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(54) Title: METHODS FOR ASSAYING DRUG SUBSTANCES AND DRUG PRODUCTS BY USING CELL LINES WITH NF-KB- INDUCIBLE REPORTER GENES

(57) Abstract: Provided herein are methods and compositions related to assaying (for example, for potency) bacterial drug substances and/or drug products comprising bacteria and/or microbial extracellular vesicles (mEVs).

METHODS FOR ASSAYING DRUG SUBSTANCES AND DRUG PRODUCTS BY USING CELL LINES WITH NF-KB- INDUCIBLE REPORTER GENES

CROSS-REFERENCE TO RELATED APPLICATIONS

[1] This application claims the benefit of U.S. Provisional Application No. 63/416,277, filed on October 14, 2022, the content of which is hereby incorporated by reference in its entirety.

SUMMARY

[2] Provided herein are methods and compositions for assaying drug product and/or drug substance that comprise bacteria and/or microbial extracellular vesicles (mEVs). For example, in some embodiments, methods and compositions provided herein can be used as a quality control process to confirm the potency of such a drug product and/or drug substance, such as prior to final formulation as a solid dose form. In some embodiments, methods and compositions provided herein can be used to test whether drug product and/or drug substance maintained its potency after being stored for a period of time. In some embodiments, methods and compositions provided herein can be used to determine the potency of a drug product and/or drug substance, such as to ensure it possesses adequate potency prior to administration to a subject (*e.g.*, a subject in need thereof).

[3] Provided herein are methods and compositions for assaying bacteria and/or microbial extracellular vesicles (mEVs) prior to preparation of a drug substance or drug product. For example, in some embodiments, methods and compositions provided herein can be used as a quality control process to assess the impact of changes in bioprocess (e.g., production parameter) development or downstream processing development. In some embodiments, methods and compositions provided herein can be used to test whether bacteria and/or microbial extracellular vesicles (mEVs) maintained their potency after a process parameter is altered. For example, the methods and compositions can be used to assay bacteria and/or microbial extracellular vesicles (mEVs) after one or more of the following process changes: change in a production parameter such as change in amount or presence of a growth media component, change in a growth condition (such as time, temperature, or optical density); or change in a downstream processing step, such as change in excipient, and change in formulating parameter (such as compression strength).

[4] In certain aspects, provided herein are methods for assaying a drug product or drug substance that comprises bacteria and/or microbial extracellular vesicles (mEVs). In some embodiments, the method comprises contacting a cell of a mammalian immune cell line (*e.g.*, a monocyte cell line, such as THP-1 cells, (*e.g.*, PMA-differentiated THP-1 cells)) with the drug product or drug substance. The cell can comprise a nucleic acid sequence operatively linked to a NF-κB regulatory sequence (i.e., a sequence that is responsive to NF-κB, such as an NF-κB response element, or a sequence that is an NF-κB promoter). The nucleic acid sequence may encode a transcription product. The method may further comprise detecting the level of expression of a transcription product encoded by the nucleic acid sequence by the cell. In some embodiments, the drug product or drug substance comprises a strain of bacteria (*e.g.*, *Prevotella histicola*, *Veillonella parvula, Harryflintia acetispora, Fournierella massiliensis, Megasphaera Sp., Selenomonas felix, Acidaminococcus intestine,* and/or *Propionospora sp.* bacteria) and/or microbial extracellular vesicles (mEVs) from a strain of bacteria (*e.g.*, *Prevotella histicola*, *Veillonella parvula, Harryflintia acetispora, Fournierella massiliensis, Megasphaera Sp., Selenomonas felix, Acidaminococcus intestine,* and/or *Propionospora sp.* microbial extracellular vesicles (mEVs)).

[5] In certain aspects, provided herein are methods for assaying bacteria and/or microbial extracellular vesicles (mEVs) prior to preparation of a drug substance or drug product. In some embodiments, the method comprises contacting a cell of a mammalian immune cell line (*e.g.*, a monocyte cell line, such as THP-1 cells, (*e.g.*, PMA-differentiated THP-1 cells)) with the bacteria and/or microbial extracellular vesicles (mEVs). The cell can comprise a nucleic acid sequence operatively linked to a NF-κB regulatory sequence (i.e., a sequence that is responsive to NF-κB, such as an NF-κB response element, or a sequence that is an NF-κB promoter). The nucleic acid sequence may encode a transcription product. The method may further comprise detecting the level of expression of a transcription product encoded by the nucleic acid sequence by the cell. In some embodiments, the bacteria are a strain of bacteria such as *Prevotella histicola*, *Veillonella parvula, Harryflintia acetispora, Fournierella massiliensis, Megasphaera Sp., Selenomonas felix, Acidaminococcus intestine,* and/or *Propionospora sp.* bacteria; and/or the microbial extracellular vesicles (mEVs) are from a strain of bacteria such as *Prevotella histicola*, *Veillonella parvula, Harryflintia acetispora, Fournierella massiliensis, Megasphaera Sp.,*

Selenomonas felix, Acidaminococcus intestine, and/or *Propionospora sp.* microbial extracellular vesicles (mEVs).

[6] In some embodiments, the drug product or drug substance comprises bacteria of a strain of *Prevotella histicola.* In some embodiments, the bacteria (*e.g., Prevotella histicola* strain bacteria) comprise at least 90%, at least 95%, at least 96%, at least 97%, or at least 98%, 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 bacteria (*e.g., Prevotella histicola* strain bacteria) comprise 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 bacteria (*e.g., Prevotella histicola* strain bacteria) is the *Prevotella* Strain B (NRRL accession number B 50329).

[7] In some embodiments, a drug product or drug substance comprises mEVs of bacteria of a strain of *Prevotella histicola.* In some embodiments, mEVs can be mEVs of bacteria (*e.g., Prevotella histicola* strain bacteria) that comprise at least 90%, at least 95%, at least 96%, at least 97%, or at least 98%, 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, mEVs can be mEVs of bacteria (*e.g., Prevotella histicola* strain bacteria) that comprise 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 of bacteria (*e.g., Prevotella histicola* strain bacteria) of the *Prevotella* Strain B (NRRL accession number B 50329).

[8] In some embodiments, a transcription product is a protein. In some embodiments, the expression of the protein is determined by an enzyme-linked immunosorbent assay (ELISA), Western blotting, radioimmunoassay, or immunocytostaining. In some embodiments, the protein is a luciferase protein, such as a firefly luciferase protein. In some embodiments, expression of the luciferase is determined by a luciferase assay. In some embodiments, the protein is a fluorescent protein, such as a green fluorescent protein, mCherry protein, a yellow fluorescent protein, a blue fluorescent protein, a cyan fluorescent protein, or a red fluorescent protein. In some embodiments, expression of the fluorescent protein is determined by detecting fluorescence.

[9] In some embodiments, a transcription product is an RNA. In some embodiments, the expression of the RNA is determined by a northern hybridization method or using reverse transcription-polymerase chain reaction (RT-PCR).

[10] In some embodiments, a NF-κB regulatory sequence is responsive to NF-κB. In some embodiments, a nucleic acid sequence is operably linked to one or more NF-κB response elements (e.g., one or more copies of an NF-κB response element, e.g., one, two three, four, five, six or more copies). In various embodiments, the one or more NF-κB response elements have different sequences. In various embodiments, the one or more NF-κB response elements have the same sequence (and in various such embodiments can be referred to as "copies" of an NF-κB response element). In various embodiments, at least two of the one or more NF-κB response elements have the same sequence. In various embodiments, at least two of the one or more NF-κB response elements have different sequences. In some embodiments, the nucleic acid sequence is operably linked to four NF-κB response elements (e.g., four copies of an NF-κB response element), e.g., positioned upstream of a promoter such as a minimal TATA promoter. In some embodiments, the NF-κB regulatory sequence is an NF-κB promoter. In some embodiments, the mammalian immune cell line is a monocyte cell line, such as THP-1 cell. In some embodiments, the monocyte cell line is differentiated (such as with Phorbol 12-myristate 13-acetate (PMA)), such as prior to contacting drug product or drug substance. In some embodiments, the method further comprises the step of contacting the cells with Phorbol 12-myristate 13-acetate (PMA), e.g., prior to contacting drug product or drug substance to the cells.

[11] In some embodiments, the method is a method of determining the potency of a drug product or drug substance, e.g., is a potency assay. In some embodiments, the method is a potency assay for batch release. In some embodiments, the method of determining the potency of a drug product or drug substance is stability indicating, e.g., the assay can be used to detect whether a drug product or drug substance (e.g., a batch thereof) has a potency that is different from (e.g., greater than or less than) a reference such as a reference standard.

[12] In some embodiments, a method further comprises serially diluting the drug product or drug substance prior to contacting the cell (e.g., THP-1 cells, such as PMA-differentiated THP-1 cells) with the drug product or drug substance.

[13] In some embodiments, a drug product or drug substance is contacted with the cells (e.g., THP-1 cells, such as PMA-differentiated THP-1 cells) for a time period sufficient for the drug

product or drug substance to induce expression of the transcription product by the cell (e.g., THP-1 cells, such as PMA-differentiated THP-1 cells). In some embodiments, the time period is at least 12 hours. In some embodiments, the time period is at least 24 hours. In some embodiments, the method further comprises determining a level of expression of the transcription product by the cell (e.g., THP-1 cells, such as PMA-differentiated THP-1 cells). In some embodiments, the method further comprises comparing level of expression of the transcription product by the cell (e.g., THP-1 cells, such as PMA-differentiated THP-1 cells) after the drug product or drug substance is contacted with the cells to the level of expression of the transcription product by the cell (e.g., THP-1 cells, such as PMA-differentiated THP-1 cells) after a reference standard is contacted with the cells. In some embodiments, the method further comprises comparing the level of expression of the transcription product to a threshold level of expression of the transcription product. In some embodiments, the method further comprises determining the threshold level by contacting a cell of the mammalian immune cell line with a reference standard of the drug product or drug substance and detecting the level of expression of the transcription product by the cell contacted with the reference standard, wherein the level of expression by the cell contacted with the reference standard is the threshold level.

[14] In some embodiments, the method further comprises discarding the drug product or drug substance if the level of expression of the transcription product by the cell (e.g., THP-1 cells, such as PMA-differentiated THP-1 cells) is below a threshold level.

[15] In some embodiments, the method further comprises identifying the drug product or drug substance as a potent drug product or drug substance if the level of expression of the transcription product by the cell (e.g., THP-1 cells, such as PMA-differentiated THP-1 cells) is at or above a threshold level.

[16] In some embodiments, the method further comprises formulating the drug product or drug substance into a solid dosage form if it has been identified as a potent drug product or drug substance (e.g., a tablet, a minitablet, a capsule, or a powder).

[17] In some embodiments, a solid dosage form is a tablet or capsule.

[18] In some embodiments, the method further comprises packaging the drug product or drug substance if it has been identified as a potent drug product or drug substance.

[19] In some embodiments, the method further comprises administering the drug product or drug substance to a subject in need thereof if it has been identified as a potent drug product or drug substance.

[20] In some embodiments, the method further comprises not discarding the drug product or drug substance if it has been identified as a potent drug product or drug substance.

[21] In some embodiments, the threshold level is about 50% to about 200%, such as 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, or 200% the level of expression of the transcription product by the cell (e.g., THP-1 cells, such as PMA-differentiated THP-1 cells) that have been contacted by a reference standard. In some embodiments, the threshold level is at least 70% the level of expression of the transcription product by cells (e.g., THP-1 cells, such as PMA-differentiated THP-1 cells) that have been contacted by a reference standard. In some embodiments, the threshold level is at most 135% the level of expression of the transcription product by cells (e.g., THP-1 cells, such as PMA-differentiated THP-1 cells) that have been contacted by a reference standard.

[22] In certain aspects, provided herein are methods of determining the potency of a drug product or drug substance, wherein potency is measured by expression of a coding nucleic acid sequence operatively linked to a regulatory sequence that is responsive to NF-κB or is an NF-κB promoter. Without wishing to be bound by any particular scientific theory, the present disclosure includes that expression of a coding nucleic acid sequence operatively linked, within a cell, to a regulatory sequence that is responsive to NF-κB or is an NF-κB promoter can correspond to and/or have a direct relationship with potency of a drug product or drug substance contacted with the cell. It will be appreciated from the present disclosure that the correspondence and/or relationship can be but need not be linear, and that in various embodiments expression can be measured by comparison to a reference such as a reference standard.

[23] In various embodiments, a method of determining the potency of a drug product or drug substance comprises i) determining a threshold level of expression of a transcription product encoded by a nucleic acid sequence by contacting a cell of a mammalian immune cell line (e.g., THP-1 cells, such as PMA-differentiated THP-1 cells) with a reference standard, wherein the cell comprises the nucleic acid sequence operatively linked to a regulatory sequence, wherein the regulatory sequence is responsive to NF-κB or is an NF-κB promoter, and the reference standard is brought into contact with the cell (e.g., THP-1 cells, such as PMA-differentiated THP-1 cells)

for a time period sufficient to allow the reference standard to induce expression of the transcription product by the cells (e.g., THP-1 cells, such as PMA-differentiated THP-1 cells). The method may further comprise ii) determining a test level of expression the transcription product of by contacting cells (e.g., THP-1 cells, such as PMA-differentiated THP-1 cells) with the drug product or drug substance, wherein the drug product or drug substance is brought into contact with the cells (e.g., THP-1 cells, such as PMA-differentiated THP-1 cells) for a time period sufficient to allow the drug product or drug substance to induce expression of the transcription product by the cells (e.g., THP-1 cells, such as PMA-differentiated THP-1 cells). The method my further comprise iii) comparing the test level of expression of the transcription product to the level of threshold level of expression of the transcription product to determine the potency of the drug product or drug substance; wherein the drug product or drug substance comprises bacteria and/or mEVs. In some embodiments, the drug product or drug substance comprises a strain of bacteria (e.g., *Prevotella histicola*, *Veillonella parvula, Harryflintia acetispora, Fournierella massiliensis, Megasphaera Sp., Selenomonas felix, Acidaminococcus intestine,* and/or *Propionospora sp.* strain bacteria) and/or microbial extracellular vesicles (mEVs) from a strain of bacteria (e.g., *Prevotella histicola*, *Veillonella parvula, Harryflintia acetispora, Fournierella massiliensis, Megasphaera Sp., Selenomonas felix, Acidaminococcus intestine,* and/or *Propionospora sp.* strain microbial extracellular vesicles (mEVs)).

[24] In some embodiments, a drug product or drug substance comprises bacteria of a strain of *Prevotella histicola.* In some embodiments, the bacteria (*e.g., Prevotella histicola* strain bacteria) comprise at least 90%, at least 95%, at least 96%, at least 97%, or at least 98%, 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 bacteria (*e.g., Prevotella histicola* strain bacteria) comprise 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 bacteria (*e.g., Prevotella histicola* strain bacteria) is the *Prevotella* Strain B (NRRL accession number B 50329).

[25] In some embodiments, a drug product or drug substance comprises mEVs of bacteria of a strain of *Prevotella histicola.* In some embodiments, mEVs can be mEVs of bacteria (*e.g., Prevotella histicola* strain bacteria) that comprise at least 90%, at least 95%, at least 96%, at least 97%, or at least 98%, 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, mEVs can be mEVs of bacteria (*e.g., Prevotella histicola* strain bacteria) that comprise 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 of bacteria (*e.g., Prevotella histicola* strain bacteria) of the *Prevotella* Strain B (NRRL accession number B 50329).

[26] In some embodiments, a transcription product is a protein. In some embodiments, the expression of the protein is determined by an enzyme-linked immunosorbent assay (ELISA), Western blotting, radioimmunoassay, or immunocytostaining. In some embodiments, the protein is a luciferase protein, such as a firefly luciferase protein. In some embodiments, expression of the luciferase is determined by a luciferase assay. In some embodiments, the protein is a fluorescent protein, such as a green fluorescent protein, mCherry protein, a yellow fluorescent protein, a blue fluorescent protein, a cyan fluorescent protein, or a red fluorescent protein. In some embodiments, expression of the fluorescent protein is determined by detecting fluorescence.

[27] In some embodiments, a transcription product is an RNA. In some embodiments, the expression of the RNA is determined by a northern hybridization method or using reverse transcription-polymerase chain reaction (RT-PCR).

[28] In some embodiments, a regulatory sequence is responsive to NF-κB. In some embodiments, the nucleic acid sequence is operably linked to one or more NF-κB response elements (e.g., one or more copies of an NF-κB response element). In some embodiments, the nucleic acid sequence is operably linked to four NF-κB response elements (e.g., four copies of an NF-κB response element) positioned upstream of a promoter such as a minimal TATA promoter. In some embodiments, a regulatory sequence is an NF-κB promoter. In some embodiments, the mammalian immune cell line is a monocyte cell line, such as THP-1 cell. In some embodiments, the monocyte cell line is differentiated (such as with Phorbol 12-myristate 13-acetate (PMA)), such as prior to contacting drug product or drug substance. In some embodiments, the method further comprises the step of contacting the cells with Phorbol 12-myristate 13-acetate (PMA), e.g., prior to contacting drug product or drug substance to the cells.

[29] In some embodiments, the method further comprises serially diluting the drug product or drug substance prior to bringing the drug product or drug substance into contact with the cells

(e.g., THP-1 cells, such as PMA-differentiated THP-1 cells). In some embodiments, the method further comprises serially diluting the reference standard prior to bringing the reference standard into contact with the cells (e.g., THP-1 cells, such as PMA-differentiated THP-1 cells).

[30] In some embodiments, a time period sufficient to allow a reference standard and/or a drug product or drug substance to induce expression of a transcription product encoded by cells (e.g., THP-1 cells, such as PMA-differentiated THP-1 cells) of an assay provided herein is at least 12 hours. In some embodiments, a time period sufficient to allow a reference standard and/or a drug product or drug substance to induce expression of a transcription product encoded by cells (e.g., THP-1 cells, such as PMA-differentiated THP-1 cells) of an assay disclosed herein is at least 24 hours.

[31] In some embodiments, a method of the present disclosure further comprises discarding the drug product or drug substance if the test level of expression of a transcription product encoded by cells of the assay is below a reference, e.g., below a threshold level of expression of the transcription product.

[32] In some embodiments, a method of the present disclosure further comprises identifying the drug product or drug substance as a potent drug product or drug substance if the test level of expression of the transcription product is at or above a threshold level of expression of the transcription product.

[33] In some embodiments, the method further comprises formulating the drug product or drug substance into a solid dosage form if it has been identified as a potent drug product or drug substance.

[34] In some embodiments, a solid dosage form is a tablet or a capsule.

[35] In some embodiments, the method further comprises packaging the drug product or drug substance if it has been identified as a potent drug product or drug substance.

[36] In some embodiments, the method further comprises administering the drug product or drug substance to a subject in need thereof it has been identified as a potent drug product or drug substance.

[37] In some embodiments, the method further comprises not discarding the drug product or drug substance if it has been identified as a potent drug product or drug substance.

[38] In some embodiments, a threshold level of expression of the transcription product is about 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%,

180%, 190%, or 200% of the level of expression of the transcription product by the cells (e.g., THP-1 cells, such as PMA-differentiated THP-1 cells) in step i). In some embodiments, the threshold level of expression of the transcription product is at least 70% of the level of expression of the transcription product by the cells (e.g., THP-1 cells, such as PMA-differentiated THP-1 cells) in step i). In some embodiments, the threshold level of expression of the transcription product is at most 135% of the level of expression of the transcription product by the cells (e.g., THP-1 cells, such as PMA-differentiated THP-1 cells) in step i).

[39] In some embodiments, a method further comprises the step of PMA-differentiating the cells (e.g., THP-1 cells, such as PMA-differentiated THP-1 cells), e.g., prior to step i).

[40] In some embodiments of an aspect provided herein, the method of determining the potency of a drug product or drug substance is a potency assay for batch release.

[41] In some embodiments, of an aspect provided herein the method of determining the potency of a drug product or drug substance is stability indicating, e.g., the method can be used to detect whether a drug product or drug substance (e.g., a batch thereof) has potency that is different from (e.g., greater than or less than) a reference such as a reference standard.

[42] In some embodiments of an aspect provided herein, a drug product or drug substance comprises bacteria.

[43] In some embodiments of an aspect provided herein, a drug product or drug substance comprises isolated bacteria (e.g., from one or more strains of bacteria (e.g., bacteria of interest)) (e.g., a therapeutically effective amount thereof). In some embodiments, 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 drug product or drug substance (e.g., pharmaceutical agent) is isolated mEVs of bacteria (e.g., bacteria of interest).

[44] In some embodiments, of an aspect provided herein a drug product or drug substance comprises isolated bacteria (e.g., from one strain of bacteria (e.g., bacteria of interest)) (e.g., a therapeutically effective amount thereof). In some embodiments, 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 drug product or drug substance (e.g., pharmaceutical agent) is isolated mEVs of bacteria (e.g., bacteria of interest).

[45] In some embodiments of an aspect provided herein, a drug product or drug substance comprises bacteria and the bacteria are from one strain.

[46] In some embodiments of an aspect provided herein, a drug product or drug substance comprises microbial extracellular vesicles (mEVs).

[47] In some embodiments of an aspect provided herein, a drug product or drug substance comprises bacteria and microbial extracellular vesicles (mEVs).

[48] In some embodiments of an aspect provided herein, a drug product or drug substance comprises isolated bacteria and/or 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 agent is the isolated bacteria and/or isolated mEVs (e.g., bacteria of interest).

[49] In some embodiments of an aspect provided herein, a drug product or drug substance comprises isolated bacteria and/or isolated mEVs (e.g., from one strain of bacteria (e.g., bacteria of interest) (e.g., a therapeutically effective amount thereof)). In some embodiments, 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 agent is the isolated bacteria and/or isolated mEVs (e.g., bacteria of interest).

[50] In some embodiments of an aspect provided herein, a drug product or drug substance comprises bacteria and/or mEVs that have been gamma irradiated, UV irradiated, heat inactivated, acid treated, or oxygen sparged.

[51] In some embodiments of an aspect provided herein, a drug product or drug substance comprises live bacteria.

[52] In some embodiments of an aspect provided herein, a drug product or drug substance comprises dead bacteria.

[53] In some embodiments of an aspect provided herein, a drug product or drug substance comprises non-replicating bacteria.

[54] In some embodiments of an aspect provided herein, a drug product or drug substance comprises one strain of bacteria or mEVs from one strain of bacteria.

[55] In some embodiments of an aspect provided herein, bacteria and/or mEVs are lyophilized (e.g., the lyophilized product further comprises a pharmaceutically acceptable excipient) (e.g., a powder form).

[56] In some embodiments of an aspect provided herein, bacteria and/or mEVs are gamma irradiated.

[57] In some embodiments of an aspect provided herein, bacteria and/or mEVs are UV irradiated.

[58] In some embodiments of an aspect provided herein, bacteria and/or mEVs are heat inactivated (e.g., at 50°C for two hours or at 90°C for two hours).

[59] In some embodiments of an aspect provided herein, bacteria and/or mEVs are acid treated.

[60] In some embodiments of an aspect provided herein, bacteria are and/or mEVs oxygen sparged (e.g., at 0.1 vvm for two hours).

[61] In some embodiments of an aspect provided herein, a drug product or drug substance 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 agent is isolated mEVs of bacteria (e.g., bacteria of interest).

[62] In some embodiments, of an aspect provided herein a drug product or drug substance comprises isolated mEVs (e.g., from one strain of bacteria (e.g., bacteria of interest)) (e.g., a therapeutically effective amount thereof). In some embodiments, 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 agent is isolated mEVs of bacteria (e.g., bacteria of interest).

[63] In some embodiments of an aspect provided herein, a drug product or drug substance comprises mEVs and the mEVs comprise secreted mEVs (smEVs).

[64] In some embodiments of an aspect provided herein, a drug product or drug substance comprises mEVs and the mEVs comprise processed mEVs (pmEVs).

[65] In some embodiments, of an aspect provided herein a drug product or drug substance comprises pmEVs and the pmEVs are produced from bacteria that have been gamma irradiated, UV irradiated, heat inactivated, acid treated, or oxygen sparged.

[66] In some embodiments of an aspect provided herein, a drug product or drug substance comprises pmEVs and the pmEVs are produced from live bacteria.

[67] In some embodiments of an aspect provided herein, a drug product or drug substance comprises pmEVs and the pmEVs are produced from dead bacteria.

[68] In some embodiments of an aspect provided herein, a drug product or drug substance comprises pmEVs and the pmEVs are produced from non-replicating bacteria.

[69] In some embodiments of an aspect provided herein, a drug product or drug substance comprises mEVs and the mEVs are from one strain of bacteria.

DETAILED DESCRIPTION

General

This disclosure is based, in part, on the need to assess the potency of drug products and/or drug substances comprising bacteria and/or microbial extracellular vesicles (mEVs). Disclosed herein are assays to demonstrate bacterial test samples (*e.g.,* drug products or drug substances comprising bacteria and/or microbial extracellular vesicles) induce expression of a transcription product in cells at levels similar to a reference standard. For example, as disclosed herein, potency of bacteria and/or microbial extracellular vesicles (e.g., *Prevotella histicola* bacteria and/or microbial extracellular vesicles) can be measured by incubating the bacteria and/or microbial extracellular vesicles with a population of one or more mammalian cells (e.g., THP-1 cells, e.g., PMA-differentiated THP-1 cells) and measuring expression of the transcription product. The expression of the transcription product can be measured by any of a variety of techniques disclosed herein or otherwise generally available to those of skill in the art. In various embodiments, expression of the transcription product can be measured by detecting expression of a nucleic acid encoding a detectable marker operably linked with a regulatory sequence, wherein the regulatory sequence is responsive to NF-κB or is an NF-κB promoter. In some embodiments, if a drug product or drug substance is determined to be not potent (e.g., to have a measured potency that is less than a potent reference or threshold of potency) according to an assay disclosed herein, the drug product or drug substance can be discarded from use. In some embodiments, if a drug product or drug substance is determined to be potent (e.g., to have a measured potency that is equal to and/or greater than a potent reference or threshold of potency) according to an assay disclosed herein, the drug product or drug substance is not discarded (*e.g.,* the drug product or drug substance is formulated into a solid dosage form (*e.g.,* capsules or tablets), packaged, and/or administered to a subject in need thereof).

Definitions

[70] The term "about" when used before a numerical value indicates that the value may vary within a reasonable range, such as within ±10%, ±5% or ±1% of the stated value.

[71] "Administration" broadly refers to a route of administration of a composition (e.g., a drug substance or drug product described herein) 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), and subcutaneous (SC) administration. A drug substance or drug product described herein can be administered in any form by any effective route, including but not limited to 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 drug substance or drug product described herein is administered orally, rectally, topically, intravesically, by injection into or adjacent to a draining lymph node, intravenously, by inhalation or aerosol, or subcutaneously. In another preferred embodiment, a drug substance or drug product described herein is administered orally, or intravenously. In another embodiment, a drug substance or drug product described herein is administered orally.

[72] 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 CnH2nOn. A carbohydrate may be a monosaccharide, a disaccharide, trisaccharide, oligosaccharide, or polysaccharide. The most basic carbohydrate is a monosaccharide, such as glucose, sucrose, 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.

[73] "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.

[74] A "combination" of bacteria from two or more strains includes the physical co-existence of the bacteria, either in the same material or product or in physically connected products, as well as the temporal co-administration or co-localization of the bacteria from the two or more strains.

[75] A "combination" of mEVs (such as smEVs and/or pmEVs) from two or more microbial (such as bacteria) strains includes the physical co-existence of the microbes from which the mEVs (such as smEVs and/or pmEVs) 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 and/or pmEVs) from the two or more strains.

[76] The term "decrease" or "deplete," and grammatical equivalents thereof, indicate qualitative or quantitative difference from a reference. For example, a decrease can refer to 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; expression of a protein, or another physical parameter (such as ear thickness (e.g., in a DTH animal model) or tumor size).

[77] The term "drug substance" or "therapeutic agent" refers to an agent for therapeutic use and comprising bacteria and/or microbial extracellular vesicles (mEVs) (such as smEVs and/or pmEVs), e.g., that can be used to treat and/or prevent a disease and/or condition. In some embodiments, the therapeutic agent is a pharmaceutical agent. In some embodiments, a medicinal product, medical food, a food product, or a dietary supplement comprises a therapeutic agent. For example, the therapeutic agent disclosed herein may be a powder comprising bacteria

and/or microbial extracellular vesicles (mEVs) (such as smEVs and/or pmEVs). In some embodiments, the therapeutic agent may further comprise an excipient.

[78] The term "drug product" or "therapeutic composition" refers to a composition that comprises a therapeutically effective amount of a therapeutic agent. In some embodiments, the therapeutic composition is (or is present in) a medicinal product, medical food, a food product, or a dietary supplement. For example, the therapeutic composition may be a tablet or capsule comprising the therapeutic agent. In some embodiments, the therapeutic composition may be a powder comprising the therapeutic agent and additional excipients. In some embodiments, a therapeutic compositioncomprises a therapeutic agent and an additional excipient.

