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(54) Title: ANTI-*PREVOTELLA* BACTERIOCIN METHODS AND COMPOSITIONS

(57) Abstract: Methods of reducing *Prevotella* load in a subject, treating or preventing inflammation in a subject, and treating or preventing an enteropathogenic bacterial infection in a subject, comprising administering an anti-*Prevotella* bacteriocin to the subject. Pharmaceutical compositions comprising an anti-*Prevotella* bacteriocin and a pharmaceutically acceptable carrier.

ANTI-*PREVOTELLA* BACTERIOCIN METHODS AND COMPOSITIONS

BACKGROUND

[0001] *Prevotella* is classically considered as a bacterial commensal due to its presence in several locations of the healthy human body including the oral cavity, gastrointestinal tract, urogenital tract and skin (*1*). The *Prevotella* genus encompasses more than 40 different culturable species of which three, *P. copri*, *P salivae* and *P. stercorea*, can be isolated from the gut. Only a few strains have been reported to be associated with opportunistic infections, e.g. periodontitis or bacterial vaginosis (*1*). *Prevotella* has been described as the major genus of one of the three reported human enterotypes (*2*). How *Prevotella* behaves in different gut ecosystems and how it interacts with other bacteria of the microbiota and/or with its host is still elusive. In addition, high levels of genomic diversity within *Prevotella* strains of the same species have been observed (*3*), which adds another layer of complexity for predicting the effects of *Prevotella* strains. Strikingly, recent studies have linked higher intestinal abundance of *P. copri* to rheumatoid arthritis (*4*), metabolic disorders, low-grade systemic inflammation (*5*) and inflammatory conditions in the context of human immunodeficiency virus (HIV) (*6, 7*), suggesting that some *Prevotella* strains may trigger and/or worsen human inflammatory diseases (*1, 8*).

[0002] The microbiota plays a central role in protecting the host from pathogens (*9*). For example, in the case of *Listeria monocytogenes*, the foodborne pathogen responsible for listeriosis, the intestinal microbiota provides protection, as germfree mice are more susceptible to bacterial infection than conventional mice (*10, 11*). Unravelling the interactions between the host, the microbiota and pathogenic bacteria is critical for the design of new therapeutic strategies *via* manipulation of the microbiota. However, identifying the specific molecules and the mechanisms used by the commensals to elicit their beneficial action is challenging due to the high complexity of the microbiomes, together with technical issues such as unculturability of many commensal species. In addition, cooperative interactions between commensals species are likely to be central to the functioning of the gut microbiota (*12*). So far, the mechanism

underlying the impact of commensals on the host has been elucidated only for a few species. Segmented filamentous bacteria (SFB) were shown to coordinate maturation of T cell responses towards Th17 cell induction (*13, 14*). Glycosphingolipids produced by the common intestinal symbiont *Bacteroides fragilis* have been found to regulate homeostasis of host intestinal natural killer T cells (*15*). A polysaccharide A (PSA) also produced by *B. fragilis* induces and expands Il-10 producing CD4+ T cells (*16-18*). Finally, the microbial anti-inflammatory molecule (MAM) secreted by *Faecalibacterium prausnitzii* impairs the nuclear-factor (NF)-κB pathway (*19*).

[0003] Conversely, enteric pathogens have developed various strategies to outcompete other species in the intestine and access nutritional and spatial niches, leading to successful infection (*20, 21*). In this regard, the contribution of bacteriocins and type VI secretion systems effectors during pathogen colonisation of the gut is an emerging field of investigation.

[0004] The recent linkage of *Prevotella* with inflammatory diseases demonstrates the need in the art for compositions and methods useful to reduce the prevalence of *Prevotella* in subjects. This invention meets this and other needs.

[0005] The citation of references in this application shall not be construed as an admission that such references are relevant or that they constitute prior art to the present disclosure.

SUMMARY OF THE INVENTION

[0006] The examples presented herein demonstrate the inventor's discovery that Lmo2776 targets *Prevotella* and reduces its abundance in the microbiota of both the mouse and human. This effect is direct and specific as (i) *Prevotella* are killed by *Listeria* culture supernatant (containing Lmo2776) *in vitro* and (ii) despite the complexity of the microbiota and its well-controlled equilibrium, no other genus of the intestinal microbiota was found to decrease in the presence of Lmo2776. The examples further demonstrate that the Lmo2776 bacteriocin targets *B. subtilis*, a Gram-positive bacterium found in the soil, suggesting that Lmo2776 could give an advantage to *Listeria* in the environment. *B. subtilis* is also found in the human gastrointestinal tract (*60*) and could also be targeted by Lmo2776 in the intestine. The

antibacterial effect of the Lmo2776 bacteriocin against *Prevotella* was confirmed using a synthetic Lmo2776 peptide. Lmo2776 peptide inhibits *Prevotella copri* growth *in vitro and in vivo* in conventional mouse microbiota. In particular, Lmo2776 peptide is capable of inhibiting the growth of *P.copri* isolates from Rheumatoid arthritis patients. These results show the therapeutic potential of Lmo2776 peptide in the treatment of inflammatory diseases and enteropathogenic bacterial infections.

[0007] In a first aspect, this invention provides methods of treating or preventing inflammation in a subject, comprising administering an anti-*Prevotella* bacteriocin to the subject. In some embodiments the anti-*Prevotella* bacteriocin is *L. monocytogenes* Lmo2776. In some embodiments the anti-*Prevotella* bacteriocin comprises an amino acid sequence that is at least 80% identical to SEQ ID NO: 1. In some embodiments the anti-*Prevotella* bacteriocin comprises the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3. In some embodiments the subject has or is at risk of developing an inflammatory disease selected from rheumatoid arthritis, metabolic syndrome, inflammatory bowel disease, and HIV infection. In some embodiments the anti-*Prevotella* bacteriocin is administered in an amount sufficient to reduce the *Prevotella* load by at least 80%.

[0008] In another aspect, this invention provides methods of treating or preventing an enteropathogenic bacterial infection in a subject, comprising administering an anti-*Prevotella* bacteriocin to the subject. In some embodiments the anti-*Prevotella* bacteriocin is *L. monocytogenes* Lmo2776. In some embodiments the anti-*Prevotella* bacteriocin comprises an amino acid sequence that is at least 80% identical to SEQ ID NO: 1. In some embodiments the anti-*Prevotella* bacteriocin comprises the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3. In some embodiments the subject has or is at risk of developing an Listeria infection. In some embodiments the anti-*Prevotella* bacteriocin is administered in an amount sufficient to reduce the *Prevotella* load by at least 80%.

[0009] In another aspect, this invention provides methods of reducing the *Prevotella* load of a subject by at least 80%, comprising administering an anti-*Prevotella* bacteriocin to the subject. In some embodiments the anti-*Prevotella* bacteriocin is *L. monocytogenes* Lmo2776. In some embodiments the anti-*Prevotella* bacteriocin comprises an amino acid sequence that is at

least 80% identical to SEQ ID NO: 1. In some embodiments the anti-*Prevotella* bacteriocin comprises the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3. In some embodiments the anti-*Prevotella* bacteriocin is administered in an amount sufficient to reduce the *Prevotella* load by at least 80%. In some embodiments the anti-*Prevotella* bacteriocin is administered in an amount sufficient to reduce the *Prevotella* load by at least 90%. In some embodiments the anti-*Prevotella* bacteriocin is administered in an amount sufficient to reduce the *Prevotella* load by at least 99%. In some embodiments the *Prevotella* load is reduced in the gut microbiota of the subject.

[0010] In another aspect, this invention provides pharmaceutical compositions comprising an anti-*Prevotella* bacteriocin and a pharmaceutically acceptable carrier. In some embodiments the anti-*Prevotella* bacteriocin is *L. monocytogenes* Lmo2776. In some embodiments the anti-*Prevotella* bacteriocin comprises an amino acid sequence that is at least 80% identical to SEQ ID NO: 1. In some embodiments the the anti-*Prevotella* bacteriocin comprises the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3.

[0011] In another aspect, this invention provides pharmaceutical compositions comprising an anti-*Prevotella* bacteriocin and a pharmaceutically acceptable carrier for use in treating or preventing inflammation in a subject. In some embodiments the anti-*Prevotella* bacteriocin is *L. monocytogenes* Lmo2776. In some embodiments the anti-*Prevotella* bacteriocin comprises an amino acid sequence that is at least 80% identical to SEQ ID NO: 1. In some embodiments the anti-*Prevotella* bacteriocin comprises the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3. In some embodiments the subject has or is at risk of developing an inflammatory disease selected from rheumatoid arthritis, metabolic syndrome, inflammatory bowel disease, and HIV infection. In some embodiments the anti-*Prevotella* bacteriocin is administered in an amount sufficient to reduce the *Prevotella* load by at least 80%.

[0012] In another aspect, this invention provides pharmaceutical compositions comprising an anti-*Prevotella* bacteriocin and a pharmaceutically acceptable carrier for use in treating or preventing an enteropathogenic bacterial infection in a subject. In some embodiments the anti-*Prevotella* bacteriocin is *L. monocytogenes* Lmo2776. In some

embodiments the anti-*Prevotella* bacteriocin comprises an amino acid sequence that is at least 80% identical to SEQ ID NO: 1. In some embodiments the anti-*Prevotella* bacteriocin comprises the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3. In some embodiments the subject has or is at risk of developing an Listeria infection. In some embodiments the anti-*Prevotella* bacteriocin is administered in an amount sufficient to reduce the *Prevotella* load by at least 80%.

[0013] In another aspect, this invention provides pharmaceutical compositions comprising an anti-*Prevotella* bacteriocin and a pharmaceutically acceptable carrier for use in reducing the *Prevotella* load of a subject. In some embodiments the anti-*Prevotella* bacteriocin is *L. monocytogenes* Lmo2776. In some embodiments the anti-*Prevotella* bacteriocin comprises an amino acid sequence that is at least 80% identical to SEQ ID NO: 1. In some embodiments the anti-*Prevotella* bacteriocin comprises the amino acid sequence of SEQ ID NO: 1 SEQ ID NO: 2 or SEQ ID NO: 3. In some embodiments the anti-*Prevotella* bacteriocin reduces the *Prevotella* load by at least 80%. In some embodiments the anti-*Prevotella* bacteriocin reduces the *Prevotella* load by at least 90%. In some embodiments the anti-*Prevotella* bacteriocin reduces the *Prevotella* load by at least 99%.

[0014] In some preferred embodiments of the above methods, pharmaceutical composition and uses thereof, said anti-*Prevotella* bacteriocin comprises or consists of the amino acid sequence of SEQ ID NO: 3.

[0015] In some embodiments of the above methods, pharmaceutical composition and uses thereof, said *Prevotella* is *Prevotella copri*.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] **Figures 1A to 1F: Lmo2776 limits *Listeria* virulence in a microbiota-dependent manner.**

(**A-C**) BALB/c mice were inoculated orally with 5×10^9 *Listeria* WT, Δ*lmo2776* or Lmo2776 complemented (*p2776*) bacteria. CFUs in the intestinal luminal content (**A**), the spleen (**B**) and the liver (**C**) were assessed at 72h post-infection.

(**D**-**F**) Germ-free C57BL/6J were infected with 5×10^9 *Listeria* WT or Δ*lmo2776* for 72h and CFUs in the intestinal luminal content (**D**), the spleen (**E**) and the liver (**F**) were assessed. Each dot represents the value for one mouse. Statistically significant differences were evaluated by the Mann–Whitney test. (*p< 0.05).

[0017] **Figures 2A to 2D: Lmo2776 targets *Prevotella* in mouse microbiota.**

(**A**) Principal coordinates analysis of the weighted Unifrac distance matrix of mice infected with WT strain (black) or Δ*lmo2776* (grey) at day 0 (top), day 1 (middle) and day 2 (bottom). Permanova: at day 0 P=0.332,at day 1 P=0.063 and at day 2 P= P=0.366.

(**B**) Relative abundance of 12 OTUs in gut microbiota of mice infected with WT or Δ*lmo2776* strains at day 0,day 1 and day 2. Each dot represents the value for one mouse.

(**C**) Heat-map analysis of the relative abundance of 12 OTUs in gut microbiota of mice infected with WT or Δ*lmo2776* strains at day 0 , day 1 and day 2. Each column represents one mouse. The white and black shades indicate low and high abundance.

(**D**) Phylogenetic tree of 16S rRNA gene alignment of 5 representative bacteria for each phylum of the bacteria domain, together with OTUs showing significantly different relative abundances in gut microbiota of mice infected with WT or Δ*lmo2776* strains at day 1. The 12 OTUs with an increased abundance in Δ*lmo2776*-infected mice compare to *Listeria* WT-infected mice are shown in grey and *Prevotella* genus is indicated in dashed line.

[0018] **Figures 3A to 3J: Lmo2776 targets *P. copri* in human microbiota and *in vitro*.**

(**A**) Principal coordinates analysis of the weighted Unifrac distance matrix of SHIME® vessels infected with WT or Δ*lmo2776* strains or non-infected () at day 0 (left) and day 1 (right). Permanova: at day 0 P=0.947 and at day 1 P=0.403.

(**B**) Relative abundance of genera in SHIME® vessels non-infected or infected with WT or Δ*lmo2776* strains at day 0 (left) and day 1 (right). The four more abundant genera are indicated.

(**C**) Relative abundance of 8 different OTUs in SHIME® vessels infected with WT or Δ*lmo2776* strains or non-infected at 24h relative to their abundance at day 0.

(**D**) Levels of propionate in SHIME® vessels infected with WT (dashed line) or Δ*lmo2776* (grey) strains or non-infected (black) overtime. Results are expressed as mean ± SEM for 2 to 3 individual vessels.

(**E-F**) Numbers of *P. copri* (**E**), *B. subtilis* or *E. coli* (**F**) after incubation with supernatant of WT (*Lm*) or Δ*lmo2776* strains. Results are expressed as mean ± SEM of a least 3 independent experiments and P-values were obtained using two-tailed unpaired Student's t-test (*p<0.05, ***p<0.005).

(**G**) Relative abundance of *P. copri* (in white) or *Bacteroides thetaiotaomicron* (in black) after 24h incubation with increasing dose of Lmo2776 peptide (+ =16.5 µg, ++=33 µg and +++ =49.5 µg) relative to their abundance without Lmo2776 peptide. Results are expressed as mean ± SEM of a least 3 independent experiments and P-values were obtained using two-tailed unpaired Student's t-test (**p<0.01, ***p<0.005).

(**H**) Relative abundance of bacteriocin 972 family targets including *B. subtilis*; various intestinal bacteria and various *Prevotella* strains including *P. copri* after incubation with the lowest concentration of Lmo2776 peptide (16.5 µg) used in (**G**) (in gray) or no peptide (in black). Results are expressed as mean ± SEM of a least 3 independent experiments and P-values were obtained using two-tailed unpaired Student's t-test (*p<0.05).

(**I**) Relative abundance of *P. copri* isolates from different Rheumatoid arthritis (RA) patients after 24h incubation with Lmo2776 peptide (16.5 µg; in gray) or no peptide (in black). Results are expressed as mean ± SEM of a least 3 independent experiments and P-values were obtained using two-tailed unpaired Student's t-test (*p<0.05).

(**J**) Relative abundance of *P. copri* in the feces of mice injected intrarectally with Lmo2776 peptide (1 mg in 100 µL of H_20) or H_20 (100 µL) at 4 hours post-injection. Each dot represents the value for one mouse.

[0019] **Figures 4A to 4F: *P. copri* controls *Listeria* infection.**

(**A**) Assessment of listerial CFUs in the intestinal luminal content of germ-free (GF) C57BL/6J mice colonized or not with *P. copri*, *B. thetaiotamicron* or *P. salivae* for 2 weeks and then infected with *L. monocytogenes* WT or Δ*lmo2776* for 72h.

(**B**) Numbers of *P. copri* CFUs in the intestinal luminal content of GF C57BL/6J mice colonized with *P. copri* and then infected with *Listeria* WT or Δ*lmo2776* for 72h.

(**C**) Distances of closest bacteria to intestinal epithelial cells per condition over 5 high-powered fields per mouse, with each dot representing a measurement.

(**D**) Germ-free (GF) C57BL/6J mice colonized or not with 10^8 *P. copri, B. thetaiotamicron* or *P. salivae* for 2 weeks were then infected with 5×10^9 *L. monocytogenes* WT or Δ*lmo2776* for 72h. Distances of closest bacteria to intestinal epithelial per condition over 5 high-powered fields per mouse Each dot represents one measurement.

(**E**) Levels of the inflammatory marker Lcn-2 in faeces of mice 2 weeks post-inoculation with *P. copri* or *B. thetaiotamicron.*

(**F**) Model depicting the effect of *Prevotella* on *Listeria* infection.

In A, B and E, each dot represents one mouse. Statistically significant differences were evaluated by the Mann–Whitney test (A), one way-ANOVA test (C and D) or two-tailed unpaired Student's t-test (B and E) (*p< 0.05, ***p<0.005).

[0020] **Figures 5A to 5C: Presence of *lmo2776* in *L. monocytogenes* strains and homologies with members of the Lactococcin 972 family.**

(**A**) Schematic representation of Listeria monocytogenes (WT) genome showing a locus comprising *lmo 2776*. Absence of *lmo 2776* locus in the non-pathogenic *L. innocua* strain CLIP 111262. Deletion of *lmo 2776* in the Δlmo2776 mutant.

(**B**) Cluster analysis based on core genome multilocus sequence typing (cgMLST) profiles of 1,096 genomes (*61*) and pattern of *lmo2774-lmo2775-lmo2776* genes presence (dark) or absence (white). The ten most frequent sublineages (SL) are highlighted. The last column corresponds to the sample source, represented by colour codes (upper left key).

(**C**) Amino acid alignment of Lmo2776 with members of the Lactococcin 972 family: L82330 from *Lactococcus lactis* subsp. *lactis* Il1403, SP_0109 bacteriocin from *Streptococcus pneumoniae* TIGR4, SAP109 from *Staphylococcus aureus* subsp. *aureus* N315, lcn972 bacteriocin 972 from *Lactococcus lactis* subsp. *lactis* and Sil from *Streptococcus iniae* SF. The conserved residues and consensus motif are indicated in Lmo2776 and on the bottom, respectively.

[0021] **Figures 6A to 6F. Effect of *lmo2776* on Listeria infection.**

(**A**) Relative expression of *lmo2774*, *lmo2775* and *lmo2776* in bacteria grown in stationary phase (white bars) compared to bacteria grown in exponential phase (black bars). The transcripts levels were normalised to the levels of *rpoB*, which were constant under all conditions, and then expressed relative to those of exponential phase. Results are expressed as mean ± SEM of a least 3 independent experiments and P-values were obtained using two-tailed unpaired Student's t-test (*$p<0.05$).

(**B**) BALB/c mice were inoculated orally with 5×10^9 *Listeria* WT or Δ*lmo2776* strains. CFUs in the intestinal luminal were assessed at 24h, 48h and 72h post-infection.

(**C**) Relative expression of *lmo2774*, *lmo2775* and *lmo2777* in *Listeria* WT (in black) and Δ*lmo2776* (in gray) strains grown in stationary phase.

(**D**) Growth curve of *Listeria* WT (in black) and Δ*lmo2776* (in gray) strains.

(**E**) BALB/c mice were inoculated intraveinously with 5×10^9 *Listeria* WT or Δ*lmo2776* strains. CFUs in the the spleen were assessed at 72h post-infection.

(**F**) BALB/c mice were inoculated intraveinously with 5×10^9 *Listeria* WT or Δ*lmo2776* *strains*. CFUs in the liver were assessed at 72h post-infection.

[0022] **Figures 7A to 7E: Effect of *Listeria* infection on mouse microbiota.**

(**A**) Principal coordinates analysis of the weighted Unifrac distance matrix of conventional mice at day 0 (red) and infected with WT strain at day 1 (blue). Permanova P= 0.002.

(**B**) Relative abundance of classes in conventional mice at day 0 (left) and infected with WT strain at day 1 (right).

(**C**) LEfSE and (**D**) histogram of the LDA scores computed for features differentially abundant between microbiota of mice at day 0 (red) and infected with WT strain at day 1 (green).

(**E**) Firmicutes/Bacteroides ratio in microbiota of mice at day 0 and infected with WT strain at day 1. Each dot represents the value for one mouse.

[0023] **Figures 8A to 8D: OTUs targeted by Lmo2776 are closely related to *Prevotella* genus.**

(**A**) Section of phylogenetic tree presented in Figure 2D. OTUs of interest are indicated in red.

(**B**) LEfSE and (**C**) histogram of the LDA scores computed for features differentially abundant between microbiota of mice infected with WT (green) or Δ*lmo2776* (red) strains at day 1 post-infection.

(**D**) Quantification of *Prevotella* (pg/mg) in the feces of conventional mice at day 0 and orally inoculated with 5×10^9 *Listeria* WT or Δ*lmo2776* strains at day 1. **Figures 9A to 9D: Production of butyrate, isobutyrate, acetate and isovalerate is not modified by Lmo2776.** Levels of butyrate (**A**), isobutyrate (**B**), acetate (**C**) and isovalerate (**D**) in SHIME® vessels infected with WT (dashed line) or Δ*lmo2776* (grey) strains or non-infected (black) overtime. Results are expressed as mean ± SEM for 2 to 3 individual vessels.

[0024] **Figures 10A to 10B: *Lm* secretes a *bona fide* bacteriocin targeting *B. subtilis*.** Numbers of *B. subtilis* (**A**) and of different *Lm* strains (**B**) were quantified after 6h of co-culture. Results are expressed as mean ± SEM of a least 3 independent experiments and P-values were obtained using two-tailed unpaired Student's t-test (***p<0.005.

[0025] **Figures 11: Lmo2776 targets *Prevotella* in mouse microbiota.** Relative abundance of OTU 355746, OTU 216524, OTU 421792, OTU 258849, OTU 331772, OTU 346870, OTU 430197, OTU 447141, OTU 465433, OTU 208409, OTU 353012, OTU 364179 in gut microbiota of mice infected with WT or Δ*lmo2776* strains at day 0, day 1 and day 2. Each dot represents the value for one mouse.

[0026] **Figures 12A to 12C: *P. copri* controls *Listeria* infection.** Assessment of listerial CFUs in the intestinal luminal content (**A**), spleen (**B**) and liver (**C**) of germ-free (GF) C57BL/6J mice colonized or not with a mixture of 12 bacteria including Verrucomicrobia (*A. muciniphila* YL44), Bacteroidetes (*B. caecimuris* I48, *M. intestinale* YL27), Proteobacteria (*T. muris* YL45), Actinobacteria (*B. longum* YL2), Fimicutes (*E. faecalis* KB1; *A. muris* KB18; *C. clostridioforme* YL32) and Firmicutes (F. plautii YL31; B. coccoides YL58; *L. reuteri* I49 and *C. innnocuum* I46) for 2 weeks and then infected with *L. monocytogenes* WT or Δ*lmo2776* for 72h.

DETAILED DESCRIPTION OF THE INVENTION

A. Definitions

[0027] Certain terms are defined in this section. Definitions of other terms are provided at locations throughout this disclosure.

[0028] The term "treatment" refers to any process, action, application, therapy, or the like, wherein a subject, including a human being, is subjected to medical aid with the object of curing a disorder, or eradicating a pathogen, or improving the subject's condition, directly or indirectly. Treatment also refers to reducing incidence, or alleviating symptoms, eliminating recurrence, preventing recurrence, preventing incidence, improving symptoms, improving prognosis or combinations thereof.

[0029] The term "preventing" includes the reducing the incidence, recurrence, spread, onset or establishment of a disorder such as a bacterial infection. It is not intended that the present disclosure be limited to complete prevention or to prevention of establishment of an infection. In some embodiments, the onset is delayed, or the severity of a subsequently contracted disease is reduced, and such constitute examples of prevention.

[0030] The term "percent amino acid sequence identity" with respect to the anti-*Prevotella* bacteriocin polypeptide sequences is defined herein as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the specific anti-*Prevotella* bacteriocin polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for example, using publicly available software such as BLAST or Megalign (DNASTAR) software.

[0031] The term "subject" refers to a subject to be treated and includes inter alia mammals, e.g., humans, dogs, cows, horses, pigs, sheep, goats, cats, mice, rabbits, rats, and transgenic non-human animals. In certain embodiments, the subject is a human.

