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(54) Title: COMPOSITIONS COMPRISING BACTERIAL STRAINS

(57) Abstract: The invention provides compositions comprising bacterial strains for treating and preventing a neurodegenerative disorder.

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COMPOSITIONS COMPRISING BACTERIAL STRAINS

TECHNICAL FIELD

This invention is in the field of compositions comprising bacterial strains isolated from the mammalian digestive tract and the use of such compositions in the treatment of disease.

BACKGROUND TO THE INVENTION

The human intestine is thought to be sterile *in utero*, but it is exposed to a large variety of maternal and environmental microbes immediately after birth. Thereafter, a dynamic period of microbial colonization and succession occurs, which is influenced by factors such as delivery mode, environment, diet and host genotype, all of which impact upon the composition of the gut microbiota, particularly during early life. Subsequently, the microbiota stabilizes and becomes adult-like [1]. The human gut microbiota contains more than 500-1000 different phylotypes belonging essentially to two major bacterial divisions, the Bacteroidetes and the Firmicutes [2]. The successful symbiotic relationships arising from bacterial colonization of the human gut have yielded a wide variety of metabolic, structural, protective and other beneficial functions. The enhanced metabolic activities of the colonized gut ensure that otherwise indigestible dietary components are degraded with release of by-products providing an important nutrient source for the host. Similarly, the immunological importance of the gut microbiota is well-recognized and is exemplified in germfree animals which have an impaired immune system that is functionally reconstituted following the introduction of commensal bacteria [3-5].

Dramatic changes in microbiota composition have been documented in gastrointestinal disorders such as inflammatory bowel disease (IBD). For example, the levels of *Clostridium* cluster XIVa bacteria are reduced in IBD patients whilst numbers of *E. coli* are increased, suggesting a shift in the balance of symbionts and pathobionts within the gut [6-9].

In recognition of the potential positive effect that certain bacterial strains may have on the animal gut, various strains have been proposed for use in the treatment of various diseases (see, for example, [10-13]). Also, certain strains, including mostly *Lactobacillus* and *Bifidobacterium* strains, have been proposed for use in treating various inflammatory and autoimmune diseases that are not directly linked to the intestines (see [14] and [15] for reviews). However, the relationship between different diseases and different bacterial strains, and the precise effects of particular bacterial strains on the gut and at a systemic level and on any particular types of diseases are poorly characterised, particularly for neurodegenerative disorders.

Recently, there has been increased interest in the art regarding alterations in the gut microbiome that may play a pathophysiological role in human brain diseases [16]. Preclinical and clinical evidence are strongly suggesting a link between brain development and microbiota [17]. A growing body of preclinical literature has demonstrated bidirectional signalling between the brain and the gut microbiome, involving multiple neurocrine and endocrine signalling systems. Indeed, increased levels

of *Clostridium* species in the microbiome have been linked to brain disorders [18], and an imbalance of the *Bacteroidetes* and *Firmicutes* phyla has also been implicated in brain development disorders [19]. Suggestions that altered levels of gut commensals, including those of *Bifidobacterium*, *Lactobacillus*, *Sutterella*, *Prevotella* and *Ruminococcus* genera and of the *Alcaligenaceae* family are involved in immune-mediated central nervous system (CNS) disorders, are questioned by studies suggesting a lack of alteration in the microbiota between patients and healthy subjects [10]. *Parabacteroides distasonis* has been proposed for treating a variety of disorders including asthma, rheumatoid arthritis and multiple sclerosis [20]

Like asthma and rheumatoid arthritis, multiple sclerosis is primarily mediated by the immune system. The immune system attacks myelinated axons in the central nervous system, destroying the myelin called plaques or lesions. Demyelination occurs in particular in the optic nerves, subpial spinal cord, brainstem, cerebellum, and juxtacortical and periventricular white matter regions.

As such, multiple sclerosis has a different pathology to other neurodegenerative diseases, such as Parkinson's disease, Alzheimer's disease or dementia. For example, multiple sclerosis is commonly diagnosed in patients in their 20s and 30s, while many other neurodegenerative diseases, such as Parkinson's disease, Alzheimer's and dementia, are diagnosed predominantly in patients aged over 65 years old.

Parkinson's disease, like many neurodegenerative diseases, is primarily mediated by the accumulation of misfolded protein. Parkinson's disease is a synucleinopathology that involves the accumulation of α-synuclein, which aggregate as insoluble fibrils in Lewy bodies within the cytoplasm of the neuronal body. The accumulation of a-synuclein is toxic and impairs the functions of mitochondria, lysosomes, and endoplasmic reticulum, and interferes with microtubule transport.

Nonsteroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen, have been tested for their efficacy in treating a variety of neurological diseases, but the clinical impact of NSAIDs on neurodegenerative diseases like Parkinson's disease remains unclear. While some studies showed that chronic NSAID use is protective against Parkinson's disease, other studies could not confirm the existence of a significant relationship. A recent meta-analysis indicated that the use of non-aspirin NSAID, particularly ibuprofen, reduces the risk of PD by 15% while the use of aspirin did not show any effect [21].

At present, the practical effect of the link between the microbiome and human brain diseases is poorly characterised. Accordingly, more direct analytical studies are required to identify the therapeutic impact of altering the microbiome on neurodegenerative disorders.

There is a requirement in the art for new methods of treating neurodegenerative disorders. There is also a requirement for the potential effects of gut bacteria to be characterised so that new therapies using gut bacteria can be developed.

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SUMMARY OF THE INVENTION

The inventors have developed new therapies for treating and preventing neurodegenerative disorders.

Accordingly, in one aspect, the present invention provides a method of treating or preventing a neurodegenerative disorder selected from the group consisting of Parkinson's disease, including progressive supranuclear palsy, progressive supranuclear palsy, Steele-Richardson-Olszewski syndrome, normal pressure hydrocephalus, vascular or arteriosclerotic parkinsonism and drug-induced parkinsonism; Alzheimer's disease, including Benson's syndrome; Huntington's disease; amyotrophic lateral sclerosis; Lou Gehrig's disease; motor neurone disease; prion disease; spinocerebellar ataxia; spinal muscular atrophy; dementia, including Lewy body, vascular and frontotemporal dementia; primary progressive aphasia; mild cognitive impairment; HIV-related cognitive impairment and corticobasal degeneration, comprising administering a composition comprising a bacterial strain of the genus *Parabacteroides* to a patient in need thereof.

In another aspect, the present invention provides the use of a composition comprising a bacterial strain of the genus *Parabacteroides*, in the manufacture of a medicament for treating or preventing a neurodegenerative disorder selected from the group consisting of Parkinson's disease, including progressive supranuclear palsy, progressive supranuclear palsy, Steele-Richardson-Olszewski syndrome, normal pressure hydrocephalus, vascular or arteriosclerotic parkinsonism and drug-induced parkinsonism; Alzheimer's disease, including Benson's syndrome; Huntington's disease; amyotrophic lateral sclerosis; Lou Gehrig's disease; motor neurone disease; prion disease; spinocerebellar ataxia; spinal muscular atrophy; dementia, including Lewy body, vascular and frontotemporal dementia; primary progressive aphasia; mild cognitive impairment; HIV-related cognitive impairment and corticobasal degeneration in a patient.

In another aspect, the present invention provides a method of treating brain injury comprising administering a composition comprising a bacterial strain of the genus *Parabacteroides* to a patient in need thereof.

In another aspect, the present invention provides the use of a composition comprising a bacterial strain of the genus *Parabacteroides*, in the manufacture of a medicament for treating brain injury in a patient.

In another aspect, the present invention provides a food product comprising the composition comprising a bacterial strain of the genus *Parabacteroides*, when used in a method of the invention.

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In another aspect, the present invention provides the use of a food product comprising the composition comprising a bacterial strain of the genus *Parabacteroides*, in the manufacture of a medicament for treating or preventing a neurodegenerative disorder in a patient.

The inventors have identified that bacterial strains from the genus *Parabacteroides* may be effective for treating neurodegenerative diseases. As described in the examples, administration of compositions comprising *Parabacteroides distasonis* can protect against reactive oxygen species and prevent inflammation, thus acting as a neuroprotectant. The inventors have also identified that treatment with *Parabacteroides distasonis* can reduce the activation of proinflammatory molecules, such as NFκB and IL-6, by LPS and mutant α-synuclein A53T. The inventors have identified that treatment with *Parabacteroides distasonis* can reduce histone deacetylation activity and lipid peroxidation *in vitro*, which can help to reduce cell death and apoptosis. The inventors have also identified that *Parabacteroides distasonis* can produce indole that can attenuate inflammation and oxidative stress. Furthermore, the inventors have demonstrated that treatment with *Parabacteroides distasonis* can increase kynurenine levels.

The inventors have also found that treatment with *Parabacteroides distasonis* increases the activation of BDNF. BDNF is a neurotrophic growth factor that has been shown to enhance neuron differentiation and survival. Thus, the compositions described herein can be used in a method of enhancing nerve cell survival in the treatment or prevention of neurodegenerative diseases.

Disclosed herein is a composition comprising a bacterial strain of the genus *Parabacteroides*, for use in a method of treating or preventing a neurodegenerative disorder.

Also disclosed herein is a composition comprising a bacterial strain of the genus *Parabacteroides*, for use in a method of treating or preventing a disease or condition selected from the group consisting of: Parkinson's disease, including progressive supranuclear palsy, progressive supranuclear palsy, Steele-Richardson-Olszewski syndrome, normal pressure hydrocephalus, vascular or arteriosclerotic parkinsonism and drug-induced parkinsonism; Alzheimer's disease, including Benson's syndrome; multiple sclerosis; Huntington's disease; amyotrophic lateral sclerosis; Lou Gehrig's disease; motor neurone disease; prion disease; spinocerebellar ataxia; spinal muscular atrophy; dementia, including Lewy body, vascular and frontotemporal dementia; primary progressive aphasia; mild cognitive impairment; HIV-related cognitive impairment and corticobasal degeneration.

Also disclosed herein is a composition comprising a bacterial strain of the genus *Parabacteroides*, for use in a method of treating or preventing Parkinson's disease, such as environmental, familial or Parkinson's associated with general inflammatory status. The inventors have identified that

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treatment with *Parabacteroides* strains can reduce the activation of proinflammatory molecules, such as NFκB and IL-6, by LPS and mutant α-synuclein A53T in *in vitro* models of environmental and familial Parkinson's. Also disclosed herein is a composition comprising a bacterial strain of the species *Parabacteroides distasonis,* for use in the treatment of Parkinson's disease. Compositions using *Parabacteroides distasonis* may be particularly effective for treating Parkinson's.

Also disclosed herein are compositions for use in a method of treating or preventing early-onset neurodegenerative disease. In some embodiments, the compositions of the invention are for use in a method of preventing or delaying onset or progression of a neurodegenerative disorder.

In preferred embodiments, the bacterial strain in the composition disclosed herein is of *Parabacteroides distasonis*. Closely related strains may also be used, such as bacterial strains that have a 16S rRNA sequence that is at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% identical to the 16S rRNA sequence of a bacterial strain of *Parabacteroides distasonis*. Preferably, the bacterial strain has a 16S rRNA sequence that is at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% identical to SEQ ID NO:1, 2, 3, 4, 5, 6, 7 8 or 9. Preferably, the sequence identity is to SEQ ID NO:9. Preferably, the bacterial strain disclosed herein has the 16S rRNA sequence represented by SEQ ID NO:9.

In certain embodiments, the composition of the invention is for oral administration. Oral administration of the strains of disclosed herein can be effective for neurodegenerative disorders. Also, oral administration is convenient for patients and practitioners and allows delivery to and / or partial or total colonisation of the intestine.

In certain embodiments, the composition of the invention comprises one or more pharmaceutically acceptable excipients or carriers.

In certain embodiments, the composition of the invention comprises a bacterial strain that has been lyophilised. Lyophilisation is an effective and convenient technique for preparing stable compositions that allow delivery of bacteria.

In certain embodiments, the disclosure provides a food product comprising the composition as described above.

In certain embodiments, the disclosure provides a vaccine composition comprising the composition as described above.

Additionally, disclosed herein is a method of treating or preventing neurodegenerative disorders, comprising administering a composition comprising a bacterial strain of the genus *Parabacteroides*.

In certain embodiments, the composition disclosed herein may be for use in treating brain injury. The neuroprotective activity of the compositions of the invention and their ability to reduce levels

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of histone deacetylase activity (HDAC) may make them useful for treating brain injury. In preferred embodiments, the compositions of disclosed herein may be for use in treating stroke, such as treating brain injury resulting from a stroke.

Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present disclosure as it existed before the priority date of each claim of this application.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1: Cell viability of neuroblastoma cells

Figure 2A: Downregulation of IL-6 secretion Figure 2B: Downregulation of IL-8 secretion

Figure 3: Inhibition of α- synuclein IL-6 and IL-8 secretion

Figure 4: Inhibition of α- synuclein induced NFκB promoter activation

Figure 5: Inhibition of LPS induced NFκB promoter activation

Figure 6: Change in antioxidant capacity

Figure 7: Change in total anti-oxidant capacity (lipid oxidation)

Figure 8: Change in histone deacetylatase (HDAC) activity

Figure 9: Level of Indole production

Figure 10: Level of Kyrunenine production

Figure 11: Neuroprotection – cell viability. Figure 11 shows the same data as Figure 1.

Figure 12: Levels of metabolite production – neurotransmitters in the brain

Figure 13: Levels of metabolite production – organic acids in the supernatant

Figure 14: Effect on intestinal barrier function

Figure 15: Level of BDNF production

Figure 16: Change in ROS levels in (A) U373 cells, (B) SH-SY5Y cells

Figure 17: Production of neurotransmitters in the brain

Figure 18: Changes in Hippocampal Receptor Expression – A) Oxytocin Receptor, B) Vasopressin Receptor, C) Glucocorticoid Receptor and D) Mineralocorticoid Receptor

Figure 19: Changes in Hippocampal Expression of A) Corticotropin-Releasing Hormone (CRH), B) BDNF Expression and C) TLR4

Figure 20: A) Changes in Hippocampal Corticotropin Releasing Hormone Receptor 1 (CRFR1) Expression and B) Corticotropin Releasing Hormone Receptor 2 (CRFR2) Expression

Figure 21: Changes in Hippocampal Expression of A) Tumour Necrosis Factor, B) Interleukin 1b and C) IL-6

Figure 22: A) Changes in Hippocampal Integrin Alpha M (CD11b) Expression and B) Changes in Hippocampal Serotonin 1A Receptor (5-HT1A receptor) Expression

Figure 23: A) Changes in Hippocampal Glutamate Ionotropic Receptor NMDA Type Subunit 2A (Grin2A) and B) Glutamate Ionotropic Receptor NMDA Type Subunit 2B (Grin2B) expression

Figure 24: Changes in Hippocampal Expression of A) Gamma-Aminobutyric Acid A Receptor 2 (GABA A2), B) Gamma-Aminobutyric Acid B Receptor 1 (GABA BR1) and C) Dopamine Receptor 1 (DRD1)

Figure 25: Changes in Amygdala Receptor Expression – A) Oxytocin Receptor, B) Vasopressin Receptor, C) Glucocorticoid Receptor and D) Mineralocorticoid Receptor

Figure 26: Changes in Amygdala Expression of A) Brain Derived Neurotrophic Factor (BDNF), B) Toll-like Receptor 4 (TLR-4), C) Corticotropin Releasing Hormone Receptor 1 (CRFR1) and D) Corticotropin Releasing Hormone Receptor 2 (CRFR2)

Figure 27: Changes in Amygdala Expression of A) Integrin Alpha M (CD11b), B) Interleukin-6 (IL-6), C) Glutamate Ionotropic Receptor NMDA Type Subunit 2A (Grin2A) and D) Glutamate Ionotropic Receptor NMDA Type Subunit 2B (Grin2B)

Figure 28: Changes in Amygdala Expression of A) GABA-A Receptor Alpha 2 Subunit (GABRA2), B) GABA-A Type B Receptor 1 Subunit (GABBR1) and C) Dopamine Receptor 1 (DRD1)

Figure 29: Changes in Prefrontal Cortex Expression of A) Oxytocin Receptor, B) Brain Derived Neurotrophic Factor (BDNF), C) Mineralocorticoid Receptor and D) Glucocorticoid Receptor

Figure 30: Changes in Prefrontal Cortex Expression of A) Toll-like Receptor 4 (TLR-4), B) Corticotropin Releasing Hormone Receptor 1 (CRFR1), C) Corticotropin Releasing Hormone Receptor 2 (CRFR2) and Integrin Alpha M (CD11b)

Figure 31: Changes in Prefrontal Cortex Expression of A) Interleukin-6 (IL-6), B) Glutamate Ionotropic Receptor NMDA Type Subunit 2A (Grin2A), C) Glutamate Ionotropic Receptor NMDA Type Subunit 2B (Grin2B) and D) GABA-A Receptor Alpha 2 Subunit (GABRA2)

Figure 32: Changes in Prefrontal Cortex Expression of A) GABA-A Receptor Type B Receptor Subunit 1 (GABBR1) and B) Dopamine Receptor 1 (DRD1)

Figure 33: Changes in Colon Expression of A) Tryptophan Hydroxylase-1 (Tph1) and B) Indoleamine2,3-Dioxygenase-1 (IDO1)

Figure 34: Changes in Ileum Expression of A) Tryptophan Hydroxylase-1 (Tph1) and B) Indoleamine2,3-Dioxygenase-1 (IDO1)

Figure 35: Changes in Circulating Tryptophan Metabolite Levels A) Kynurenine, B) Tryptophan and C) Kynurenine/ Tryptophan Index of metabolism

Figure 36: Effect on Interferon-γ Production from mouse Splenocytes from mice fed with MRx0005 and MRx0029

Figure 37: Effect on Interleukin-1β Production from Splenocytes

Figure 38: Effect on Interleukin-6 Production from Splenocytes

Figure 39: Effect on Tumour Necrosis Factor Production from Splenocytes

Figure 40: Effect on Interleukin-10 Production from Splenocytes

Figure 41: Effect on Chemoattractant CXCL1 Production from Splenocytes

Figure 42: Changes in Caecal Short Chain Fatty Acid Levels

Figure 43: MRx0029 and MRX005-induced changes in gene expression levels of Actin, Villin, Occludin TJP1, TJP2, MAP2, DRD2, GABRB3, SYP, PINK1, PARK7 and NSE.

Figure 44: SHSY5Y cell differentiation induced by MRx0005 and MRx0029. (A-C) Representative images of immuno labelled cells with Phalloidin and MAP2. (D—F) images of A-C merged with DAPI images. (G—I) β3 tubulin immunolabelled cells. (J-L) merged with DAPI images. Magnification x630. J-K) Western blot analysis of effects of MRx0005 and MRx0029 treatment on SHSY5Y cells. Western blot membranes were probed with antibodies to MAP2 (M) and b3 tubulin (N). Actin was used as a loading control. Lower panels: representative blots from one of six separate experiments; upper panels: relative densitometric intensity.

DISCLOSURE OF THE INVENTION

Bacterial strains

The compositions of the invention comprise a bacterial strain of the genus *Parabacteroides*. The examples demonstrate that bacteria of this genus are useful for treating or preventing neurodegenerative disorders. The preferred bacterial strains are of the species *Parabacteroides distasonis.*

Examples of *Parabacteroides* species for use in the invention include *Parabacteroides distasonis*, *Parabacteroides goldsteinii*, *Parabacteroides merdae* and *Parabacteroides johnsonii*. The *Parabacteroides* resemble the *Bacteroides* and are Gram-negative, obligately anaerobic, non-spore-forming, non-motile and rod-shaped, and 0.8–1.6×1.2–12μm in size. *Parabacteroides distasonis* is one of the most common species in human faeces. The type strain of *P. distasonis* is JCM 5825^T (=CCUG 4941^T=DSM 20701^T=ATCC 8503^T) The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *P. distasonis* strains JCM 5825T, JCM 13400, JCM 13401, JCM 13402, JCM 13403 and JCM 13404 and *P. merdae* strains JCM 9497T and JCM 13405 are AB238922–AB238929, respectively (disclosed herein as SEQ ID NOs:1-8). Exemplary strains are also described in [22].

The *Parabacteroides distasonis* bacterium deposited under accession number NCIMB 42382 was tested in the Examples and is also referred to herein as strain 755 or MRx0005. A 16S rRNA sequence for the 755 strain that was tested is provided in SEQ ID NO:9. Strain 755 was deposited with the international depositary authority NCIMB, Ltd. (Ferguson Building, Aberdeen, AB21 9YA, Scotland) by GT Biologics Ltd. (Life Sciences Innovation Building, Aberdeen, AB25 2ZS, Scotland) on 12th March 2015 as "*Parabacteroides sp* 755" and was assigned accession number NCIMB 42382. GT Biologics Ltd. Subsequently changed its name to 4D Pharma Research Limited.

WO 2016/203220 describes administration of strain 755 to mice and shows that it can affect disease processes outside of the gut (such as asthma and arthritis). Strain 755 also affects disease processes outside of the gut in the treatment of neurodegenerative disorders described herein.

A genome sequence for strain 755 is provided in SEQ ID NO:10. This sequence was generated using the PacBio RS II platform.

Bacterial strains closely related to the strain tested in the examples are also expected to be effective for treating or preventing neurodegenerative disorders. In certain embodiments, the bacterial strain for use in the invention has a 16s rRNA sequence that is at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% identical to the 16s rRNA sequence of a bacterial strain of *Parabacteroides distasonis*. Preferably, the bacterial strain for use in the invention has a 16s rRNA sequence that is at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% identical to SEQ ID NO:1, 2, 3, 4, 5, 6, 7, 8 or 9. Preferably, the sequence identity is to SEQ ID NO:9. Preferably, the bacterial strain for use in the invention has the 16s rRNA sequence represented by SEQ ID NO:9.

Bacterial strains that are biotypes of the bacterium deposited under accession number 42382 are also expected to be effective for treating or preventing neurodegenerative disorders. A biotype is a closely related strain that has the same or very similar physiological and biochemical characteristics.

Strains that are biotypes of the bacterium deposited under accession number NCIMB 42382 and that are suitable for use in the invention may be identified by sequencing other nucleotide sequences for the bacterium deposited under accession number NCIMB 42382. For example, substantially the whole genome may be sequenced and a biotype strain for use in the invention may have at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% sequence identity across at least 80% of its whole genome (e.g. across at least 85%, 90%, 95% or 99%, or across its whole genome). Other suitable sequences for use in identifying biotype strains may include hsp60 or repetitive sequences such as BOX, ERIC, $(GTG)_5$, or REP or [23]. Biotype strains may have sequences with at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% sequence identity to the corresponding sequence of the bacterium deposited under accession number NCIMB 42382.

In certain embodiments, the bacterial strain for use in the invention has a genome with sequence identity to SEQ ID NO:10. In preferred embodiments, the bacterial strain for use in the invention has a genome with at least 90% sequence identity (e.g. at least 92%, 94%, 95%, 96%, 97%, 98%, 99% or

100% sequence identity) to SEQ ID NO:10 across at least 60% (e.g. at least 65%, 70%, 75%, 80%, 85%, 95%, 96%, 97%, 98%, 99% or 100%) of SEQ ID NO:10. For example, the bacterial strain for use in the invention may have a genome with at least 90% sequence identity to SEQ ID NO:10 across 70% of SEQ ID NO:10, or at least 90% sequence identity to SEQ ID NO:10 across 80% of SEQ ID NO:10, or at least 90% sequence identity to SEQ ID NO:10 across 90% of SEQ ID NO:10, or at least 90% sequence identity to SEQ ID NO:10 across 100% of SEQ ID NO:10, or at least 95% sequence identity to SEQ ID NO:10 across 70% of SEQ ID NO:10, or at least 95% sequence identity to SEQ ID NO:10 across 80% of SEQ ID NO:10, or at least 95% sequence identity to SEQ ID NO:10 across 90% of SEQ ID NO:10, or at least 95% sequence identity to SEQ ID NO:10 across 100% of SEQ ID NO:10, or at least 98% sequence identity to SEQ ID NO:10 across 70% of SEQ ID NO:10, or at least 98% sequence identity to SEQ ID NO:10 across 80% of SEQ ID NO:10, or at least 98% sequence identity to SEQ ID NO:10 across 90% of SEQ ID NO:10, or at least 98% sequence identity to SEQ ID NO:10 across 100% of SEQ ID NO:10.

Alternatively, strains that are biotypes of the bacterium deposited under accession number NCIMB 42382 and that are suitable for use in the invention may be identified by using the accession number NCIMB 42382 deposit and restriction fragment analysis and/or PCR analysis, for example by using fluorescent amplified fragment length polymorphism (FAFLP) and repetitive DNA element (rep)-PCR fingerprinting, or protein profiling, or partial 16S or 23S rDNA sequencing. In preferred embodiments, such techniques may be used to identify other *Parabacteroides distasonis* strains.

In certain embodiments, strains that are biotypes of the bacterium deposited under accession number NCIMB 42382 and that are suitable for use in the invention are strains that provide the same pattern as the bacterium deposited under accession number NCIMB 42382 when analysed by amplified ribosomal DNA restriction analysis (ARDRA), for example when using Sau3AI restriction enzyme (for exemplary methods and guidance see, for example [24]). Alternatively, biotype strains are identified as strains that have the same carbohydrate fermentation patterns as the bacterium deposited under accession number NCIMB 42382.

Other *Parabacteroides* strains that are useful in the compositions and methods of the invention, such as biotypes of the bacteria deposited under accession number NCIMB 42382, may be identified using any appropriate method or strategy, including the assays described in the examples. For instance, strains for use in the invention may be identified by culturing with neuroblastoma cells and then assessing cytokine levels and levels of neuroprotection or neuro-proliferation. In particular, bacterial strains that have similar growth patterns, metabolic type and/or surface antigens to the bacterium deposited under accession number NCIMB 42382 may be useful in the invention. A useful strain will have comparable immune modulatory activity to the NCIMB 42382 strain. In particular, a biotype strain will elicit comparable effects on the neurodegenerative disease models and comparable effects on cytokine levels to the effects shown in the Examples, which may be identified by using the culturing and administration protocols described in the Examples.

A particularly preferred strain of the invention is the *Parabacteroides distasonis* strain deposited under accession number NCIMB 42382. This is the exemplary 755 strain tested in the examples and shown to be effective for treating disease. Therefore, the invention provides a cell, such as an isolated cell, of the *Parabacteroides distasonis* strain deposited under accession number NCIMB 42382, or a derivative thereof. The invention also provides a composition comprising a cell of the *Parabacteroides distasonis* strain deposited under accession number NCIMB 42382, or a derivative thereof. The invention also provides a biologically pure culture of the *Parabacteroides distasonis* strain deposited under accession number NCIMB 42382. The invention also provides a cell of the *Parabacteroides distasonis* strain deposited under accession number NCIMB 42382, or a derivative thereof, for use in therapy, in particular for the diseases described herein.

A derivative of the strain deposited under accession number NCIMB 42382 may be a daughter strain (progeny) or a strain cultured (subcloned) from the original. A derivative of a strain of the invention may be modified, for example at the genetic level, without ablating the biological activity. In particular, a derivative strain of the invention is therapeutically active. A derivative strain will have comparable immune modulatory activity to the original NCIMB 42382 strain. In particular, a derivative strain will elicit comparable effects on the neurodegenerative disease models and comparable effects on cytokine levels to the effects shown in the Examples, which may be identified by using the culturing and administration protocols described in the Examples. A derivative of the NCIMB 42382 strain will generally be a biotype of the NCIMB 42382 strain.