[79] "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.

[80] 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.

[81] The term "gene" refers to a DNA sequence that is or includes coding sequence (i.e., a DNA sequence that encodes an expression product, such as an RNA product and/or a polypeptide product), optionally together with some or all of regulatory sequences that control expression of the coding sequence. In some embodiments, a gene includes non-coding sequence such as, without limitation, introns. In some embodiments, a gene may include both coding (e.g.,

exonic) and non-coding (e.g., intronic) sequences. In some embodiments, a gene includes a regulatory sequence that is a promoter or a transcription factor response element. In some embodiments, a gene includes one or both of a (i) DNA nucleotides extending a predetermined number of nucleotides upstream of the coding sequence in a reference context, such as a source genome, and (ii) DNA nucleotides extending a predetermined number of nucleotides downstream of the coding sequence in a reference context, such as a source genome. In various embodiments, the predetermined number of nucleotides can be 500 bp, 1 kb, 2 kb, 3 kb, 4 kb, 5 kb, 10 kb, 20 kb, 30 kb, 40 kb, 50 kb, 75 kb, or 100 kb. As used herein, a "transgene" refers to a gene that is not endogenous or native to a reference context in which the gene is present or into which the gene may be placed by engineering.

[82] "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.)).

[83] 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).

[84] The term "increase," and grammatical equivalents thereof, indicates qualitative or quantitative difference from a reference. For example, an increase can refer to 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; expression of a protein, or another physical parameter (such as ear thickness (e.g., in a DTH animal model) or tumor size).

[85] "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, TLR10 and TLR11. 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).

[86] The "internal transcribed spacer" or "ITS" is a piece of non-functional RNA positioned 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.

[87] The term "isolated" or "enriched" encompasses a microbe (such as a bacterium), mEV (such as an smEV and/or pmEV), 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. As used herein, a substance is "pure" if it is 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 mEVs 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, and a purified microbe or microbial population or mEVs 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 microbial population 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. In the instance of microbial compositions provided herein, the one or more microbial or mEV 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.

[88] 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).

[89] 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).

[90] "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.

[91] "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. 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.

[92] "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.*

[93] "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 or treatment conditions (*e.g.*, antibiotic treatment, exposure to different microbes). In some embodiments, the microbiome occurs at a mucosal surface. In some embodiments, the microbiome is a gut microbiome.

[94] "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. mEVs can be modified, e.g., as described herein.

[95] "Operably linked" refers to the association of at least a first element and a second element such that the component elements are in a relationship permitting them to function in their intended manner. For example, a nucleic acid sequence or amino acid sequence is operably linked with another sequence if it modifies the expression, structure, or activity of the linked sequence, e.g., in an intended manner. In many cases, two nucleic acid sequences are operably linked if they contribute to the expression, structure, or activity of a gene or encoded polypeptide. For example, a nucleic acid regulatory sequence is "*operably linked*" to a nucleic acid coding sequence if the regulatory sequence and coding sequence are associated in a manner that permits control of expression of the coding sequence by the regulatory sequence. In some

embodiments, an "*operably linked*" regulatory sequence is directly or indirectly covalently associated with a coding sequence (e.g., in a single nucleic acid). In some embodiments, a regulatory sequence controls expression of a coding sequence in *trans* and inclusion of the regulatory sequence in the same nucleic acid as the coding sequence is not a requirement of operable linkage.

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

[97] 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.

[98] As used herein, the term "preventing" a disease or condition in a subject refers to administering to the subject to a pharmaceutical treatment, e.g., the administration of one or more agents (e.g., pharmaceutical agent), such that onset of at least one symptom of the disease or condition is delayed or prevented.

[0001] As used herein, a "promoter" or "promoter sequence" can be a DNA regulatory sequence that directly or indirectly (e.g., through promoter-bound proteins or substances) participates in initiation and/or processivity of transcription of a coding sequence. A promoter

may, under suitable conditions, initiate transcription of a coding sequence upon binding of one or more transcription factors and/or regulatory moieties with the promoter. A promoter that participates in initiation of transcription of a coding sequence can be "operably linked" to the coding sequence. Promoters can be positioned in a nucleic acid near to and upstream of the transcription start sites of genes (5' of a transcription start site). In certain instances, a promoter can be or include a DNA regulatory sequence that extends from a transcription start site (at its 3' terminus) to an upstream (5' direction) position such that the sequence so designated includes one or both of a minimum number of bases or elements necessary to initiate a transcription event. A promoter can be about 100-3000 or about 100–1000 base pairs in length. A promoter may be, include, or be operably associated with or operably linked to, expression control sequences such as enhancer and repressor sequences.

[99] As used herein, "protein expression" and grammatical equivalents thereof refers individually and/or cumulatively to one or more biological process that result in production from a nucleic acid sequence of an encoded agent, such as a polypeptide. Expression specifically includes either or both of transcription and translation. Expression of a protein can be detected directly (i.e., by measuring the level of the protein in a relevant context) or indirectly (e.g., by measuring an activity of the protein in a relevant context, or by measuring a surrogate for expression of the protein such as expression of a transgene that includes a nucleic acid sequence encoding a detectable marker operably linked with a promoter native to the protein of interest). Accordingly, expression of a protein can refer to, and/or be detected by measuring, the level or production of the protein, level or production of mRNA encoding the protein, or activity of a promoter that controls expression of the protein.

[100] 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 and/or a pmEV) 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 and/or a pmEV) 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 and/or pmEVs) 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 a smEV and/or a pmEV) compositions (or preparations) are, e.g., purified from residual habitat products.

[101] As used herein, the term "purified mEV composition" or "mEV composition" refers to a preparation that includes mEVs (such as smEVs and/or pmEVs) 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 and/or pmEVs) 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 and/or pmEVs) 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.

[102] "Reference" refers to a standard or control relative to which a comparison is performed. For example, in some embodiments, an agent, sample, sequence, cell, subject, animal, or individual, or population thereof, or a measure or characteristic representative thereof, is compared with a reference, an agent, sample, sequence, cell, subject, animal, or individual, or population thereof, or a measure or characteristic representative thereof. In some embodiments, a reference is a measured value. In some embodiments, a reference is an established standard or expected value. In some embodiments, a reference is a historical reference. A reference can be quantitative of qualitative. Typically, as would be understood by those of skill in the art, a reference and the value to which it is compared represent assessments under comparable conditions. Those of skill in the art will appreciate when sufficient similarities are present to justify reliance on and/or comparison. In some embodiments, an appropriate reference may be an agent, sample, sequence, cell, subject, animal, or individual, or population thereof, under conditions those of skill in the art will recognize as comparable, e.g., for the purpose of assessing one or more particular variables (e.g., potency), or a measure or characteristic representative thereof.

[103] As used herein, "reference standard" means a normalized value obtained from a standardized sample, and in the case of analyzing the potency of a drug product or drug substance, can mean normalized expression of a transcription product (e.g., a reporter mRNA or

protein) encoded by a nucleic acid sequence operatively linked to a regualtory sequence measured in a reference sample of known potency (e.g., as measured in parallel with measurement of the same parameter in a test sample to which the reference is compared, with same or comparable steps and conditions applied to both the reference sample and the test sample). In some embodiments, a reference standard is a sample of a drug product or drug substance that has previously been confirmed to be potent. For example, a reference standard can be a preparation of the same bacteria or mEV as in a drug product or drug substance being tested, wherein the reference standard causes a certain level of transcription product expression in the assay.

[104] As used herein in the context of expression of a nucleic acid coding sequence, a "regulatory sequence" refers to a nucleic acid sequence that controls expression of a coding sequence. In some embodiments, a regulatory sequence can control or impact one or more aspects of gene expression (e.g., cell-type-specific expression, inducible expression, etc.).

[105] "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.

[106] "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 one regulatory sequence (e.g., a promoter, a response element, 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 sequence (e.g., a promoter, a response element, 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.

[107] 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.

[108] As used herein, a "systemic effect" in a subject treated with a pharmaceutical composition containing bacteria or mEVs (e.g., a pharmaceutical agent comprising bacteria or mEVs) provided herein means a physiological effect occurring at one or more sites outside the gastrointestinal tract. Systemic effect(s) can result from immune modulation (e.g., via an increase and/or a reduction of one or more immune cell types or subtypes (e.g., CD8+ T cells) and/or one or more cytokines). Such systemic effect(s) may be the result of the modulation by bacteria or mEVs provided herein on immune or other cells (such as epithelial cells) in the gastrointestinal tract which then, directly or indirectly, result in the alteration of activity (activation and/or deactivation) of one or more biochemical pathways outside the gastrointestinal tract. The systemic effect may include treating or preventing a disease or condition in a subject.

[109] 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.

Bacteria

[110] The drug products and drug substances disclosed herein can comprise a pharmaceutical agent comprising bacteria and/or microbial extracellular vesicles (mEVs) (such as smEVs and/or pmEVs). For example, the drug products and drug substances disclosed herein can comprise a powder comprising bacteria and/or microbial extracellular vesicles (mEVs) (such as smEVs and/or pmEVs) (e.g., the powder can be resuspended prior to use in a method described herein). Within a pharmaceutical agent that contains bacteria and mEVs, the mEVs can be from the same bacterial origin (e.g., same strain) as the bacteria of the pharmaceutical agent (e.g., drug substance). Drug products (e.g., pharmaceutical compositions) and drug substances (e.g., pharmaceutical agents) can contain bacteria and/or mEVs from one or more strains. Drug products (e.g., pharmaceutical compositions) and drug substances (e.g., pharmaceutical agents) can contain bacteria and/or mEVs from one strain.

[111] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are modified to reduce toxicity or other adverse effects, to enhance delivery) (e.g., oral delivery) (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 bacteria and/or mEVs (e.g., either alone or in combination with another therapeutic agent), and/or to enhance immune activation or suppression by the bacteria and/or mEVs (such as smEVs and/or pmEVs) (e.g., through modified production of polysaccharides, pili, fimbriae, adhesins). In some embodiments, the engineered bacteria described herein are modified to improve bacteria and/or mEV (such as smEV and/or pmEV) 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 result 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.

[112] Examples of taxonomic groups (e.g., class, order, family, genus, species or strain) of bacteria that can be used as a source of bacteria and/or mEVs (such as smEVs and/or pmEVs) for a pharmaceutical agent described herein are provided herein (e.g., listed in Table 1, Table 2, Table 3, and/or Table 4 and/or elsewhere in 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 herein. In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are oncotrophic bacteria. In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are immunomodulatory bacteria. In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are immunostimulatory bacteria. In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are immunosuppressive bacteria. In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are generated from one of the bacterial strains provided herein. In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are generated from a combination of the 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 the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are from bacterial strains listed herein 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 herein (e.g., listed in Table 1, Table 2, Table 3, and/or Table 4 and/or elsewhere in the specification). In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are generated from a bacterial strain provided herein. In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are

obtained are from a bacterial strain listed herein (e.g., listed in Table 1, Table 2, Table 3, and/or Table 4 and/or elsewhere in the specification) and/or 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 herein (e.g., listed in Table 1, Table 2, Table 3, and/or Table 4 and/or elsewhere in the specification).

[113] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are Gram negative bacteria.

[114] 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 EVs from this class were investigated. It was found that on a per cell basis these bacteria produce a high number of vesicles (10-150 EVs/cell). The EVs 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 *Negativicutes* class includes the families *Veillonellaceae, Selenomonadaceae, Acidaminococcaceae*, and *Sporomusaceae*. The *Negativicutes* class 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.*

[115] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are Gram positive bacteria.

[116] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are aerobic bacteria.

[117] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are anaerobic bacteria. In some embodiments, the anaerobic bacteria comprise obligate anaerobes. In some embodiments, the anaerobic bacteria comprise facultative anaerobes.

[118] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are acidophile bacteria.

[119] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are alkaliphile bacteria.

[120] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are neutralophile bacteria.

[121] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are fastidious bacteria.

[122] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are nonfastidious bacteria.

[123] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained or the mEVs themselves are lyophilized.

[124] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained or the mEVs themselves are gamma irradiated (e.g., at 17.5 or 25 kGy).

[125] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained or the mEVs themselves are UV irradiated.

[126] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained or the mEVs themselves are heat inactivated (e.g., at 50°C for two hours or at 90°C for two hours).

[127] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained or the mEVs themselves are acid treated.

[128] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained or the mEVs themselves are oxygen sparged (e.g., at 0.1 vvm for two hours).

[129] The phase of growth can affect the amount or properties of bacteria and/or mEVs produced by bacteria. For example, in the methods of mEVs preparation provided herein, mEVs 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.

[130] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent 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*.

[131] The *Negativicutes* class includes the families *Veillonellaceae, Selenomonadaceae, Acidaminococcaceae,* and *Sporomusaceae*. The *Negativicutes* class includes the genera *Megasphaera, Selenomonas, Propionospora,* and *Acidaminococcus*. Exemplary *Negativicutes* species include, but are not limited to, *Megasphaera sp., Selenomonas felix, Acidaminococcus intestini,* and *Propionospora* sp.

[132] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the *Negativicutes* class.

[133] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the *Veillonellaceae* family.

[134] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the *Selenomonadaceae* family.

[135] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the *Acidaminococcaceae* family.

[136] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the *Sporomusaceae* family.

[137] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the *Megasphaera* genus.

[138] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the *Selenomonas* genus.

[139] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the *Propionospora* genus.

[140] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the *Acidaminococcus* genus.

[141] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are *Megasphaera sp.* bacteria.

[142] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are *Selenomonas felix* bacteria.

[143] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are *Acidaminococcus intestini* bacteria.

[144] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are *Propionospora sp.* bacteria.

[145] The *Oscillospriraceae* family within the *Clostridia* class of microorganisms are common commensal organisms of vertebrates.

[146] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the *Clostridia* class.

[147] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the *Oscillospriraceae* family.

[148] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the *Faecalibacterium* genus.

[149] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the *Fournierella* genus.

[150] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the *Harryflintia* genus.

[151] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the *Agathobaculum* genus.

[152] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are *Faecalibacterium prausnitzii* (e.g., *Faecalibacterium prausnitzii* Strain A) bacteria.

[153] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are *Fournierella massiliensis* (e.g., *Fournierella massiliensis* Strain A) bacteria.

[154] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are *Harryflintia acetispora* (e.g., *Harryflintia acetispora* Strain A) bacteria.

[155] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are *Agathobaculum sp.* (e.g., *Agathobaculum sp.* Strain A) bacteria.

[156] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are bacteria of a genus selected from the group consisting of *Escherichia, Klebsiella, Lactobacillus, Shigella,* and *Staphylococcus*.

[157] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are 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*.

[158] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are 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.*

[159] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are 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 bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are 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 of the strain of bacteria deposited with the ATCC Deposit number as provided in Table 3.

[160] The *Negativicutes* class includes the families *Veillonellaceae, Selenomonadaceae, Acidaminococcaceae,* and *Sporomusaceae*. The *Negativicutes* class includes the genera *Megasphaera, Selenomonas, Propionospora,* and *Acidaminococcus*. Exemplary *Negativicutes* species include, but are not limited to, *Megasphaera sp., Selenomonas felix, Acidaminococcus intestini,* and *Propionospora* sp.

[161] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the *Negativicutes* class.

[162] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the *Veillonellaceae* family.

[163] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the *Selenomonadaceae* family.

[164] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the *Acidaminococcaceae* family.

[165] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the *Sporomusaceae* family.

[166] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the *Megasphaera* genus.

[167] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the *Selenomonas* genus.

[168] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the *Propionospora* genus.

[169] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the *Acidaminococcus* genus.

[170] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are *Megasphaera sp.* bacteria.

[171] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are *Selenomonas felix* bacteria.

[172] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are *Acidaminococcus intestini* bacteria.

[173] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are *Propionospora sp.* bacteria.

[174] The *Oscillospriraceae* family within the *Clostridia* class of microorganisms are common commensal organisms of vertebrates.

[175] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the *Clostridia* class.

[176] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the *Oscillospriraceae* family.

[177] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the *Faecalibacterium* genus.

[178] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the *Fournierella* genus.

[179] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the *Harryflintia* genus.

[180] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the *Agathobaculum* genus.

[181] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are *Faecalibacterium prausnitzii* (e.g., *Faecalibacterium prausnitzii* Strain A) bacteria.

[182] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are *Fournierella massiliensis* (e.g., *Fournierella massiliensis* Strain A) bacteria.

[183] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are *Harryflintia acetispora* (e.g., *Harryflintia acetispora* Strain A) bacteria.

[184] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are *Agathobaculum sp.* (e.g., *Agathobaculum sp.* Strain A) bacteria.

[185] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are a strain of *Agathobaculum sp.* In some embodiments, the *Agathobaculum sp.* strain is a strain comprising 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 nucleotide sequence (e.g., genomic sequence, 16S sequence, CRISPR sequence) of the *Agathobaculum sp.* Strain A (ATCC Deposit Number PTA-125892). In some embodiments, the *Agathobaculum sp.* strain is the Agathobaculum sp. Strain A (ATCC Deposit Number PTA- 125892).

[186] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the class *Bacteroidia* [phylum *Bacteroidota*]. In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are bacteria of order *Bacteroidales*. In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the family *Porphyromonoadaceae*. In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the family *Prevotellaceae*. In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are bacteria of the class *Bacteroidia* wherein the cell envelope structure of the bacteria is diderm. In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are bacteria of the class *Bacteroidia* that stain Gram negative. In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are bacteria of the class *Bacteroidia* wherein the bacteria is diderm and the bacteria stain Gram negative.

[187] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are bacteria of the class *Clostridia* [phylum *Firmicutes*]. In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the order *Eubacteriales*. In some

embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the family *Oscillispiraceae*. In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the family *Lachnospiraceae*. In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the family *Peptostreptococcaceae*. In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the family *Clostridiales family XIII/ Incertae sedis 41*. In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the class *Clostridia* wherein the cell envelope structure of the bacteria is monoderm. In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the class *Clostridia* that stain Gram negative. In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the class *Clostridia* that stain Gram positive. In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the class *Clostridia* wherein the cell envelope structure of the bacteria is monoderm and the bacteria stain Gram negative. In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the class *Clostridia* wherein the cell envelope structure of the bacteria is monoderm and the bacteria stain Gram positive.

[188] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the class *Negativicutes* [phylum *Firmicutes*]. In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the order *Veillonellales*. In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the family *Veillonelloceae*. In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the order *Selenomonadales*. In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are bacteria of the family *Selenomonadaceae*. In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of

the family *Sporomusaceae*. In some embodiments, t the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the class *Negativicutes* wherein the cell envelope structure of the bacteria is diderm. In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are the EVs are from bacteria of the class *Negativicutes* wherein the cell envelope structure of the bacteria is diderm and the bacteria stain Gram negative.

[189] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the class *Synergistia* [phylum *Synergistota*]. In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the order *Synergistales*. In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the family *Synergistaceae*. In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the class *Synergistia* wherein the cell envelope structure of the bacteria is diderm. In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the class *Synergistia* that stain Gram negative. In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the class *Synergistia* wherein the cell envelope structure of the bacteria is diderm and the bacteria stain Gram negative.

[190] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are from one strain of bacteria, e.g., a strain provided herein.

[191] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are from one strain of bacteria (e.g., a strain provided herein) or from more than one strain provided herein.

[192] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are *Lactococcus lactis cremoris* bacteria, e.g., 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 bacteria of the pharmaceutical agent or from which the

mEVs of the pharmaceutical agent are obtained are *Lactococcus* bacteria, e.g., *Lactococcus lactis cremoris* Strain A (ATCC designation number PTA-125368).

[193] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are *Prevotella* bacteria, e.g., 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 bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are *Prevotella* bacteria, e.g., *Prevotella* Strain B 50329 (NRRL accession number B 50329).

[194] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are *Prevotella histicola* bacteria, e.g., a strain comprising at least 90% or at least 99% genomic, 16S and/or CRISPR sequence identity to the nucleotide sequence of the *Prevotella histicola* ATCC designation number PTA-126140. In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are *Prevotella histicola* bacteria, e.g., *Prevotella histicola* ATCC designation number PTA-126140.

[195] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are *Bifidobacterium* bacteria, e.g., 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 bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are *Bifidobacterium* bacteria, e.g., *Bifidobacterium* bacteria deposited as ATCC designation number PTA-125097.

[196] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are *Veillonella* bacteria, e.g., 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 bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are *Veillonella* bacteria, e.g., *Veillonella* bacteria deposited as ATCC designation number PTA-125691.

[197] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are *Ruminococcus gnavus* bacteria. In some embodiments, the *Ruminococcus gnavus* bacteria are 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 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 *Ruminococcus gnavus* bacteria deposited as ATCC designation number PTA-126695.

[198] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are *Megasphaera sp.* bacteria. In some embodiments, the *Megasphaera sp.* bacteria are 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 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 *Megasphaera sp.* bacteria deposited as ATCC designation number PTA-126770.

[199] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are *Fournierella massiliensis* bacteria. In some embodiments, the *Fournierella massiliensis* bacteria are 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-126696. In some embodiments, the *Fournierella massiliensis* bacteria are 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-126696. In some embodiments, the *Fournierella massiliensis* bacteria are *Fournierella massiliensis* bacteria deposited as ATCC designation number PTA-126696.

[200] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are *Harryflintia acetispora* bacteria. In some

embodiments, the *Harryflintia acetispora* bacteria are 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-126694. In some embodiments, the *Harryflintia acetispora* bacteria are 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-126694. In some embodiments, the *Harryflintia acetispora* bacteria are *Harryflintia acetispora* bacteria deposited as ATCC designation number PTA-126694.

[201] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are bacteria that produce metabolites, e.g., the bacteria produce butyrate, iosine, proprionate, or tryptophan metabolites.

[202] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are bacteria that produce butyrate. In some embodiments, the bacteria are from the genus *Blautia; Christensella; Copracoccus; Eubacterium; Lachnosperacea; Megasphaera;* or *Roseburia*.

[203] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are bacteria that produce iosine. In some embodiments, the bacteria are from the genus *Bifidobacterium; Lactobacillus;* or *Olsenella*.

[204] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are bacteria that produce proprionate. In some embodiments, the bacteria are from the genus *Akkermansia; Bacteriodes; Dialister; Eubacterium; Megasphaera; Parabacteriodes; Prevotella; Ruminococcus;* or *Veillonella*.

[205] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are bacteria that produce tryptophan metabolites. In some embodiments, the bacteria are from the genus *Lactobacillus* or *Peptostreptococcus*.

[206] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are bacteria that produce inhibitors of histone deacetylase 3 (HDAC3). In some embodiments, the bacteria are from the species *Bariatricus massiliensis, Faecalibacterium prausnitzii, Megasphaera massiliensis* or *Roseburia intestinalis.*

[207] In some embodiments, the bacteria are from the genus *Alloiococcus*; *Bacillus*; *Catenibacterium*; *Corynebacterium*; *Cupriavidus*; *Enhydrobacter*; *Exiguobacterium*;

Faecalibacterium; *Geobacillus*; *Methylobacterium*; *Micrococcus*; *Morganella*; *Proteus*; *Pseudomonas*; *Rhizobium*; or *Sphingomonas*. In some embodiments, the bacteria are from the genus *Cutibacterium*. In some embodiments, the bacteria are from the species *Cutibacterium avidum*. In some embodiments, the bacteria are from the genus *Lactobacillus*. In some embodiments, the bacteria are from the species *Lactobacillus gasseri*. In some embodiments, the bacteria are from the genus *Dysosmobacter*. In some embodiments, the bacteria are from the species *Dysosmobacter welbionis*.

[208] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the genus *Alloiococcus*; *Bacillus*; *Catenibacterium*; *Corynebacterium*; *Cupriavidus*; *Enhydrobacter*; *Exiguobacterium*; *Faecalibacterium*; *Geobacillus*; *Methylobacterium*; *Micrococcus*; *Morganella*; *Proteus*; *Pseudomonas*; *Rhizobium*; or *Sphingomonas*.

[209] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the *Cutibacterium* genus. In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are *Cutibacterium avidum* bacteria.

[210] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the genus *Leuconostoc*.

[211] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the genus *Lactobacillus*.

[212] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are of the genus *Akkermansia*; *Bacillus*; *Blautia*; *Cupriavidus*; *Enhydrobacter*; *Faecalibacterium*; *Lactobacillus*; *Lactococcus*; *Micrococcus*; *Morganella*; *Propionibacterium*; *Proteus*; *Rhizobium*; or *Streptococcus*.

[213] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are *Leuconostoc holzapfelii* bacteria.

[214] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are *Akkermansia muciniphila; Cupriavidus metallidurans; Faecalibacterium prausnitzii; Lactobacillus casei; Lactobacillus plantarum; Lactobacillus paracasei; Lactobacillus plantarum; Lactobacillus rhamnosus; Lactobacillus sakei;* or *Streptococcus pyogenes* bacteria.

[215] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are *Lactobacillus casei; Lactobacillus plantarum; Lactobacillus paracasei; Lactobacillus plantarum; Lactobacillus rhamnosus;* or *Lactobacillus sakei* bacteria.

[216] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are *Megasphaera sp.* bacteria (e.g., from the strain with accession number NCIMB 43385, NCIMB 43386 or NCIMB 43387).

[217] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are *Megasphaera massiliensis* bacteria (e.g., from the strain with accession number NCIMB 42787, NCIMB 43388 or NCIMB 43389).

[218] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are *Megasphaera massiliensis* bacteria (e.g., from the strain with accession number DSM 26228).

[219] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are *Bacillus amyloliquefaciens* bacteria (e.g., from the strain with accession number NCIMB 43088, NCIMB 43087, or NCIMB 43086).

[220] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are *Parabacteroides distasonis* bacteria (e.g., from the strain with accession number NCIMB 42382).

[221] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are *Megasphaera massiliensis* bacteria (e.g., from the strain with accession number NCIMB 43388 or NCIMB 43389), or a derivative thereof. See, e.g., WO 2020/120714. In some embodiments, the *Megasphaera massiliensis* bacteria is a strain comprising at least 80%, at least 85%, at least 90%, 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 nucleotide sequence (e.g., genomic sequence, 16S sequence, and/or CRISPR sequence) of *Megasphaera massiliensis* bacteria from the strain with accession number NCIMB 43388 or NCIMB 43389. In some embodiments, the *Megasphaera massiliensis* bacteria is the strain with accession number NCIMB 43388 or NCIMB 43389.

[222] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are *Megasphaera massiliensis* bacteria strain deposited under accession number NCIMB 42787, or a derivative thereof. See, e.g., WO 2018/229216. In some embodiments, the *Megasphaera massiliensis* bacteria is a strain comprising at least 80%, at least 85%, at least 90%, 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 nucleotide sequence (e.g., genomic sequence, 16S sequence, and/or CRISPR sequence) of the *Megasphaera massiliensis* bacteria strain deposited under accession number NCIMB 42787. In some embodiments, the *Megasphaera massiliensis* bacteria is the strain deposited under accession number NCIMB 42787.

[223] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are *Megasphaera sp.* bacteria from the strain with accession number NCIMB 43385, NCIMB 43386 or NCIMB 43387, or a derivative thereof. See, e.g., WO 2020/120714. In some embodiments, the *Megasphaera sp.* bacteria is a strain comprising at least 80%, at least 85%, at least 90%, 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 nucleotide sequence (e.g., genomic sequence, 16S sequence, and/or CRISPR sequence) of the *Megasphaera sp.* from a strain with accession number NCIMB 43385, NCIMB 43386 or NCIMB 43387. In some embodiments, the *Megasphaera sp.* bacteria is the strain with accession number NCIMB 43385, NCIMB 43386 or NCIMB 43387.

[224] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are *Parabacteroides distasonis* bacteria deposited under accession number NCIMB 42382, or a derivative thereof. See, e.g., WO 2018/229216. In some embodiments, the *Parabacteroides distasonis* bacteria is a strain comprising at least 80%, at least 85%, at least 90%, 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 nucleotide sequence (e.g., genomic sequence, 16S

sequence, and/or CRISPR sequence) of the *Parabacteroides distasonis* bacteria deposited under accession number NCIMB 42382. In some embodiments, the *Parabacteroides distasonis* bacteria is the strain deposited under accession number NCIMB 42382.

[225] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are *Megasphaera massiliensis* bacteria deposited under accession number DSM 26228, or a derivative thereof. See, e.g., WO 2018/229216. In some embodiments, the *Megasphaera massiliensis* bacteria is a strain comprising at least 80%, at least 85%, at least 90%, 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 nucleotide sequence (e.g., genomic sequence, 16S sequence, and/or CRISPR sequence) of *Megasphaera massiliensis* bacteria deposited under accession number DSM 26228. In some embodiments, the *Megasphaera massiliensis* bacteria is the strain deposited under accession number DSM 26228.