[0032] The phrase "inhibiting growth" in reference to *Prevotella* refers to any mechanism that reduces the accumulation of *Prevotella* cells in an area over time. For example, any mechanism that causes *Prevotella* cells in a culture to accumulate at a slower rate than *Prevotella* cells in a control culture, or any mechanism that that causes *Prevotella* cells in an in vivo context (e.g., gut microbiota, lung microbiota) to accumulate at a slower rate than *Prevotella* cells in a control. In this context "inhibiting" includes without limitation mechanisms reducing the rate of cell division and induction of cell death such as by cell lysis.

[0033] The term "*Prevotella* load" refers to a measurable quantity of bacteria in an object, organism, or organism compartment. In some embodiments the *Prevotella* load is determined at least in part by measuring the proportion of total bacteria present that are viable *Prevotella*.

B. Anti-*Prevotella* Bacteriocins

[0034] Bacteriocins are proteinaceous or peptidic toxins produced by bacteria to inhibit the growth of similar or closely related bacterial strain(s). They are similar to yeast and paramecium killing factors, and are structurally, functionally, and ecologically diverse.

[0035] As used herein an "anti-*Prevotella* bacteriocin" is a bacteriocin that inibits the growth of *P. copri* in an *in vitro* culture. An exemplary assay is demonstrated in Example 3. In that assay bacteriocin is obtained by culturing *L. monocytogenes* Lmo2776 and collecting the supernatant of the culture that comprises bacteriocin. *P. copri* is then grown at 37°C in anaerobic conditions in the presence of the supernatant. A skilled artisan will appreciate that the assay may be adapted to use bacteriocin from any suitable source at a defined concentration in the culture media.

[0036] In an exemplary assay a candidate bacteriocin is added to culture media at a concentration of from 10 nM to 80 μM and the effect of the candidate bacteriocin on growth of *P. copri* colonies is measured. In some embodiments a candidate bacteriocin that inhibits the growth of *P. copri* colonies when present in culture media at a concentration within this concentration range is identified as a anti-*Prevotella* bacteriocin. In some embodiments a candidate bacteriocin that inhibits the growth of *P. copri* colonies when present in culture media at a concentration of from 10 nM to 100 nM is identified as a anti-*Prevotella* bacteriocin. In some embodiments a candidate bacteriocin that inhibits the growth of *P. copri* colonies when present in culture media at a concentration of from 100 nM to 1μM is identified as a anti-*Prevotella* bacteriocin. In some embodiments a candidate bacteriocin that inhibits the growth of *P. copri* colonies when present in culture media at a concentration of from 1μM to 10 μM is identified as a anti-*Prevotella* bacteriocin. In some embodiments a candidate bacteriocin that inhibits the growth of *P. copri* colonies when present in culture media at a concentration of from 10 μM to 80 μM is identified as a anti-*Prevotella* bacteriocin.

[0037] In some embodiments of this invention the anti-*Prevotella* bacteriocin is an *L. monocytogenes* bacteriocin. In some embodiments the anti-*Prevotella* bacteriocin is *L.*

monocytogenes Lmo2776. In some embodiments the anti-*Prevotella* bacteriocin is *L. monocytogenes* EGD-e Lmo2776, which has the following sequence:

MKKKVCVLAIALLTVLSPMGALAAQNLNEGETSLDSLMSANKTGEKVIFS
ETTTGKKPLLKAAQSAGGGTFWVTWGQDRHYSNYQHTKKTHRSSASNYRA
TERSSWKAKNNLATAWIKSSLWGNKANWATK **(SEQ ID NO: 1)**

[0038] In some embodiments the anti-*Prevotella* bacteriocin is a fragment of *L. monocytogenes* EGD-e Lmo2776. For example, in some embodiments the anti-*Prevotella* bacteriocin is a fragment of *L. monocytogenes* EGD-e Lmo2776 from which the signal peptide has been cleaved. Bacteriocin of the lactococcin 972 family are cleaved following a GG motif (positions 67-68 of SEQ ID NO: 1; underlined) to the mature form. Thus, in some embodiments the fragment comprises or consists of the following sequence:

QNLNEGETSLDSLMSANKTGEKVIFSETTTGKKPLLKAAQSAGGGTFWVT
WGQDRHYSNYQHTKKTHRSSASNYRATERSSWKAKNNLATAWIKSSLWGN
KANWATK **(SEQ ID NO: 2)**

[0039] In some embodiments the fragment comprises or consists of the following sequence:

GTFWVTWGQDRHYSNYQHTKKTHRSSASNYRATERSSWKAKNNLATAWIK
SSLWGNKANWATK **(SEQ ID NO: 3)**

[0040] In some embodiments the anti-*Prevotella* bacteriocin comprises an amino acid sequence that is at least 70% identical to a sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3. In some embodiments the anti-*Prevotella* bacteriocin comprises an amino acid sequence that is at least 75% identical to a sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3. In some embodiments the anti-*Prevotella* bacteriocin comprises an amino acid sequence that is at least 80% identical to a sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3. In some embodiments the anti-*Prevotella* bacteriocin comprises an amino acid sequence that is at least 85% identical to a sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3. In some embodiments the anti-*Prevotella* bacteriocin comprises an amino acid sequence that is at least 90% identical to a

sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3. In some embodiments the anti-*Prevotella* bacteriocin comprises an amino acid sequence that is at least 91% identical to a sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3. In some embodiments the anti-*Prevotella* bacteriocin comprises an amino acid sequence that is at least 92% identical to a sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3. In some embodiments the anti-*Prevotella* bacteriocin comprises an amino acid sequence that is at least 93% identical to a sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3. In some embodiments the anti-*Prevotella* bacteriocin comprises an amino acid sequence that is at least 94% identical to a sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3. In some embodiments the anti-*Prevotella* bacteriocin comprises an amino acid sequence that is at least 95% identical to a sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3. In some embodiments the anti-*Prevotella* bacteriocin comprises an amino acid sequence that is at least 96% identical to a sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3. In some embodiments the anti-*Prevotella* bacteriocin comprises an amino acid sequence that is at least 97% identical to a sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3. In some embodiments the anti-*Prevotella* bacteriocin comprises an amino acid sequence that is at least 98% identical to a sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3. In some embodiments the anti-*Prevotella* bacteriocin comprises an amino acid sequence that is at least 98% identical to a sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3. In some embodiments the anti-*Prevotella* bacteriocin comprises the amino acid sequence of SEQ ID NO: 1. In some embodiments the anti-*Prevotella* bacteriocin consists of the amino acid sequence of SEQ ID NO: 1. In some embodiments the anti-*Prevotella* bacteriocin comprises the amino acid sequence of SEQ ID NO: 2. In some embodiments the anti-*Prevotella* bacteriocin consists of the amino acid sequence of SEQ ID NO: 2. In some embodiments the anti-*Prevotella* bacteriocin comprises the amino acid sequence of SEQ ID NO: 3. In some embodiments the anti-*Prevotella* bacteriocin consists of the amino acid sequence of SEQ ID NO: 3.

[0041] In some preferred embodiments, the anti-*Prevotella* bacteriocin comprises an amino acid sequence that is at least 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,

97% or 98% identical to SEQ ID NO: 3. In some more preferred embodiments, the anti-*Prevotella* bacteriocin comprises or consists of the amino acid sequence of SEQ ID NO: 3.

[0042] The invention encompasses variants of *L. monocytogenes* Lmo2776 including natural and synthetic variants thereof. Natural variants are found in the various serotypes, strains and isolates of *L. monocytogenes*. Synthetic variants can be made from any one of SEQ ID NO: 1, 2, and 3 by routine techniques in the art and screened for activity (i.e. *anti-Prevotella* bacteriocin activity) using the assays described herein such as the exemplary assay disclosed in Example 3 or other similar assays.

[0043] Bacteriocins may be produced using any suitable reagents and methods known in the art. In some embodiments, a bacteriocin-producing strain of bacteria is cultured and the bacteriocin is recovered from the culture. In come embodiments the bacteriocin is recovered by collecting culture media from the culture and then optionally purifying the bacteriocin from the culture media. In some embodiments the bacteriocin-producing strain is a *Listeria* strain. In some embodiments the bacteriocin-producing strain is an *E coli* strain.

[0044] In an exemplary embodiment for producing bacteriocins, *lmo2776* was cloned in pGEX4T1 plasmid and the recombinant plasmid transformed into *E. coli* BL21(DE3) Gold competent cells (Novagen). The bacteria were grown at 37°C up to an OD of 0.7-0.8 and expression of GST-Lmo2776 was induced with 0.6 mM IPTG at 16°C for 16h prior to collection of cell pellets by centrifugation. Cells were resuspended in 20 mM Tris pH 8.5, 150 mM NaCl, 1 mM DTT and lysed by sonication or by passage through a French press. Soluble fractions were recovered from cell debris by ultracentrifugation at 40,000g and subsequently incubated with glutathione-Sepharose beads for 2h at 4°C. Beads were washed with PBS and 40 mM Tris, 100mM NaCl, pH 8.0. Lmo2776 was then cleaved with thrombin, dialysed in 40 mM Tris, pH7.4 and concentrated using Amicon-10 filter devices (Millipore). Protein was flash frozen in liquid nitrogen and stored at -80°C. Skilled artisans will be aware that variations on this method may also be used.

[0045] In another exemplary embodiment for producing bacteriocins, Lmo2776 peptide as defined above is synthesized using standard peptide synthesis methods that are well-known in the art such as for example solid-phase synthesis methods.

C. Pharmaceutical Compositions

[0046] Also provided are pharmaceutical compositions comprising an anti-*Prevotella* bacteriocin and a pharmaceutically acceptable carrier. In some embodiments, the pharmaceutical composition is a solution, a suspension, an emulsion, an inhalable powder, an aerosol, or a spray. In some embodiments, the pharmaceutical composition further comprises one or more antibiotics suitable for the treatment of bacterial infection. In some embodiments, the pharmaceutical composition further comprises one or more antibiotics suitable for the treatment of Gram-negative and/or Gram-positive bacterial infection. In some embodiments, the pharmaceutical composition further comprises one or more antibiotics suitable for the treatment of Gram-negative bacterial infection. In some embodiments, the pharmaceutical composition further comprises one or more anti-inflammatory agents for the treatment of inflammation.

[0047] The term "pharmaceutically acceptable carrier" includes any and all solvents, additives, excipients, dispersion media, solubilizing agents, coatings, preservatives, isotonic and absorption delaying agents, surfactants, propellants and the like that are physiologically compatible. The carrier(s) must be "acceptable" in the sense of not being deleterious to the subject to be treated in amounts typically used in medicaments. Pharmaceutically acceptable carriers are compatible with the other ingredients of the composition without rendering the composition unsuitable for its intended purpose. Furthermore, pharmaceutically acceptable carriers are suitable for use with subjects as provided herein without undue adverse side effects (such as toxicity, irritation, and allergic response). Side effects are "undue" when their risk outweighs the benefit provided by the composition. Non-limiting examples of pharmaceutically acceptable carriers or excipients include any of the standard pharmaceutical carriers such as phosphate buffered saline solutions, water, and emulsions such as oil/water emulsions and microemulsions.

[0048] For solid compositions comprising a lyophilized bacteriocin polypeptide, excipients such as urea or mesna can be included to improve stability. Other excipients include bulking agents, buffering agents, tonicity modifiers, surfactants, preservatives and co-solvents.

[0049] For solid oral compositions comprising a bacteriocin, suitable pharmaceutically acceptable excipients include, but are not limited to, starches, sugars, diluents, granulating agents, lubricants, binders, disintegrating agents and the like.

[0050] For liquid oral compositions, suitable pharmaceutically acceptable excipients include, but are not limited to, water, glycols, oils, alcohols, flavoring agents, preservatives, and the like.

[0051] For topical solid compositions such as creams, gels, foams, ointments, or sprays, suitable excipients include, but are not limited to a cream, a cellulosic or oily base, emulsifying agents, stiffening agents, rheology modifiers or thickeners, surfactants, emollients, preservatives, humectants, alkalizing or buffering agents, and solvents.

[0052] Suitable excipients for the formulation of the foam base include, but are not limited to, propylene glycol, emulsifying wax, cetyl alcohol, and glyceryl stearate. Potential preservatives include methylparaben and propylparaben.

[0053] The compositions of the present disclosure can take the form of solutions, suspensions, emulsion, tablets, pills, pellets, capsules, capsules containing liquids, powders, sustained-release formulations, suppositories, tampon applications emulsions, aerosols, sprays, suspensions, lozenges, troches, candies, injectants, chewing gums, ointments, smears, a time-release patches, a liquid absorbed wipes, and combinations thereof.

[0054] Administration of the compositions of the present disclosure or pharmaceutically acceptable forms thereof may be topical, i.e., the pharmaceutical composition is applied directly where its action is desired, or systemic. In turn, systemic administration can be enteral or oral, i.e., substance is given via the digestive tract, parenteral, i.e., substance is given by other routes than the digestive tract such as by injection or inhalation. Thus, the bacteriocin polypeptides of the present disclosure can be administered to a subject orally, parenterally, by inhalation,

topically, rectally, nasally, buccally or via an implanted reservoir or by any other known method. The bacteriocin polypeptides of the present disclosure can also be administered by means of sustained release dosage forms.

[0055] For oral administration, the bacteriocin polypeptides of the present disclosure can be formulated into solid or liquid preparations, for example tablets, capsules, powders, solutions, suspensions and dispersions. The compound can be formulated with excipients such as, e.g., lactose, sucrose, corn starch, gelatin, potato starch, alginic acid and/or magnesium stearate.

[0056] For preparing solid compositions such as tablets and pills, a bacteriocin polypeptide of the present disclosure is mixed with a pharmaceutical excipient to form a solid preformulation composition. If desired, tablets may be sugar coated or enteric coated by standard techniques. The tablets or pills may be coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action. For example, the tablet or pill can include an inner dosage and an outer dosage component, the latter being in the form of an envelope over the former. The two components can be separated by enteric layer, which serves to resist disintegration in the stomach and permit the inner component to pass intact into the duodenum or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, such materials including a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol, and cellulose acetate.

[0057] The bacteriocin of the present disclosure may also be administered by injection of a therapeutic agent comprising the appropriate amount of bacteriocin polypeptide and a carrier. For example, the bacteriocin polypeptides can be administered intramuscularly, intrathecally, subdermally, subcutaneously, or intravenously. The carrier may be comprised of distilled water, a saline solution, albumin, a serum, or any combinations thereof. Additionally, pharmaceutical compositions of parenteral injections can comprise pharmaceutically acceptable aqueous or nonaqueous solutions of bacteriocin polypeptides in addition to one or more of the following: pH buffered solutions, adjuvants (e.g., preservatives, wetting agents, emulsifying agents, and dispersing agents), liposomal formulations, nanoparticles, dispersions, suspensions

or emulsions, as well as sterile powders for reconstitution into sterile injectable solutions or dispersions just prior to use.

[0058] In cases where parenteral injection is the chosen mode of administration, an isotonic formulation is preferably used. Generally, additives for isotonicity can include sodium chloride, dextrose, mannitol, sorbitol, and lactose. In some cases, isotonic solutions such as phosphate buffered saline are preferred. Stabilizers can include gelatin and albumin. A vasoconstriction agent can be added to the formulation. The pharmaceutical preparations according to this type of application are provided sterile and pyrogen free.

[0059] The diluent may further comprise one or more other excipient such as, e.g., ethanol, propylene glycol, an oil or a pharmaceutically acceptable emulsifier or surfactant.

[0060] In another embodiment, the compositions of the present disclosure are inhalable compositions. The inhalable compositions of the present disclosure can further comprise a pharmaceutically acceptable carrier. In one embodiment, bacteriocin polypeptide(s) of the present disclosure are advantageously formulated as a dry, inhalable powder. In specific embodiments, bacteriocin polypeptides inhalation solution may further be formulated with a propellant for aerosol delivery. In certain embodiments, solutions may be nebulized.

[0061] A surfactant can be added to an inhalable pharmaceutical composition of the present disclosure in order to lower the surface and interfacial tension between the medicaments and the propellant. Where the medicaments, propellant and excipient are to form a suspension, a surfactant may or may not be required. Where the medicaments, propellant and excipient are to form a solution, a surfactant may or may not be necessary, depending in part, on the solubility of the particular medicament and excipient. The surfactant may be any suitable, non-toxic compound which is non-reactive with the medicament and which substantially reduces the surface tension between the medicament, the excipient and the propellant and/or acts as a valve lubricant.

[0062] Examples of suitable surfactants include, but are not limited to: oleic acid; sorbitan trioleate; cetyl pyridinium chloride; soya lecithin; polyoxyethylene(20) sorbitan monolaurate; polyoxy ethylene (10) stearyl ether; poly oxy ethylene (2) oleyl ether; poly

oxypropylene-polyoxy ethylene ethylene diamine block copolymers; polyoxyethylene(20) sorbitan monostearate; polyoxyethylene(20) sorbitan monooleate; polyoxypropylene-polyoxyethylene block copolymers; castor oil ethoxylate; and combinations thereof.

[0063] Examples of suitable propellants include, but are not limited to: dichlorodifluoromethane, trichlorofluoromethane, dichloro-tetrafluoroethane and carbon dioxide.

[0064] Examples of suitable excipients for use in inhalable compositions include, but are not limited to: lactose, starch, propylene glycol diesters of medium chain fatty acids; triglyceride esters of medium chain fatty acids, short chains, or long chains, or any combination thereof; perfluorodimethylcyclobutane; perfluorocyclobutane; polyethylene glycol; menthol; lauroglycol; diethylene glycol monoethylether; polyglycolized glycerides of medium chain fatty acids; alcohols; eucalyptus oil; short chain fatty acids; and combinations thereof.

[0065] In some embodiments, the compositions of the present disclosure comprise nasal applications. Nasal applications include for instance nasal sprays, nasal drops, nasal ointments, nasal washes, nasal injections, nasal packings, bronchial sprays and inhalers, or indirectly through use of throat lozenges, mouthwashes or gargles, or through the use of ointments applied to the nasal nares, or the face or any combination of these and similar methods of application.

[0066] In another embodiment, the pharmaceutical compositions of the present disclosure contain a complementary agent, including one or more antimicrobial agents, one or more conventional antibiotics, and/or one or more anti-inflammatory agents.

[0067] .In order to accelerate the treatment of the infection, or augment the antibacterial effect, the therapeutic agent containing one or more bacteriocin polypeptides of the present disclosure may further include at least one complementary agent which can also potentiate the bactericidal activity of the bacteriocin polypeptide. The complementary agent may be one or more antibiotics.

[0068] In some preferred embodiments, the pharmaceutical compositions of the present disclosure comprise an anti-*Prevotella* bacteriocin comprising an amino acid sequence that is at

least 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97% or 98% identical to SEQ ID NO: 3; preferably an anti-*Prevotella* bacteriocin comprising or consisting of the amino acid sequence of SEQ ID NO: 3.

D. Methods of Inhibiting Growth of *Prevotella* and Reducing *Prevotella* Load

[0069] Examples 1 and 2 demonstrate that Lmo2776 reduces the amount of *Prevotella* in the human gut microbiota. Example 3 demonstrates that Lmo2776 is a *bona fide* bacteriocin that targets directly *P. copri* in an *in vitro* assay. Accordingly, this disclosure provides methods of inhibiting growth of *Prevotella* and/or methods of reducing *Prevotella* load.

[0070] Accordingly this invention also provides methods of inhibiting growth of *Prevotella*, comprising contacting *Prevotella* with an anti-*Prevotella* bacteriocin. In some embodiments the anti-*Prevotella* bacteriocin is *L. monocytogenes* Lmo2776. In some embodiments the anti-*Prevotella* bacteriocin comprises an amino acid sequence that is at least 80% identical to a sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3. In some embodiments the anti-*Prevotella* bacteriocin comprises an amino acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3. In some preferred the anti-*Prevotella* bacteriocin comprises an amino acid sequence that is at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97% or 98% identical to SEQ ID NO: 3; preferably the anti-*Prevotella* bacteriocin comprises or consists of the amino acid sequence of SEQ ID NO: 3. In some embodiments the anti-*Prevotella* bacteriocin is in an amount sufficient to reduce the growth of *Prevotella* by at least 80%. In some embodiments the anti-*Prevotella* bacteriocin is in an amount sufficient to reduce the growth of *Prevotella* by at least 90%. In some embodiments the anti-*Prevotella* bacteriocin is in an amount sufficient to reduce the growth of *Prevotella* by at least 95%. In some embodiments the anti-*Prevotella* bacteriocin is in an amount sufficient to reduce the growth of *Prevotella* by at least 99%.

[0071] This invention also provides methods of inhibiting growth of *Prevotella* in a subject, comprising administering an anti-*Prevotella* bacteriocin to the subject. In some embodiments the anti-*Prevotella* bacteriocin is *L. monocytogenes* Lmo2776. In some embodiments the anti-*Prevotella* bacteriocin comprises an amino acid sequence that is at least

80% identical to a sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3. In some embodiments the anti-*Prevotella* bacteriocin comprises an amino acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3. In some preferred the anti-*Prevotella* bacteriocin comprises an amino acid sequence that is at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97% or 98% identical to SEQ ID NO: 3; preferably the anti-*Prevotella* bacteriocin comprises or consists of the amino acid sequence of SEQ ID NO: 3. In some embodiments the anti-*Prevotella* bacteriocin is in an amount sufficient to reduce the growth of *Prevotella* by at least 80%. In some embodiments the anti-*Prevotella* bacteriocin is in an amount sufficient to reduce the growth of *Prevotella* by at least 90%. In some embodiments the anti-*Prevotella* bacteriocin is in an amount sufficient to reduce the growth of *Prevotella* by at least 95%. In some embodiments the anti-*Prevotella* bacteriocin is in an amount sufficient to reduce the growth of *Prevotella* by at least 99%.

[0072] This invention also provides methods of reducing the *Prevotella* load in a subject, comprising administering an anti-*Prevotella* bacteriocin to the subject. In some embodiments the anti-*Prevotella* bacteriocin is *L. monocytogenes* Lmo2776. In some embodiments the anti-*Prevotella* bacteriocin comprises an amino acid sequence that is at least 80% identical to a sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3. In some embodiments the anti-*Prevotella* bacteriocin comprises an amino acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3. In some preferred the anti-*Prevotella* bacteriocin comprises an amino acid sequence that is at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97% or 98% identical to SEQ ID NO: 3; preferably the anti-*Prevotella* bacteriocin comprises or consists of the amino acid sequence of SEQ ID NO: 3. In some embodiments the anti-*Prevotella* bacteriocin is in an amount sufficient to reduce the *Prevotella* load by at least 80%. In some embodiments the anti-*Prevotella* bacteriocin is in an amount sufficient to reduce the *Prevotella* load by at least 90%. In some embodiments the anti-*Prevotella* bacteriocin is in an amount sufficient to reduce the *Prevotella* load by at least 95%. In some embodiments the anti-*Prevotella* bacteriocin is in an amount sufficient to reduce the *Prevotella* load by at least 99%. In some embodiments the *Prevotella* load in the gut microbiota of a subject is reduced. In some embodiments the *Prevotella* load in the lung microbiota of a subject is reduced. In some embodiments the *Prevotella* load in an organ or another

compartment of a subject is reduced. In some embodiments the *Prevotella* load in at least one first compartment of a subject is reduced while the *Prevotella* load in at least one first compartment of a subject is not reduced.

[0073] In some embodiments of the above methods, said *Prevotella* is *Prevotella copri*.

E. Methods of Treating or Preventing Inflammation

[0074] *Prevotella* bacteria are associated with inflammation. As shown in Example 5, the presence of *Prevotella* in the intestine is associated with a thinner mucus layer and increased levels of faecal Lipocalin-2 (LCN-2). These results indicate that *Prevotella* is a bacterium promoting a pro-inflammatory phenotype. Thus, the identification in this disclosure of anti-*Prevotella* bacteriocin provides a new and useful reagent for treating and/or preventing inflammation and diseases having an inflammation component. Thus, this invention provides methods of treating and/or preventing inflammation in a subject.