References to cells of the *Parabacteroides distasonis* strain deposited under accession number NCIMB 42382 encompass any cells that have the same safety and therapeutic efficacy characteristics as the strains deposited under accession number NCIMB 42382, and such cells are encompassed by the invention.

In preferred embodiments, the bacterial strains in the compositions of the invention are viable and capable of partially or totally colonising the intestine.

The inventors have found that *Parabacteroides distasonis* strains reduce the secretion of pro-inflammatory cytokines such as IL-6 and IL-8. IL-8 has been implicated in myelin sheath formation. Chronic inflammation induced by IL-6 can ultimately lead to cell death. Therefore, the bacterial strains of the invention are particularly useful in the treatment or prevention of neurodegenerative disorders. In some embodiments, the bacterial strains are useful in the treatment of conditions characterised by the enhanced activation of IL-6. In some embodiments, the compositions of the invention are for use in the treatment or prevention of neurodegenerative diseases characterised by demyelination. Many neurodegenerative diseases are characterised by demyelination. Demyelination impedes the propagation of action potentials within neurons, impairing effective communication within the nervous system. IL-8 has been shown to contribute positively to myelin sheath formation and repair. MRx0029 increases *per se* secretion of IL-8. Therefore, the compositions of the invention are particularly

beneficial in the treatment or prevention of neurodegenerative disorders characterised by demyelination.

The inventors have also found that the bacterial strains of invention increase the activation of BDNF. BDNF is a neurotrophic growth factor that has been shown to enhance neuron differentiation and survival. Thus, the compositions of the invention can be used in a method of enhancing nerve cell survival in the treatment or prevention of neurodegenerative diseases.

A further bacteria that may be used in the compositions of the invention is the species *Megasphaera massiliensis*. The examples demonstrate that *Parabacteroides distasonis* and *Megasphaera massiliensis* both have neuroprotective activities, but produce different metabolites and may have different mechanisms of action and specific neuroprotective activities. Therefore, these species may be particularly effective when used in combination. In preferred embodiments, the composition comprises a strain of the species *Parabacteroides distasonis* and a strain of the species *Megasphaera massiliensis.*

The *Parabacteroides distasonis* bacterium deposited under accession number NCIMB 42382 was tested in the Examples and is also referred to herein as strain MRx0005. MRX0005, MRX005, MRx005 and MRx0005 are used herein interchangeably. A 16S rRNA sequence for the MRx0005 strain that was tested is provided in SEQ ID NO:9. Strain MRx0005 was deposited with the international depositary authority NCIMB, Ltd. (Ferguson Building, Aberdeen, AB21 9YA, Scotland) by GT Biologics Ltd. (Life Sciences Innovation Building, Aberdeen, AB25 2ZS, Scotland) on 12th March 2015 as "*Parabacteroides sp* 755" and was assigned accession number NCIMB 42382. GT Biologics Ltd. Subsequently changed its name to 4D Pharma Research Limited.

The *Megasphaera massiliensis* strain deposited under accession number NCIMB 42787 is the exemplary MRx0029 strain tested in the examples and shown to be effective for treating disease. Strain NCIMB 42787 was deposited with the international depositary authority NCIMB, Ltd. (Ferguson Building, Aberdeen, AB21 9YA, Scotland) by 4D Pharma Research Ltd. (Life Sciences Innovation Building, Aberdeen, AB25 2ZS, Scotland) on 13th July 2017 as "*Megasphaera massiliensis MRx0029*" and was assigned accession number NCIMB 42787. Therefore, the invention provides a cell, such as an isolated cell, of the *Megasphaera massiliensis* strain deposited under accession number NCIMB 42787, or a derivative thereof. The invention also provides a composition comprising a cell of the *Megasphaera massiliensis* strain deposited under accession number NCIMB 42787, or a derivative thereof. The invention also provides a biologically pure culture of the *Megasphaera massiliensis* strain deposited under accession number NCIMB 42787. The invention also provides a cell of the *Megasphaera massiliensis* strain deposited under accession number NCIMB 42787, or a derivative thereof, for use in therapy, in particular for the diseases described herein.

In some embodiments, the invention provides a composition comprising the strain deposited at NCIMB under accession number NCIMB 42787, or a derivative or biotype thereof, and the strain deposited at

NCIMB under accession number NCIMB 42382, or a derivative or biotype thereof, preferably for use in therapy, preferably for use in treating a neurodegenerative disease such as Parkinson's disease.

Therapeutic uses

As demonstrated in the examples, the bacterial compositions of the invention are effective for treating neurodegenerative disorders. In particular, treatment with compositions of the invention increase neuroproliferation and act as a neuroprotectant against agents that destroy dopaminergic neurons. Therefore, the compositions of the invention may be useful for treating or preventing neurodegenerative disorders that are the result of neuron death.

Compositions of the invention can decrease the activation of the NFκB promoter, which activates cytokine production, for example IL-1β, IL-1α, IL-18, TNFα and IL-6. Treating cells with mutant α-synuclein is a model for familial Parkinson's. A point mutation at position 53 from adenine to threonine leads to α-synuclein mis-folding. The incorrectly folded α-synuclein subsequently aggregates into insoluble fibrils which form Lewy bodies. Therefore, the compositions of the invention may be useful for treating or preventing neurodegenerative disorders that are the result of neuroinflammation, protein misfolding and/or environmental exposure. Compositions of the invention can be used for treatment of familial Parkinson's. Activation of the NFκB promoter is mediated through the TLR4 ligand. TL4 is known to mediate cell death in the mouse model MPTP, which simulates Parkinson's disease. Compositions of the invention can be used to inhibit the ability of TLR4 signalling to activate the NFκB promoter. Of particular relevance for PD, both TLR2 and TLR4 were found to be upregulated in brains of PD patients [25]. Moreover α-syn has been described as a ligand for TLR2 [26] and we have demonstrated that α-syn is also a ligand for TLR4 using HEK-TLR4 cells.

Compositions of the invention decrease the secretion of pro-inflammatory cytokines such as IL-6, which can be induced by lipopolysaccharide (LPS). Treatment of cells with LPS simulates Parkinson's caused by environmental factors. Compositions of the invention can be used to decrease IL-6 secretion. Compositions of the invention can be used for treatment of environmental Parkinson's.

Chemokines have been postulated to have important functions in the central nervous system (CNS), in addition to their principal role of directional migration of leukocytes. In a murine oligodendrocyte precursor-like cell line the chemokine MCP-1 did not increase oligodendrocyte precursor proliferation. In primary myelinating cultures MCP-1 did not enhance myelin segment formation in this system. The inventors have found that MRx0005 promotes MCP-1 levels. In certain embodiments, the compositions of the invention are for use in increasing MCP-1 levels in the treatment of disease.

Examples of neurodegenerative diseases to be treated by compositions of the invention include: Parkinson's disease, including progressive supranuclear palsy, progressive supranuclear palsy, Steele-Richardson-Olszewski syndrome, normal pressure hydrocephalus, vascular or arteriosclerotic

parkinsonism and drug-induced parkinsonism; Alzheimer's disease, including Benson's syndrome; multiple sclerosis; Huntington's disease; amyotrophic lateral sclerosis; Lou Gehrig's disease; motor neurone disease; prion disease; spinocerebellar ataxia; spinal muscular atrophy; dementia, including Lewy body, vascular and frontotemporal dementia; primary progressive aphasia; mild cognitive impairment; HIV-related cognitive impairment, and corticobasal degeneration. A further neurodegenerative diseases to be treated by compositions of the invention is progressive inflammatory neuropathy.

In certain embodiments, the compositions of the invention can be effective for treating neurodegenerative disorders that occur in elderly patients. The examples show that compositions of the invention can treat Parkinson's disease which is predominantly diagnosed in patients aged over 65 years old. In preferred embodiments, the compositions of the invention are for treating patients 65 years or older. In other certain embodiments, the patients are between 40 to 65 years old. In other embodiments, the patients are older than 40 years. In certain embodiments, the compositions of the invention are for use in treating a disease associated with old age, for example, a disease diagnosed after 50 years of age.

In certain embodiments, the compositions of the invention are for use in treating a neurodegenerative disorder mediated or characterised by the accumulation of protein, in particular mis-folded protein.

In certain embodiments, the compositions of the invention are for use in treating a neurodegenerative disorder associated with grey matter neuronal loss. In certain embodiments, the compositions of the invention are for treating a neurodegenerative disorder that is not associated with white matter lesions.

In certain embodiments, the compositions of the invention are for use in treating a neurodegenerative disorder associated with permanent symptoms.

In certain embodiments, the compositions of the invention are for use in treating a neurodegenerative disorder that is not an auto-immune disorder. In certain embodiments, the compositions of the invention are for use in treating a neurodegenerative disorder that is not multiple sclerosis.

In certain embodiments, the compositions of the invention are for use in reducing neuron death, in particular, in the treatment of neurodegenerative disorders. In certain embodiments, the compositions of the invention are for use in protecting neurons, in particular in the treatment of neurodegenerative disorders.

The neuroprotective properties of the compositions of the invention, as shown in the examples, mean that the compositions may be particularly effective for preventing or delaying onset or progression of neurodegenerative disorders. In certain embodiments, the compositions of the invention are for use in delaying onset or progression of a neurodegenerative disorders.

Compositions of the invention can increase the secretion of IL-8. IL-8 has been shown to play a role in neuron myelination. In some embodiments, compositions of the invention can be used to increase IL-8 secretion.

The therapeutic compositions of the invention can increase the activation of BDNF. BDNF acts on certain neurons of the central nervous system to support the survival of existing neurons and help the growth and development of new neurons and synapses. BDNF is active in the hippocampus, cortex and basal forebrain, and is important for long-term memory. The compositions of the invention can therefore be used to increase the secretion of BDNF. The compositions may therefore be used in the treatment of neurodegenerative diseases associated with the impairment of long-term memory. The compositions of the invention may be used for improving long-term memory, in particular for improving long-term memory that is impaired by a neurodegenerative disease.

In certain embodiments, the compositions of the invention increase the mitochondria metabolic activity in neuronal cells.

Modulation of the microbiota-gut-brain axis

Communication between the gut and the brain (the microbiota-gut-brain axis) occurs via a bidirectional neurohumoral communication system. Recent evidence shows that the microbiota that resides in the gut can modulate brain development and produce behavioural phenotypes via the microbiota-gut-brain axis. Indeed, a number of reviews suggest a role of the microbiota-gut-brain axis in maintaining central nervous system functionality and implicate dysfunction of the microbiota-gut-brain axis in the development of central nervous system disorders and conditions [16-27].

The bidirectional communication between the brain and the gut (*i.e.* the-gut-brain axis) includes the central nervous system, neuroendocrine and neuroimmune systems, including the hypothalamus-pituitary-adrenal (HPA) axis, sympathetic and parasympathetic arms of the autonomic nervous system (ANS), including the enteric nervous system (ENS) and the vagus nerve, and the gut microbiota.

As demonstrated in the examples, the compositions of the present invention can modulate the microbiota-gut-brain axis and reduce cell death associated with neurodegenerative disorders. Accordingly, the compositions of the invention may be useful for treating or preventing neurodegenerative disorders, in particular those disorders and conditions associated with dysfunction of the microbiota-gut-brain axis.

In particular embodiments, the compositions of the invention may be useful for treating or preventing a disease or condition selected from the group consisting of: Parkinson's disease, including progressive supranuclear palsy, progressive supranuclear palsy, Steele-Richardson-Olszewski syndrome, normal pressure hydrocephalus, vascular or arteriosclerotic parkinsonism and drug-induced parkinsonism; Alzheimer's disease, including Benson's syndrome; multiple sclerosis; Huntington's disease; amyotrophic lateral sclerosis; Lou Gehrig's disease; motor neurone disease; prion disease;

spinocerebellar ataxia; spinal muscular atrophy; dementia; including Lewy body; vascular and frontotemporal dementia; primary progressive aphasia; mild cognitive impairment; HIV-related cognitive impairment and corticobasal degeneration.

The compositions of the invention may be particularly useful for treating or preventing chronic disease, treating or preventing disease in patients that have not responded to other therapies (such as treatment with Levodopa, dopamine agonists, MAO-B inhibitors, COMT inhibitors, Glutamate antagonists, and/or anticholinergics), and/or treating or preventing the tissue damage and symptoms associated with dysfunction of the microbiota-gut-brain axis.

In certain embodiments, the compositions of the invention modulate the CNS. In some embodiments, the compositions of the invention modulate the autonomic nervous system (ANS). In some embodiments, the compositions of the invention modulate the enteric nervous system (ENS). In some embodiments, the compositions of the invention modulate the hypothalamic, pituitary, adrenal (HPA) axis. In some embodiments, the compositions of the invention modulate the neuroendocrine pathway. In some embodiments, the compositions of the invention modulate the neuroimmune pathway. In some embodiments, the compositions of the invention modulate the CNS, the ANS, the ENS, the HPA axis and/or the neuroendocrine and neuroimmune pathways. In certain embodiments, the compositions of the invention module the levels of commensal metabolites and/or the gastrointestinal permeability of a subject. In certain embodiments, the compositions of the invention may be used to modulate the dopaminergic system.

The signalling of the microbiota-gut-brain axis is modulated by neural systems. Accordingly, in some embodiments, the compositions of the invention modulate signalling in neural systems. In certain embodiments, the compositions of the invention modulate the signalling of the central nervous system. In some embodiments, the compositions of the invention modulate signalling in sensory neurons. In other embodiments, the compositions of the invention modulate signalling in motor neurons. In some embodiments, the compositions of the invention modulate the signalling in the ANS. In some embodiments, the ANS is the parasympathetic nervous system. In preferred embodiments, the compositions of the invention modulate the signalling of the vagus nerve. In other embodiments, the ANS is the sympathetic nervous system. In other embodiments, the compositions of the invention modulate the signalling in the enteric nervous system. In certain embodiments, the signalling of ANS and ENS neurons responds directly to luminal contents of the gastrointestinal tract. In other embodiments, the signalling of ANS and ENS neurons responds indirectly to neurochemicals produced by luminal bacteria. In other embodiments, the signalling of ANS and ENS neurons responds to neurochemicals produced by luminal bacteria or enteroendocrine cells. In certain preferred embodiments, the neurons of the ENS activate vagal afferents that influence the functions of the CNS. In some embodiments, the compositions of the invention regulate the activity of enterochromaffin cells.

Neurodegenerative diseases

Tauopathies are neurodegenerative diseases associated with the pathological aggregation of tau protein in neurofibrillary or gliofibrillary tangles in the human brain. Alzheimer's disease is an example of a tauopathology. Synucleinopathies (also called α-Synucleinopathies) are neurodegenerative diseases characterised by the abnormal accumulation of aggregates of α-synuclein in neurons, nerve fibres or glial cells. Parkinson's disease is an example of a synucleinopathology.

There is clinical and pathological overlap between these two pathologies. Parkinson's disease patients frequently have dementia and Alzheimer's disease patients often manifest parkinsonism [28]. For example, progressive supranuclear palsy (also known as Steele-Richardson-Olszewski syndrome) has a tauopathology, but also leads to prominent parkinsonism [29]. Mutations in *LRRK2* known to cause parkinsonism are associated with the accumulation of synuclein, tau, neither, or both proteins [30].

Lewy body disease (LBD) is a neurodegenerative disease that is one of the most common causes of dementia in the elderly. LBD exemplifies the existence of a continuum between tau- and synuclein-pathologies. LBD shares clinical and pathological features with Parkinson disease, Parkinson disease dementia and Alzheimer disease [28].

In particular embodiments, the compositions of the invention may be useful for treating or preventing tauopathies and/or synucleinopathies. In particular embodiments, the compositions of the invention may be useful for treating or preventing tauopathies. In particular embodiments, the compositions of the invention may be useful for treating or preventing synucleinopathies. In certain embodiments, the compositions of the invention may be useful for treating or preventing a disease or condition selected from the group consisting of: Parkinson's disease, including progressive supranuclear palsy, progressive supranuclear palsy, Steele-Richardson-Olszewski syndrome, normal pressure hydrocephalus, vascular or arteriosclerotic parkinsonism and drug-induced parkinsonism; Alzheimer's disease, including Benson's syndrome; and dementia; including Lewy body; vascular and frontotemporal dementia.

In preferred embodiments, the compositions of the invention may be useful for treating or preventing Parkinson's disease, including progressive supranuclear palsy, progressive supranuclear palsy, Steele-Richardson-Olszewski syndrome, normal pressure hydrocephalus, vascular or arteriosclerotic parkinsonism and drug-induced parkinsonism. In preferred embodiments, the compositions of the invention may be useful for treating or preventing Alzheimer's disease, including Benson's syndrome. In further preferred embodiments, the compositions of the invention may be useful for treating or preventing dementia; including Lewy body; vascular and frontotemporal dementia.

Parkinson's disease

Parkinson's disease is a common neurodegenerative disease neuropathologically characterised by degeneration of heterogeneous populations of neural cells (dopamine-producing cells). The clinical

diagnosis of Parkinson's disease requires bradykinesia and at least one of the following core symptoms: resting tremor; muscle rigidity and postural reflex impairment. Other signs and symptoms that may be present or develop during the progression of the disease are autonomic disturbances (sialorrhoea, seborrhoea, constipation, micturition disturbances, sexual functioning, orthostatic hypotension, hyperhydrosis), sleep disturbances and disturbances in the sense of smell or sense of temperature. Parkinson's disease is a neurodegenerative diseases that may develop or persist due to dysfunction of the microbiota-gut-brain axis. Therefore, in preferred embodiments, the compositions of the invention are for use in treating or preventing Parkinson's disease in a subject.

In further preferred embodiments, the invention provides a composition comprising a bacterial strain of the genus *Parabacteroides*, for use in a method of treating or preventing Parkinson's disease. Compositions comprising a bacterial strain of the genus *Parabacteroides* may improve motor and cognitive functions in models of Parkinson's disease. Treatment with *Parabacteroides* strains may modulate signalling in the central, autonomic and enteric nervous systems; may modulate the activity of the HPA axis pathway; may modulate neuroendocrine and/or neuroimmune pathways; and may modulate the levels of commensal metabolites, inflammatory markers and/or gastrointestinal permeability of a subject, all of which are implicated in the neuropathology of Parkinson's disease. In preferred embodiments, the invention provides a composition comprising a bacterial strain of the species *Parabacteroides distasonis* for use in a method of treating or preventing Parkinson's disease. Compositions using *Parabacteroides distasonis* may be particularly effective for treating Parkinson's disease.

In preferred embodiments, the compositions of the invention prevent, reduce or alleviate one or more of the symptoms of Parkinson's disease in a subject. In preferred embodiments, the compositions of the invention prevent, reduce or alleviate one or more core symptoms of Parkinson's disease in a subject. In certain embodiments, the compositions of the invention prevent, reduce or alleviate bradykinesia in a subject. In certain embodiments, the compositions of the invention prevent, reduce or alleviate resting tremor; muscle rigidity and/or postural reflex impairment in a subject. In certain embodiments, the compositions of the invention prevent, reduce or alleviate one or more symptoms associated with Parkinson's disease progression selected from autonomic disturbances (sialorrhoea, seborrhoea, constipation, micturition disturbances, sexual functioning, orthostatic hypotension, hyperhydrosis), sleep disturbances and disturbances in the sense of smell or sense of temperature.

In preferred embodiments, the compositions of the invention prevent, reduce or alleviate depressive symptoms comorbid with Parkinson's disease. In certain embodiments, the compositions of the invention improve verbal memory and/or executive functions. In certain embodiments, the compositions of the invention improve attention, working memory, verbal fluency and/or anxiety.

In other preferred embodiments, the compositions of the invention prevent, reduce or alleviate cognitive dysfunctions comorbid with Parkinson's disease.

In certain embodiments, the compositions of the invention prevent, reduce or alleviate Parkinson's disease progression. In certain embodiments, the compositions of the invention prevent, reduce or alleviate later motor complications. In certain embodiments, the compositions of the invention prevent, reduce or alleviate late motor fluctuations. In certain embodiments, the compositions of the invention prevent, reduce or alleviate neuronal loss. In certain embodiments, the compositions of the invention improve symptoms of Parkinson's disease dementia (PDD). In certain embodiments, the compositions of the invention prevent, reduce or alleviate impairment of executive function, attention and/or working memory. In certain embodiments, the compositions of the invention improve dopaminergic neurotransmission. In certain embodiments, the compositions of the invention prevent, reduce or alleviate impaired dopaminergic neurotransmission.

In some embodiments, the compositions of the invention improve the symptoms of Parkinson's disease according to a symptomatic or diagnostic scale. In certain embodiments, the tests for assessing symptomatic improvement of motor function in Parkinson's disease is the Unified Parkinson's Disease Rating Scale. In particular, UPDRS II considers the activity of daily life and UPDRS III considers motor-examination.

In some embodiments, the compositions of the invention improve the symptoms associated the PDD according to a symptomatic or diagnostic test and/or scale. In certain embodiments, the test or scale is selected from the Hopkins Verbal Learning Test – Revised (HVLT-R); the Delis-Kaplan Executive Function System (D-KEFS) Color-Word Interference Test; the Hamilton Depression Rating Scale (HAM-D 17; depression); the Hamilton Anxiety Rating Scale (HAM-A; anxiety) and the Unified Parkinson's Disease Rating Scale (UPDRS; PD symptom severity).

In some embodiments, the compositions of the invention improve the Clinical Global Impression – Global Improvement (CGI-I) scale for assessing psychiatric and neurological disorders. In some embodiments, the compositions of the invention display a positive effect on global social and occupational impairment of the subject with Parkinson's disease.

Alzheimer's disease and dementia

In DSM-5, the term dementia was replaced with the terms major neurocognitive disorder and mild neurocognitive disorder. Neurocognitive disorder is a heterogeneous class of psychiatric diseases. The most common neurocognitive disorder is Alzheimer's disease, followed by vascular dementias or mixed forms of the two. Other forms of neurodegenerative disorders (*e.g.* Lewy body disease, frontotemporal dementia, Parkinson's dementia, Creutzfeldt-Jakob disease, Huntington's disease, and Wernicke-Korsakoff syndrome) are accompanied by dementia.

Alzheimer's disease and dementia are also characterised by neuronal loss, so the neuroprotective and neuroproliferative effects shown in the examples for the compositions of the invention indicate that they may be useful for treating or preventing these conditions.

The symptomatic criteria for dementia under DSM-5 are evidence of significant cognitive decline from a previous level of performance in one or more cognitive domains selected from: learning and memory; language; executive function; complex attention; perceptual-motor and social cognition. The cognitive deficits must interfere with independence in everyday activities. In addition, the cognitive deficits do not occur exclusively in the context of a delirium and are not better explained by another mental disorder (for example MDD or schizophrenia).

In addition to the primary symptom, subjects with neurodegenerative disorders display behavioural and psychiatric symptoms including agitation, aggression, depression, anxiety, apathy, psychosis and sleep-wake cycle disturbances.

Neurodegenerative disorders may develop or persist due to dysfunction of the microbiota-gut-brain axis. Therefore, in preferred embodiments, the compositions of the invention are for use in treating or preventing neurodegenerative disorders in a subject. In preferred embodiments, the neurodegenerative disorder is Alzheimer's disease. In other embodiments, the neurodegenerative disorder is selected from vascular dementias; mixed form Alzheimer's disease and vascular dementia; Lewy body disease; frontotemporal dementia; Parkinson's dementia; Creutzfeldt-Jakob disease; Huntington's disease; and Wernicke-Korsakoff syndrome.

In preferred embodiments, the compositions of the invention prevent, reduce or alleviate one or more of the symptoms of neurodegenerative disorders in a subject. In certain embodiments, the compositions of the invention prevent, reduce or alleviate the occurrence of cognitive decline in a subject. In certain embodiments, the compositions of the invention improve the level of performance of a subject with neurodegenerative disorders in one or more cognitive domains selected from: learning and memory; language; executive function; complex attention; perceptual-motor and social cognition. In some embodiments, the compositions of the invention prevent, reduce or alleviate the occurrence of one or more behavioural and psychiatric symptoms associated with neurodegenerative disorders selected from agitation, aggression, depression, anxiety, apathy, psychosis and sleep-wake cycle disturbances.

In certain embodiments, the compositions of the invention prevent, reduce or alleviate symptomatic disease by intervention in suspected pathogenic mechanisms at a preclinical stage. In certain embodiments, the compositions of the invention improve disease modification, with slowing or arrest of symptom progression. In some embodiments, the slowing or arrest of symptom progression correlates with evidence in delaying the underlying neuropathological process. In preferred embodiments, the compositions of the invention improve symptoms of neurodegenerative disorders comprising enhanced cognitive and functional improvement. In preferred embodiments, the compositions of the invention improve the behavioural and psychiatric symptoms of dementia (BPSD). In preferred embodiments, the compositions of the invention improve the ability of a subject with neurodegenerative disorder to undertake everyday activities.

In preferred embodiments, the compositions of the invention improve both cognition and functioning in a subject with Alzheimer's disease. In some embodiments, the composition of the invention improves the cognitive endpoint in a subject with Alzheimer's disease. In some embodiments, the compositions of the invention improve the functional endpoint in a subject with Alzheimer's disease. In preferred embodiments, the compositions of the invention improve the cognitive and functional endpoint in a subject with Alzheimer's disease. In yet further preferred embodiments, the compositions of the invention improve the overall clinical response (the global endpoint) in a subject with Alzheimer's disease.

In some embodiments, the compositions of the invention improve the symptoms of neurodegenerative disorders according to a symptomatic or diagnostic test. In certain embodiments, the tests for assessing symptomatic improvement of Alzheimer's disease (and other neurodegenerative disorders) are selected from objective cognitive, activities of daily living, global assessment of change, health related quality of life tests and tests assessing behavioural and psychiatric symptoms of neurodegenerative disorders.

In certain embodiments, the objective cognitive tests for assessment of symptomatic improvement use the Alzheimer's disease Assessment Scale cognitive subscale (ADAS-cog) and the classic ADAS scale. In certain embodiments, symptomatic improvement of cognition is assessed using the Neurophysiological Test Battery for Use in Alzheimer's Disease (NTB).

In some embodiments, the global assessment of change test uses the Clinical Global Impression – Global Improvement (CGI-I) scale for assessing psychiatric and neurological disorders. In some embodiments, the global scale is the Clinician's Interview Based Impression of Change plus (CIBIC-plus). In some embodiments, the global scale is the Alzheimer's Disease Cooperative Study Unit Clinician's Global Impression of Change (ADCS-CGIC).

In certain embodiments, the health related quality of life measures are the Alzheimer's Disease-Related QOL (ADRQL) and the QOL-Alzheimer's Disease (QOL-AD).

In certain embodiments, the tests assessing behavioural and psychiatric symptoms of neurodegenerative disorders are selected from the Behavioural pathology in Alzheimer's Disease Rating Scale (BEHAVE-AD); the Behavioural Rating Scale for Dementia (BRSD); the Neuropsychiatric Inventory (NPI); and the Cohen-Mansfield Agitation Inventory (CMAI).