[226] In some embodiments, the bacteria of the pharmaceutical agent or from which the mEVs of the pharmaceutical agent are obtained are *Bacillus amyloliquefaciens* bacteria (e.g., from the strain with accession number NCIMB 43088, NCIMB 43087, or NCIMB 43086, or a derivative thereof. See, e.g., WO 2019/236806. In some embodiments, the *Bacillus amyloliquefaciens* bacteria is a strain comprising at least 80%, at least 85%, at least 90%, 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 nucleotide sequence (e.g., genomic sequence, 16S sequence, and/or CRISPR sequence) of *Bacillus amyloliquefaciens* bacteria from the strain with accession number NCIMB 43088, NCIMB 43087, or NCIMB 43086. In some embodiments, the *Bacillus amyloliquefaciens* bacteria is the strain with accession number NCIMB 43088, NCIMB 43087, or NCIMB 43086. In some embodiments, the *Bacillus amyloliquefaciens* bacteria is the strain with accession number NCIMB 43088.

Table 1: Bacteria by Class

Class	Order	Family	Genus*	Species
Actinobacter	*Actinomycetales*	*Mycobacteriaceae*	*Mycobacterium*	

		Streptomycetaceae	*Streptomyces (S.)*	*S. lividans, S coelicolor, S sudanesis, S somaliensis*
	Bifidobacteriales	*Bifidobacteriaceae*	*Bifidobacterium (B.)*	*B. adolescentis, B.animalis, B.bifidum, B. breve, B. lactis, B. longum, B. pseudocatenulatum*
	Coriobacteriales	*Coriobacteriaceae*	*Collinsella*	*Collinsella aerofaciens*
			Olsenella	*Olsenella faecalis*
	Propionibacteriales	*Propionibacteraceae*	*Propionibacterium*	
Bacilli	*Bacillales*	*Bacillales incertae sedis family XI*	*Gemella (G.)*	*G. haemolysans, G. morbillorum*
		Listeraceae	*Listeria (L.)*	*L. monocytogenes, L. welshimeri*
	Lactobacillales	*Enterococcaceae*	*Enterococcus (E.)*	*E. durans, E. faecium, E. faecalis, E. gallinarum, E. villorum,*
			Lactobacillus (L.)	*L. casei, L. fermentum, L. mucosae, L. plantarum, L. reuteri, L. rhamnosus, L. salvarius*
		Streptococcaceae	*Lactococcus*	*Lactococcus lactis cremoris*
			Staphylococcus	*Staphylococcus aureus*
			Streptococcus (S.)	*S. agalactiae, S. aureus, S. australi, S. mutans, S. parasanguinis, S. pneumoniae, S. pyogenes, S. salivarius*
Bacteriodes	*Bacteroidales*	*Bacteriodaceae*	*Bacteriodes (B.)*	*B. caccae, B. cellulosilyticus, B. coprocola, B. dorei, B. fragilis, B. ovatus, B. putredinis, B. salanitronis, B. thetaiotaomicron, B. vulgatus*
		Odoribacteraceae	*Odoribacter*	*Odoribacter splanchnicus*
		Porphyromonadaceae	*Parabacteriodes (P.)*	*P. distasonis, P. goldsteinii, P. merdae*
			Porphyromonas	*Porphyromonas gingivalis*
		Prevotellaceae	*Prevotella (P.)*	*P. albensis, P. amnii, P. aurantiaca, P. baroniae, P. bergensis, P. bivia, P. brevis, P. bryantii, P. buccae, P. buccalis, P.*

				colorans, P. corporis, P. copri, P. dentalis, P. dentasini, P. denticola, P. disiens,, P. enoeca, P. falsenii, P. fusca, P. heparinolytica, P. histicola, P. intermedia, P. jejuni, , P. loescheii, P. maculosa, P. marshii, P. melaninogenica, P. micans, P. multiformis, P. multisaccharivorax, P. nanceiensis, P. nigrescens, P. oralis, P. oris, , P. oryzae, P. oulorum, P. pallens, P. paludivivens, P. pleuritidis P. ruminicola, P. saccharolytica, P. salivae, P. scopos, P. shahii, P. stercorea, P. tannerae, P. timonensis, P. veroralis, P. zoogleoformans
		Rikenellaceae	*Alstipes (A.)*	*A. communis, A. dispar, A. finegoldii, A. indistinctus, A. ihumii, A. inops, A. massiliensis, A. megaguti, A. obesi , A. onderdonkii, A. provencensis, A. putredinis, A. senegalensis, A. shahii, A. timonensis*
Betaproteobacteria	*Burkholderiales*	*Alcaligenaceae*	*Paenalcaligenes*	*Paenalcaligenes hominis*
			Bordella	*Bordella pertussis*
		Burkholderiaceae	*Burkholderia (B.)*	*B. mallei, B. pseudomallei*
			Ralstonia	*Ralstonia solanacearum*
		Neisseriaceae	*Neisseria*	*Neisseria meningitidis*
		Sutterellaceae	*Sutterella (S.)*	*S. parvirubra, S. stercoricanis, S. wadsworthensis*
Clostridia	*Clostridiales*	*Catabacteriaceae*	*Catabacter*	*Catabacter hongkongensis*
		Clostridiaceae	*Aminiphila*	*Anaerosphaera aminiphila*
			Christensenellaceae (C.)	*C. massiliensis, C. minuta, C. timonensis*

			Hungatella	*Hungatella effluvia*
		Eubacteriaceae	*Eubacterium (E.)*	*E. contortum, E. eligens, E. faecium, E. hadrum, E. hallii, E. limosum, E. ramulus, E. rectale*
		Lachnospiraceae	*Anaerostipes (A.)*	*A. caccae, A. hadrus*
			Blautia (B.)	*B. hydrogenotrophica, B. massiliensis, B. stercoris, B. wexlerae*
			Catonella	*Catonella morbi*
			Coprococcus (C.)	*C. catus, C. comes, C. eutactus*
			Dialister (D.)	*D. invisus, D. micraeophilus, D. succinatiphilus*
			Dorea (D.)	*D. formicigenerans, D. longicatena*
			Johnsonella	*Johnsonella ignava*
			Oribacterium (O.)	*O. parvum, O. sinus*
			Lachnobacterium	
			Lachnoclostridium	
			Lacrimispora (L.)	*L. sacchaarolytica*
			Roseburia (R.)	*R. hominis, R. intestinalis*
			Tyzzerella	*Tyzzerella nexilis*
		Oscillospiraceae	*Oscillibacter*	*Oscillibacter valericigenes*
			Harryflintia	*Harryflinta acetispora*
		Peptococcaceae		
		Peptostreptococcaceae	*Paraclostridium*	*Paraclostridium benzoelyticum*
			Peptostreptococcus	*Peptostreptococcus russellii*
		Ruminococcaceae	*Agathobaculum*	*Agathobaculum sp.*
			Fournierella	*Fournierella masssiliensis*
			Ruminococcus (R.)	*R. albus, R. bromii, R. callidus, R. gnavus, R. inulinivorans, R. obeum, R. torques*
			Faecalibacterium	*Faecalibacterium prasusnitzii*
		Clostridiales family XIII/ Incertae sedis		*Intestimonas butyriciproducens*
Fusobacteria	*Fusobacteriales*	*Fusobacteriaceae*	*Fusobacterium (F.)*	*F. nucleatum, F. naviforme*
		Leptotrichiaceae	*Leptotrichia*	
			Sneathia	

Gammaproteobacteria	***Enterobacterales***	***Enterobacteriaceae***	***Klebsiella (K.)***	***K. oxytoca, K. pneumoniae, K. quasipneumoniae subsp. Similipneumoniae,***
			Escherichia (E.)	***E. coli strain Nissle 1917 (EcN), E. coli strain ECOR12, E. coli strain ECOR63***
			Shigella	
Negativicutes		***Acidaminococcaceae***	***Acidaminococcus (A.)***	***A. fermentans, A. intestine***
			Phascolarctobacterium (P.)	***P. faecium, P. succinatutens***
		Selenomonadaceae	***Selenomonas (S.)***	***S. felix, S. incertae sedis, S. sputigena***
		Sporomusaceae	***Selenomonadales***	
		Veillonellaceae	***Allisonella***	
			Anaeroglobus	***Anaeroglobus germinatus***
			Caecibacter	
			Colibacter	
			Megasphaera (M.)	***M. elsedenii, M. massiliensis, M. micronuciformis, Megasphaera sp***
			Massilibacillus	***Massilibacillus massiliensis***
			Propionispira	
			Negativicoccus	***Negativicoccus succinicivornas***
			Veillonella (V.)	***V. dispar, V. parvula, V. ratti, V. tobetsuensis***
	Synergistales	***Synergistaceae***	***Aminobacterium***	***Aminobacterium mobile***
			Cloacibacillus	***Cloacibacillus evryensis***
			Rarimicrobium	***Rarimicrobium hominis***
Verrucomicrobia	***Verrucomicrobiales***	***Akkermansiaceae***	***Akkermansia***	***Akkermansia mucinophila***

* The abbreviation given in the parenthetical is for the species in the row in which it is listed.

Table 2: Exemplary Bacterial Strains

OTU	**Public DB Accession**	**OTU**	**Public DB Accession**
Actinobacillus actinomycetemcomitans	AY362885	*Lactobacillus murinus*	NR_042231

Actinobacillus minor	ACFT01000025	*Lactobacillus nodensis*	NR_041629
Actinobacillus pleuropneumoniae	NR_074857	*Lactobacillus oeni*	NR_043095
Actinobacillus succinogenes	CP000746	*Lactobacillus oris*	AEKL01000077
Actinobacillus ureae	AEVG01000167	*Lactobacillus parabrevis*	NR_042456
Actinobaculum massiliae	AF487679	*Lactobacillus parabuchneri*	NR_041294
Actinobaculum schaalii	AY957507	*Lactobacillus paracasei*	ABQV01000067
Actinobaculum sp. BM#101342	AY282578	*Lactobacillus parakefiri*	NR_029039
Actinobaculum sp. P2P_19 P1	AY207066	*Lactobacillus pentosus*	JN813103
Akkermansia muciniphila	CP001071	*Lactobacillus perolens*	NR_029360
Alistipes finegoldii	NR_043064	*Lactobacillus plantarum*	ACGZ02000033
Alistipes indistinctus	AB490804	*Lactobacillus pontis*	HM218420
Alistipes onderdonkii	NR_043318	*Lactobacillus reuteri*	ACGW02000012
Alistipes putredinis	ABFK02000017	*Lactobacillus rhamnosus*	ABWJ01000068
Alistipes shahii	FP929032	*Lactobacillus rogosae*	GU269544
Alistipes sp. HGB5	AENZ01000082	*Lactobacillus ruminis*	ACGS02000043
Alistipes sp. JC50	JF824804	*Lactobacillus sakei*	DQ989236
Alistipes sp. RMA 9912	GQ140629	*Lactobacillus salivarius*	AEBA01000145
Anaerostipes caccae	ABAX03000023	*Lactobacillus saniviri*	AB602569
Anaerostipes sp. 3 2 56FAA	ACWB01000002	*Lactobacillus senioris*	AB602570
Bacillus aeolius	NR_025557	*Lactobacillus sp. 66c*	FR681900
Bacillus aerophilus	NR_042339	*Lactobacillus sp. BT6*	HQ616370
Bacillus aestuarii	GQ980243	*Lactobacillus sp. KLDS 1.0701*	EU600905
Bacillus alcalophilus	X76436	*Lactobacillus sp. KLDS 1.0702*	EU600906
Bacillus amyloliquefaciens	NR_075005	*Lactobacillus sp. KLDS 1.0703*	EU600907
Bacillus anthracis	AAEN01000020	*Lactobacillus sp. KLDS 1.0704*	EU600908
Bacillus atrophaeus	NR_075016	*Lactobacillus sp. KLDS 1.0705*	EU600909
Bacillus badius	NR_036893	*Lactobacillus sp. KLDS 1.0707*	EU600911
Bacillus cereus	ABDJ01000015	*Lactobacillus sp. KLDS 1.0709*	EU600913
Bacillus circulans	AB271747	*Lactobacillus sp. KLDS 1.0711*	EU600915
Bacillus clausii	FN397477	*Lactobacillus sp. KLDS 1.0712*	EU600916

Bacillus coagulans	DQ297928	*Lactobacillus sp. KLDS 1.0713*	EU600917
Bacillus firmus	NR_025842	*Lactobacillus sp. KLDS 1.0716*	EU600921
Bacillus flexus	NR_024691	*Lactobacillus sp. KLDS 1.0718*	EU600922
Bacillus fordii	NR_025786	*Lactobacillus sp. KLDS 1.0719*	EU600923
Bacillus gelatini	NR_025595	*Lactobacillus sp. oral clone HT002*	AY349382
Bacillus halmapalus	NR_026144	*Lactobacillus sp. oral clone HT070*	AY349383
Bacillus halodurans	AY144582	*Lactobacillus sp. oral taxon 052*	GQ422710
Bacillus herbersteinensis	NR_042286	*Lactobacillus tucceti*	NR_042194
Bacillus horti	NR_036860	*Lactobacillus ultunensis*	ACGU01000081
Bacillus idriensis	NR_043268	*Lactobacillus vaginalis*	ACGV01000168
Bacillus lentus	NR_040792	*Lactobacillus vini*	NR_042196
Bacillus licheniformis	NC_006270	*Lactobacillus vitulinus*	NR_041305
Bacillus megaterium	GU252124	*Lactobacillus zeae*	NR_037122
Bacillus nealsonii	NR_044546	*Lactococcus garvieae*	AF061005
Bacillus niabensis	NR_043334	*Lactococcus lactis*	CP002365
Bacillus niacini	NR_024695	*Lactococcus raffinolactis*	NR_044359
Bacillus pocheonensis	NR_041377	*Listeria grayi*	ACCR02000003
Bacillus pumilus	NR_074977	*Listeria innocua*	JF967625
Bacillus safensis	JQ624766	*Listeria ivanovii*	X56151
Bacillus simplex	NR_042136	*Listeria monocytogenes*	CP002003
Bacillus sonorensis	NR_025130	*Listeria welshimeri*	AM263198
Bacillus sp. 10403023 MM10403188	CAET01000089	*Megasphaera elsdenii*	AY038996
Bacillus sp. 2_A_57_CT2	ACWD01000095	*Megasphaera genomosp. C1*	AY278622
Bacillus sp. 2008724126	GU252108	*Megasphaera genomosp. type_1*	ADGP01000010
Bacillus sp. 2008724139	GU252111	*Megasphaera micronuciformis*	AECS01000020
Bacillus sp. 7_16AIA	FN397518	*Megasphaera sp. BLPYG_07*	HM990964
Bacillus sp. 9_3AIA	FN397519	*Megasphaera sp. UPII 199_6*	AFIJ01000040
Bacillus sp. AP8	JX101689	*Microbacterium gubbeenense*	NR_025098
Bacillus sp. B27(2008)	EU362173	*Microbacterium lacticum*	EU714351

Bacillus sp. BT1B_CT2	ACWC01000034	*Mitsuokella jalaludinii*	NR_028840
Bacillus sp. GB1.1	FJ897765	*Mitsuokella multacida*	ABWK02000005
Bacillus sp. GB9	FJ897766	*Mitsuokella sp. oral taxon 521*	GU413658
Bacillus sp. HU19.1	FJ897769	*Mitsuokella sp. oral taxon G68*	GU432166
Bacillus sp. HU29	FJ897771	*Mycobacterium abscessus*	AGQU01000002
Bacillus sp. HU33.1	FJ897772	*Mycobacterium africanum*	AF480605
Bacillus sp. JC6	JF824800	*Mycobacterium alsiensis*	AJ938169
Bacillus sp. oral taxon F26	HM099642	*Mycobacterium avium*	CP000479
Bacillus sp. oral taxon F28	HM099650	*Mycobacterium chelonae*	AB548610
Bacillus sp. oral taxon F79	HM099654	*Mycobacterium colombiense*	AM062764
Bacillus sp. SRC_DSF1	GU797283	*Mycobacterium elephantis*	AF385898
Bacillus sp. SRC_DSF10	GU797292	*Mycobacterium gordonae*	GU142930
Bacillus sp. SRC_DSF2	GU797284	*Mycobacterium intracellulare*	GQ153276
Bacillus sp. SRC_DSF6	GU797288	*Mycobacterium kansasii*	AF480601
Bacillus sp. tc09	HQ844242	*Mycobacterium lacus*	NR_025175
Bacillus sp. zh168	FJ851424	*Mycobacterium leprae*	FM211192
Bacillus sphaericus	DQ286318	*Mycobacterium lepromatosis*	EU203590
Bacillus sporothermodurans	NR_026010	*Mycobacterium mageritense*	FR798914
Bacillus subtilis	EU627588	*Mycobacterium mantenii*	FJ042897
Bacillus thermoamylovorans	NR_029151	*Mycobacterium marinum*	NC_010612
Bacillus weihenstephanensis	NR_074926	*Mycobacterium microti*	NR_025234
Bacteroidales bacterium ph8	JN837494	*Mycobacterium neoaurum*	AF268445
Bacteroidales genomosp. P1	AY341819	*Mycobacterium parascrofulaceum*	ADNV01000350
Bacteroidales genomosp. P2 oral clone MB1_G13	DQ003613	*Mycobacterium paraterrae*	EU919229
Bacteroidales genomosp. P3 oral clone MB1_G34	DQ003615	*Mycobacterium phlei*	GU142920
Bacteroidales genomosp. P4 oral clone MB2_G17	DQ003617	*Mycobacterium seoulense*	DQ536403
Bacteroidales genomosp. P5 oral clone MB2_P04	DQ003619	*Mycobacterium smegmatis*	CP000480

Bacteroidales genomosp. P6 oral clone MB3_C19	DQ003634	*Mycobacterium sp. 1761*	EU703150
Bacteroidales genomosp. P7 oral clone MB3_P19	DQ003623	*Mycobacterium sp. 1776*	EU703152
Bacteroidales genomosp. P8 oral clone MB4_G15	DQ003626	*Mycobacterium sp. 1781*	EU703147
Bacteroides acidifaciens	NR_028607	*Mycobacterium sp. 1791*	EU703148
Bacteroides barnesiae	NR_041446	*Mycobacterium sp. 1797*	EU703149
Bacteroides caccae	EU136686	*Mycobacterium sp. AQ1GA4*	HM210417
Bacteroides cellulosilyticus	ACCH01000108	*Mycobacterium sp. B10_07.09.0206*	HQ174245
Bacteroides clarus	AFBM01000011	*Mycobacterium sp. GN_10546*	FJ497243
Bacteroides coagulans	AB547639	*Mycobacterium sp. GN_10827*	FJ497247
Bacteroides coprocola	ABIY02000050	*Mycobacterium sp. GN_11124*	FJ652846
Bacteroides coprophilus	ACBW01000012	*Mycobacterium sp. GN_9188*	FJ497240
Bacteroides dorei	ABWZ01000093	*Mycobacterium sp. GR_2007_210*	FJ555538
Bacteroides eggerthii	ACWG01000065	*Mycobacterium sp. HE5*	AJ012738
Bacteroides faecis	GQ496624	*Mycobacterium sp. NLA001000736*	HM627011
Bacteroides finegoldii	AB222699	*Mycobacterium sp. W*	DQ437715
Bacteroides fluxus	AFBN01000029	*Mycobacterium tuberculosis*	CP001658
Bacteroides fragilis	AP006841	*Mycobacterium ulcerans*	AB548725
Bacteroides galacturonicus	DQ497994	*Mycobacterium vulneris*	EU834055
Bacteroides helcogenes	CP002352	*Mycoplasma agalactiae*	AF010477
Bacteroides heparinolyticus	JN867284	*Mycoplasma amphoriforme*	AY531656
Bacteroides intestinalis	ABJL02000006	*Mycoplasma arthritidis*	NC_011025
Bacteroides massiliensis	AB200226	*Mycoplasma bovoculi*	NR_025987
Bacteroides nordii	NR_043017	*Mycoplasma faucium*	NR_024983
Bacteroides oleiciplenus	AB547644	*Mycoplasma fermentans*	CP002458
Bacteroides ovatus	ACWH01000036	*Mycoplasma flocculare*	X62699
Bacteroides pectinophilus	ABVQ01000036	*Mycoplasma genitalium*	L43967
Bacteroides plebeius	AB200218	*Mycoplasma hominis*	AF443616
Bacteroides pyogenes	NR_041280	*Mycoplasma orale*	AY796060
Bacteroides salanitronis	CP002530	*Mycoplasma ovipneumoniae*	NR_025989
Bacteroides salyersiae	EU136690	*Mycoplasma penetrans*	NC_004432

Bacteroides sp. 1_1_14	ACRP01000155	*Mycoplasma pneumoniae*	NC_000912
Bacteroides sp. 1_1_30	ADCL01000128	*Mycoplasma putrefaciens*	U26055
Bacteroides sp. 1_1_6	ACIC01000215	*Mycoplasma salivarium*	M24661
Bacteroides sp. 2_1_22	ACPQ01000117	*Mycoplasmataceae genomosp. P1 oral clone MB1_G23*	DQ003614
Bacteroides sp. 2_1_56FAA	ACWI01000065	*Neisseria bacilliformis*	AFAY01000058
Bacteroides sp. 2_2_4	ABZZ01000168	*Neisseria cinerea*	ACDY01000037
Bacteroides sp. 20_3	ACRQ01000064	*Neisseria elongata*	ADBF01000003
Bacteroides sp. 3_1_19	ADCJ01000062	*Neisseria flavescens*	ACQV01000025
Bacteroides sp. 3_1_23	ACRS01000081	*Neisseria genomosp. P2 oral clone MB5_P15*	DQ003630
Bacteroides sp. 3_1_33FAA	ACPS01000085	*Neisseria gonorrhoeae*	CP002440
Bacteroides sp. 3_1_40A	ACRT01000136	*Neisseria lactamica*	ACEQ01000095
Bacteroides sp. 3_2_5	ACIB01000079	*Neisseria macacae*	AFQE01000146
Bacteroides sp. 315_5	FJ848547	*Neisseria meningitidis*	NC_003112
Bacteroides sp. 31SF15	AJ583248	*Neisseria mucosa*	ACDX01000110
Bacteroides sp. 31SF18	AJ583249	*Neisseria pharyngis*	AJ239281
Bacteroides sp. 35AE31	AJ583244	*Neisseria polysaccharea*	ADBE01000137
Bacteroides sp. 35AE37	AJ583245	*Neisseria sicca*	ACKO02000016
Bacteroides sp. 35BE34	AJ583246	*Neisseria sp. KEM232*	GQ203291
Bacteroides sp. 35BE35	AJ583247	*Neisseria sp. oral clone AP132*	AY005027
Bacteroides sp. 4_1_36	ACTC01000133	*Neisseria sp. oral clone JC012*	AY349388
Bacteroides sp. 4_3_47FAA	ACDR02000029	*Neisseria sp. oral strain B33KA*	AY005028
Bacteroides sp. 9_1_42FAA	ACAA01000096	*Neisseria sp. oral taxon 014*	ADEA01000039
Bacteroides sp. AR20	AF139524	*Neisseria sp. SMC_A9199*	FJ763637
Bacteroides sp. AR29	AF139525	*Neisseria sp. TM10_1*	DQ279352
Bacteroides sp. B2	EU722733	*Neisseria subflava*	ACEO01000067
Bacteroides sp. D1	ACAB02000030	*Odoribacter laneus*	AB490805
Bacteroides sp. D2	ACGA01000077	*Odoribacter splanchnicus*	CP002544
Bacteroides sp. D20	ACPT01000052	*Oscillibacter sp. G2*	HM626173
Bacteroides sp. D22	ADCK01000151	*Oscillibacter valericigenes*	NR_074793
Bacteroides sp. F_4	AB470322	*Oscillospira guilliermondii*	AB040495

Bacteroides sp. NB_8	AB117565	*Paenibacillus barcinonensis*	NR_042272
Bacteroides sp. WH2	AY895180	*Paenibacillus barengoltzii*	NR_042756
Bacteroides sp. XB12B	AM230648	*Paenibacillus chibensis*	NR_040885
Bacteroides sp. XB44A	AM230649	*Paenibacillus cookii*	NR_025372
Bacteroides stercoris	ABFZ02000022	*Paenibacillus durus*	NR_037017
Bacteroides thetaiotaomicron	NR_074277	*Paenibacillus glucanolyticus*	D78470
Bacteroides uniformis	AB050110	*Paenibacillus lactis*	NR_025739
Bacteroides ureolyticus	GQ167666	*Paenibacillus lautus*	NR_040882
Bacteroides vulgatus	CP000139	*Paenibacillus pabuli*	NR_040853
Bacteroides xylanisolvens	ADKP01000087	*Paenibacillus polymyxa*	NR_037006
Bacteroidetes bacterium oral taxon D27	HM099638	*Paenibacillus popilliae*	NR_040888
Bacteroidetes bacterium oral taxon F31	HM099643	*Paenibacillus sp. CIP 101062*	HM212646
Bacteroidetes bacterium oral taxon F44	HM099649	*Parabacteroides distasonis*	CP000140
Barnesiella intestinihominis	AB370251	*Parabacteroides goldsteinii*	AY974070
Bifidobacteriaceae genomosp. C1	AY278612	*Parabacteroides gordonii*	AB470344
Bifidobacterium adolescentis	AAXD02000018	*Parabacteroides johnsonii*	ABYH01000014
Bifidobacterium angulatum	ABYS02000004	*Parabacteroides merdae*	EU136685
Bifidobacterium animalis	CP001606	*Parabacteroides sp. D13*	ACPW01000017
Bifidobacterium bifidum	ABQP01000027	*Parabacteroides sp. NS31_3*	JN029805
Bifidobacterium breve	CP002743	*Peptococcus niger*	NR_029221
Bifidobacterium catenulatum	ABXY01000019	*Peptococcus sp. oral clone JM048*	AY349389
Bifidobacterium dentium	CP001750	*Peptococcus sp. oral taxon 167*	GQ422727
Bifidobacterium gallicum	ABXB03000004	*Peptoniphilus asaccharolyticus*	D14145
Bifidobacterium infantis	AY151398	*Peptoniphilus duerdenii*	EU526290
Bifidobacterium kashiwanohense	AB491757	*Peptoniphilus harei*	NR_026358
Bifidobacterium longum	ABQQ01000041	*Peptoniphilus indolicus*	AY153431
Bifidobacterium pseudocatenulatum	ABXX02000002	*Peptoniphilus ivorii*	Y07840
Bifidobacterium pseudolongum	NR_043442	*Peptoniphilus lacrimalis*	ADDO01000050

Bifidobacterium scardovii	AJ307005	*Peptoniphilus sp. gpac007*	AM176517
Bifidobacterium sp. HM2	AB425276	*Peptoniphilus sp. gpac018A*	AM176519
Bifidobacterium sp. HMLN12	JF519685	*Peptoniphilus sp. gpac077*	AM176527
Bifidobacterium sp. M45	HM626176	*Peptoniphilus sp. gpac148*	AM176535
Bifidobacterium sp. MSX5B	HQ616382	*Peptoniphilus sp. JC140*	JF824803
Bifidobacterium sp. TM_7	AB218972	*Peptoniphilus sp. oral taxon 386*	ADCS01000031
Bifidobacterium thermophilum	DQ340557	*Peptoniphilus sp. oral taxon 836*	AEAA01000090
Bifidobacterium urinalis	AJ278695	*Peptostreptococcaceae bacterium ph1*	JN837495
Blautia coccoides	AB571656	*Peptostreptococcus anaerobius*	AY326462
Blautia glucerasea	AB588023	*Peptostreptococcus micros*	AM176538
Blautia glucerasei	AB439724	*Peptostreptococcus sp. 9succ1*	X90471
Blautia hansenii	ABYU02000037	*Peptostreptococcus sp. oral clone AP24*	AB175072
Blautia hydrogenotrophica	ACBZ01000217	*Peptostreptococcus sp. oral clone FJ023*	AY349390
Blautia luti	AB691576	*Peptostreptococcus sp. P4P_31 P3*	AY207059
Blautia producta	AB600998	*Peptostreptococcus stomatis*	ADGQ01000048
Blautia schinkii	NR_026312	*Porphyromonadaceae bacterium NML 060648*	EF184292
Blautia sp. M25	HM626178	*Porphyromonas asaccharolytica*	AENO01000048
Blautia stercoris	HM626177	*Porphyromonas endodontalis*	ACNN01000021
Blautia wexlerae	EF036467	*Porphyromonas gingivalis*	AE015924
Bordetella bronchiseptica	NR_025949	*Porphyromonas levii*	NR_025907
Bordetella holmesii	AB683187	*Porphyromonas macacae*	NR_025908
Bordetella parapertussis	NR_025950	*Porphyromonas somerae*	AB547667
Bordetella pertussis	BX640418	*Porphyromonas sp. oral clone BB134*	AY005068
Borrelia afzelii	ABCU01000001	*Porphyromonas sp. oral clone F016*	AY005069