[0075] Accordingly, also provided are methods of treating or preventing inflammation in a subject, comprising administering an anti-*Prevotella* bacteriocin to the subject. In some embodiments the anti-*Prevotella* bacteriocin is *L. monocytogenes* Lmo2776. In some embodiments the anti-*Prevotella* bacteriocin comprises an amino acid sequence that is at least 80% identical to a sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3. In some embodiments the anti-*Prevotella* bacteriocin comprises an amino acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3. In some preferred the anti-*Prevotella* bacteriocin comprises an amino acid sequence that is at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97% or 98% identical to SEQ ID NO: 3; preferably the anti-*Prevotella* bacteriocin comprises or consists of the amino acid sequence of SEQ ID NO: 3. In some embodiments the subject has or is at risk of developing an inflammatory disease selected from rheumatoid arthritis, metabolic syndrome, inflammatory bowel disease, HIV infection and lung disorders, in particular cytic fibrosis. In some embodiments administration of the anti-*Prevotella* bacteriocin to the subject reduces inflammation in the subject. In some embodiments the reduction in inflammation results in treatment and/or prevention of an inflammatory disease selected from rheumatoid arthritis, metabolic syndrome, inflammatory bowel disease, HIV

infection and lung disorders, in particular cystic fibrosis in the subject. In some embodiments the anti-*Prevotella* bacteriocin is administered in an amount sufficient to reduce the *Prevotella* load by at least 80% in the subject.

[0076] Accordingly, also provided are methods of treating or preventing an inflammatory disease selected from rheumatoid arthritis, metabolic syndrome, inflammatory bowel disease, HIV infection and lung disorders, in particular cystic fibrosis in a subject, comprising administering an anti-*Prevotella* bacteriocin to the subject. In some embodiments the anti-*Prevotella* bacteriocin is *L. monocytogenes* Lmo2776. In some embodiments the anti-*Prevotella* bacteriocin comprises an amino acid sequence that is at least 80% identical to a sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3. In some embodiments the anti-*Prevotella* bacteriocin comprises an amino acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3. In some preferred the anti-*Prevotella* bacteriocin comprises an amino acid sequence that is at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97% or 98% identical to SEQ ID NO: 3; preferably the anti-*Prevotella* bacteriocin comprises or consists of the amino acid sequence of SEQ ID NO: 3. In some embodiments administration of the anti-*Prevotella* bacteriocin to the subject reduces inflammation in the subject. In some embodiments the reduction in inflammation results in treatment and/or prevention of an inflammatory disease selected from rheumatoid arthritis, metabolic syndrome, inflammatory bowel disease, HIV infection and lung disorders, in particular cystic fibrosis in the subject. In some embodiments the anti-*Prevotella* bacteriocin is administered in an amount sufficient to reduce the *Prevotella* load by at least 80% in the subject.

[0077] In some embodiments of the above methods, said *Prevotella* is *Prevotella copri*.

F. Methods of Treating or Preventing Enteropathogenic Bacterial Infection

[0078] Example 4 demonstrates that the ability of the Δ*lmo2776* mutant to better grow in the intestine compared to the WT is dependent on the presence of *Prevotella* in the microbiota of the intestine, as it is observed either in conventional mice or mice colonized with *P. copri*. Example 5 demonstrates that the presence of *Prevotella* in the intestine is associated with a thinner mucus layer and increased levels of faecal Lipocalin-2 (LCN-2). These results

indicate that *Prevotella* is a bacterium promoting a pro-inflammatory phenotype. These results also strongly indicate that *P. copri*, by modifying the mucus layer thickness and changing the gut inflammatory condition, promotes infection. This suggests that people with high abundance of intestinal *Prevotella* are at risk for enteropathogens infection. Thus, this invention provides methods of treating and/or preventing an enteropathogenic bacterial infection in a subject.

[0079] Accordingly, this invention also provides methods of treating or preventing an enteropathogenic bacterial infection in a subject, comprising administering an anti-*Prevotella* bacteriocin to the subject. In some embodiments the anti-*Prevotella* bacteriocin is *L. monocytogenes* Lmo2776. In some embodiments the anti-*Prevotella* bacteriocin comprises an amino acid sequence that is at least 80% identical to a sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3. In some embodiments the anti-*Prevotella* bacteriocin comprises an amino acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3. In some preferred the anti-*Prevotella* bacteriocin comprises an amino acid sequence that is at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97% or 98% identical to SEQ ID NO: 3; preferably the anti-*Prevotella* bacteriocin comprises or consists of the amino acid sequence of SEQ ID NO: 3. In some embodiments the subject has or is at risk of developing an Listeria infection. In some embodiments the anti-*Prevotella* bacteriocin is administered in an amount sufficient to reduce the *Prevotella* load of the subject by at least 80%.

[0080] In some embodiments of the above methods, said *Prevotella* is *Prevotella copri*.

EXAMPLES

Materials and Methods

[0081] The following materials and methods were used in performing the examples that follow.

[0082] **Bacterial strains and plasmids:** For standard experiments, *Lm* EGD-e, *E. coli* and *B. subtilis* were grown at 37°C with shaking in Brain Heart Infusion (BHI) medium (Difco) and Luria-Bertani (LB) medium (BD). If needed, *Lm* were selected on Oxford medium (Oxoid). *P. copri* (DSM18205) and *B. thetaiotamicron* (CIP 104206T, CRB Institut Pasteur) were grown

in M20 medium at 37°C under anaerobic conditions (Genbag Anaer, Biomérieux or AnaeroGen, ThermoScientific). The Lmo2776 deletion mutant was constructed using the pMAD shuttle plasmid (*22*) as described previously (*23*) and confirmed by DNA sequencing. For the construction of pAD-based plasmid, fragment obtained by PCR with EGD-e genomic DNA as template, was cloned into the SmaI/SalI sites of the pAD vector (*24*) derived previously from the pPL2 vector (*25*). Plasmid was verified by sequencing with primers pPL2-Rv and pPL2-Fw and were transformed into Δ*lmo2776* by electroporation. Integration in the chromosome was verified by PCR using primers NC16 and PL95 (*24*).

[0083] **Mice:** 9- to 12-week-old female BALB/C conventional (Charles River), C57BL6/J conventional (Charles River), C57BL6/J germfree (CDTA or Pasteur) or Oligo-Mouse-Microbiota (Oligo MM12) C57BL6/J (Brugiroux *et al.,* Nature Microbiology, 2016, 16131) wild-type mice were used for experiments. Germfree mice were housed in plastic gnotobiotic isolators.

[0084] All animal experiments were carried out in strict accordance with the French national and European laws and conformed to the Council Directive on the approximation of laws, regulations, and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes (86/609/Eec). Experiments that relied on laboratory animals were performed in strict accordance with the Institut Pasteur's regulations for animal care and use protocol, which was approved by the Animal Experiment Committee of the Institut Pasteur (approval no. 03-49)

[0085] **Mice infection:** Lm overnight cultures were diluted in BHI and bacteria were grown to an optical density at 600 nm (OD600) of 1. Bacterial cultures were centrifuged at 3,500 × g for 15 min and washed three times in phosphate-buffered saline (PBS). Mice were infected orally with 5×10^9 bacteria diluted in 200 µl of PBS supplemented with 300 µl of CaCO3 (50 mg/ml). Serial dilutions of the inoculum were plated to control the number of bacteria inoculated.

[0086] Mice were killed at subsequent time points, and intestines, mesenteric lymph nodes, spleens, and livers were removed. The small intestine was opened, and the luminal

content was recovered in a 1.5-mL tube and weighed. The small intestine (duodenum, jejunum, and ileum) tissue was washed four times in DMEM (ThermoFisher), incubated for 2 h in DMEM supplemented with 40 µg/mL gentamycin, and finally washed three four in DMEM. All of the organs and the intestinal luminal content were homogenized, serially diluted, and plated onto BHI plates or Oxford plates and grown overnight at 37 °C for 48–72 h. CFU were enumerated to assess bacterial load. At least three independent experiments were carried out with four or five mice per group in each experiment. Statistically significant differences were evaluated by the Mann–Whitney test, and differences were considered statistically significant when P values were <0.05.

[0087] In case of intra-veinous infection, mice were infected with 5 x 10^3 bacteria diluted in 100ul of PBS. Mice were killed at subsequent time points, and spleens, and livers were removed and processed as described above.

[0088] For mice colonisation, *P. copri, P. salivae* and *B. thetaiotamicron* were grown to log phase under anaerobic conditions in M20 liquid media and 107 CFU were used to inoculate mice. Feces were collected at 2 weeks post-gavage to confirm colonization. Feces were homogenized, serially diluted and plated on M20 agar plates

[0089] **Fecal microbiota analysis by 16S rRNA gene sequencing using Illumina technology:** Before infection, 8 BALB/c conventional mice were co-housed for 5 weeks in order to stabilize and homogenize their microbiota. After oral infection, animals were single-housed. Feces were collected and frozen at -20°C. 16S rRNA gene amplification and sequencing were done using the Illumina MiSeq technology following the protocol of Earth Microbiome Project with their modifications to the MOBIO PowerSoil DNA Isolation Kit procedure for extracting DNA (www.earthmicrobiome.org/emp-standard-protocols) (*27, 28*). Bulk DNA were extracted from frozen extruded feces using a PowerSoil DNA Isolation kit (MoBio Laboratories) with mechanical disruption (bead-beating). The 16S rRNA genes, region V4, were PCR amplified from each sample as described in Chassaing *et al.*, 2015 (*29*). Four independent PCRs were performed for each sample, combined, purified with Ampure magnetic purification beads (Agencourt), and products were visualized by gel electrophoresis. Products were then quantified (BIOTEK Fluorescence Spectrophotometer) using Quant-iT PicoGreen

dsDNA assay. A master DNA pool was generated from the purified products in equimolar ratios. The pooled products were quantified using Quant-iT PicoGreen dsDNA assay and then sequenced using an Illumina MiSeq sequencer (paired-end reads, 2 × 250 bp) at Cornell University (Ithaca, USA).

[0090] **16S rRNA gene sequence analysis :** Analysis of the 16S rRNA gene sequence was performed exactly as previously described (*29*).

[0091] **Quatification of bacteria in feces :** after DNA extraction, quantitative PCR were performed using SYBR Green master mix and primers specific to *Prevotella*. Reaction cycling and quantification was carried out in an C1000 touch Thermal cycler (CFX384, Biorad) using known amounts of *Prevotella* DNA as standard. Expression levels were normalized to the 16S gene. Samples were evaluated in triplicate and results represent at least three independent experiments.

[0092] **M-SHIME:** M-SHIME system is a dynamic in vitro model which simulates the lumen-associated and mucus-associated human intestinal microbial ecosystem (ProDigest, Ghent University, Belgium) (30-32). It consists of consecutive pH-controlled, stirred, airtight, double-jacketed glass vessels kept at 37°C and under anaerobic conditions. The system was operated as described earlier (33) with minor modifications The set-up used here consisted of 3 stomach-small intestine vessels and nine proximal colon vessels in parallel. The colon vessels were inoculated at the start with 40 mL fresh human faecal suspension in 500 mL sterile nutritional medium. Every 2 days, half of the mucin agar-covered microcosms were replaced by fresh sterile ones under a flow of N2 to prevent disruption of anaerobic conditions. Fourteen days after inoculation, 3 colon vessels were infected with 106 WT bacteria , 3 colon vessels with 106 Δlmo2776 and 3 were left uninfected. Lumen (10 ml) and mucin agar samples were taken at 6, 24, 48 and 72h. Mucin agar-covered microcosms were washed with sterile PBS to remove lumen bacteria. Mucin agar was removed from microcosms, homogenised and stored immediately at −20°C until further analysis.

[0093] For 16S rRNA Gene Sequencing, DNA was extracted from 1 ml of lumen samples or 250 mg of mucin agar samples using a PowerSoil DNA Isolation kit and 16S rRNA

gene sequencing was analysed as decribed above. Our full 16S rRNA gene sequence data are deposited under Study ID YYYY in the QIIME-DB database (http://www.microbio.me/qiime/).

[0094] For SCFA analysis, lumen samples were diluted 1:2 in demineralized water. Acetate, propionate, butyrate, isobutyrate and isovalerate were extracted and quantified as described (34).

[0095] **RNA extraction and qRT:** A total of 25 ml cultures of bacteria (Lm in stationary phase or Lm in exponential phase) was grown in medium (BHI, LB or M20) overnight. Cultures were subsequently pelleted for 20 min at 3000g. Pellets were resuspended in 1 ml TRI Reagent (Sigma), transferred to 2-ml Lysing Matrix tubes (MP Biomedicals) and mechanically lysed by bead beating in a FastPrep apparatus (twice 45s, speed 6.5). Tubes were then centrifuged for 5 min at 8000g at 4°C to separate beads from lysates. The lysates were drawn off and transferred to a 2-ml Eppendorf tube. 200 uL chloroform was added to the lysate, shaken and incubated 10 min at room temperature, followed by centrifugation for 15 min at 13 000g at 4°C. The aqueous phase was transferred to a new tube and RNA was precipitated by the addition of 500 uL isopropanol and incubation at room temperature for 10 min. RNA was pelleted by centrifuging for 10 min at 13000 g at 4°C, washed twice with 75% ethanol. RNA pellets were resuspended in 50 to 100 uL water. For each sample, 10 ug of RNA was treated with Dnase (Turbo DNA-free, Ambion) following manufacturer's instructions. cDNA was synthetiszed from 1 ug of RNA using QuantiTect Reverse Transcription (Qiagen) and reactions were subsenquently diluted with 180 ul of water. qRT-PCR reactions were prepared with SYBR Green master mix. Reaction cycling and quantification was carried out in an C1000 touch Thermal cycler (CFX384, Biorad). Expression levels were normalized to the rpoB gene. Samples were evaluated in triplicate and results represent at least three independent experiments.

[0096] **Co-culture assays:** For co-culture assays, 10^7 bacteria from overnight cultures were inoculated into 5 mL of fresh BHI either in single culture or in coculture with another strain as indicated in the figures and incubated at 37°C for 6 hours. Serial dilutions were plated on selective media for CFU enumeration.

[0097] For culture of target in presence of *Listeria* supernatant, 25 ml of overnight culture of *Listeria* were centrifuged at 13000g and the supernatants were collected and centrifuged further to remove cells and cells debris. Supernatants were filtered through a 0.20um pore size filter. 10^7 bacterial targets were inoculated into 2.5 ml of listerial supernatant and 2.5 ml of fresh medium at 37°C. At 16h after inoculation, cultures were serially diluted and plated on selective media.

[0098] **Peptide synthesis:** Lmo2776 (SEQ ID NO : 3) was prepared by solid-phase synthesis (Polypeptide Group) with a purity of more than 95 %. The molecular weight of the peptide was confirmed by mass spectrometry (7402.5 ES+).

[0099] **Effect of the peptide**: For *in vitro* experiments, 10^7 bacteria from overnight cultures were inoculated into 5 mL of fresh medium either in single culture or in presence of peptide as indicated in the figures and incubated at 37°C for 24 hours. Serial dilutions were plated on selective media for CFU enumeration. For *in vivo* experiments, conventional BALB/C mice were anaesthetized with an intraperitoneal injection of 65 mg ketamine kg −1 and 13 mg xylazine kg −1. Mice were inoculated intra-rectally with Lmo2776 peptide (1 mg in 100 ul of water) or water (100 ul). Feces were collected between 1 and 4h post-administration. After DNA extraction, levels of total bacteria and *P. copri* were quantified in feces by quantitative PCR as described above.

[00100] **Immunostaining of mucins and localization of bacteria by FISH:** Mucus immunostaining was paired with fluorescent in situ hybridization (FISH), as previously described (33, 35). Observations were performed with a Zeiss LSM 700 confocal microscope. The software Zen 2011 version 7.1 was used to measure the distance between bacteria and epithelial cell monolayer.

[00101] **Quantification of fecal Lcn-2 by ELISA:** Fecal samples were weighted, reconstituted in PBS-0.1% Tween 20 to a final concentration of 100 mg/mL and homogenized. Samples were then centrifuged for 10 min at 14 000 g and 4°C and supernatants were collected and stored at −20°C until analysis. Lcn-2 levels were measured using DuoSet mouse Lipocalin-2/NGAL ELISA kit (R&D Systems).

[00102] **Core genome MLST:** cgMLST analysis was performed as previously described (53).

[00103] **Statistical analysis:** Statistical tests are reported and described in the figure legends. Differences denoted in the text as significant fall below a p-value of 0.05.

Example 1: Lmo2776 limits *Listeria* intestinal colonization and virulence in a microbiota-dependent manner

[00104] A recent reannotation of the genome of the *Listeria* strain EGD-e indicated the presence of three genes, *lmo2774*, *lmo2775* and *lmo2776*, absent in the non-pathogenic *L. innocua* strain CLIP 11262 and potentially related to bacteriocin production and transport (36), (**Figure 5A**). This locus is present in Lineage I strains responsible for the majority of *Listeria* clinical cases (37) and in some Lineage II strains, such as EGD-e (**Figure 5B**). Conserved domain search indicated that Lmo2776 is predicted to be a secreted bacteriocin of 107 amino acids (36, 38), belonging to the bacteriocin 972 family, which contains bacteriocins secreted by *Lactococcus lactis* (39) and also by pathogenic *Streptococcus pneumoniae, Staphylococcus aureus* and *Streptococcus iniae* (**Figure 5C**). Bacteriocin of the lactococcin 972 family are cleaved following a GG motif to the mature form, indicating that Lmo2776 mature form is predicted to be a peptide of 63 amino acids (SEQ ID NO: 3). Lmo2776 shares between 38 to 47% overall sequence similarity with the other members of the bacteriocin 972 family. Lmo2774 and Lmo2775 have sequence similarity with ABC transporters and immunity proteins of the related bacteriocin 972 family members, respectively.

[00105] We observed that during EGD-e growth at 37 °C in BHI medium, expression of *lmo2774, lmo2775* and *lmo2776* genes significantly increases in stationary phase compared to exponential phase (**Figure 6A**). Expression of *lmo2774* (upstream) and *lmo2777* (downstream) genes in stationary and exponential phases were not significatively different in Δ*lmo2776* strain compared to the wild type, indicating that *lmo2776* deletion does not affect expression of the surrounding genes (**Figure 6C**). All experiments were thus conducted with *Listeria* grown up to stationary phase. We then examined lmo2776 expression in mice orally infected with Listeria. Upon inoculation of conventional BALB/c mice with a Listeria strain expressing a

chromosomal copy of the Lux operon under the control of the putative promoter region of *lmo2776* (Lm p*lmo2776*:lux), a bioluminescent signal was detected in the abdomen of animals 3 days post-infection, indicating that Lmo2776 is expressed in vivo.

[00106] To elucidate the effect of Lmo2776 *in vivo*, we infected mice orally with either the WT, the Δ*lmo2776* or the Lmo2776 complemented strains and compared *Listeria* loads in the intestinal content and target organs 72h post-infection. The Δ*lmo2776* strain displayed significantly higher bacterial loads in the small intestinal content compared to the WT or to the Lmo2776 complemented strains (**Figure 1A**). The bacterial loads of Δ*lmo2776* were also significantly higher in the spleen and liver at 72h post-infection compared to the WT and the Lmo2776 complemented strains (**Figure 1B and C**). The Δ*lmo2776* strain displayed significantly higher bacterial loads in the small intestinal content compared to the WT strain at 24h, 48h and 72h post-infection (**Figure 6B**). No difference was observed between the growth curves of the Δ*lmo2776* strain and the wild-type *in vitro* in BHI medium, indicating that *lmo2776* deletion does not affect bacterial growth (**Figure 6D**). Similar differences were observed in C57BL/6J mice (data not shown). Altogether, these data indicate a key role for Lmo2776 in bacterial growth in the intestine and deeper organs. The higher number of Δ*lmo2776* strain in the spleen and the liver directly correlate with the high number of bacteria in the intestinal content, crossing the intestinal barrier and consequently reaching deeper organs such as the spleen and the liver.

[00107] Upon intravenous infection of BALB/c mice with 5.10^3 bacteria, the bacterial loads at 72h post-infection of WT and Δ*lmo2776* were similar in the spleen (p=0.2854) and liver (p=0.224), indicating that Lmo2776 is only important for the intestinal phase of infection.

[00108] As lmo2776 is predicted to encode a bacteriocin and as we have shown that it plays a role in the intestinal phase of infection, we hypothesized that Lmo2776 could target intestinal bacteria and that this targeting of the intestinal microbiota was critical for Listeria virulence. We thus orally inoculated germ-free mice with WT and Δ*lmo2776* strains and compared bacterial burden loads at 72h post-infection. Strikingly, no significant difference was observed between WT and Δ*lmo2776* strains in the content of the small intestine (**Figure 1D**),

nor in spleen and liver (**Figure 1E and F**). These results strongly reveal that the Lmo2776 bacteriocin limits the virulence of wild-type *Listeria* in a microbiota-dependent manner.

Example 2: Lmo2776 specifically targets *Prevotella* in mouse and human microbiota

[00109] We compared the microbiota compositions of mice orally infected with WT or Δ*lmo2776* strains. We verified by 16S rRNA gene sequencing that the fecal microbiota composition of all mice was undistinguishable at day 0 (**Figure 2A**). As expected, the microbiota composition at day 1 post-infection was dramatically altered by infection with *Listeria* WT (**Figure 7A**). These alterations in microbiota composition included reduced levels of Bacteroidetes phylum (relative abundance before infection: 65.4% and at day 1 post-infection: 42.4%) and increased levels of Firmicutes (relative abundance before infection: 29.9% and at day 1 post infection: 54.0%) (**Figure 7B to E**). The increased levels in the Firmicutes were mainly due to an increase of the Clostridia class (relative abundance before infection: 27.4% and at day 1 post-infection: 50.7%). Of note, the relative abundance of Listeria was around 0.1% and cannot therefore explain by itself the increased levels of Firmicutes observed between day 0 and day 1. At 24h and 48h post-infection, microbial community compositions of the intestine differed in mice orally infected with the Δ*lmo2776* strain compared to the WT strain (**Figure 2A and 11**). We focussed on operational taxonomic units (OTUs) for which the relative abundance was constant before the infection with *Listeria* strains (day -3 to day 0) and was at least 2 fold different at day 1 post-infection in mice infected with Δ*lmo2776*compared to mice infected with WT strain. We observed that the relative abundance of 12 operational taxonomic units (OTUs) was lower in mice infected with the WT strain compared to the Δ*lmo2776* mutant (**Figure 2B and C**) (OTU 355746, 216524, 421792, 258849, 331772, , 346870, 430194, 447141, 465433, , 208409, 353012 and 364179). Phylogenetic analyses indicated that all these 12 OTUs belong to the *Prevotella* cluster (**Figure 2D and 8A**). The amount of *Prevotella* in feces was increased at day 1 post-infection in mice orally infected with Δ*lmo2776* compared to mice infected with WT strain (**Figure 8D**). Linear discriminant analysis (LDA) effect size (LEfSe) analysis (40) confirmed a positive association between Δ*lmo2776 Listeria* strain and *Prevotella* genus (**Figure 8B and C**), strongly suggesting that Lmo2776 targets *Prevotella*.

[00110] Important differences exist between mouse and human gut microbiota composition. Indeed, *Prevotella* abundance is known to be low in the mouse feces (less than 1%) and can reach up to 50 % in the human feces (41, 42). As *Listeria* is a human pathogen, we investigated the impact of Lmo2776 on human intestinal microbiota. For this purpose, we used the model M-SHIME®, a mucosal simulator of the human intestinal microbial ecosystem. This ex vivo model allows to stably maintain a human microbiota in vitro, in the absence of host cells (30-33). The microbiota of a healthy human volunteer with high levels of Prevotella was inoculated into the system, which was then infected with WT or Δ*lmo2776* bacteria. Application of 16S sequencing to luminal M-SHIME® samples indicated that the bacterial composition of all vessels was similar before infection (**Figure 3A and B**). In contrast, at day 1 post-infection, microbial community compositions were different in vessels infected with the WT bacteria compared to non-infected vessels or vessels infected with the Δ*lmo2776* strain (**Figure 3A and B**). The relative abundance of 8 OTUs (313121, 518820, 346938, 588929, New.0.ReferenceOTU20, 181539, 173565 and 89083) was lower with the WT strain compared to the non-infected condition or infection with the Δ*lmo2776* strain (**Figure 3C**). These 8 OTUs correspond to *Prevotella copri*, revealing that Lmo2776 targets *P. copri* in human microbiota, in a host-independent manner.

[00111] Furthermore, short-chain fatty acid levels serve as a general read-out for gut microbiota metabolism. Analysis of luminal M-SHIME® samples indicated a specific decrease in propionate production upon infection with WT bacteria as early as 6h post-infection (**Figure 3D**) compared to non-infected and Δlmo2776-infected vessels. This difference was continuously observed up to 3 days post-infection, while no significant difference was observed for butyrate, isobutyrate, acetate and isovalerate (**Figure 9**). Although propionate is produced by many bacteria, the decrease in propionate production observed during infection of M-SHIME® with WT *Listeria* is in agreement with the decrease in *Prevotella* population, as *Prevotella* are known to produce this short chain fatty acid (43).