In some embodiments, the compositions of the invention are particularly effective at preventing, reducing or alleviating neurodegenerative disorders when used in combination with another therapy for treating neurodegenerative disorders. In certain embodiments, such therapies include acetylcholinesterase inhibitors including donepezil (Aricept®), galantamine (Razadyne®) and rivastigmine (Exelon ®), and memantine.

Multiple Sclerosis

Multiple sclerosis (MS) is a demyelinating disease in which the myelin sheath surrounding neurons in the brain and spinal cord are damaged. The exact underlying causes of MS are unknown, but are thought to vary between individuals. Certain forms of MS are hereditary. Environmental factors are also thought to contribute to MS. In some individuals, a combination of both genetic and environmental factors may trigger the onset of MS.

There are a wide variety of symptoms associated with MS. Subjects may exhibit almost any neurological symptom associated with the impairment of autonomic, visual, motor or sensory control. The exact symptoms will vary depending on the site of neuronal damage/demyelination.

IL-8 has been implicated in the formation of myelin sheaths. The compositions of the invention may therefore be for use in the remyelination of neurons in subjects with MS. The compositions of the invention may also be used to protect neurons from demyelination. In other words, the compositions of the invention may be for use in a method of treating or preventing multiple sclerosis by restoring or preventing loss of neuron myelin sheaths.

In some embodiments, the compositions of the invention prevent, reduce or alleviate one or more symptoms of MS in a subject. In some embodiments, the compositions of the invention prevent, reduce or alleviate fatigue in a subject. In certain embodiments, the compositions of the invention prevent, reduce or alleviate resting tremor, muscle weakness, muscle spasms, muscle stiffness, paraesthesia and/or ataxia in a subject. In certain embodiments, the compositions of the invention prevent, reduce or alleviate one or more symptoms associated with MS progression selected from the list consisting of autonomic disturbances: constipation, micturition disturbances, sexual functioning, dysphagia, dysarthria, syncope, vertigo and/or dizziness; sleep disturbances; and disturbances in the sense of smell or sense of temperature. In some embodiments, the compositions of the invention prevent, reduce or alleviate one or more ocular symptoms associated with MS. In some embodiments, the ocular symptom is selected from the list consisting of loss of vision, eye pain, colour blindness, double vision and/or involuntary eye movements in a subject.

In some embodiments, the compositions of the invention prevent, reduce or alleviate dizziness, vertigo, neuropathic pain, musculoskeletal pain, cognitive dysfunction, bowel incontinence, dysphagia, dysarthria, or any combination thereof.

In some embodiments, the compositions of the invention prevent, reduce or alleviate depressive symptoms or anxiety comorbid with MS.

In some embodiments, the improvement of symptoms are determined using the 2017 McDonald criteria for diagnosing MS.

In certain embodiments, treatment with the compositions of the invention results in a reduction in MS incidence or MS severity. In certain embodiments, the compositions of the invention are for use in

reducing relapse incidence or relapse severity. In certain embodiments, treatment with the compositions of the invention prevents a decline in motor function or results in improved motor function associated with MS. In certain embodiments, the compositions of the invention are for use in preventing a decline in motor function or for use in improving motor function in the treatment of MS. In certain embodiments, treatment with the compositions of the invention prevents the development of paralysis in MS. In certain embodiments, the compositions of the invention are for use in preventing paralysis in the treatment of MS.

In certain embodiments the compositions of the invention are for use in preventing multiple sclerosis in a patient that has been identified as at risk of multiple sclerosis, or that has been diagnosed with early-stage multiple sclerosis or "relapsing-remitting" multiple sclerosis. The compositions of the invention may be useful for preventing the development of MS. The compositions of the invention may be useful for preventing the progression of MS. In certain embodiments, the compositions of the invention are for use in a patient identified as having a genetic predisposition to MS, such as major histocompatibility complex (MHC) class II phenotype, human leukocyte antigen (HLA)-DR2 or HLA-DR4.

The compositions of the invention may be useful for managing or alleviating multiple sclerosis. The compositions of the invention may be particularly useful for reducing symptoms associated with multiple sclerosis. Treatment or prevention of multiple sclerosis may refer to, for example, an alleviation of the severity of symptoms or a reduction in the frequency of exacerbations or the range of triggers that are a problem for the patient. In certain embodiments, the compositions of the invention slow or stop progression of the disease.

In certain embodiments, the compositions of the invention are for use in treating relapsing-remitting MS. In alternative embodiments, the compositions of the invention are for use in treating progressive MS, such as secondary progressive MS (SPMS), which develops over time following diagnosis of RRMS, primary progressive MS (PPMS) which exhibits gradual continuous neurologic deterioration and progressive relapsing MS (PRMS), which is similar to PPMS but with overlapping relapses.

In certain embodiments, the compositions of the invention are for use in treating one or more of symptoms of MS selected from the group consisting of: fatigue, vision problems, numbness, tingling, muscle spasms, muscle stiffness, muscle weakness, mobility problems, pain, problems with thinking, learning and planning, depression and anxiety, sexual problems, bladder problems, bowel problems, speech and swallowing difficulties.

Neurochemical factors, neuropeptides and neurotransmitters and the microbiota-gut-brain axis

As outlined above, the microbiota-gut-brain axis is modulated by a number of different physiological systems. The microbiota-gut-brain axis is modulated by a number of signalling molecules. Alterations in the levels of these signalling molecules results in neurodegenerative diseases. The experiments performed by the inventors indicate that administration of *Parabacteroides* species, and in particular

Parabacteroides distasonis, can modulate levels of indole and kynurenine. Dysregulation of these metabolites can lead to neurodegenerative diseases, such as Parkinson's disease.

In certain embodiments, the compositions of the invention modulate the levels of brain monoamines and metabolites thereof. In preferred embodiments the metabolite is kynurenine. In certain embodiments, the compositions of the invention modulate kynurenine, which is the main route of tryptophan metabolism, which serves as a route to nicotinamide adenine dinucleotide (NAD+) production. Kynurenine can be metabolized to neuroactive compounds such as kynurenic acid (KYNA) and 3-hydroxy-l-kynurenine (3-OH-l-KYN), and in further steps to quinolinic acid (QUIN). Dysregulation of the kynurenine pathway can lead to activation of the immune system and the accumulation of potentially neurotoxic compounds. Alterations in the kynurenine metabolism may be involved in the development of Parkinson's diseases. Kynurenine levels have been demonstrated to be decreased in the frontal cortex, putamen and substantia nigra pars compacta of patients with PD [31]. Therefore, in certain embodiments the compositions of the invention are for use in increasing the levels of kynurenine in the treatment of Parkinson's disease.

In certain embodiments of the invention the compositions of the invention can increase the levels of kynurenin. Increased levels of kynurenine have been shown to attenuated MPP+-induced neuronal cell death *in vitro* in a human dopaminergic neuroblastoma cell line [32]. In certain embodiments kynurenine and kynurenic acid, can activate GI aryl hydrocarbon receptor (Ahr) and GPR35 receptors. Activation of Ahr receptor induces IL-22 production, which can inhibit local inflammation. Activation of GPR35 inducing the production of inositol triphosphate and Ca2+ mobilization.

In certain embodiments, the compositions of the invention modulate the levels of indole. In preferred embodiments the metabolite is kynurenine. In certain embodiments, the compositions of the invention modulate kynurenine routes, which is the main route of tryptophan metabolism.

The signalling of the microbiota-gut-brain axis is modulated by levels of neurochemical factors, neuropeptides and neurotransmitters. Accordingly, in certain embodiments, the compositions of the invention modulates levels of neurochemical factors, neuropeptides and neurotransmitters. Accordingly, in certain preferred embodiments, the compositions of the invention directly alter CNS biochemistry.

The signalling of the microbiota-gut-brain axis is modulated by levels of γ-aminobutyric acid (GABA). Accordingly, in preferred embodiments, the compositions of the invention modulate the levels of GABA. GABA is an inhibitory neurotransmitter that reduces neuronal excitability. In certain embodiments, the compositions of the invention increase the levels of GABA. In certain embodiments, the compositions of the invention decrease the levels of GABA. In certain embodiments, the compositions of the invention alter GABAergic neurotransmission. In certain embodiments, the compositions of the invention modulate the level of GABA transcription in different regions of the central nervous system. In certain embodiments, the commensal derived GABA crosses the blood-

brain barrier and affects neurotransmission directly. In certain embodiments, the compositions of the invention lead to a reduction of GABA in the hippocampus, amygdala and/or locus coeruleus. In certain embodiments, the compositions of the invention lead to an increase of GABA in cortical regions.

Immune response

The signalling of the microbiota-gut-brain axis is modulated by alterations in the immune response and inflammatory factors and markers. Accordingly, in certain embodiments, the compositions of the invention may modulate the immune response. In certain embodiments, the compositions of the invention modulate the systemic levels of circulating neuroimmune signalling molecules. In certain preferred embodiments, the compositions of the invention modulate pro-inflammatory cytokine production and inflammation. In certain embodiments, the compositions of the invention modulate the inflammatory state. In certain embodiments, the compositions of the invention decrease IL-6 production and secretion. In certain embodiments, the compositions of the invention decrease the activation of the NFκB promoter. In certain embodiments, the compositions of the invention are able to modulate the activation of IL-6 production by the potent pro-inflammatory endotoxin lipopolysaccharide (LPS). In certain embodiments, the compositions of the invention are able to modulate the activation of the NFκB promoter by LPS and α-synuclein mutant proteins such as A53T. Increased circulating levels of cytokines are closely associated with various neurodegenerative disorders, including Parkinson's, dementia and Alzheimer's. In certain embodiments, the compositions of the invention are for use in reducing IL-6 levels and/or NFκB levels in the treatment of a neurodegenerative disorder.

In some embodiments, the compositions of the invention increase the secretion of IL-8. IL-8 has been shown to induce myelin sheath formation and restore or preserve effective neuronal communication. Thus, in some embodiments, the compositions of the invention are for use in inducing myelin sheath formation in the treatment of neurodegenerative diseases. In some embodiments, the compositions of the invention are for use in restoring neuronal communication. In some embodiments, the compositions of the invention are for use in preserving neuronal communication.

The signalling of the microbiota-gut-brain axis is modulated by levels of commensal metabolites. Accordingly, in certain embodiments, the compositions of the invention modulate the systemic levels of microbiota metabolites. In certain preferred embodiments, the compositions of the invention modulate the level of short chain fatty acids (SCFAs). In certain embodiments the level of SCFAs is increased or decreased. In some embodiments, the SCFA is butyric acid (BA) (or butyrate). In some embodiments, the SCFA is propionic acid (PPA). In some embodiments, the SCFA is acetic acid. In certain embodiments, the compositions of the invention modulate the ability of SCFAs to cross the blood-brain barrier.

Histone acetylation and deacetylation are important epigenetic regulators of gene expression. An imbalance in histone acetylation and deacetylation can result in apoptosis. Dysregulation of such histone acetyltransferases has been implicated in the pathogenesis associated with age-associated neurodegenerative diseases, such as Parkinson's disease, Huntington's disease, Alzheimer's disease, amyotrophic lateral sclerosis and cognitive decline [33]. Accordingly, in certain embodiments, the compositions of the invention can modulate histone deacetylatase activity. In certain embodiments, the compositions of the invention can reduce histone deacetylatase activity. In certain embodiments, the compositions of the invention can reduce histone acetylatase activity.

Patients with neurodegenerative diseases, including Parkinson's disease, Huntington's disease, Alzheimer's disease and amyotrophic lateral sclerosis, exhibit high levels of lipid peroxidation. Lipid are vulnerable to oxidation by reactive oxygen species, and the brain is rich in polyunsaturated fatty acids. Accordingly, in certain embodiments, the compositions of the invention can modulate lipid peroxidation. In certain embodiments, the compositions of the invention can reduce lipid peroxidation. Reducing the oxidative damage caused by reactive oxygen species can be used to target early the stages neurodegenerative diseases. Accordingly, in certain embodiments, the compositions of the invention are for use in treating early stage neurodegeneration. Also accordingly, in certain embodiments, the compositions of the invention are for use in preventing the development of a neurodegenerative disorder. In such embodiments, the compositions of the invention may be for use in a patient that has been identified as at risk of developing a neurodegenerative disorder.

The signalling of the microbiota-gut-brain axis is modulated by levels of gastrointestinal permeability. Accordingly, in some embodiments, the compositions of the invention alter the integrity of the gastrointestinal tract epithelium. In certain embodiments, the compositions of the invention modulate the permeability of the gastrointestinal tract. In certain embodiments, the compositions of the invention modulate the barrier function and integrity of the gastrointestinal tract. In certain embodiments, the compositions of the invention modulate gastrointestinal tract motility. In certain embodiments, the compositions of the invention modulate the translocation of commensal metabolites and inflammatory signalling molecules into the bloodstream from the gastrointestinal tract lumen.

The signalling of the microbiota-gut-brain axis is modulated by microbiome composition in the gastrointestinal tract. Accordingly, in certain embodiments, the compositions of the invention modulates the microbiome composition of the gastrointestinal tract. In certain embodiments, the compositions of the invention prevents microbiome dysbiosis and associated increases in toxic metabolites (e.g. LPS). In certain embodiments, the compositions of the invention modulate the levels of Clostridium in the gastrointestinal tract. In preferred embodiments, the compositions of the invention reduce the level of Clostridium in the gastrointestinal tract. In certain embodiments, the compositions of the invention reduce the levels of Campylobacter jejuni. In certain embodiments, the compositions of the invention modulate the proliferation of harmful anaerobic bacteria and the production of neurotoxins produced by these bacteria. In certain embodiments, the compositions of the

invention modulate the microbiome levels of Lactobacillus and/or Bifidobacterium. In certain embodiments, the compositions of the invention modulate the microbiome levels of Sutterella, Prevotella, Ruminococcus genera and/or the Alcaligenaceae family. In certain embodiments, the compositions of the invention increase the level of Lactobacillus plantarum and/or Saccharomyces boulardii.

Brain injury

The examples demonstrate that the compositions of the invention are neuroprotective and have HDAC inhibitory activity. HDAC2 is a crucial target for functional recovery from stroke [34] and HDAC inhibition can prevent white matter injury [35], so the compositions of the invention may be useful in the treatment of brain injury.

In certain embodiments, the compositions of the invention are for use in treating brain injury. In some embodiments, the brain injury is a traumatic brain injury. In some embodiments, the brain injury is an acquired brain injury. In some embodiments, the compositions of the invention are for use in treating brain injury resulting from trauma. In some embodiments, the compositions of the invention are for use in treating brain injury resulting from a tumour. In some embodiments, the compositions of the invention are for use in treating brain injury resulting from a stroke. In some embodiments, the compositions of the invention are for use in treating brain injury resulting from a brain haemorrhage. In some embodiments, the compositions of the invention are for use in treating brain injury resulting from encephalitis. In some embodiments, the compositions of the invention are for use in treating brain injury resulting from cerebral hypoxia. In some embodiments, the compositions of the invention are for use in treating brain injury resulting from cerebral anoxia.

In preferred embodiments, the compositions of the invention are for use in treating stroke. The effects shown in the examples are particularly relevant to the treatment of stroke. Stroke occurs when blood flow to at least a part of the brain is interrupted. Without an adequate supply of blood to provide oxygen and nutrients to the brain tissue and to remove waste products from the brain tissue, brain cells rapidly begin to die. The symptoms of stroke are dependent on the region of the brain which is affected by the inadequate blood flow. Symptoms include paralysis, numbness or weakness of the muscles, loss of balance, dizziness, sudden severe headaches, speech impairment, loss of memory, loss of reasoning ability, sudden confusion, vision impairment, coma or even death. A stroke is also referred to as a brain attack or a cerebrovascular accident (CVA). The symptoms of stroke may be brief if adequate blood flow is restored within a short period of time. However, if inadequate blood flow continues for a significant period of time, the symptoms can be permanent.

In some embodiments, the stroke is cerebral ischemia. Cerebral ischemia results when there is insufficient blood flow to the tissues of the brain to meet metabolic demand. In some embodiments, the cerebral ischemia is focal cerebral ischemia, i.e. confined to a specific region of the brain. In some embodiments the cerebral ischemia is global cerebral ischemia, i.e. encompassing a wide area of the

brain tissue. Focal cerebral ischemia commonly occurs when a cerebral vessel has become blocked, either partially or completely, reducing the flow of blood to a specific region of the brain. In some embodiments the focal cerebral ischemia is ischemic stroke. In some embodiments, the ischemic stroke is thrombotic, i.e. caused by a thrombus or blood clot, which develops in a cerebral vessel and restricts or blocks blood flow. In some embodiments the ischemic stroke is a thrombotic stroke. In some embodiments, the ischemic stroke is embolic, i.e. caused by an embolus, or an unattached mass that travels through the bloodstream and restricts or blocks blood flow at a site distant from its point of origin. In some embodiments the ischemic stroke is an embolic stroke. Global cerebral ischemia commonly occurs when blood flow to the brain as a whole is blocked or reduced. In some embodiments the global cerebral ischemia is caused by hypoperfusion, i.e. due to shock. In some embodiments the global cerebral ischemia is a result of a cardiac arrest.

In some embodiments the subject diagnosed with brain injury has suffered cerebral ischemia. In some embodiments, the subject diagnosed with brain injury has suffered focal cerebral ischemia. In some embodiments, the subject diagnosed with brain injury has suffered an ischemic stroke. In some embodiments, the subject diagnosed with brain injury has suffered a thrombotic stroke. In some embodiments, the subject diagnosed with brain injury has suffered an embolic stroke. In some embodiments, the subject diagnosed with brain injury has suffered global cerebral ischemia. In some embodiments, the subject diagnosed with brain injury has suffered hypoperfusion. In some embodiments, the subject diagnosed with brain injury has suffered a cardiac arrest.

In some embodiments, the compositions of the invention are for use in treating cerebral ischemia. In some embodiments, the compositions of the invention are for use in treating focal cerebral ischemia. In some embodiments, the compositions of the invention are for use treating ischemic stroke. In some embodiments, the compositions of the invention are for use in treating thrombotic stroke. In some embodiments, the compositions of the invention are for use in treating embolic stroke. In some embodiments, the compositions of the invention are for use in treating global cerebral ischemia. In some embodiments, the compositions of the invention are for use in treating hypoperfusion.

In some embodiments, the stroke is hemorrhagic stroke. Hemorrhagic stroke is caused by bleeding into or around the brain resulting in swelling, pressure and damage to the cells and tissues of the brain. Hemorrhagic stroke is commonly a result of a weakened blood vessel that ruptures and bleeds into the surrounding brain. In some embodiments, the hemorrhagic stroke is an intracerebral hemorrhage, i.e. caused by bleeding within the brain tissue itself. In some embodiments the intracerebral hemorrhage is caused by an intraparenchymal hemorrhage. In some embodiments the intracerebral hemorrhage is caused by an intraventricular hemorrhage. In some embodiments the hemorrhagic stroke is a subarachnoid hemorrhage i.e. bleeding that occurs outside of the brain tissue but still within the skull. In some embodiments, the hemorrhagic stroke is a result of cerebral amyloid angiopathy. In some embodiments, the hemorrhagic stroke is a result of a brain aneurysm. In some embodiments, the hemorrhagic stroke is a result of cerebral arteriovenous malformation (AVM).

In some embodiments the subject diagnosed with brain injury has suffered hemorrhagic stroke. In some embodiments, the subject diagnosed with brain injury has suffered an intracerebral hemorrhage. In some embodiments, the subject diagnosed with brain injury has suffered an intraparenchymal hemorrhage. In some embodiments, the subject diagnosed with brain injury has suffered an intraventricular hemorrhage. In some embodiments, the subject diagnosed with brain injury has suffered a subarachnoid hemorrhage. In some embodiments, the subject diagnosed with brain injury has suffered cerebral amyloid angiopathy. In some embodiments, the subject diagnosed with brain injury has suffered a brain aneurysm. In some embodiments, the subject diagnosed with brain injury has suffered cerebral AVM.

In some embodiments, the compositions of the invention are for use in treating hemorrhagic stroke. In some embodiments, the compositions of the invention are for use in treating an intracerebral hemorrhage. In some embodiments, the compositions of the invention are for use in treating an intraparenchymal hemorrhage. In some embodiments, the compositions of the invention are for use in treating an intraventricular hemorrhage. In some embodiments, the compositions of the invention are for use in treating a subarachnoid hemorrhage. In some embodiments, the compositions of the invention are for use in treating a cerebral amyloid angiopathy. In some embodiments, the compositions of the invention are for use in treating a brain aneurysm. In some embodiments, the compositions of the invention are for use in treating cerebral AVM.

Restoration of adequate blood flow to the brain after a period of interruption, though effective in alleviating the symptoms associated with stroke, can paradoxically result in further damage to the brain tissue. During the period of interruption, the affected tissue suffers from a lack of oxygen and nutrients, and the sudden restoration of blood flow can result in inflammation and oxidative damage through the induction of oxidative stress. This is known as reperfusion injury, and is well documented not only following stroke, but also following a heart attack or other tissue damage when blood supply returns to the tissue after a period of ischemia or lack of oxygen. In some embodiments the subject diagnosed with brain injury has suffered from reperfusion injury as a result of stroke. In some embodiments, the compositions of the invention are for use in treating reperfusion injury as a result of stroke.

A transient ischemic attack (TIA), often referred to as a mini-stroke, is a recognised warning sign for a more serious stroke. Subjects who have suffered one or more TIAs are therefore at greater risk of stroke. In some embodiments the subject diagnosed with brain injury has suffered a TIA. In some embodiments, the compositions of the invention are for use in treating a TIA. In some embodiments, the compositions of the invention are for use in treating brain injury in a subject who has suffered a TIA.

High blood pressure, high blood cholesterol, a familial history of stroke, heart disease, diabetes, brain aneurysms, arteriovenous malformations, sickle cell disease, vasculitis, bleeding disorders, use of nonsteroidal anti-inflammatory drugs (NSAIDs), smoking tobacco, drinking large amounts of alcohol,

illegal drug use, obesity, lack of physical activity and an unhealthy diet are all considered to be risk factors for stroke. In particular, lowering blood pressure has been conclusively shown to prevent both ischemic and hemorrhagic strokes [36, 37]. In some embodiments, the compositions of the invention are for use in treating brain injury in a subject who has at least one risk factor for stroke. In some embodiments the subject has two risk factors for stroke. In some embodiments the subject has three risk factors for stroke. In some embodiments the subject has four risk factors for stroke. In some embodiments the subject has more than four risk factors for stroke. In some embodiments the subject has high blood pressure. In some embodiments the subject has high blood cholesterol. In some embodiments the subject has a familial history of stroke. In some embodiments the subject has heart disease. In some embodiments the subject has diabetes. In some embodiments the subject has a brain aneurysm. In some embodiments the subject has arteriovenous malformations. In some embodiments the subject has vasculitis. In some embodiments the subject has sickle cell disease. In some embodiments the subject has a bleeding disorder. In some embodiments the subject has a history of use of nonsteroidal anti-inflammatory drugs (NSAIDs). In some embodiments the subject smokes tobacco. In some embodiments the subject drinks large amounts of alcohol. In some embodiments the subject uses illegal drugs. In some embodiments the subject is obese. In some embodiments the subject is overweight. In some embodiments the subject has a lack of physical activity. In some embodiments the subject has an unhealthy diet.

The examples indicate that the compositions of the invention may be useful for treating brain injury and aiding recovery when administered before the injury event occurs. Therefore, the compositions of the invention may be particularly useful for treating brain injury when administered to subjects at risk of brain injury, such as stroke.

In certain embodiments, the compositions of the invention are for use in reducing the damage caused by a potential brain injury, preferably a stroke. The compositions may reduce the damage caused when they are administered before the potential brain injury occurs, in particular when administered to a patient identified as at risk of a brain injury.

The examples indicate that the compositions of the invention may be useful for treating brain injury and aiding recovery when administered after the injury event occurs. Therefore, the compositions of the invention may be particularly useful for treating brain injury when administered to subjects following a brain injury, such as stroke.

In some embodiments, the compositions of the invention treat brain injury by reducing motoric damage. In some embodiments, the compositions of the invention treat brain injury by improving motor function. In some embodiments, the compositions of the invention treat brain injury by improving muscle strength. In some embodiments, the compositions of the invention treat brain injury by improving memory. In some embodiments, the compositions of the invention treat brain injury by

improving social recognition. In some embodiments, the compositions of the invention treat brain injury by improving neurological function.

Treatment of brain injury may refer to, for example, an alleviation of the severity of symptoms. Treatment of brain injury may also refer to reducing the neurological impairments following stroke. Compositions of the invention for use in treating stroke may be provided to the subject in advance of the onset of stroke, for example in a patient identified as being at risk of stroke. Compositions of the invention for use in treating stroke may be provided after a stroke has occurred, for example, during recovery. Compositions of the invention for use in treating stroke may be provided during the acute phase of recovery (i.e. up to one week after stroke). Compositions of the invention for use in treating stroke may be provided during the subacute phase of recovery (i.e. from one week up to three months after stroke). Compositions of the invention for use in treating stroke may be provided during the chronic phase of recovery (from three months after stroke).

In certain embodiments, the compositions of the invention are for use in combination with a secondary active agent. In certain embodiments, the compositions of the invention are for use in combination with aspirin or tissue plasminogen activator (tPA). Other secondary agents include other antiplatelets (such as clopidogrel), anticoagulants (such as heparins, warfarin, apixaban, dabigatran, edoxaban or rivaroxaban), antihypertensives (such as diuretics, ACE inhibitors, calcium channel blockers, beta-blockers or alpha-blockers) or statins. The compositions of the invention may improve the patient's response to the secondary active agent.

In certain embodiments, the compositions of the invention reduce the effect of ischemia on tissues. In certain embodiments, the compositions of the invention reduce the amount of damage to tissues caused by ischemia. In certain embodiments, the tissues damaged by ischemia are the cerebral tissues. In certain embodiments, the compositions of the invention reduce necrosis or the number of necrotic cells. In certain embodiments, the compositions of the invention reduce apoptosis or the number of apoptotic cells. In certain embodiments, the compositions of the invention reduce the number of necrotic and apoptotic cells. In certain embodiments, the compositions of the invention prevent cell death by necrosis and/or apoptosis. In certain embodiments, the compositions of the invention prevent cell death by necrosis and/or apoptosis caused by ischemia. In certain embodiments, the compositions of the invention improve the recovery of the tissue damaged by ischemia. In certain embodiments, the compositions of the invention improve the speed of clearance of necrotic cells and/or apoptotic cells. In certain embodiments, the compositions of the invention improve the efficacy of the clearance of necrotic cells and/or apoptotic cells. In certain embodiments, the compositions of the invention improve the replacement and/or regeneration of cells within tissues. In certain embodiments, the compositions of the invention improve the replacement and/or regeneration of cells within tissues damaged by ischemia. In certain embodiments, the compositions of the invention improve the overall histology of the tissue (for example upon a biopsy).

Modes of administration

Preferably, the compositions of the invention are to be administered to the gastrointestinal tract in order to enable delivery to and / or partial or total colonisation of the intestine with the bacterial strain of the invention. Generally, the compositions of the invention are administered orally, but they may be administered rectally, intranasally, or via buccal or sublingual routes.

In certain embodiments, the compositions of the invention may be administered as a foam, as a spray or a gel.