Borrelia burgdorferi	ABGI01000001	*Porphyromonas sp. oral clone P2PB_52 P1*	AY207054
Borrelia crocidurae	DQ057990	*Porphyromonas sp. oral clone P4GB_100 P2*	AY207057
Borrelia duttonii	NC_011229	*Porphyromonas sp. UQD 301*	EU012301
Borrelia garinii	ABJV01000001	*Porphyromonas uenonis*	ACLR01000152
Borrelia hermsii	AY597657	*Prevotella albensis*	NR_025300
Borrelia hispanica	DQ057988	*Prevotella amnii*	AB547670
Borrelia persica	HM161645	*Prevotella bergensis*	ACKS01000100
Borrelia recurrentis	AF107367	*Prevotella bivia*	ADFO01000096
Borrelia sp. NE49	AJ224142	*Prevotella brevis*	NR_041954
Borrelia spielmanii	ABKB01000002	*Prevotella buccae*	ACRB01000001
Borrelia turicatae	NC_008710	*Prevotella buccalis*	JN867261
Borrelia valaisiana	ABCY01000002	*Prevotella copri*	ACBX02000014
Brucella ovis	NC_009504	*Prevotella corporis*	L16465
Brucella sp. 83_13	ACBQ01000040	*Prevotella dentalis*	AB547678
Brucella sp. BO1	EU053207	*Prevotella denticola*	CP002589
Brucella suis	ACBK01000034	*Prevotella disiens*	AEDO01000026
Burkholderia ambifaria	AAUZ01000009	*Prevotella genomosp. C1*	AY278624
Burkholderia cenocepacia	AAHI01000060	*Prevotella genomosp. C2*	AY278625
Burkholderia cepacia	NR_041719	*Prevotella genomosp. P7 oral clone MB2_P31*	DQ003620
Burkholderia mallei	CP000547	*Prevotella genomosp. P8 oral clone MB3_P13*	DQ003622
Burkholderia multivorans	NC_010086	*Prevotella genomosp. P9 oral clone MB7_G16*	DQ003633
Burkholderia oklahomensis	DQ108388	*Prevotella heparinolytica*	GQ422742
Burkholderia pseudomallei	CP001408	*Prevotella histicola*	JN867315
Burkholderia rhizoxinica	HQ005410	*Prevotella intermedia*	AF414829
Burkholderia sp. 383	CP000151	*Prevotella loescheii*	JN867231
Burkholderia xenovorans	U86373	*Prevotella maculosa*	AGEK01000035
Burkholderiales bacterium 1_1_47	ADCQ01000066	*Prevotella marshii*	AEEI01000070
Butyrivibrio crossotus	ABWN01000012	*Prevotella melaninogenica*	CP002122
Butyrivibrio fibrisolvens	U41172	*Prevotella micans*	AGWK01000061
Chlamydia muridarum	AE002160	*Prevotella multiformis*	AEWX01000054
Chlamydia psittaci	NR_036864	*Prevotella multisaccharivorax*	AFJE01000016
Chlamydia trachomatis	U68443	*Prevotella nanceiensis*	JN867228
Chlamydiales bacterium NS11	JN606074	*Prevotella nigrescens*	AFPX01000069

Citrobacter amalonaticus	FR870441	*Prevotella oralis*	AEPE01000021
Citrobacter braakii	NR_028687	*Prevotella oris*	ADDV01000091
Citrobacter farmeri	AF025371	*Prevotella oulorum*	L16472
Citrobacter freundii	NR_028894	*Prevotella pallens*	AFPY01000135
Citrobacter gillenii	AF025367	*Prevotella ruminicola*	CP002006
Citrobacter koseri	NC_009792	*Prevotella salivae*	AB108826
Citrobacter murliniae	AF025369	*Prevotella sp. BI_42*	AJ581354
Citrobacter rodentium	NR_074903	*Prevotella sp. CM38*	HQ610181
Citrobacter sedlakii	AF025364	*Prevotella sp. ICM1*	HQ616385
Citrobacter sp. 30_2	ACDJ01000053	*Prevotella sp. ICM55*	HQ616399
Citrobacter sp. KMSI_3	GQ468398	*Prevotella sp. JCM 6330*	AB547699
Citrobacter werkmanii	AF025373	*Prevotella sp. oral clone AA020*	AY005057
Citrobacter youngae	ABWL02000011	*Prevotella sp. oral clone ASCG10*	AY923148
Cloacibacillus evryensis	GQ258966	*Prevotella sp. oral clone ASCG12*	DQ272511
Clostridiaceae bacterium END_2	EF451053	*Prevotella sp. oral clone AU069*	AY005062
Clostridiaceae bacterium JC13	JF824807	*Prevotella sp. oral clone CY006*	AY005063
Clostridiales bacterium 1_7_47FAA	ABQR01000074	*Prevotella sp. oral clone DA058*	AY005065
Clostridiales bacterium 9400853	HM587320	*Prevotella sp. oral clone FL019*	AY349392
Clostridiales bacterium 9403326	HM587324	*Prevotella sp. oral clone FU048*	AY349393
Clostridiales bacterium oral clone P4PA_66 P1	AY207065	*Prevotella sp. oral clone FW035*	AY349394
Clostridiales bacterium oral taxon 093	GQ422712	*Prevotella sp. oral clone GI030*	AY349395
Clostridiales bacterium oral taxon F32	HM099644	*Prevotella sp. oral clone GI032*	AY349396
Clostridiales bacterium ph2	JN837487	*Prevotella sp. oral clone GI059*	AY349397
Clostridiales bacterium SY8519	AB477431	*Prevotella sp. oral clone GU027*	AY349398
Clostridiales genomosp. BVAB3	CP001850	*Prevotella sp. oral clone HF050*	AY349399
Clostridiales sp. SM4_1	FP929060	*Prevotella sp. oral clone ID019*	AY349400
Clostridiales sp. SS3_4	AY305316	*Prevotella sp. oral clone IDR_CEC_0055*	AY550997

Clostridiales sp. SSC_2	FP929061	*Prevotella sp. oral clone IK053*	AY349401
Clostridium acetobutylicum	NR_074511	*Prevotella sp. oral clone IK062*	AY349402
Clostridium aerotolerans	X76163	*Prevotella sp. oral clone P4PB_83 P2*	AY207050
Clostridium aldenense	NR_043680	*Prevotella sp. oral taxon 292*	GQ422735
Clostridium aldrichii	NR_026099	*Prevotella sp. oral taxon 299*	ACWZ01000026
Clostridium algidicarnis	NR_041746	*Prevotella sp. oral taxon 300*	GU409549
Clostridium algidixylanolyticum	NR_028726	*Prevotella sp. oral taxon 302*	ACZK01000043
Clostridium aminovalericum	NR_029245	*Prevotella sp. oral taxon 310*	GQ422737
Clostridium amygdalinum	AY353957	*Prevotella sp. oral taxon 317*	ACQH01000158
Clostridium argentinense	NR_029232	*Prevotella sp. oral taxon 472*	ACZS01000106
Clostridium asparagiforme	ACCJ01000522	*Prevotella sp. oral taxon 781*	GQ422744
Clostridium baratii	NR_029229	*Prevotella sp. oral taxon 782*	GQ422745
Clostridium bartlettii	ABEZ02000012	*Prevotella sp. oral taxon F68*	HM099652
Clostridium beijerinckii	NR_074434	*Prevotella sp. oral taxon G60*	GU432133
Clostridium bifermentans	X73437	*Prevotella sp. oral taxon G70*	GU432179
Clostridium bolteae	ABCC02000039	*Prevotella sp. oral taxon G71*	GU432180
Clostridium botulinum	NC_010723	*Prevotella sp. SEQ053*	JN867222
Clostridium butyricum	ABDT01000017	*Prevotella sp. SEQ065*	JN867234
Clostridium cadaveris	AB542932	*Prevotella sp. SEQ072*	JN867238
Clostridium carboxidivorans	FR733710	*Prevotella sp. SEQ116*	JN867246
Clostridium carnis	NR_044716	*Prevotella sp. SG12*	GU561343
Clostridium celatum	X77844	*Prevotella sp. sp24*	AB003384
Clostridium celerecrescens	JQ246092	*Prevotella sp. sp34*	AB003385
Clostridium cellulosi	NR_044624	*Prevotella stercorea*	AB244774
Clostridium chauvoei	EU106372	*Prevotella tannerae*	ACIJ02000018
Clostridium citroniae	ADLJ01000059	*Prevotella timonensis*	ADEF01000012
Clostridium clariflavum	NR_041235	*Prevotella veroralis*	ACVA01000027

Clostridium clostridiiformes	M59089	*Prevotellaceae bacterium P4P_62 P1*	AY207061
Clostridium clostridioforme	NR_044715	*Propionibacteriaceae bacterium NML 02_0265*	EF599122
Clostridium coccoides	EF025906	*Propionibacterium acidipropionici*	NC_019395
Clostridium cochlearium	NR_044717	*Propionibacterium acnes*	ADJM01000010
Clostridium cocleatum	NR_026495	*Propionibacterium avidum*	AJ003055
Clostridium colicanis	FJ957863	*Propionibacterium freudenreichii*	NR_036972
Clostridium colinum	NR_026151	*Propionibacterium granulosum*	FJ785716
Clostridium difficile	NC_013315	*Propionibacterium jensenii*	NR_042269
Clostridium disporicum	NR_026491	*Propionibacterium propionicum*	NR_025277
Clostridium estertheticum	NR_042153	*Propionibacterium sp. 434_HC2*	AFIL01000035
Clostridium fallax	NR_044714	*Propionibacterium sp. H456*	AB177643
Clostridium favososporum	X76749	*Propionibacterium sp. LG*	AY354921
Clostridium felsineum	AF270502	*Propionibacterium sp. oral taxon 192*	GQ422728
Clostridium frigidicarnis	NR_024919	*Propionibacterium sp. S555a*	AB264622
Clostridium gasigenes	NR_024945	*Propionibacterium thoenii*	NR_042270
Clostridium ghonii	AB542933	*Pseudomonas aeruginosa*	AABQ07000001
Clostridium glycolicum	FJ384385	*Pseudomonas fluorescens*	AY622220
Clostridium glycyrrhizinilyticum	AB233029	*Pseudomonas gessardii*	FJ943496
Clostridium haemolyticum	NR_024749	*Pseudomonas mendocina*	AAUL01000021
Clostridium hathewayi	AY552788	*Pseudomonas monteilii*	NR_024910
Clostridium hiranonis	AB023970	*Pseudomonas poae*	GU188951
Clostridium histolyticum	HF558362	*Pseudomonas pseudoalcaligenes*	NR_037000
Clostridium hylemonae	AB023973	*Pseudomonas putida*	AF094741
Clostridium indolis	AF028351	*Pseudomonas sp. 2_1_26*	ACWU01000257
Clostridium innocuum	M23732	*Pseudomonas sp. G1229*	DQ910482

Clostridium irregulare	NR_029249	*Pseudomonas sp. NP522b*	EU723211
Clostridium isatidis	NR_026347	*Pseudomonas stutzeri*	AM905854
Clostridium kluyveri	NR_074165	*Pseudomonas tolaasii*	AF320988
Clostridium lactatifermentans	NR_025651	*Pseudomonas viridiflava*	NR_042764
Clostridium lavalense	EF564277	*Ralstonia pickettii*	NC_010682
Clostridium leptum	AJ305238	*Ralstonia sp. 5_7_47FAA*	ACUF01000076
Clostridium limosum	FR870444	*Roseburia cecicola*	GU233441
Clostridium magnum	X77835	*Roseburia faecalis*	AY804149
Clostridium malenominatum	FR749893	*Roseburia faecis*	AY305310
Clostridium mayombei	FR733682	*Roseburia hominis*	AJ270482
Clostridium methylpentosum	ACEC01000059	*Roseburia intestinalis*	FP929050
Clostridium nexile	X73443	*Roseburia inulinivorans*	AJ270473
Clostridium novyi	NR_074343	*Roseburia sp. 11SE37*	FM954975
Clostridium orbiscindens	Y18187	*Roseburia sp. 11SE38*	FM954976
Clostridium oroticum	FR749922	*Rothia aeria*	DQ673320
Clostridium paraputrificum	AB536771	*Rothia dentocariosa*	ADDW01000024
Clostridium perfringens	ABDW01000023	*Rothia mucilaginosa*	ACVO01000020
Clostridium phytofermentans	NR_074652	*Rothia nasimurium*	NR_025310
Clostridium piliforme	D14639	*Rothia sp. oral taxon 188*	GU470892
Clostridium putrefaciens	NR_024995	*Ruminobacter amylophilus*	NR_026450
Clostridium quinii	NR_026149	*Ruminococcaceae bacterium D16*	ADDX01000083
Clostridium ramosum	M23731	*Ruminococcus albus*	AY445600
Clostridium rectum	NR_029271	*Ruminococcus bromii*	EU266549
Clostridium saccharogumia	DQ100445	*Ruminococcus callidus*	NR_029160
Clostridium saccharolyticum	CP002109	*Ruminococcus champanellensis*	FP929052
Clostridium sardiniense	NR_041006	*Ruminococcus flavefaciens*	NR_025931
Clostridium sartagoforme	NR_026490	*Ruminococcus gnavus*	X94967
Clostridium scindens	AF262238	*Ruminococcus hansenii*	M59114
Clostridium septicum	NR_026020	*Ruminococcus lactaris*	ABOU02000049
Clostridium sordellii	AB448946	*Ruminococcus obeum*	AY169419
Clostridium sp. 7_2_43FAA	ACDK01000101	*Ruminococcus sp. 18P13*	AJ515913
Clostridium sp. D5	ADBG01000142	*Ruminococcus sp. 5_1_39BFAA*	ACII01000172
Clostridium sp. HGF2	AENW01000022	*Ruminococcus sp. 9SE51*	FM954974
Clostridium sp. HPB_46	AY862516	*Ruminococcus sp. ID8*	AY960564
Clostridium sp. JC122	CAEV01000127	*Ruminococcus sp. K_1*	AB222208

Clostridium sp. L2 50	AAYW02000018	*Ruminococcus torques*	AAVP02000002
Clostridium sp. LMG 16094	X95274	*Salmonella bongori*	NR_041699
Clostridium sp. M62 1	ACFX02000046	*Salmonella enterica*	NC_011149
Clostridium sp. MLG055	AF304435	*Salmonella enterica*	NC_011205
Clostridium sp. MT4 E	FJ159523	*Salmonella enterica*	DQ344532
Clostridium sp. NMBHI 1	JN093130	*Salmonella enterica*	ABEH02000004
Clostridium sp. NML 04A032	EU815224	*Salmonella enterica*	ABAK02000001
Clostridium sp. SS2 1	ABGC03000041	*Salmonella enterica*	NC_011080
Clostridium sp. SY8519	AP012212	*Salmonella enterica*	EU118094
Clostridium sp. TM 40	AB249652	*Salmonella enterica*	NC_011094
Clostridium sp. YIT 12069	AB491207	*Salmonella enterica*	AE014613
Clostridium sp. YIT 12070	AB491208	*Salmonella enterica*	ABFH02000001
Clostridium sphenoides	X73449	*Salmonella enterica*	ABEM01000001
Clostridium spiroforme	X73441	*Salmonella enterica*	ABAM02000001
Clostridium sporogenes	ABKW02000003	*Salmonella typhimurium*	DQ344533
Clostridium sporosphaeroides	NR_044835	*Salmonella typhimurium*	AF170176
Clostridium stercorarium	NR_025100	*Selenomonas artemidis*	HM596274
Clostridium sticklandii	L04167	*Selenomonas dianae*	GQ422719
Clostridium straminisolvens	NR_024829	*Selenomonas flueggei*	AF287803
Clostridium subterminale	NR_041795	*Selenomonas genomosp. C1*	AY278627
Clostridium sulfidigenes	NR_044161	*Selenomonas genomosp. C2*	AY278628
Clostridium symbiosum	ADLQ01000114	*Selenomonas genomosp. P5*	AY341820
Clostridium tertium	Y18174	*Selenomonas genomosp. P6 oral clone MB3 C41*	DQ003636
Clostridium tetani	NC_004557	*Selenomonas genomosp. P7 oral clone MB5 C08*	DQ003627
Clostridium thermocellum	NR_074629	*Selenomonas genomosp. P8 oral clone MB5 P06*	DQ003628
Clostridium tyrobutyricum	NR_044718	*Selenomonas infelix*	AF287802
Clostridium viride	NR_026204	*Selenomonas noxia*	GU470909
Clostridium xylanolyticum	NR_037068	*Selenomonas ruminantium*	NR_075026
Collinsella aerofaciens	AAVN02000007	*Selenomonas sp. FOBRC9*	HQ616378
Collinsella intestinalis	ABXH02000037	*Selenomonas sp. oral clone FT050*	AY349403
Collinsella stercoris	ABXJ01000150	*Selenomonas sp. oral clone GI064*	AY349404

Collinsella tanakaei	AB490807	*Selenomonas sp. oral clone GT010*	AY349405
Coprobacillus cateniformis	AB030218	*Selenomonas sp. oral clone HU051*	AY349406
Coprobacillus sp. 29_1	ADKX01000057	*Selenomonas sp. oral clone IK004*	AY349407
Coprobacillus sp. D7	ACDT01000199	*Selenomonas sp. oral clone IQ048*	AY349408
Coprococcus catus	EU266552	*Selenomonas sp. oral clone JI021*	AY349409
Coprococcus comes	ABVR01000038	*Selenomonas sp. oral clone JS031*	AY349410
Coprococcus eutactus	EF031543	*Selenomonas sp. oral clone OH4A*	AY947498
Coprococcus sp. ART55_1	AY350746	*Selenomonas sp. oral clone P2PA_80 P4*	AY207052
Dialister invisus	ACIM02000001	*Selenomonas sp. oral taxon 137*	AENV01000007
Dialister micraerophilus	AFBB01000028	*Selenomonas sp. oral taxon 149*	AEEJ01000007
Dialister microaerophilus	AENT01000008	*Selenomonas sputigena*	ACKP02000033
Dialister pneumosintes	HM596297	*Serratia fonticola*	NR_025339
Dialister propionicifaciens	NR_043231	*Serratia liquefaciens*	NR_042062
Dialister sp. oral taxon 502	GQ422739	*Serratia marcescens*	GU826157
Dialister succinatiphilus	AB370249	*Serratia odorifera*	ADBY01000001
Dorea formicigenerans	AAXA02000006	*Serratia proteamaculans*	AAUN01000015
Dorea longicatena	AJ132842	*Shigella boydii*	AAKA01000007
Enhydrobacter aerosaccus	ACYI01000081	*Shigella dysenteriae*	NC_007606
Enterobacter aerogenes	AJ251468	*Shigella flexneri*	AE005674
Enterobacter asburiae	NR_024640	*Shigella sonnei*	NC_007384
Enterobacter cancerogenus	Z96078	*Sphingobacterium faecium*	NR_025537
Enterobacter cloacae	FP929040	*Sphingobacterium mizutaii*	JF708889
Enterobacter cowanii	NR_025566	*Sphingobacterium multivorum*	NR_040953
Enterobacter hormaechei	AFHR01000079	*Sphingobacterium spiritivorum*	ACHA02000013
Enterobacter sp. 247BMC	HQ122932	*Sphingomonas echinoides*	NR_024700
Enterobacter sp. 638	NR_074777	*Sphingomonas sp. oral clone FI012*	AY349411
Enterobacter sp. JC163	JN657217	*Sphingomonas sp. oral clone FZ016*	AY349412

Enterobacter sp. SCSS	HM007811	*Sphingomonas sp. oral taxon A09*	HM099639
Enterobacter sp. TSE38	HM156134	*Sphingomonas sp. oral taxon F71*	HM099645
Enterobacteriaceae bacterium 9_2_54FAA	ADCU01000033	*Staphylococcaceae bacterium NML 92_0017*	AY841362
Enterobacteriaceae bacterium CF01Ent_1	AJ489826	*Staphylococcus aureus*	CP002643
Enterobacteriaceae bacterium Smarlab 3302238	AY538694	*Staphylococcus auricularis*	JQ624774
Enterococcus avium	AF133535	*Staphylococcus capitis*	ACFR01000029
Enterococcus caccae	AY943820	*Staphylococcus caprae*	ACRH01000033
Enterococcus casseliflavus	AEWT01000047	*Staphylococcus carnosus*	NR_075003
Enterococcus durans	AJ276354	*Staphylococcus cohnii*	JN175375
Enterococcus faecalis	AE016830	*Staphylococcus condimenti*	NR_029345
Enterococcus faecium	AM157434	*Staphylococcus epidermidis*	ACHE01000056
Enterococcus gallinarum	AB269767	*Staphylococcus equorum*	NR_027520
Enterococcus gilvus	AY033814	*Staphylococcus fleurettii*	NR_041326
Enterococcus hawaiiensis	AY321377	*Staphylococcus haemolyticus*	NC_007168
Enterococcus hirae	AF061011	*Staphylococcus hominis*	AM157418
Enterococcus italicus	AEPV01000109	*Staphylococcus lugdunensis*	AEQA01000024
Enterococcus mundtii	NR_024906	*Staphylococcus pasteuri*	FJ189773
Enterococcus raffinosus	FN600541	*Staphylococcus pseudintermedius*	CP002439
Enterococcus sp. BV2CASA2	JN809766	*Staphylococcus saccharolyticus*	NR_029158
Enterococcus sp. CCRI_16620	GU457263	*Staphylococcus saprophyticus*	NC_007350
Enterococcus sp. F95	FJ463817	*Staphylococcus sciuri*	NR_025520
Enterococcus sp. RfL6	AJ133478	*Staphylococcus sp. clone bottae7*	AF467424
Enterococcus thailandicus	AY321376	*Staphylococcus sp. H292*	AB177642
Erysipelotrichaceae bacterium 3_1_53	ACTJ01000113	*Staphylococcus sp. H780*	AB177644
Erysipelotrichaceae bacterium 5_2_54FAA	ACZW01000054	*Staphylococcus succinus*	NR_028667
Escherichia albertii	ABKX01000012	*Staphylococcus vitulinus*	NR_024670
Escherichia coli	NC_008563	*Staphylococcus warneri*	ACPZ01000009
Escherichia fergusonii	CU928158	*Staphylococcus xylosus*	AY395016

Escherichia hermannii	HQ407266	*Streptobacillus moniliformis*	NR_027615
Escherichia sp. 1_1_43	ACID01000033	*Streptococcus agalactiae*	AAJO01000130
Escherichia sp. 4_1_40B	ACDM02000056	*Streptococcus alactolyticus*	NR_041781
Escherichia sp. B4	EU722735	*Streptococcus anginosus*	AECT01000011
Escherichia vulneris	NR_041927	*Streptococcus australis*	AEQR01000024
Eubacteriaceae bacterium P4P_50 P4	AY207060	*Streptococcus bovis*	AEEL01000030
Eubacterium barkeri	NR_044661	*Streptococcus canis*	AJ413203
Eubacterium biforme	ABYT01000002	*Streptococcus constellatus*	AY277942
Eubacterium brachy	U13038	*Streptococcus cristatus*	AEVC01000028
Eubacterium budayi	NR_024682	*Streptococcus downei*	AEKN01000002
Eubacterium callanderi	NR_026330	*Streptococcus dysgalactiae*	AP010935
Eubacterium cellulosolvens	AY178842	*Streptococcus equi*	CP001129
Eubacterium contortum	FR749946	*Streptococcus equinus*	AEVB01000043
Eubacterium coprostanoligenes	HM037995	*Streptococcus gallolyticus*	FR824043
Eubacterium cylindroides	FP929041	*Streptococcus genomosp. C1*	AY278629
Eubacterium desmolans	NR_044644	*Streptococcus genomosp. C2*	AY278630
Eubacterium dolichum	L34682	*Streptococcus genomosp. C3*	AY278631
Eubacterium eligens	CP001104	*Streptococcus genomosp. C4*	AY278632
Eubacterium fissicatena	FR749935	*Streptococcus genomosp. C5*	AY278633
Eubacterium hadrum	FR749933	*Streptococcus genomosp. C6*	AY278634
Eubacterium hallii	L34621	*Streptococcus genomosp. C7*	AY278635
Eubacterium infirmum	U13039	*Streptococcus genomosp. C8*	AY278609
Eubacterium limosum	CP002273	*Streptococcus gordonii*	NC_009785
Eubacterium moniliforme	HF558373	*Streptococcus infantarius*	ABJK02000017
Eubacterium multiforme	NR_024683	*Streptococcus infantis*	AFNN01000024
Eubacterium nitritogenes	NR_024684	*Streptococcus intermedius*	NR_028736
Eubacterium nodatum	U13041	*Streptococcus lutetiensis*	NR_037096

Eubacterium ramulus	AJ011522	*Streptococcus massiliensis*	AY769997
Eubacterium rectale	FP929042	*Streptococcus milleri*	X81023
Eubacterium ruminantium	NR_024661	*Streptococcus mitis*	AM157420
Eubacterium saburreum	AB525414	*Streptococcus mutans*	AP010655
Eubacterium saphenum	NR_026031	*Streptococcus oligofermentans*	AY099095
Eubacterium siraeum	ABCA03000054	*Streptococcus oralis*	ADMV01000001
Eubacterium sp. 3_1_31	ACTL01000045	*Streptococcus parasanguinis*	AEKM01000012
Eubacterium sp. AS15b	HQ616364	*Streptococcus pasteurianus*	AP012054
Eubacterium sp. OBRC9	HQ616354	*Streptococcus peroris*	AEVF01000016
Eubacterium sp. oral clone GI038	AY349374	*Streptococcus pneumoniae*	AE008537
Eubacterium sp. oral clone IR009	AY349376	*Streptococcus porcinus*	EF121439
Eubacterium sp. oral clone JH012	AY349373	*Streptococcus pseudopneumoniae*	FJ827123
Eubacterium sp. oral clone JI012	AY349379	*Streptococcus pseudoporcinus*	AENS01000003
Eubacterium sp. oral clone JN088	AY349377	*Streptococcus pyogenes*	AE006496
Eubacterium sp. oral clone JS001	AY349378	*Streptococcus ratti*	X58304
Eubacterium sp. oral clone OH3A	AY947497	*Streptococcus salivarius*	AGBV01000001
Eubacterium sp. WAL 14571	FJ687606	*Streptococcus sanguinis*	NR_074974
Eubacterium tenue	M59118	*Streptococcus sinensis*	AF432857
Eubacterium tortuosum	NR_044648	*Streptococcus sp. 16362*	JN590019
Eubacterium ventriosum	L34421	*Streptococcus sp. 2_1_36FAA*	ACOI01000028
Eubacterium xylanophilum	L34628	*Streptococcus sp. 2285_97*	AJ131965
Eubacterium yurii	AEES01000073	*Streptococcus sp. 69130*	X78825
Fusobacterium canifelinum	AY162222	*Streptococcus sp. AC15*	HQ616356
Fusobacterium genomosp. C1	AY278616	*Streptococcus sp. ACS2*	HQ616360
Fusobacterium genomosp. C2	AY278617	*Streptococcus sp. AS20*	HQ616366
Fusobacterium gonidiaformans	ACET01000043	*Streptococcus sp. BS35a*	HQ616369
Fusobacterium mortiferum	ACDB02000034	*Streptococcus sp. C150*	ACRI01000045
Fusobacterium naviforme	HQ223106	*Streptococcus sp. CM6*	HQ616372

Fusobacterium necrogenes	X55408	*Streptococcus sp. CM7*	HQ616373
Fusobacterium necrophorum	AM905356	*Streptococcus sp. ICM10*	HQ616389
Fusobacterium nucleatum	ADVK01000034	*Streptococcus sp. ICM12*	HQ616390
Fusobacterium periodonticum	ACJY01000002	*Streptococcus sp. ICM2*	HQ616386
Fusobacterium russii	NR_044687	*Streptococcus sp. ICM4*	HQ616387
Fusobacterium sp. 1_1_41FAA	ADGG01000053	*Streptococcus sp. ICM45*	HQ616394
Fusobacterium sp. 11_3_2	ACUO01000052	*Streptococcus sp. M143*	ACRK01000025
Fusobacterium sp. 12_1B	AGWJ01000070	*Streptococcus sp. M334*	ACRL01000052
Fusobacterium sp. 2_1_31	ACDC02000018	*Streptococcus sp. OBRC6*	HQ616352
Fusobacterium sp. 3_1_27	ADGF01000045	*Streptococcus sp. oral clone ASB02*	AY923121
Fusobacterium sp. 3_1_33	ACQE01000178	*Streptococcus sp. oral clone ASCA03*	DQ272504
Fusobacterium sp. 3_1_36A2	ACPU01000044	*Streptococcus sp. oral clone ASCA04*	AY923116
Fusobacterium sp. 3_1_5R	ACDD01000078	*Streptococcus sp. oral clone ASCA09*	AY923119
Fusobacterium sp. AC18	HQ616357	*Streptococcus sp. oral clone ASCB04*	AY923123
Fusobacterium sp. ACB2	HQ616358	*Streptococcus sp. oral clone ASCB06*	AY923124
Fusobacterium sp. AS2	HQ616361	*Streptococcus sp. oral clone ASCC04*	AY923127
Fusobacterium sp. CM1	HQ616371	*Streptococcus sp. oral clone ASCC05*	AY923128
Fusobacterium sp. CM21	HQ616375	*Streptococcus sp. oral clone ASCC12*	DQ272507
Fusobacterium sp. CM22	HQ616376	*Streptococcus sp. oral clone ASCD01*	AY923129
Fusobacterium sp. D12	ACDG02000036	*Streptococcus sp. oral clone ASCD09*	AY923130
Fusobacterium sp. oral clone ASCF06	AY923141	*Streptococcus sp. oral clone ASCD10*	DQ272509
Fusobacterium sp. oral clone ASCF11	AY953256	*Streptococcus sp. oral clone ASCE03*	AY923134
Fusobacterium ulcerans	ACDH01000090	*Streptococcus sp. oral clone ASCE04*	AY953253
Fusobacterium varium	ACIE01000009	*Streptococcus sp. oral clone ASCE05*	DQ272510
Gemella haemolysans	ACDZ02000012	*Streptococcus sp. oral clone ASCE06*	AY923135

