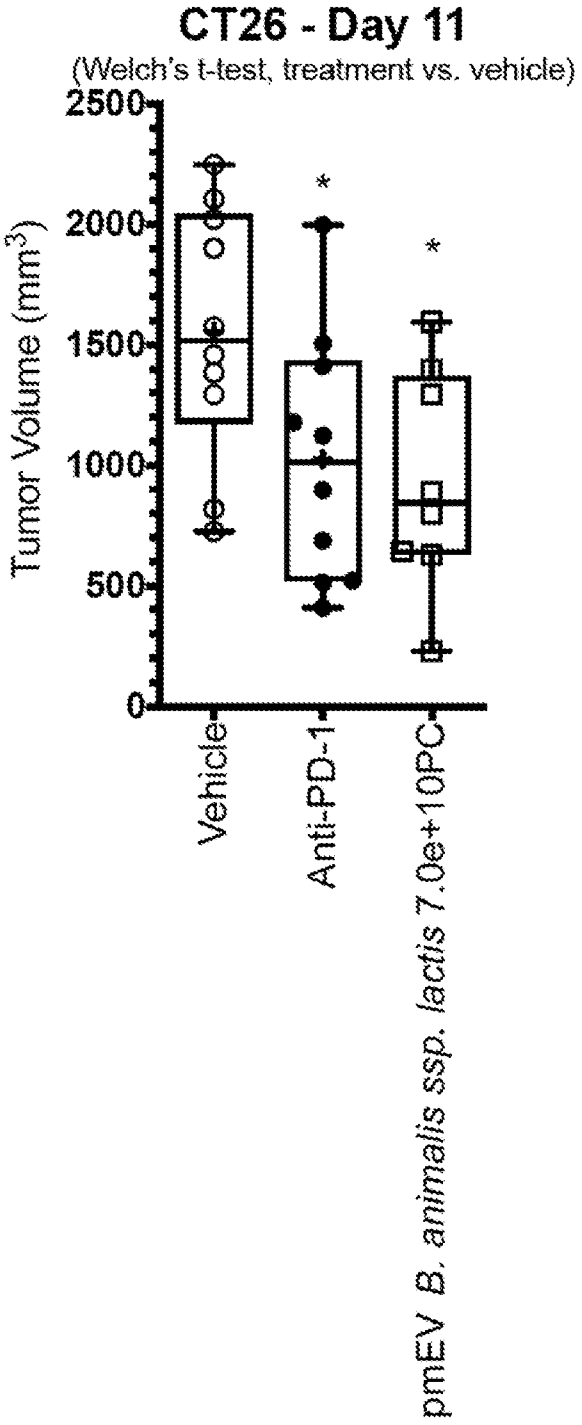

CT26 - Day 11
(Welch's t-test, treatment vs. vehicle)
Tumor Volume (mm3)
2500
2000
1500
1000
500
0
*
*
Vehicle
Anti-PD-1
pmEV B. animalis ssp. lactis 7.0e+10PC

Figure 2

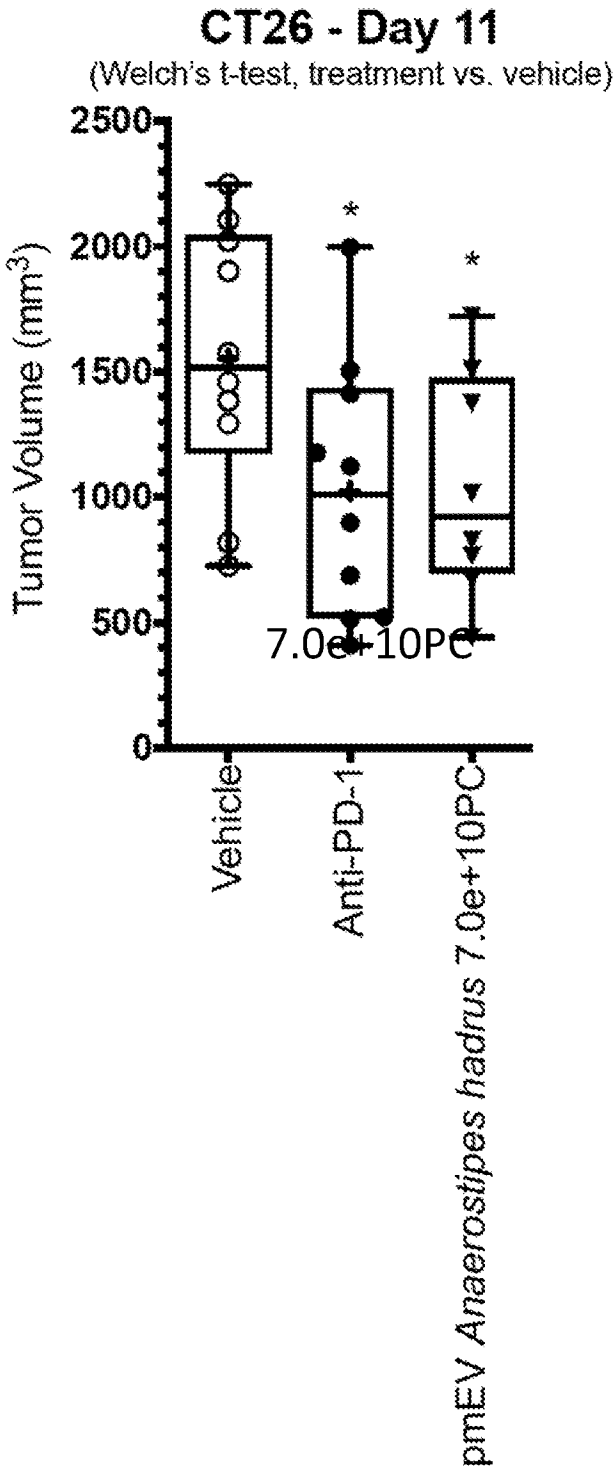

CT26 - Day 11
(Welch's t-test, treatment vs. vehicle)
Tumor Volume (mm3)
2500
2000
1500
1000
500
0
*
*
7.0e+10PC
Vehicle
Anti-PD-1
pmEV Anaerostipes hadrus 7.0e+10PC

Figure 3

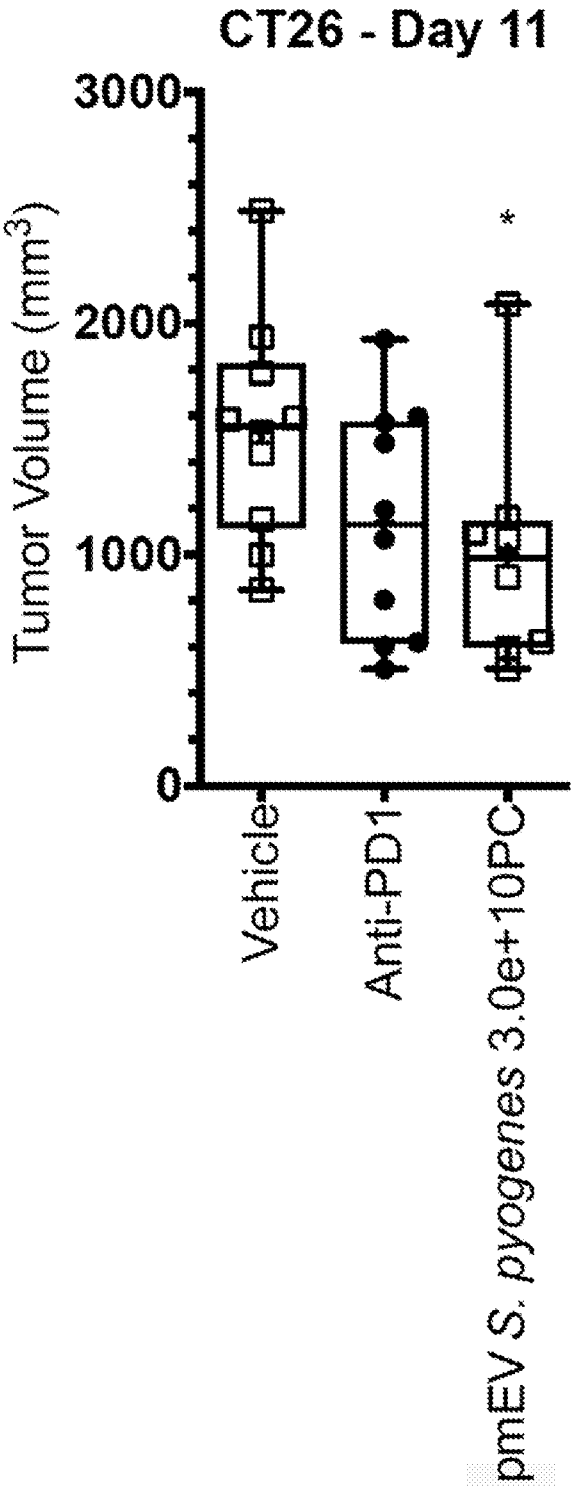

CT26 - Day 11
Tumor Volume (mm3)
3000
2000
1000
0
*
Vehicle
Anti-PD1
pmEV S. pyogenes 3.0e+10PC

Figure 4

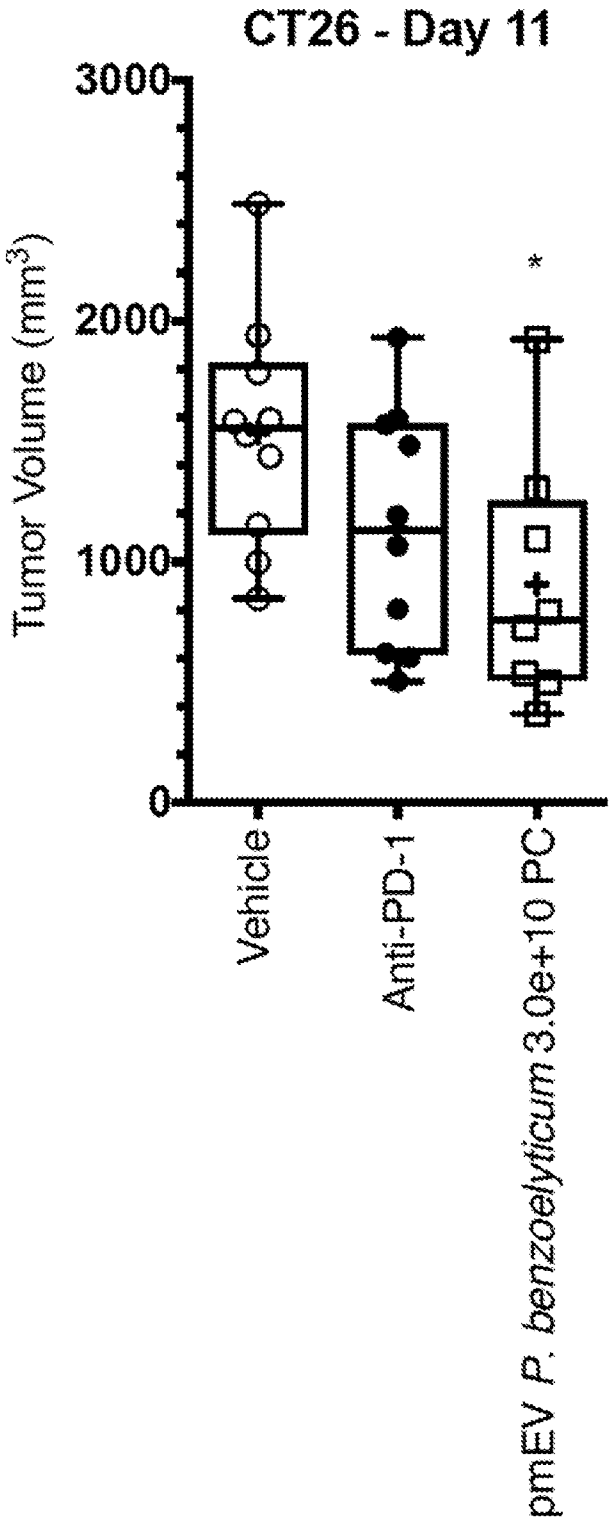

CT26 - Day 11
Tumor Volume (mm3)
3000
2000
1000
0
*
Vehicle
Anti-PD-1
pmEV P. benzoelyticum 3.0e+10 PC