Example 3: Lmo2776 targets *P. copri in vitro*

[00112] We addressed the direct inhibitory activity of Lmo2776 on *P. copri* by growing *P. copri* at 37 °C in anaerobic conditions in the presence of culture supernatants of *Listeria*

strains and counting the viable CFUs on agar plates. Growth of *P. copri* dramatically decreased (up to 3 Log) in the presence of the WT strain supernatant compared to the Δ*lmo2776* strain supernatant (**Figure 3E**), demonstrating that Lmo2776 targets directly *P. copri*.

[00113] The effects of *Listeria* strains or that of their supernatants were also tested on the growth of other bacteria that might be encountered by *Listeria* in the environment. Growth of *Bacillus subtilis* was specifically and significantly reduced in the presence of WT *Listeria* and of p*2776* strains compared to the Δ*lmo2776* strain (**Figure 10**). Moreover, addition of the culture supernatant of WT *Listeria* to *B. subtilis* significantly decreased the number of *B. subtilis* compared to the addition of Δ*lmo2776* culture supernatant (**Figure 3F**). Interestingly, *B. subtilis* is also targeted by the bacteriocin Sil, produced by the fish pathogen *Streptococcus iniae*, and a member of the bacteriocin 972 family, as Lmo2776 (*44*). No effect of the WT *Listeria* culture supernatant was observed on the growth of other tested bacteria, including *E. coli* (**Figure 3F**), *Bacillus thuringiensis*, different *Staphylococcus aureus* strains, *S. epidermidis*, *Streptococcus carnosus*, *S. sanguinis*, *S. pneumoniae*, *Enterococcus faecalis*, different *Lactococcus lactis* strains and *L. monocytogenes* 10403S. These results indicate that Lmo2776 is a *bona fide* bacteriocin that targets directly both *P. copri* and *B. subtilis in vitro*.

Example 4: Colonization of germ-free mice by *P. copri* recapitulates the effect of the microbiota on *Listeria* intestinal growth in conventional mice

[00114] To decipher the role of *P. copri* during *Listeria* infection *in vivo*, germ-free C57BL/6J mice were inoculated orally with *P. copri*, *P. salivae, B. thetaiotaomicron,* another commensal bacterium, or a mixture of 12 bacteria including Verrucomicrobia (*A. muciniphila* YL44), Bacteroidetes (*B. caecimuris* I48, *M. intestinale* YL27), Proteobacteria (*T. muris* YL45), Actinobacteria (*B. longum* YL2), Fimicutes (*E. faecalis* KB1; *A. muris* KB18; *C. clostridioforme* YL32) and Firmicutes (F. plautii YL31; B. coccoides YL58; *L. reuteri* I49 and *C. innnocuum* I46). Analysis of viable bacteria from fecal samples 2 weeks post-inoculation revealed robust intestinal colonization by each bacterium (data not shown). These mice were then orally infected with WT *Listeria* or Δ*lmo2776* strains and bacterial loads in the intestinal content were compared 72h post-infection. The Δ*lmo2776* strain displayed significantly higher loads in the intestinal content compared to the WT in mice colonized with *P. copri* (**Figure 4A**),

while no difference between the two strains was observed in mice monocolonized with *B. thetaiotaomicron* or *P. salivae* (**Figure4A**), or colonized with the mixture of 12 bacteria **(Figure 12).**

[00115] In addition, the number of *P. copri* significantly decreased in WT-infected animals compared to Δ*lmo2776*-infected animals (**Figure 4B**). Altogether, these results indicate that the ability of the Δ*lmo2776* mutant to better grow in the intestine compared to the WT dependent on the presence of *Prevotella* in the microbiota, as it is observed either in conventional mice or mice colonized with *P. copri*.

Example 5: *P. copri* modifies the intestinal environment

[00116] Mucus in the intestine of conventional animals forms a physical barrier of about 30µm that is able to keep bacteria at a distance from the epithelium (*45*). *Prevotella*, through production of sulfatases that induce actively mucus degradation (*46*), might impair the mucosal barrier function and therefore contribute to local inflammation and better accessibility to intestinal epithelial cells for pathogens. To test this hypothesis, we compared the mucus layer thickness of conventional mice infected with WT *Listeria* or Δ*lmo2776* by confocal microscopy, using mucus-preserving Carnoy fixation and FISH (*35*). The average distance of bacteria from colonic epithelial cells was significantly smaller in mice infected with Δ*lmo2776* compared to mice infected with WT *Listeria* at 24 and 48h (**Figure 4D**), strongly suggesting that *Prevotella* present in the microbiota of mice infected with Δ*lmo2776* decreases the mucus layer thickness. Of note, these distances were also smaller than in uninfected mice, indicating that *Listeria* can affect the mucus layer thickness in different ways. Disruption of the mucosal barrier function by *Prevotella* could favour invasion of the host by pathogenic bacteria and contribute to intestinal inflammation. Lipocalin-2 (LCN-2) is a small secreted innate immune protein which is critical for iron homeostasis and which increases during inflammation. Levels of faecal LCN-2 are used as a marker of intestinal inflammation (*47*). Faecal LCN-2 levels were thus analysed after colonization of germ-free mice with *P. copri* compared to non-colonized mice or mice colonized with *B. thetaiotaomicron*. A significant increase of faecal LCN-2 was observed in germ-free mice monocolonized with *P. copri* compared to non-colonised animals or to animals monocolonized with *B. thetaiotaomicron* (**Figure 4E**), revealing that *P. copri*

induces intestinal inflammation. Altogether, these results showed that presence of *Prevotella* in the intestine is associated with a thinner mucus layer and increased levels of faecal LCN-2. They are consistent with previous reports describing *Prevotella*, as a bacterium promoting a pro-inflammatory phenotype (*1, 4, 48*).

[00117] To demonstate the direct role of *P. copri* on mucus layer thickness, germ-free C57BL/6J mice were inoculated orally with *P. copri*, *P. salivae* or *B. thetaiotaomicron*, two weeks later mice were orally infected with WT *Listeria* or Δ*lmo2776* strains and mucus layer thickness was compared 72h post-infection. The average distance of bacteria from colonic epithelial cells was significantly smaller in *P. copri* colonized mice infected with Δ*lmo2776* compared to *P. copri* colonized mice infected with WT *Listeria* at 72h (**Figure 4D**), non-colonised animals or to animal monocolonized with *B. thetaiotaomicron* or *P. salivae,* showing that *Prevotella* decreases specifically the mucus layer thickness.

Example 6: Lmo2776 peptide inhibits *P. copri* growth *in vitro* and *in vivo*

[00118] Lmo2776 peptide of SEQ ID NO: 3 was synthesized and tested *in vitro* and *in vivo*. *In vitro*, Lmo2776 peptide inhibits *Prevotella copri* growth in a dose dependent manner and has no effect on the growth of *B. thetaiotaomicron* (**Figure 3G**). Lmo2776 peptide also inhibits *B. subtilis* growth but has no effect on the growth of other *Prevotella* strains, intestinal bacteria or Lactic acid bacteria (IPLA) (**Figure 3H**). In addition, conventional mice treated with Lmo2776 peptide displayed significantly lower number of *P. copri* in the feces compared to untreated mice (**Figure 3J**). Furthermore, Lmo2776 peptide is also capable of inhibiting the growth of various *P. copri* isolates from Rheumatoid arthritis (RA) patients (**Figure 3I**). These results confirm that Lmo2776 is a *bona fide* bacteriocin that targets directly both *P. copri* and *B. subtilis in vitro* and *in vivo*. These results show the therapeutic potential of Lmo2776 peptide in the treatment of inflammatory diseases and enteropathogenic bacterial infections.

Discussion:

[00119] Outcompeting intestinal microbiota is one of the first challenging issues for enteropathogens. These bacteria may secrete diffusible molecules such as bacteriocins or T6SS effectors to target commensals and consequently promote colonization and virulence. However,

in most cases, the molecular mechanisms underlying the interplay between pathogenic and commensal bacteria in the intestine remain elusive. Nevertheless, we previously reported that epidemic *Listeria* strains, such as F2365 strain, secrete a bacteriocin that promotes intestinal colonisation by *Listeria* (*49*). When overexpressed in mice gut, this bacteriocin, named Listeriolysin S (LLS), decreases *Allobaculum* and *Alloprevotella* genera known to produce butyrate or acetate, two SCFAs reported to inhibit transcription of virulence factors or growth of *Listeria* (*50, 51*). However, whether physiological concentrations of LLS have a direct or an indirect role on these genera is still under investigation. In the case of *Salmonella enterica* serovar Typhimurium infection, killing of intestinal *Klebsiella oxytoca via* the *Salmonella* T6SS is essential for colonisation of the murine gut (*52*), but whether *K. oxytoca* and other members of the gut microbiota are targeted by the *Salmonella* T6SS in conventional mice is unknown. Finally, *Shigella sonnei* uses a T6SS to outcompete *E. coli in vivo* but the effectors responsible for this effect are unknown (*53*).

[00120] Here, we demonstrated that the Lmo2776 *Listeria* bacteriocin targets *Prevotella* in mouse and human microbiota. This effect is direct and specific as (i) *Prevotella* are killed by *Listeria* culture supernatant *in vitro* and (ii) despite the complexity of the microbiota and its well-controlled equilibrium, no other genus of the intestinal microbiota was found to decrease in the presence of Lmo2776. By studying Lmo2776, we report for the first time a role for intestinal *Prevotella* in controlling bacterial infection. The intestinal microbiota, in some cases, has been reported to trigger bacterial virulence by producing metabolites that enhance pathogens virulence gene expression and colonisation in the gut (*20, 21*). For example, *B. thetaiotaomicron* enhances *Clostridium rodentium* colonization by producing succinate (*54, 55*) and *Akkermansia muciniphila* exacerbates *S. Typhimurium*-induced intestinal inflammation by disturbing host mucus homeostasis (*56*). *P. copri* decreases the mucus layer thickness and increases propionate concentration and levels of fecal LCN-2 (**Figure 4F**), in agreement with previous studies reporting that *Prevotella* exacerbates inflammation (*4, 48*). In addition, *Prevotella* enrichment within the lung microbiome of HIV-infected patients has been observed and is associated with Th17 inflammation (*57*). *Prevotella* sp. have also been associated with bacterial vaginosis and the role in pathogenicity has been linked to the production of sialidase, an enzyme involved in mucin degradation and increased levels of pro-inflammatory cytokines

(*58, 59*). Our data strongly indicate that *P. copri*, by modifying the mucus layer thickness and changing the gut inflammatory condition, promotes infection. We can therefore speculate that people with high abundance of intestinal *Prevotella* might be more susceptible to enteropathogens infection.

[00121] We also showed that the Lmo2776 bacteriocin targets *B. subtilis*, a Gram-positive bacterium found in the soil, suggesting that Lmo2776 could give an advantage to *Listeria* in the environment. Importantly, as the natural reservoir for *Listeria* is at present unknown, it is possible that Lmo2776 is critical for the strain survival and replication in this special niche or animal species, consequently favoring transmission or dissemination, thereby explaining its presence in clinical strains. *B. subtilis* is also found in the human gastrointestinal tract (*60*) and could also be targeted by Lmo2776 in the intestine. *B. subtilis* is also targeted by Sil, another member of the bacteriocin 972 family (*44*). The role of the homologs of bacteriocin 972 in other human pathogenic bacteria such as *S. pneumoniae* and *S. aureus* remains to be determined. The conservation of the bacteriocin in different pathogenic bacteria strongly suggests an important role.

[00122] Taken together, our data indicate that *Prevotella* may behave as pathobiont that can participate in human disease. Consequently, Lmo2776 might represent an effective therapeutic tool to reduce specifically *P. copri* abundance in the gut without a profound effect on the commensal population.

[00123] The antibacterial effect of the Lmo2776 bacteriocin against *Prevotella* was confirmed using a synthetic Lmo2776 peptide. Lmo2776 peptide inhibits *Prevotella copri* growth *in vitro and in vivo* in conventional mouse microbiota. In particular, Lmo2776 peptide is capable of inhibiting the growth of *P.copri* isolates from Rheumatoid arthritis patients. These results show the therapeutic potential of Lmo2776 peptide in the treatment of inflammatory diseases and enteropathogenic bacterial infections.

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CLAIMS

1. A pharmaceutical composition comprising an anti-*Prevotella* bacteriocin and a pharmaceutically acceptable carrier.

2. The pharmaceutical composition of claim 1, wherein the anti-*Prevotella* bacteriocin is *L. monocytogenes* Lmo2776.

3. The pharmaceutical composition of claim 1 or 2, wherein the anti-*Prevotella* bacteriocin comprises an amino acid sequence that is at least 80% identical to a sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3.

4. The pharmaceutical composition of claim 3, wherein the anti-*Prevotella* bacteriocin comprises an amino acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3.

5. The pharmaceutical composition of claim 4, wherein the anti-*Prevotella* bacteriocin comprises or consists of the amino acid sequence of SEQ ID NO: 3.

6. The pharmaceutical composition of any one claims 1 to 5, wherein said *Prevotella* is *Prevotella copri*.

7. A pharmaceutical composition according to any one of claims 1 to 6 for use in treating or preventing inflammation in a subject.

8. The pharmaceutical composition for use according to claim 7, wherein the subject has or is at risk of developing an inflammatory disease selected from rheumatoid arthritis, metabolic syndrome, inflammatory bowel disease, and HIV infection.

9. A pharmaceutical composition according to any one of claims 1 to 6 for use in treating or preventing an enteropathogenic bacterial infection in a subject.

10. The pharmaceutical composition for use according to claim 9, wherein the subject has or is at risk of developing a Listeria infection.

11. A pharmaceutical composition according to any one of claims 1 to 6 for use in reducing the *Prevotella* load in a subject.

12. The pharmaceutical composition for use according to claim 11, wherein said *Prevotella* is *Prevotella copri*.

13. The pharmaceutical composition for use according to any one of claims 7 to 12, wherein the anti-*Prevotella* bacteriocin is administered in an amount sufficient to reduce the *Prevotella* load by at least 80%.

14. The pharmaceutical composition for use according to any one of claims 7 to 12, wherein the anti-*Prevotella* bacteriocin is administered in an amount sufficient to reduce the *Prevotella* load by at least 90%.

15. The pharmaceutical composition for use according to claim 13 or 14, wherein said *Prevotella* is *Prevotella copri*.

Figure 1

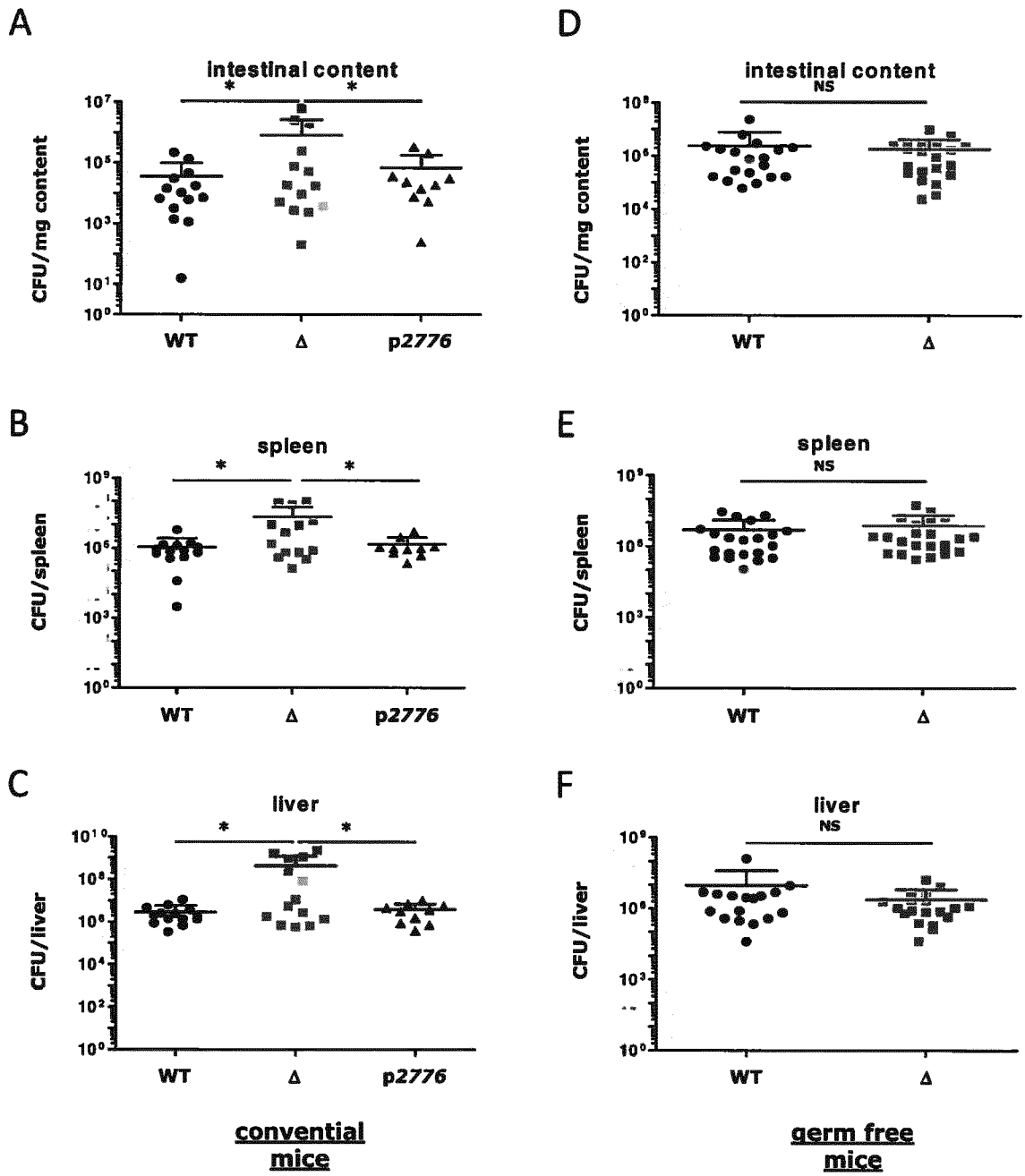
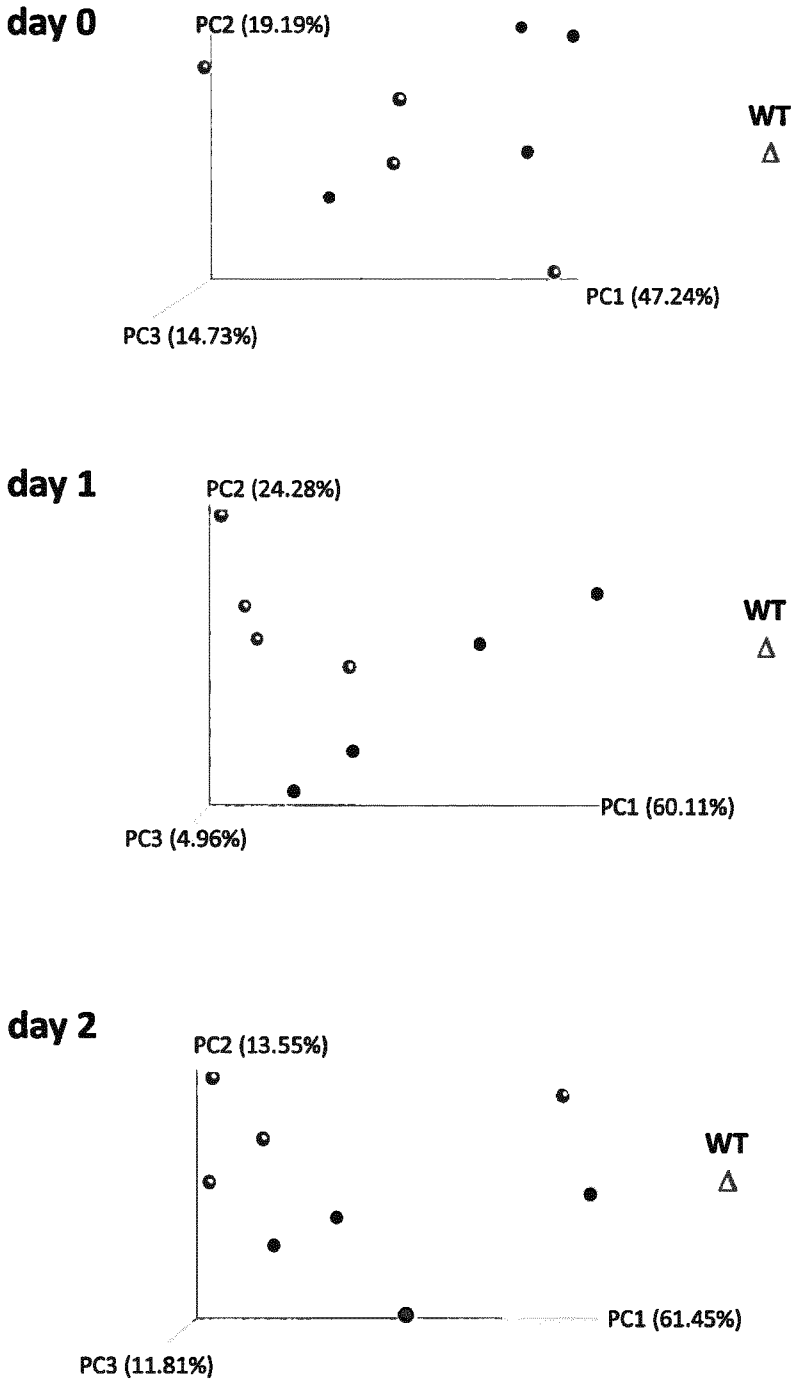

A
intestinal content
CFU/mg content
WT
Δ
p2776
B
spleen
CFU/spleen
C
liver
CFU/liver
convential mice
D
intestinal content
NS
E
spleen
F
liver
germ free mice

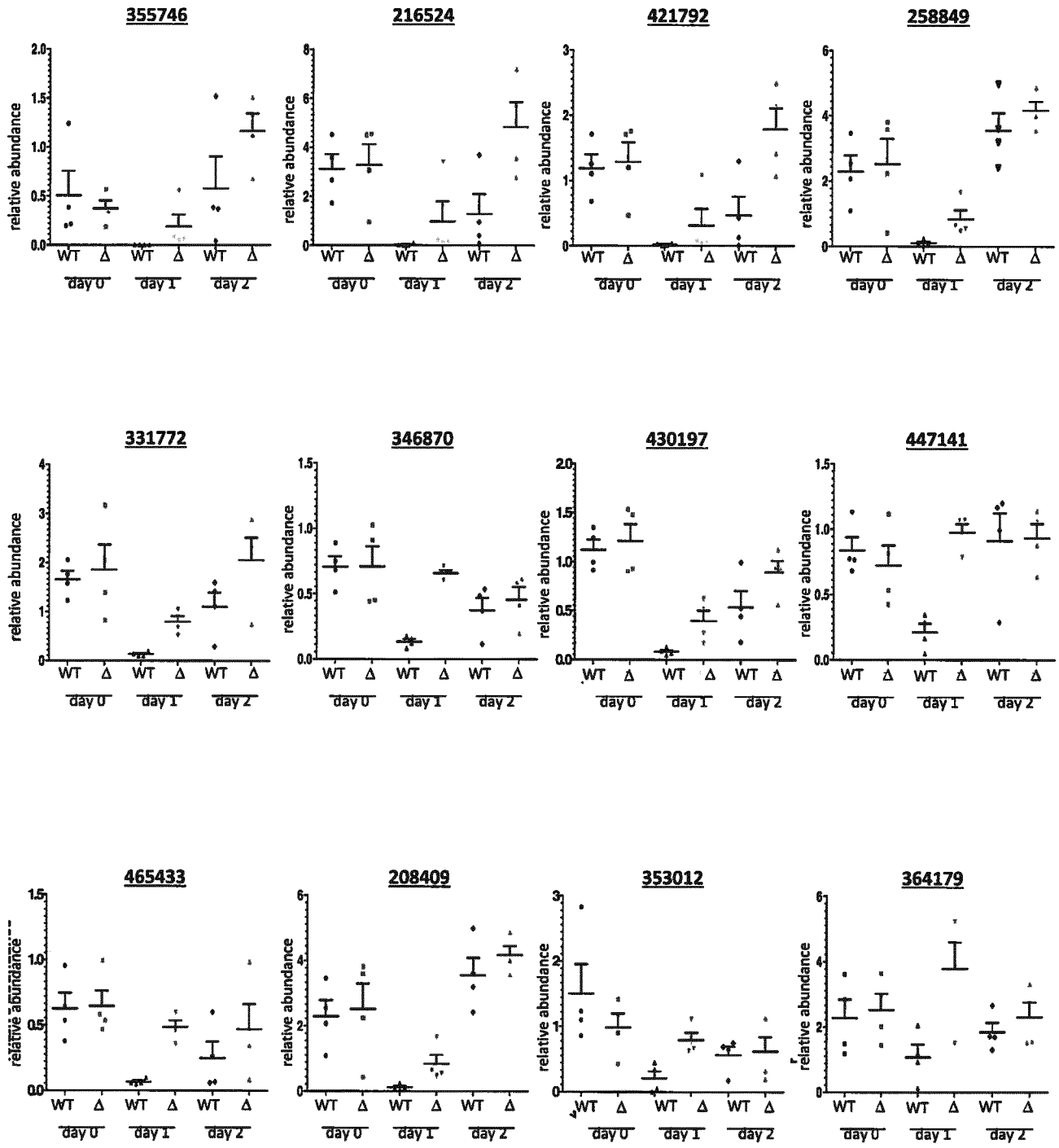
Figure 2

A


day 0
PC2 (19.19%)
WT
Δ
PC1 (47.24%)
PC3 (14.73%)
day 1
PC2 (24.28%)
WT
Δ
PC1 (60.11%)
PC3 (4.96%)
day 2
PC2 (13.55%)
WT
Δ
PC1 (61.45%)
PC3 (11.81%)