In certain embodiments, the compositions of the invention may be administered as a suppository, such as a rectal suppository, for example in the form of a theobroma oil (cocoa butter), synthetic hard fat (e.g. suppocire, witepsol), glycero-gelatin, polyethylene glycol, or soap glycerin composition.

In certain embodiments, the composition of the invention is administered to the gastrointestinal tract via a tube, such as a nasogastric tube, orogastric tube, gastric tube, jejunostomy tube (J tube), percutaneous endoscopic gastrostomy (PEG), or a port, such as a chest wall port that provides access to the stomach, jejunum and other suitable access ports.

The compositions of the invention may be administered once, or they may be administered sequentially as part of a treatment regimen. In certain embodiments, the compositions of the invention are to be administered daily.

In certain embodiments of the invention, treatment according to the invention is accompanied by assessment of the patient's gut microbiota. Treatment may be repeated if delivery of and / or partial or total colonisation with the strain of the invention is not achieved such that efficacy is not observed, or treatment may be ceased if delivery and / or partial or total colonisation is successful and efficacy is observed.

In certain embodiments, the composition of the invention may be administered to a pregnant animal, for example a mammal such as a human in order to prevent an inflammatory or autoimmune disease developing in her child *in utero* and / or after it is born.

The compositions of the invention may be administered to a patient that has been diagnosed with a neurodegenerative disease, or that has been identified as being at risk of a neurodegenerative disease. The compositions may also be administered as a prophylactic measure to prevent the development of neurodegenerative disease in a healthy patient.

The compositions of the invention may be administered to a patient that has been identified as having an abnormal gut microbiota. For example, the patient may have reduced or absent colonisation by *Parabacteroides*, and in particular *Parabacteroides distasonis.*

The compositions of the invention may be administered as a food product, such as a nutritional supplement.

Generally, the compositions of the invention are for the treatment of humans, although they may be used to treat animals including monogastric mammals such as poultry, pigs, cats, dogs, horses or rabbits. The compositions of the invention may be useful for enhancing the growth and performance of animals. If administered to animals, oral gavage may be used.

Compositions

Generally, the composition of the invention comprises bacteria. In preferred embodiments of the invention, the composition is formulated in freeze-dried form. For example, the composition of the invention may comprise granules or gelatin capsules, for example hard gelatin capsules, comprising a bacterial strain of the invention.

Preferably, the composition of the invention comprises lyophilised bacteria. Lyophilisation of bacteria is a well-established procedure and relevant guidance is available in, for example, references [38-40].

Alternatively, the composition of the invention may comprise a live, active bacterial culture.

In some embodiments, the bacterial strain in the composition of the invention has not been inactivated, for example, has not been heat-inactivated. In some embodiments, the bacterial strain in the composition of the invention has not been killed, for example, has not been heat-killed. In some embodiments, the bacterial strain in the composition of the invention has not been attenuated, for example, has not been heat-attenuated. For example, in some embodiments, the bacterial strain in the composition of the invention has not been killed, inactivated and/or attenuated. For example, in some embodiments, the bacterial strain in the composition of the invention is live. For example, in some embodiments, the bacterial strain in the composition of the invention is viable. For example, in some embodiments, the bacterial strain in the composition of the invention is capable of partially or totally colonising the intestine. For example, in some embodiments, the bacterial strain in the composition of the invention is viable and capable of partially or totally colonising the intestine.

In some embodiments, the composition comprises a mixture of live bacterial strains and bacterial strains that have been killed.

In preferred embodiments, the composition of the invention is encapsulated to enable delivery of the bacterial strain to the intestine. Encapsulation protects the composition from degradation until delivery at the target location through, for example, rupturing with chemical or physical stimuli such as pressure, enzymatic activity, or physical disintegration, which may be triggered by changes in pH. Any appropriate encapsulation method may be used. Exemplary encapsulation techniques include entrapment within a porous matrix, attachment or adsorption on solid carrier surfaces, self-aggregation by flocculation or with cross-linking agents, and mechanical containment behind a microporous membrane or a microcapsule. Guidance on encapsulation that may be useful for preparing compositions of the invention is available in, for example, references [41] and [42].

The composition may be administered orally and may be in the form of a tablet, capsule or powder. Encapsulated products are preferred because *Parabacteroides* are anaerobes. Other ingredients (such as vitamin C, for example), may be included as oxygen scavengers and prebiotic substrates to improve the delivery and / or partial or total colonisation and survival *in vivo*. Alternatively, the probiotic composition of the invention may be administered orally as a food or nutritional product, such as milk or whey based fermented dairy product, or as a pharmaceutical product.

The composition may be formulated as a probiotic.

A composition of the invention includes a therapeutically effective amount of a bacterial strain of the invention. A therapeutically effective amount of a bacterial strain is sufficient to exert a beneficial effect upon a patient. A therapeutically effective amount of a bacterial strain may be sufficient to result in delivery to and / or partial or total colonisation of the patient's intestine.

A suitable daily dose of the bacteria, for example for an adult human, may be from about 1 x 10^3 to about 1 x 10^{11} colony forming units (CFU); for example, from about 1 x 10^7 to about 1 x 10^{10} CFU; in another example from about 1 x 10^6 to about 1 x 10^{10} CFU.

In certain embodiments, the composition contains the bacterial strain in an amount of from about 1 x 10^6 to about 1 x 10^{11} CFU/g, respect to the weight of the composition; for example, from about 1 x 10^8 to about 1 x 10^{10} CFU/g. The dose may be, for example, 1 g, 3g, 5g, and 10g.

Typically, a probiotic, such as the composition of the invention, is optionally combined with at least one suitable prebiotic compound. A prebiotic compound is usually a non-digestible carbohydrate such as an oligo- or polysaccharide, or a sugar alcohol, which is not degraded or absorbed in the upper digestive tract. Known prebiotics include commercial products such as inulin and transgalacto-oligosaccharides.

In certain embodiments, the probiotic composition of the present invention includes a prebiotic compound in an amount of from about 1 to about 30% by weight, respect to the total weight composition, (e.g. from 5 to 20% by weight). Carbohydrates may be selected from the group consisting of: fructo- oligosaccharides (or FOS), short-chain fructo-oligosaccharides, inulin, isomalt-oligosaccharides, pectins, xylo-oligosaccharides (or XOS), chitosan-oligosaccharides (or COS), beta-glucans, arable gum modified and resistant starches, polydextrose, D-tagatose, acacia fibers, carob, oats, and citrus fibers. In one aspect, the prebiotics are the short-chain fructo-oligosaccharides (for simplicity shown herein below as FOSs-c.c); said FOSs-c.c. are not digestible carbohydrates, generally obtained by the conversion of the beet sugar and including a saccharose molecule to which three glucose molecules are bonded.

In certain embodiments, the compositions of the invention are used in combination with another therapeutic compound for treating or preventing the neurodegenerative disorder. In some embodiments, the compositions of the invention are administered with nutritional supplements that

modulate neuroprotection or neuroproliferation. In preferred embodiments, the nutritional supplements comprise or consist of nutritional vitamins. In certain embodiments, the vitamins are vitamin B6, magnesium, dimethylglycine (vitamin B16) and vitamin C. In certain embodiments, the compositions of the invention are administered in combination with another probiotic.

In certain embodiments, the compositions of the invention are for use in enhancing the effect of a second agent on a neurodegenerative disease. The immune modulatory effects of the compositions of the invention may make the brain more susceptible to conventional therapies such as Levodopa, dopamine agonists, MAO-B inhibitors, COMT inhibitors, Glutamate antagonists, or anticholinergics, which are exemplary secondary agents to be administered in combination (sequentially or contemporaneously) with the compositions of the invention.

The compositions of the invention may comprise pharmaceutically acceptable excipients or carriers. Examples of such suitable excipients may be found in the reference [43]. Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art and are described, for example, in reference [44]. Examples of suitable carriers include lactose, starch, glucose, methyl cellulose, magnesium stearate, mannitol, sorbitol and the like. Examples of suitable diluents include ethanol, glycerol and water. The choice of pharmaceutical carrier, excipient or diluent can be selected with regard to the intended route of administration and standard pharmaceutical practice. The pharmaceutical compositions may comprise as, or in addition to, the carrier, excipient or diluent any suitable binder(s), lubricant(s), suspending agent(s), coating agent(s), solubilising agent(s). Examples of suitable binders include starch, gelatin, natural sugars such as glucose, anhydrous lactose, free-flow lactose, beta-lactose, corn sweeteners, natural and synthetic gums, such as acacia, tragacanth or sodium alginate, carboxymethyl cellulose and polyethylene glycol. Examples of suitable lubricants include sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride and the like. Preservatives, stabilizers, dyes and even flavouring agents may be provided in the pharmaceutical composition. Examples of preservatives include sodium benzoate, sorbic acid and esters of p-hydroxybenzoic acid. Antioxidants and suspending agents may be also used.

The compositions of the invention may be formulated as a food product. For example, a food product may provide nutritional benefit in addition to the therapeutic effect of the invention, such as in a nutritional supplement. Similarly, a food product may be formulated to enhance the taste of the composition of the invention or to make the composition more attractive to consume by being more similar to a common food item, rather than to a pharmaceutical composition. In certain embodiments, the composition of the invention is formulated as a milk-based product. The term "milk-based product" means any liquid or semi-solid milk- or whey- based product having a varying fat content. The milk-based product can be, e.g., cow's milk, goat's milk, sheep's milk, skimmed milk, whole milk, milk recombined from powdered milk and whey without any processing, or a processed product, such as yoghurt, curdled milk, curd, sour milk, sour whole milk, butter milk and other sour milk products. Another important group includes milk beverages, such as whey beverages, fermented milks,

condensed milks, infant or baby milks; flavoured milks, ice cream; milk-containing food such as sweets.

In some embodiments, the compositions of the invention comprise one or more bacterial strains of the genus *Parabacteroides* and do not contain bacteria from any other genera, or which comprise only *de minimis* or biologically irrelevant amounts of bacteria from another genera. Thus, in some embodiments, the invention provides a composition comprising one or more bacterial strains of the genus *Parabacteroides*, which does not contain bacteria from any other genera or which comprises only *de minimis* or biologically irrelevant amounts of bacteria from another genera, for use in therapy.

In some embodiments, the compositions of the invention comprise one or more bacterial strains of the species *Parabacteroides distasonis* and do not contain bacteria from any other species, or which comprise only *de minimis* or biologically irrelevant amounts of bacteria from another species. Thus, in some embodiments, the invention provides a composition comprising one or more bacterial strains of the species *Parabacteroides distasonis*, which does not contain bacteria from any other species or which comprises only *de minimis* or biologically irrelevant amounts of bacteria from another species, for use in therapy.

In some embodiments, the compositions of the invention comprise one or more bacterial strains of the species *Parabacteroides distasonis* and do not contain bacteria from any other *Parabacteroides* species, or which comprise only *de minimis* or biologically irrelevant amounts of bacteria from another *Parabacteroides* species. Thus, in some embodiments, the invention provides a composition comprising one or more bacterial strains of the species *Parabacteroides distasonis*, which does not contain bacteria from any other *Parabacteroides* species or which comprises only *de minimis* or biologically irrelevant amounts of bacteria from another *Parabacteroides* species, for use in therapy.

In certain embodiments, the compositions of the invention contain a single bacterial strain or species and do not contain any other bacterial strains or species. Such compositions may comprise only *de minimis* or biologically irrelevant amounts of other bacterial strains or species. Such compositions may be a culture that is substantially free from other species of organism.

In some embodiments, the invention provides a composition comprising a single bacterial strain of the genus *Parabacteroides,* which does not contain bacteria from any other strains or which comprises only *de minimis* or biologically irrelevant amounts of bacteria from another strain for use in therapy.

In some embodiments, the invention provides a composition comprising a single bacterial strain of the species *Parabacteroides distasonis,* which does not contain bacteria from any other strains or which comprises only *de minimis* or biologically irrelevant amounts of bacteria from another strain for use in therapy.

In some embodiments, the compositions of the invention comprise more than one bacterial strain. For example, in some embodiments, the compositions of the invention comprise more than one strain from

within the same species (e.g. more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40 or 45 strains), and, optionally, do not contain bacteria from any other species. In some embodiments, the compositions of the invention comprise less than 50 strains from within the same species (e.g. less than 45, 40, 35, 30, 25, 20, 15, 12, 10, 9, 8, 7, 6, 5, 4 or 3 strains), and, optionally, do not contain bacteria from any other species. In some embodiments, the compositions of the invention comprise 1-40, 1-30, 1-20, 1-19, 1-18, 1-15, 1-10, 1-9, 1-8, 1-7, 1-6, 1-5, 1-4, 1-3, 1-2, 2-50, 2-40, 2-30, 2-20, 2-15, 2-10, 2-5, 6-30, 6-15, 16-25, or 31-50 strains from within the same species and, optionally, do not contain bacteria from any other species. The invention comprises any combination of the foregoing.

In some embodiments, the composition comprises a microbial consortium. For example, in some embodiments, the composition comprises the *Parabacteroides* bacterial strain as part of a microbial consortium. For example, in some embodiments, the *Parabacteroides* bacterial strain is present in combination with one or more (e.g. at least 2, 3, 4, 5, 10, 15 or 20) other bacterial strains from other genera with which it can live symbiotically *in vivo* in the intestine. For example, in some embodiments, the composition comprises a bacterial strain of *Parabacteroides* in combination with a bacterial strain from a different genus. In some embodiments, the microbial consortium comprises two or more bacterial strains obtained from a faeces sample of a single organism, e.g. a human. In some embodiments, the microbial consortium is not found together in nature. For example, in some embodiments, the microbial consortium comprises bacterial strains obtained from faeces samples of at least two different organisms. In some embodiments, the two different organisms are from the same species, e.g. two different humans. In some embodiments, the two different organisms are an infant human and an adult human. In some embodiments, the two different organisms are a human and a non-human mammal.

In some embodiments, the composition of the invention additionally comprises a bacterial strain that has the same safety and therapeutic efficacy characteristics as strain MRX005, but which is not MRx0005, or which is not a *Parabacteroides distasonis*.

In some embodiments in which the composition of the invention comprises more than one bacterial strain, species or genus, the individual bacterial strains, species or genera may be for separate, simultaneous or sequential administration. For example, the composition may comprise all of the more than one bacterial strain, species or genera, or the bacterial strains, species or genera may be stored separately and be administered separately, simultaneously or sequentially. In some embodiments, the more than one bacterial strains, species or genera are stored separately but are mixed together prior to use.

In some embodiments, the bacterial strain for use in the invention is obtained from human adult faeces. In some embodiments in which the composition of the invention comprises more than one bacterial strain, all of the bacterial strains are obtained from human adult faeces or if other bacterial strains are

present they are present only in *de minimis* amounts. The bacteria may have been cultured subsequent to being obtained from the human adult faeces and being used in a composition of the invention.

In some embodiments, the bacterial strain for use in the invention is obtained from human infant faeces. In some embodiments in which the composition of the invention comprises more than one bacterial strain, all of the bacterial strains are obtained from human infant faeces or if other bacterial strains are present they are present only in *de minimis* amounts. The bacteria may have been cultured subsequent to being obtained from the human infant faeces and being used in a composition of the invention

As mentioned above, in some embodiments, the one or more *Parabacteroides* bacterial strains is/are the only therapeutically active agent(s) in a composition of the invention. In some embodiments, the bacterial strain(s) in the composition is/are the only therapeutically active agent(s) in a composition of the invention.

The compositions for use in accordance with the invention may or may not require marketing approval.

In certain embodiments, the invention provides the above pharmaceutical composition, wherein said bacterial strain is lyophilised. In certain embodiments, the invention provides the above pharmaceutical composition, wherein said bacterial strain is spray dried. In certain embodiments, the invention provides the above pharmaceutical composition, wherein the bacterial strain is lyophilised or spray dried and wherein it is live. In certain embodiments, the invention provides the above pharmaceutical composition, wherein the bacterial strain is lyophilised or spray dried and wherein it is viable. In certain embodiments, the invention provides the above pharmaceutical composition, wherein the bacterial strain is lyophilised or spray dried and wherein it is capable of partially or totally colonising the intestine. In certain embodiments, the invention provides the above pharmaceutical composition, wherein the bacterial strain is lyophilised or spray dried and wherein it is viable and capable of partially or totally colonising the intestine.

In some cases, the lyophilised bacterial strain is reconstituted prior to administration. In some cases, the reconstitution is by use of a diluent described herein.

The compositions of the invention can comprise pharmaceutically acceptable excipients, diluents or carriers.

In certain embodiments, the invention provides a pharmaceutical composition comprising: a bacterial strain of the invention; and a pharmaceutically acceptable excipient, carrier or diluent; wherein the bacterial strain is in an amount sufficient to treat a neurodegenerative disorder when administered to a subject in need thereof.

In certain embodiments, the invention provides pharmaceutical composition comprising: a bacterial strain of the invention; and a pharmaceutically acceptable excipient, carrier or diluent; wherein the bacterial strain is in an amount sufficient to treat or prevent a neurodegenerative disorder.

In certain embodiments, the invention provides the above pharmaceutical composition, wherein the amount of the bacterial strain is from about 1×10^3 to about 1×10^{11} colony forming units per gram with respect to a weight of the composition.

In certain embodiments, the invention provides the above pharmaceutical composition, wherein the composition is administered at a dose of 1 g, 3 g, 5 g or 10 g.

In certain embodiments, the invention provides the above pharmaceutical composition, wherein the composition is administered by a method selected from the group consisting of oral, rectal, subcutaneous, nasal, buccal, and sublingual.

In certain embodiments, the invention provides the above pharmaceutical composition, comprising a carrier selected from the group consisting of lactose, starch, glucose, methyl cellulose, magnesium stearate, mannitol and sorbitol.

In certain embodiments, the invention provides the above pharmaceutical composition, comprising a diluent selected from the group consisting of ethanol, glycerol and water.

In certain embodiments, the invention provides the above pharmaceutical composition, comprising an excipient selected from the group consisting of starch, gelatin, glucose, anhydrous lactose, free-flow lactose, beta-lactose, corn sweetener, acacia, tragacanth, sodium alginate, carboxymethyl cellulose, polyethylene glycol, sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate and sodium chloride.

In certain embodiments, the invention provides the above pharmaceutical composition, further comprising at least one of a preservative, an antioxidant and a stabilizer.

In certain embodiments, the invention provides the above pharmaceutical composition, comprising a preservative selected from the group consisting of sodium benzoate, sorbic acid and esters of p-hydroxybenzoic acid.

In certain embodiments, the invention provides the above pharmaceutical composition, wherein said bacterial strain is lyophilised.

In certain embodiments, the invention provides the above pharmaceutical composition, wherein when the composition is stored in a sealed container at about 4◦C or about 25◦C and the container is placed in an atmosphere having 50% relative humidity, at least 80% of the bacterial strain as measured in colony forming units, remains after a period of at least about: 1 month, 3 months, 6 months, 1 year, 1.5 years, 2 years, 2.5 years or 3 years.

In some embodiments, the composition of the invention is provided in a sealed container comprising a composition as described herein. In some embodiments, the sealed container is a sachet or bottle. In some embodiments, the composition of the invention is provided in a syringe comprising a composition as described herein.

The composition of the present invention may, in some embodiments, be provided as a pharmaceutical formulation. For example, the composition may be provided as a tablet or capsule. In some embodiments, the capsule is a gelatine capsule ("gel-cap").

In some embodiments, the compositions of the invention are administered orally. Oral administration may involve swallowing, so that the compound enters the gastrointestinal tract, and/or buccal, lingual, or sublingual administration by which the compound enters the blood stream directly from the mouth.

Pharmaceutical formulations suitable for oral administration include solid plugs, solid microparticulates, semi-solid and liquid (including multiple phases or dispersed systems) such as tablets; soft or hard capsules containing multi- or nano-particulates, liquids (e.g. aqueous solutions), emulsions or powders; lozenges (including liquid-filled); chews; gels; fast dispersing dosage forms; films; ovules; sprays; and buccal/mucoadhesive patches.

In some embodiments the pharmaceutical formulation is an enteric formulation, i.e. a gastro-resistant formulation (for example, resistant to gastric pH) that is suitable for delivery of the composition of the invention to the intestine by oral administration. Enteric formulations may be particularly useful when the bacteria or another component of the composition is acid-sensitive, e.g. prone to degradation under gastric conditions.

In some embodiments, the enteric formulation comprises an enteric coating. In some embodiments, the formulation is an enteric-coated dosage form. For example, the formulation may be an enteric-coated tablet or an enteric-coated capsule, or the like. The enteric coating may be a conventional enteric coating, for example, a conventional coating for a tablet, capsule, or the like for oral delivery. The formulation may comprise a film coating, for example, a thin film layer of an enteric polymer, e.g. an acid-insoluble polymer.

In some embodiments, the enteric formulation is intrinsically enteric, for example, gastro-resistant without the need for an enteric coating. Thus, in some embodiments, the formulation is an enteric formulation that does not comprise an enteric coating. In some embodiments, the formulation is a capsule made from a thermogelling material. In some embodiments, the thermogelling material is a cellulosic material, such as methylcellulose, hydroxymethylcellulose or hydroxypropylmethylcellulose (HPMC). In some embodiments, the capsule comprises a shell that does not contain any film forming polymer. In some embodiments, the capsule comprises a shell and the shell comprises hydroxypropylmethylcellulose and does not comprise any film forming polymer (e.g. see [45]). In some embodiments, the formulation is an intrinsically enteric capsule (for example, Vcaps® from Capsugel).

In some embodiments, the formulation is a soft capsule. Soft capsules are capsules which may, owing to additions of softeners, such as, for example, glycerol, sorbitol, maltitol and polyethylene glycols, present in the capsule shell, have a certain elasticity and softness. Soft capsules can be produced, for example, on the basis of gelatine or starch. Gelatine-based soft capsules are commercially available

from various suppliers. Depending on the method of administration, such as, for example, orally or rectally, soft capsules can have various shapes, they can be, for example, round, oval, oblong or torpedo-shaped. Soft capsules can be produced by conventional processes, such as, for example, by the Scherer process, the Accogel process or the droplet or blowing process.

Culturing methods

The bacterial strains for use in the present invention can be cultured using standard microbiology techniques as detailed in, for example, references [46-48].

The solid or liquid medium used for culture may be YCFA agar or YCFA medium. YCFA medium may include (per 100ml, approximate values): Casitone (1.0 g), yeast extract (0.25 g), $NaHCO_3$ (0.4 g), cysteine (0.1 g), K_2HPO_4 (0.045 g), KH_2PO_4 (0.045 g), NaCl (0.09 g), $(NH_4)_2SO_4$ (0.09 g), $MgSO_4 \cdot 7H_2O$ (0.009 g), $CaCl_2$ (0.009 g), resazurin (0.1 mg), hemin (1 mg), biotin (1 μg), cobalamin (1 μg), *p*-aminobenzoic acid (3 μg), folic acid (5 μg), and pyridoxamine (15 μg).

Bacterial strains for use in vaccine compositions

The inventors have identified that the bacterial strains of the invention are useful for treating or preventing neurodegenerative disorders. This is likely to be a result of the effect that the bacterial strains of the invention have on the host immune system. Therefore, the compositions of the invention may also be useful for preventing neurodegenerative disorders, when administered as vaccine compositions. In certain such embodiments, the bacterial strains of the invention may be killed, inactivated or attenuated. In certain such embodiments, the compositions may comprise a vaccine adjuvant. In certain embodiments, the compositions are for administration via injection, such as via subcutaneous injection.

General

The practice of the present invention will employ, unless otherwise indicated, conventional methods of chemistry, biochemistry, molecular biology, immunology and pharmacology, within the skill of the art. Such techniques are explained fully in the literature. See, *e.g.*, references [49] and [50-56], *etc.*

The term "comprising" encompasses "including" as well as "consisting" *e.g.* a composition "comprising" X may consist exclusively of X or may include something additional *e.g.* X + Y.

The term "about" in relation to a numerical value *x* is optional and means, for example, $x \pm 10\%$.

The word "substantially" does not exclude "completely" *e.g.* a composition which is "substantially free" from Y may be completely free from Y. Where necessary, the word "substantially" may be omitted from the definition of the invention.

References to a percentage sequence identity between two nucleotide sequences means that, when aligned, that percentage of nucleotides are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in

the art, for example those described in section 7.7.18 of ref. [57]. A preferred alignment is determined by the Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 2, BLOSUM matrix of 62. The Smith-Waterman homology search algorithm is disclosed in ref. [58].

Unless specifically stated, a process or method comprising numerous steps may comprise additional steps at the beginning or end of the method, or may comprise additional intervening steps. Also, steps may be combined, omitted or performed in an alternative order, if appropriate.

Various embodiments of the invention are described herein. It will be appreciated that the features specified in each embodiment may be combined with other specified features, to provide further embodiments. In particular, embodiments highlighted herein as being suitable, typical or preferred may be combined with each other (except when they are mutually exclusive).

MODES FOR CARRYING OUT THE INVENTION

Example 1 – Efficacy of bacterial inocula to act as a neuroprotectant

Summary

Neuroblastoma cells were treated with compositions comprising bacterial strains according to the invention. The SH-SY5Y neuroblastoma cells used are dopamine producing and well-established as an *in vitro* model for studying neurodegenerative diseases. The ability of the bacterial strains to increase neuroproliferation was observed. The neuroblastoma cells were treated also treated dopaminergic neurotoxin 1-methyl-4-phenylpyridinium (MPP), which induces permanent symptoms of Parkinson's disease in neuroblastoma cells. The ability of the bacterial strains to act as a neuroprotectant against MPP was investigated.

Material and Methods

Bacterial strain

755: *Parabacteroides distasonis, Megasphaera massiliensis* MRx0029

Cell line

SH-SY5Y neuroblastoma cells were purchased from ECCACC (Cat. no: 94030304) and were grown in MEM (Sigma Aldrich, cat n. M2279) supplemented with Nutrient Mixture F-12 Ham (Sigma Aldrich, cat n. N4888).

Method

Once grown the SH-SY5Y neuroblastoma cells were plated on 96-well plate at 11,000 cells/well and incubated for 2 days. The cells were then transferred to differentiation medium (which contains FBS at 1%) and 10 uM retinoic acid (Sigma Aldrich, cat. n. R2625-100MG). Differentiation medium was replaced every other day and cells were harvested at 7 day of differentiation. Cells were pre-treated

with or without MPP (Sigma Aldrich, cat. n. D048-1G) for 8 hours. Subsequently, cells were treated with 10% bacterial supernatant and incubated overnight. Cell viability was measured by using CCK-8 reagent (Sigma Aldrich, Cell Counting Kit – 8, cat. n. 96992-3000TESTS-F) and read at 450nm wavelength.

Results

The results of these experiments are shown in Figure 1. Treatment of neuroblastoma cells with MRx0005 or MRx0029 led to an increase in the proliferation of neurons. Neuroblastoma cells that were treated with MPP together with the bacterial strain had increased cell viability compared to the cells treated with MPP alone (which had decreased viability). These data show that the bacterial strains can act as a neuroprotectant.

Example 2A – Efficacy of bacterial inocula to reduce IL-6 secretion.

Summary

Activation of proinflammatory cytokines has been associated with neuron damage in neurodegenerative disease. Lipopolysaccharide (LPS) is a known stimulator of the proinflammatory cytokine IL-6. Human glioblastoma astrocytoma cells were treated with compositions comprising bacterial strains according to the invention in combination with LPS to observe their ability to modulate the levels of IL-6.