Figure 5

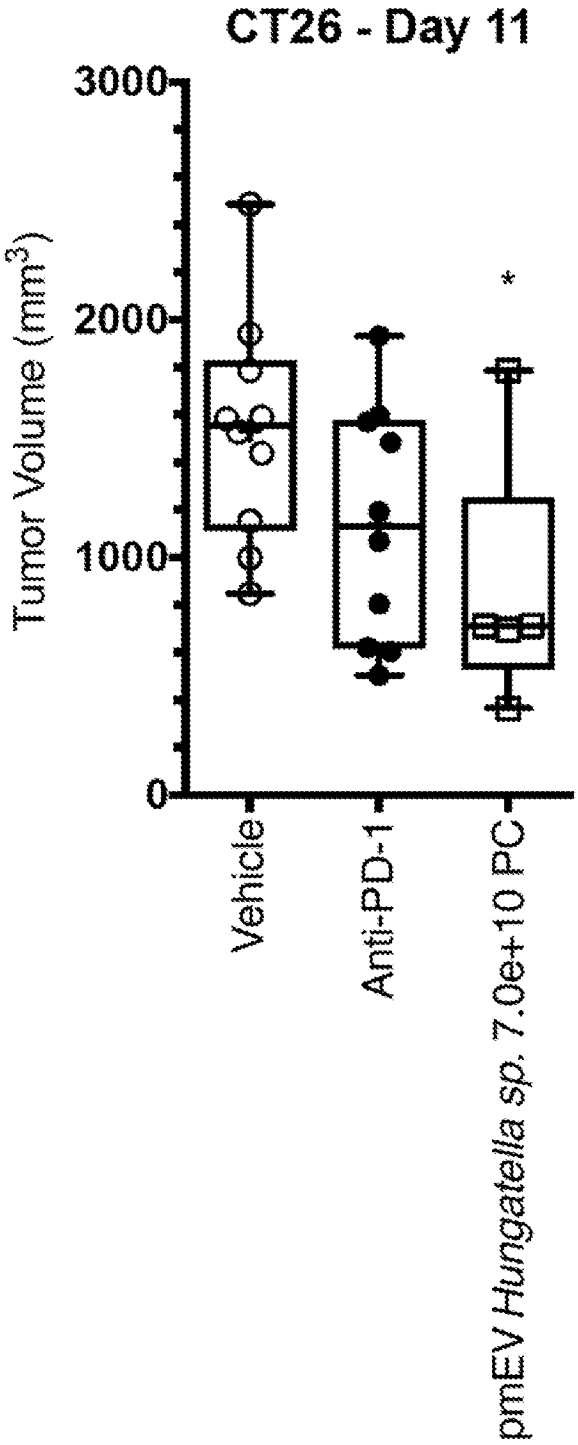

CT26 - Day 11
Tumor Volume (mm3)
3000
2000
1000
0
*
Vehicle
Anti-PD-1
pmEV Hungatella sp. 7.0e+10 PC

Figure 6

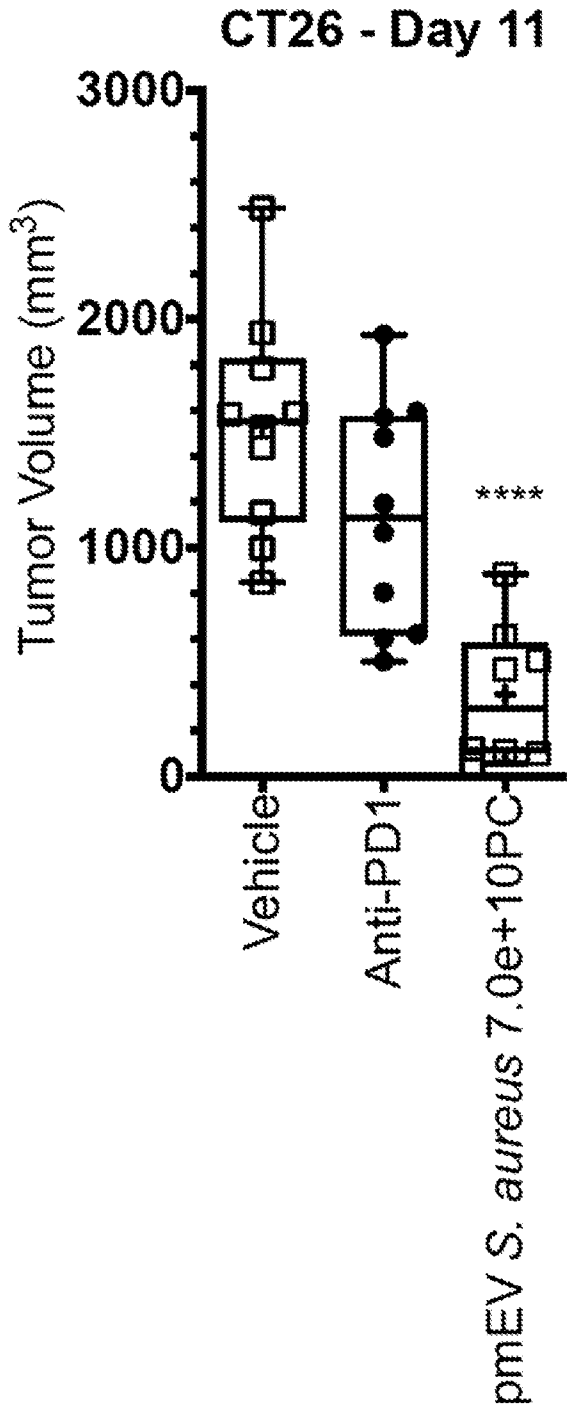

CT26 - Day 11
Tumor Volume (mm3)
3000
2000
1000
0
****
Vehicle
Anti-PD1
pmEV S. aureus 7.0e+10PC

Figure 7

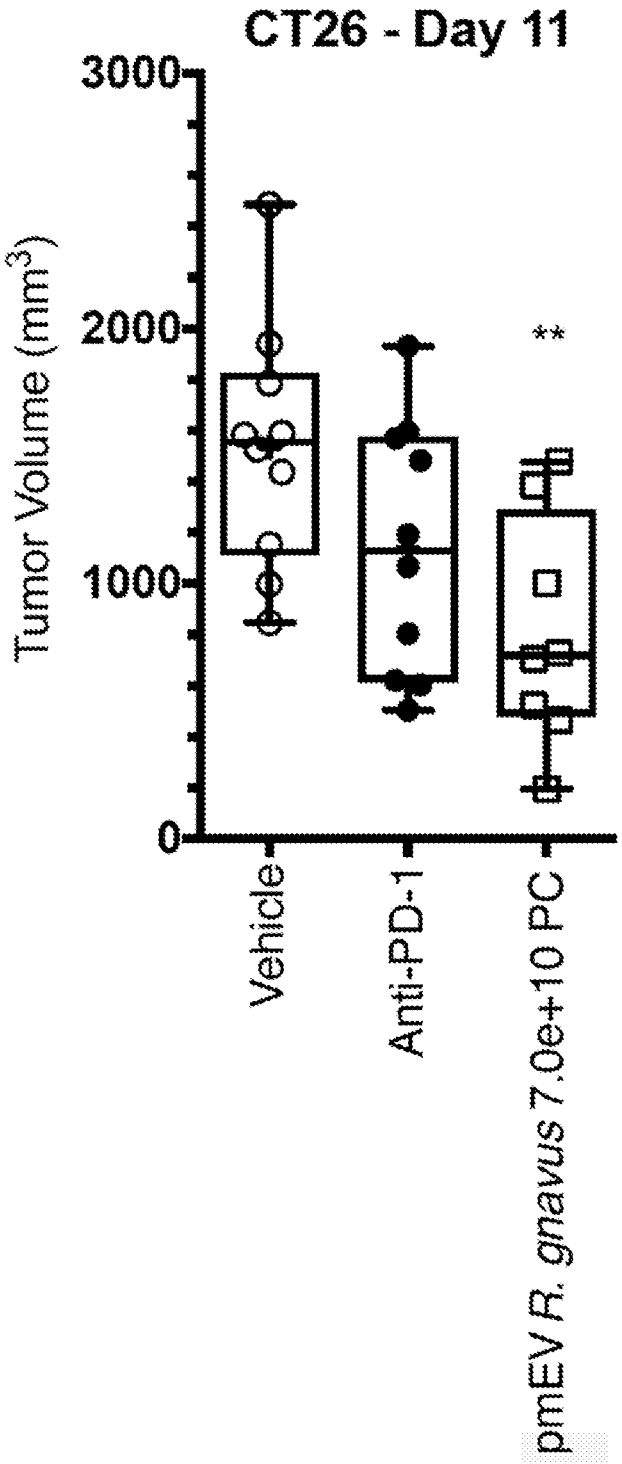

CT26 - Day 11
Tumor Volume (mm3)
3000
2000
1000
0
**
Vehicle
Anti-PD-1
pmEV R. gnavus 7.0e+10 PC

Figure 8

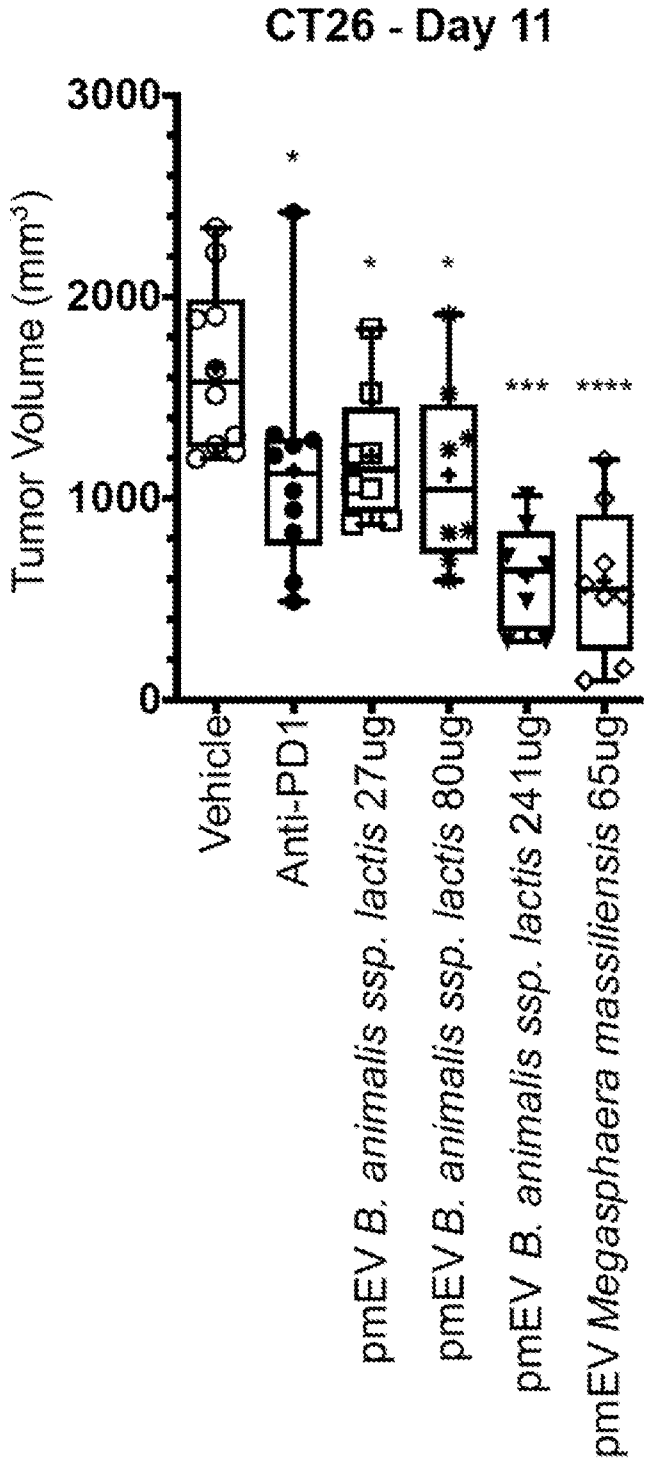

CT26 - Day 11
Tumor Volume (mm³)
3000
2000
1000
0
*
*
*
***
****
Vehicle
Anti-PD1
pmEV B. animalis ssp. lactis 27ug
pmEV B. animalis ssp. lactis 80ug
pmEV B. animalis ssp. lactis 241ug
pmEV Megasphaera massiliensis 65ug

Figure 9

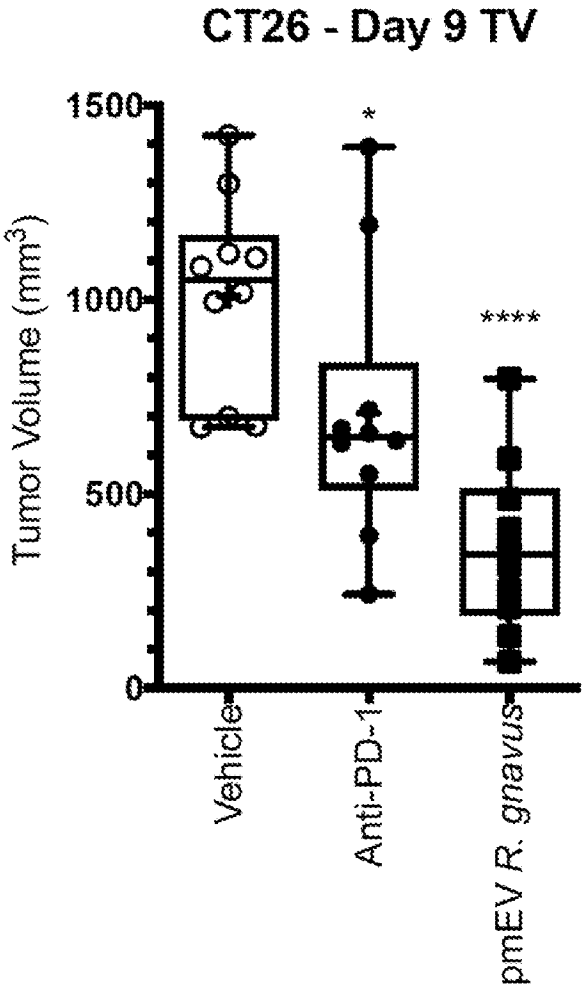

CT26 - Day 9 TV
Tumor Volume (mm3)
1500
1000
500
0
*
****
Vehicle
Anti-PD-1
pmEV R. gnavus