Gemella morbillorum	NR_025904	*Streptococcus sp. oral clone ASCE09*	AY923136
Gemella morbillorum	ACRX01000010	*Streptococcus sp. oral clone ASCE10*	AY923137
Gemella sanguinis	ACRY01000057	*Streptococcus sp. oral clone ASCE12*	AY923138
Gemella sp. oral clone ASCE02	AY923133	*Streptococcus sp. oral clone ASCF05*	AY923140
Gemella sp. oral clone ASCF04	AY923139	*Streptococcus sp. oral clone ASCF07*	AY953255
Gemella sp. oral clone ASCF12	AY923143	*Streptococcus sp. oral clone ASCF09*	AY923142
Gemella sp. WAL 1945J	EU427463	*Streptococcus sp. oral clone ASCG04*	AY923145
Klebsiella oxytoca	AY292871	*Streptococcus sp. oral clone BW009*	AY005042
Klebsiella pneumoniae	CP000647	*Streptococcus sp. oral clone CH016*	AY005044
Klebsiella sp. AS10	HQ616362	*Streptococcus sp. oral clone GK051*	AY349413
Klebsiella sp. Co9935	DQ068764	*Streptococcus sp. oral clone GM006*	AY349414
Klebsiella sp. enrichment culture clone SRC_DSD25	HM195210	*Streptococcus sp. oral clone P2PA_41 P2*	AY207051
Klebsiella sp. OBRC7	HQ616353	*Streptococcus sp. oral clone P4PA_30 P4*	AY207064
Klebsiella sp. SP_BA	FJ999767	*Streptococcus sp. oral taxon 071*	AEEP01000019
Klebsiella sp. SRC_DSD1	GU797254	*Streptococcus sp. oral taxon G59*	GU432132
Klebsiella sp. SRC_DSD11	GU797263	*Streptococcus sp. oral taxon G62*	GU432146
Klebsiella sp. SRC_DSD12	GU797264	*Streptococcus sp. oral taxon G63*	GU432150
Klebsiella sp. SRC_DSD15	GU797267	*Streptococcus sp. SHV515*	Y07601
Klebsiella sp. SRC_DSD2	GU797253	*Streptococcus suis*	FM252032
Klebsiella sp. SRC_DSD6	GU797258	*Streptococcus thermophilus*	CP000419
Klebsiella variicola	CP001891	*Streptococcus uberis*	HQ391900
Lachnobacterium bovis	GU324407	*Streptococcus urinalis*	DQ303194
Lachnospira multipara	FR733699	*Streptococcus vestibularis*	AEKO01000008
Lachnospira pectinoschiza	L14675	*Streptococcus viridans*	AF076036

Lachnospiraceae bacterium 1 1 57FAA	ACTM01000065	*Sutterella morbirenis*	AJ832129
Lachnospiraceae bacterium 1 4 56FAA	ACTN01000028	*Sutterella parvirubra*	AB300989
Lachnospiraceae bacterium 2 1 46FAA	ADLB01000035	*Sutterella sanguinus*	AJ748647
Lachnospiraceae bacterium 2 1 58FAA	ACTO01000052	*Sutterella sp. YIT 12072*	AB491210
Lachnospiraceae bacterium 3 1 57FAA CT1	ACTP01000124	*Sutterella stercoricanis*	NR_025600
Lachnospiraceae bacterium 4 1 37FAA	ADCR01000030	*Sutterella wadsworthensis*	ADMF01000048
Lachnospiraceae bacterium 5 1 57FAA	ACTR01000020	*Synergistes genomosp. C1*	AY278615
Lachnospiraceae bacterium 5 1 63FAA	ACTS01000081	*Synergistes sp. RMA 14551*	DQ412722
Lachnospiraceae bacterium 6 1 63FAA	ACTV01000014	*Synergistetes bacterium ADV897*	GQ258968
Lachnospiraceae bacterium 8 1 57FAA	ACWQ01000079	*Synergistetes bacterium LBVCM1157*	GQ258969
Lachnospiraceae bacterium 9 1 43BFAA	ACTX01000023	*Synergistetes bacterium oral taxon 362*	GU410752
Lachnospiraceae bacterium A4	DQ789118	*Synergistetes bacterium oral taxon D48*	GU430992
Lachnospiraceae bacterium DJF VP30	EU728771	*Turicibacter sanguinis*	AF349724
Lachnospiraceae bacterium ICM62	HQ616401	*Veillonella atypica*	AEDS01000059
Lachnospiraceae bacterium MSX33	HQ616384	*Veillonella dispar*	ACIK02000021
Lachnospiraceae bacterium oral taxon 107	ADDS01000069	*Veillonella genomosp. P1 oral clone MB5 P17*	DQ003631
Lachnospiraceae bacterium oral taxon F15	HM099641	*Veillonella montpellierensis*	AF473836
Lachnospiraceae genomosp. C1	AY278618	*Veillonella parvula*	ADFU01000009
Lactobacillus acidipiscis	NR_024718	*Veillonella sp. 3 1 44*	ADCV01000019
Lactobacillus acidophilus	CP000033	*Veillonella sp. 6 1 27*	ADCW01000016
Lactobacillus alimentarius	NR_044701	*Veillonella sp. ACP1*	HQ616359
Lactobacillus amylolyticus	ADNY01000006	*Veillonella sp. AS16*	HQ616365
Lactobacillus amylovorus	CP002338	*Veillonella sp. BS32b*	HQ616368
Lactobacillus antri	ACLL01000037	*Veillonella sp. ICM51a*	HQ616396
Lactobacillus brevis	EU194349	*Veillonella sp. MSA12*	HQ616381
Lactobacillus buchneri	ACGH01000101	*Veillonella sp. NVG 100cf*	EF108443

Lactobacillus casei	CP000423	*Veillonella sp. OK11*	JN695650
Lactobacillus catenaformis	M23729	*Veillonella sp. oral clone ASCA08*	AY923118
Lactobacillus coleohominis	ACOH01000030	*Veillonella sp. oral clone ASCB03*	AY923122
Lactobacillus coryniformis	NR_044705	*Veillonella sp. oral clone ASCG01*	AY923144
Lactobacillus crispatus	ACOG01000151	*Veillonella sp. oral clone ASCG02*	AY953257
Lactobacillus curvatus	NR_042437	*Veillonella sp. oral clone OH1A*	AY947495
Lactobacillus delbrueckii	CP002341	*Veillonella sp. oral taxon 158*	AENU01000007
Lactobacillus dextrinicus	NR_036861	*Veillonellaceae bacterium oral taxon 131*	GU402916
Lactobacillus farciminis	NR_044707	*Veillonellaceae bacterium oral taxon 155*	GU470897
Lactobacillus fermentum	CP002033	*Vibrio cholerae*	AAUR01000095
Lactobacillus gasseri	ACOZ01000018	*Vibrio fluvialis*	X76335
Lactobacillus gastricus	AICN01000060	*Vibrio furnissii*	CP002377
Lactobacillus genomosp. C1	AY278619	*Vibrio mimicus*	ADAF01000001
Lactobacillus genomosp. C2	AY278620	*Vibrio parahaemolyticus*	AAWQ01000116
Lactobacillus helveticus	ACLM01000202	*Vibrio sp. RC341*	ACZT01000024
Lactobacillus hilgardii	ACGP01000200	*Vibrio vulnificus*	AE016796
Lactobacillus hominis	FR681902	*Yersinia aldovae*	AJ871363
Lactobacillus iners	AEKJ01000002	*Yersinia aleksiciae*	AJ627597
Lactobacillus jensenii	ACQD01000066	*Yersinia bercovieri*	AF366377
Lactobacillus johnsonii	AE017198	*Yersinia enterocolitica*	FR729477
Lactobacillus kalixensis	NR_029083	*Yersinia frederiksenii*	AF366379
Lactobacillus kefiranofaciens	NR_042440	*Yersinia intermedia*	AF366380
Lactobacillus kefiri	NR_042230	*Yersinia kristensenii*	ACCA01000078
Lactobacillus kimchii	NR_025045	*Yersinia mollaretii*	NR_027546
Lactobacillus leichmannii	JX986966	*Yersinia pestis*	AE013632
Lactobacillus mucosae	FR693800	*Yersinia pseudotuberculosis*	NC_009708
		Yersinia rohdei	ACCD01000071

Table 3: Exemplary Bacterial Strains

Strain	**Deposit Number**

Parabacteroides goldsteinii	PTA-126574
Bifidobacterium animalis ssp. lactis Strain A	PTA-125097
Blautia Massiliensis Strain A	PTA-125134
Prevotella Strain B	NRRL accession Number B 50329
Prevotella Histicola	PTA-126140
Blautia Strain A	PTA-125346
Lactococcus lactis cremoris Strain A	PTA-125368
Lactobacillus salivarius	PTA-125893
Ruminococcus gnavus strain	PTA-125706
Tyzzerella nexilis strain	PTA-125707
Paraclostridium benzoelyticum	PTA-125894
Ruminococcus gnavus (also referred to as Mediterraneibacter gnavus)	PTA-126695
Veillonella parvula	PTA-125710
Veillonella atypica Strain A	PTA-125709
Veillonella atypica Strain B	PTA-125711
Veillonella parvula Strain A	PTA-125691
Veillonella parvula Strain B	PTA-125711
Veillonella tobetsuensis Strain A	PTA-125708
Agathobaculum sp.	PTA-125892
Turicibacter sanguinis	PTA-125889
Klebsiella quasipneumoniae subsp. similipneumoniae	PTA-125891
Klebsiella oxytoca	PTA-125890
Megasphaera Sp. Strain A	PTA-126770
Megasphaera Sp.	PTA-126837
Harryflintia acetispora	PTA-126694
Fournierella massiliensis	PTA-126696

Table 4. Exemplary Bacterial Strains

Escherichia coli	NCIMB 12210
Enterococcus faecalis	NCIMB 13280
Bacteroides fragilis	DSM 2151
Bacteroides vulgatus	DSM 1447
Bacteroides ovatus	DSM 1896
Megasphaera massiliensis	DSM 26228
Megasphaera elsdenii	NCIMB 8927
Megasphaera massiliensis	NCIMB 42787
Bifidobacterium breve	DSM 20213
Bifidobacterium longum subsp. longum	DSM 20219
Faecalibacterium prausnitzii	DSM17677
Anaerostipes hadrus	DSM 3319
Blautia coccoides	DSM 935
Dorea longicatena	DSM 13814
Parabacteroides distasonis	DSM 20701
Faecalicatena contorta	DSM3982
Ruminococcus gnavus	ATCC29149
Megasphaera massiliensis	NCIMB 43388
Megasphaera massiliensis	NCIMB 43389
Megasphaera spp.	NCIMB 43385
Megasphaera spp.	NCIMB 43386
Megasphaera spp.	NCIMB 43387
Parabacteroides distasonis (also referred to as "Parabacteroides sp 755")	NCIMB 42382
Bacillus amyloliquefaciens	NCIMB 43088
Bacillus amyloliquefaciens	NCIMB 43087
Bacillus amyloliquefaciens	NCIMB 43086

Modified Bacteria and mEVs

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

[228] 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 some embodiments, co-administration of the cancer-specific moiety with the pharmaceutical agent (either in combination or in separate administrations) increases the targeting of the pharmaceutical agent to the cancer cells.

[229] In some embodiments, the bacteria and/or mEVs described herein can be 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 a bacteria- or a mEV-binding moiety that binds to the bacteria or mEV. In some embodiments, the bacteria- or mEV-binding moiety is a fragment of or a full-length peptidoglycan recognition protein, such as PGRP. In some embodiments the bacteria- or mEV-binding moiety has binding specificity for the bacteria or mEV (e.g., by having binding specificity for a bacterial antigen). In some embodiments, the bacteria- or mEV-binding moiety comprises an antibody or antigen binding fragment thereof. In some embodiments, the bacteria- or mEV-binding moiety comprises a T cell receptor or a chimeric antigen receptor (CAR). In some embodiments, the bacteria- or mEV-binding moiety comprises a ligand for a receptor expressed on the surface of a cancer cell or a receptor-binding fragment thereof. In some embodiments, co-administration of the magnetic and/or paramagnetic moiety with the bacteria or 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 Processed Microbial Extracellular Vesicles (pmEVs)

[230] In certain aspects, the pmEVs described herein can be prepared using any method known in the art.

[231] In some embodiments, the pmEVs are prepared without a pmEV purification step. For example, in some embodiments, bacteria from which the pmEVs described herein are released are killed using a method that leaves the bacterial pmEVs intact, and the resulting bacterial components, including the pmEVs, 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.

[232] In some embodiments, the pmEVs described herein are purified from one or more other bacterial components. Methods for purifying pmEVs from bacteria (and optionally, other bacterial components) are known in the art. In some embodiments, pmEVs are prepared from bacterial cultures using methods described in Thein, *et al.* (*J. Proteome Res.* 9(12):6135-6147 (2010)) or Sandrini, *et al.* (*Bio-protocol* 4(21): e1287 (2014)), 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- 15,000 x g for 10- 15 min at room temperature or 4°C). In some embodiments, the supernatants are discarded and cell pellets are frozen at -80°C. In some embodiments, cell pellets are thawed on ice and resuspended in 100 mM Tris-HCl, pH 7.5 supplemented with 1 mg/mL DNase I. In some embodiments, cells are lysed using an Emulsiflex C-3 (Avestin, Inc.) under conditions recommended by the manufacturer. In some embodiments, debris and unlysed cells are pelleted by centrifugation at 10,000 x g for 15 min at 4°C. In some embodiments, supernatants are then centrifuged at 120,000 x g for 1 hour at 4°C. In some embodiments, pellets are resuspended in ice-cold 100 mM sodium carbonate, pH 11, incubated with agitation for 1 hr at 4°C, and then centrifuged at 120,000 x g for 1 hour at 4°C. In some embodiments, pellets are resuspended in 100 mM Tris-HCl, pH 7.5, re-centrifuged at 120,000 x g for 20 min at 4°C, and then resuspended in 0.1 M Tris-HCl, pH 7.5 or in PBS. In some embodiments, samples are stored at -20°C.

[233] In certain aspects, pmEVs are obtained by methods adapted from Sandrini et al, 2014. In some embodiments, bacterial cultures are centrifuged at 10,000-15,500 x g for 10-15 min at room temp or at 4°C. In some embodiments, cell pellets are frozen at -80°C and

supernatants are discarded. In some embodiments, 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. In some embodiments, samples are incubated with mixing at room temp or at 37°C for 30 min. In some embodiments, samples are re-frozen at -80°C and thawed again on ice. In some embodiments, DNase I is added to a final concentration of 1.6 mg/mL and MgCl2 to a final concentration of 100 mM. In some embodiments, samples are sonicated using a QSonica Q500 sonicator with 7 cycles of 30 sec on and 30 sec off. In some embodiments, debris and unlysed cells are pelleted by centrifugation at 10,000 x g for 15 min. at 4°C. In some embodiments, supernatants are then centrifuged at 110,000 x g for 15 min at 4°C. In some embodiments, pellets are resuspended in 10 mM Tris-HCl, pH 8.0, 2% Triton X-100 and incubated 30-60 min with mixing at room temperature. In some embodiments, samples are centrifuged at 110,000 x g for 15 min at 4°C. In some embodiments, pellets are resuspended in PBS and stored at -20°C.

[234] In certain aspects, a method of forming (e.g., preparing) isolated bacterial pmEVs, described herein, comprises the steps of: (a) centrifuging a bacterial culture, thereby forming a first pellet and a first supernatant, wherein the first pellet comprises cells; (b) discarding the first supernatant;(c) resuspending the first pellet in a solution; (d) lysing the cells; (e) centrifuging the lysed cells, thereby forming a second pellet and a second supernatant; (f) discarding the second pellet and centrifuging the second supernatant, thereby forming a third pellet and a third supernatant; (g) discarding the third supernatant and resuspending the third pellet in a second solution, thereby forming the isolated bacterial pmEVs.

[235] In some embodiments, the method further comprises the steps of: (h) centrifuging the solution of step (g), thereby forming a fourth pellet and a fourth supernatant; (i) discarding the fourth supernatant and resuspending the fourth pellet in a third solution. In some embodiments, the method further comprises the steps of: (j) centrifuging the solution of step (i), thereby forming a fifth pellet and a fifth supernatant; and (k) discarding the fifth supernatant and resuspending the fifth pellet in a fourth solution.

[236] In some embodiments, the centrifugation of step (a) is at 10,000 x g. In some embodiments the centrifugation of step (a) is for 10-15 minutes. In some embodiments, the centrifugation of step (a) is at 4 °C or room temperature. In some embodiments, step (b) further comprises freezing the first pellet at -80 °C. In some embodiments, the solution in step (c) is 100mM Tris-HCl, pH 7.5 supplemented with 1mg/ml DNaseI. In some embodiments, the

solution in step (c) is 10mM Tris-HCl, pH 8.0, 1mM EDTA, supplemented with 0.1 mg/ml lysozyme. In some embodiments, step (c) further comprises incubating for 30 minutes at 37 °C or room temperature. In some embodiments, step (c) further comprises freezing the first pellet at -80 °C. In some embodiments, step (c) further comprises adding DNase I to a final concentration of 1.6mg/ml. In some embodiments, step (c) further comprises adding $MgCl_2$ to a final concentration of 100mM. In some embodiments, the cells are lysed in step (d) via homogenization. In some embodiments, the cells are lysed in step (d) via emulsiflex C3. In some embodiments, the cells are lysed in step (d) via sonication. In some embodiments, the cells are sonicated in 7 cycles, wherein each cycle comprises 30 seconds of sonication and 30 seconds without sonication. In some embodiments, the centrifugation of step (e) is at 10,000 x g. In some embodiments, the centrifugation of step (e) is for 15 minutes. In some embodiments, the centrifugation of step (e) is at 4 °C or room temperature.

[237] In some embodiments, the centrifugation of step (f) is at 120,000 x g. In some embodiments, the centrifugation of step (f) is at 110,000 x g. In some embodiments, the centrifugation of step (f) is for 1 hour. In some embodiments, the centrifugation of step (f) is for 15 minutes. In some embodiments, the centrifugation of step (f) is at 4 °C or room temperature. In some embodiments, the second solution in step (g) is 100 mM sodium carbonate, pH 11. In some embodiments, the second solution in step (g) is 10mM Tris-HCl pH 8.0, 2% triton X-100. In some embodiments, step (g) further comprises incubating the solution for 1 hour at 4 °C. In some embodiments, step (g) further comprises incubating the solution for 30-60 minutes at room temperature. In some embodiments, the centrifugation of step (h) is at 120,000 x g. In some embodiments, the centrifugation of step (h) is at 110,000 x g. In some embodiments, the centrifugation of step (h) is for 1 hour. In some embodiments, the centrifugation of step (h) is for 15 minutes. In some embodiments, the centrifugation of step (h) is at 4 °C or room temperature. In some embodiments, the third solution in step (i) is 100mM Tris-HCl, pH 7.5. In some embodiments, the third solution in step (i) is PBS. In some embodiments, the centrifugation of step (j) is at 120,000 x g. In some embodiments, the centrifugation of step (j) is for 20 minutes. In some embodiments, the centrifugation of step (j) is at 4 °C or room temperature. In some embodiments, the fourth solution in step (k) is 100mM Tris-HCl, pH 7.5 or PBS.

[238] pmEVs obtained by methods provided herein may be further purified by size based column chromatography, by affinity 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 35% Optiprep in PBS. 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 35-60% discontinuous sucrose gradient and centrifuged at 200,000 x g for 3-24 hours at 4°C.

[239] In some embodiments, to confirm sterility and isolation of the pmEV preparations, pmEVs 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 pmEVs may be DNase or proteinase K treated.

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

[241] In some embodiments select pmEVs are isolated and enriched by chromatography and binding surface moieties on pmEVs. In other embodiments, select pmEVs 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.

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

[243] In some embodiments, pmEVs are lyophilized.

[244] In some embodiments, pmEVs are gamma irradiated (e.g., at 17.5 or 25 kGy).

[245] In some embodiments, pmEVs are UV irradiated.

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

[247] In some embodiments, pmEVs are acid treated.

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

[249] The phase of growth can affect the amount or properties of bacteria. In the methods of pmEV preparation provided herein, pmEVs 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.

Production of Secreted Microbial Extracellular Vesicles (smEVs)

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

[251] In some embodiments, the smEVs are prepared without a 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.

[252] 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.

[253] 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.

[254] 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.

[255] 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.

[256] 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.

[257] 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.

[258] In some 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.

[259] 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.

[260] 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.

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

[262] In some embodiments, smEVs are lyophilized.

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

[264] In some embodiments, smEVs are UV irradiated.

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

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

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

[268] 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.

[269] 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 5.

Table 5: Culture Techniques to Increase smEV Production

smEV inducement	smEV inducer	Acts on
Temperature		
	Heat RT to 37 °C temp change 37 to 40 °C temp change	stress response simulates infection febrile infection

ROS		
	Plumbagin Cumene hydroperoxide Hydrogen Peroxide	oxidative stress response oxidative stress response oxidative stress response
Antibiotics		
	Ciprofloxacin Gentamycin Polymyxin B D-cylcloserine	bacterial SOS response protein synthesis outer membrane cell wall
Osmolyte		
	NaCl	osmotic stress
Metal Ion Stress		
	Iron Chelation EDTA Low Hemin	iron levels removes divalent cations iron levels
Media additives or removal		
Other mechanisms	Lactate Amino acid deprivation Hexadecane Glucose Sodium bicarbonate PQS Diamines+ DFMO High nutrients Low nutrients Oxygen No Cysteine Inducing biofilm or floculation Diauxic Growth Phage Urea	growth stress stress growth ToxT induction vesiculator (from bacteria) membrane anchoring (negativicutes only) enhanced growth oxygen stress in anaerobe oxygen stress in anaerobe

[270] In the methods of smEVs preparation provided herein, the method can optionally include exposing a culture of bacteria to a smEV inducer prior to isolating smEVs from the bacterial culture. The culture of bacteria can be exposed to a 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.

Methods of Making Enhanced Bacteria

[271] In certain aspects, provided herein are methods of making engineered bacteria for the production of the bacteria and/or mEVs (such as smEVs and/or pmEVs) 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 bacteria and/or mEVs (such as smEVs and/or pmEVs) (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 and/or pmEV) 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.

[272] 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).

Expression of a Transcription Product: Potency Assay

[273] The present disclosure includes, among other things, assays useful for determining the potency of a drug product or drug substance, based at least in part on measuring expression of a transcription product in cells contacted with the drug product or drug substance.

In various embodiments, the drug product or drug substance can be bacteria and/or microbial extracellular vesicles (e.g., *Prevotella histicola* bacteria and/or microbial extracellular vesicles). Without wishing to be bound by any particular scientific theory, expression of the transcription product by cells contacted with the drug product or drug substance (e.g., with bacteria and/or microbial extracellular vesicles) is representative of biological activity thereof. Without wishing to be bound by any particular scientific theory, expression of the transcription product by cells contacted with the drug product or drug substance (e.g., with bacteria and/or microbial extracellular vesicles) is relevant to and/or indicative of a desired physiological effect to be achieved by delivery and/or administration of the drug product or drug substance, e.g., to a subject to benefit therefrom.

[274] In certain aspects, provided herein are methods for assaying a drug product or drug substance (e.g., a drug product or drug substance that is or includes bacteria and/or microbial extracellular vesicles) that includes contacting (e.g., incubating) cells (*e.g.,* THP-1 cells) with the drug product or drug substance and detecting expression of the transcription product by the cells, wherein the drug product or drug substance comprises a strain of bacteria (*e.g., Prevotella histicola* strain bacteria) and/or microbial extracellular vesicles (mEVs) from a strain of bacteria (*e.g., Prevotella histicola* strain microbial extracellular vesicles (mEVs)). In some embodiments, the bacterial strain is a strain of *Prevotella histicola* having 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 histicola* bacteria are of *Prevotella* Strain B (NRRL accession number B 50329). In some embodiments, the bacterial strain is a strain of *Prevotella histicola* having at least 99% genomic, 16S and/or CRISPR sequence identity to the nucleotide sequence of *Prevotella histicola* ATCC designation number PTA-126140. In some embodiments, the *Prevotella histicola* bacteria are of *Prevotella histicola* ATCC designation number PTA-126140.

[275] The drug product or drug substance may comprise bacteria and/or microbial extracellular vesicles (mEVs). In some embodiments, the drug product or drug substance comprises a strain of bacteria (*e.g., Prevotella histicola, Veillonella parvula, Harryflintia acetispora, Fournierella massiliensis, Megasphaera Sp., Selenomonas felix, Acidaminococcus intestine,* and/or *Propionospora sp.* bacteria) and/or microbial extracellular vesicles (mEVs) from a strain of bacteria (*e.g., Prevotella histicola, Veillonella parvula, Harryflintia acetispora,*

Fournierella massiliensis, Megasphaera Sp., Selenomonas felix, Acidaminococcus intestine, and/or *Propionospora sp.* microbial extracellular vesicles (mEVs)).

[276] In certain aspects, provided herein are methods for assaying a drug product or drug substance comprising contacting a cell of a mammalian immune cell line (*e.g.,* THP-1 cells, such as PMA-differentiated THP-1 cells) with the drug product or drug substance. The cell may comprise a nucleic acid sequence operatively linked to a regulatory sequence, wherein the regulatory sequence is responsive to NF-κB or is an NF-κB promoter. The method may further comprise detecting the level of expression of a transcription product encoded by the nucleic acid sequence by the cell.

[277] In various embodiments, a regulatory sequence that is responsive to NF-κB is or includes an NF-κB response element. An NF-κB response element, as used herein, can refer to a sequence that is bound by NF-κB (e.g., a sequence that is in a promoter or other regulatory sequence of a coding sequence, e.g., where expression of the coding sequences is regulated and/or controlled by NF-κB), and/or to an NF-κB response element sequence as disclosed herein or known in the art. The consensus sequence for NF-κB response element is known in art (for example Mulero, M.C., et al. Nucleic Acids Res. 2019 Nov 4;47(19):9967-9989). In various embodiments, an NF-κB response element can have the sequence TGGGGACTTTCCGC (SEQ ID NO: 1). In various embodiments, an NF-κB response element can have a sequence according to GGGRNWYYCC (where R is a purine, Y is a pyrimidine, W is an adenine or thymine and N is an unspecified base (SEQ ID NO: 2), an example of which is the NF-κB response element GGGAATTCCC (SEQ ID NO: 3)). In various embodiments, an NF-κB response element can have a sequence that is a portion of any one of SEQ ID NOs: 1-3, e.g., that comprises at least 6, 7, 8, 9, 10, 11, 12, 13, or 14 contiguous nucleotides of any of SEQ ID NOs: 1-3. In various embodiments, an NF-κB response element can have a sequence that differs from SEQ ID NO: 1, 2 or 3 in that it includes at least 1, 2, 3, 4, or 5 nucleotide sequence differences, where the difference can be, e.g., an insertion, deletion or substitution of a nucleotide to or of SEQ ID NO: 1, 2 or 3. In various embodiments, an NF-κB response element can have a sequence that is identical to a portion of any of SEQ ID NOs: 1-3, e.g., that comprises at least 6, 7, 8, 9, 10, 11, 12, 13, or 14 contiguous nucleotides of any of SEQ ID NOs: 1-3, except in that the portion includes at least 1, 2, 3, 4, or 5 nucleotide sequence differences from a corresponding portion of any of SEQ ID NOs: 1-3.