Material and Methods

Bacterial strain

755: *Parabacteroides distasonis*

Cell line

MG U373 is a human glioblastoma astrocytoma derived from a malignant tumour and were purchased from Sigma-Aldrich (cat n. 08061901-1VL). MG U373 human glioblastoma astrocytoma cells were grown in MEM (Sigma Aldrich, cat n. M-2279) supplemented with 10% FBS, 1% Pen Strep, 4mM L-Glut, 1X MEM Non essential Amino Acid solution and 1X Sodium Piruvate.

Method

Once grown the MG U373 cells were plated on 24-well plate at 100,000 cells/well. The cells were treated with LPS (1ug/mL) alone or with 10% of bacteria supernatant from MRx0005 for 24h. A control was also performed where the cells were incubated in untreated media. Afterwards the cell free supernatants were collected, centrifuged at 10,000g for 3min at 4°C. IL-6 was measured using the Human IL-6 ELISA Kit from Peprotech (cat n.#900-K16) according to manufacturer instructions.

Results

The results of these experiments are shown in Figure 2A. Treatment of neuroblastoma cells with LPS and the bacteria strain led to a decrease in the level of IL-6 secreted.

Example 2B – Efficacy of bacterial inocula to modulate IL-8 secretion.

Summary

As neuro-inflammation plays a pivotal role in neurodegenerative diseases and IL-8 has been shown to have neuro-positive effects, the effect of compositions comprising bacterial strains of the invention and LPS on the activation of IL-8 were assessed. Human glioblastoma astrocytoma cells were treated with compositions comprising bacterial strains according to the invention in combination with LPS to observe their ability to modulate the levels of IL-8.

Material and Methods

Bacterial strains

Megasphaera massiliensis MRX0029; *Parabacteroides distasonis* MRX0005

Cell line

MG U373 is a human glioblastoma astrocytoma derived from a malignant tumour and were purchased from Sigma-Aldrich (cat n. 08061901-1VL). MG U373 human glioblastoma astrocytoma cells were grown in MEM (Sigma Aldrich, cat n. M-2279) supplemented with 10% FBS, 1% Pen Strep, 4mM L-Glut, 1X MEM Non essential Amino Acid solution and 1X Sodium Piruvate.

Method

Once grown the MG U373 cells were plated on 24-well plate at 100,000 cells/well. The cells were treated with LPS (1ug/mL) alone or with 10% of bacteria supernatant from MRX0029 for 24h. Afterwards the cell free supernatants were collected, centrifuged at 10,000g for 3min at 4°C. IL-8 was measured using Human IL-8 ELISA Kit from Peprotech (cat n.#900-K18) according to manufacturer instruction.

Results

The results of these experiments are shown in Figure 2B.

Example 2C – Efficacy of bacterial inocula to reduce α-synuclein-induced inflammation.

Summary

Neuroinflammation plays a pivotal role in Parkinson's disease and α-synuclein has been shown to induce neuroinflammation *in vivo*. Therefore, the ability of the bacteria strains of the invention to inhibit α-synuclein-induced neuroinflammation was assessed. A co-culture of human glioblastoma

astrocytoma cells and neuroblastoma cells were exposed to wild-type α-synuclein and the mutant isoforms E46K and A53T and treated with compositions comprising bacterial strains according to the invention. The ability of the bacteria strains to inhibit α-synuclein-induced secretion of IL-6 was then tested.

Material and Methods

Bacterial strains

Megasphaera massiliensis MRX0029*; Parabacteroides distasonis* MRX0005

Cell line

MG U373 is a human glioblastoma astrocytoma derived from a malignant tumour and were purchased from Sigma-Aldrich (cat n. 08061901-1VL). MG U373 human glioblastoma astrocytoma cells were grown in MEM (Sigma Aldrich, cat n. M-2279) supplemented with 10% FBS, 1% Pen Strep, 4mM L-Glut, 1X MEM Non essential Amino Acid solution and 1X Sodium Piruvate.

SH-SY5Y is a human neurobastoma cell line derived from a malignant neuroblastoma and can be purchased from Sigma-Aldrich (cat n. 94030304-1VL). The cells were grown in 50 % MEM and 50% Nutrient Mixture F-12 Ham media supplemented with 2mM L-Glutamine, 10% heat inactivated FBS, 100 U/ml penicillin, 100 µg/ml streptomycin. Cells on growth medium were plated on 96-well plate at 11,000 cells/well and placed in the incubator. After 2 days, media were replaced with differentiation medium (growth medium containing 1% FBS) and 10 µM retinoic acid. Differentiation medium was replaced every other day and cells were used after 7 days of differentiation.

Method

SHSY5Y cells were plated on 12 well plates at a density of 50,000 cells/well. The cells were grown in 50 % MEM and 50% Nutrient Mixture F-12 Ham media supplemented with 2mM L-Glutamine, 10% heat inactivated FBS, 100 U/ml penicillin, 100 µg/ml streptomycin. Cells on growth medium were plated on 96-well plate at 11,000 cells/well and placed in the incubator. After 2 days, media were replaced with differentiation medium (growth medium containing 1% FBS) and 10 µM retinoic acid. Differentiation medium was replaced every other day and cells were used after 7 days of differentiation. U373 were plated on 12 transwell plates (0.4µm polyester membrane, Costar) at a density of 50,000cells/well for 72 hrs. Cells were co-cultured together for 24hrs before treatment in differentiation medium (growth medium containing 1% FBS without retinoic acid).

Thereafter cells were treated with 25µg/ml α-synuclein (Wt, A53T, E46K) in the presence or absence of 10% bacteria supernatant for 48 hrs. Cell free Supernatants were collected, spun-dwon at 10000g for 3 min at 4°C, aliquoted and stored at -80 0C. Human IL-6 and IL-8 were measured as described above.

Results

The results of these experiments are shown in Figure 3. Treatment of cells with wild-type α-synuclein and the mutant isoforms E46K and A53T induced moderate secretion of IL-6. The α-syn-induced secretion of IL-6 was inhibited in cells treated with the bacteria strains.

Example 3 – Efficacy of bacterial inocula to reduce NFκB activation

Summary

Activation of the NFκB promoter leads to the production of proinflammatory cytokines including IL-1β, IL-1α, IL-18, TNFα and IL-6. The NFκB promoter can be activated by α-synuclein and LPS by stimulating the TLR4 ligand. Mutations in α-synuclein, such as α-synuclein A53T, are implicated in familial Parkinson's. Treatment of neuronal cells with LPS simulates Parkinson's caused by environmental factors. The ability of compositions comprising bacterial strains according to the invention to inhibit the activation of the NFκB promoter was investigated.

Material and Methods

Bacterial strain

755: *Parabacteroides distasonis*

Cell line

Human Hek blue TLR4 were purchased from InvivoGen (cat n. hkb-htlr4). Human Hek blue TLR4 were grown in DMEM high glucose (Sigma Aldrich, cat n. D-6171) supplemented with 10% FBS, 1% Pen Strep, 4mM L-Glut, Normocin and 1X HEK Blue selection solution.

Method

Once grown the Human Hek blue cells were plated in 96 well plates at 25,000 cells/well in 4 replicates. One set of cells were treated with α-synuclein A53T (1ug/mL) alone or with 10% of bacteria supernatant from MRx0005 for 22h. The second set of cells were treated with LPS (10 ng/mL, from Salmonella enterica serotype Typhimurium, Sigma Aldrich, cat n. L6143) alone or with 10% of bacteria supernatant from MRx0005 for 22h. The cells were subsequently spun down and 20ul of the supernatant was mixed with 200ul of Quanti Blue reagent (InvivoGen, cat n. rep-qb2), incubated for 2 h and absorbance read at 655nm.

Results

The results of these experiments are shown in Figure 4 and 5. Figure 4 shows that the activation of the NFκB promoter by α-synuclein is inhibited by MRx0005. Figure 5 shows that the activation of the NFκB promoter by LPS is inhibited by MRx0005.

Example 4 – Efficacy of bacterial inocula to alter antioxidant capacity

Summary

The ability of compositions comprising bacterial strains according to the invention to alter the antioxidant capacity. The antioxidant capacity of the bacterial strain was established using the well-known ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) assay.

Bacterial strain

755: *Parabacteroides distasonis*

Method

Bacterial cells (10^6 or greater) were collected and centrifuged. They were resuspended in assay buffer (using three times the pellet volume). The suspension was sonicated on ice for 5 minutes and then spun down at 12,000 x g for 10 minutes. The supernatant was removed and measured using the ABTS assay kit produced by Sigma Aldrich (code CS0790), in accordance with manufacturer's instructions.

Results

The results of these experiments are shown in Figure 6. Figure 6 shows that MRx0005 has an antioxidant capacity of approximately 0.5mM compared to Trolox.

Example 5 – Efficacy of bacterial inocula to alter lipid peroxidation levels

Summary

The ability of compositions comprising bacterial strains according to the invention to alter lipid peroxidation levels was investigated. The thiobarbituric reactive substances assay (TBARs) was used to measure the by-products of lipid peroxidation.

Material and Methods

Bacterial strain

755: *Parabacteroides distasonis*

Method

Bacterial cells (10^6 or greater) were collected and centrifuged, a wash step was performed with isotonic saline before the pellet was re-suspensed in potassium chloride assay buffer. The suspension was sonicated on ice for 10 minutes and then spun down at 10,000 x g for 10 minutes. The supernatant was removed and the level of lipid peroxidation evaluated using the thiobarbituric reactive substances assay.

Results

The results of the experiments are shown in Figure 7. Figure 7 shows that MRx0005 is able to inhibit lipid peroxidation by approximately 20 %, which is a higher antioxidant capacity than the positive control, butylated hydroxytoluene (1% w/v).

Example 6 – Efficacy of bacterial inocula on histone deacetylatase activity

Summary

The ability of compositions comprising bacterial strains according to the invention to alter histone deacetylatase activity was investigated. Dysregulation of histone deacetylatase has been implicated in the pathogenesis associated with age-associated neurodegenerative diseases.

Material and Methods

Bacterial strain

755: *Parabacteroides distasonis*

Cell line

The cell line HT-29 was used because histone deacetylase is present.

Method

Cell free supernatants of stationary phase bacterial cultures were isolated by centrifugation and filtering in a 0.22 uM filter. HT-29 cells were used 3 days' post confluence and stepped down in 1 mL DTS 24 hours prior to commencement of the experiment. The HT-29 cells were challenged with 10 % cell free supernatant diluted in DTS and this was left to incubate for 48 hours. Nuclease proteins were then extracted using the Sigma Aldrich Nuclease extraction kit and samples were snap frozen prior to HDAC activity measurement. HDAC activity was assessed fluorometrically using the Sigma Aldrich (UK) kit.

Results

The results of the experiments are shown in Figure 8. Figure 8 shows that MRx0005 is able reduce the levels of histone deacetylase activity.

Example 7 – Level of indole production in bacteria

Summary

The ability of the bacteria of the invention to produce indole was investigated. Indole has been implicated in attenuating inflammation and oxidative stress.

Material and Methods

Bacterial strain

755: *Parabacteroides distasonis*

ATCC 11775 is a bacterial reference strain that is known to produce indole.

Method

Intact bacterial cells in stationary phase were incubated with 6mM Tryptophan for 48 hours. Bacterial species which possess the enzyme tryptophanase will utilise tryptophan as a substrate to produce indole. Following the 48 hour incubation period, the supernatant was removed and added to Kovac's reagent for quantification of indole. Standards, stock solutions and reagents were prepared using standardised methods validated in-house.

Results

The results of the experiments are shown in Figure 9. Figure 9 shows that MRx0005 has the capacity to produce indole from tryptophan, at concentrations of approximately 0.2mM.

Example 8 – Level of kynurenine production in bacteria

Summary

The ability of the bacteria of the invention to produce kynurenine was investigated. Dysregulation of the kynurenine pathway can lead to activation of the immune system and the accumulation of potentially neurotoxic compounds. Alterations in the kynurenine metabolism may be involved in the development of Parkinson's diseases.

Bacterial strain

755: *Parabacteroides distasonis*

DSM 17136 is a strain of Bacteroides copricola that is known to produce kynurenine.

Method

Cell free supernatants of stationary phase bacterial cultures were isolated by centrifugation and filtering in a 0.22 uM filter and frozen until use. Kynurenine standards, stock solutions and reagents were prepared using standardised methods validated in-house. Sample were treated with trichloroacetic acid and centrifuged at 10,000xg for 10 minutes at 4°C. The supernatant was collected and dispensed into a 96 well plate. Ehrlich's reagent was used for kynurenine detection and added at a ratio of 1:1.

Results

The results of the experiments are shown in Figure 10. Figure 10 shows that MRx0005 has the capacity to produce kynurenine at a concentration of approximately 70 μM.

Example 9 – neuroprotection

RA-differentiated SHSY-5Y cells were treated with MPP+, the active metabolite of MPTP, a chemical widely used to mimic *in vitro* and *in vivo* some of the features of PD pathology. Cell viability was measured as the rate of mitochondria respiration (Figure 11). Both MRx0005 and MRx0029 showed

significant effects and promote *per se* an increase of the mitochondria metabolic activity in SHSY-5Y cells. MRx0005 protection was about 20% compared to YCFA-MPP+ treated sample, about the same observed for the quercetin positive control (Fig. 11).

Example 10 – Metabolite production – metabolites in the brain

Background

Metabolites present in bacteria supernatants can directly influence the host response to oxidative stress, cell-to-cell communication and neuroprotection. Metabolites that play a key role in neurological processes were measured during the *ex vivo* screening in brain tissue of mice fed with MRx0005 and MRx0029.

Methods

Animals

BALBc (Envigo, UK) adult male mice were group housed under a 12 h light-dark cycle; standard rodent chow and water were available ad libitum. All experiments were performed in accordance with European guidelines following approval by University College Cork Animal Ethics Experimentation Committee. Animals were 8 weeks old at the start of the experiment.

Study Design

Animals were allowed to habituate to their holding room for one week after arrival into the animal unit. They receive oral gavage (200µL dose) of live biotherapeutics at a dose of 1 X 10^9 CFU for 6 consecutive days between 15:00 and 17:00. On day 7, the animals are decapitated, and tissues are harvested for experimentation.

Tissue Collection

Animals were sacrificed in a random fashion regarding treatment and testing condition; sampling occurred between 9.00 a.m. and 1:00 p.m. Trunk blood was collected in potassium EDTA (Ethylene Diamine Tetra Acetic Acid) tubes and spun for 15 min at 4000 g. Plasma was isolated and stored at −80 °C for further analysis. The brain was quickly excised, dissected and each brain region was snap-frozen on dry ice and stored at −80 °C for further analysis. Spleen was removed and processed immediately after culls for *ex-vivo* immune stimulation. Intestinal tissue (2 cm segments of ileum and colon closest to the caecum were excised, and the furthest 1cm of tissue from the caecum were used) were mounted into the Ussing chambers for intestinal permeability assay. The caecum was removed, weighted and stored at −80 °C for SCFAs analysis.

Monoamine Analysis

Neurotransmitter concentration was analysed by HPLC on samples from the brainstem. Briefly, brainstem tissue was sonicated in 500 µl of chilled mobile phase spiked with 4 ng/40 µl of N-Methyl

5-HT (Sigma Chemical Co., UK) as internal standard. The mobile phase contained 0.1 M citric acid, 5.6 mM octane-1-sulphonic acid (Sigma), 0.1 M sodium dihydrogen phosphate, 0.01 mM EDTA (Alkem/Reagecon, Cork) and 9% (v/v) methanol (Alkem/Reagecon) and was adjusted to pH 2.8 using 4 N sodium hydroxide (Alkem/Reagecon). Homogenates were then centrifuged for 15 min at 22,000 × g at 4 °C and 40 µl of the supernatant injected onto the HPLC system which consisted of a SCL 10-Avp system controller, LECD 6A electrochemical detector (Shimadzu), a LC-10AS pump, a CTO-10A oven, a SIL-10A autoinjector (with sample cooler maintained at 40 C) and an online Gastorr Degasser (ISS, UK). A reverse-phase column (Kinetex 2.6 u C18 100 × 4.6 mm, Phenomenex) maintained at 30 °C was employed in the separation (Flow rate 0.9 ml/min). The glassy carbon working electrode combined with an Ag/AgCl reference electrode (Shimdazu) operated a +0.8 V and the chromatograms generated were analyzed using Class-VP 5 software (Shimadzu). The neurotransmitters were identified by their characteristic retention times as determined by standard injections, which run at regular intervals during the sample analysis. The ratios of peak heights of analyte versus internal standard were measured and compared with standard injection. Results were expressed as ng of neurotransmitter per g fresh weight of tissue.

Metabolite analysis

For GC-metabolite analysis, samples of bacterial supernatants were derivatized with methyl chloroformate using a slightly modified version of the protocol described by Smart et al. (DOI: 10.1038/nprot.2010.108). All samples were analyzed in a randomized order. Analysis was performed using GC (7890B, Agilent) coupled with a quadropole detector (59977B, Agilent). The system was controlled by ChemStation (Agilent). Raw data was converted to netCDF format using Chemstation (Agilent), before the data was imported and processed in Matlab R2014b (Mathworks, Inc.) using the PARADISe software described by Johnsen et. al (DOI: 10.1016/j.chroma.2017.04.052).

For fatty acid analysis samples were acidified using hydrochloride acid, and deuterium labelled internal standards where added. All samples were analyzed in a randomized order. Analysis was performed using a high polarity column (Zebron™ ZB-FFAP, GC Cap. Column 30 m x 0.25 mm x 0.25 µm) installed in a GC (7890B, Agilent) coupled with a quadropole detector (59977B, Agilent). The system was controlled by ChemStation (Agilent). Raw data was converted to netCDF format using Chemstation (Agilent), before the data was imported and processed in Matlab R2014b (Mathworks, Inc.) using the PARADISe software described by Johnsen et al (DOI: 10.1016/j.chroma.2017.04.052).

Results – neurotransmitter production

The results are shown in Figure 12, which shows that in brains of mice fed with MRx0029, noradrenaline levels are increased (p=0.0507), accompanied with a slight increase of serotonin and 5-HIAA. These data support the metabolite analysis set out below, suggesting that MRx00029 is a major producer of 4-hydroxyphenylacetic acid, a known antioxidant (Weon et al, 2016). More importantly, 4-hydroxyphenylacetic acid is a synthetic intermediate of dopamine and norepinephrine and an

important bio-active molecule (Huot et al, Parkinson's Disese 2015). In fact, in PD, degenerative changes extend beyond the dopaminergic system, affecting equally the serotonergic and noradrenergic systems, which in turn leads to decreased levels of serotonin (5-hydroxytryptamine, 5-HT) and noradrenaline (norepinephrine) in both striatal and extra-striatal structures (Scatton B, Javoy-Agid F, Rouquier L, Dubois B, Agid Y Brain Res. 1983 Sep 26; 275(2):321-8.). L-DOPA targets mainly the dopamine-related features of PD, however it does not address the decreases in both 5-HT and noradrenaline. Adding to this is that the longer is the duration of L-DOPA treatment, the more visible are a range of motor and nonmotor complications (e.g. dyskinesia, psychiatric symptoms) (Hely MA, Morris JG, Reid WG, Trafficante R, Mov Disord. 2005 Feb; 20(2):190-9.) Therefore, these data demonstrate that bacteria that produce organic acids, such as 4-hydroxyphenylacetic acid or succinic acid, may be useful in therapy, in particular in the treatment of neurodegenerative diseases.

Results – metabolite production

Metabolites present in bacteria supernatants can directly influence the host response to oxidative stress, cell-to-cell communication and neuroprotection in the specific. Metabolites in the supernatant of cultures of MRX0029 and MRX0005 were analysed and the results are shown in Figure 13.

A few metabolites showed a striking difference between the two strains analysed. The concentration of succinic acid was particularly elevated in MRx0005. Interestingly, the ratio sample/media for 4-hydroxyphenylacetic acid was significantly higher in MRx0029 (Fig. 13).

Fatty acid analysis in the supernatants revealed an interesting dichotomy in the two strains: MRx0005 produced mainly acetic and propanoic acid, while MRx0029 produced butanoic, pentanoic and hexanoic acid, both in the linear and branched forms (Fig. 14B). The two strains looked very different and in particular, the production of succinic acid and 4-hydroxyphenylacetic acid by MRx0005 and MRx0029 respectively was notable (Figure 14A). Furthermore, MRx0005 seems to produce more C2 and C3 short chain fatty acids, while MRx00029 produced more C4 (butyrate) and both linear and branched medium chain fatty acids, including hexanoic acid.

Succinic acid is a Krebs cycle metabolite involved in oxidative phosphorylation. Oxidative phosphorylation complex is a key step for synaptic trafficking of proteins and vesicles to proximal and distal regions (Budd SL and Nichols, 1998). Its dysfunction has been reported in neurodegenerative disorders including Alzheimer's disease, Parkinson's disease and Spinocerebellar ataxia type 1 (Manczak M et al. 2004; Ebadi et al, 2001). These findings are particularly interesting as succinic acid can augment mitochondrial activity and support vulnerable neurons in neurodegenerative disease related to misfolded proteins including PD (Ferroet al, Plos one, 2017). BDNF and succinic acid have both a similar protective activity not only in neuro-degeneration but also in mental disorders like depression and anxiety, which are quite common amongst patients diagnosed with PD or AD.

Figure 14B also demonstrates that MRX0029 is a butyrate (butanoic acid) producer. This may be significant because butyrate has a known role is reducing impermeability of the blood brain barrier, which has a neuroprotective effect [59]. This property of MRx0029 (and other neuroprotective bacteria) may contribute to its efficacy.

Example 11 - Modulation of the mRNA expression of tight junction proteins

Since recent evidence suggests that intestinal dysfunction and inflammation is a non-motor symptom associated with PD, the ability of the bacterial strains of the invention to cause any intestinal barrier dysfunction was investigated. HT29-mtx epithelial, mucin-producing cell monolayers (Gagnon et al, J Microbiological Methods, 2013) were used as an *in vitro* model to evaluate gut barrier disruption and immune stimulation following treatment with MRx0005 and MRx0029. Differentiated HT29-mtx cells exposed to phorbol 12-myristate-13-acetate (PMA) secreted a significant amount of IL-8; in contrast treatment for 24h with MRx005 and MRx0029 bacterial supernatants, induced an even lower secretion of IL-8 compared than both untreated and YCFA-treated cells (Fig. 14A).

The ability of MRx0005 and MRx0029 to regulate epithelial permeability by modifying intracellular signal transduction involved in the expression and localization of proteins involved in the gut barrier formation was then investigated.

RNA was isolated and Quantitative RT-PCR (qRT-PCR) analysis was performed to characterize the changes in gene expression of tight junction proteins during incubation with MRx0005 and MRx0029. The administration of MRx0029 enhanced Occludin, Vlillin, Tight Junction Protein 1 and 2 (respectively TJP1 and TJP2) mRNA expression after 2h incubation (Fig. 14B). In contrast, exposure to MRx0005 did not alter the gene expression of tight junction proteins indicating that the two strains act differentially on the intestinal barrier.

The *in vitro* results were compared with data from the *ex vivo* parallel analysis on the gut of mice fed with MRx0005 and MRx0029. Gene expression of TJP2 and occludin was quantified in the colon and ileum. The *ex vivo* data perfectly mirror the *in vitro* data as MRx0029 was able to significantly up-regulate TJP1 and Occludin (p=0.073) in the colon region of the murine intestine (Fig. 14C+14D). MRx0029 was also able to decrease the permeability function in the colon of the same mice (Fig. 14E+14F).

Materials and methods - RNA extraction and qPCR analysis

Total RNA was extracted using the RNeasy mini kit (Qiagen, Manchester, JUK) according to the manufacturer's instructions, and the RNA concentration determined by absorbance at 260/280 nm using a spectrophotometer (nano-Drop ND-1000; Thermo Scientific, Wilmington, DE). For mRNA expression analysis, cDNA was prepared from 2000 ng of total RNA using the High-Capacity cDNA

reverse transcription kit (Thermo Fisher, Loughborough) according to the manufacturer's instructions. The reverse transcription reactions were performed in a thermo cycler (Biometra, Germany) at 25°C for 10 min, 37°C for 120 min, and 85°C for 5 min. Resulting cDNA was amplified in duplicates by the SYBR-Green PCR assay, and products were detected on QuantStudio 7 real-time PCR machine (Applied Biosystems, UK) using a standardised profile (initial denaturation of 95°C for 10 minutes, followed by 40 cycles of 10 seconds of denaturation at 95°C and 30 seconds of annealing/extension at 60°C). A dissociation stage was added after the 40 cycles to generate a melting curve. Analysis was performed using the Applied Biosystems QuantStudio Real-Time PCR Software v1.2. The primer sequences for Actin, Villin, Occludin TJP1 and TJP2 are provided in the sequence listing.

Example 12 – Level of BDNF secretion in SHSY-5Y cells

Background

Brain-derived neurotrophic factor (BDNF) is a ubiquitous molecule in the brain associated with neural development, neuro-protection and neuro-regeneration. BDNF not only protects against neurodegeneration but also mental disorders like depression and anxiety, which are quite common amongst patients diagnosed with PD or AD.

Methods

SH-SY5-SY were plated in 24 wells plate at density of 60,000 cells/well and placed in the incubator. After 24 h, media were replaced with differentiation medium (growth medium containing 1% FBS) and 10 µM retinoic acid. Differentiation medium was replaced every other day and cells were used on day 10 of differentiation. For the treatment differentiation medium was removed and replaced with 450ul of full growth media and 50 µl of bacteria SN was added to the treated wells or YCFA+ was added as negative Control.

Results

The results are shown in Figure 15, which shows that administration of MRX0005 in combination with retinoic acid increases the secretion of BDNF from differentiated neuroblastoma cells.

Example 13 – Efficacy of bacterial inocula to reduce oxidative levels in cells

Background

The generation of reactive oxygen species contributes to the pathology of neurodegenerative diseases. The ability of bacterial strains to protect differentiated SHSY-5Y and U373 cells from reactive oxygen species (ROS) generated by treatment with Tert-Butyl Hydrogen Peroxide (TBHP) was investigated.

Material and Methods

Bacterial strain

Megasphaera massiliensis MRX0029

Method

SHSY-5Y cells were plated in black flat bottom 96 well plate at density of 5000 cells/well and placed in the CO2 incubator. After 24 h, media were replaced with differentiation medium (growth medium containing 1% FBS) and 10 μM retinoic acid. Differentiation medium was replaced every other day. On Day 10 the differentiation medium was removed and cells were washed with pre-warmed PBS and stained with 10uM DCFDA molecular probe for 20 mins in growth medium containing 1% FBS. Then cells were washed with pre-warmed PBS again and treated with 100uM TBHP in the presence or absence of 10% bacteria supernatant for 2h. Fluorescence intensity was measured using TECAN plate reader at Ex/Em 485/530 nm.

Results

The results of the experiments are shown in Figure 16. Figure 16b shows that MRX0005 is able to inhibit ROS production in differentiated SHSY-5Y neuroblastoma cells. MRX0005 also reduces the generation of ROS in astroglioblastoma cells (Figure 16a). This shows that MRX0005 has general antioxidant activity.