Figure 10

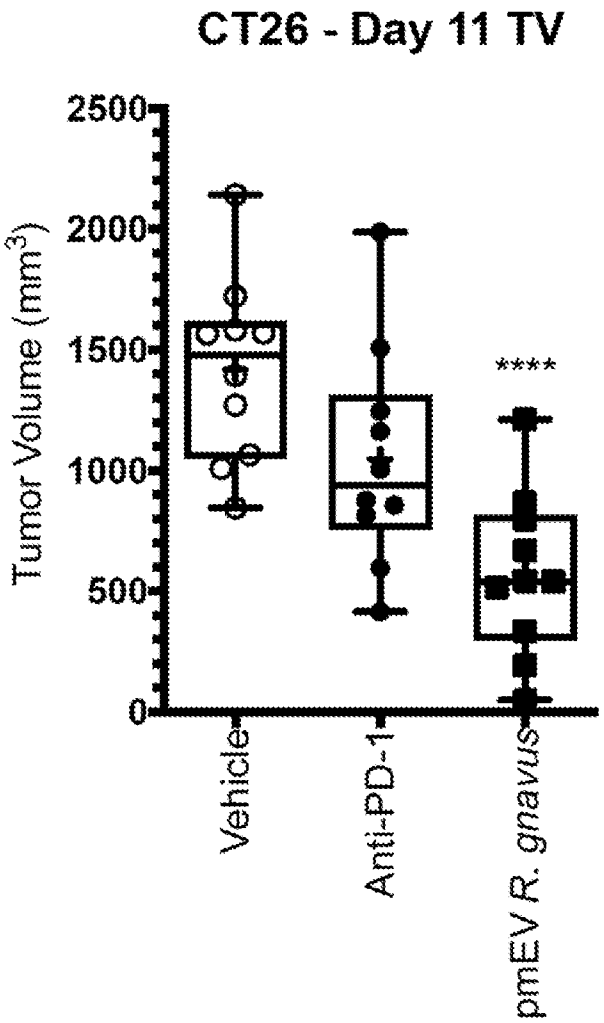

CT26 - Day 11 TV
Tumor Volume (mm3)
2500
2000
1500
1000
500
0
****
Vehicle
Anti-PD-1
pmEV R. gnavus

Figure 11

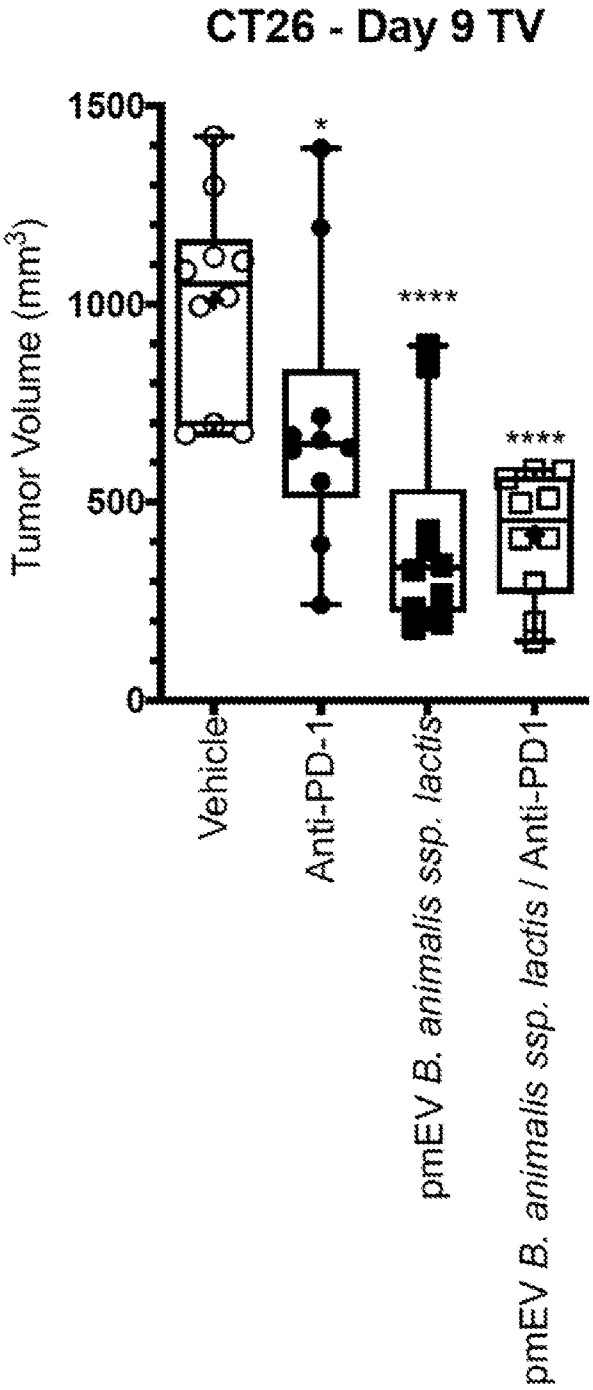

CT26 - Day 9 TV
Tumor Volume (mm3)
1500
1000
500
0
*
****
****
Vehicle
Anti-PD-1
pmEV B. animalis ssp. lactis
pmEV B. animalis ssp. lactis / Anti-PD1

Figure 12

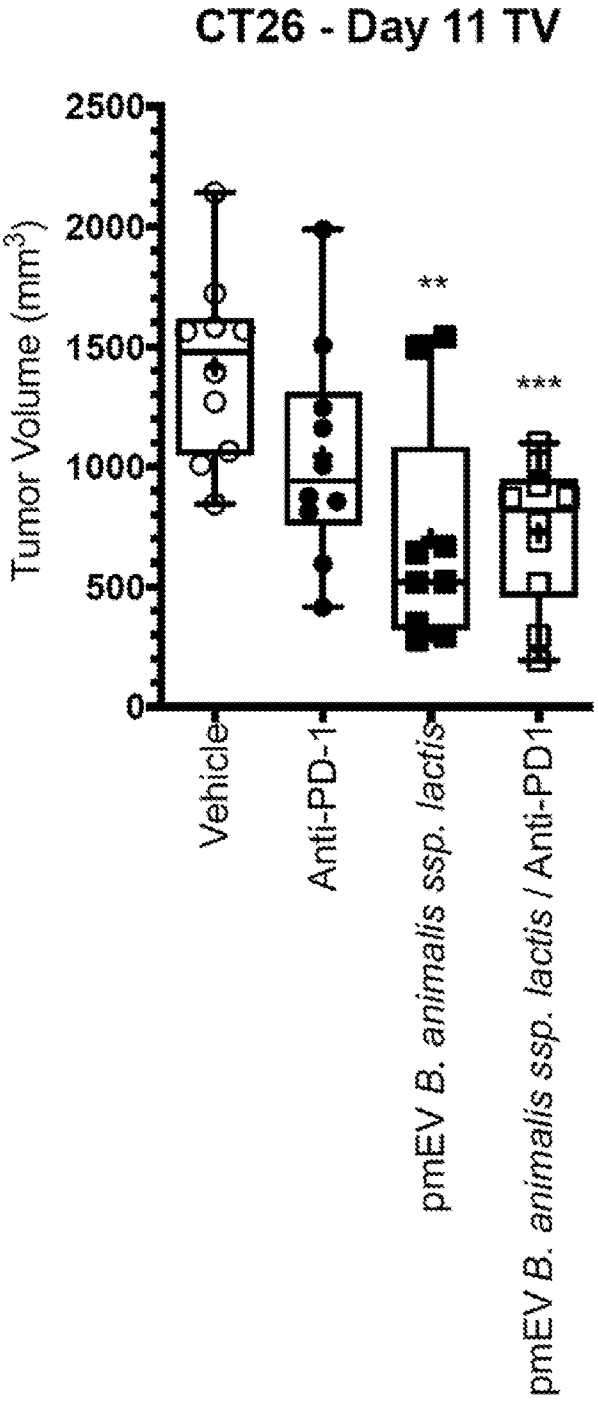

CT26 - Day 11 TV
Tumor Volume (mm3)
2500
2000
1500
1000
500
0
**
***
Vehicle
Anti-PD-1
pmEV B. animalis ssp. lactis
pmEV B. animalis ssp. lactis / Anti-PD1

Figure 13

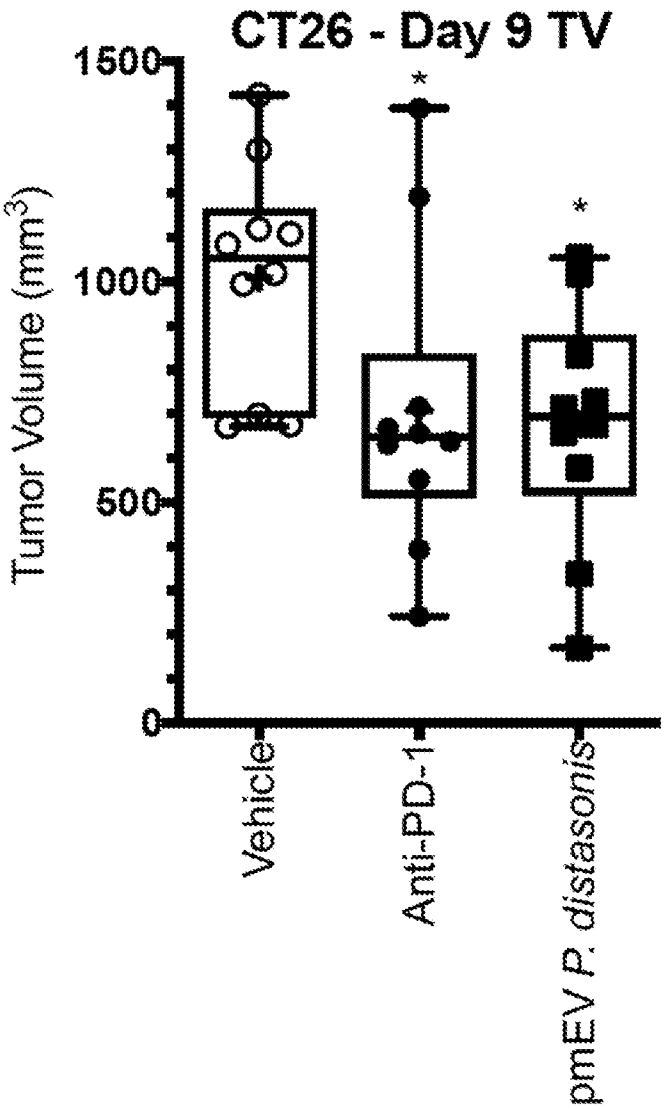

CT26 - Day 9 TV
Tumor Volume (mm3)
1500
1000
500
0
*
*
Vehicle
Anti-PD-1
pmEV P. distasonis

Figure 14

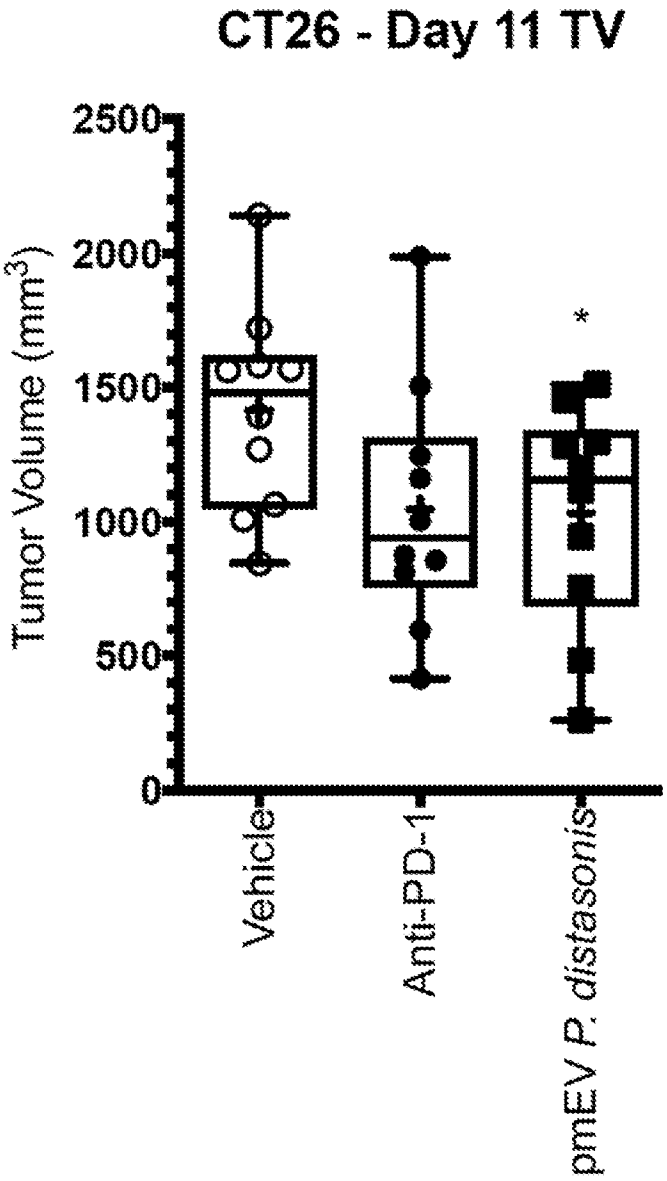

CT26 - Day 11 TV
Tumor Volume (mm3)
2500
2000
1500
1000
500
0
*
Vehicle
Anti-PD-1
pmEV P. distasonis