[278] In various embodiments in which a regulatory sequence includes a plurality of NF-κB response elements, the NF-κB response elements can be positioned contiguously or such that one or more of the plurality of NF-κB response elements are separated by one or more intervening nucleotides, the number of intervening nucleotides between any two response elements in each case independently selected from, e.g., at least 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, or 50 nucleotides. In various embodiments in which a regulatory sequence includes one or more NF-κB response elements and a promoter, the NF-κB response element nearest to the promoter can be adjacent to the promoter or separated from the promoter by one or more intervening nucleotides (e.g., by at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, or 50 nucleotides). In various embodiments, the regulatory sequence includes 1-30 NF-κB response elements. In various embodiments, the regulatory sequence includes 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 NF-κB response elements. In various embodiments, the regulatory sequence includes four NF-κB response elements.

[279] In various embodiments, an NF-κB promoter can refer to a sequence that has at least 80% identity (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) identity with a sequence that is positioned near to and upstream of the transcription start site of an NF-κB coding sequence (e.g., in the genome of a wild type mammalian cell). In various embodiments, a sequence that is is positioned near to and upstream of the transcription start site of an NF-κB coding sequence extends from a position that is immediately adjacent to or not more than 500 basepairs upstream (5') from the NF-κB transcription start site to a further upstream position. In various embodiments, a sequence that is is positioned near to and upstream of the transcription start site of an NF-κB coding sequence has a length of, e.g, about 100-3000 nucleotides, e.g., about 100-1000 nucleotides.

[280] Bacteria and/or microbial extracellular vesicles assayed for potency using methods and compositions disclosed herein can be, or be present in, a drug product or drug substance. Bacteria and/or microbial extracellular vesicles assayed for potency using methods and compositions disclosed herein can be any bacteria and/or microbial extracellular vesicles disclosed herein, and/or any bacteria and/or microbial extracellular vesicles produced according to methods disclosed herein. In various embodiments, bacteria and/or microbial extracellular vesicles assayed for potency using methods and compositions disclosed herein can be *Prevotella histicola* bacteria and/or microbial extracellular vesicles.

[281] In various embodiments, a potency assay of the present disclosure includes contacting cells with a drug product or drug substance. In various embodiments, the cells are or include a population of cells, such as a culture of cells. In various embodiments, the cells are or include mammalian cells.

[282] In various embodiments, a potency assay of the present disclosure includes contacting a drug product or drug substance with one or more cells that are or include white blood cells and/or cells derived (e.g., differentiated) therefrom. In various embodiments, a potency assay of the present disclosure includes contacting a drug product or drug substance with one or more cells that are or include immune cells and/or cells derived (e.g., differentiated) therefrom. In some embodiments, cells derived from a lineage, or from cells, comprise an immortalized cell line.

[283] In various embodiments, a potency assay of the present disclosure includes contacting a drug product or drug substance with one or more cells that are or include cells of a monocyte lineage and/or cells derived (e.g., differentiated) therefrom (such as monocytes, macrophages, or dendritic cells). In various embodiments, a potency assay of the present disclosure includes contacting a drug product or drug substance with one or more cells that are or include cells differentiated from a monocyte lineage, such as monocytes, macrophages, or dendritic cells. In some embodiments, cells derived from a lineage, or from cells, comprise an immortalized cell line. Monocyte-derived macrophages can be polarized into M1, M2a, M2b, and M2c cells.

[284] In various embodiments, a potency assay of the present disclosure includes contacting a drug product or drug substance with one or more cells that are or include immortalized cells and/or cells derived (e.g., differentiated) therefrom. In various embodiments, a potency assay of the present disclosure includes contacting a drug product or drug substance with one or more cells that are or include cancer or cancer-derived cells, and/or cells derived (e.g., differentiated) therefrom. In various embodiments, a potency assay of the present disclosure includes contacting a drug product or drug substance with one or more cells that are or include cells that are, or are derived from, cells of a human leukemia, and/or cells derived (e.g., differentiated) therefrom.

[285] In various embodiments, a potency assay of the present disclosure includes contacting a drug product or drug substance with one or more cells that are or include

immortalized monocytes and/or cells derived (e.g., differentiated) therefrom. In various embodiments, a potency assay of the present disclosure includes contacting a drug product or drug substance with one or more cells that are or include cancer or cancer-derived monocytes and/or cells derived (e.g., differentiated) therefrom. In various embodiments, a potency assay of the present disclosure includes contacting a drug product or drug substance with one or more cells that are or include monocytes that are, or are derived from, cells of a human leukemia or lymphoma, and/or cells derived (e.g., differentiated) therefrom.

[286] In various embodiments, a potency assay of the present disclosure includes contacting a drug product or drug substance with one or more cells that are or include myeloid cells, cells of myeloid lineage, and/or cells derived from myeloid cells, and/or cells derived (e.g., differentiated) therefrom. In various embodiments, a potency assay of the present disclosure includes contacting a drug product or drug substance with one or more cells that are or include T cells and/or cells derived (e.g., differentiated) therefrom. In various embodiments, a potency assay of the present disclosure includes contacting a drug product or drug substance with one or more cells that are or include Jurkat cells and/or cells derived (e.g., differentiated) therefrom. In various embodiments, a potency assay of the present disclosure includes contacting a drug product or drug substance with one or more cells that are or include B cells and/or cells derived (e.g., differentiated) therefrom. In various embodiments, a potency assay of the present disclosure includes contacting a drug product or drug substance with one or more cells that are or include Raji cells and/or cells derived (e.g., differentiated) therefrom. In any of the various embodiments of a potency assay of the present disclosure, cells with which a drug product or drug substance are contacted can be or include human cells.

[287] In various embodiments, a potency assay of the present disclosure includes contacting a drug product or drug substance with one or more cells that are or include U937 cells and/or cells derived (e.g., differentiated) therefrom. In various embodiments, U937 is a known myeloid lineage cancer cell line that exhibits monocyte morphology and was derived in the 1970's from malignant cells. Without wishing to be bound by any particular scientific theory, U937 cells have been described as a pro-monocytic, human myeloid leukaemia cell line. U937 cells can differentiate into macrophages or dendritic cells (antigen-presenting cells). U937 cells can differentiate along a monocyte/macrophage lineage when contacted with TPA. U937 cells can develop into macrophage-like cells when contacted with phorbol 12-myristate 13-acetate

(PMA). In various embodiments, a potency assay of the present disclosure includes contacting a drug product or drug substance with one or more cells that are or include K562 cells and/or cells derived (e.g., differentiated) therefrom. In various embodiments, K562 cells are a known immortalized line of lymphoblast cells isolated from the bone marrow of a 53-year-old chronic myelogenous leukemia patient. Without wishing to be bound by any particular scientific theory, K562 blasts are multipotential, hematopoietic malignant cells that differentiate into recognizable progenitors of the erythrocyte, granulocyte and monocytic series.

[288] In various embodiments, a potency assay of the present disclosure includes contacting a drug product or drug substance with one or more cells that are or include THP-1 cells and/or cells derived (e.g., differentiated) therefrom. In certain preferred embodiments, the cells are or include THP-1 cells. In various embodiments, THP-1 is a known human monocytic leukaemia cell line established in 1980, derived from the blood of a patient with acute monocytic leukaemia. Without wishing to be bound by any particular scientific theory, THP-1 cells have been characterized as having morphology and differentiation properties characteristic of primary monocytes and macrophages. THP-1 cells show a large, round single-cell morphology and express distinct monocytic markers. THP-1 cells start to adhere to culture plates and differentiate into macrophages after exposure to phorbol-12-myristate-13-acetate (PMA, also known as TPA,12-O-tetradecanoylphorbol-13-acetate). THP-1 cells can also be differentiated by exposure to Phorbol 12, 13-dibutyrate (PDBu). Monocyte-derived macrophages can be polarized into M1, M2a, M2b, and M2c cells.

[289] Various cell types are known to those of skill in the art have characteristics similar to THP-1 cells. These include U937 cells, K562 cells, ML-2 cells, HL-60 cells, and Mono Mac 6 cells. Although U937 and THP-1 are derived from separate origins and maturation stage, at least in that U937 cells are of tissue origin while THP-1 cells originate from a blood leukaemia, the present disclosure includes that both are suitable for use in assays disclosed herein. The present disclosure further includes that THP-1 and/or U937 are representative of classes of cell types useful in the present disclosure, which classes include without limitation mammalian cells, human cells, immune cells, myeloid cells, myeloid lineage cells, cells exhibiting monocyte morphology, monocytes, immortalized cells, cancer cells, cancer-derived cells, leukemia-derived monocytes, cancer-derived monocytes, and/or leukemia-derived

monocytes as well as other types and clases of cells disclosed herein, and/or cells derived (e.g., differentiated) therefrom.

[290] In some embodiments, a potency assay of the present disclosure includes contacting a drug product or drug substance with differentiated cells (e.g., any of the cell types disclosed herein, and/or a cell type disclosed herein differentiated as disclosed herein). In various embodiments, diffentiation can be induced by conditions such as culture conditions, including without limitation contacting cells with an agent that induces differentiation. In various embodiments, steps to cause differentiation of cells are undertaken prior to contacting the cells with a drug product or drug substance. To provide a non-limiting example, in some embodiments, a potency assay of the present disclosure includes contacting a drug product or drug substance with cells differentiated to a monocytic phenotype (e.g., THP-1 can be treated with PMA to differentiate the cells to a macrophage phenotype).

[291] In various embodiments, cells of a potency assay disclosed herein can be engineered to encode and/or express a detectable marker. For the avoidance of doubt, a cell line, cell type, or category or class of cells disclosed herein can be referred to by the same terminology and recognized as being of the same line, type, category, class or other other grouping whether such detectable marker is present or absent.

[292] In various embodiments of the present disclosure, cells of a potency assay disclosed herein can be engineered to encode and/or express a detectable marker that produces a signal that correspondonds to expression of the transcription product. In various embodiments, cells of a potency assay disclosed herein can be engineered to encode and/or express a detectable expression product from a coding nucleic acid sequence that is operably linked with a regulatory sequence, such as a regulatory sequence that is responsive to NF-κB or is an NF-κB promoter. In various embodiments, cells of a potency assay disclosed herein can be engineered to encode and/or express a detectable expression product from a coding nucleic acid sequence that is operably linked with a regulatory sequence that is or includes at least one NF-κB response element. In various embodiments, a coding sequence that encodes a detectable expression product can be operably linked with a regulatory sequence that is or includes at least one (e.g., one, two, three, four, or five) NF-κB response elements. In various embodiments, a regulatory sequence can include a promoter such as a minimal TATA promoter. In some embodiments, a regulatory sequence can be or include at least one (e.g., one, two, three, four, or five) NF-κB

response elements and a promoter such as a minimal TATA promoter, e.g., such that the regulatory sequence includes the at least one (e.g., four) NF-κB response elements positioned upstream of a promoter such as a minimal TATA promoter. In various embodiments, cells of a potency assay disclosed herein can be engineered to encode and/or express a detectable expression product from a coding nucleic acid sequence that is operably linked with an NF-κB promoter.

[293] The present disclosure includes that a promoter can be adjancent to, but is not necessarily adjacent to, a transcription start site. In various embodiments, a promoter can be positioned upstream of a transcription start site, e.g., at least or about 1, 2, 3, 4, 5, 10, 15, 20, 25, 50, 100, 150, 200, 250, 500, 1,000 or more base pairs upstream of a transcription start site. The present disclosure includes that an NF-κB response element can be adjancent to, but is not necessarily adjacent to, a transcription start site and/or promoter. In various embodiments, an NF-κB response element can be positioned upstream or downstream of a transcription start site and/or promoter, e.g., at least or about 1, 2, 3, 4, 5, 10, 15, 20, 25, 50, 100, 150, 200, 250, 500, 1,000 or more base pairs upstream or downstream of a transcription start site. In various embodiments including two or more NF-κB response elements (e.g., copies of an NF-κB response element), the present disclosure includes that the sequences of any two NF-κB response elements of the two or more NF-κB response elements can be adjancent to each other or non-adjacent. In various embodiments, a first NF-κB response element can be upstream or downstream of a second NF-κB response element, e.g., at least or about 1, 2, 3, 4, 5, 10, 15, 20, 25, 50, 100, 150, 200, 250, 500, 1,000 or more base pairs upstream or downstream of the second NF-κB response element. In various embodiments, one or more NF-κB response elements and/or a promoter are operatively linked with a coding sequence and/or can be readily determined as being operatively linked with a coding sequence by methods familiar to those of skill in the art (e.g., by methods including measurement of expression of an associated and/or operatively linked coding sequence).

[294] In various embodiments, a detectable expression product can be a detectable expression product such as a fluorescent label, luminescent label, enzyme, or detectable tag. In various embodiments, a fluorescent label can be, e.g., yellow fluorescent protein (YFP), cyan fluorescent protein (CFP), green fluorescent protein (GFP), red fluorescent protein (RFP), blue fluorescent protein (BFP), modified red fluorescent protein (mRFP), mCherry, red fluorescent

protein tdimer2 (RFP tdimer2), and/or HcRED fluorescent protein. In various embodiments, a luminescent label can be, e.g., luciferase (e.g., firefly luciferase, Renilla luciferase, or NanoLuc Luciferase). In various embodiments, a detectable expression product that is an enzyme can be, e.g., horseradish peroxidase, alkaline phosphatase, or β-galactosidase. In various embodiments, a detectable expression product can be a tag or ligand such as avidin, streptavidin, a FLAG tag, or a His tag.

[295] In some embodiments, mammalian cells comprise a sequence encoding a fluorescent transcription product operably linked with more than one NF-κB response elements (e.g., more than one copy of an NF-κB response element, such as four copies of an NF-κB response element) positioned upstream of promoter such as a minimal TATA promoter. In some embodiments, mammalian cells comprise a sequence encoding a fluorescent transcription product operably linked with more than one NF-κB response elements (e.g., more than one copy of an NF-κB response element, such as four copies of an NF-κB response element) positioned upstream of a promoter such as a minimal TATA promoter. In some embodiments, the fluorescent transcription product is a green fluorescent polypeptide, mCherry polypeptide, yellow fluorescent polypeptide, blue fluorescent polypeptide, cyan fluorescent polypeptide, or red fluorescent polypeptide. In some embodiments, the expression of the transcription product is determined by a fluorescence assay. In some embodiments, mammalian cells comprise a luciferase coding sequence operably linked with more than one NF-κB response elements (e.g., more than one copy of an NF-κB response element, such as four copies of an NF-κB response element) positioned upstream of promoter such as a minimal TATA promoter. In some embodiments, the luciferase is a firefly luciferase and/or expression is determined by a luciferase assay.

[296] In some embodiments, mammalian cells comprise a sequence encoding a fluorescent transcription product operably linked with an NF-κB romoter. In some embodiments, the fluorescent transcription product operably linked with an NF-κB romoter is a green fluorescent polypeptide, mCherry polypeptide, yellow fluorescent polypeptide, blue fluorescent polypeptide, cyan fluorescent polypeptide, or red fluorescent polypeptide. In some embodiments, the expression of the transcription product operably linked with an NF-κB romoter is determined by a fluorescence assay. In someembodiments, mammalian cells comprise a

luciferase coding sequence operably linked with an NF-κB romoter. In some embodiments, the luciferase is a firefly luciferase and/or expression is determined by a luciferase assay.

[297] In various embodiments, a potency assay of the present disclosure includes contacting (e.g., incubating) a drug product or drug substance with cells (e.g., THP-1 cells, such as PMA-differentiated THP-1 cells) for a time period that is sufficient for the drug product or drug substance, and/or for a reference drug product or drug substance, to induce detectable expression of the transcription product by the cells. In various embodiments, a drug product or drug substance is incubated with cells for a period of at least or about 1, at least or about 2, at least or about 3, at least or about 4, at least or about 5, at least or about 6, at least or about 8, at least or about 10, at least or about 12, at least or about 14, at least or about 16, at least or about 18, at least or about 20, at least or about 22, at least or about 24, at least or about 36, at least or about 48 hours, or at least or about 72 hours. In various embodiments, a drug product or drug substance is incubated with cells for a period having a lower boundary of at least or about 1, at least or about 2, at least or about 3, at least or about 4, at least or about 5, at least or about 6, at least or about 8, at least or about 10, at least or about 12, at least or about 14, at least or about 16, at least or about 18, at least or about 20, at least or about 22, or at least or about 24 hours, and an upper boundary of at least or about 12, at least or about 14, at least or about 16, at least or about 18, at least or about 20, at least or about 22, at least or about 24, at least or about 36, at least or about 48 hours, or at least or about 72 hours. In various embodiments, a drug product or drug substance is incubated with cells for a period of 1 to 12 hours, 6 to 12 hours, 12 to 24 hours, 24 to 48 hours, or 48 to 72 hours.

[298] In various embodiments, a potency assay of the present disclosure includes, subsequent to contacting (e.g., incubating) a drug product or drug substance with cells, detecting and/or measuring expression of the transcription product by the cells. A variety of approaches to directly or indirectly measuring expression of a protein are known to those of skill in the art. Examples include, without limitation, measuring, detecting, and/or sequencing mRNA molecules from the cells (e.g., mRNA molecules encoding NF-κB or encoding a detectable protein) or protein from the cells. The transcription product mRNA can be measured or detected, e.g., by Northern blot, RT-PCR (reverse transcription-polymerase chain reaction), RNAseq, or microarray. The transcription product protein can be measured or detected, e.g., by a Western Blot, immunoassay, enzyme-linked immunosorbent assay (ELISA), meso scale discovery (MSD)

assay, radioimmunoassay, or immunocytostaining. In some embodiments, expression of the transcription product is detected by immunohistochemistry. In some embodiments, expression of the transcription product is detected by immunostaining with a labeled antibody that binds to the expression of the transcription product.

[299] In some embodiments, antibodies specific for the transcription product or NF-κB are immobilized on a surface (e.g., are reactive elements on an array, such as a microarray, or are on another surface, such as used for surface plasmon resonance (SPR)-based technology, such as Biacore), and the transcription product or NF-κB in a sample are detected by virtue of their ability to bind specifically to the antibodies. Alternatively, the transcription product or NF-κB in the sample can be immobilized on a surface, and detected by virtue of their ability to bind specifically to the antibodies. Methods of preparing the surfaces and performing the analyses, including conditions effective for specific binding, are conventional and well-known in the art.

[300] Any of a variety of antibodies can be used in methods of the detection. Such antibodies include, for example, polyclonal, monoclonal (mAbs), recombinant, humanized or partially humanized, single chain, Fab, and fragments thereof. The antibodies can be of any isotype, e.g., IgM, various IgG isotypes such as IgG1, IgG2a, etc., and they can be from any animal species that produces antibodies, including goat, rabbit, mouse, chicken or the like.

[301] In various embodiments in which the cells are engineered to include or encode a detectable expression product operably linked to a regulatory sequence, wherein the regulatory sequence is responsive to NF-κB or is an NF-κB promoter, detection of the detectable expression product detects and/or measures expression of the transcription product by the cells. Thus, in various embodiments in which a detectable expression product is fluorescent or luminescent, transcription product expression by the cells can be detected and/or measured by the fluorescence or luminescence. In some embodiments, the expression of the transcription product is detected using fluorescence activated cell sorting (FACS). In some embodiments, expression of the transcription product is detected using microscopy (e.g., fluorescence microscopy). In various embodiments in which a detectable expression product is an enzyme, transcription product expression by the cells can be detected and/or measured by the activity of the enzyme. In various embodiments in which a detectable expression product is a tag or ligand, expression of the transcription product by the cells can be detected and/or measured by interaction of binding agents (such as anti-tag antibodies, e.g., labeled anti-tag antibodies) with the tag or ligand.

[302] In various embodiments, potency is determined by quantitative, semi-quantitative, or qualitative comparison of measured expression of the transcription product of a drug product or drug substance to a reference. In various embodiments, the reference is a sample known to have, determined to have, or designated as having a target level of potency. In various embodiments, the reference can be a standard value or threshold value.

[303] In various embodiments, a drug product or drug substance and/or a reference (e.g., a reference standard) is assayed for potency according to the present disclosure using serial dilution prior to contacting the drug product(s) and/or drug substance(s) with cells. In some embodiments, the ability of the cell type to express the transcription product is confirmed, e.g., by use of positive controls, e.g., contacting an agent that is known to induce the expression of the transcription product with cells of the cell type, and/or assaying the cells for the transcription product mRNA or protein expression.

[304] In certain aspects, provided herein are methods of determining the potency of a drug product or drug substance. The method may comprise i) determining a threshold level of expression of a transcription product encoded by a nucleic acid sequence by contacting a cell of a mammalian immune cell line (e.g., THP-1 cells, such as PMA-differentiated THP-1 cells) with a reference standard, wherein the cell comprises the nucleic acid sequence operatively linked to a regulatory sequence, wherein the regulatory sequence is responsive to NF-κB or is an NF-κB promoter, and the reference standard is brought into contact with the cell (e.g., THP-1 cells, such as PMA-differentiated THP-1 cells) for a time period sufficient to allow the reference standard to induce expression of the transcription product by the cells (e.g., THP-1 cells, such as PMA-differentiated THP-1 cells). The method may further comprise ii) determining a test level of expression the transcription product of by contacting cells (e.g., THP-1 cells, such as PMA-differentiated THP-1 cells) with the drug product or drug substance, wherein the drug product or drug substance is brought into contact with the cells (e.g., THP-1 cells, such as PMA-differentiated THP-1 cells) for a time period sufficient to allow the drug product or drug substance to induce expression of the transcription product by the cells (e.g., THP-1 cells, such as PMA-differentiated THP-1 cells). The method my further comprise iii) comparing the test level of expression of the transcription product to the level of threshold level of expression of the transcription product to determine the potency of the drug product or drug substance; wherein the drug product or drug substance comprises bacteria or mEVs. In some embodiments, the drug

product or drug substance comprises a strain of bacteria (e.g., *Prevotella histicola*, *Veillonella parvula, Harryflintia acetispora, Fournierella massiliensis, Megasphaera Sp., Selenomonas felix, Acidaminococcus intestine,* and/or *Propionospora sp.* strain bacteria) and/or microbial extracellular vesicles (mEVs) from a strain of bacteria (e.g., *Prevotella histicola*, *Veillonella parvula, Harryflintia acetispora, Fournierella massiliensis, Megasphaera Sp., Selenomonas felix, Acidaminococcus intestine,* and/or *Propionospora sp.* strain microbial extracellular vesicles (mEVs)).

[305] In some embodiments, the threshold above which a drug product or drug substance is identified as potent, and/or below which a drug product or drug substance is identified as not being potent, is expression of the transcription product that is equal to or greater than about 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, or 200% the level of expression of the transcription product by cells (e.g., THP-1 cells) that have been contacted by a reference standard. In some embodiments, the threshold above which a drug product or drug substance is identified as potent, and/or below which a drug product or drug substance is identified as not being potent, is expression of the transcription product that is equal to or greater than 70% the level of expression of the transcription product by cells (e.g., THP-1 cells) that have been contacted by a reference standard. In some embodiments, the threshold above which a drug product or drug substance is identified as potent, and/or below which a drug product or drug substance is identified as not being potent, is expression of the transcription product that is at most 135% the level of expression of the transcription product by cells (e.g., THP-1 cells) that have been contacted by a reference standard.

[306] In some embodiments, the threshold above which a drug product or drug substance is identified as potent, and/or below which a drug product or drug substance is identified as not being potent, is expression of the transcription product that is equal to or greater than about 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, or 200% of a determined threshold level of expression of the transcription product (e.g., from step (i) of certain exemplary methods provided herein). In some embodiments, the threshold above which a drug product or drug substance is identified as potent, and/or below which a drug product or drug substance is identified as not being potent, is expression of the transcription product that is equal to or greater than about 70% of a determined threshold level of

expression of the transcription product (e.g., from step (i) of certain exemplary methods provided herein). In some embodiments, the threshold above which a drug product or drug substance is identified as potent, and/or below which a drug product or drug substance is identified as not being potent, is expression of the transcription product that is at most 135% of a determined threshold level of expression of the transcription product (e.g., from step (i) of certain exemplary methods provided herein).

[307] In various embodiments, a drug product or drug substance is identified as a potent drug product or drug substance if the level of expression of the transcription product by the cells (e.g., THP-1 cells) is at or above a reference level. In various embodiments, the reference level is determined by comparison to a reference sample known to have, determined to have, or designated as having a target level of potency. In various embodiments, the reference level is a threshold level or value.

[308] In some embodiments, the present disclosure includes formulating the drug product or drug substance into a solid dosage form if it has been identified as a potent drug product or drug substance (e.g., a tablet, a minitablet, a capsule, or a powder). In some embodiments, the solid dosage form is a tablet or capsule. In some embodiments, the present disclosure includes packaging a drug product or drug substance (e.g., a formulated drug product or drug substance) if it has been identified as a potent drug product or drug substance. In some embodiments, the present disclosure includes administering a drug product or drug substance (e.g., a formulated drug product or drug substance) to a subject in need thereof if it has been identified as a potent drug product or drug substance.

[309] In some embodiments, the present disclosure includes discarding a drug product or drug substance if it has been identified as a drug product or drug substance that is not potent. In some embodiments, the present disclosure includes discarding a drug product or drug substance if a measured sample's level of expression of the transcription product is below a selected threshold level of expression of the transcription product. In some embodiments, the present disclosure includes not discarding a drug product or drug substance if it has been identified as a potent drug product or drug substance. In some embodiments, the present disclosure includes not discarding a drug product or drug substance if a measured sample's level of expression of the transcription product is equal to or greater than a selected threshold level of expression of the transcription product.

[310] In some embodiments, a potency assay of the present disclosure is applied to a drug product or drug substance for batch release. In some embodiments, a potency assay of the present disclosure is applied to a drug product or drug substance in order to measure, determine, and/or monitor storage stability. In some embodiments, a potency assay of the present disclosure is applied to bacteria and/or microbial extracellular vesicles (mEVs) prior to preparation of a drug substance or drug product, for example, during the manufacturing process, such as a process control, in order to measure, determine, and/or monitor the effects of a process (e.g., a step thereof) on the potency of bacteria and/or microbial extracellular vesicles (mEVs). For example, the potency assay can be used to assess the effects of changing a process parameter on the potency of bacteria and/or microbial extracellular vesicles (mEVs). Examples of process parameters include change in amount or presence of a growth media component, change in a growth condition (such as time, temperature, or optical density), change in a downstream processing step, change in excipient, and change in formulating parameter (such as compression strength).

Solid Dosage Form Compositions

[311] In certain aspects, provided herein are solid dosage forms (*e.g.*, pharmaceutical products having a solid dosage form) comprising a pharmaceutical agent (e.g., drug substance) that contains bacteria and/or microbial extracellular vesicles (mEVs) (such as *Prevotella histicola* bacteria and/or mEVs). In some embodiments, the pharmaceutical agent can optionally contain one or more additional components, such as a cryoprotectant. The pharmaceutical agent can be lyophilized (e.g., resulting in a powder). The pharmaceutical agent can be combined with one or more excipients (e.g., pharmaceutically acceptable excipients) in the solid dose form.

[312] In certain aspects, provided herein are solid dosage forms of pharmaceutical compositions. In some embodiments, the solid dosage form comprises a pharmaceutical agent, wherein the pharmaceutical agent comprises bacteria and/or microbial extracellular vesicles (mEVs) (such as *Prevotella histicola* bacteria and/or mEVs (e.g., bacteria and/or mEVs, and/or a powder comprising bacteria and/or mEVs)) and a diluent.

[313] In some embodiments, the solid dosage form provided herein comprises a lubricant.

[314] In some embodiments, a solid dosage form provided herein comprises a glidant.

[315] Thus, in some embodiments, provided herein are solid dosage forms comprising a pharmaceutical agent that contains bacteria and/or microbial extracellular vesicles (mEVs) (such as *Prevotella histicola* bacteria and/or mEVs). The bacteria can be live bacteria (e.g., powder or biomass thereof); non-live (dead) bacteria (e.g., powder or biomass thereof); non replicating bacteria (e.g., powder or biomass thereof); gamma-irradiated bacteria (e.g., powder or biomass thereof); and/or lyophilized bacteria (e.g., powder or biomass thereof).

[316] In some embodiments, the pharmaceutical agents comprise lyophilized bacteria and/or microbial extracellular vesicles (mEVs) (such as *Prevotella histicola* bacteria and/or mEVs).

[317] In some embodiments, total cell count (TCC) can be used to quantify the numbers of bacteria (such as *Prevotella histicola* bacteria) present in a sample. In some embodiments, to quantify the numbers of bacteria (such as *Prevotella histicola* bacteria) present in a sample, electron microscopy (e.g., EM of ultrathin frozen sections) can be used to visualize the 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 in a given sample. Coulter counting reveals the numbers of particles with diameters of 0.7-10 um. For most bacterial samples, the Coulter counter alone can reveal the number of bacteria 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.

[318] In some embodiments, the bacteria and/or microbial extracellular vesicles (mEVs) (such as *Prevotella histicola* bacteria and/or mEVs) may be quantified based on particle count. For example, total particle count of a bacteria and/or microbial extracellular vesicles (mEVs) (such as *Prevotella histicola* bacteria and/or mEVs) preparation can be measured using NTA.