Example 14 – Stability testing

A composition described herein containing at least one bacterial strain described herein is stored in a sealed container at 25°C or 4°C and the container is placed in an atmosphere having 30%, 40%, 50%, 60%, 70%, 75%, 80%, 90% or 95% relative humidity. After 1 month, 2 months, 3 months, 6 months, 1 year, 1.5 years, 2 years, 2.5 years or 3 years, at least 50%, 60%, 70%, 80% or 90% of the bacterial strain shall remain as measured in colony forming units determined by standard protocols.

Example 15

Methods

Animals

The animals and study design used were the same as for Example 10.

Bacterial strains

- 755: *Parabacteroides distasonis (MRX005)*
- *Megasphaera massiliensis (MRX0029)*

Tissue Collection

Animals were sacrificed in a random fashion regarding treatment and testing condition; sampling occurred between 9.00 a.m. and 2:30 p.m. Trunk blood was collected in potassium EDTA (Ethylene Diamine Tetra Acetic Acid) tubes and spun for 15 min at 4000 g. Plasma was isolated and stored at −80 °C for further analysis. The brain was quickly excised, dissected and each brain region was snap-frozen on dry ice and stored at −80 °C for further analysis. Spleen was removed, collected in 5 mL

RPMI media (with L-glutamine and sodium bicarbonate, R8758 Sigma + 10 % FBS (F7524, Sigma) + 1% Pen/Strep (P4333, Sigma)) and processed immediately after culls for ex-vivo immune stimulation. Intestinal tissue (2 3cm segments of ileum and colon closest to the caecum were excised, and the furthest 1cm 2cm of tissue from the caecum were used) were mounted into the Ussing chambers for intestinal permeability assay. The caecum was removed, weighted and stored at −80 °C for SCFAs analysis.

Monoamine Analysis

The neurotransmitter concentration was analysed as described in Example 10

Spleen Cytokine Assay

Spleens were collected immediately in 5mL RPMI media following sacrifice and cultured immediately. Spleen cells were first homogenised in this RPMI media, followed by 5 mins incubation with 1ml of RBC lysis buffer (11814389001 ROCHE, Sigma). A further 10 ml of RPMI media was added, followed by 200G centrifugation for 5 mins. The supernatant was then filtered through 40um strainer. Cells were counted and seeded (4,000,000/mL media). After 2.5 h of adaptation, cells were stimulated with lipopolysaccharide (LPS-2 μg/ml) or concanavalin A (ConA-2.5 μg/ml) for 24 h. Following stimulation, the supernatants were harvested to assess the cytokine release using Proinflammatory Panel 1 (mouse) V-PLEX Kit (Meso Scale Discovery, Maryland, USA) for TNFα, IL-10, IL-1β, Interferon γ, CXCL2 and IL6. The analyses were performed using MESO QuickPlex SQ 120, SECTOR Imager 2400, SECTOR Imager 6000, SECTOR S 600.

Gene Expression Analysis

Total RNA was extracted using the mirVana™ miRNA Isolation kit (Ambion/Llife technologies, Paisley, UK) and DNase treated (Turbo DNA-free, Ambion/life technologies) according to the manufacturers recommendations. RNA was quantified using NanoDrop™ spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, Delaware, USA) according to the manufacturer's instructions. RNA quality was assessed using the Agilent Bioanalyzer (Agilent, Stockport, UK) according to the manufacturer's procedure and an RNA integrity number (RIN) was calculated. RNA with RIN value >7 was used for subsequent experiments. RNA was reverse transcribed to cDNA using the Applied Biosystems High Capacity cDNA kit (Applied Biosystems, Warrington, UK) according to manufacturer's instructions. Briefly, Multiscribe Reverse Transcriptase (50 U/μL) (1)(2)(1)(10) was added as part of RT master mix, incubated for 25°C for 10 min, 37°C for 2 h, 85°C for 5 min and stored at 4°C. Quantitative PCR was carried out using probes (6 carboxy fluorescein - FAM) designed by Applied Biosystems to mouse specific targeted genes, while using β-actin as an endogenous control. Amplification reactions contained 1 μl cDNA, 5 μl of the 2X PCR Master mix (Roche), 900 nM of each primer and were brought to a total of 10 μl by the addition of RNase-free water. All reactions were performed in triplicate using 96-well plates on the LightCycler®480 System. Thermal cycling conditions were as recommended by the manufacturer (Roche) for 55 cycles. To check for amplicon

contamination, each run contained no template controls in triplicate for each probe used. Cycle threshold (Ct) values were recorded. Data was normalized using β-actin and transformed using the 2−ΔΔCT method and presented as a fold change vs. control group.

Short Chain Fatty Acids Analysis in the Caecal Content

Caecum content was mixed and vortexed with MilliQ water and incubated at room temperature for 10 min. Supernatants were obtained by centrifugation (10000 g, 5 min, 4 °C) to pellet bacteria and other solids and filtration by 0.2μm. It was transferred to a clear GC vial and 2-Ethylbutyric acid (Sigma) was used as the internal standard. The concentration of SCFA was analyzed using a Varian 3500 GC flame-ionization system, fitted with a with a ZB-FFAP column (30 m x 0.32 mm x 0.25 mm; Phenomenex). A standard curve was built with different concentrations of a standard mix containing acetate, propionate, iso-butyrate, n-butyrate, isovalerate and valerate (Sigma). Peaks were integrated by using the Varian Star Chromatography Workstation version 6.0 software. All SCFA data are expressed as μmol/g.

Statistical Analysis

Normally distributed data are presented as mean ± SEM; Non-parametric datasets are presented as median with inter-quartile range. Unpaired two-tailed t-test were applied to analyse parametric data and Mann-Whitney test was used for non-parametric. Spearman's rank correlation coefficient was employed for the correlation analysis in the pooled datasets. A p value < 0.05 was deemed significant in all cases.

Results – Neurotransmitter production

The results in Figure 17 show the effect of MRx005 treatment on the concentration of neurotransmitters in the brain of mice. Most notably, treatment with MRx005 leads to a decrease in dopamine.

Results – Gene expression

Expression of genes for neurotransmitter receptors [serotonin receptor 1a(5-HTR1a), dopamine D1 receptor, GABA receptor subunit B1, GABAA receptor, NMDA2A (Grin2A) and NMDA2B (Grin2b) receptor], inflammatory markers [IL-1β, IL6, CD11b, TNFα and TLR4], and endocrine markers [corticosterone releasing factor (CRF), corticosterone releasing factor receptors 1 and 2 (CRFR1, CRFR2), brain-derived neurotrophin factor (BDNF), vasopressin receptor, oxytocin receptor, glucocorticoid receptor and mineralocorticoid receptor] were analysed in brain tissue from the hippocampus, amygdala and prefrontal cortex.

Figures 18-32 show the changes in gene expression after MRX005 or MRX0029 treatment in the hippocampal, amygdala and prefrontal cortex. Treatment with MRx0029 led to an increase in glucocorticoid receptor expression in the amygdala (Figure 25C). Figure 26A shows that MRx005

significantly increased the expression of BDNF in the amygdala, while treatment with MRx0029 significantly increased the expression of TLR4 in the amygdala (Figure 26B).

Both MRx005 and MRx0029 can increase expression of CD11b in the amygdala (Figure 27A), while the expression of IL-6, Grin2a and Grin2b is reduced after MRx005 treatment (Figures 27B-D). In addition, MRx005 and MRx0029 significantly increased the expression of GABRA2 and increased the expression of GABBR1 in the amygdala.

Treatment with MRx005 led to a significant increase in the expression of BDNF in the prefrontal cortex (Figure 29B).

Discussion

MRx005 and MRx0029 administration caused changes in gene expression , especially in the amygdala.

Results – Effect on Tph1 and IDO-1 expression

Figure 33 shows that MRx0029 can significantly increase the expression tryptophan hydroxylase- 1 (Tph1) in the colon and that MRX005 treatment can increase IDO-1 expression in the colon. Treatment with MRX005 increased the expression of Tph1 and IDO1 in the ileum, while MRX029 had no effect on the expression of these genes in the ileum (Figure 34).

Indoleamine-pyrrole 2,3-dioxygenase-1 (IDO-1) the first and rate-limiting enzyme in the tryptophan/kynurenine pathway while tryptophan hydroxylase 1 (Tph1), an isoform of the enzyme tryptophan hydroxylase, responsible for the synthesis of serotonin. These data suggest that MRx0029 and MRx005 may affect serotonin levels and the tryptophan/kynurenine pathway.

Results – Effect on tryptophan metabolite levels

Figure 35 shows the effect of treatment with MRx005 on the levels of circulating kynurenine and tryptophan.

Results – Effect on cytokine expression from splenocytes

The ex-vivo splenocyte assay involves challenging the splenocytes (cells isolated from the spleen - a main organ involved in immune defence), with a bacterio- or viral-mimetic challenge.

MRX005 significantly reduced the levels of interferon-γ in splenocytes following a challenge with LPS (Figure 36). In addition, MRX005 reduced the levels of interleukin-6 and tumour necrosis factor after a challenge with LPS (Figures 38 and 39, respectively). Treatment with MRx0029 led to a reduction in interferon-γ, interleukin-1β and interleukin-6 following a challenge with LPS (Figures 36, 37 and 38, respectively).

Treatment with MRx005 and MRx0029 led to an increase in the levels of the chemoattractant CXCL1 (Figure 41).

Results – Effect on Caecal Short Chain Fatty Acid Levels

Short chain fatty acids (SCFAs) are produced when non-digestible fibres from the diet are fermented by bacteria in the gut. The effects of MRX005 administration are shown in Figure 42.

Example 16 – Further analysis of MRX029 and MRX005 changes in gene expression levels

Methods

Cell line

SH-SY5Y cells

Bacterial strains

- 755: *Parabacteroides distasonis (MRX005)*
- *Megasphaera massiliensis (MRX0029)*

qPCR

SH-SY5Y were plated in 10cm petri dishes a density of $2x10^6$ cells. After 24h cells were treated in differentiation medium (growth medium containing 1% FBS without RA) with 10% bacteria supernatants or YCFA+, 10uM RA, 200uM hexanoic acid or 200uM valproic acid, for 17 hrs. There after representative images were taken using phase contrast EVOS XL core microscope at 40X/0.65 magnification. Cells were collected, and total RNA was isolated according to RNeasy mini kit protocol (Qiagen). cDNAs were made using the high capacity cDNA reverse transcription kit (Applied Biosystems). Gene expression was measured using qPCR. GAPDH was used as internal control. Fold change was calculated according to the $2^{(-\Delta\Delta ct)}$ method. The primer sequences for MAP2, DRD2, GABRB3, SYP, PINK1, PARK7 and NSE are provided in the sequence listing.

Immuno-labelling and cell imaging

Cells were seeded onto 8-well chamber slides (Marienfeld Laboratory Glassware) at $5x10^4$ cells/well overnight and were treated with 10% bacterial supernatant for 24 h. For differentiation, cells were treated with 10 nM RA for 5 days before treating with cell-free bacterial supernatant for 24 h. Afterwards, the cells were fixed with 4% paraformaldehyde in PBS for 20 minutes at room temperature (RT). Fixed cells were washed with PBS, and permeabilized with 1% Triton X-100 in PBS for 10 minutes. After washing with PBS, the slides were incubated with blocking buffer (4% BSA/PBS) for 1 h at RT before adding anti-MAP2 antibody or β3-tubulin (sc-74421 and sc-80005 respectively, Santa Cruz Biotechnology Inc) diluted in 1% BSA/PBS for 12 h at 4°C. They were then washed twice with PBS, followed by incubation with Alexa Flour 488 conjugated anti-mouse (Molecular Probes Inc) and Alexa Flour 594 conjugated Phalloidin (ab176757, Abcam) for 1 h at RT. After washing 3X with PBS, the slides were staining with DAPI and mounted with Vectashield® (Vector Laboratories). Slides were viewed using a Axioskop 50 microscope (Zeiss) equipped with a 63x/1.2 W Korr objective and filter sets suitable for detection of the fluorochromes used. Manual exposure times for the digital acquisition

of images immuno-labelled with MAP-2 were kept constant allowing comparison between different wells and treatments. Phalloidin (F-actin) and DAPI exposure times varied to suit the field of view. Randomised fields of view were acquired using a QImaging camera controlled by Image Pro Plus software. Images were saved as TIFF files and opened in Adobe Photoshop CC 2015.1.2. Images of the MAP-2, DAPI and Phalloidin images were then overlaid and merged. Representative images were selected to illustrate the differences in abundance and location of the proteins examined.

Immunoblotting

SH-SY5Y cells cultured under the indicated conditions described above, treated with MRx0005 and MRx0029 for 24h and then lysed in RIPA buffer containing cocktail of protease inhibitors (Roche Diagnostics, UK). Protein concentration was estimated using the BCA protein assay kit (Pierce Biotechnology, Rockford, IL), separated by SDS-PAGE and transferred to a PVDF membrane. Membranes were then blocked with 5% non-fat dry milk or 5% BSA and incubated overnight at 4°C with the primary antibodies (respectively MAP2 and β3-tubulin). The blots were then incubated with the appropriate horseradish peroxidase (HRP)-conjugated secondary antibody, and proteins were detected by chemiluminescence detection kit (Pierce Biotechnology, Rockford, IL). For both MAP2 and β3-tubulin, β-actin served as a control to monitor protein loading variability amongst samples.

Results and Discussion

Gene expression

Figure 43 shows the MRx0029 and MRX005-induced changes in expression levels of Actin, Villin, Occludin TJP1, TJP2, MAP2, DRD2, GABRB3, SYP, PINK1, PARK7 and NSE.

Results – Microscopy and Immunoblotting

Figure 44 shows the change in the level of expression of MAP2 in SHSY5Y cells as determined by confocal microscopy. The expression levels of MAP2 and B3-tubulin were also quantified by immunoblot analysis. The results shown in Figure 44M and N indicate that MRX029 induces an increase in the level expression of MAP2

Sequences

Additional primers used in qPCR (with SEQ ID NO in brackets)

Gene ID	Forward sequence	Reverse sequence
NSE	CCCTGTATCGTAAGAACGGT (30)	GCCACCATTGATCACGTTGA (31)

PINK1	CCCAAGCAACTAGCCCCTC (32)	GGCAGCACATCAGGGTAGTC (33)
PARK7	GTAGCCGTGATGTGGTCATTT (34)	CTGTGCGCCCAGATTACCT (35)
SYP	CTCGGCTTTGTGAAGGTGCT (36)	GGCTTCATGGCATCAACTTCA (37)

Sequences

SEQ ID NO:1 (*Parabacteroides distasonis* gene for 16S ribosomal RNA, partial sequence, strain: JCM 5825 - AB238922)

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   1 agagtttgat cctggctcag gatgaacgct agcgacaggc ttaacacatg caagtcgagg
  61 ggcagcgggg tgtagcaata caccgccggc gaccggcgca cgggtgagta acgcgtatgc
 121 aacttgccta tcagaggggg ataacccggc gaaagtcgga ctaataccgc atgaagcagg
 181 gatcccgcat gggaatattt gctaaagatt catcgctgat agataggcat gcgttccatt
 241 aggcagttgg cggggtaacg gcccaccaaa ccgacgatgg atagggggttc tgagaggaag
 301 gtcccccaca ttggtactga gacacggacc aaactcctac gggaggcagc agtgaggaat
 361 attggtcaat gggcgtaagc ctgaaccagc caagtcgcgt gagggatgaa ggttctatgg
 421 atcgtaaacc tcttttataa gggaataaag tgcgggacgt gtcccgtttt gtatgtacct
 481 tatgaataag gatcggctaa ctccgtgcca gcagccgcgg taatacggag gatccgagcg
 541 ttatccggat ttattgggtt taaagggtgc gtaggcggcc ttttaagtca gcggtgaaag
 601 tctgtggctc aaccatagaa ttgccgttga aactgggggg cttgagtatg tttgaggcag
 661 gcggaatgcg tggtgtagcg gtgaaatgca tagatatcac gcagaacccc gattgcgaag
 721 gcagcctgcc aagccattac tgacgctgat gcacgaaagc gtggggatca aacaggatta
 781 gataccctgg tagtccacgc agtaaacgat gatcactagc tgtttgcgat acactgtaag
 841 cggcacagcg aaagcgttaa gtgatccacc tggggagtac gccggcaacg gtgaaactca
 901 aaggaattga cgggggcccg cacaagcgga ggaacatgtg gtttaattcg atgatacgcg
 961 aggaacctta cccgggtttg aacgcattcg gaccgaggtg gaaacacctt ttctagcaat
1021 agccgtttgc gaggtgctgc atggttgtcg tcagctcgtg ccgtgaggtg tcggcttaag
1081 tgccataacg agcgcaaccc ttgccactag ttactaacag gttaggctga ggactctggt
1141 gggactgcca gcgtaagctg cgaggaaggc ggggatgacg tcaaatcagc acggcccttа
1201 catccggggc gacacacgtg ttacaatggc gtggacaaag ggaggccacc tggcgacagg
1261 gagcgaatcc ccaaaccacg tctcagttcg gatcggagtc tgcaacccga ctccgtgaag
1321 ctggattcgc tagtaatcgc gcatcagcca tggcgcggtg aatacgttcc cgggccttgt
1381 acacaccgcc cgtcaagcca tgggagccgg gggtacctga agtccgtaac cgaaaggatc
1441 ggcctagggt aaaactggtg actggggcta agtcgtaaca aggtaacc
```

SEQ ID NO:2 (*Parabacteroides distasonis* gene for 16S ribosomal RNA, partial sequence, strain: JCM 13400 - AB238923)

```
   1 agagtttgat cctggctcag gatgaacgct agcgacaggc ttaacacatg caagtcgagg
  61 ggcagcacag gtagcaatac cgggtggcga ccggcgcacg ggtgagtaac gcgtatgcaa
```

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 121 cttacctatc agagggggat aacccggcga aagtcggact aataccgcat gaagcagggg
 181 ccccgcatgg ggatatttgc taaagattca tcgctgatag ataggcatgc gttccattag
 241 gcagttggcg gggtaacggc ccaccaaacc gacgatggat aggggttctg agaggaaggt
 301 cccccacatt ggtactgaga cacggaccaa actcctacgg gaggcagcag tgaggaatat
 361 tggtcaatgg gcgtaagcct gaaccagcca agtcgcgtga gggatgaagg ttctatggat
 421 cgtaaacctc ttttataagg gaataaagtg cgggacgtgt cctgttttgt atgtacctta
 481 tgaataagga tcggctaact ccgtgccagc agccgcggta atacggagga tccgagcgtt
 541 atccggattt attgggttta aagggtgcgt aggcggcctt ttaagtcagc ggtgaaagtc
 601 tgtggctcaa ccatagaatt gccgttgaaa ctgggggct tgagtatgtt tgaggcaggc
 661 ggaatgcgtg gtgtagcggt gaaatgctta gatatcacgc agaaccccga ttgcgaaggc
 721 agcctgccaa gccatgactg acgctgatgc acgaaagcgt ggggatcaaa caggattaga
 781 taccctggta gtccacgcag taaacgatga tcactagctg tttgcgatac agtgtaagcg
 841 gcacagcgaa agcgttaagt gatccacctg gggagtacgc cggcaacggt gaaactcaaa
 901 ggaattgacg ggggcccgca caagcggagg aacatgtggt ttaattcgat gatacgcgag
 961 gaaccttacc cgggtttgaa cgcattcgga ccgaggtgga aacacctttt ctagcaatag
1021 ccgtttgcga ggtgctgcat ggttgtcgtc agctcgtgcc gtgaggtgtc ggcttaagtg
1081 ccataacgag cgcaaccctt gccactagtt actaacaggt aaagctgagg actctggtgg
1141 gactgccagc gtaagctgcg aggaaggcgg ggatgacgtc aaatcagcac ggcccttaca
1201 tccggggcga cacacgtgtt acaatggcgt ggacaaaggg aagccacctg gcgacaggga
1261 gcgaatcccc aaaccacgtc tcagttcgga tcggagtctg caacccgact ccgtgaagct
1321 ggattcgcta gtaatcgcgc atcagccatg gcgcggtgaa tacgttcccg ggccttgtac
1381 acaccgcccg tcaagccatg ggagccgggg gtacctgaag tccgtaaccg aaaggatcgg
1441 cctagggtaa aactggtgac tggggctaag tcgtaacaag gtaacc
```

SEQ ID NO:3 (*Parabacteroides distasonis* gene for 16S ribosomal RNA, partial sequence, strain: JCM 13401 - AB238924)

```
   1 agagtttgat cctggctcag gatgaacgct agcgacaggc ttaacacatg caagtcgagg
  61 ggcagcacag gtagcaatac ccgccggcga ccggcgcacg ggtgagtaac gcgtatgcaa
 121 cttgcctatc agaggggat aacccggcga aagtcggact aataccgcat gaagcagggg
 181 ccccgcatgg ggatatttgc taaagattca tcgctgatag ataggcatgc gttccattag
 241 gcagttggcg gggtaacggc ccaccaaacc gacgatggat aggggttctg agaggaaggt
 301 cccccacatt ggtactgaga cacggaccaa actcctacgg gaggcagcag tgaggaatat
 361 tggtcaatgg gcgtaagcct gaaccagcca agtcgcgtga gggatgaagg ttctatggat
 421 cgtaaacctc ttttataagg gaataaagtg tgggacgtgt cctgttttgt atgtacctta
 481 tgaataagga tcggctaact ccgtgccagc agccgcggta atacggagga tccgagcgtt
 541 atccggattt attgggttta aagggtgcgt aggcggcctt ttaagtcagc ggtgaaagtc
 601 tgtggctcaa ccatagaatt gccgttgaaa ctgggaggct tgagtatgtt tgaggcaggc
 661 ggaatgcgtg gtgtagcggt gaaatgctta gatatcacgc agaaccccga ttgcgaaggc
 721 agcctgccaa gccatgactg acgctgatgc acgaaagcgt ggggatcaaa caggattaga
 781 taccctggta gtccacgcag taaacgatga tcactagctg tttgcgatac actgtaagcg
 841 gcacagcgaa agcgttaagt gatccacctg gggagtacgc cggcaacggt gaaactcaaa
 901 ggaattgacg ggggcccgca caagcggagg aacatgtggt ttaattcgat gatacgcgag
 961 gaaccttacc cgggtttgaa cgcattcgga ccgaggtgga aacacctttt ctagcaatag
```

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1021 ccgtttgcga ggtgctgcat ggttgtcgtc agctcgtgcc gtgaggtgtc ggcttaagtg
1081 ccataacgag cgcaaccctt gccactagtt actaacaggt gatgctgagg actctggtgg
1141 gactgccagc gtaagctgcg aggaaggcgg ggatgacgtc aaatcagcac ggcccttaca
1201 tccggggcga cacacgtgtt acaatggcgt ggacaaaggg atgccacctg gcgacaggga
1261 gcgaatcccc aaaccacgtc tcagttcgga tcggagtctg caacccgact ccgtgaagct
1321 ggattcgcta gtaatcgcgc atcagccatg gcgcggtgaa tacgttcccg ggccttgtac
1381 acaccgcccg tcaagccatg ggagccgggg gtacctgaag tccgtaaccg aaaggatcgg
1441 cctagggtaa aactggtgac tggggctaag tcgtaacaag gtaacc
```

SEQ ID NO:4 (*Parabacteroides distasonis* gene for 16S ribosomal RNA, partial sequence, strain: JCM 13402 - AB238925)

```
   1 agagtttgat cctggctcag gatgaacgct agcgacaggc ttaacacatg caagtcgagg
  61 ggcagcacag gtagcaatac cgggtggcga ccggcgcacg ggtgagtaac gcgtatgcaa
 121 cttacctatc agagggggat aacccggcga aagtcggact aataccgcat gaagcagggg
 181 ccccgcatgg ggatatttgc taaagattca tcgctgatag ataggcatgc gttccattag
 241 gcagttggcg gggtaacggc ccaccaaacc gacgatggat agggggttctg agaggaaggt
 301 cccccacatt ggtactgaga cacggaccaa actcctacgg gaggcagcag tgaggaatat
 361 tggtcaatgg gcgtaagcct gaaccagcca agtcgcgtga gggatgaagg ttctatggat
 421 cgtaaacctc ttttataagg gaataaagtg cgggacgtgt cccgttttgt atgtacctta
 481 tgaataagga tcggctaact ccgtgccagc agccgcggta atacggagga tccgagcgtt
 541 atccggattt attgggttta aagggtgcgt aggcggcctt ttaagtcagc ggtgaaagtc
 601 tgtggctcaa ccatagaatt gccgttgaaa ctgggaggct tgagtatgtt tgaggcaggc
 661 ggaatgcgtg gtgtagcggt gaaatgctta gatatcacgc agaaccccga ttgcgaaggc
 721 agcctgccaa gccatgactg acgctgatgc acgaaagcgt ggggatcaaa caggattaga
 781 taccctggta gtccacgcag taaacgatga tcactagctg tttgcgatac actgtaagcg
 841 gcacagcgaa agcgttaagt gatccacctg gggagtacgc cggcaacggt gaaactcaaa
 901 ggaattgacg gggcccgca caagcggagg aacatgtggt ttaattcgat gatacgcgag
 961 gaaccttacc cgggtttgaa cgcattcgga ccgaggtgga aacacctttt ctagcaatag
1021 ccgtttgcga ggtgctgcat ggttgtcgtc agctcgtgcc gtgaggtgtc ggcttaagtg
1081 ccataacgag cgcaaccctt gccactagtt actaacaggt aaagctgagg actctggtgg
1141 gactgccagc gtaagctgcg aggaaggcgg ggatgacgtc aaatcagcac ggcccttaca
1201 tccggggcga cacacgtgtt acaatggcgt ggacaaaggg aggccacctg gcgacaggga
1261 gcgaatcccc aaaccacgtc tcagttcgga tcggagtctg caacccgact ccgtgaagct
1321 ggattcgcta gtaatcgcgc atcagccatg gcgcggtgaa tacgttcccg ggccttgtac
1381 acaccgcccg tcaagccatg ggagccgggg gtacctgaag tccgtaaccg aaaggatcgg
1441 cctagggtaa aactggtgac tggggctaag tcgtaacaag gtaacc
```

SEQ ID NO:5 (*Parabacteroides distasonis* gene for 16S ribosomal RNA, partial sequence, strain: JCM 13403 - AB238926)