Figure 15

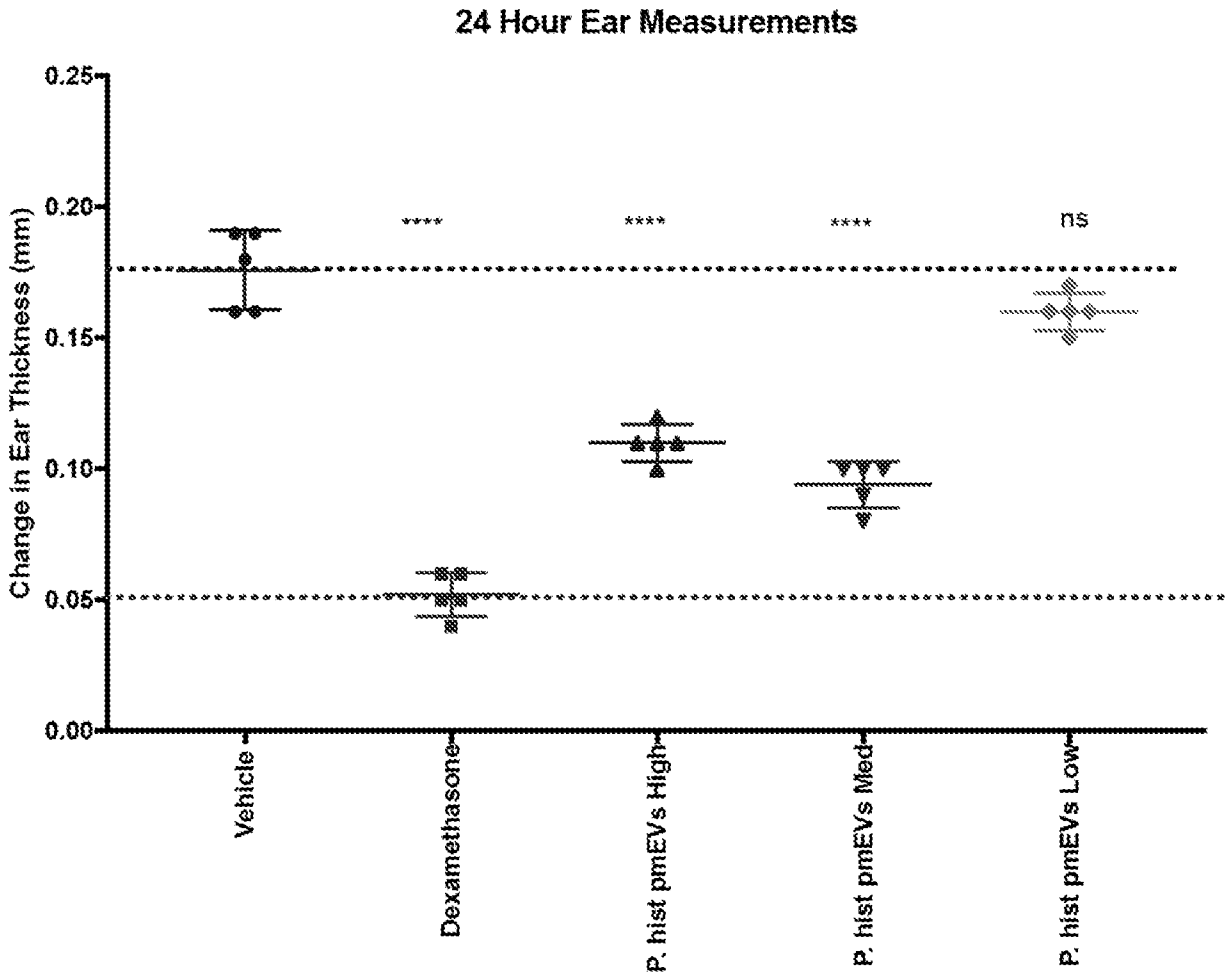

24 Hour Ear Measurements
Change in Ear Thickness (mm)
0.25
0.20
0.15
0.10
0.05
0.00
****
****
****
ns
Vehicle
Dexamethasone
P. hist pmEVs High
P. hist pmEVs Med
P. hist pmEVs Low

Figure 16

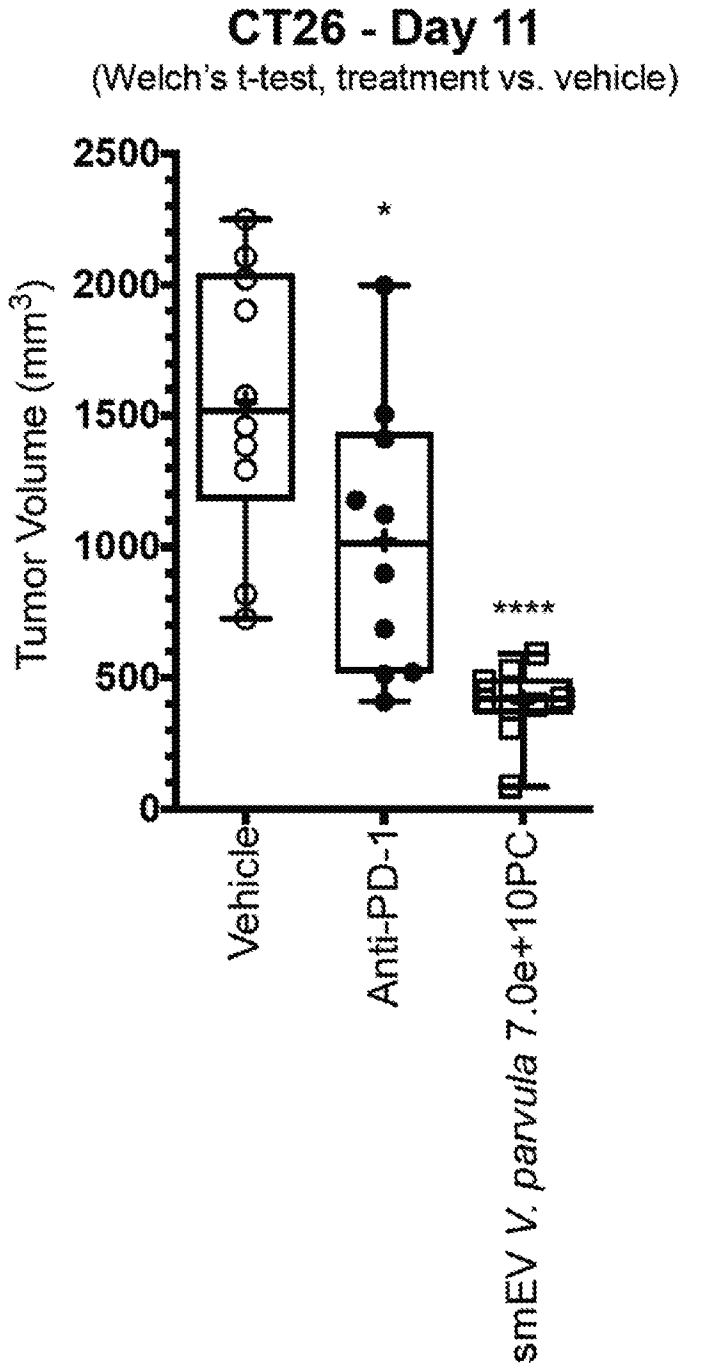

CT26 - Day 11
(Welch's t-test, treatment vs. vehicle)
Tumor Volume (mm3)
2500
2000
1500
1000
500
0
*
****
Vehicle
Anti-PD-1
smEV V. parvula 7.0e+10PC

Figure 17

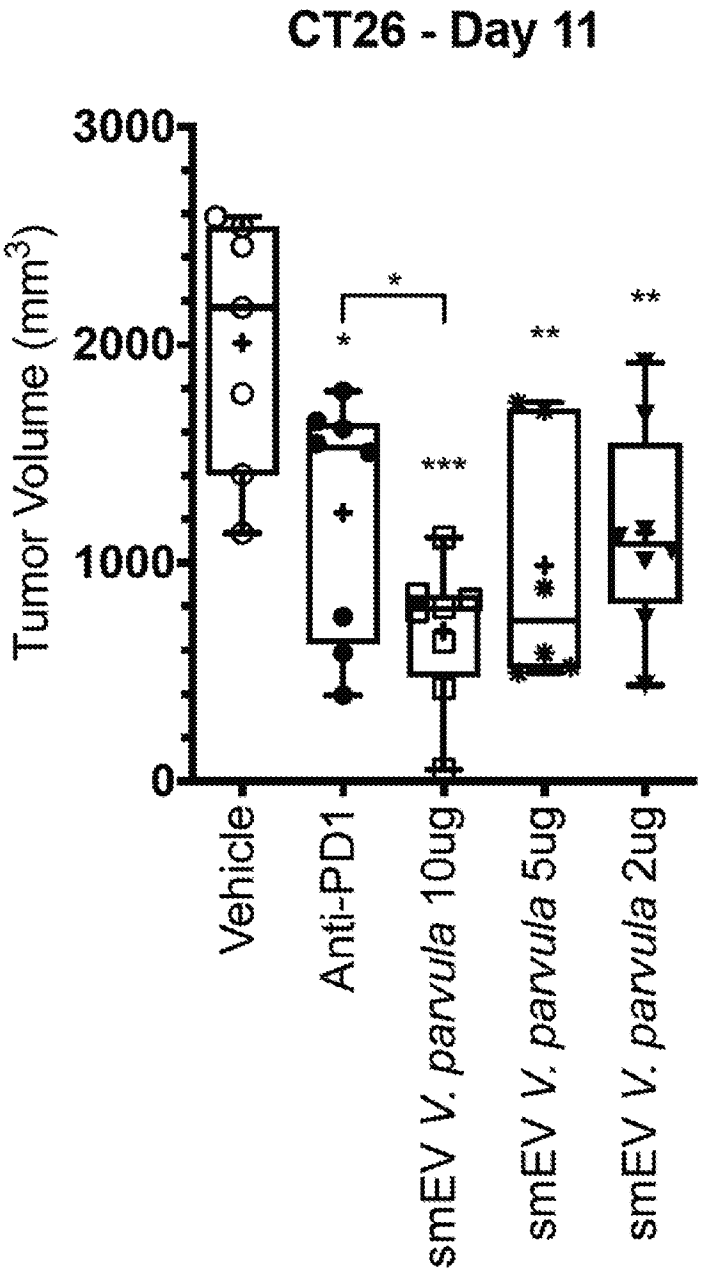

CT26 - Day 11
Tumor Volume (mm3)
3000
2000
1000
0
*
*
***
**
**
Vehicle
Anti-PD1
smEV V. parvula 10ug
smEV V. parvula 5ug
smEV V. parvula 2ug

Figure 18

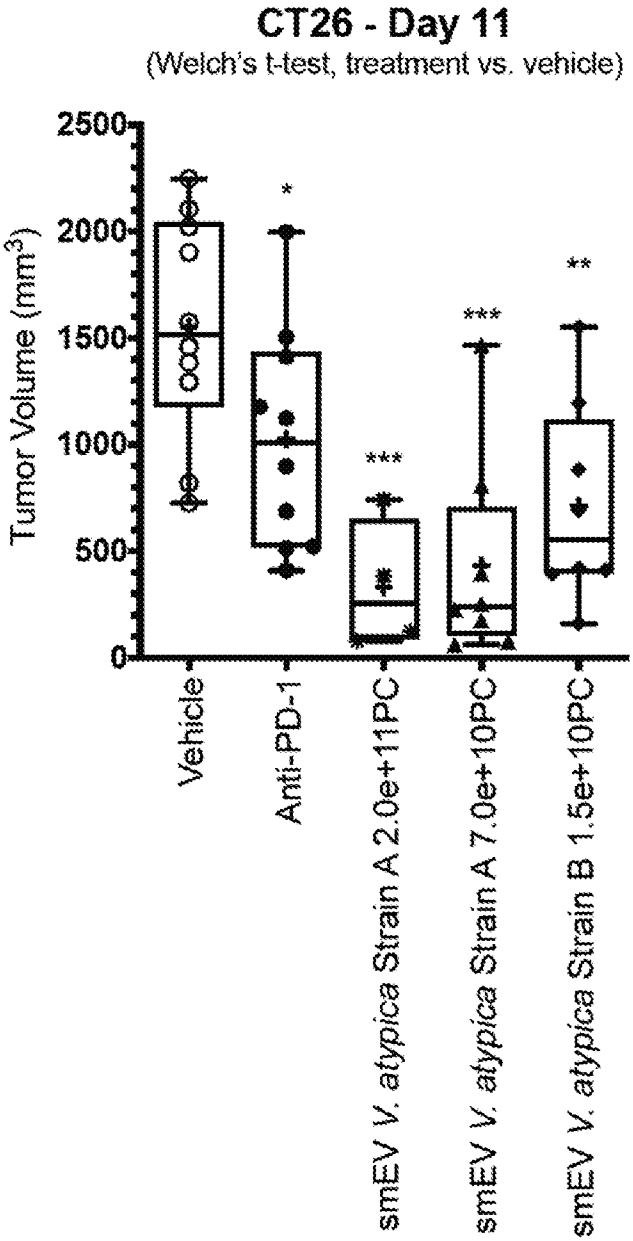

CT26 - Day 11
(Welch's t-test, treatment vs. vehicle)
Tumor Volume (mm3)
2500
2000
1500
1000
500
0
*
***
***
**
Vehicle
Anti-PD-1
smEV V. atypica Strain A 2.0e+11PC
smEV V. atypica Strain A 7.0e+10PC
smEV V. atypica Strain B 1.5e+10PC