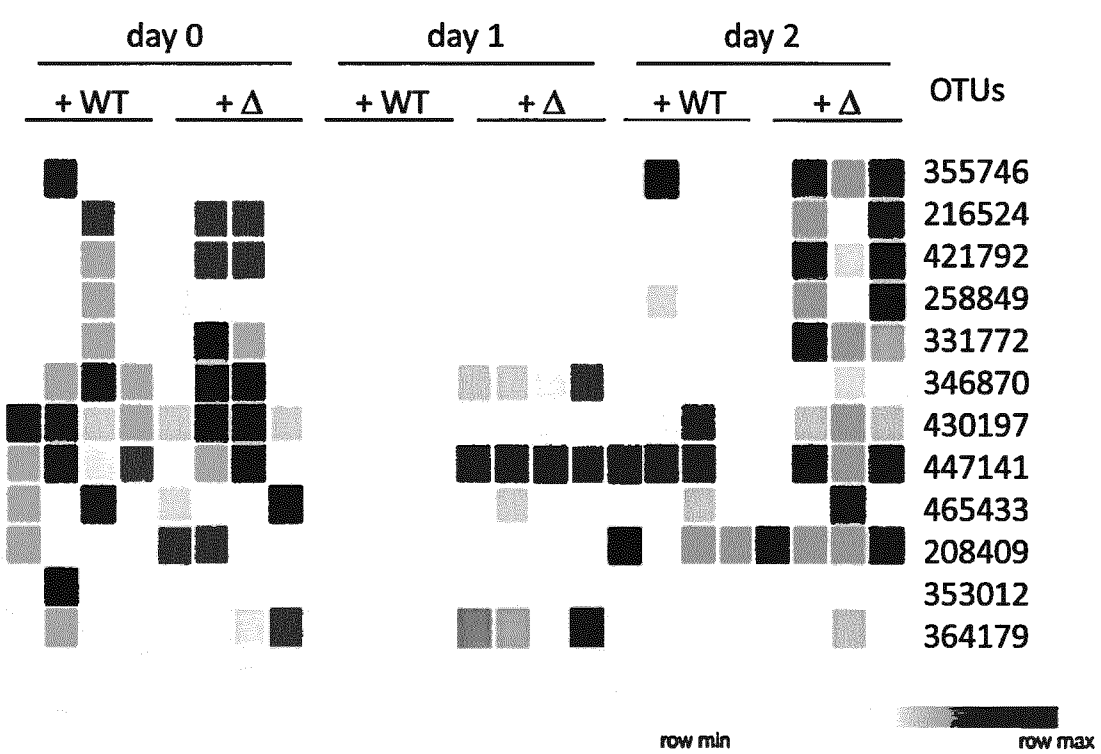
Continuation of figure 2

B



Continuation of figure 2

C



D

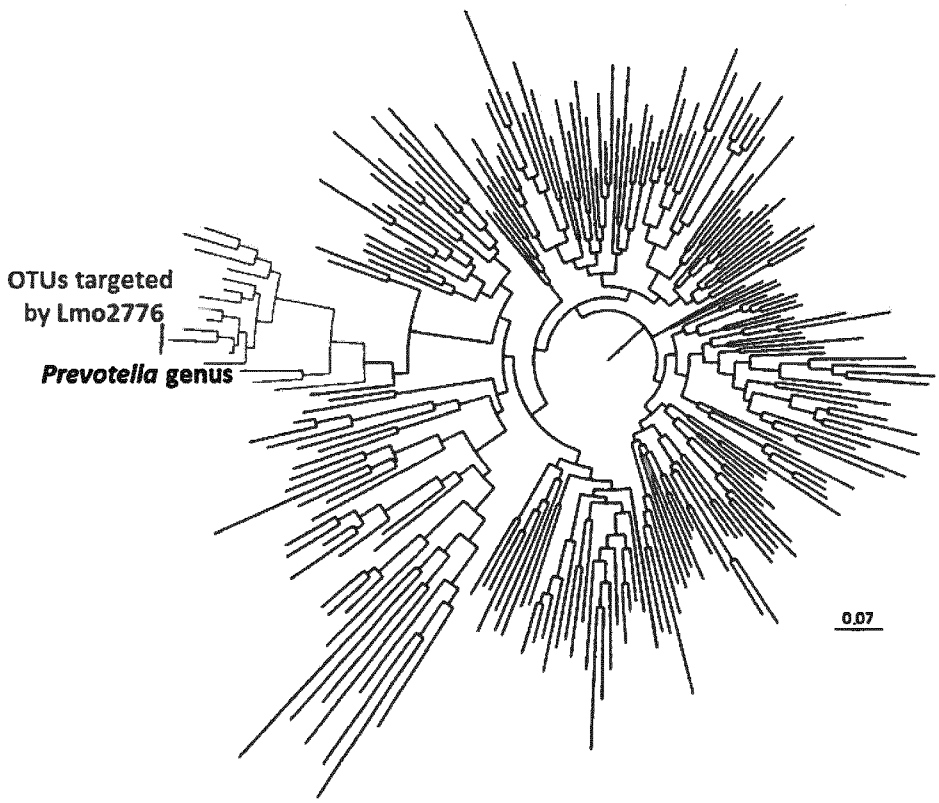
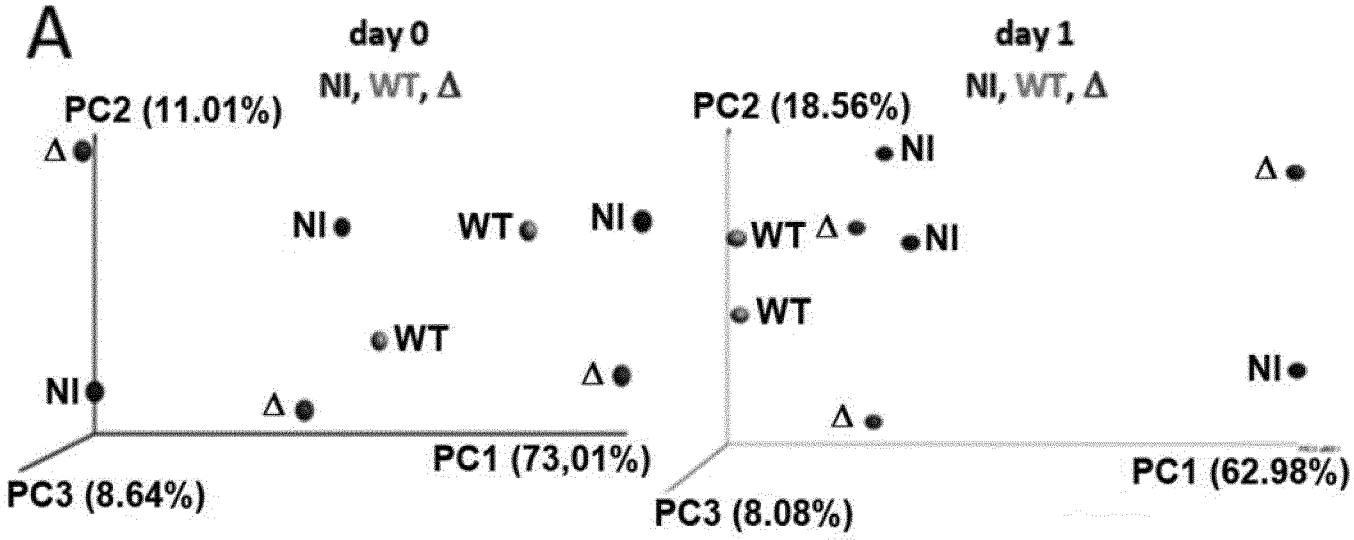
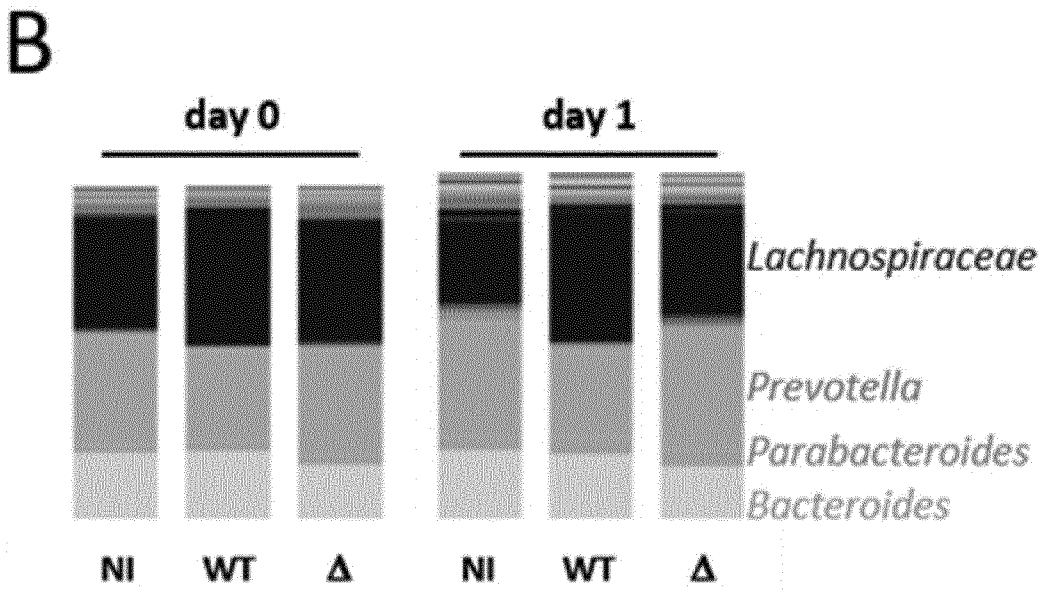


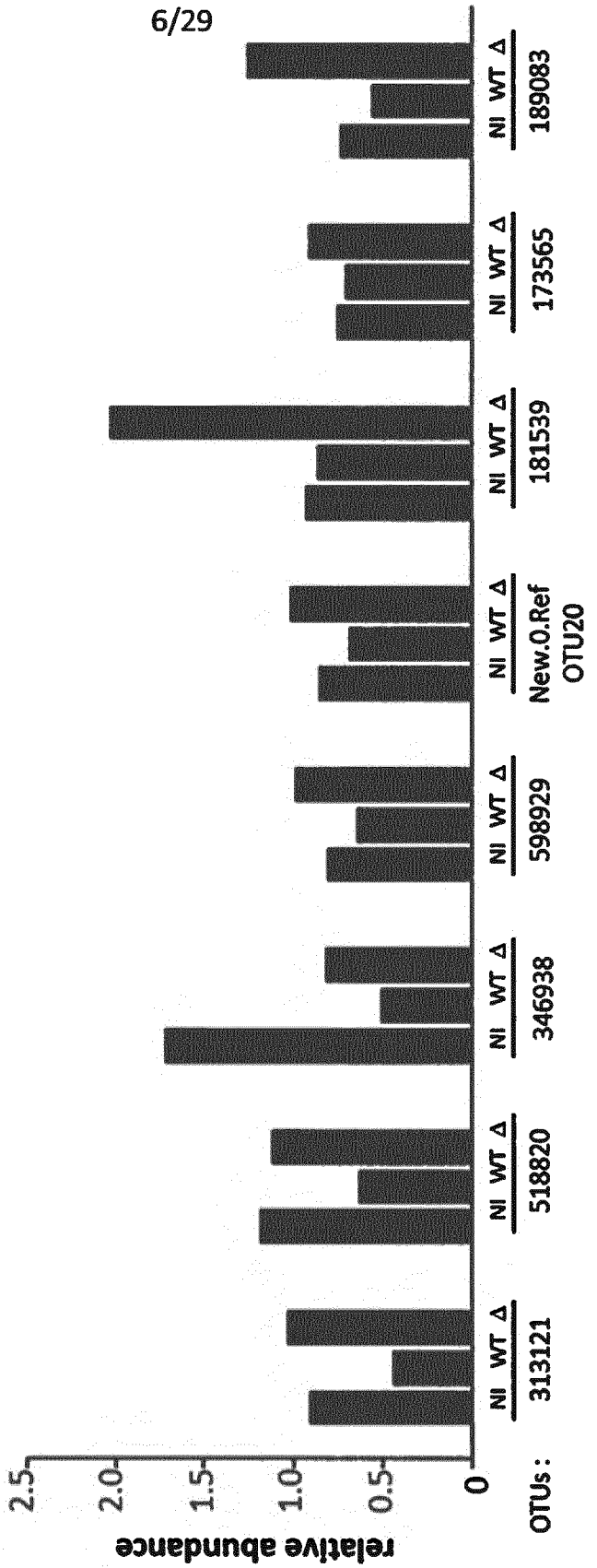
Figure 3


A
day 0
NI, WT, Δ
PC2 (11.01%)
PC1 (73,01%)
PC3 (8.64%)
day 1
NI, WT, Δ
PC2 (18.56%)
PC1 (62.98%)
PC3 (8.08%)


B
day 0
day 1
Lachnospiraceae
Prevotella
Parabacteroides
Bacteroides
NI
WT
Δ
NI
WT
Δ

Continuation of figure 3

C



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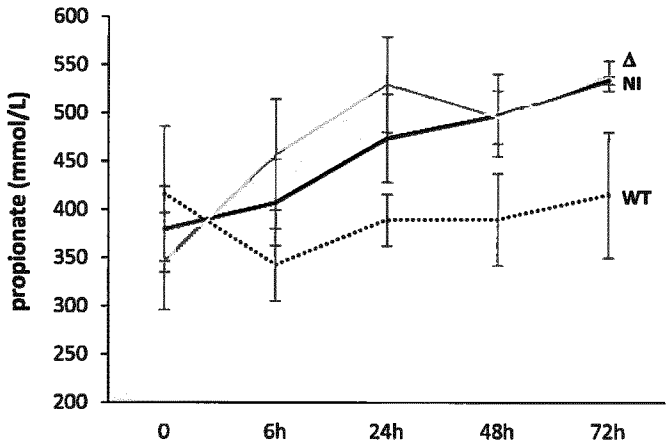

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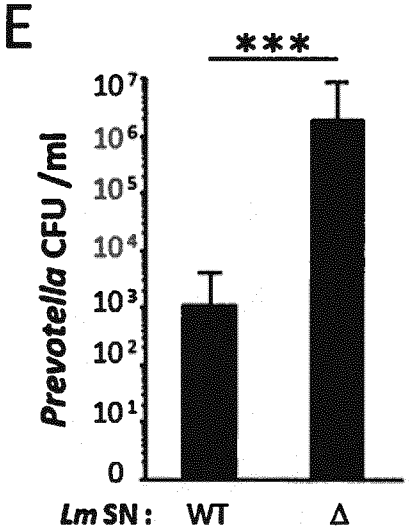

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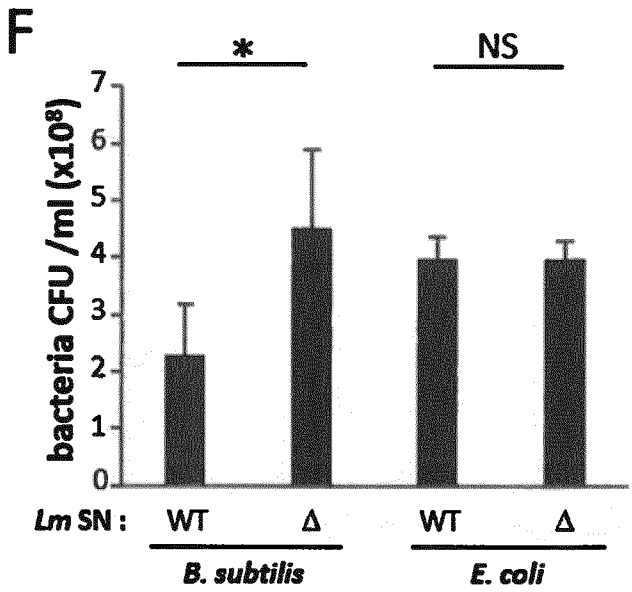

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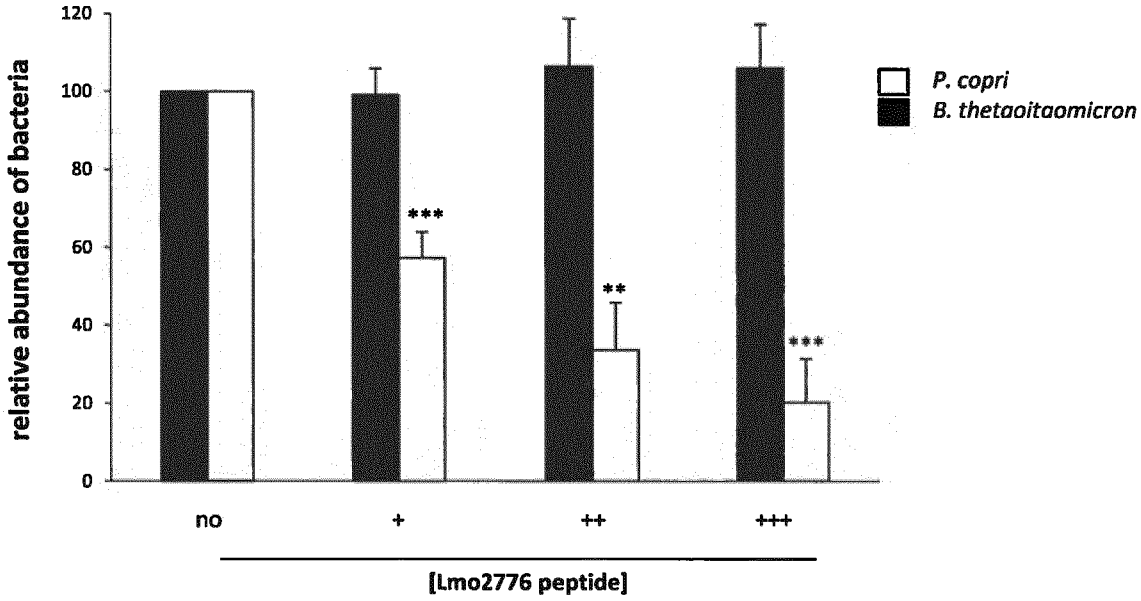
G



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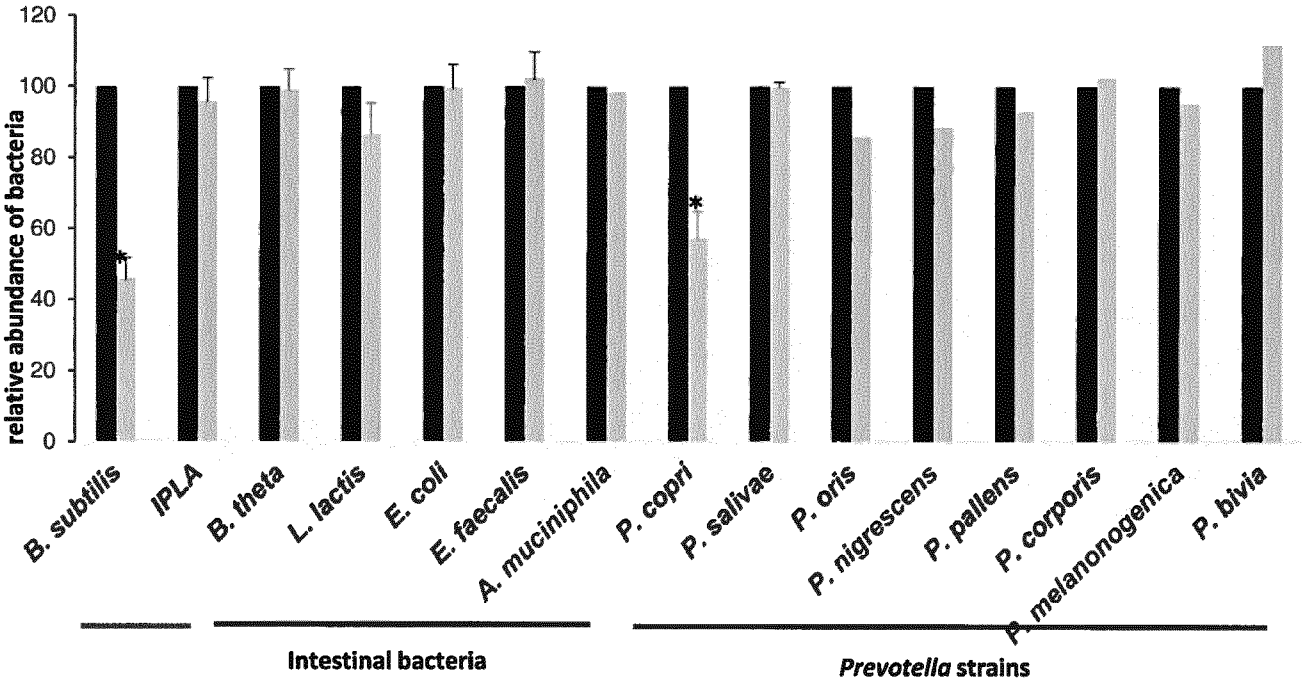
H



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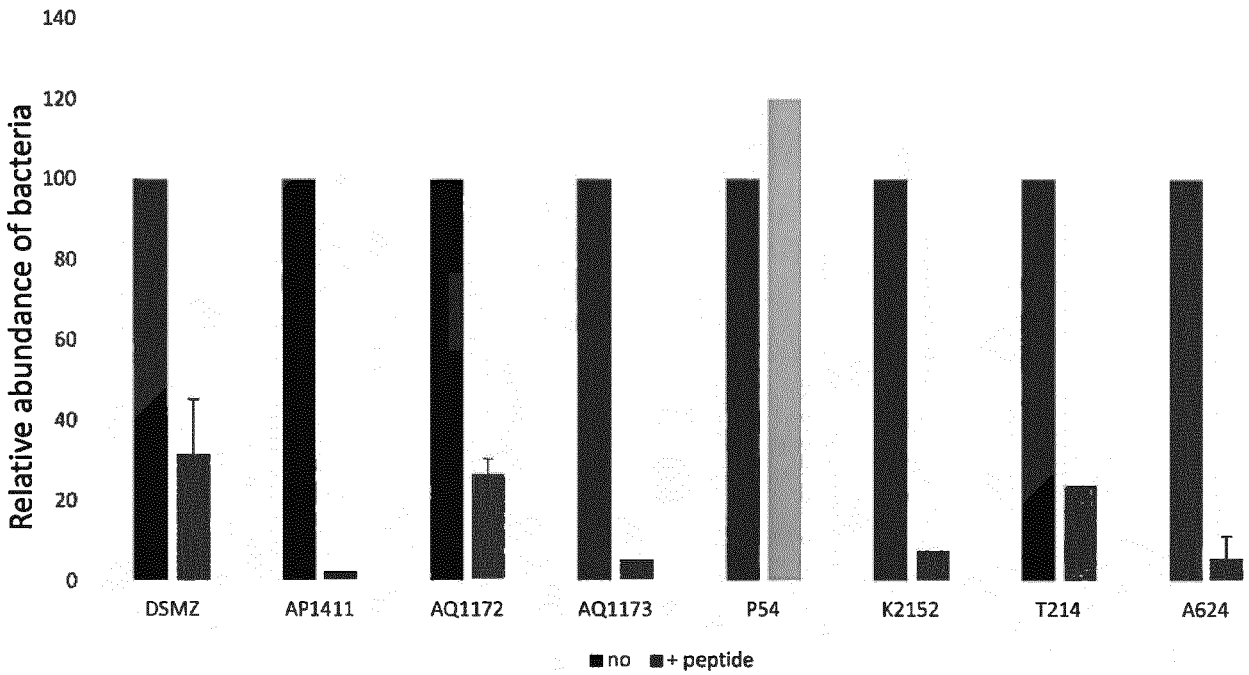
I