```
   1 agagtttgat cctggctcag gatgaacgct agcgacaggc ttaacacatg caagtcgagg
  61 ggcagcacag gtagcaatac cgggtggcga ccggcgcacg ggtgagtaac gcgtatgcaa
```

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 121 cttacctatc agagggggat aacccggcga aagtcggact aataccgcat gaagcagggg
 181 ccccgcatgg ggatatttgc taaagattca tcgctgatag ataggcatgc gttccattag
 241 gcagttggcg gggtaacggc ccaccaaacc gacgatggat aggggttctg agaggaaggt
 301 cccccacatt ggtactgaga cacggaccaa actcctacgg gaggcagcag tgaggaatat
 361 tggtcaatgg gcgtaagcct gaaccagcca agtcgcgtga gggatgaagg ttctatggat
 421 cgtaaacctc ttttataagg gaataaagtg tgggacgtgt cccgttttgt atgtacctta
 481 tgaataagga tcggctaact ccgtgccagc agccgcggta atacggagga tccgagcgtt
 541 atccggattt attgggttta aagggtgcgt aggcggcctt ttaagtcagc ggtgaaagtc
 601 tgtggctcaa ccatagaatt gccgttgaaa ctgggaggct tgagtatgtt tgaggcaggc
 661 ggaatgcgtg gtgtagcggt gaaatgctta gatatcacgc agaaccccga ttgcgaaggc
 721 agcctgccaa gccatgactg acgctgatgc acgaaagcgt ggggatcaaa caggattaga
 781 taccctggta gtccacgcag taaacgatga tcactagctg tttgcgatac attgtaagcg
 841 gcacagcgaa agcgttaagt gatccacctg gggagtacgc cggcaacggt gaaactcaaa
 901 ggaattgacg ggggcccgca caagcggagg aacatgtggt ttaattcgat gatacgcgag
 961 gaaccttacc cgggtttgaa cgcattcgga ccgaggtgga aacacctttt ctagcaatag
1021 ccgtttgcga ggtgctgcat ggttgtcgtc agctcgtgcc gtgaggtgtc ggcttaagtg
1081 ccataacgag cgcaaccctt gccactagtt actaacaggt aaagctgagg actctggtgg
1141 gactgccagc gtaagctgcg aggaaggcgg ggatgacgtc aaatcagcac ggcccttaca
1201 tccggggcga cacacgtgtt acaatggcgt ggacaaaggg aggccacctg gcgacaggga
1261 gcgaatcccc aaaccacgtc tcagttcgga tcggagtctg caacccgact ccgtgaagct
1321 ggattcgcta gtaatcgcgc atcagccatg gcgcggtgaa tacgttcccg ggccttgtac
1381 acaccgcccg tcaagccatg ggagccgggg gtacctgaag tccgtaaccg aaaggatcgg
1441 cctagggtaa aactggtgac tggggctaag tcgtaacaag gtaacc
```

SEQ ID NO:6 (*Parabacteroides distasonis* gene for 16S ribosomal RNA, partial sequence, strain: JCM 13404 - AB238927)

```
   1 agagtttgat cctggctcag gatgaacgct agcgacaggc ttaacacatg caagtcgagg
  61 ggcagcacag gtagcaatac cgggtggcga ccggcgcacg ggtgagtaac gcgtatgcaa
 121 cttacctatc agaggggat aacccggcga aagtcggact aataccgcat gaagcagggg
 181 ccccgcatgg ggatatttgc taaagattca tcgctgatag ataggcatgc gttccattag
 241 gcagttggcg gggtaacggc ccaccaaacc gacgatggat aggggttctg agaggaaggt
 301 cccccacatt ggtactgaga cacggaccaa actcctacgg gaggcagcag tgaggaatat
 361 tggtcaatgg gcgtaagcct gaaccagcca agtcgcgtga gggatgaagg ttctatggat
 421 cgtaaacctc ttttataagg gaataaagtg tgggacgtgt cccgttttgt atgtacctta
 481 tgaataagga tcggctaact ccgtgccagc agccgcggta atacggagga tccgagcgtt
 541 atccggattt attgggttta aagggtgcgt aggcggcctt ttaagtcagc ggtgaaagtc
 601 tgtggctcaa ccatagaatt gccgttgaaa ctgggaggct tgagtatgtt tgaggcaggc
 661 ggaatgcgtg gtgtagcggt gaaatgctta gatatcacgc agaaccccga ttgcgaaggc
 721 agcctgccaa gccatgactg acgctgatgc acgaaagcgt ggggatcaaa caggattaga
 781 taccctggta gtccacgcag taaacgatga tcactagctg tttgcgatac attgtaagcg
 841 gcacagcgaa agcgttaagt gatccacctg gggagtacgc cggcaacggt gaaactcaaa
 901 ggaattgacg ggggcccgca caagcggagg aacatgtggt ttaattcgat gatacgcgag
 961 gaaccttacc cgggtttgaa cgcattcgga ccgaggtgga aacacctttt ctagcaatag
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1021 ccgtttgcga ggtgctgcat ggttgtcgtc agctcgtgcc gtgaggtgtc ggcttaagtg
1081 ccataacgag cgcaaccctt gccactagtt actaacaggt aaagctgagg actctggtgg
1141 gactgccagc gtaagctgcg aggaaggcgg ggatgacgtc aaatcagcac ggcccttaca
1201 tccggggcga cacacgtgtt acaatggcgt ggacaaaggg aggccacctg gcgacaggga
1261 gcgaatcccc aaaccacgtc tcagttcgga tcggagtctg caacccgact ccgtgaagct
1321 ggattcgcta gtaatcgcgc atcagccatg gcgcggtgaa tacgttcccg ggccttgtac
1381 acaccgcccg tcaagccatg ggagccgggg gtacctgaag tccgtaaccg aaaggatcgg
1441 cctagggtaa aactggtgac tggggctaag tcgtaacaag gtaacc
```

SEQ ID NO:7 (*Parabacteroides merdae* gene for 16S ribosomal RNA, partial sequence, strain: JCM 9497 - AB238928)

```
   1 agagtttgat cctggctcag gatgaacgct agcgacaggc ttaacacatg caagtcgagg
  61 ggcagcatga tttgtagcaa tacagattga tggcgaccgg cgcacgggtg agtaacgcgt
 121 atgcaactta cctatcagag ggggatagcc cggcgaaagt cggattaata ccccataaaa
 181 caggggtccc gcatgggaat atttgttaaa gattcatcgc tgatagatag gcatgcgttc
 241 cattaggcag ttggcggggt aacggcccac caaaccgacg atggataggg gttctgagag
 301 gaaggtcccc cacattggta ctgagacacg gaccaaactc ctacgggagg cagcagtgag
 361 gaatattggt caatggccga gaggctgaac cagccaagtc gcgtgaagga agaaggatct
 421 atggtttgta aacttctttt ataggggaat aaagtggagg acgtgtcctt ttttgtatgt
 481 accctatgaa taagcatcgg ctaactccgt gccagcagcc gcggtaatac ggaggatgcg
 541 agcgttatcc ggatttattg ggtttaaagg gtgcgtaggt ggtgatttaa gtcagcggtg
 601 aaagtttgtg gctcaaccat aaaattgccg ttgaaactgg gttacttgag tgtgtttgag
 661 gtaggcggaa tgcgtggtgt agcggtgaaa tgcatagata tcacgcagaa ctccgattgc
 721 gaaggcagct tactaaacca taactgacac tgaagcacga aagcgtgggg atcaaacagg
 781 attagatacc ctggtagtcc acgcagtaaa cgatgattac taggagtttg cgatacaatg
 841 taagctctac agcgaaagcg ttaagtaatc cacctgggga gtacgccggc aacggtgaaa
 901 ctcaaaggaa ttgacggggg cccgcacaag cggaggaaca tgtggtttaa ttcgatgata
 961 cgcgaggaac cttacccggg tttgaacgta gtctgaccgg agtggaaaca ctccttctag
1021 caatagcaga ttacgaggtg ctgcatggtt gtcgtcagct cgtgccgtga ggtgtcggct
1081 taagtgccat aacgagcgca acccttatca ctagttacta acaggtgaag ctgaggactc
1141 tggtgagact gccagcgtaa gctgtgagga aggtggggat gacgtcaaat cagcacggcc
1201 cttacatccg gggcgacaca cgtgttacaa tggcatggac aaagggcagc tacctggcga
1261 caggatgcta atctccaaac catgtctcag ttcggatcgg agtctgcaac tcgactccgt
1321 gaagctggat tcgctagtaa tcgcgcatca gccatggcgc ggtgaatacg ttcccgggcc
1381 ttgtacacac cgcccgtcaa gccatgggag ccgggggtac ctgaagtccg taaccgcaag
1441 gatcggccta gggtaaaact ggtgactggg gctaagtcgt aacaaggtaa cc
```

SEQ ID NO:8 (*Parabacteroides merdae* gene for 16S ribosomal RNA, partial sequence, strain: JCM 13405 - AB238929)

```
   1 agagtttgat cctggctcag gatgaacgct agcgacaggc ttaacacatg caagtcgagg
  61 ggcagcatga tttgtagcaa tacagattga tggcgaccgg cgcacgggtg agtaacgcgt
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 121 atgcaactta cctatcagag ggggatagcc cggcgaaagt cggattaata ccccataaaa
 181 caggggttcc gcatgggaat atttgttaaa gattcatcgc tgatagatag gcatgcgttc
 241 cattaggcag ttggcggggt aacggcccac caaaccgacg atggataggg gttctgagag
 301 gaaggtcccc cacattggta ctgagacacg gaccaaactc ctacgggagg cagcagtgag
 361 gaatattggt caatggccga gaggctgaac cagccaagtc gcgtgaagga agaaggatct
 421 atggtttgta aacttctttt ataggggaat aaagtggagg acgtgtcctt ttttgtatgt
 481 accctatgaa taagcatcgg ctaactccgt gccagcagcc gcggtaatac ggaggatgcg
 541 agcgttatcc ggatttattg ggtttaaagg gtgcgtaggt ggtgatttaa gtcagcggtg
 601 aaagtttgtg gctcaaccat aaaattgccg ttgaaactgg gttacttgag tgtgtttgag
 661 gtaggcggaa tgcgtggtgt agcggtgaaa tgcatagata tcacgcagaa ctccgattgc
 721 gaaggcagct tactaaacca taactgacac tgaagcacga aagcgtgggg atcaaacagg
 781 attagatacc ctggtagtcc acgcagtaaa cgatgattac taggagtttg cgatacaatg
 841 taagctctac agcgaaagcg ttaagtaatc cacctgggga gtacgccggc aacggtgaaa
 901 ctcaaaggaa ttgacggggg cccgcacaag cggaggaaca tgtggtttaa ttcgatgata
 961 cgcgaggaac cttacccggg tttgaacgta gtctgaccgg agtggaaaca ctccttctag
1021 caatagcaga ttacgaggtg ctgcatggtt gtcgtcagct cgtgccgtga ggtgtcggct
1081 taagtgccat aacgagcgca acccttatca ctagttacta acaggtgaag ctgaggactc
1141 tggtgagact gccagcgtaa gctgtgagga aggtggggat gacgtcaaat cagcacggcc
1201 cttacatccg gggcgacaca cgtgttacaa tggcatggac aaagggcagc tacctggcga
1261 caggatgcta atctccaaac catgtctcag ttcggatcgg agtctgcaac tcgactccgt
1321 gaagctggat tcgctagtaa tcgcgcatca gccatggcgc ggtgaatacg ttcccgggcc
1381 ttgtacacac cgcccgtcaa gccatgggag ccgggggtac ctgaagtccg taaccgcaag
1441 gatcggccta gggtaaaact ggtgactggg gctaagtcgt aacaaggtaa cc
```

SEQ ID NO:9 (consensus 16S rRNA sequence for *Parabacteroides distasonis* strain 755)

```
AMCCGGGTGGCGACCGGCGCACGGGTGAGTAACGCGTATGCAACTTGCCTATCAGAGGGGGATAACCCGGCGAAAGT
CGGACTAATACCGCATGAAGCAGGGATCCCGCATGGGAATATTTGCTAAAGATTCATCGCTGATAGATAGGCATGCG
TTCCATTAGGCAGTTGGCGGGGTAACGGCCCACCAAACCGACGATGGATAGGGGTTCTGAGAGGAAGGTCCCCCACA
TTGGTACTGAGACACGGACCAAACTCCTACGGGAGGCAGCAGTGAGGAATATTGGTCAATGGGCGTGAGCCTGAACC
AGCCAAGTCGCGTGAGGGATGAAGGTTCTATGGATCGTAAACCTCTTTTATAAGGGAATAAAGTGCGGGACGTGTCC
CGTTTTGTATGTACCTTATGAATAAGGATCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGATCCGAGCGT
TATCCGGATTTATTGGGTTTAAAGGGTGCGTAGGCGGCCTTTTAAGTCAGCGGTGAAAGTCTGTGGCTCAACCATAG
AATTGCCGTTGAAACTGGGAGGCTTGAGTATGTTTGAGGCAGGCGGAATGCGTGGTGTAGCGGTGAAATGCATAGAT
ATCACGCAGAACCCCGATTGCGAAGGCAGCCTGCCAAGCCATTACTGACGCTGATGCACGAAAGCGTGGGGATCAAA
CAGGATTAGATACCCTGGTAGTCCACGCAGTAAACGATGATCACTAGCTGTTTGCGATACACTGTAAGCGGCACAGC
GAAAGCGTTAAGTGATCCACCTGGGGAGTACGCCGGCAACGGTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAG
CGGAGGAACATGTGGTTTAATTCGATGATACGCGAGGAACCTTACCCGGGTTTGAACGCATTCGGACMGAKGTGGAA
ACACATTTTCTAGCAATAGCCATTTGCGAGGTGCTGCATGGTTGTCGTCAGCTCGTGCCGTGAGGTGTCGGCTTAAG
TGCCATAACGAGCGCAACCCTTGCCACTAGTTACTAACAGGTAAAGCTGAGGACTCTGGTGGGACTGCCAGCGTAAG
CTGCGAGGAAGGCGGGGATGACGTCAAATCAGCACGGCCCTTACATCCGGGGCGACACACGTGTTACAATGGCGTGG
ACAAAGGGAAGCCACCTGGCGACAGGGAGCGAATCCCCAAACCACGTCTCAGTTCGGATCGGAGTCTGCAACCCGAC
TCCGTGAAGCTGGATTCGCTAGTAATCGCGCATCAGCCATGGCGCGGTGAATACGTTCCCGGGCCTTGTACACACCG
CCCGTCAAGCCATGGGAGCCGGGGGTACCTGAAGTCCGTAACCGCGAGGATCGGCCTAGGGTAAAACTGGTGACTGG
GGCTAAGTCGTACGGGG
```

SEQ ID NO:10 (strain 755 genome sequence) – see electronic sequence listing.

SEQ ID NO:11 (consensus 16S rRNA sequence for *Megasphaera massiliensis* strain MRX0029)

TGAGAAGCTTGCTTCTTATCGATTCTAGTGGCAAACGGGTGAGTAACGCGTAAGCAACCTGCCCTTCAGATGGGGAC
AACAGCTGGAAACGGCTGCTAATACCGAATACGTTCTTTCCGCCGCATGACGGGAAGAAGAAAGGGAGGCCTTCGGG
CTTTCGCTGGAGGAGGGGCTTGCGTCTGATTAGCTAGTTGGAGGGGTAACGGCCCACCAAGGCGACGATCAGTAGCC
GGTCTGAGAGGATGAACGGCCACATTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATCTT
CCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAACGATGACGGCCTTCGGGTTGTAAAGTTCTGTTATATG
GGACGAACAGGACATCGGTTAATACCCGGTGTCTTTGACGGTACCGTAAGAGAAAGCCACGGCTAACTACGTGCCAG
CAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGGGCGCGCAGGCGGCATCGCAAGT
CGGTCTTAAAAGTGCGGGGCTTAACCCCGTGAGGGGACCGAAACTGTGAAGCTCGAGTGTCGGAGAGGAAAGCGGAA
TTCCTAGTGTAGCGGTGAAATGCGTAGATATTAGGAGGAACACCAGTGGCGAAAGCGGCTTTCTGGACGACAACTGA
CGCTGAGGCGCGAAAGCCAGGGGAGCAAACGGGATTAGATACCCCGGTAGTCCTGGCCGTAAACGATGGATACTAGG
TGTAGGAGGTATCGACTCCTTCTGTGCCGGAGTTAACGCAATAAGTATCCCGCCTGGGGAGTACGGCCGCAAGGCTG
AAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGTATGTGGTTTAATTCGACGCAACGCGAAGAACCTTA
CCAAGCCTTGACATTGATTGCTACGGAAAGAGATTTCCGGTTCTTCTTCGGAAGACAAGAAAACAGGTGGTGCACGG
CTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCCTATCTTCTGTTGCCAGCACC
TCGGGTGGGGACTCAGAAGAGACTGCCGCAGACAATGCGGAGGAAGGCGGGGATGACGTCAAGTCATCATGCCCCTT
ATGGCTTGGGCTACACACGTACTACAATGGCTCTTAATAGAGGGAAGCGAAGGAGCGATCCGGAGCAAACCCCAAAA
ACAGAGTCCCAGTTCGGATTGCAGGCTGCAACTCGCCTGCATGAAGCAGGAATCGCTAGTAATCGCAGGTCAGCATA
CTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAAAGTCATTCACACCCGAAGCCGGTGA
GGCAACCGCAAG

Primers used for qPCR (with SEQ ID NO in brackets)

Name	Forward sequence	Reverse sequence
ACTB	GATCAAGATCATTGCTCCTC (12)	TTGTCAAGAAAGGGTGTAAC (13)
GAPDH	GGTATCGTGGAAGGACTCATG (14)	ATGCCAGTGAGCTTCCCGTTC (15)
MAP2	CTCAGCACCGCTAACAGAGG (16)	CATTGGCGCTTCTCTCCTC (17)
Occludin	AAGAGGAATTTTGACACTGG (18)	GCCATGTACTCTTCACTTTC (19)
TJ1	AAGTCACACTGGTGAAATCC (20)	CTCTTGCTGCCAAACTATCT (21)
TJP2	CCCTCCCCTGGATCAGGAT (22)	GCCATCAAACTCGTCCATCA (23)
Villin	CATTACCTGCTCTACGTTTG (24)	AGATGGACATAAGATGAGGTG (25)

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1-3-2	Address of depositary institution	NCIMB Ltd, Ferguson Building, Craibstone Estate, Bucksburn, Aberdeen AB21 9YA, United Kingdom
1-3-3	Date of deposit	12 March 2015 (12.03.2015)
1-3-4	Accession Number	NCIMB 42382
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2-3-1	Name of depositary institution	NCIMB National Collections of Industrial, Food and Marine Bacteria (NCIMB)
2-3-2	Address of depositary institution	NCIMB Ltd, Ferguson Building, Craibstone Estate, Bucksburn, Aberdeen AB21 9YA, United Kingdom
2-3-3	Date of deposit	13 July 2017 (13.07.2017)
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2018285445 13 Jan 2020

CLAIMS

1. A method of treating or preventing a neurodegenerative disorder selected from the group consisting of Parkinson's disease, including progressive supranuclear palsy, progressive supranuclear palsy, Steele-Richardson-Olszewski syndrome, normal pressure hydrocephalus, vascular or arteriosclerotic parkinsonism and drug-induced parkinsonism; Alzheimer's disease, including Benson's syndrome; Huntington's disease; amyotrophic lateral sclerosis; Lou Gehrig's disease; motor neurone disease; prion disease; spinocerebellar ataxia; spinal muscular atrophy; dementia, including Lewy body, vascular and frontotemporal dementia; primary progressive aphasia; mild cognitive impairment; HIV-related cognitive impairment and corticobasal degeneration, comprising administering a composition comprising a bacterial strain of the genus *Parabacteroides* to a patient in need thereof.

2. Use of a composition comprising a bacterial strain of the genus *Parabacteroides*, in the manufacture of a medicament for treating or preventing a neurodegenerative disorder selected from the group consisting of Parkinson's disease, including progressive supranuclear palsy, progressive supranuclear palsy, Steele-Richardson-Olszewski syndrome, normal pressure hydrocephalus, vascular or arteriosclerotic parkinsonism and drug-induced parkinsonism; Alzheimer's disease, including Benson's syndrome; Huntington's disease; amyotrophic lateral sclerosis; Lou Gehrig's disease; motor neurone disease; prion disease; spinocerebellar ataxia; spinal muscular atrophy; dementia, including Lewy body, vascular and frontotemporal dementia; primary progressive aphasia; mild cognitive impairment; HIV-related cognitive impairment and corticobasal degeneration in a patient.

3. The method of claim 1, wherein the method is a method of treating or preventing Parkinson's disease; or the use of claim 2, wherein the medicament is for treating or preventing Parkinson's disease.

4. The method of claim 1 or claim 3, wherein the method is a method of treating or preventing early-onset neurodegenerative disease; or the use of claim 2 or claim 3, wherein the medicament is for treating or preventing early-onset neurodegenerative disease.

5. The method of any one of claims 1, 3 or 4, wherein the method is a method of preventing or delaying onset or progression of a neurodegenerative disorder; or the use of any one of claims 2-4, wherein the medicament is for preventing or delaying onset or progression of a neurodegenerative disorder.

6. A method of treating brain injury comprising administering a composition comprising a bacterial strain of the genus *Parabacteroides* to a patient in need thereof.

7. Use of a composition comprising a bacterial strain of the genus *Parabacteroides*, in the manufacture of a medicament for treating brain injury in a patient.

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8. The method of claim 6 or the use of claim 7, wherein the brain injury is stroke, such as cerebral ischemia, focal cerebral ischemia, ischemic stroke or hemorrhagic stroke.

9. The method of any one of claims 1, 3-6 or 8 or the use of any one of claims 2-5 or 7-8, wherein the bacterial strain is of *Parabacteroides distasonis*.

10. The method of any one of claims 1, 3-6 or 8-9 or the use of any one of claims 2-5 or 7-9, wherein the bacterial strain has a 16s rRNA sequence that is at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% identical to the 16s rRNA sequence of a bacterial strain of *Parabacteroides distasonis*.

11. The method of any one of claims 1, 3-6 or 8-9 or the use of any one of claims 2-5 or 7-9, wherein the bacterial strain has a 16s rRNA sequence that is at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% identical to SEQ ID NO:1, 2, 3, 4, 5, 6, 7 8 or 9.

12. The method or the use of claim 11, wherein the bacterial strain has a 16s rRNA sequence that is at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% identical to SEQ ID NO:9, or wherein the bacterial strain has the 16s rRNA sequence represented by SEQ ID NO:9.

13. The method of claim 1 or claim 3, wherein the composition comprises a bacterial strain of the species *Parabacteroides distasonis*; or the use of claim 2 or claim 3, wherein the medicament comprises a bacterial strain of the species *Parabacteroides distasonis*.

14. The method of any one of claims 1, 3-6 or 8-13, composition is administered orally; or the use of any one of claims 2-5 or 7-13, wherein the medicament is formulated for oral administration.

15. The method of any one of claims 1, 3-6 or 8-14 or the use of any one of claims 2-5 or 7-14, wherein the composition comprises one or more pharmaceutically acceptable excipients or carriers.

16. The method of any one of claims 1, 3-6 or 8-15 or the use of any one of claims 2-5 or 7-15, wherein the bacterial strain is lyophilised.

17. A food product comprising the composition comprising a bacterial strain of the genus *Parabacteroides*, when used in a method of any one of claims 1, 3-6 or 8-16.

18. Use of a food product comprising the composition comprising a bacterial strain of the genus *Parabacteroides*, in the manufacture of a medicament for treating or preventing a neurodegenerative disorder in a patient.

19. The use of claim 18, modified by the features of any one of claims 2-5 or 7-16.

20. The method of any one of claims 1 or 3-5 or the use of any one of claims 2-5, wherein the bacterial strain is a cell of the *Parabacteroides distasonis* strain deposited under accession number NCIMB 42382.

FIG. 1 Protection against neurotoxicity

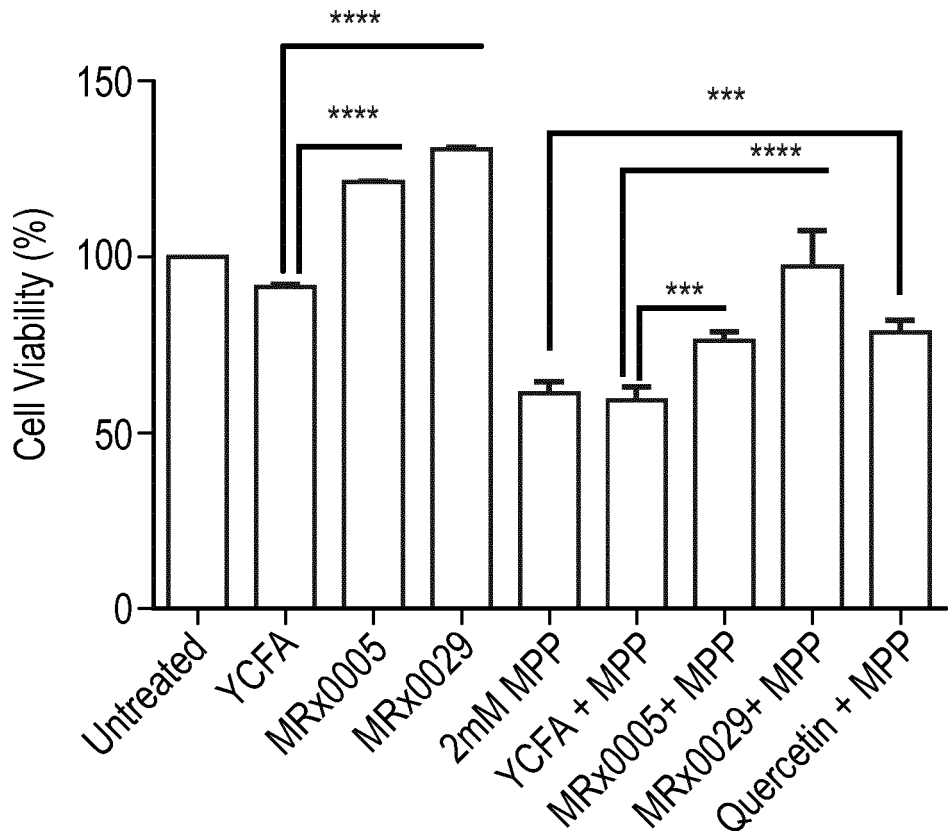


FIG. 2A Inhibition of LPS-induced IL-6 secretion in MG U373 cells

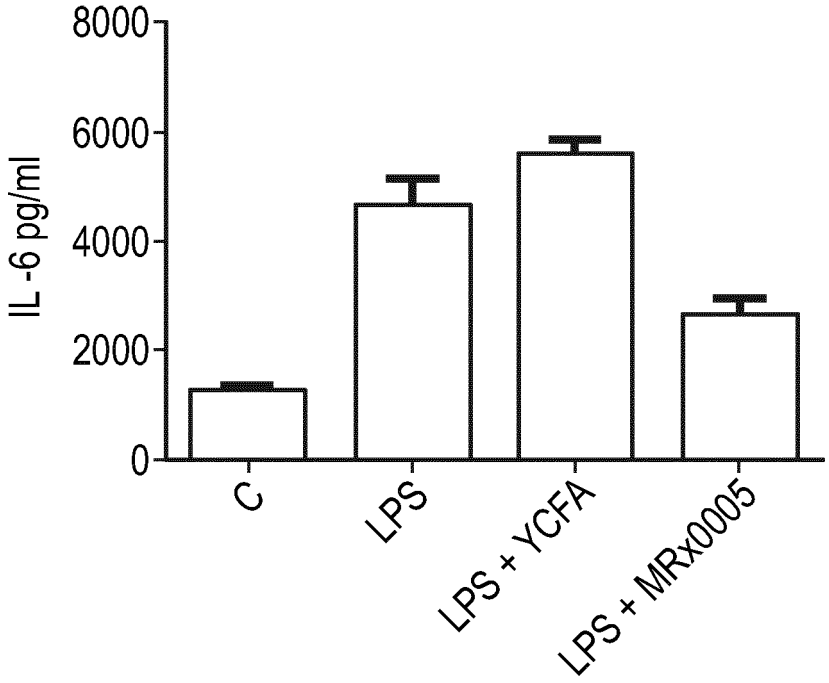
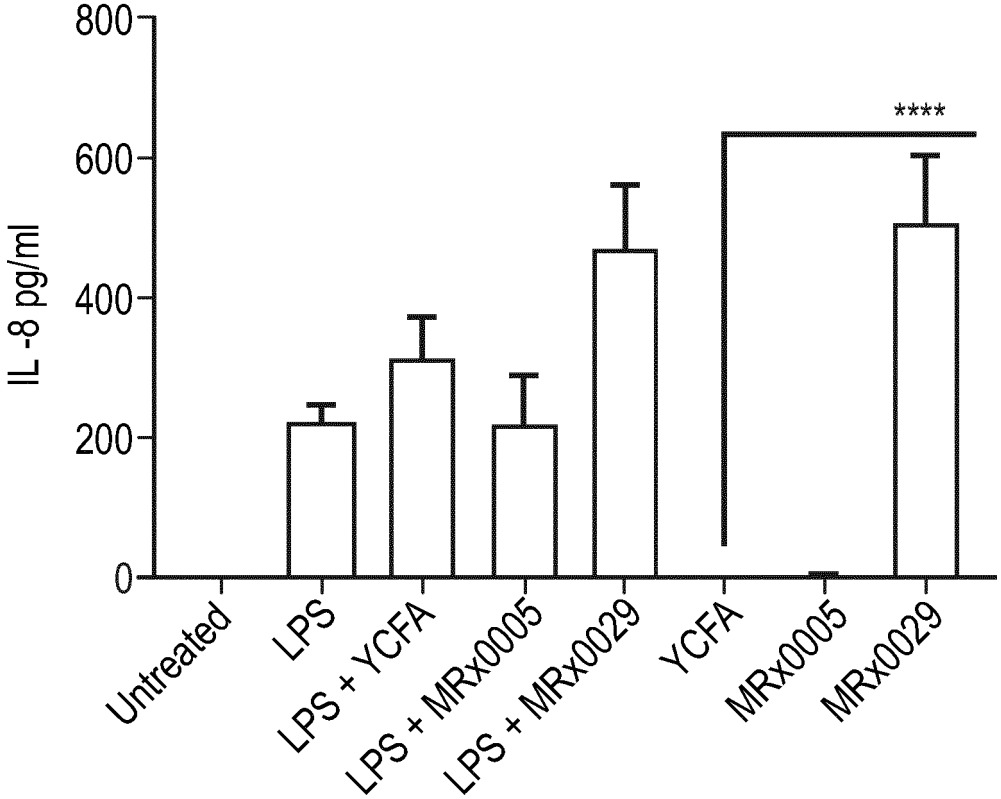