[319] In some embodiments, the bacteria and/or microbial extracellular vesicles (mEVs) (such as *Prevotella histicola* bacteria and/or mEVs) may be quantified based on the amount of

protein, lipid, or carbohydrate. For example, total protein content of bacteria and/or microbial extracellular vesicles (mEVs) (such as *Prevotella histicola* bacteria and/or mEVs), and/or preparations thereof, can be measured using the Bradford assay or BCA. In some embodiments, bacteria and/or microbial extracellular vesicles (mEVs) (such as *Prevotella histicola* bacteria and/or mEVs) may be quantified based on a lipid assay. See, e.g., WO2023114293.

[320] In certain aspects, provided herein are solid dosage forms comprising pharmaceutical agents that comprise bacteria and/or microbial extracellular vesicles (mEVs) (such as *Prevotella histicola* bacteria and/or mEVs) useful for the treatment and/or prevention of disease (e.g., an autoimmune disease, an inflammatory disease, a metabolic disease, or a dysbiosis), as well as methods of making and/or identifying such bacteria, and methods of using pharmaceutical agents and solid dosage forms thereof (e.g., for the treatment of an autoimmune disease, an inflammatory disease, or a metabolic disease, either alone or in combination with other therapeutics). In some embodiments, the pharmaceutical agents comprise bacteria (such as *Prevotella histicola* bacteria) (e.g., whole bacteria) (e.g., live bacteria, dead (e.g., killed) bacteria, non-replicating bacteria, gamma-irradiated bacteria; attenuated bacteria). In some embodiments, the pharmaceutical agents comprise bacteria (microbial extracellular vesicles (mEVs) (such as *Prevotella histicola* mEVs).

[321] In certain aspects, provided are pharmaceutical agents for administration to a subject (e.g., human subject). In some embodiments, the pharmaceutical agents 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 agent is combined with an adjuvant such as an immuno-adjuvant (e.g., a STING agonist, a TLR agonist, or a NOD agonist).

[322] In some embodiments, the solid dosage form comprises at least one carbohydrate.

[323] In some embodiments, the solid dosage form 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).

[324] In some embodiments, the solid dosage form comprises at least one 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.

[325] In some embodiments, the solid dosage form comprises at least one 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.

[326] In some embodiments, the solid dosage form 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.

[327] Suitable excipients that can be included in the solid dosage form can be one or more pharmaceutically acceptable excipients known in the art. For example, see Rowe, Sheskey, and Quinn, eds., *Handbook of Pharmaceutical Excipients*, sixth ed.; 2009; Pharmaceutical Press and American Pharmacists Association.

Solid Dosage Forms

[328] The solid dosage form described herein can be a capsule.

[329] The solid dosage forms of a pharmaceutical agent as described herein can comprise capsules. In some embodiments, the capsule is a size 00, size 0, size 1, size 2, size 3, size 4, or size 5 capsule. In some embodiments, the capsule comprises HPMC (hydroxyl propyl methyl cellulose) or gelatin. In some embodiments, the capsule comprises HPMC (hydroxyl propyl methyl cellulose). In some embodiments, the capsule is banded.

[330] In some embodiments, the solid dosage form is enterically coated (e.g., comprises an enteric coating; e.g., is coated with an enteric coating).

[331] In some embodiments, the solid dosage form comprises a tablet (> 4mm) (e.g., 5mm-17mm). For example, the tablet is a 5mm, 6mm, 7mm, 8mm, 9mm, 10mm, 11mm, 12mm, 13mm, 14mm, 15mm, 16mm, 17mm, or 18mm tablet. The size refers to the diameter of the tablet, as is known in the art. As used herein, the size of the tablet refers to the size of the tablet prior to application of an enteric coating.

[332] In some embodiments, the solid dosage form comprises a minitablet. The minitablet can be in the size range of 1mm-4 mm range. E.g., the minitablet can be a 1mm minitablet, 1.5 mm minitablet, 2mm minitablet, 3mm minitablet, or 4mm minitablet. The size refers to the diameter of the minitablet, as is known in the art. As used herein, the size of the minitablet refers to the size of the minitablet prior to application of an enteric coating.

[333] The minitablets can be in a capsule. The capsule can be a size 00, size 0, size 1, size 2, size 3, size 4, or size 5 capsule. The capsule that contains the minitablets can comprise HPMC (hydroxyl propyl methyl cellulose) or gelatin. The minitablets can be inside a capsule: the number of minitablets inside a capsule will depend on the size of the capsule and the size of the minitablets. As an example, a size 0 capsule can contain 31-35 (an average of 33) minitablets that are 3mm minitablets. In some embodiments, the capsule is banded after loading. In some embodiments, the capsule is banded with an HPMC-based banding solution.

Coating

[334] The solid dosage form (e.g., capsule or tablet or minitablet) described herein can be enterically coated. The enteric coating allows for release of the pharmaceutical agent, e.g., in the small intestine, e.g., upper small intestine, e.g., duodenum and/or jejunum. In some embodiments, the solid dosage form is enteric coated to dissolve at pH 5.5.

[335] Release of the pharmaceutical agent in the small intestine, e.g., in the upper small intestine, e.g., in the duodenum, or in the jejunum, allows the pharmaceutical agent to target and affect cells (e.g., epithelial cells and/or immune cells) located at these specific locations, e.g., which can cause a local effect in the small intestine and/or cause a systemic effect (e.g., an effect outside of the gastrointestinal tract).

[336] 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.

[337] Examples of other materials that can be used in the enteric coating (e.g., the one enteric coating or the inner enteric coating and/or the outer enteric coating) include cellulose acetate phthalate (CAP), cellulose acetate trimellitate (CAT), poly(vinyl acetate phthalate) (PVAP), hydroxypropyl methylcellulose phthalate (HPMCP), fatty acids, waxes, shellac (esters of aleurtic acid), plastics, plant fibers, zein, AQUA-ZEIN® (an aqueous zein formulation containing no alcohol), amylose starch, starch derivatives, dextrins, methyl acrylate-methacrylic acid copolymers, cellulose acetate succinate, hydroxypropyl methyl cellulose acetate succinate (hypromellose acetate succinate), methyl methacrylate-methacrylic acid copolymers, and/or sodium alginate.

[338] The enteric coating can include a polymethacrylate-based copolymer.

[339] The enteric coating can include poly(methacrylic acid-co-ethyl acrylate).

[340] The enteric coating can include a methacrylic acid ethyl acrylate (MAE) copolymer (1:1).

[341] The enteric coating can include methacrylic acid ethyl acrylate (MAE) copolymer (1:1) (such as Kollicoat MAE 100P).

[342] The enteric coating can include a Eudragit copolymer, e.g., a Eudragit L (e.g., Eudragit L 100-55; Eudragit L 30 D-55), a Eudragit S, a Eudragit RL, a Eudragit RS, a Eudragit E, or a Eudragit FS (e.g., Eudragit FS 30 D).

[343] Other examples of materials that can be used in the enteric coating (e.g., the one enteric coating or the inner enteric coating and/or the outer enteric coating) include those described in, e.g., U.S. 6312728; U.S. 6623759; U.S. 4775536; U.S. 5047258; U.S. 5292522; U.S. 6555124; U.S. 6638534; U.S. 2006/0210631; U.S. 2008/200482; U.S. 2005/0271778; U.S. 2004/0028737; WO 2005/044240.

[344] See also, e.g., U.S. 9233074, which provides pH dependent, enteric polymers that can be used with the solid dosage forms provided herein, including methacrylic acid copolymers, polyvinylacetate phthalate, hydroxypropylmethyl cellulose acetate succinate, hydroxypropylmethyl cellulose phthalate and cellulose acetate phthalate; suitable methacrylic acid copolymers include: poly(methacrylic acid, methyl methacrylate) 1:1 sold, for example,

under the Eudragit L100 trade name; poly(methacrylic acid, ethyl acrylate) 1:1 sold, for example, under the Eudragit L100-55 trade name; partially-neutralized poly(methacrylic acid, ethyl acrylate) 1:1 sold, for example, under the Kollicoat MAE-100P trade name; and poly(methacrylic acid, methyl methacrylate) 1:2 sold, for example, under the Eudragit S100 trade name.

Methods of Use

[345] The solid dosage forms, pharmaceutical agents (e.g., drug substances) and pharmaceutical compositions (e.g., drug products) described herein allow, e.g., for oral administration of a pharmaceutical agent contained therein.

[346] The solid dosage forms, pharmaceutical agents and pharmaceutical compositions described herein can be used in the treatment and/or prevention of inflammation, autoimmunity, a metabolic condition, or a dysbiosis.

[347] The solid dosage forms, pharmaceutical agents and pharmaceutical compositions described herein can be used in the treatment and/or prevention of bacterial septic shock, cytokine storm and/or viral infection (such as a coronavirus infection, an influenza infection, and/or a respiratory syncytial virus infection).

[348] The solid dosage forms, pharmaceutical agents and pharmaceutical compositions described herein can be used to decrease inflammatory cytokine expression (*e.g.,* decreased IL-8, IL-6, IL-1β, and/or TNFα expression levels).

[349] Methods of using a solid dosage form and/or pharmaceutical composition (e.g., for oral administration) (e.g., for pharmaceutical use) comprising a pharmaceutical agent (e.g., a therapeutically effective amount thereof), wherein the pharmaceutical agent comprises bacteria and/or microbial extracellular vesicles (mEVs) (such as *Prevotella histicola* bacteria and/or mEVs), and wherein the solid dosage form and/or pharmaceutical composition further comprises the disclosed components are described herein.

[350] The methods and administered solid dosage forms and/or pharmaceutical compositions described herein allow, e.g., for oral administration of a pharmaceutical agent contained therein. The solid dosage form and/or pharmaceutical composition can be administered to a subject is a fed or fasting state. The solid dosage form and/or pharmaceutical composition can be administered, e.g., on an empty stomach (e.g., one hour before eating or two hours after

eating). The solid dosage form and/or pharmaceutical composition can be administered one hour before eating. The solid dosage form and/or pharmaceutical composition can be administered two hours after eating.

[351] A solid dosage form, pharmaceutical agent, and/or pharmaceutical composition for use in the treatment and/or prevention of inflammation, autoimmunity, a metabolic condition, a cancer, or a dysbiosis is provided herein.

[352] A solid dosage form, pharmaceutical agent, and/or pharmaceutical composition for use in the treatment and/or prevention of bacterial septic shock, cytokine storm and/or viral infection (such as a coronavirus infection, an influenza infection, and/or a respiratory syncytial virus infection) is provided herein.

[353] A solid dosage form, pharmaceutical agent, and/or pharmaceutical composition for use in decrease inflammatory cytokine expression (e.g., decreased IL-8, IL-6, IL-1β, and/or TNFα expression levels) is provided herein.

[354] Use of a solid dosage form, pharmaceutical agent, and/or pharmaceutical composition for the preparation of a medicament for the treatment and/or prevention of inflammation, autoimmunity, a metabolic condition, a cancer, or a dysbiosis is provided herein.

[355] Use of a solid dosage form, pharmaceutical agent, and/or pharmaceutical composition for the preparation of a medicament for the treatment and/or prevention of bacterial septic shock, cytokine storm and/or viral infection (such as a coronavirus infection, an influenza infection, and/or a respiratory syncytial virus infection) is provided herein.

[356] Use of a solid dosage form, pharmaceutical agent, and/or pharmaceutical composition for the preparation of a medicament for decreasing inflammatory cytokine expression (*e.g.*, decreased IL-8, IL-6, IL-1β, and/or TNFα expression levels) is provided herein.

Method of Making Solid Dosage Forms

[357] Methods of preparing a solid dosage form of a drug substance or drug product can comprise, among other things, prepration of a tablet, a minitablet, a capsule, or a powder, and steps thereof. Methods of preparing a solid dosage form of a drug substance or drug product can comprise blending, encapsulation, banding, and/or coating of a dosage form such as a tablet, a minitablet, or a capsule.

[358] In certain aspects, provided herein are methods of preparing a solid dosage form of a pharmaceutical composition, the method comprising combining (e.g., blending) into a pharmaceutical composition a pharmaceutical agent (*e.g.,* bacteria and/or microbial extracellular vesicles (mEVs) (such as *Prevotella histicola* bacteria and/or mEVs) or a powder comprising bacteria and/or microbial extracellular vesicles (mEVs) (such as *Prevotella histicola* bacteria and/or mEVs)) and one or more additional components described herein. In certain aspects, provided herein are methods of preparing a solid dosage form of a pharmaceutical composition, the method comprising combining into a pharmaceutical composition a pharmaceutical agent (*e.g.,* bacteria and/or microbial extracellular vesicles (mEVs) (such as *Prevotella histicola* bacteria and/or mEVs) or a powder comprising bacteria and/or microbial extracellular vesicles (mEVs) (such as *Prevotella histicola* bacteria and/or mEVs)) and a diluent.

[359] In some embodiments, the method further comprises loading the pharmaceutical composition into a capsule (e.g., encapsulation).

[360] In some embodiments, the method further comprises banding the capsule after loading.

[361] In some embodiments, the method further comprises enterically coating the capsule.

[362] In some embodiments, the method further comprises compressing the pharmaceutical composition into a tablet.

[363] In some embodiments, the method further comprises enterically coating the tablet.

[364] In some embodiments, the method further comprises compressing the pharmaceutical composition into a minitablet.

[365] In some embodiments, the method further comprises enterically coating the minitablet.

Additional Aspects of the Solid Dosage Forms

[366] The solid dosage forms and/or pharmaceutical compositions, e.g., as described herein, comprising a pharmaceutical agent (e.g., a therapeutically effective amount thereof), wherein the pharmaceutical agent comprises *Prevotella histicola* bacteria, and wherein the solid dosage form and/or pharmaceutical composition further comprises the described components, can provide a therapeutically effective amount of the pharmaceutical agent to a subject, e.g., a human.

[367] The solid dosage forms and/or pharmaceutical compositions, e.g., as described herein, comprising a pharmaceutical agent (e.g., a therapeutically effective amount thereof), wherein the pharmaceutical agent comprises bacteria and/or microbial extracellular vesicles (mEVs) (such as *Prevotella histicola* bacteria and/or mEVs), and wherein the solid dosage form further comprises the described components, can provide a non-natural amount of the therapeutically effective components (e.g., present in the pharmaceutical agent) to a subject, e.g., a human.

[368] The solid dosage forms and/or pharmaceutical compositions, e.g., as described herein, comprising a pharmaceutical agent (e.g., a therapeutically effective amount thereof), wherein the pharmaceutical agent comprises bacteria and/or microbial extracellular vesicles (mEVs) (such as *Prevotella histicola* bacteria and/or mEVs), and wherein the solid dosage form and/or pharmaceutical composition further comprises the described components, can provide an unnatural quantity of the therapeutically effective components (e.g., present in the pharmaceutical agent) to a subject, e.g., a human.

[369] The solid dosage forms and/or pharmaceutical compositions, e.g., as described herein, comprising a pharmaceutical agent (e.g., a therapeutically effective amount thereof), wherein the pharmaceutical agent comprises bacteria and/or microbial extracellular vesicles (mEVs) (such as *Prevotella histicola* bacteria and/or mEVs), and wherein the solid dosage form and/or pharmaceutical composition further comprises the described components, 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.

The solid dosage forms and/or pharmaceutical compositions, e.g., as described herein, comprising a pharmaceutical agent (e.g., a therapeutically effective amount thereof), wherein the pharmaceutical agent comprises bacteria and/or microbial extracellular vesicles (mEVs) (such as *Prevotella histicola* bacteria and/or mEVs), and wherein the solid dosage form and/or pharmaceutical composition further comprises the described components, 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.

Administration

[370] In certain aspects, provided herein is a method of delivering a solid dosage form and/or pharmaceutical composition described herein to a subject. In some embodiments of the methods provided herein, the solid dosage form and/or pharmaceutical composition is

administered in conjunction with the administration of an additional therapeutic agent. In some embodiments, the solid dosage form and/or pharmaceutical composition comprises a pharmaceutical agent co-formulated with the additional therapeutic agent. In some embodiments, the solid dosage form and/or pharmaceutical composition is co-administered with the additional therapeutic agent. In some embodiments, the additional therapeutic agent is administered to the subject before administration of the solid dosage form (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 solid dosage form and/or pharmaceutical composition (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 solid dosage form and/or pharmaceutical composition and the additional therapeutic agent. In some embodiments, different modes of delivery are used to administer the solid dosage form and/or pharmaceutical composition and the additional therapeutic agent. For example, in some embodiments the solid dosage form and/or pharmaceutical composition is administered orally while the additional therapeutic agent is administered via injection (e.g., an intravenous and/or intramuscular).

[371] 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, 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 agent (e.g., in a solid dosage form) 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.

[372] In some embodiments, the dose administered to a subject is sufficient to prevent disease (e.g., autoimmune disease, inflammatory disease, or metabolic disease), 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., pharmaceutical 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 pharmaceutical agent and the desired physiological effect.

[373] 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 no more 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.

[374] In accordance with the above, in therapeutic applications, the dosages of the pharmaceutical agents used as provided herein 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. 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.

[375] 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 solid dosage form and/or 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.

[376] 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 no more than the time period for a subject to mount an immune response, such as no more than about one week, no more than about ten days, no more than about two weeks, or no more than about a month.

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

[378] 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.

[379] 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 heartbeat, 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.

Exemplary Embodiments

1. A method for assaying a drug product or drug substance, the method comprising:

(a) contacting a cell of a mammalian immune cell line with the drug product or drug substance, wherein:

(i) the cell comprises a nucleic acid sequence operatively linked to a NF-κB regulatory sequence, wherein the NF-κB regulatory sequence is a sequence that is responsive to NF-κB or is an NF-κB promoter; and

(ii) the drug product or drug substance comprises bacteria (*e.g., Prevotella histicola, Veillonella parvula, Harryflintia acetispora, Fournierella massiliensis, Megasphaera Sp., Selenomonas felix, Acidaminococcus intestine,* and/or *Propionospora sp.* bacteria) and/or microbial extracellular vesicles (mEVs) (*e.g.,* from *Prevotella histicola, Veillonella parvula, Harryflintia acetispora, Fournierella massiliensis, Megasphaera Sp., Selenomonas felix, Acidaminococcus intestine,* and/or *Propionospora sp.* bacteria); and

(b) detecting the level of expression of a transcription product encoded by the nucleic acid sequence by the cell.

2. The method of embodiment 1, wherein the bacteria and/or mEVs are from a strain of *Prevotella histicola.*

3. The method of embodiment 2, wherein the *Prevotella histicola* strain comprises 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).

4. The method of embodiment 3, wherein the *Prevotella histicola* strain is the *Prevotella* Strain B (NRRL accession number B 50329).

5. The method of any one of embodiments 1-4, wherein the transcription product is a protein.

6. The method of embodiment 5, wherein the expression of the protein is determined by an enzyme-linked immunosorbent assay (ELISA), Western blotting, radioimmunoassay, or immunocytostaining.

7. The method of embodiment 5, wherein the protein is a luciferase protein.

8. The method of embodiment 7, wherein the luciferase protein is a firefly luciferase protein.

9. The method of embodiment 7 or embodiment 8, wherein expression of the luciferase is determined by a luciferase assay.

10. The method of embodiment 5, wherein the protein is a fluorescent protein.

11. The method of embodiment 10, wherein the fluorescent protein is a green fluorescent protein, mCherry protein, a yellow fluorescent protein, a blue fluorescent protein, a cyan fluorescent protein, or a red fluorescent protein.

12. The method of embodiment 10 or embodiment 11, wherein expression of the fluorescent protein is determined by detecting fluorescence.

13. The method of any one of embodiments 1-4, wherein the transcription product is an RNA.

14. The method of embodiment 13, wherein the expression of the RNA is determined by a northern hybridization method or using reverse transcription-polymerase chain reaction (RT-PCR).

15. The method of any one of embodiment 1-14, wherein the NF-κB regulatory sequence is a sequence that is responsive to NF-κB.

16. The method of embodiment 15, wherein the sequence that is responsive to NF-κB is an NF-κB response element.

17. The method of embodiment 16, wherein the NF-κB response element comprises a sequence according to SEQ ID NO: 1, 2, or 3.

18. The method of any one of embodiments 15-17, wherein the sequence that is responsive to NF-κB is positioned upstream of a promoter.

19. The method of embodiment 18, wherein the promoter is a minimal TATA promoter.

20. The method of any one of embodiments 15-19, wherein the nucleic acid sequence is operably linked to at least two NF-κB response elements.

21. The method of embodiment 20, wherein each of the at least two NF-κB response elements comprises a sequence according to SEQ ID NO: 1, 2, or 3.

22. The method of embodiment 20 or 21, wherein each of the at least two NF-κB response elements has the same sequence.

23. The method of embodiment 20 or 21, wherein each of the at least two NF-κB response elements has a distinct sequence.

24. The method of any one of embodiments 20-23, wherein the at least two NF-κB response elements are positioned upstream of a promoter.

25. The method of embodiment 24, wherein the promoter is a minimal TATA promoter.

26. The method of any one of embodiment 1-14, wherein the NF-κB regulatory sequence is an NF-κB promoter.

27. The method of any one of embodiments 1-26, wherein the mammalian immune cell line is a monocyte cell line.

28. The method of embodiment 27, wherein the cell is a THP-1 cell.

29. The method of embodiment 27 or 28, further comprising the step of contacting the cells with Phorbol 12-myristate 13-acetate (PMA).

30. The method of any one of embodiments 1-29, further comprising serially diluting the drug product or drug substance prior to contacting the cell with the drug product or drug substance.

31. The method of any one of embodiments 30, wherein the drug product or drug substance is contacted with the cell for a time period sufficient for the drug product or drug substance to induce expression of the transcription product by the cell.

32. The method of any one of embodiment 1-31, further comprising comparing the level of expression of the transcription product to a threshold level of expression of the transcription product.

33. The method of embodiment 32, further comprising determining the threshold level by contacting a cell of the mammalian immune cell line with a reference standard of the drug product or drug substance and detecting the level of expression of the transcription product by

the cell contacted with the reference standard, wherein the level of expression by the cell contacted with the reference standard is the threshold level.

34. The method of embodiment 32, wherein the threshold level is about 50%-200% the level of expression of the transcription product by a cell of the mammalian immune cell line that has been contacted by a reference standard.

35. The method of embodiment 32, wherein the threshold level is about 70%-135% the level of expression of the transcription product by a cell of the mammalian immune cell line that has been contacted by a reference standard.

36. The method any one of embodiments 32-35, wherein the method further comprises discarding the drug product or drug substance if level of expression of the transcription product is below the threshold level.

37. The method of any one of embodiments 32-36, further comprising identifying the drug product or drug substance as a potent drug product or drug substance if the level of expression of the transcription product is at or above the threshold level.

38. The method of embodiment 37, further comprising formulating the drug product or drug substance into a solid dosage form if it has been identified as a potent drug product or drug substance.

39. The method of embodiment 38, wherein the solid dosage form is a tablet or a capsule.

40. The method of any one of embodiments 37-39, further comprising packaging the drug product or drug substance if it has been identified as a potent drug product or drug substance.

41. The method of any one of embodiments 37-40, further comprising administering the drug product or drug substance to a subject in need thereof if it has been identified as a potent drug product or drug substance.

42. The method of any one of embodiments 37-41, further comprising not discarding the drug product or drug substance if it has been identified as a potent drug product or drug substance.

43. A method of determining the potency of a drug product or drug substance, comprising the steps of:

i) determining a threshold level of expression of a transcription product encoded by a nucleic acid sequence by contacting a cell of a mammalian immune cell line with a reference standard, wherein the cell comprises the nucleic acid sequence operatively

linked to a NF-κB regulatory sequence, wherein the NF-κB regulatory sequence is a sequence that is responsive to NF-κB or is an NF-κB promoter, and the reference standard is brought into contact with the cell for a time period sufficient to allow the reference standard to induce expression of the transcription product by the cell;

ii) determining a test level of expression of the transcription product by contacting the cell with the drug product or drug substance, wherein the drug product or drug substance is brought into contact with the cell for a time period sufficient to allow the drug product or drug substance to induce expression of the transcription product by the cell; and

iii) comparing the test level of expression of the transcription product to the level of threshold level of expression of the transcription product to determine the potency of the drug product or drug substance;

wherein the drug product or drug substance comprises bacteria (*e.g., Prevotella histicola, Veillonella parvula, Harryflintia acetispora, Fournierella massiliensis, Megasphaera Sp., Selenomonas felix, Acidaminococcus intestine,* and/or *Propionospora sp.* bacteria) and/or microbial extracellular vesicles (mEVs) (*e.g.,* from *Prevotella histicola, Veillonella parvula, Harryflintia acetispora, Fournierella massiliensis, Megasphaera Sp., Selenomonas felix, Acidaminococcus intestine,* and/or *Propionospora sp.* bacteria).

44. The method of embodiment 43, wherein the bacteria and/or mEVs are from a strain of *Prevotella histicola.*

45. The method of embodiment 44, wherein the *Prevotella histicola* strain comprises 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).

46. The method of embodiment 44, wherein the *Prevotella histicola* strain is the *Prevotella* Strain B (NRRL accession number B 50329).

47. The method of any one of embodiments 43-46, wherein the transcription product is a protein.

48. The method of embodiment 47, wherein the expression of the protein is determined by an enzyme-linked immunosorbent assay (ELISA), Western blotting, radioimmunoassay, or immunocytostaining.

49. The method of embodiment 47, wherein the protein is a luciferase protein.

50. The method of embodiment 49, wherein the luciferase protein is a firefly luciferase protein.

51. The method of embodiment 49 or embodiment 50, wherein expression of the luciferase is determined by a luciferase assay.

52. The method of embodiment 47, wherein the protein is a fluorescent protein.

53. The method of embodiment 52, wherein the fluorescent protein is a green fluorescent protein, mCherry protein, a yellow fluorescent protein, a blue fluorescent protein, a cyan fluorescent protein, or a red fluorescent protein.

54. The method of embodiment 52 or embodiment 53, wherein expression of the fluorescent protein is determined by detecting fluorescence.

55. The method of any one of embodiments 43-46, wherein the transcription product is an RNA.

56. The method of embodiment 55, wherein the expression of the RNA is determined by a northern hybridization method or using reverse transcription-polymerase chain reaction (RT-PCR).

57. The method of any one of embodiment 43-56, wherein the NF-κB regulatory sequence is a sequence that is responsive to NF-κB.

58. The method of embodiment 57, wherein the sequence that is responsive to NF-κB is an NF-κB response element.

59. The method of embodiment 58, wherein the NF-κB response element comprises a sequence according to SEQ ID NO: 1, 2, or 3.

60. The method of any one of embodiments 57-59, wherein the sequence that is responsive to NF-κB is positioned upstream of a promoter.

61. The method of embodiment 60, wherein the promoter is a minimal TATA promoter.

62. The method of embodiment 58, wherein the nucleic acid sequence is operably linked to at least two NF-κB response elements.

63. The method of embodiment 62, wherein each of the at least two NF-κB response elements comprises a sequence according to SEQ ID NO: 1, 2, or 3.

64. The method of embodiment 62 or 63, wherein each of the at least two NF-κB response elements has the same sequence.

65. The method of embodiment 62 or 63, wherein each of the at least two NF-κB response elements has a distinct sequence.

66. The method of any one of embodiments 62-65, wherein the at least two NF-κB response elements are positioned upstream of a promoter.

67. The method of embodiment 66, wherein the promoter is a minimal TATA promoter.

68. The method of any one of embodiment 43-56, wherein the NF-κB regulatory sequence is an NF-κB promoter.

69. The method of any one of embodiments 43-68, wherein the mammalian immune cell line is a monocyte cell line.

70. The method of embodiment 69, wherein the cell is a THP-1 cell.

71. The method of embodiment 69 or 70, further comprising the step of contacting the cell with Phorbol 12-myristate 13-acetate (PMA).

72. The method of any one of embodiments 43-71, further comprising serially diluting the drug product or drug substance prior to bringing the drug product or drug substance into contact with the cell.

73. The method of one of embodiments 43-72, wherein the time period sufficient to allow the reference standard and the drug product or drug substance to induce expression of the transcription product by the cell is at least 24 hours.

74. The method of any one of embodiments 43-73, wherein the threshold level of transcription product expression is about 50-200% of the level of transcription product expression by the mammalian cells in step i).

75. The method of any one of embodiments 43-74, wherein the threshold level of transcription product expression is about 70-135% of the level of transcription product expression by the mammalian cells in step i).

76. The method of any one of embodiments 43-75, wherein the method further comprises discarding the drug product or drug substance if the test level of expression of the transcription product is below the threshold level of expression of the transcription product.

77. The method of any one of embodiments 43-76, further comprising identifying the drug product or drug substance as a potent drug product or drug substance if the test level of expression of the transcription product is at or above a threshold level of expression of the transcription product.

78. The method of embodiment 77, further comprising formulating the drug product or drug substance into a solid dosage form if it has been identified as a potent drug product or drug substance.