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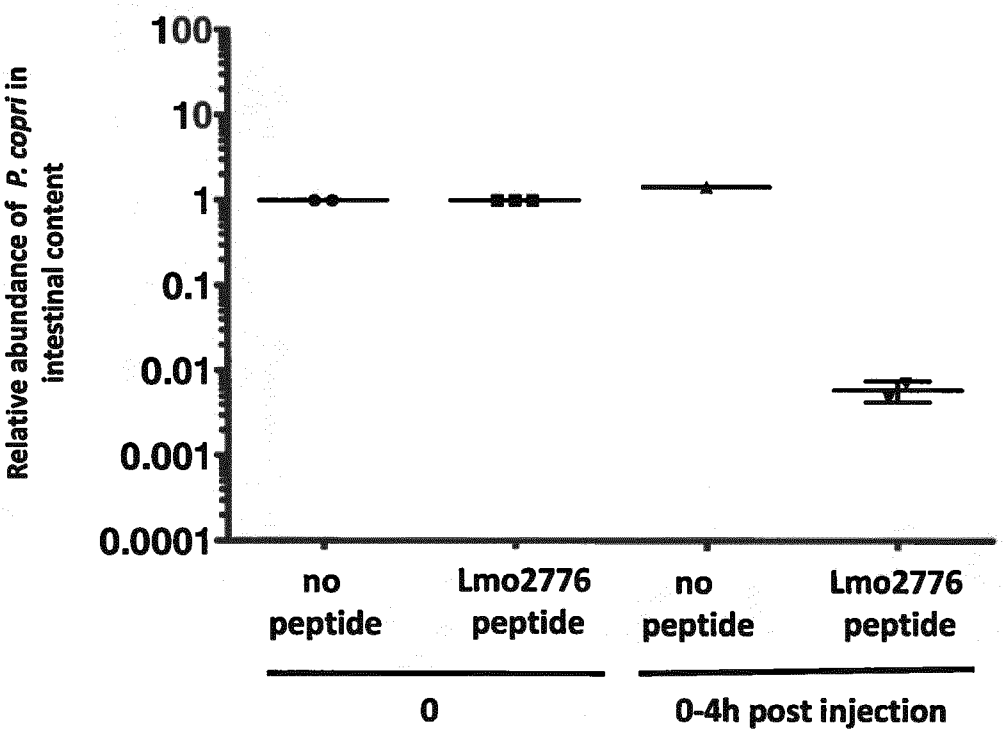
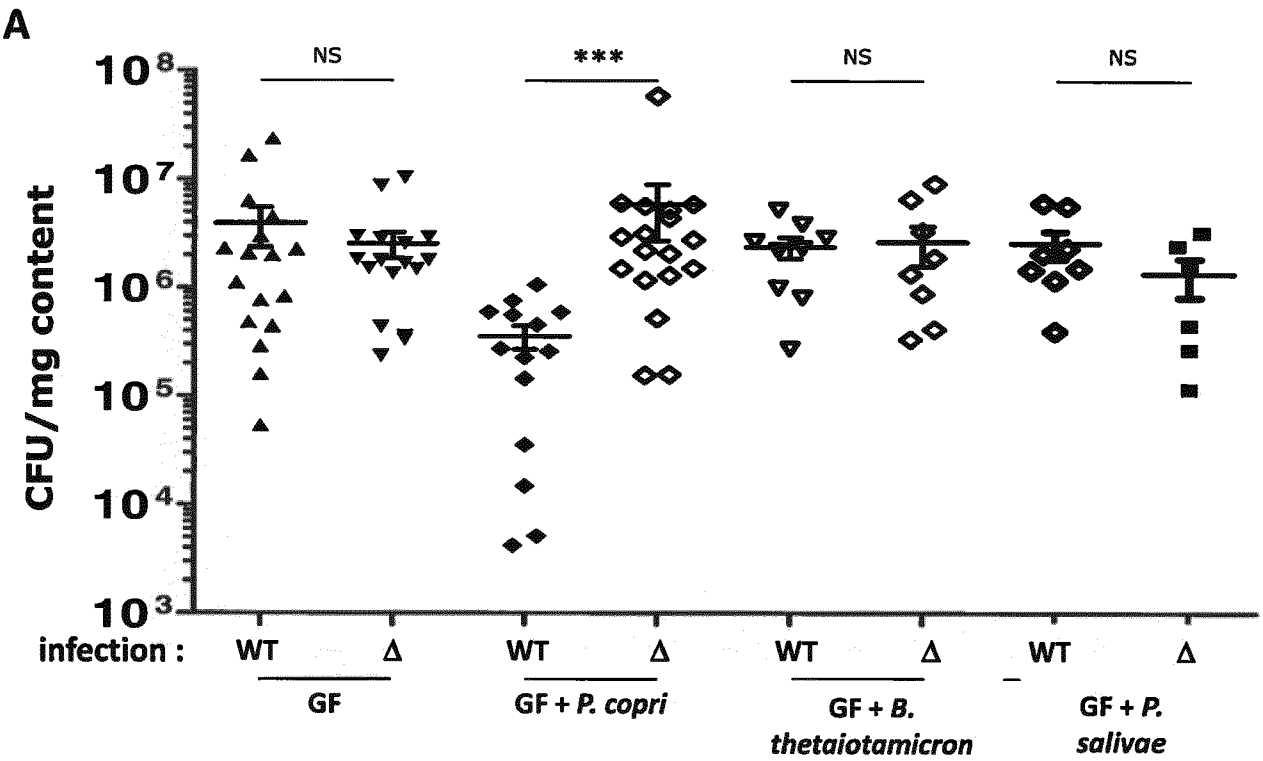
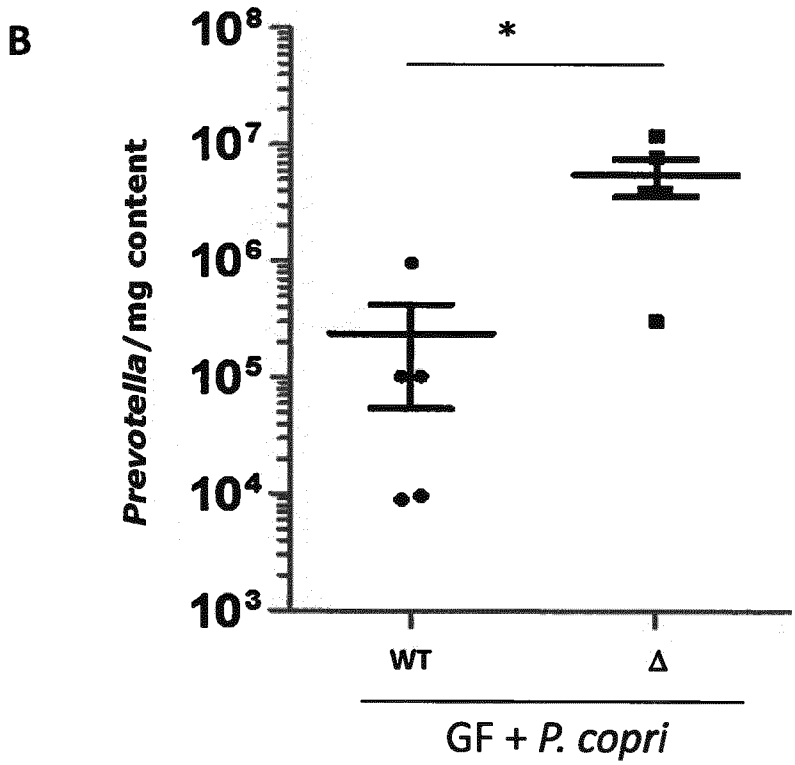


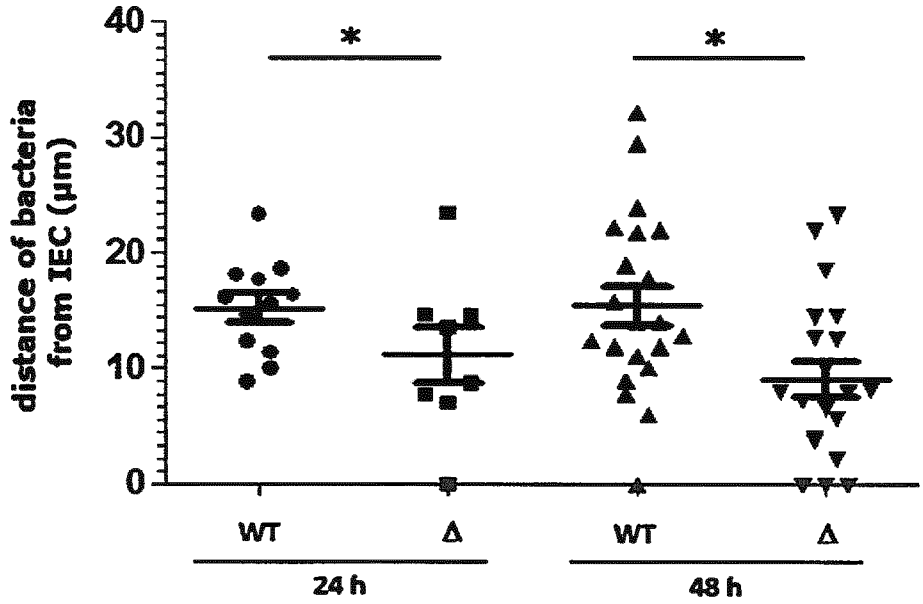
Figure 4


A
NS
***
NS
NS
10^8
10^7
10^6
10^5
10^4
10^3
CFU/mg content
infection :
WT
Δ
WT
Δ
WT
Δ
WT
Δ
GF
GF + P. copri
GF + B. thetaiotamicron
GF + P. salivae


B
*
10^8
10^7
10^6
10^5
10^4
10^3
Prevotella/mg content
WT
Δ
GF + P. copri

Continuation of figure 4

C



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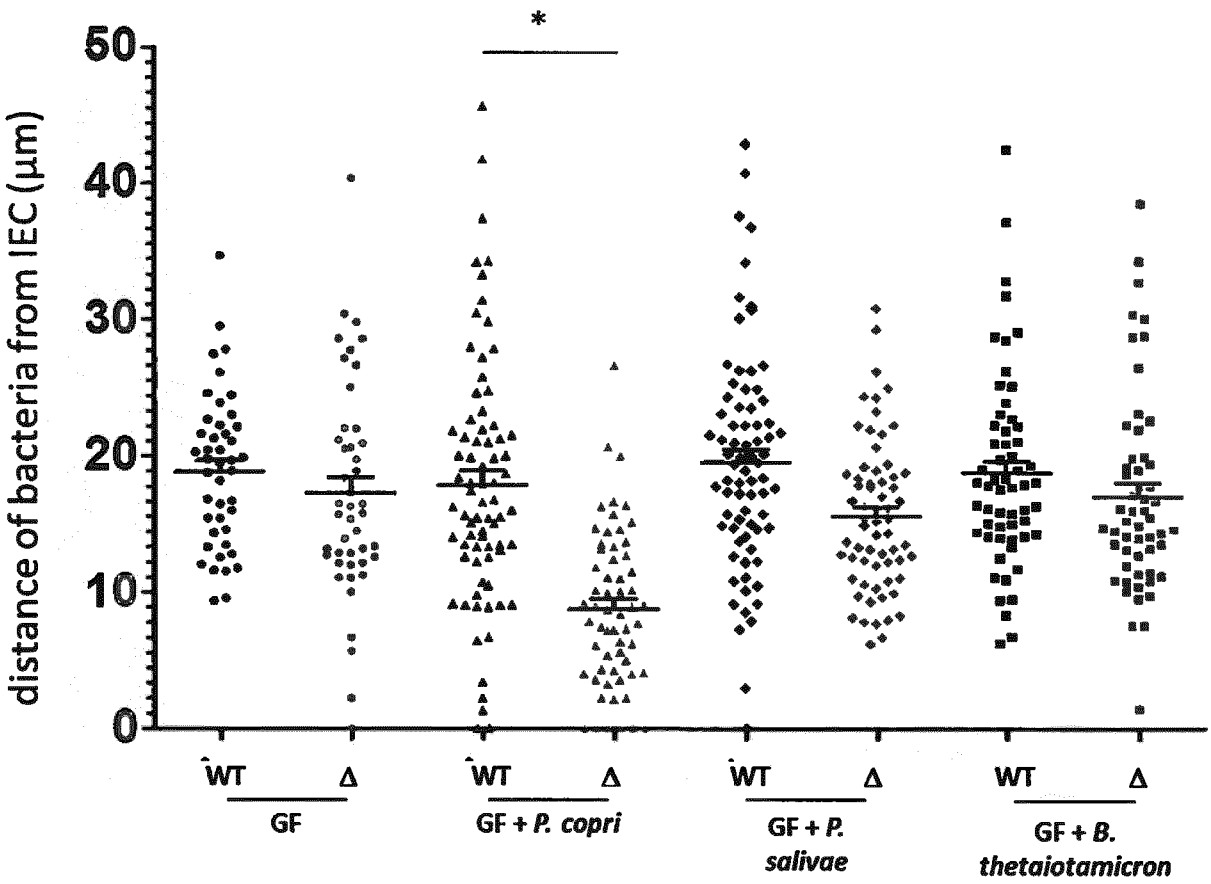

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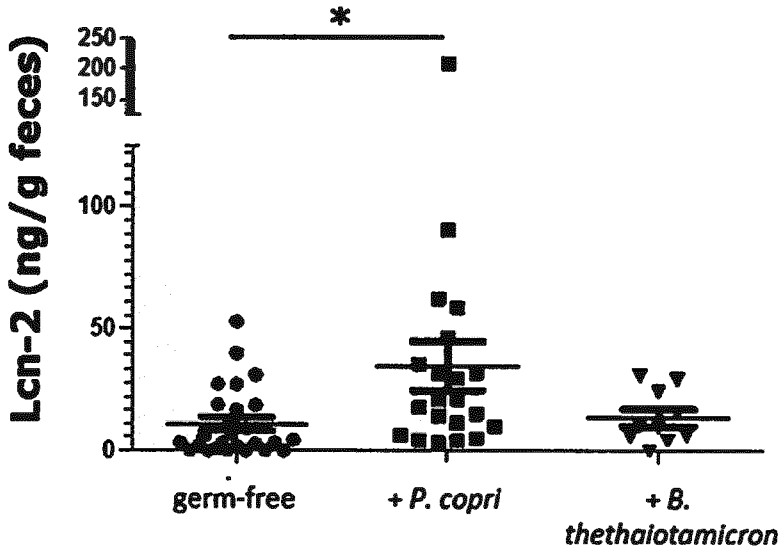
E



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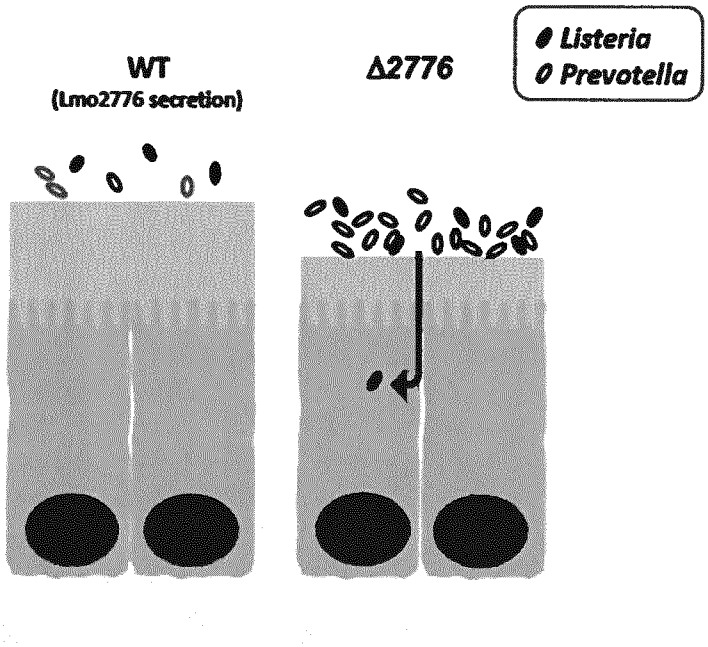
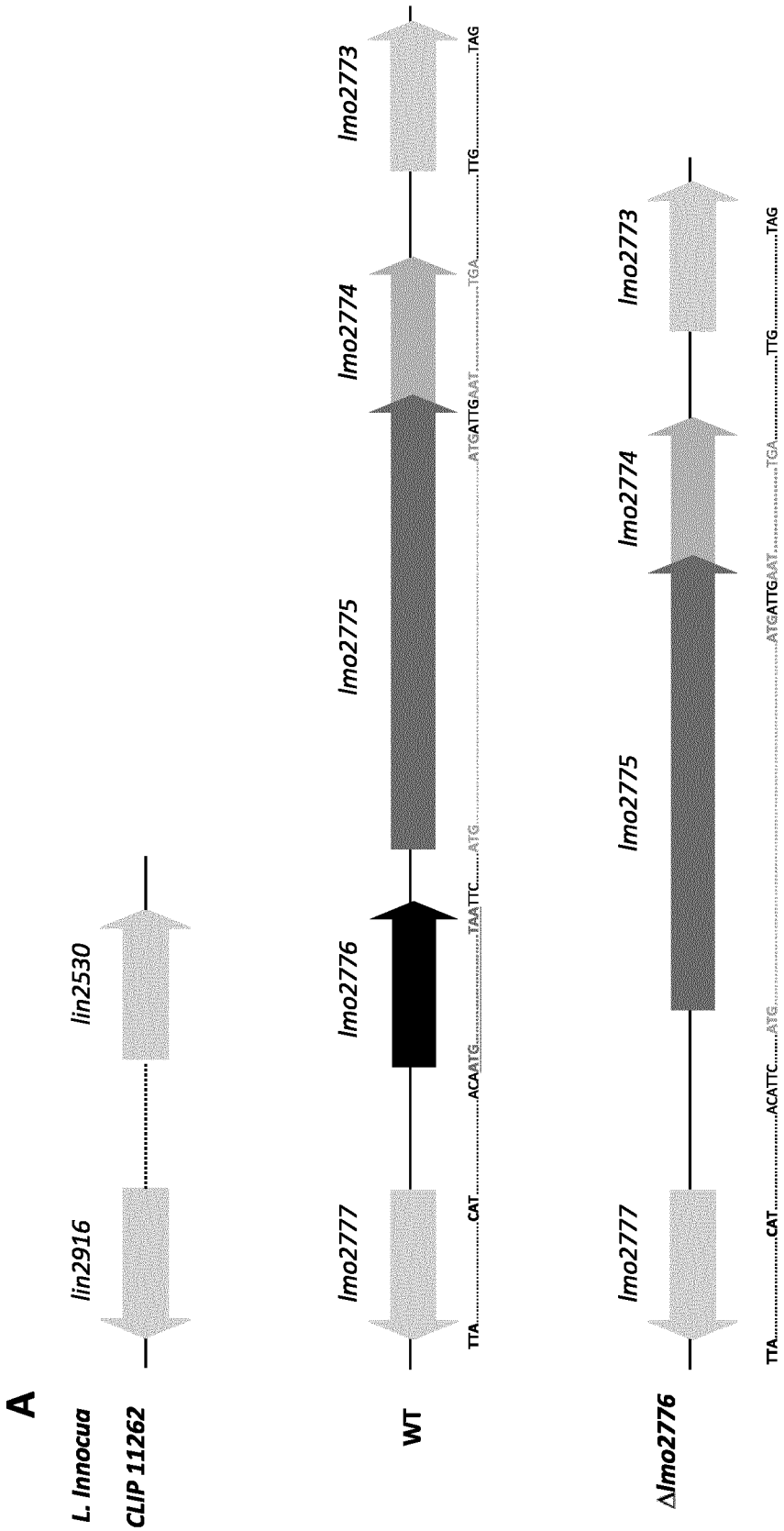
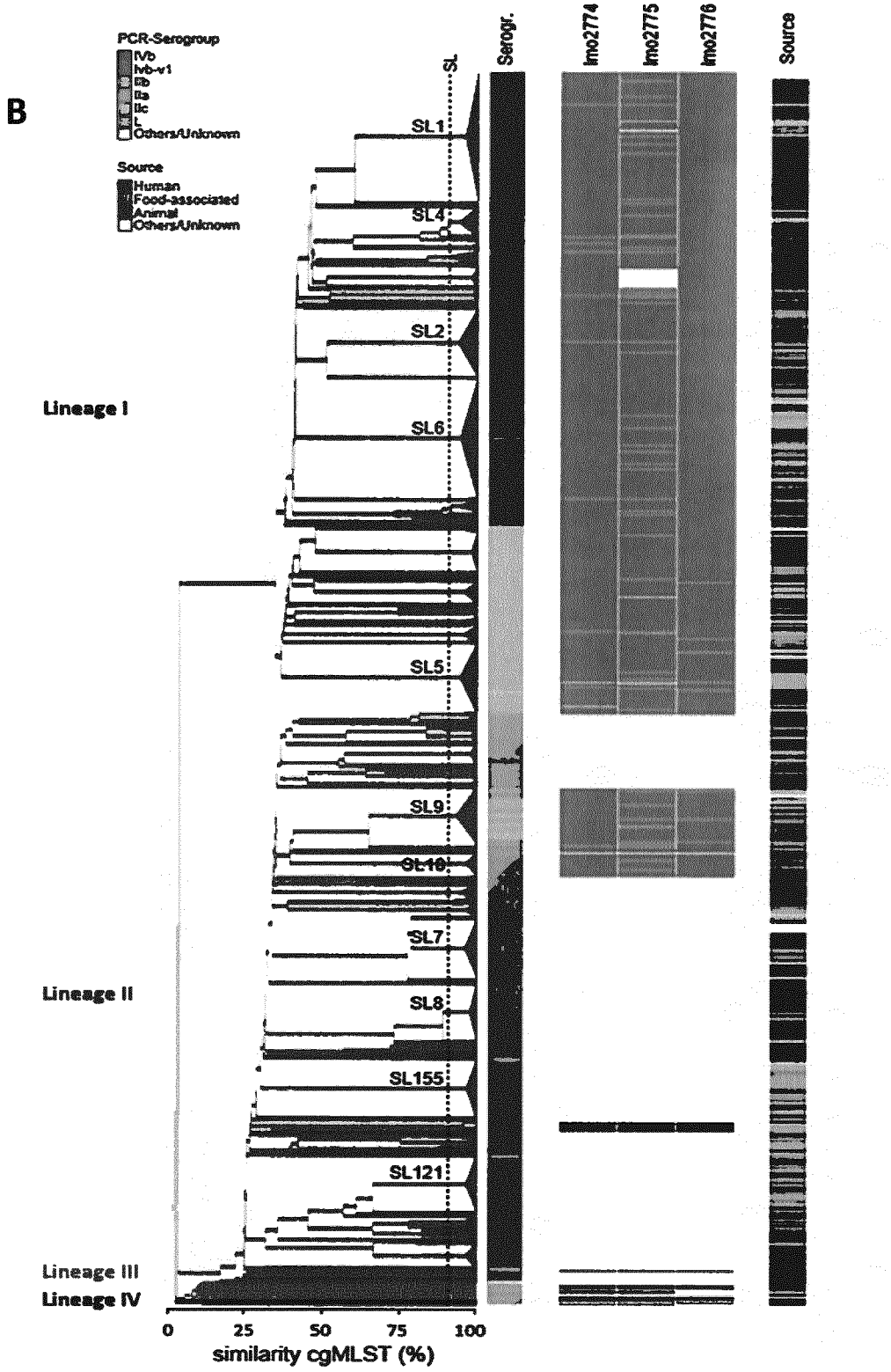


Figure 5


A
L. Innocua CLIP 11262
lin2916
lin2530
WT
lmo2777
lmo2776
lmo2775
lmo2774
lmo2773
TTA.....CAT.....ACAATG.....TAATTC.....ATG.....ATGATTGAAT.....TGA.....TTG.....TAG
ΔImo2776
lmo2777
lmo2775
lmo2774
lmo2773
TTA.....CAT.....ACATTC.....ATG.....ATGATTGAAT.....TGA.....TTG.....TAG