Figure 19

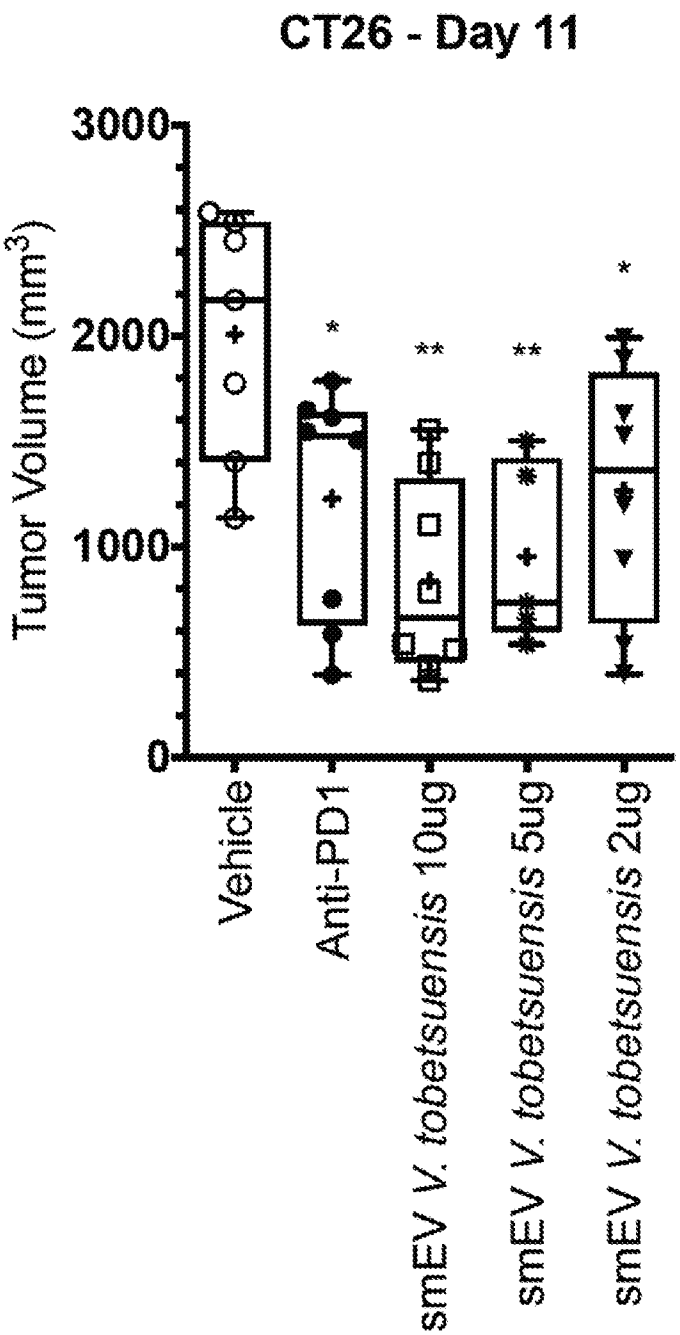

CT26 - Day 11
Tumor Volume (mm3)
3000
2000
1000
0
*
**
**
*
Vehicle
Anti-PD1
smEV V. tobetsuensis 10ug
smEV V. tobetsuensis 5ug
smEV V. tobetsuensis 2ug

Figure 20

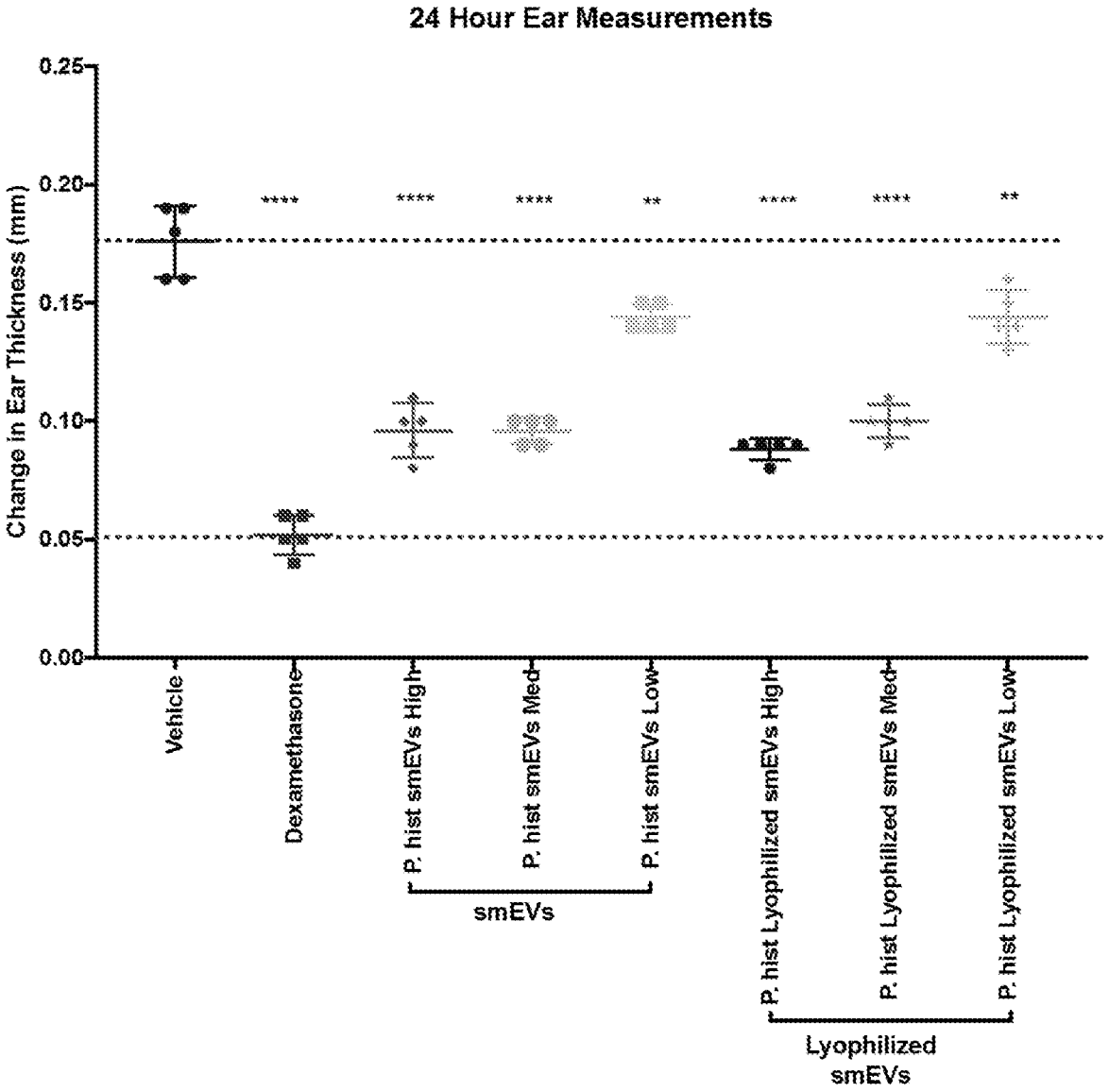

24 Hour Ear Measurements
Change in Ear Thickness (mm)
0.25
0.20
0.15
0.10
0.05
0.00
****
****
****
**
****
****
**
Vehicle
Dexamethasone
P. hist smEVs High
P. hist smEVs Med
P. hist smEVs Low
smEVs
P. hist Lyophilized smEVs High
P. hist Lyophilized smEVs Med
P. hist Lyophilized smEVs Low
Lyophilized smEVs

Figure 21

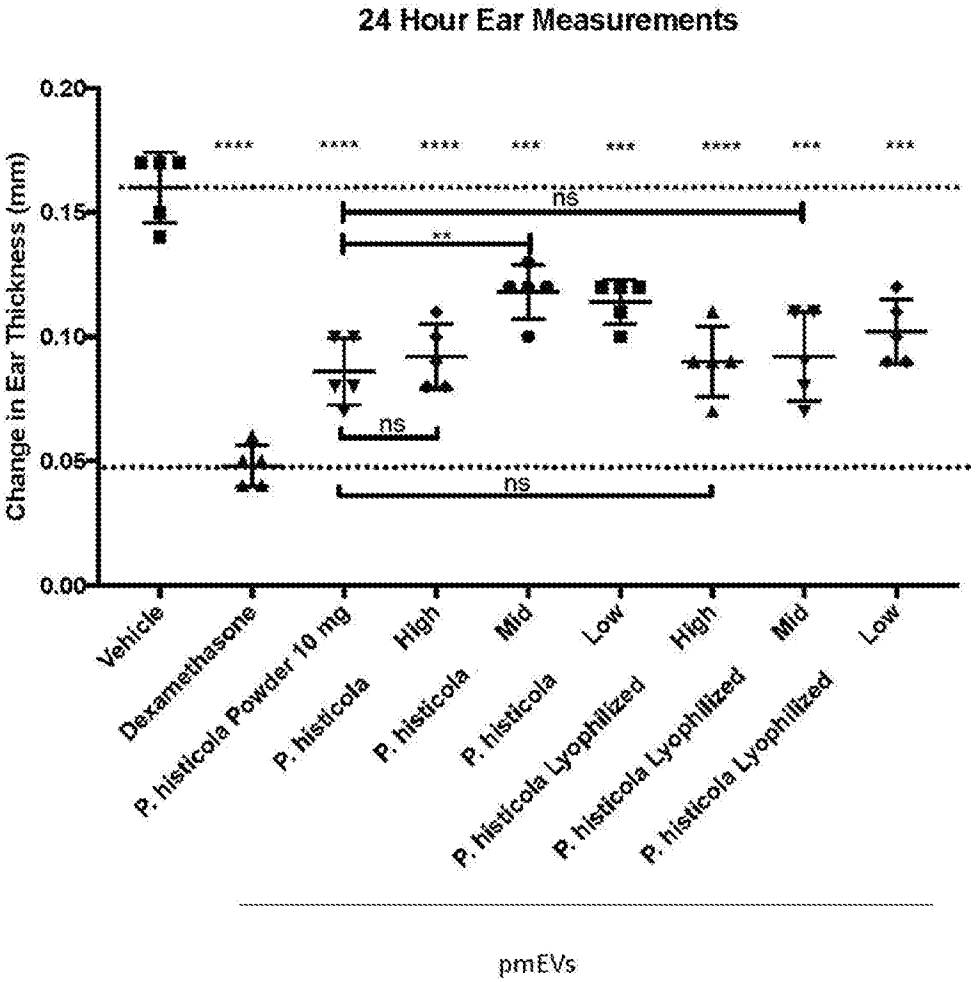

24 Hour Ear Measurements
Change in Ear Thickness (mm)
0.20
0.15
0.10
0.05
0.00
****
****
****
***
***
****
***
***
ns
**
ns
ns
Vehicle
Dexamethasone
P. histicola Powder 10 mg
P. histicola High
P. histicola Mid
P. histicola Low
P. histicola Lyophilized High
P. histicola Lyophilized Mid
P. histicola Lyophilized Low
pmEVs