79. The method of embodiment 78, wherein the solid dosage form is a tablet or a capsule.

80. The method of any one of embodiments 77-79, further comprising packaging the drug product or drug substance it has been identified as a potent drug product or drug substance.

81. The method of any one of embodiments 77-80, further comprising administering the drug product or drug substance to a subject in need thereof if it has been identified as a potent drug product or drug substance.

82. The method of any one of embodiments 77-81, further comprising not discarding the drug product or drug substance if it has been identified as a potent drug product or drug substance.

83. The method of any one of embodiments 1 to 82, wherein the drug product or drug substance comprises bacteria.

84. The method of any one of embodiments 1 to 83, wherein the drug product or drug substance comprises bacteria and/or mEVs that have been gamma irradiated, UV irradiated, heat inactivated, acid treated, or oxygen sparged.

85. The method of any one of embodiments 83 to 84, wherein the drug product or drug substance comprises live bacteria.

86. The method of any one of embodiments 83 to 84, wherein the drug product or drug substance comprises dead bacteria.

87. The method of any one of embodiments 83 to 86, wherein the drug product or drug substance comprises non-replicating bacteria.

88. The method of any one of embodiments 1 to 87, wherein the drug product or drug substance comprises one strain of bacteria or mEVs from one strain of bacteria.

89. The method of any one of embodiments 1 to 88, wherein the bacteria and/or mEVs are lyophilized (e.g., the lyophilized product further comprises a pharmaceutically acceptable excipient) (e.g., a powder form).

90. The method of any one of embodiments 1 to 89, wherein the bacteria and/or mEVs are gamma irradiated.

91. The method of any one of embodiments 1 to 89, wherein the bacteria and/or mEVs are UV irradiated.

92. The method of any one of embodiments 1 to 91, wherein the bacteria and/or mEVs are heat inactivated (e.g., at 50°C for two hours or at 90°C for two hours).

93. The method of any one of embodiments 1 to 92, wherein the bacteria and/or mEVs are acid treated.

94. The method of any one of embodiments 1 to 93, wherein the bacteria and/or mEVs are oxygen sparged (e.g., at 0.1 vvm for two hours).

95. The method of any one of embodiments 1 to 94, wherein the drug product or drug substance comprises microbial extracellular vesicles (mEVs).

96. The method of embodiment 95, wherein the drug product or drug substance comprises isolated mEVs (e.g., from one or more strains of bacteria (e.g., bacteria of interest)) (e.g., a therapeutically effective amount thereof).

97. The method of embodiments 95 or 96, wherein the mEVs comprise secreted mEVs (smEVs).

98. The method of embodiments 95 or 96, wherein the mEVs comprise processed mEVs (pmEVs).

99. The method of embodiment 98, wherein the pmEVs are produced from bacteria that have been gamma irradiated, UV irradiated, heat inactivated, acid treated, or oxygen sparged.

100. The method of embodiment 98 or 99, wherein the pmEVs are produced from live bacteria.

101. The method of any one of embodiments 98 to 100, wherein the pmEVs are produced from dead bacteria.

102. The method of any one of embodiments 98 to 99, wherein the pmEVs are produced from non-replicating bacteria.

103. The method of any one of embodiments 1 to 102, wherein the mEVs are from one strain of bacteria.

104. The method of any one of embodiments 1 to 102, wherein the bacteria are from one strain of bacteria.

105. A method for assaying a drug product or drug substance, the method comprising:

(a) contacting a cell of THP-1 cell line with the drug product or drug substance, wherein:

(i) the cell comprises a nucleic acid sequence operatively linked to a NF-κB regulatory sequence, wherein the NF-κB regulatory sequence is a sequence that is responsive to NF-κB or is an NF-κB promoter; and

(ii) the drug product or drug substance comprises bacteria and/or microbial extracellular vesicles (mEVs), wherein the bacteria and/or mEVs are from a strain of *Prevotella histicola* (such as *Prevotella* Strain B 50329 (NRRL accession number B 50329); and

(b) detecting the level of expression of a transcription product encoded by the nucleic acid sequence by the cell.

106. A method of determining the potency of a drug product or drug substance, comprising the steps of:

i) determining a threshold level of expression of a transcription product encoded by a nucleic acid sequence by contacting a cell of THP-1 cell line with a reference standard, wherein the cell comprises the nucleic acid sequence operatively linked to a NF-κB regulatory sequence, wherein the NF-κB regulatory sequence is a sequence that is responsive to NF-κB or is an NF-κB promoter, and the reference standard is brought into contact with the cell for a time period sufficient to allow the reference standard to induce expression of the transcription product by the cell;

ii) determining a test level of expression of the transcription product by contacting the cell with the drug product or drug substance, wherein the drug product or drug substance is brought into contact with the cell for a time period sufficient to allow the drug product or drug substance to induce expression of the transcription product by the cell; and

iii) comparing the test level of expression of the transcription product to the level of threshold level of expression of the transcription product to determine the potency of the drug product or drug substance;

wherein the drug product or drug substance comprises bacteria and/or microbial extracellular vesicles (mEVs), wherein the bacteria and/or mEVs are from a strain of *Prevotella histicola* (such as *Prevotella* Strain B 50329 (NRRL accession number B 50329).

EXAMPLES

Example 1: Measuring the relative potency of mEVs

[380] The present Example demonstrates the determination of potency of secreted microbial extracellular vesicles (smEVs) derived from a *Prevotella histicola* microbial strain in a *Prevotella* extracellular vesicle (smEV) drug substance (DS) formulation. In this Example, the *Prevotella* extracellular vesicles (smEVs) were isolated from *Prevotella* Strain B (NRRL accession number B 50329). The smEVs were contacted with differentiated THP-1 NF-κB luciferase (luc) reporter cells.

[381] The NF-κB reporter (Luc)-THP-1 cell line includes a transgene that encodes a firefly luciferase gene driven by four copies of the NF-κB response element positioned upstream of a minimal TATA promoter. After activation, endogenous NF-κB transcription factors bind to the DNA response elements, inducing transcription of the luciferase reporter gene.

[382] THP-1 NF-κB luc reporter cells were cultured using complete RPMI-1640 cell culture media containing puromycin selection marker. THP-1 NF-κB luc reporter cells were passaged twice a week for up to 30 passages, maintaining a $0.4 – 1.6\times10^6$ cells/mL density. Cultured THP-1 NF-κB luc reporter cells were seeded in four white opaque tissue culture treated flat bottom 96-well plates at 7.0×10^4 cells/well. THP-1 cells were incubated in the presence of PMA 50 nM for 24 hours at 37°C, 5% CO_2. Differentiated THP-1 cells were rested without PMA for 24 hours at 37°C, 5% CO_2.

[383] *Prevotella* Strain B smEVs DS reference standard and test sample powders were resuspended in THP-1 cell culture media. *Prevotella* Strain B smEVs reference standard and test sample DS suspensions were serially diluted to create eleven multiplicity-of-infection (MOI; that is, the ratio of EVs to cells) dilution points. Serial dilutions of *Prevotella* Strain B smEVs reference standard and DS test samples were added to four 96-well plates containing PMA-differentiated THP-1 cells. Differentiated THP-1 cells were cultured with *Prevotella* Strain B smEVs reference standard and DS test sample dilutions for 4 hours at 37°C, 5% CO_2.

[384] Steady-Glo® reagent was added to the four plates to lyse the THP-1 cells and generate the luminescence signal. Luminescence intensity generated by NF-κB luc reporter was quantified for each dilution of *Prevotella* Strain B smEVs reference standard and test DS samples using a luminescence microplate reader. Luminescence values were plotted against the corresponding MOI dilution values to obtain a sigmoidal dose response curve using four-

parameter logistics (4-PL) regression. The EC50 values calculated for *Prevotella* Strain B smEVs reference standard and test sample DS samples were used to calculate the relative potency value for *Prevotella* Strain B smEVs test DS samples.

[385] Experiments of this Example were carried out according to the protocol provided below.

Reagent Preparation

[386] Preparation of PMA 5 mg/mL (8.1 mM) stock solution: Reconstitute lyophilized PMA powder by adding 2 mL of sterile DMSO to a vial containing 10 mg of PMA to form a 5 mg/mL stock concentration, equivalent to 8.1 mM stock concentration. Prepare 50 µL aliquots of PMA 8.1 mM stock solution. Assign a 6-month expiration date from the date of preparation. Store at ≤ −20°C.

[387] Preparation of THP-1 cell culture media: Combine 900 mL of RPMI 1640 media, 100 mL of heat-inactivated FBS (10% final concentration) and 0.1 mL of puromycin 10 mg/mL (1 µg/mL final concentration) inside a biosafety cabinet. Sterilize the complete THP-1 cell culture media using a 0.2 µm vacuum filtration system. Assign a 1-month expiration date from the date of preparation. Store at 2 – 8°C.

[388] Preparation of Steady-Glo® reagent: Reconstitute lyophilized Steady-Glo® reagent by adding 100 mL of included buffer to the bottle. Mix by inverting the bottle until the lyophilized Steady-Glo® reagent is fully resuspended. Aliquot the resuspended Steady-Glo® reagent into amber 50 mL conical tubes. Assign a 1-month expiration date from the date of preparation. Keep at −20°C.

Day 1: Seed THP-1 Cells and Differentiate with PMA for 24 Hours

[389] Count THP-1 cells: Measure the pre-spin THP-1 cell density and viability using NucleoCounter NC-200 or a similar cell counter. Transfer the required volume of cell suspension from a T175 tissue culture flask to the 50 mL conical tube. Centrifuge the 50 mL conical tube at 300 × *g* for 5 minutes at room temperature in a swinging bucket rotor. Aspirate the supernatant using vacuum. Resuspend the cell pellet in THP-1 cell culture media. Measure the post-spin THP-1 cell density and viability. Adjust the final concentration of THP-1 cells to 7.0×10^5cells/mL using THP-1 cell culture media.

[390] Differentiate THP-1 cells with PMA: Thaw an aliquot of PMA 8.1 mM (equivalent to 5 mg/mL) stock solution at room temperature. Label a sterile 1.5 mL microcentrifuge tube as

"PMA". Transfer 990 µL of THP-1 cell culture media to the "PMA" labeled tube. Prepare PMA 81 µM working solution by transferring 10 µL of PMA 8.1 mM (5 mg/mL) stock solution to the "PMA" labeled tube to create a 1:100 dilution. Add PMA to the THP-1 cell suspension to achieve a 50 nM final PMA concentration. For example, add 22.2 µL of PMA 81 µM working solution to 36 mL of THP-1 cell suspension.

[391] Plate THP-1 cells + PMA in four white 96-well tissue culture treated plates: Homogenize the THP-1 7.0×10^5 cells/mL suspension containing PMA 50 nM inside the 50 mL conical tube by pipetting up and down with a 25 mL serological pipette. Transfer the cell suspension a 50 mL sterile reservoir. Mix the cell suspension before addition to plates. Add 100 µL of cell suspension to rows B – G of four white tissue culture treated flat-bottom 96-well plates to achieve 7.0×10^4 cells per well. Transfer 25 mL of sterile PBS pH 7.4 to a 50 mL sterile reservoir. Add 200 µL of PBS to rows A and H of each 96-well plate. Incubate the four 96-well plates for 24 hours at 37°C, 5% CO_2.

Day 2: Media Exchange

[392] Warm the THP-1 cell culture media inside a 37°C water bath. Transfer 70 mL of THP-1 cell culture media to 100 mL sterile reservoir. Remove PMA-containing cell culture media from each 96-well plate using a P200 multichannel pipette. Hold the 96-well plate at a 45° angle and aspirate from the corner of the wells. Be careful not to disrupt the adherent THP-1 cells. Add 200 µL of fresh THP-1 cell culture media to each well using a P200 multichannel pipette. Incubate the differentiated THP-1 cells for additional 24 hours at 37°C, 5% CO_2.

Day 3 – Morning: Addition of *Prevotella* Strain B smEVs DS Reference Standard and Test Lot Dilutions

[393] The dilutions of *Prevotella* Strain B smEVs DS reference standard and test samples will be prepared using THP-1 cell culture media inside a designated biosafety cabinet using aseptic techniques. Preparation of *Prevotella* Strain B smEVs reference standard suspension. Resuspend 50 mg of *Prevotella* Strain B smEVs DS powder in 1 mL of THP-1 cell culture media. Mix by vortexing at full speed until the *Prevotella* Strain B smEVs DS powder is fully resuspended. Calculate the concentration (EVs per mL) of the *Prevotella* Strain B smEVs DS suspension, using the following equation:

$$\frac{\begin{array}{c}\textit{Prevotella}\text{ Strain B smEVs}\\ \text{powder weight}\\ (0.05\ grams)\end{array} \times \begin{array}{c}1.15\times10^{13}\\ \text{EV count}\\ (EVs/gram)\end{array}}{\begin{array}{c}\text{Volume of THP}-1\text{ cell culture media}\\ (1\ mL)\end{array}} = \begin{array}{c}5.75\times10^{11}\\ \text{concentration of}\\ \textit{Prevotella}\text{ Strain B smEVs}\\ \text{DS suspension}\\ (EVs/mL)\end{array}$$

[394] Calculate the initial multiplicity of infection (MOI, that is ratio of EVs to cells) value for the *Prevotella* Strain B smEVs DS reference standard suspension, using the following equation:

$$\frac{\begin{array}{c}\text{Concentration of}\\ \textit{Prevotella}\text{ Strain B smEVs}\\ \text{DS suspension}\\ (5.75\times10^{11}\ EVs/mL)\end{array} \times \begin{array}{c}\text{Volume of}\\ \text{MOI dilutions}\\ \text{per well}\\ (0.1\ mL)\end{array}}{\begin{array}{c}\text{Number of THP1 cells per well}\\ (7.0\times10^{4}\ cells)\end{array}} = 8.21\times10^{5}\ \begin{array}{c}\text{Initial MOI}\\ \text{value of}\\ \textit{Prevotella}\text{ Strain B smEVs}\\ \text{DS reference}\\ \text{standard}\\ \text{suspension}\end{array}$$

[395] Keep the *Prevotella* Strain B smEVs DS suspension on ice.

Preparation of *Prevotella* Strain B smEVs DS test sample suspension

[396] Weigh out 50 – 100 mg of the *Prevotella* Strain B smEVs DS test sample powder inside a screw-cap vial using an analytical balance. Resuspend the weighted *Prevotella* Strain B smEVs DS test sample powder in 2 mL of THP-1 cell culture media. Mix by vortexing at full speed until the *Prevotella* Strain B smEVs DS test sample powder is fully resuspended. Calculate the concentration (EVs per mL) of the *Prevotella* Strain B smEVs DS test sample suspension, using the following equation:

$$\frac{\begin{array}{c}\text{Weight of }\textit{Prevotella}\text{ Strain B smEVs DS}\\ \text{test sample powder}\\ (grams)\end{array} \times \begin{array}{c}\text{EV count of }\textit{Prevotella}\text{ Strain B smEVs DS}\\ \text{test sample powder}\\ (EVs/gram)\end{array}}{\begin{array}{c}\text{Volume of THP}-1\text{ cell culture media}\\ (2\ mL)\end{array}} = \begin{array}{c}\text{Concentration}\\ \text{of }\textit{Prevotella}\text{ Strain B smEVs}\\ \text{DS test sample}\\ \text{suspension}\\ (EVs/mL)\end{array}$$

[397] For example, resuspending 100 mg of *Prevotella* Strain B smEVs DS test lot powder, which has 1.6E+14 EVs/gram packing density, in 2 mL of THP-1 cell culture media will result in 8.0E+12 EVs/mL suspension.

[398] Calculate the initial multiplicity of infection (MOI) value for the *Prevotella* Strain B smEVs DS test sample, using the following equation:

$$\frac{\text{Concentration of } \textit{Prevotella} \text{ Strain B smEVs DS test sample suspension } (EVs/mL) \times \text{Volume of MOI dilutions per well } (0.1\ mL)}{\text{Number of THP1 cells per well } (7.0 \times 10^4\ cells)} = \text{Initial MOI value of } \textit{Prevotella} \text{ Strain B smEVs test sample suspension}$$

[399] Keep the *Prevotella* Strain B smEVs DS test sample suspension on ice.

Preparation of preliminary *Prevotella* Strain B smEVs DS reference standard MOI dilutions

[400] Two independently prepared MOI dilution series of *Prevotella* Strain B smEVs DS reference standard are created, acting as reference standard 1 (RS1) and reference standard 2 (RS2) for the relative potency analysis of *Prevotella* Strain B smEVs DS test samples. 2 mL deep-well block #1 is used for preparing preliminary dilutions of *Prevotella* Strain B smEVs DS reference standard suspensions and test sample suspensions. Prepare three preliminary dilutions of *Prevotella* Strain B smEVs DS reference standard suspension in a 2 mL deep-well block #1, based on the initial MOI value calculated above.

[401] Mix each dilution by pipetting up and down 5 – 10 times using a P1000 multichannel pipette. Change tips after every transfer.

[402] Preparation of preliminary *Prevotella* Strain B smEVs DS test sample MOI dilutions. Depending on the strength of *Prevotella* Strain B smEVs DS test sample, the first preliminary MOI dilution may be 1.0×10^6 or 1.0×10^5, based on the initial MOI value of *Prevotella* Strain B smEVs DS test sample suspension. Prepare preliminary dilutions of *Prevotella* Strain B smEVs DS test sample suspensions in a 2 mL deep-well block #1, based on the initial MOI value calculated previously. Mix each dilution by pipetting up and down 5 – 10 times using a P1000 multichannel pipette. Change tips after every transfer.

Preparation of *Prevotella* Strain B smEVs DS reference standard and test sample dilutions

[403] Prepare 4-fold serial dilutions of *Prevotella* Strain B smEVs DS reference standard and test sample suspensions in a 2 mL deep-well block #2 to create samples with indicated MOI values.

[404] Mix by pipetting up and down 5 – 10 times using P1000 multichannel pipette. Change pipette tips after each sample transfer. Position of *Prevotella* Strain B smEVs reference standard and test sample MOI dilutions.

Addition of reference standard and test sample MOI dilutions to PMA-differentiated THP-1 cells.

[405] Handle one row of the 96-well plate at a time. Change tips after every transfer. Remove cell culture media from each 96-well plate using a P300 multichannel pipette. Hold the 96-well plate at a 45° angle and aspirate from the corner of the wells. Be careful not to disrupt the adherent THP-1 cells. Follow the plate map shown below for the addition of *Prevotella* Strain B smEVs DS reference standard and *Prevotella* Strain B smEVs DS test sample MOI dilutions to the four 96-well plates. Transfer 100 µL of *Prevotella* Strain B smEVs DS reference standard 1 (RS1) MOI dilutions to row C using P200 multichannel pipette. Transfer 100 µL of *Prevotella* Strain B smEVs DS reference standard 2 (RS2) MOI dilutions to row F using P200 multichannel pipette. Transfer 100 µL of *Prevotella* Strain B smEVs DS test sample 1 (S1) MOI dilutions to row B on plate 1, row D on plate 2, row E on plate 3 and row G on plate 4 using P200 multichannel pipette.

[406] Incubate the four 96-well plates for 4 hours ± 15 minutes at 37°C and 5% CO_2. Transfer an aliquot of the Steady-Glo® reagent from −20°C to the bench.

Day 3 – Afternoon: Analysis of NF-κB Luciferase Reporter Activity using Promega Steady-Glo® Luciferase Assay System

[407] Remove the four 96-well plates from the microbial cell culture 37°C incubator. Remove the lid from each plate. Incubate the four 96-well plates at room temperature for 10 – 15 minutes. Add 50 µL of Steady-Glo® reagent to rows B – G of each plate using P300 multichannel pipette. Immediately cover each plate with a black lid. Shake the four 96-well plates at 450 rpm at room temperature for 20 – 30 minutes. Launch the software for the spectrophotometer. Create a new experiment using a luminescence protocol template. Measure the luminescence of the four 96-well plates in the same order that the Steady-Glo® reagent was added. Export the results of each experiment as an Excel file.

Example 2: Measuring the relative potency of additional mEVs

[408] The assay detailed in Example 1 was used to determine the potency of smEVs from two additional bacterial strains: *Veillonella* bacteria deposited as ATCC designation number PTA-125691 and *Fournierella massiliensis* bacteria deposited as ATCC designation number PTA-126696.

[409] The general assay steps of number of cells used for seeding plates, concentration and incubation time for PMA differentiation, media exchange and rest period incubation time, incubation time of smEVs with differentiated THP-1 NF-κB luc cells were kept the same as in Example 1.

[410] For each of the two additional smEVs, the required smEV dilution range was determined by performing an extended (24 dose point) dose finding experiment.

[411] smEVs were derived from *Veillonella* bacteria deposited as ATCC designation number PTA-125691. Uses for the potency assay for these smEVs include assessment of bioprocess development, such as comparison of batch culture conditions.

[412] smEVs were derived from *Fournierella massiliensis* bacteria deposited as ATCC designation number PTA-126696. Uses for the potency assay for these smEVs include drug substance stability assessments.

Incorporation by Reference

[413] 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

[414] 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.

CLAIMS

What is claimed is:

1. A method for assaying a drug product or drug substance, the method comprising:

(a) contacting a cell of a mammalian immune cell line with the drug product or drug substance, wherein:

(i) the cell comprises a nucleic acid sequence operatively linked to a NF-κB regulatory sequence, wherein the NF-κB regulatory sequence is a sequence that is responsive to NF-κB or is an NF-κB promoter; and

(ii) the drug product or drug substance comprises bacteria and/or microbial extracellular vesicles (mEVs); and

(b) detecting the level of expression of a transcription product encoded by the nucleic acid sequence by the cell.

2. The method of claim 1, further comprising comparing the level of expression of the transcription product to a threshold level of expression of the transcription product.

3. The method of claim 1 or 2, further comprising determining the threshold level by contacting a cell of the mammalian immune cell line with a reference standard of the drug product or drug substance and detecting the level of expression of the transcription product by the cell contacted with the reference standard, wherein the level of expression by the cell contacted with the reference standard is the threshold level.

4. A method of determining the potency of a drug product or drug substance, comprising the steps of:

i) determining a threshold level of expression of a transcription product encoded by a nucleic acid sequence by contacting a cell of a mammalian immune cell line with a reference standard, wherein the cell comprises the nucleic acid sequence operatively linked to a NF-κB regulatory sequence, wherein the NF-κB regulatory sequence is a sequence that is responsive to NF-κB or is an NF-κB promoter, and the reference standard is brought into contact with the cell for a time period sufficient to allow the reference standard to induce expression of the transcription product by the cell;

ii) determining a test level of expression of the transcription product by contacting the cell with the drug product or drug substance, wherein the drug product or drug substance is brought into contact with the cell for a time period sufficient to allow the

drug product or drug substance to induce expression of the transcription product by the cell; and

iii) comparing the test level of expression of the transcription product to the level of threshold level of expression of the transcription product to determine the potency of the drug product or drug substance;

wherein the drug product or drug substance comprises bacteria and/or microbial extracellular vesicles (mEVs).

5. The method of any one of claims 1 to 4, wherein the bacteria and/or mEVs are from a strain of *Prevotella histicola*.

6. The method of claim 5, wherein the *Prevotella histicola* strain comprises 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).

7. The method of claim 5, wherein the *Prevotella histicola* strain is the *Prevotella* Strain B (NRRL accession number B 50329).

8. The method of any one of claims 1 to 7, wherein the transcription product is a protein.

9. The method of claim 8, wherein the expression of the protein is determined by an enzyme-linked immunosorbent assay (ELISA), Western blotting, radioimmunoassay, or immunocytostaining.

10. The method of any one of claims 1 to 7, wherein the transcription product is an RNA.

11. The method of claim 10, wherein the expression of the RNA is determined by a northern hybridization method or using reverse transcription-polymerase chain reaction (RT-PCR).

12. The method of any one of claims 1 to 11, wherein the mammalian immune cell line is a monocyte cell line.

13. The method of claim 12, wherein the cell is a THP-1 cell.

14. The method of any one of claims 1 to 13, further comprising identifying the drug product or drug substance as a potent drug product or drug substance if the test level of expression of the transcription product is at or above a threshold level of expression of the transcription product.

15. The method of claim 14, further comprising formulating the drug product or drug substance into a solid dosage form if it has been identified as a potent drug product or drug substance.

16. The method of any one of claims 1 to 15, further comprising administering the drug product or drug substance to a subject in need thereof if it has been identified as a potent drug product or drug substance.

17. The method of any one of claims 1 to 16, wherein the drug product or drug substance comprises bacteria.

18. The method of any one of claims 1 to 17, wherein the drug product or drug substance comprises one strain of bacteria or mEVs from one strain of bacteria.

19. The method of any one of claims 1 to 18, wherein the bacteria and/or mEVs are lyophilized.

20. The method of any one of claims 1 to 19, wherein the drug product or drug substance comprises microbial extracellular vesicles (mEVs).

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2023/035125

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/50 C12Q1/6897 G01N33/569
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)
G01N C12Q

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CLAIRE BATTIN ET AL: "A human monocytic NF-[kappa]B fluorescent reporter cell line for detection of microbial contaminants in biological samples", PLOS ONE, vol. 12, no. 5, 24 May 2017 (2017-05-24), page e0178220, XP055771727, DOI: 10.1371/journal.pone.0178220	1-4,8-13
Y	p. 4, par.:THP-1 reporter assays; p. 6-7, par.: THP-1 NF-kB-eGFP cells exhibit selective sensitivity towards TLR ligands; Fig. 1;	14-20
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[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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 February 2024	06/03/2024

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2023/035125

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	US 2017/119643 A1 (HAUGHT JOHN CHRISTIAN [US] ET AL) 4 May 2017 (2017-05-04) abstract; Examples 11 and 12; par. 0169; Example 5 and Fig. 2A -----	1-4,8-13 14-20
X	HAILE LYDIA ASRAT ET AL: "Detection of Innate Immune Response Modulating Impurities in Therapeutic Proteins", PLOS ONE, vol. 10, no. 4, 22 April 2015 (2015-04-22), page e0125078, XP093125598, US ISSN: 1932-6203, DOI: 10.1371/journal.pone.0125078	1-4,8,12
Y	abstract and p. 7-8, par.:Use of RAW-BLUE, THP-1 and MM6 cells to screen products for IIRMI;p. 3-4, par.: Stimulation of NF-kB reporter cells with TLR ligands; Fig. 3; Table 2 -----	14-20
Y	RAMANI KRITIKA ET AL: "Regulation of Peripheral Inflammation by a Non-Viable, Non-Colonizing Strain of Commensal Bacteria", FRONTIERS IN IMMUNOLOGY, vol. 13, 2 February 2022 (2022-02-02), XP093125492, Lausanne, CH ISSN: 1664-3224, DOI: 10.3389/fimmu.2022.768076 p. 8-9, par.: "The Anti-Inflammatory Effects Driven by EDP1867 Require Trafficking of Immune Cells From the Gut to Periphery to Resolve Inflammation"; Supplementary Figure 6: (p. 12, par.: Dosing With EDP1867 and Controls In Vivo; p. 15, Delayed-Type Hypersensitivity Mouse Model, Imiquimod-Induced Psoriasis-Like Skin Inflammation Protocol, Experimental Autoimmune Encephalomyelitis & Ramani Kritika: "Supplementary Figures: Regulation of Peripheral Inflammation by a Non-Viable, Non-Colonizing Strain of Commensal Bacteria", , 2 February 2022 (2022-02-02), XP093126084, Retrieved from the Internet: URL:https://www.frontiersin.org/articles/10.3389/fimmu.2022.768076/full#supplementary-material [retrieved on 2024-01-31] the whole document -/--	5-7, 14-20

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2023/035125

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	& Ramani Kritika: "Supplementary Materials: Regulation of Peripheral Inflammation by a Non-Viable, Non-Colonizing Strain of Commensal Bacteria", , 2 February 2022 (2022-02-02), XP093126089, Retrieved from the Internet: URL:https://www.frontiersin.org/articles/10.3389/fimmu.2022.768076/full#supplementary-material [retrieved on 2024-01-31] the whole document	

Y	ITANO ANDREA ET AL: "Orally-administered EDP1815, a single strain of Prevotella histicola, has potent systemic anti-inflammatory effects in Type 1, Type 2, and Type 3 inflammatory models", EADV 2020 VIRTUAL CONGRESS, 1 January 2020 (2020-01-01), XP093126575, abstract	5-7

Y	Maslin Douglas ET AL: "Mechanism and Proof of Concept for a Novel, Orally Delivered, Gut-Restricted Drug Candidate for the Treatment of Psoriasis and Other Inflammatory Diseases", , 1 January 2022 (2022-01-01), XP093126585, Retrieved from the Internet: URL:https://www.researchgate.net/publication/361610920_Mechanism_and_Proof_of_Concept_for_a_Novel_Orally_Delivered_Gut-Restricted_Drug_Candidate_for_the_Treatment_of_Psoriasis_and_Other_Inflammatory_Diseases [retrieved on 2024-02-01] abstract	5-7

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2023/035125

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