Continuation of figure 5



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C

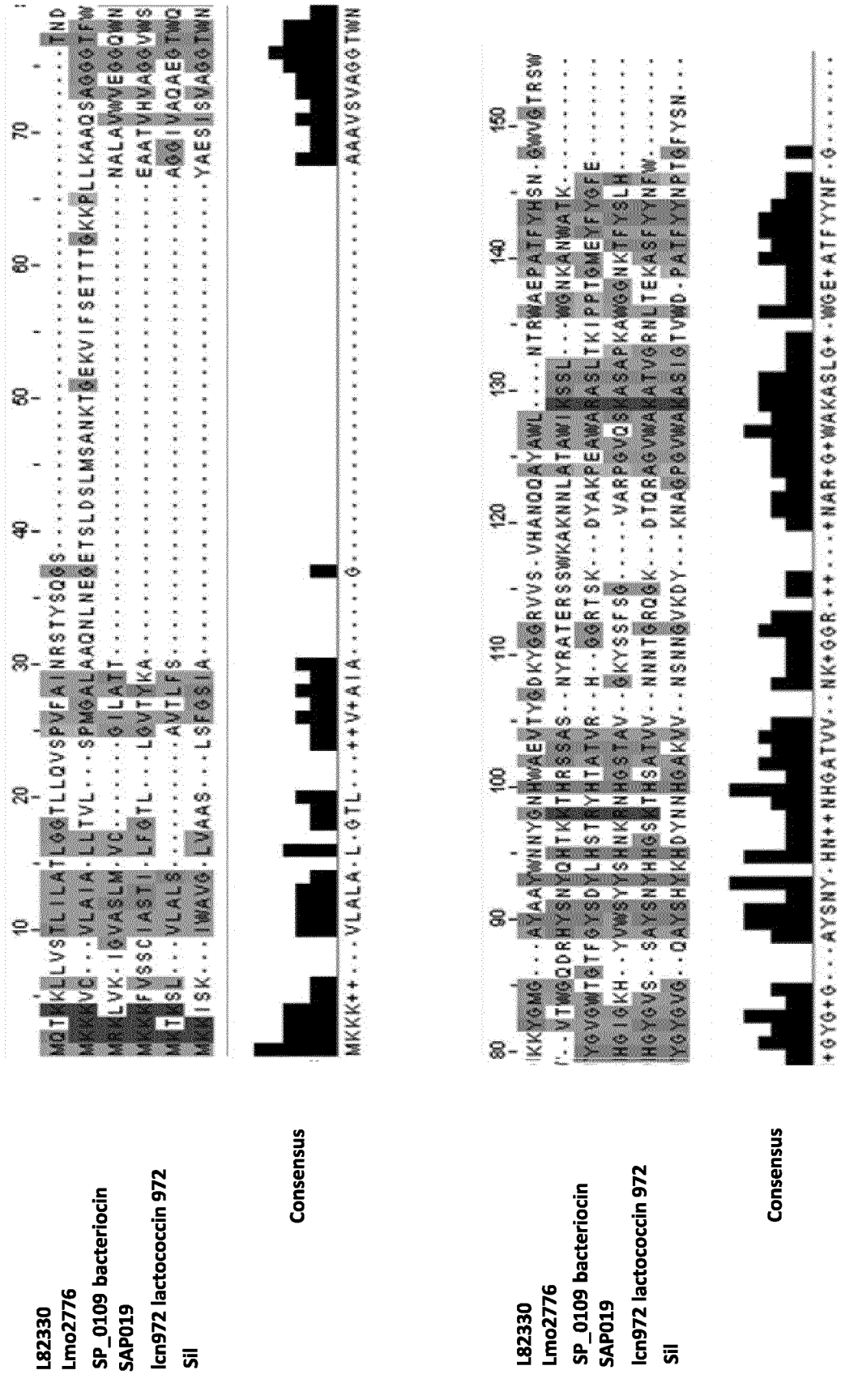


Figure 6

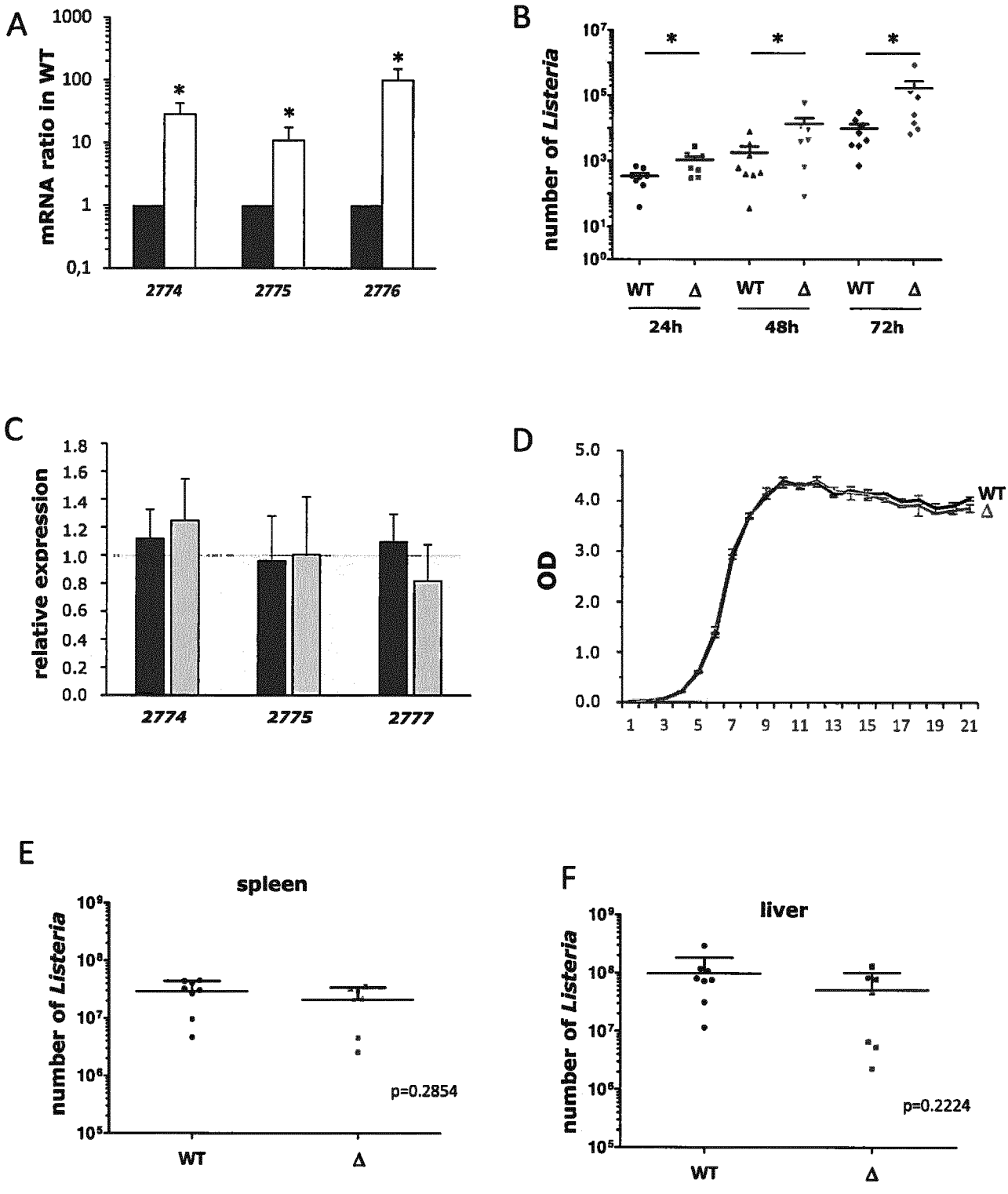
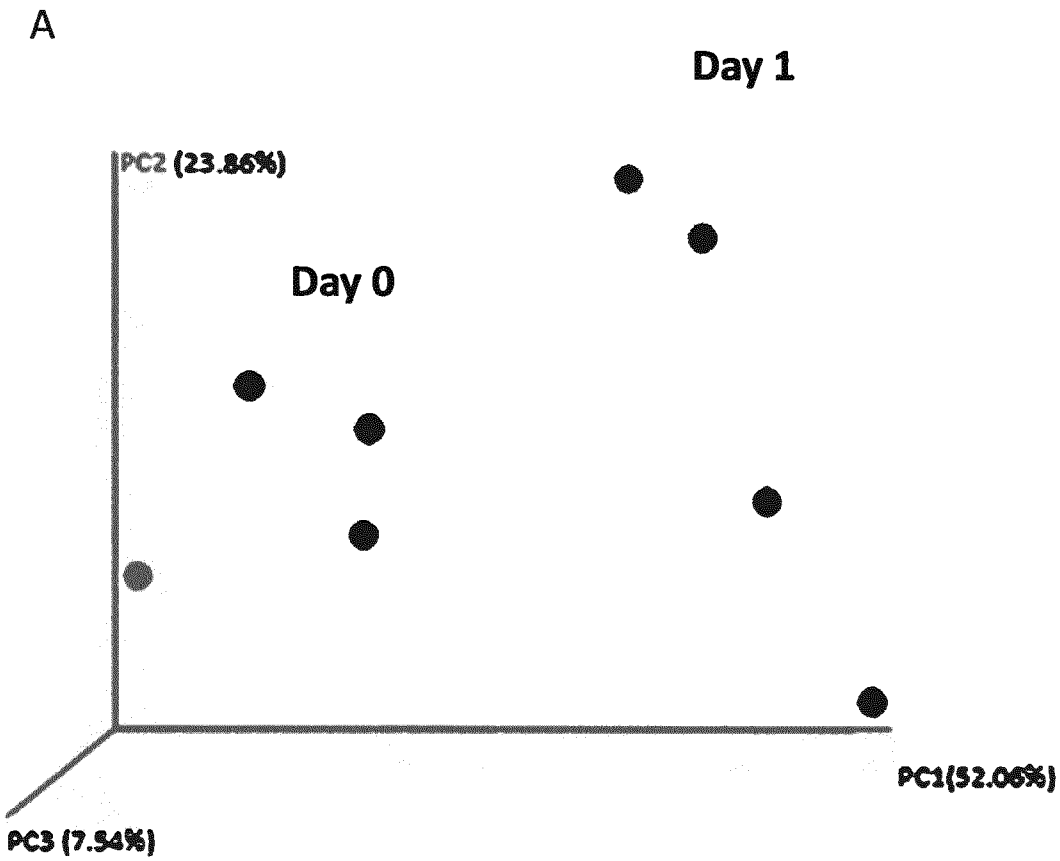
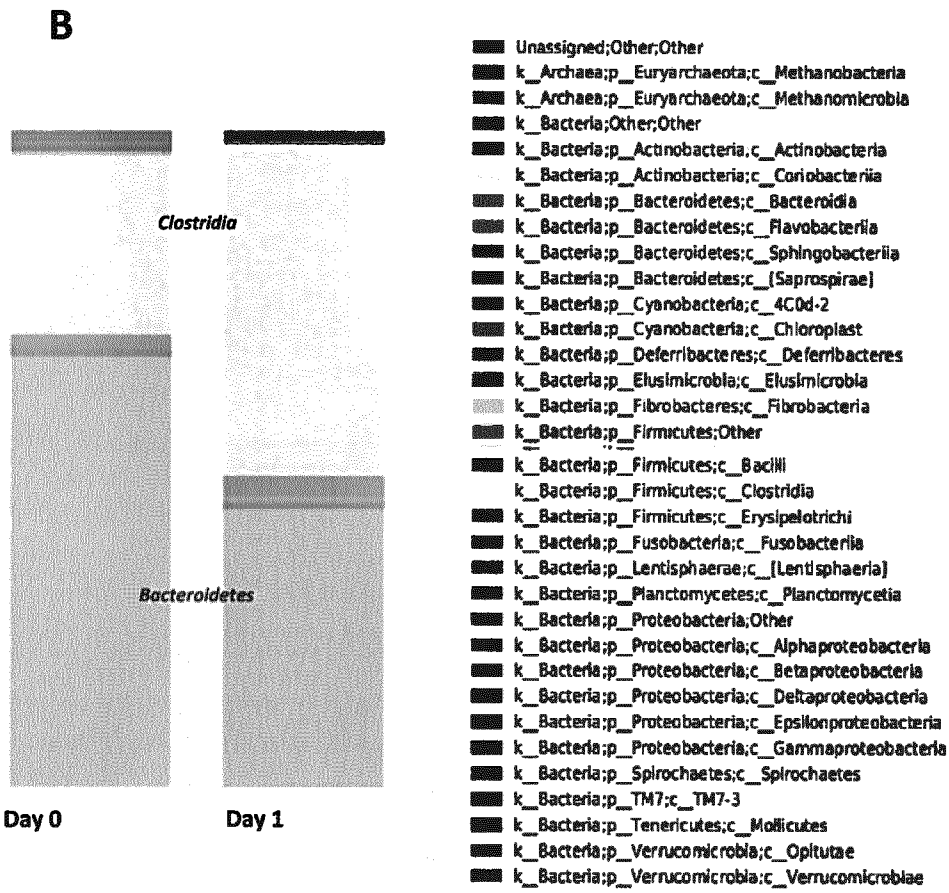

A
mRNA ratio in WT
1000
100
10
1
0,1
2774
2775
2776
B
number of Listeria
WT
Δ
24h
48h
72h
C
relative expression
2774
2775
2777
D
OD
WT
Δ
E
spleen
number of Listeria
p=0.2854
WT
Δ
F
liver
number of Listeria
p=0.2224
WT
Δ