Figure 22

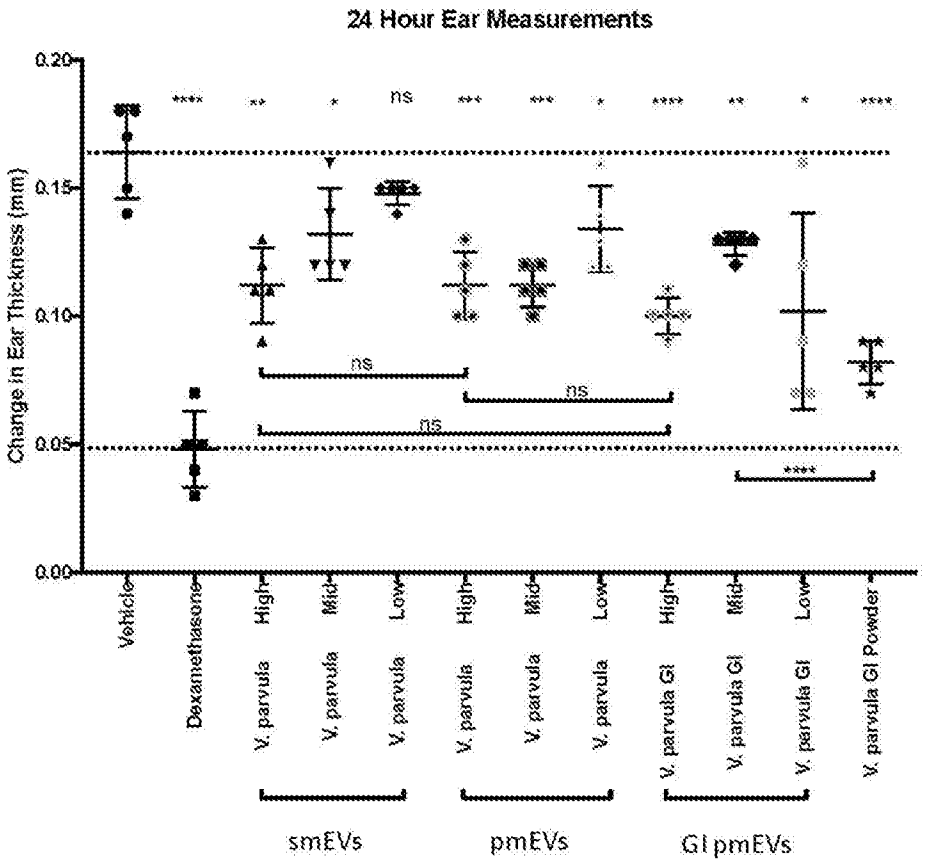

24 Hour Ear Measurements
Change in Ear Thickness (mm)
0.20
0.15
0.10
0.05
0.00
****
**
*
ns
***
***
*
****
**
*
****
ns
ns
ns
****
Vehicle
Dexamethasone
High
Mid
Low
V. parvula
V. parvula
V. parvula
High
Mid
Low
V. parvula
V. parvula
V. parvula
High
Mid
Low
V. parvula GI
V. parvula GI
V. parvula GI
V. parvula GI Powder
smEVs
pmEVs
GI pmEVs

Figure 23

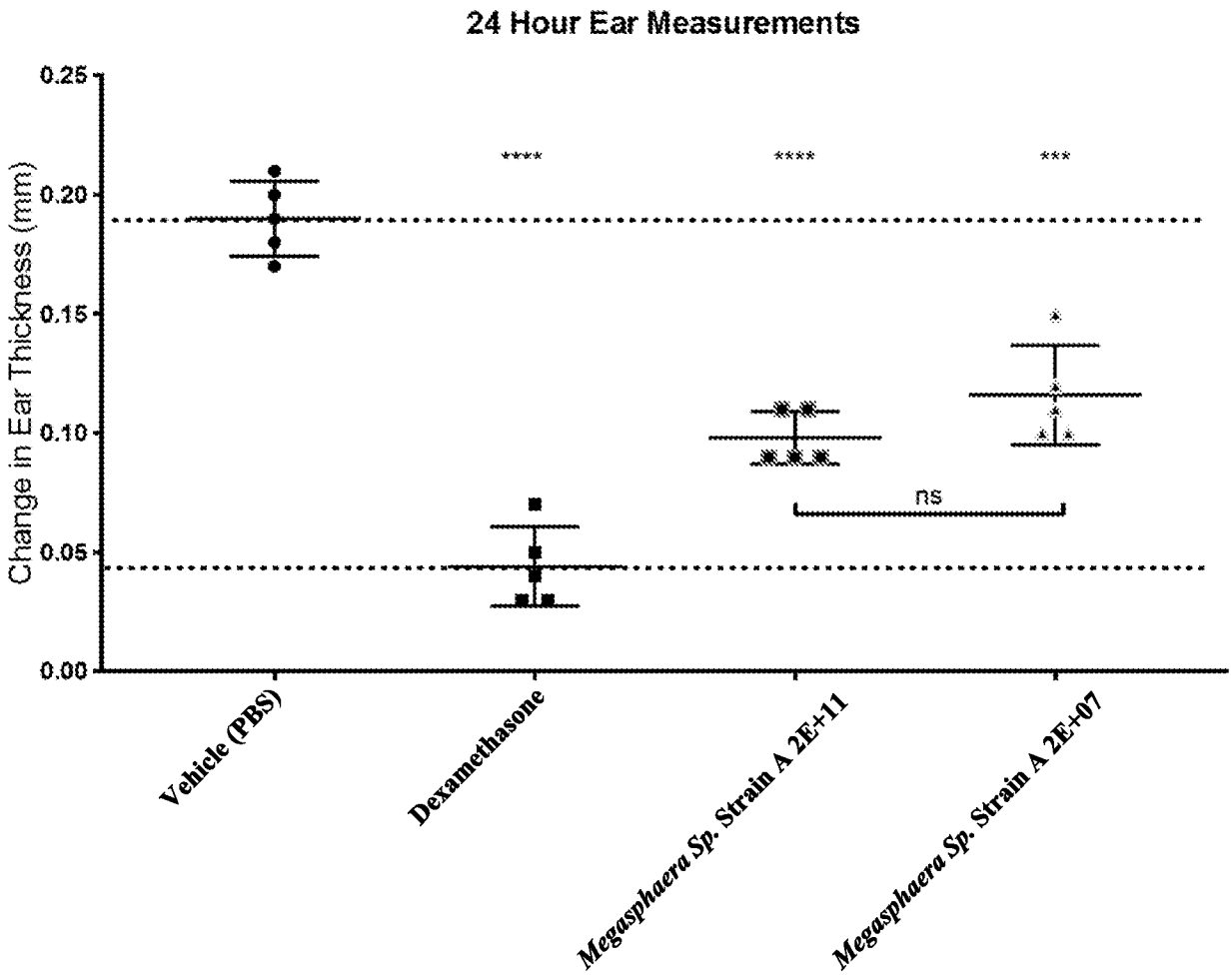

24 Hour Ear Measurements
Change in Ear Thickness (mm)
0.25
0.20
0.15
0.10
0.05
0.00
****
****
***
ns
Vehicle (PBS)
Dexamethasone
Megasphaera Sp. Strain A 2E+11
Megasphaera Sp. Strain A 2E+07

Figure 24

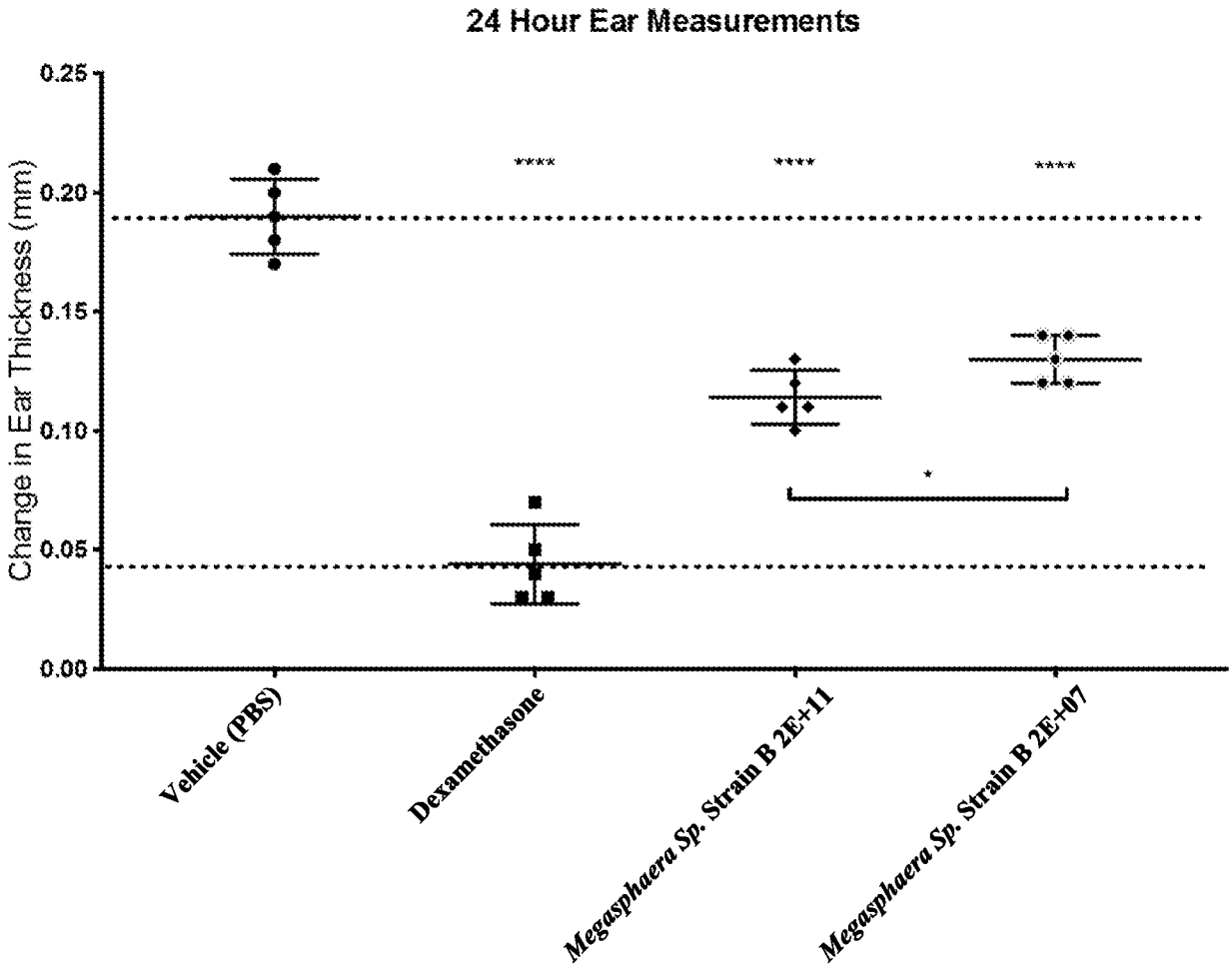

24 Hour Ear Measurements
Change in Ear Thickness (mm)
0.25
0.20
0.15
0.10
0.05
0.00
****
****
****
*
Vehicle (PBS)
Dexamethasone
Megasphaera Sp. Strain B 2E+11
Megasphaera Sp. Strain B 2E+07

Figure 25

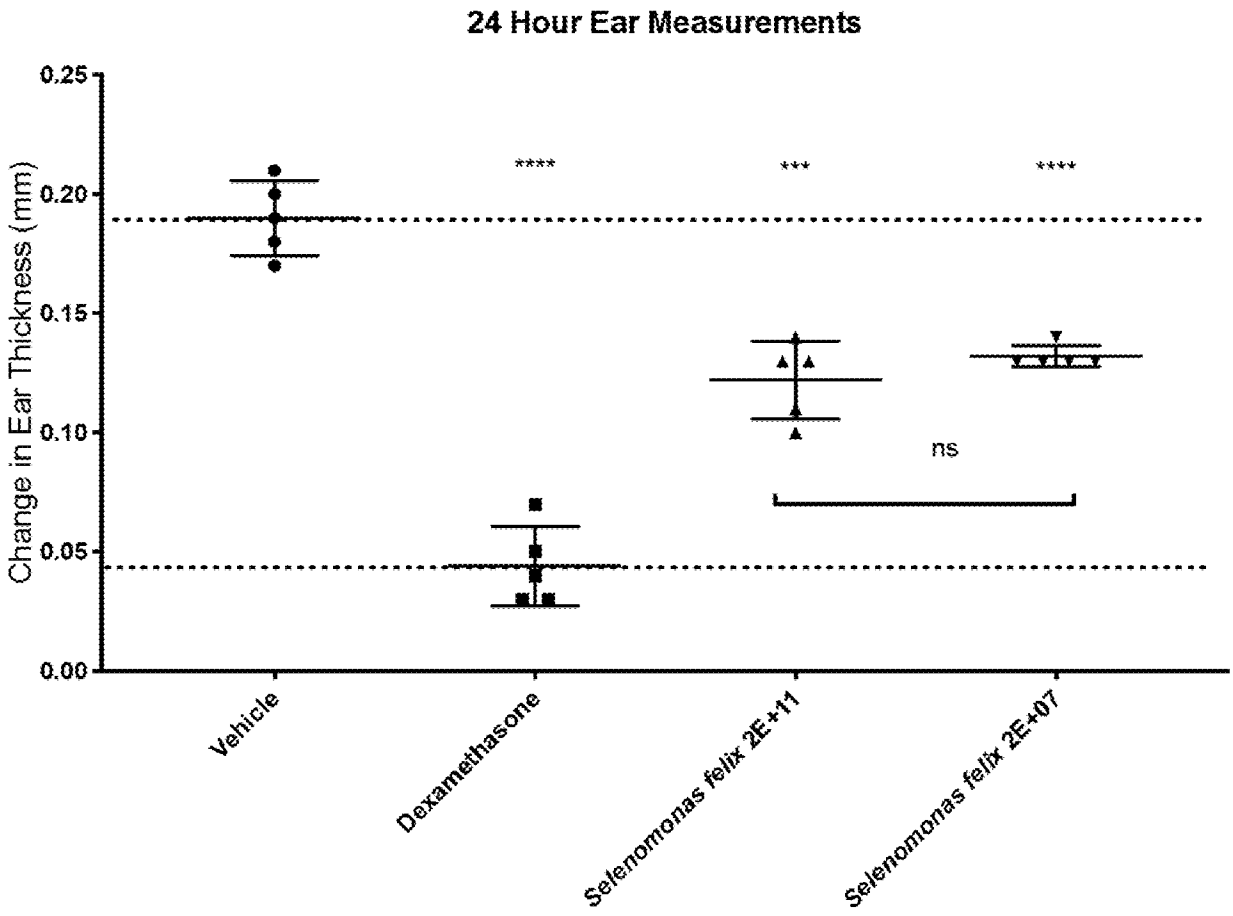

24 Hour Ear Measurements
Change in Ear Thickness (mm)
0.25
0.20
0.15
0.10
0.05
0.00
****
***
****
ns
Vehicle
Dexamethasone
Selenomonas felix 2E+11
Selenomonas felix 2E+07

Figure 26

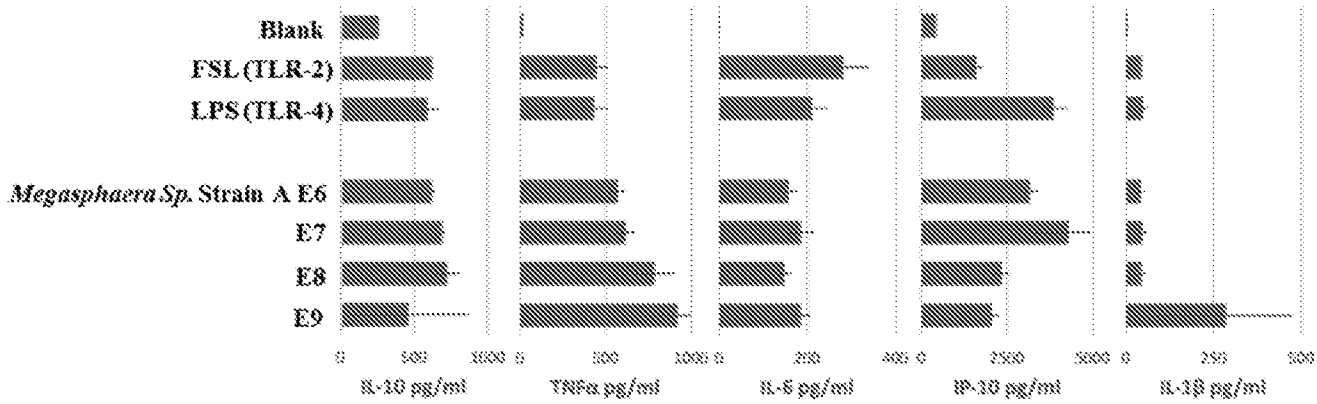

Blank
FSL (TLR-2)
LPS (TLR-4)
Megasphaera Sp. Strain A E6
E7
E8
E9
IL-10 pg/ml
TNFα pg/ml
IL-6 pg/ml
IP-10 pg/ml
IL-1β pg/ml

Figure 27A

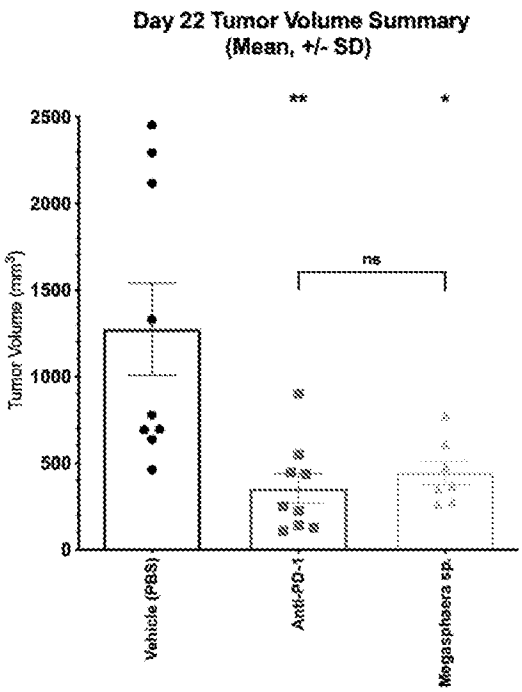



Figure 27B

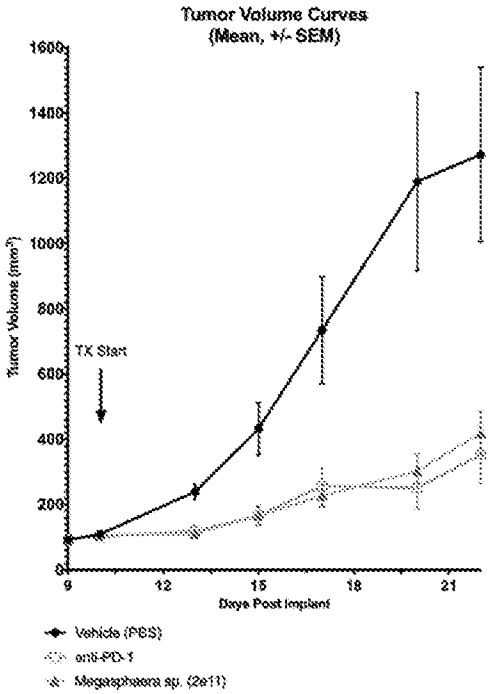


Figure 28A

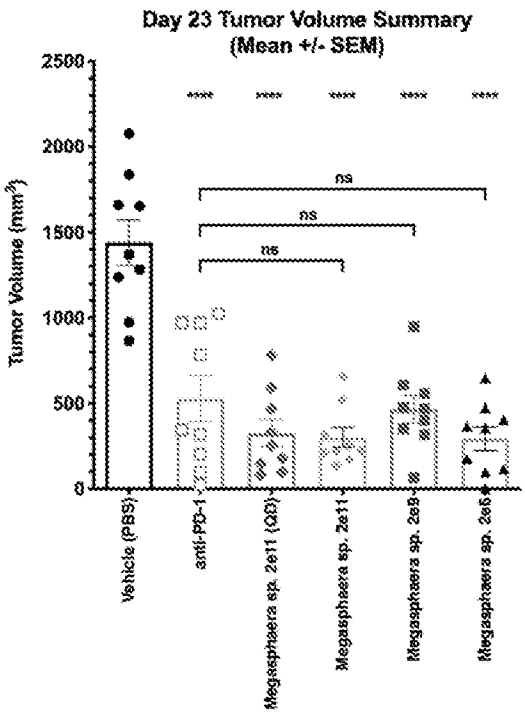



Figure 28B

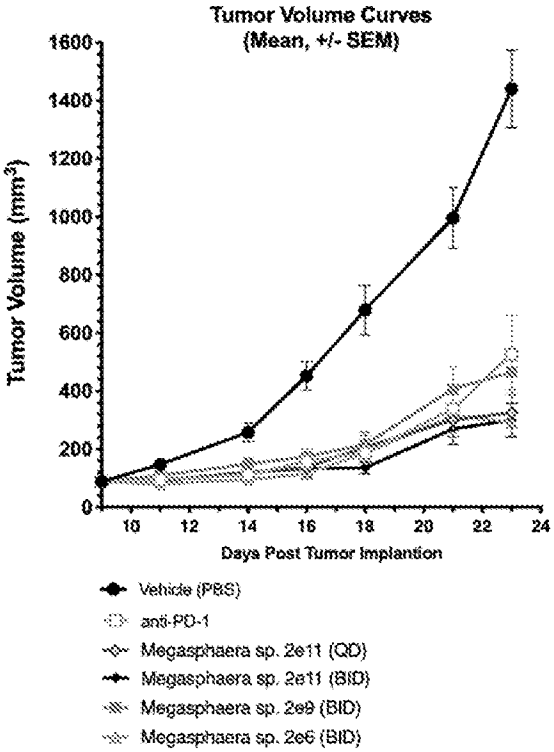


Figure 29

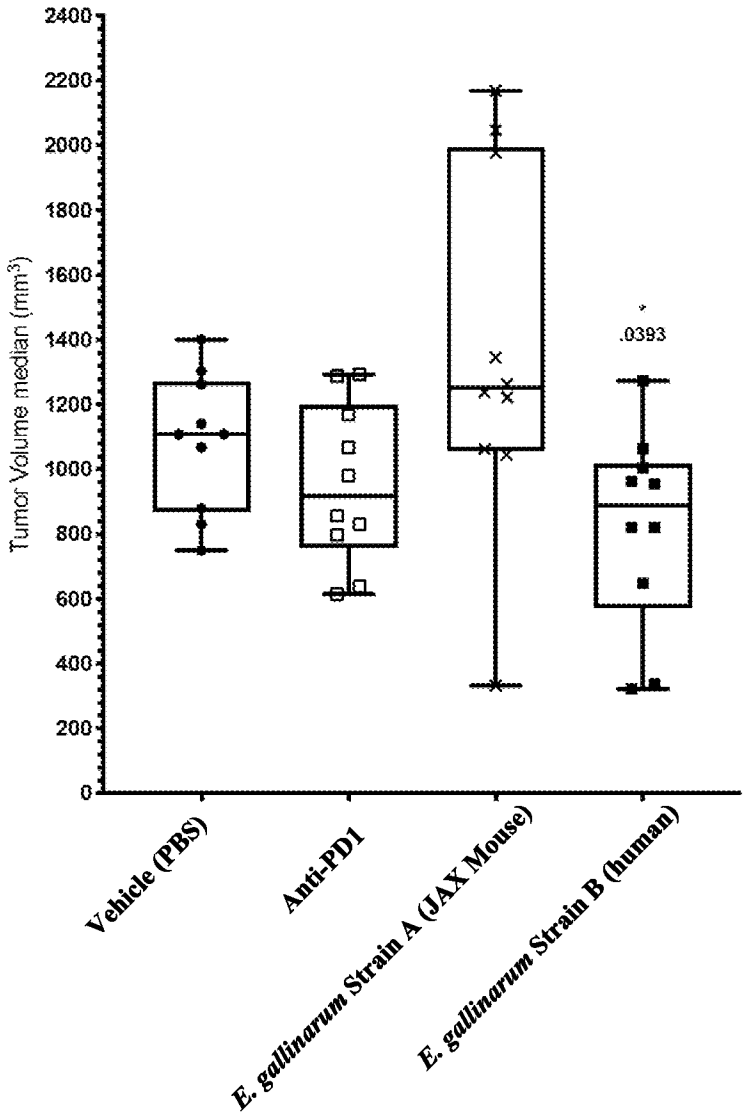

Tumor Volume median (mm3)
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
.0393
Vehicle (PBS)
Anti-PD1
E. gallinarum Strain A (JAX Mouse)
E. gallinarum Strain B (human)

Figure 30

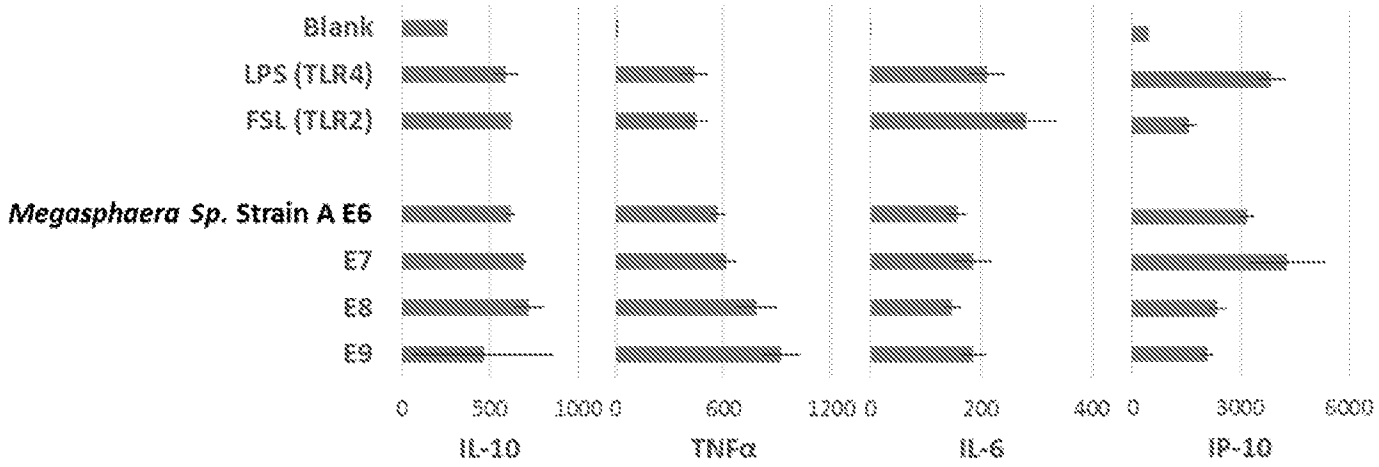

Blank
LPS (TLR4)
FSL (TLR2)
Megasphaera Sp. Strain A E6
E7
E8
E9
0
500
1000
IL-10
0
600
1200
TNFα
0
200
400
IL-6
0
3000
6000
IP-10