Figure 7

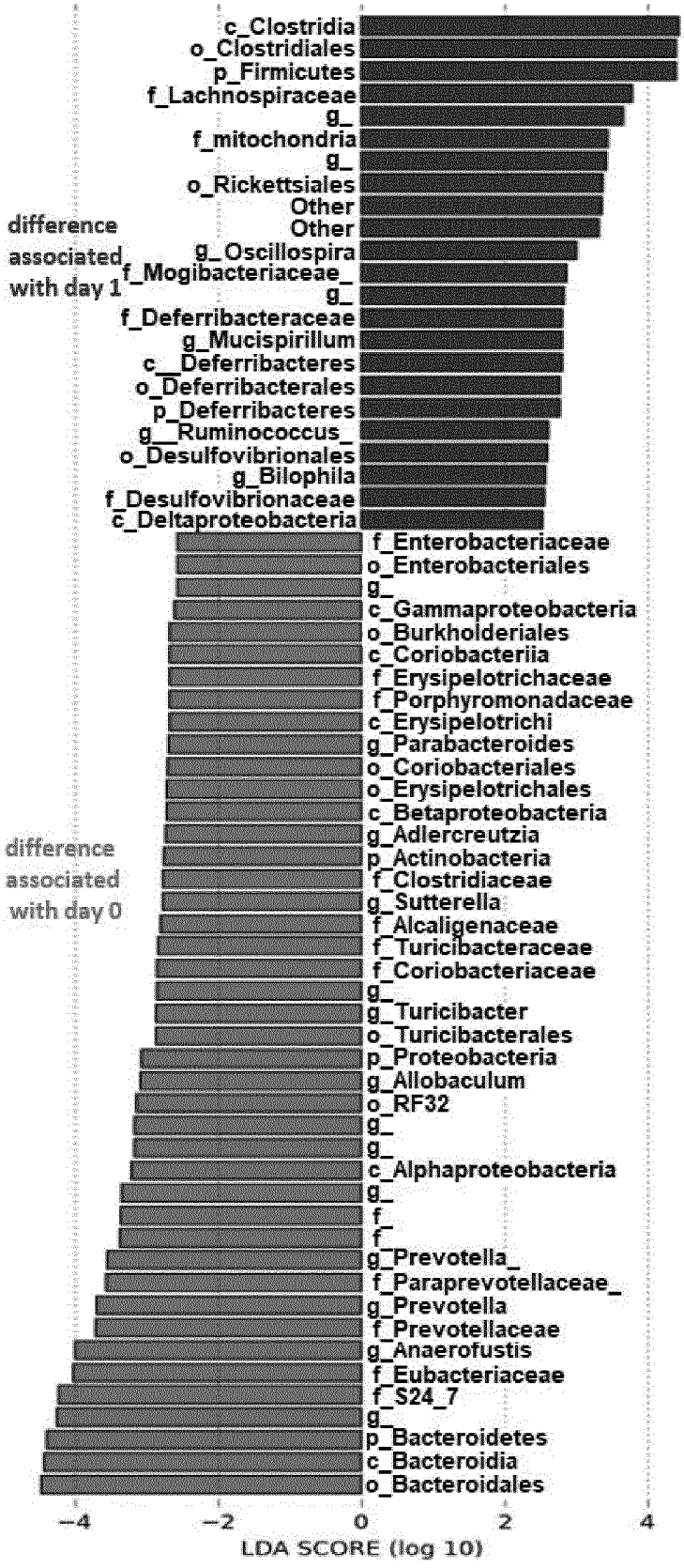


Continuation of figure 7



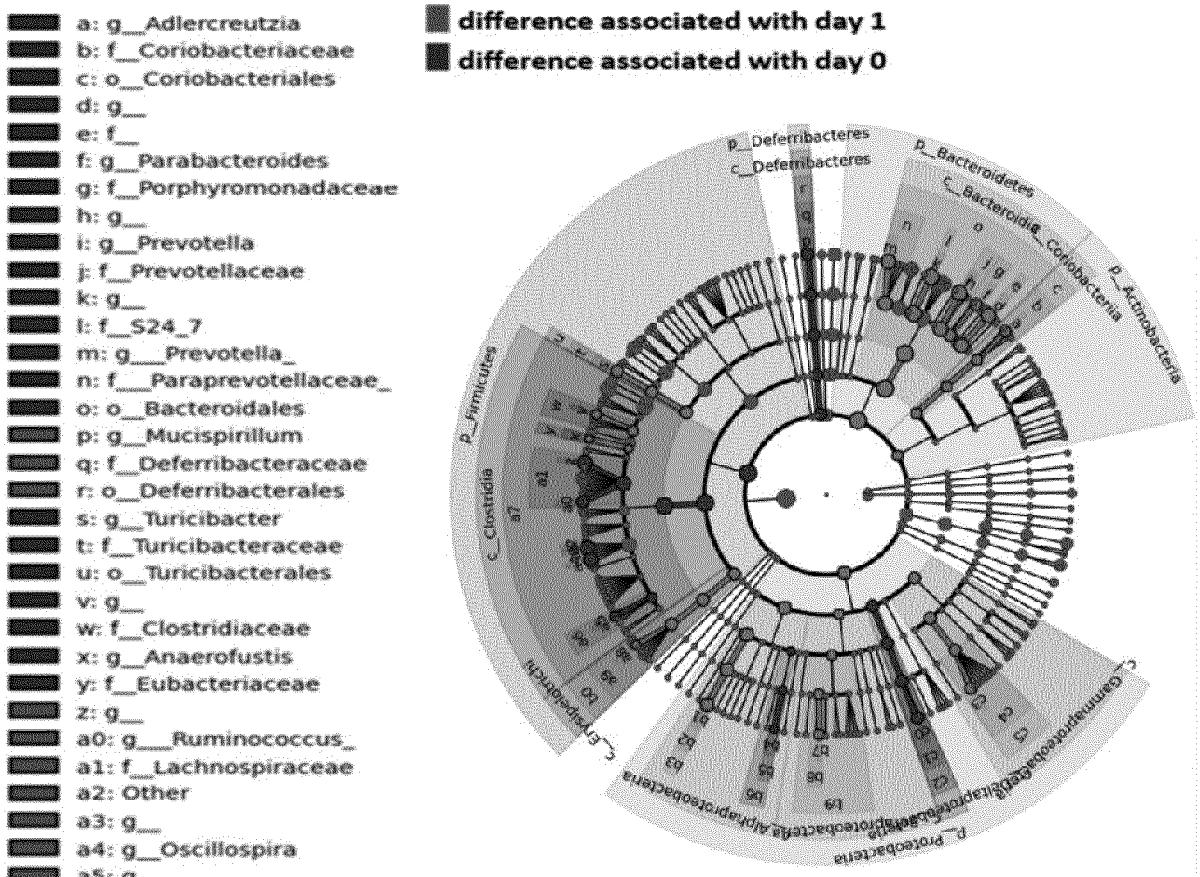
Continuation of figure 7

C



Continuation of figure 7

D



E

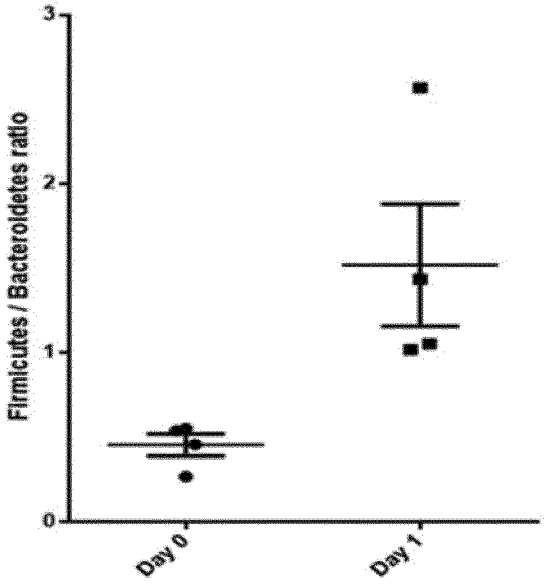
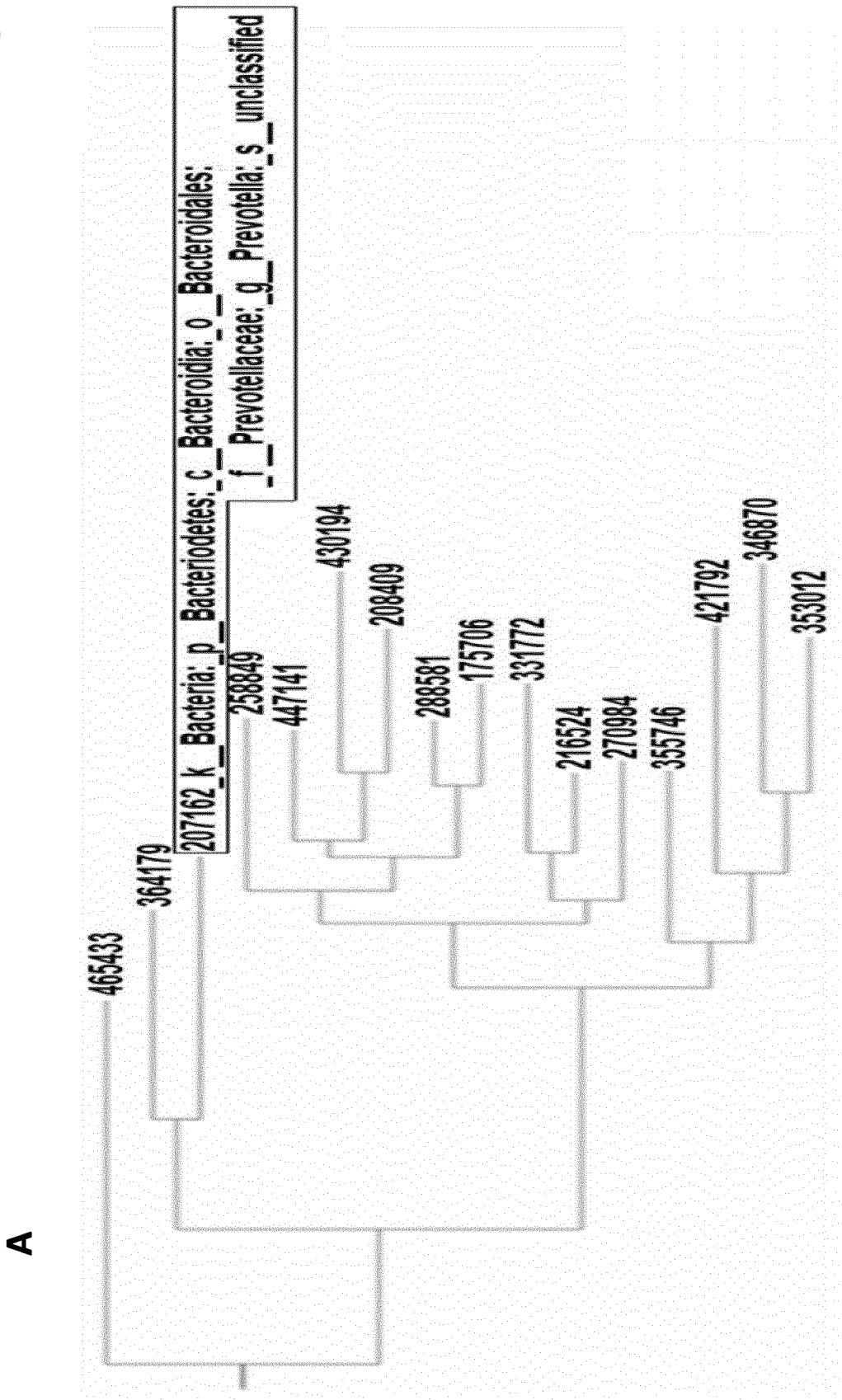
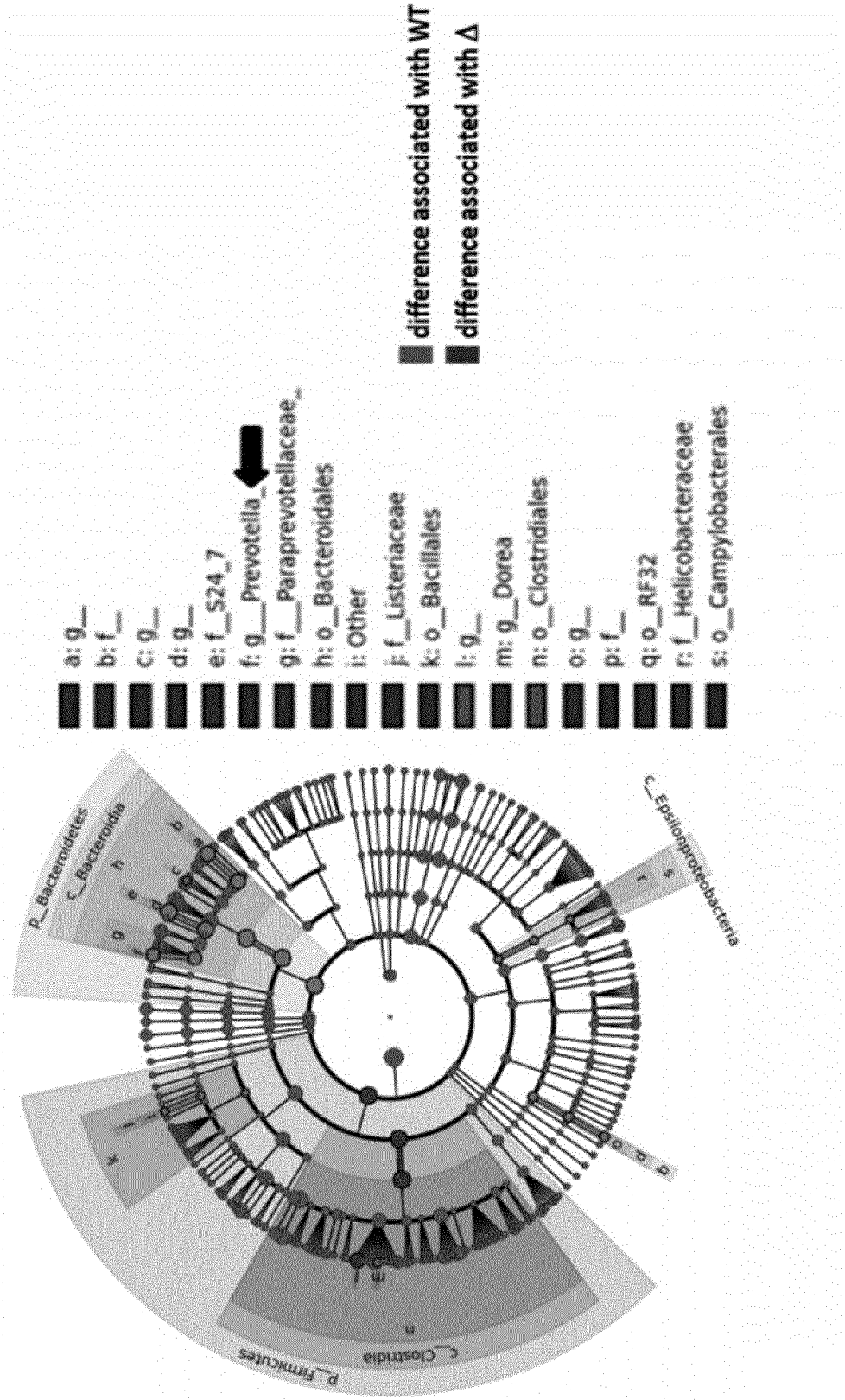


Figure 8

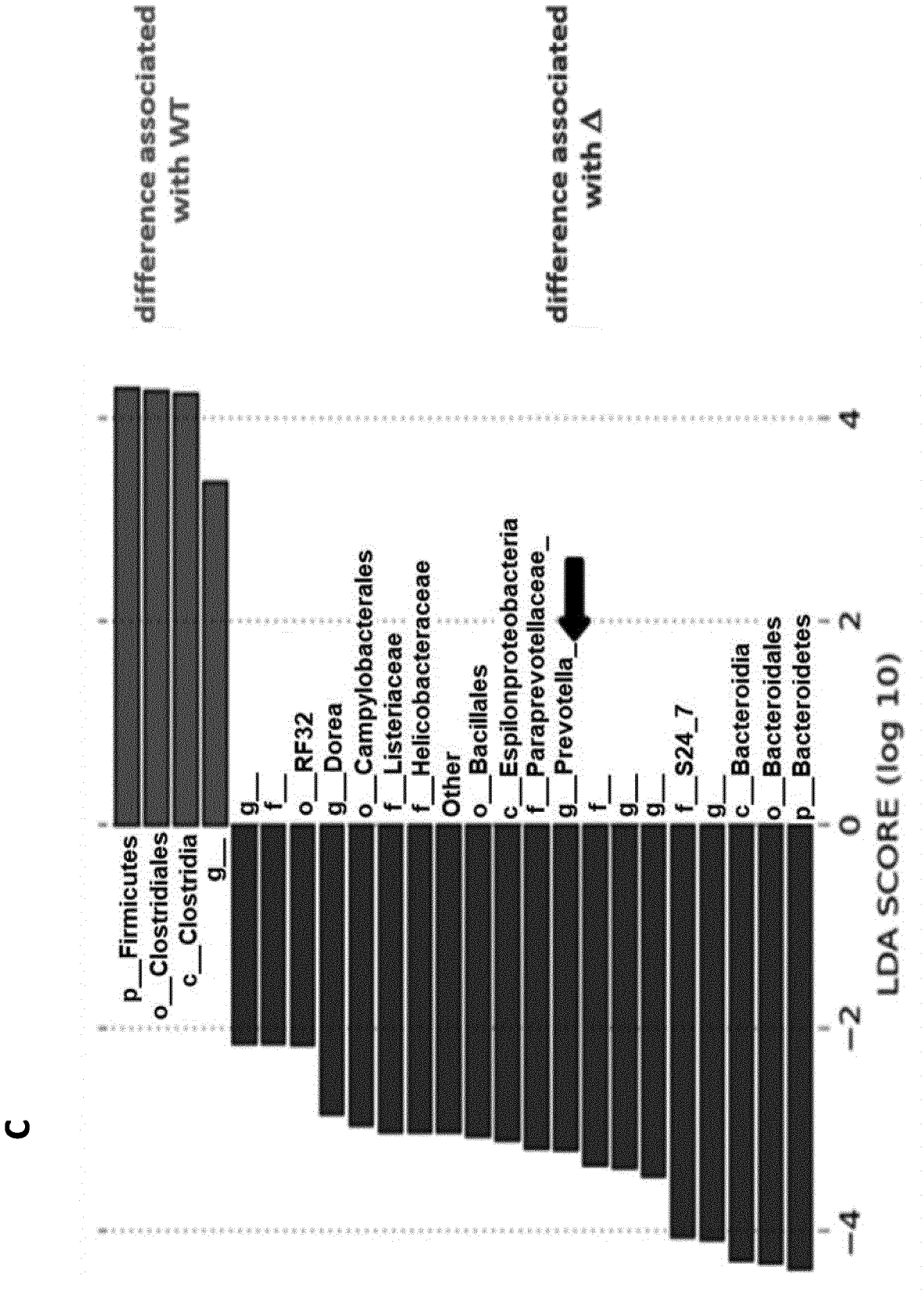

A
207162 k__Bacteria; p__Bacteriodetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__unclassified
364179
465433
258849
447141
430194
208409
288581
175706
331772
216524
270984
355746
421792
346870
353012

Continuation of figure 8

B



Continuation of figure 8



Continuation of figure 8

D

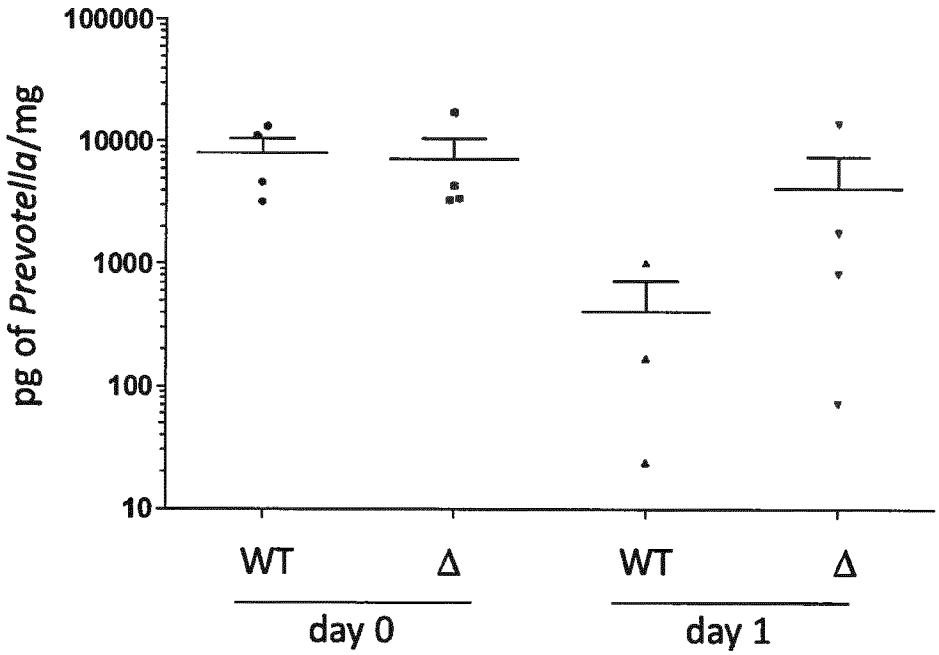


Figure 9

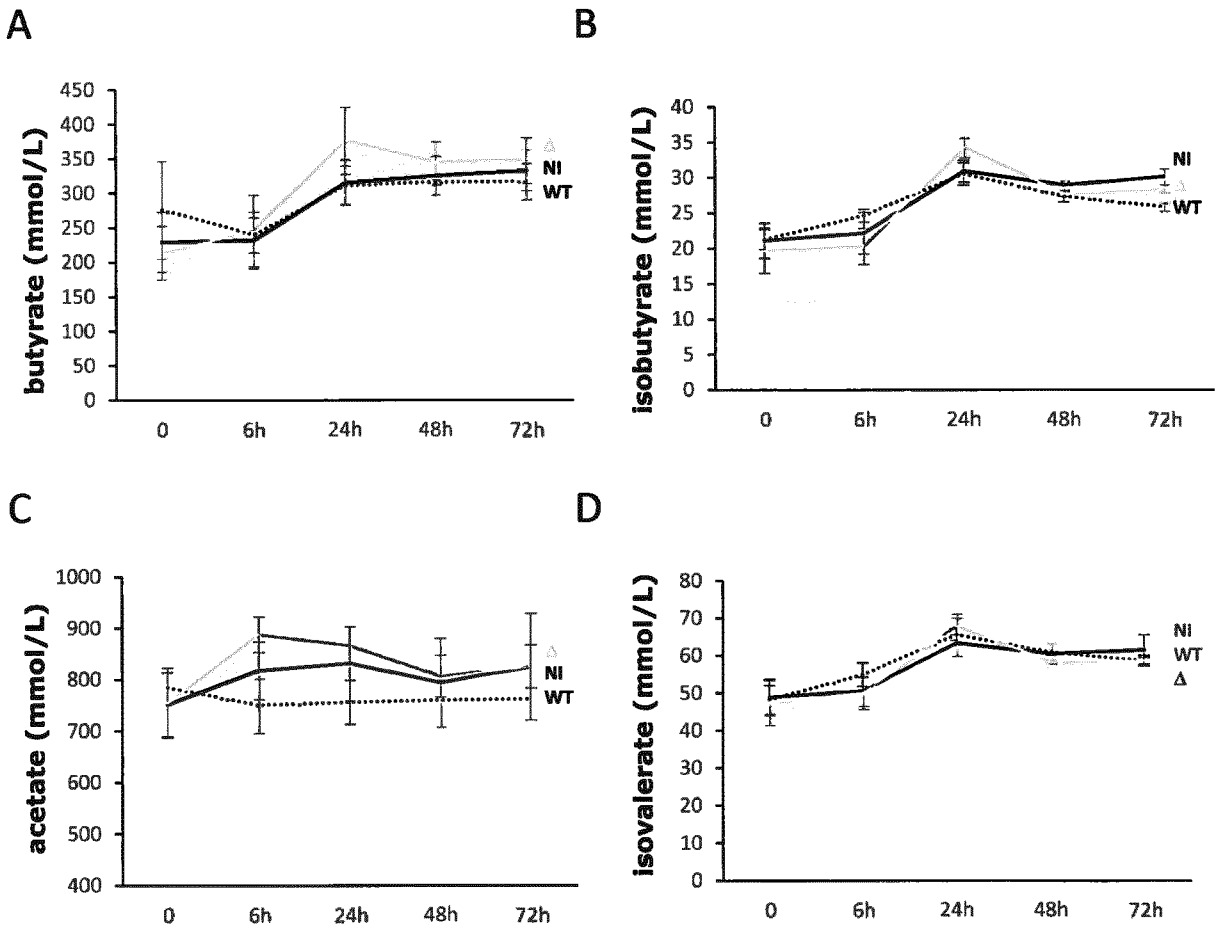

A
butyrate (mmol/L)
450
400
350
300
250
200
150
100
50
0
0
6h
24h
48h
72h
NI
WT
Δ
B
isobutyrate (mmol/L)
40
35
30
25
20
15
10
5
0
0
6h
24h
48h
72h
NI
WT
Δ
C
acetate (mmol/L)
1000
900
800
700
600
500
400
0
6h
24h
48h
72h
NI
WT
Δ
D
isovalerate (mmol/L)
80
70
60
50
40
30
20
10
0
0
6h
24h
48h
72h
NI
WT
Δ

Figure 10

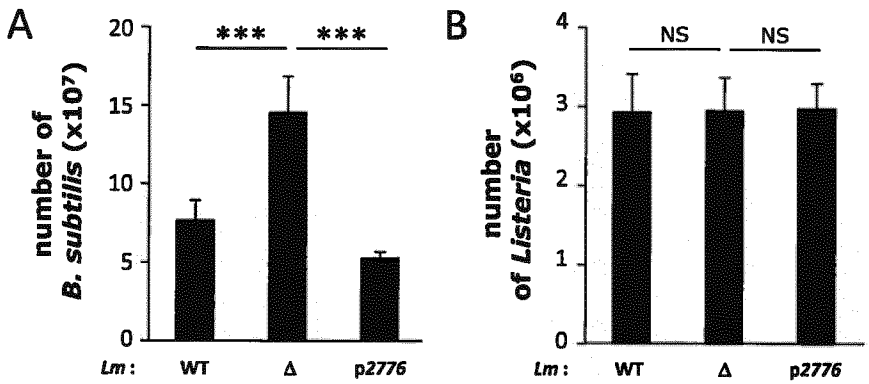

A
number of B. subtilis (x10⁷)
20
15
10
5
0
***
***
Lm :
WT
Δ
p2776
B
number of Listeria (x10⁶)
4
3
2
1
0
NS
NS
Lm :
WT
Δ
p2776

Figure 11

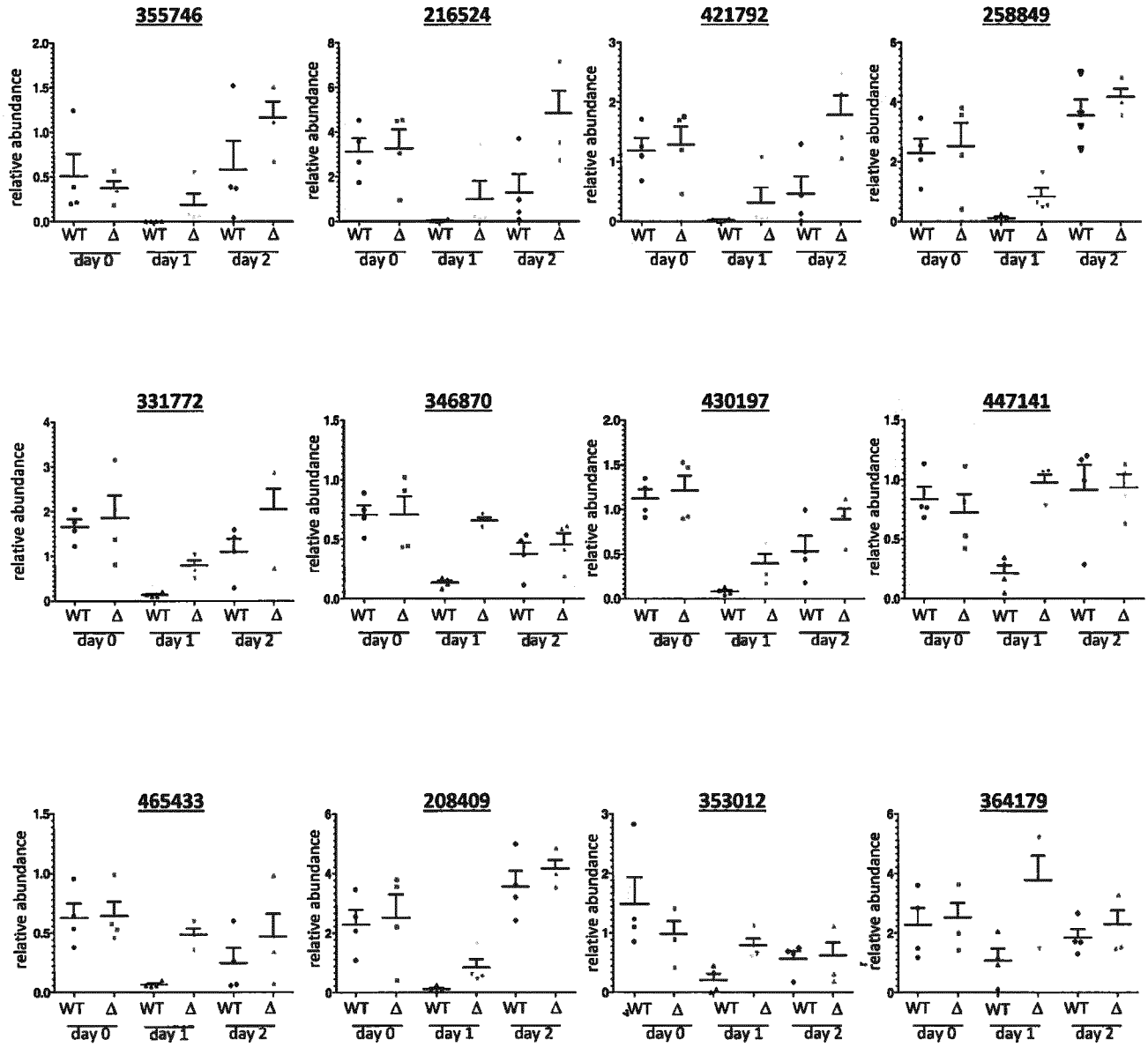
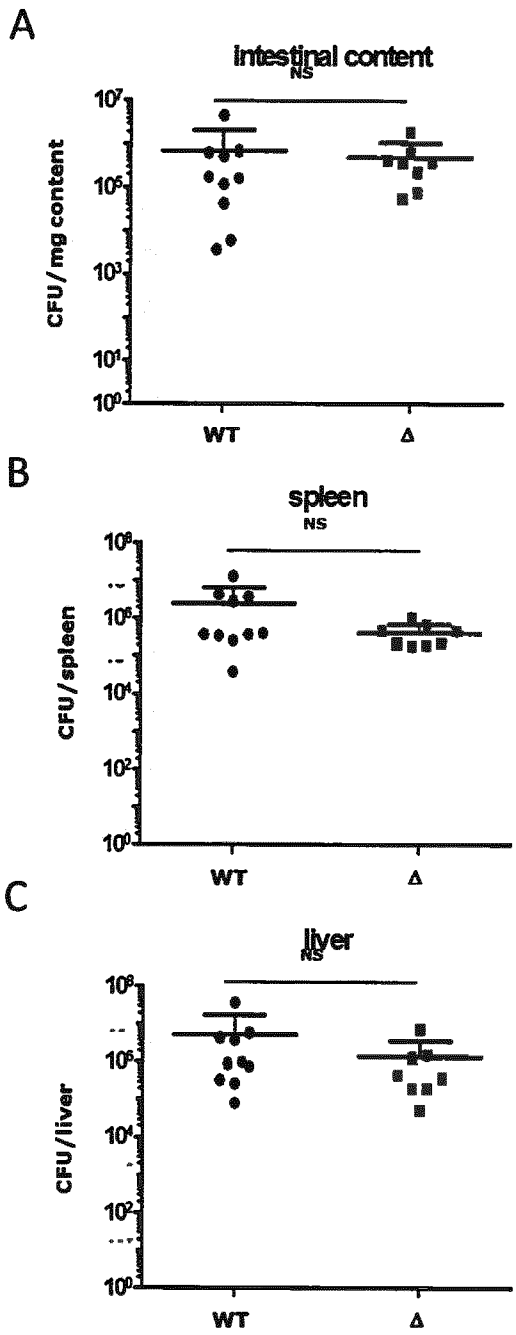

355746
216524
421792
258849
331772
346870
430197
447141
465433
208409
353012
364179
relative abundance
WT
Δ
day 0
day 1
day 2

Figure 12


A
Intestinal content
NS
CFU/mg content
10^7
10^5
10^3
10^1
10^0
WT
Δ
B
spleen
NS
CFU/spleen
10^6
10^4
10^2
10^0
WT
Δ
C
liver
NS
CFU/liver
10^6
10^4
10^2
10^0
WT
Δ

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2019/053170

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/07 A61P31/04 A61K38/16
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

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, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2010/149792 A2 (UNIV LEUVEN KATH [BE]; LYSANDO HOLDING AG [LI] ET AL.) 29 December 2010 (2010-12-29)	1,9,11
Y	the whole document pages 7-9 and 20-23	12-15

X	WO 01/77335 A2 (PASTEUR INSTITUT [FR]) 18 October 2001 (2001-10-18) the whole document in particular, SEQ:2632 and claim 115	1-6

X	EP 2 682 463 A1 (UNIV HIROSHIMA [JP]) 8 January 2014 (2014-01-08)	7-11
Y	the whole document in particular, pages 5-6	12-15

	-/--	

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"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

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 May 2019	27/05/2019

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Pérez-Mato, Isabel

2

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	AHMAD VARISH ET AL: "Antimicrobial potential of bacteriocins: in therapy, agriculture and food preservation", INTERNATIONAL JOURNAL OF ANTIMICROBIAL AGENTS, ELSEVIER, AMSTERDAM, NL, vol. 49, no. 1, 29 September 2016 (2016-09-29), pages 1-11, XP029862502, ISSN: 0924-8579, DOI: 10.1016/J.IJANTIMICAG.2016.08.016 the whole document in particular, sections 1 and 11 -----	1-15
A	RIADH HAMMAMI ET AL: "Anti-infective properties of bacteriocins: an update", CELLULAR AND MOLECULAR LIFE SCIENCES, vol. 70, no. 16, 1 August 2013 (2013-08-01), pages 2947-2967, XP055116653, ISSN: 1420-682X, DOI: 10.1007/s00018-012-1202-3 the whole document in particular, page 2956 -----	1-15
A	JOSE U SCHER ET AL: "Expansion of intestinal Prevotella copri correlates with enhanced susceptibility to arthritis", ELIFE, vol. 2, 5 November 2013 (2013-11-05), pages 1-20, XP055475267, GB ISSN: 2045-5267, DOI: 10.7554/eLife.01202 the whole document -----	7,8

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2019/053170

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