Figure 31

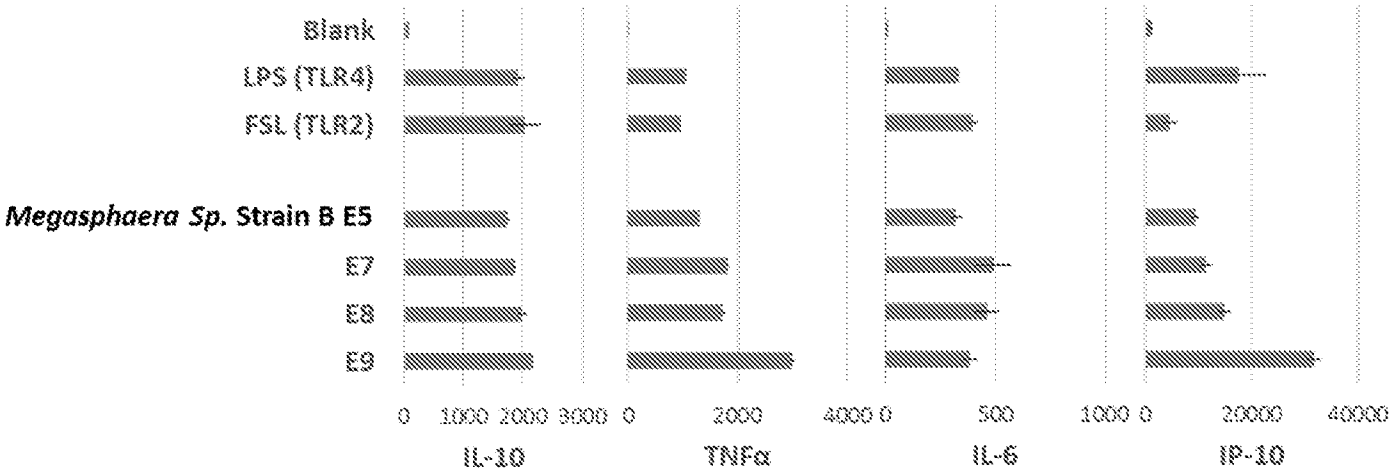

Blank
LPS (TLR4)
FSL (TLR2)
Megasphaera Sp. Strain B E5
E7
E8
E9
0 1000 2000 3000
IL-10
0 2000 4000
TNFα
0 500 1000
IL-6
0 20000 40000
IP-10

Figure 32

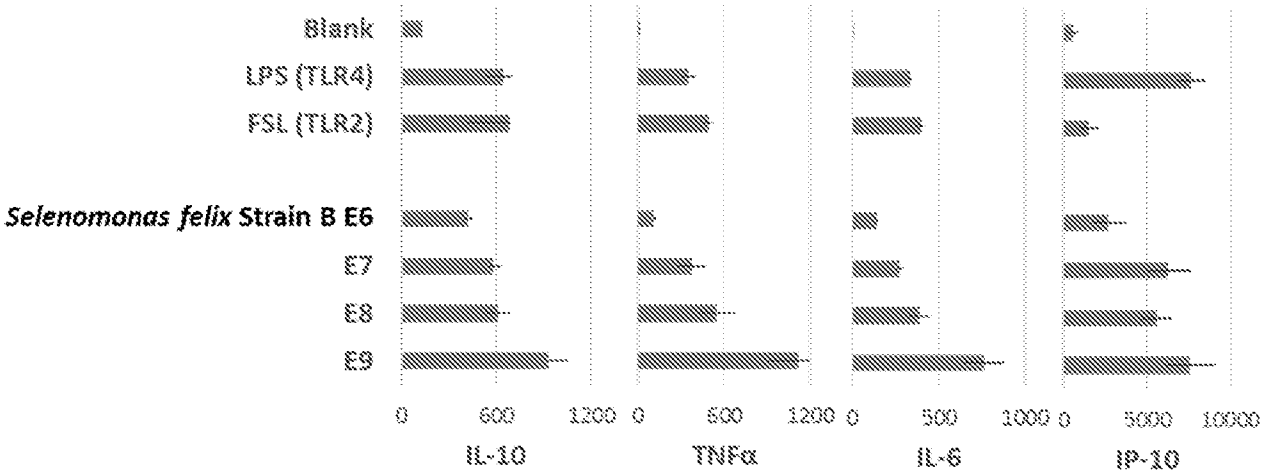

Blank
LPS (TLR4)
FSL (TLR2)
Selenomonas felix Strain B E6
E7
E8
E9
0
600
1200
IL-10
0
600
1200
TNFα
0
500
1000
IL-6
0
5000
10000
IP-10

Figure 33

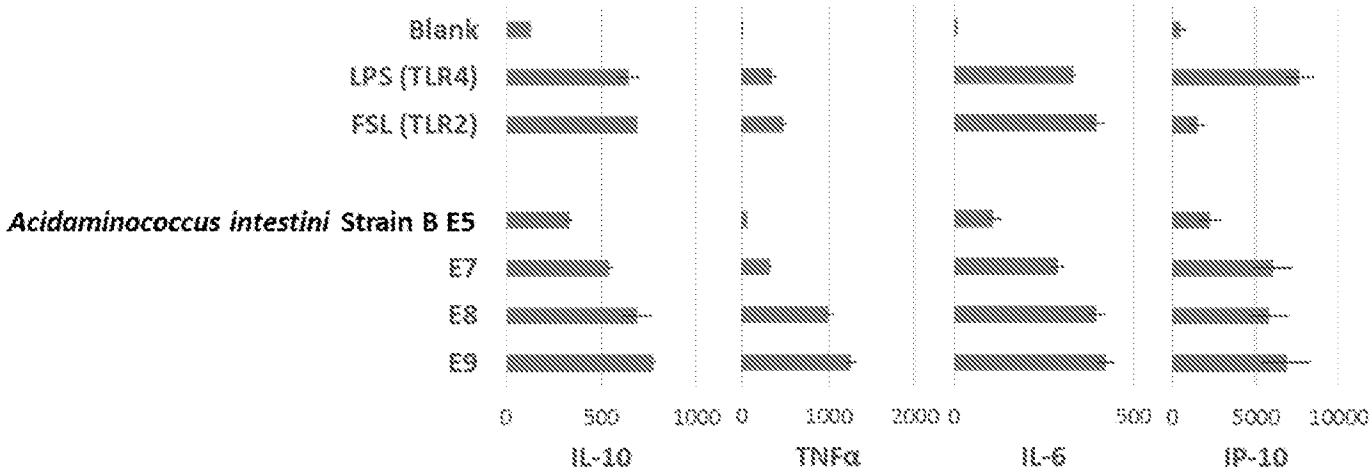
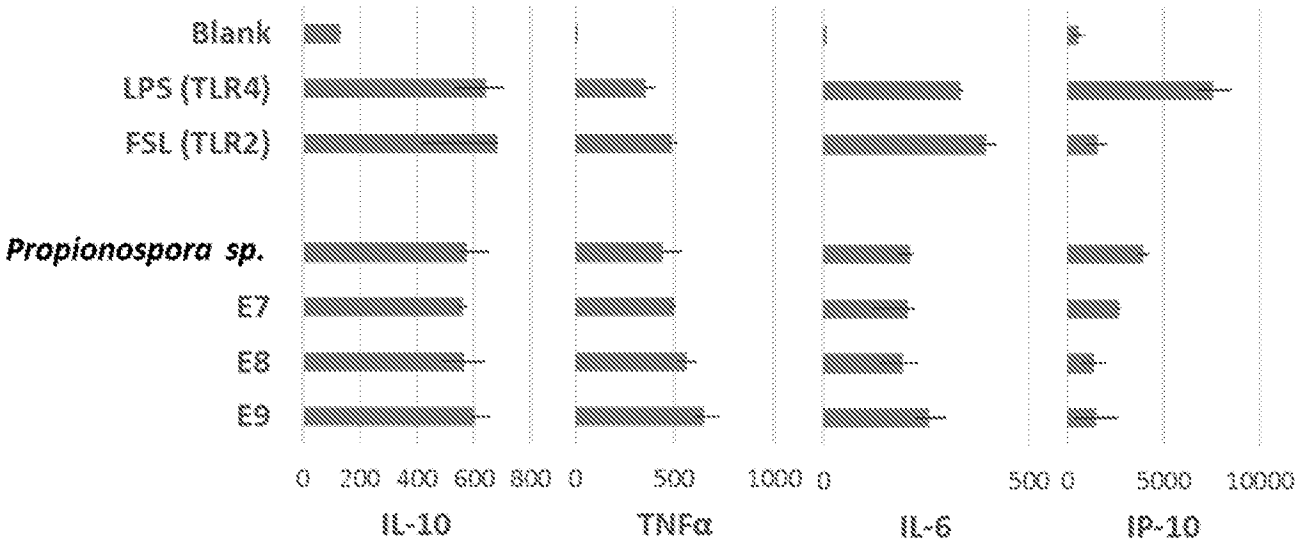

Blank
LPS (TLR4)
FSL (TLR2)
Acidaminococcus intestini Strain B E5
E7
E8
E9
0
500
1000
IL-10
0
1000
2000
TNFα
0
500
IL-6
0
5000
10000
IP-10

Figure 34


Blank
LPS (TLR4)
FSL (TLR2)
Propionospora sp.
E7
E8
E9
0 200 400 600 800
IL-10
0 500 1000
TNFα
0
IL-6
500 0 5000 10000
IP-10

# INTERNATIONAL SEARCH REPORT

International application No
PCT/US2020/037210

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K35/74 A61K35/745 A61K35/744 A61P37/00 A61P35/00
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | EP 3 403 670 A1 (MD HEALTHCARE INC [KR]) 21 November 2018 (2018-11-21) paragraphs [0006], [0011], [0017], [0042]; claims 1-11 | 1-76 |
| X | WO 2019/051380 A1 (EVELO BIOSCIENCES INC [US]) 14 March 2019 (2019-03-14) Examples; paragraphs [0002], [0081], [0300], [0332]; claims 1-161; table 1 | 1-76 |
| X | WO 2019/051381 A1 (EVELO BIOSCIENCES INC [US]) 14 March 2019 (2019-03-14) Examples; claims 1-181 | 1-76 |

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 29 October 2020 | 06/11/2020 |

| Name and mailing address of the ISA/ | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040,<br>Fax: (+31-70) 340-3016 | Nurmi, Jussi |

1

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | WO 2019/099482 A1 (EVELO BIOSCIENCES INC [US]) 23 May 2019 (2019-05-23) Examples; paragraphs [0060], [0061], [0066], [0097] - [0100], [0243]; claims 1-129 | 1-76 |
| X | WO 2019/099682 A1 (EVELO BIOSCIENCES INC [US]) 23 May 2019 (2019-05-23) Examples; claims 1-94 | 1-76 |
| X | WO 2019/010255 A1 (EVELO BIOSCIENCES INC [US]) 10 January 2019 (2019-01-10) Examples; paragraph [0005]; claims 1-133 | 1-76 |
| X | CHIL-SUNG KANG ET AL: "Extracellular Vesicles Derived from Gut Microbiota, Especially Akkermansia muciniphila, Protect the Progression of Dextran Sulfate Sodium-Induced Colitis", PLOS ONE, vol. 8, no. 10, 1 January 2013 (2013-01-01), pages e76520-e76520, XP055106720, ISSN: 1932-6203, DOI: 10.1371/journal.pone.0076520 abstract Introduction and Discussion | 1-76 |

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2020/037210

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| EP 3403670 A1 | 21-11-2018 | CN 108697784 A | 23-10-2018 |
| | | EP 3403670 A1 | 21-11-2018 |
| | | JP 6646155 B2 | 14-02-2020 |
| | | JP 2019501926 A | 24-01-2019 |
| | | KR 20170085943 A | 25-07-2017 |
| | | US 2019247465 A1 | 15-08-2019 |
| WO 2019051380 A1 | 14-03-2019 | AR 113011 A1 | 15-01-2020 |
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| | | CN 111148531 A | 12-05-2020 |
| | | CO 2020004233 A2 | 24-04-2020 |
| | | EP 3678693 A1 | 15-07-2020 |
| | | KR 20200053531 A | 18-05-2020 |
| | | TW 201919673 A | 01-06-2019 |
| | | US 2020254028 A1 | 13-08-2020 |
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