FIG. 2B Secretion of IL-8 from U373



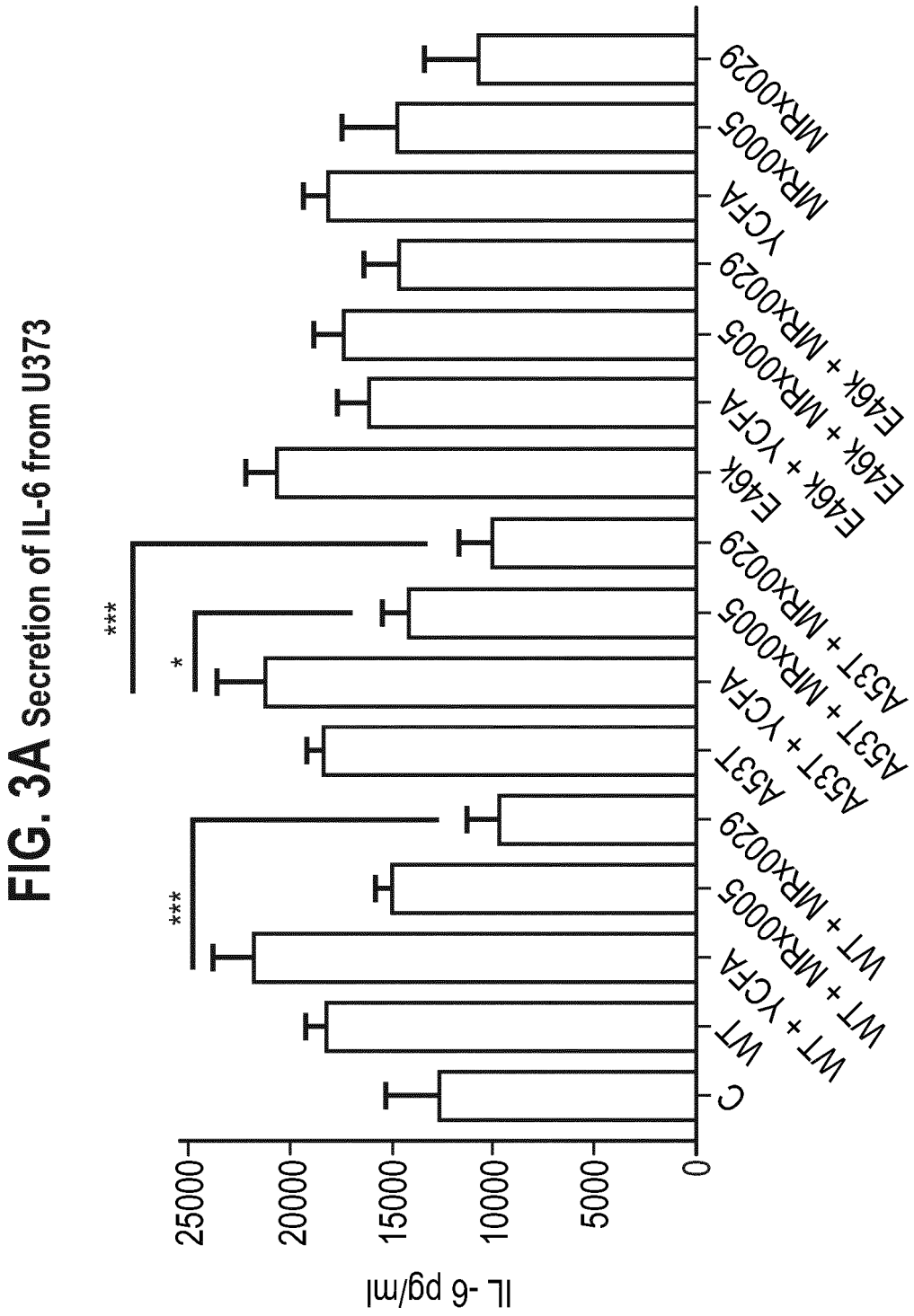


FIG. 3A Secretion of IL-6 from U373
IL -6 pg/ml
0
5000
10000
15000
20000
25000
***
*
***
C
WT
WT + VCFA
WT + MRX0005
WT + MRX0029
A53T
A53T + VCFA
A53T + MRX0005
A53T + MRX0029
E46K
E46K + VCFA
E46K + MRX0005
E46K + MRX0029
VCFA
MRX0005
MRX0029

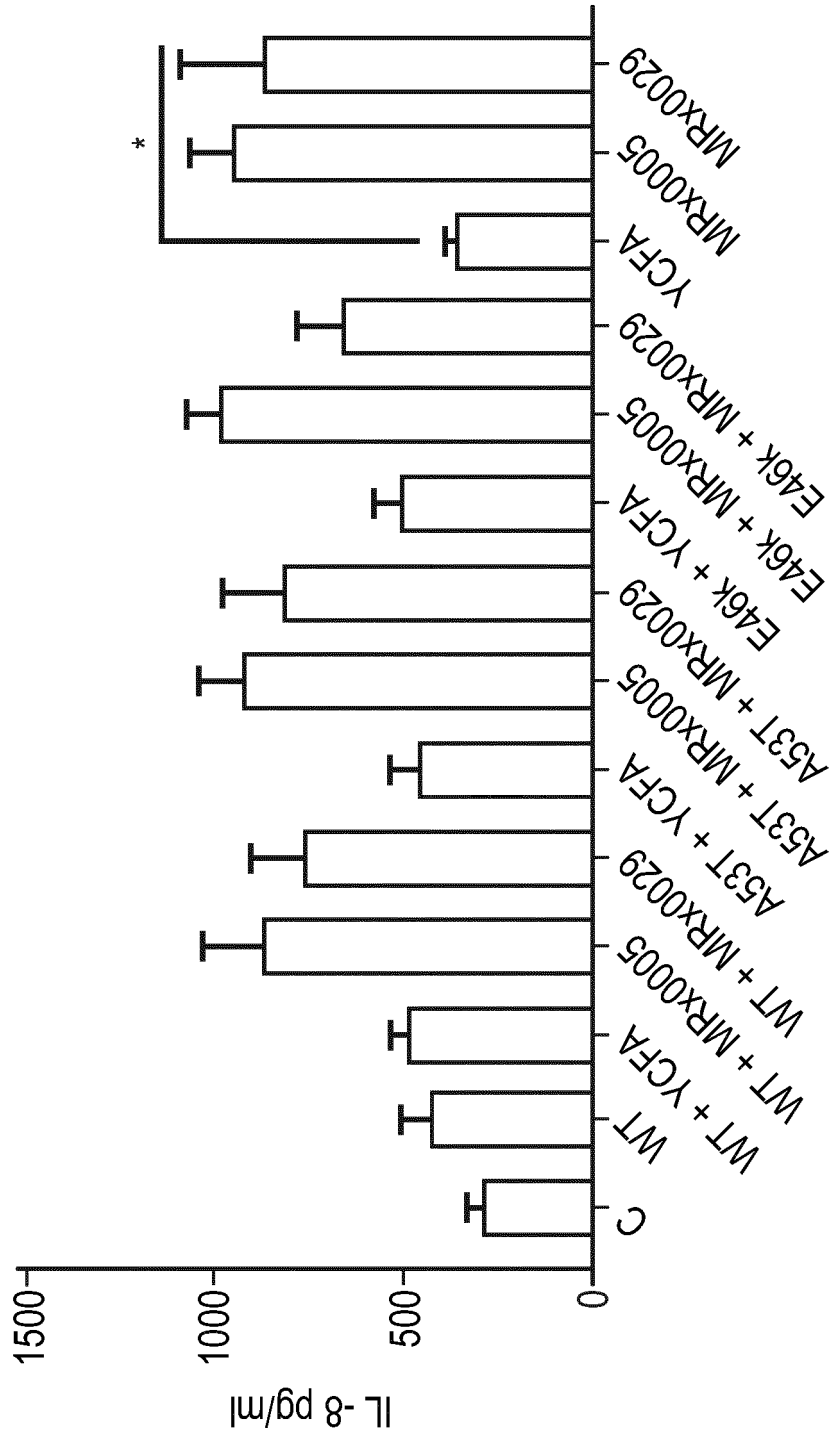


FIG. 3B Secretion of IL-8 from U373

FIG. 4 Inhibition of a-synuclein-induced NFkB-AP1 activation in HEK-TLR4

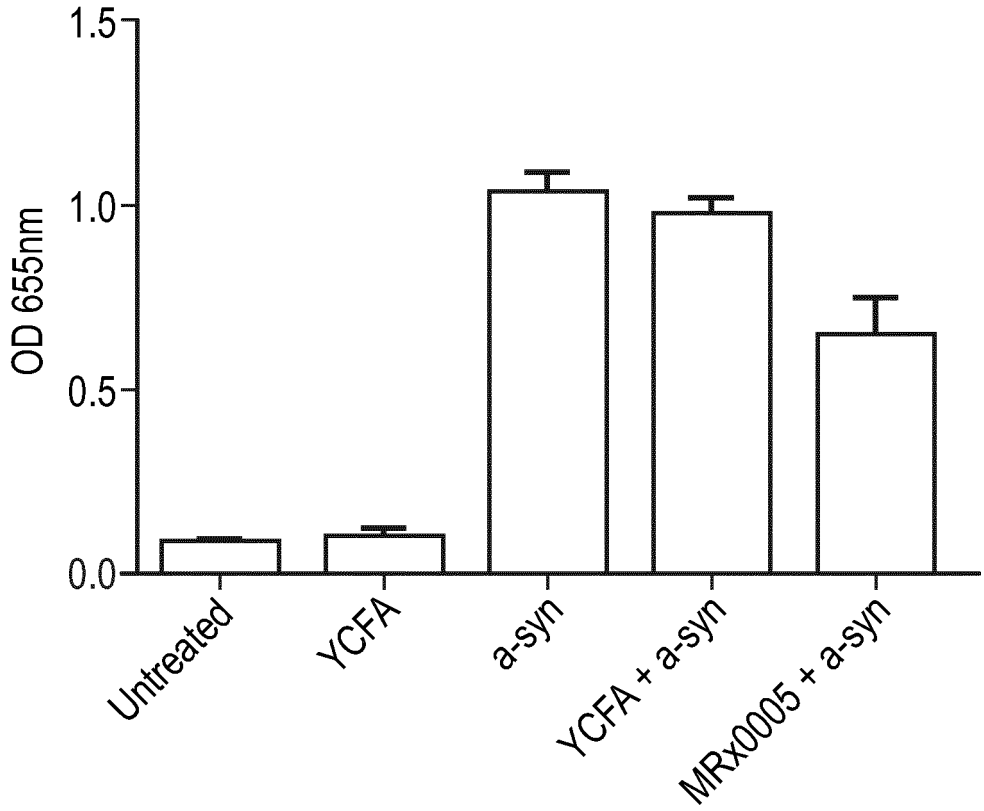


FIG. 5 Inhibition of LPS-induced NFkB-AP1 activation in HEK-TLR4

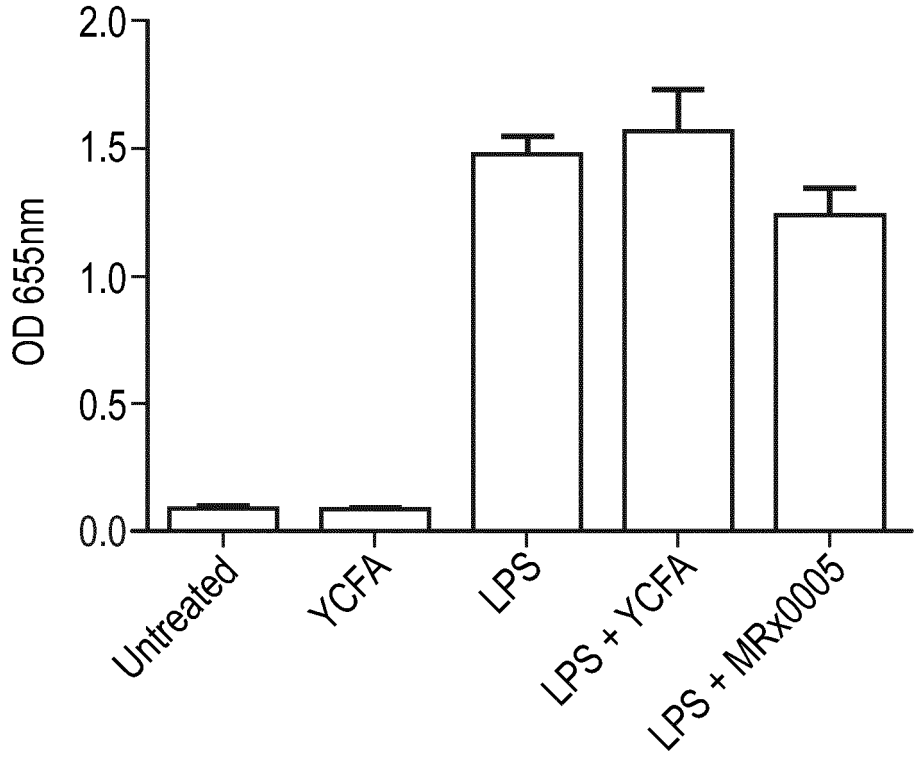


FIG. 6 Antioxidant Capacity Assay

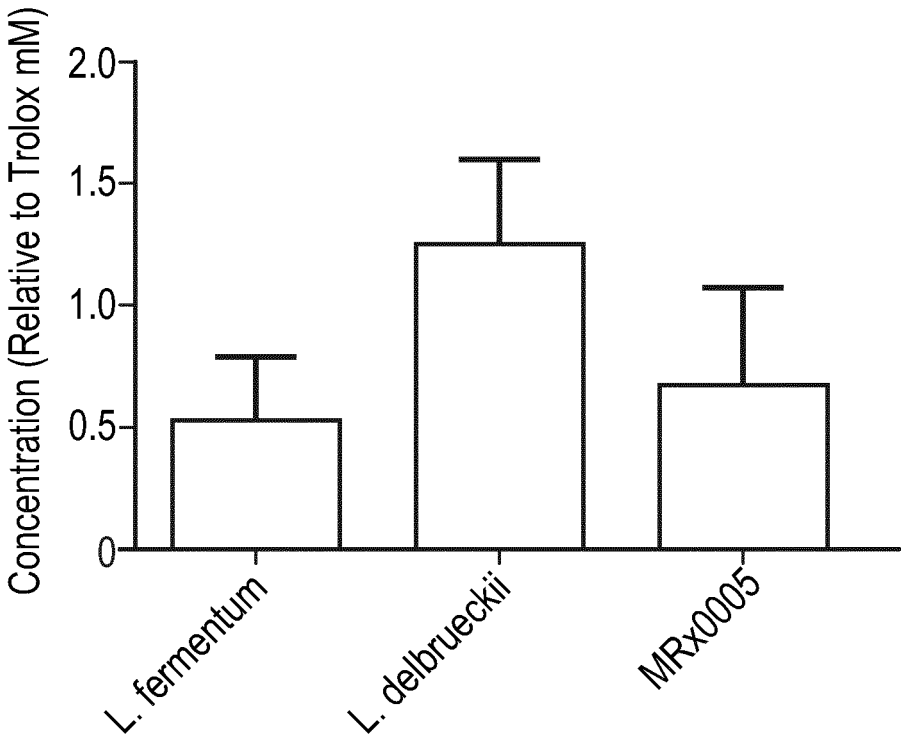
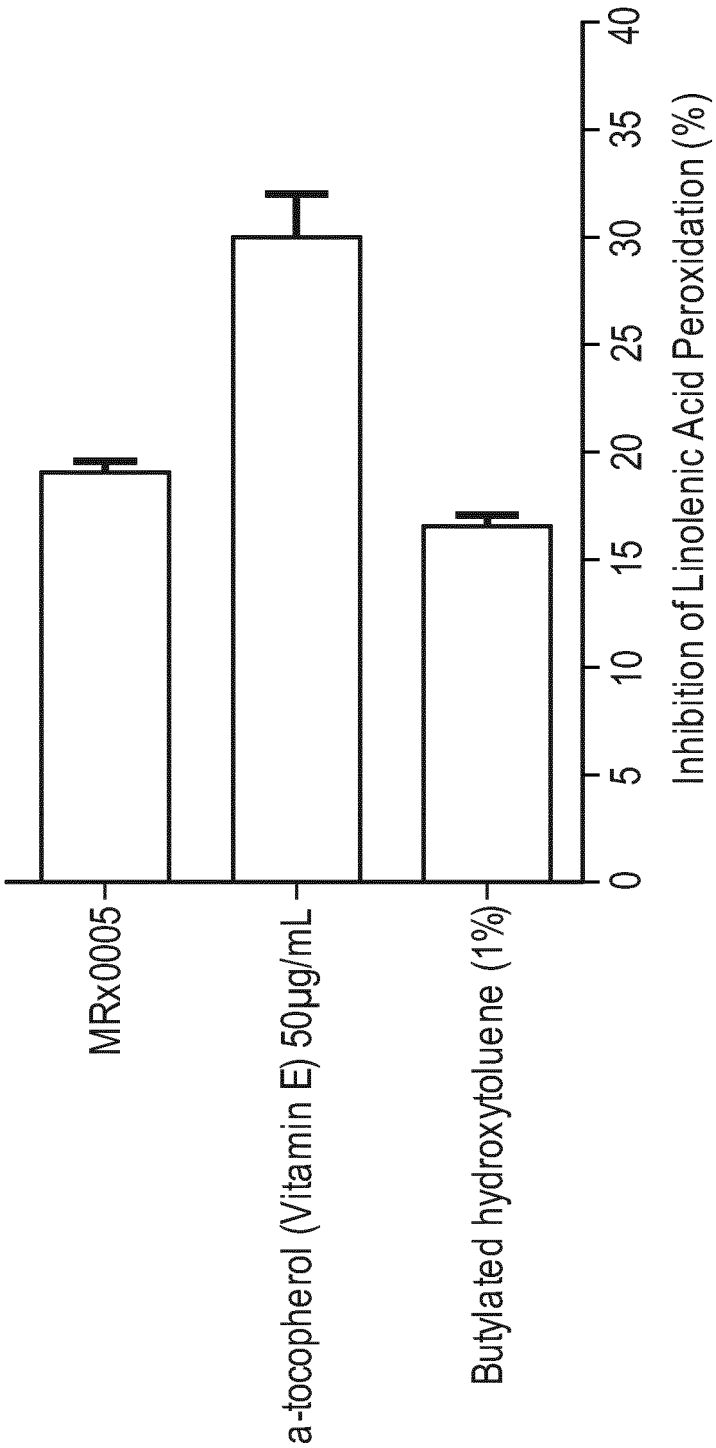


FIG. 7 Total Antioxidant Capacity



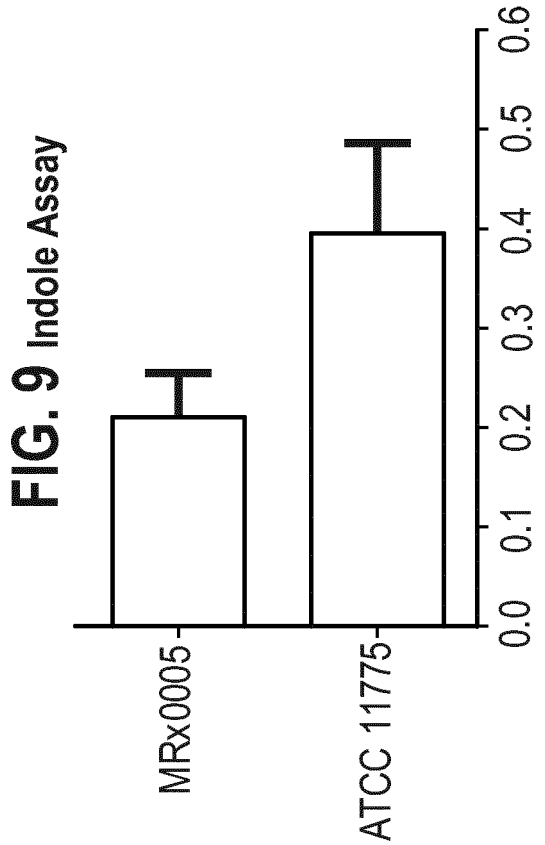


FIG. 9 Indole Assay

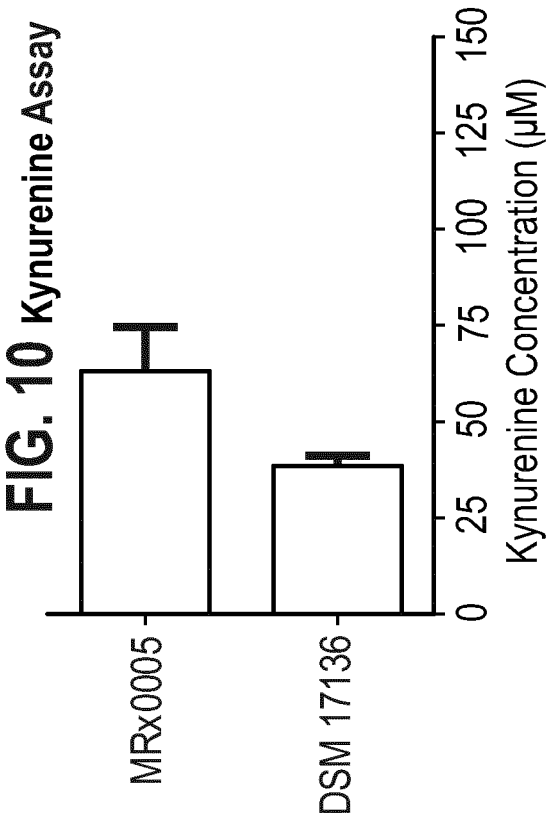


FIG. 10 Kynurenine Assay

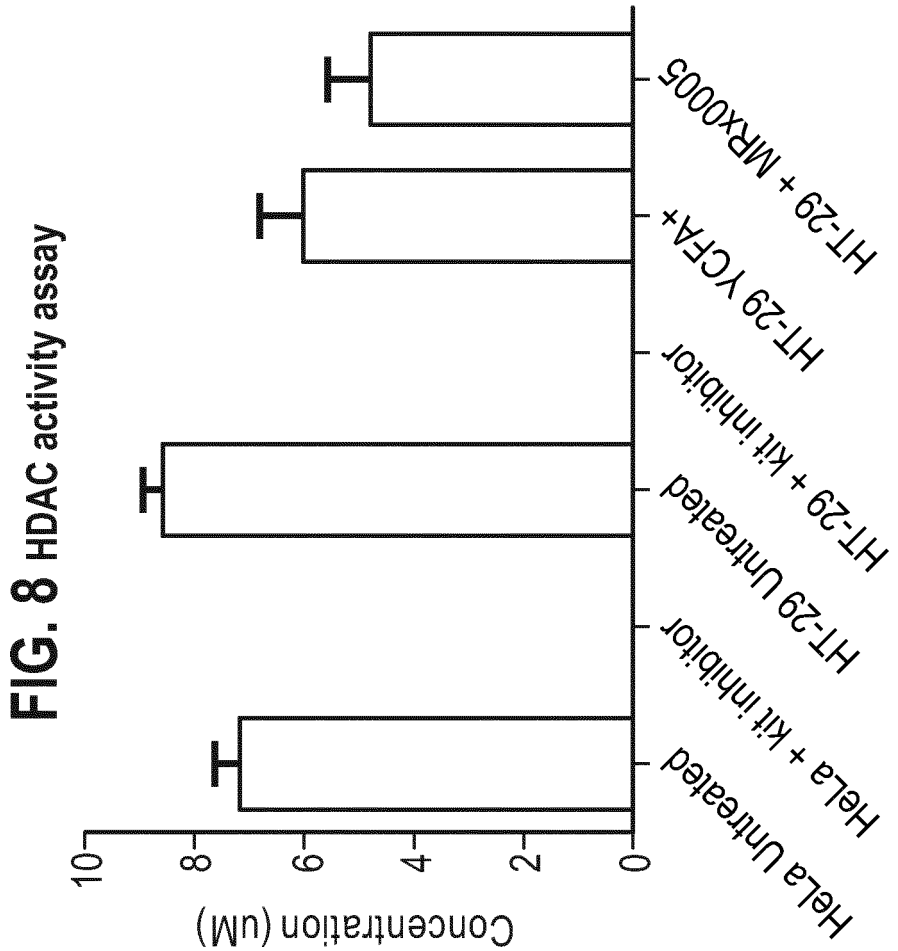


FIG. 8 HDAC activity assay

FIG. 11 Neuroprotection – cell viability

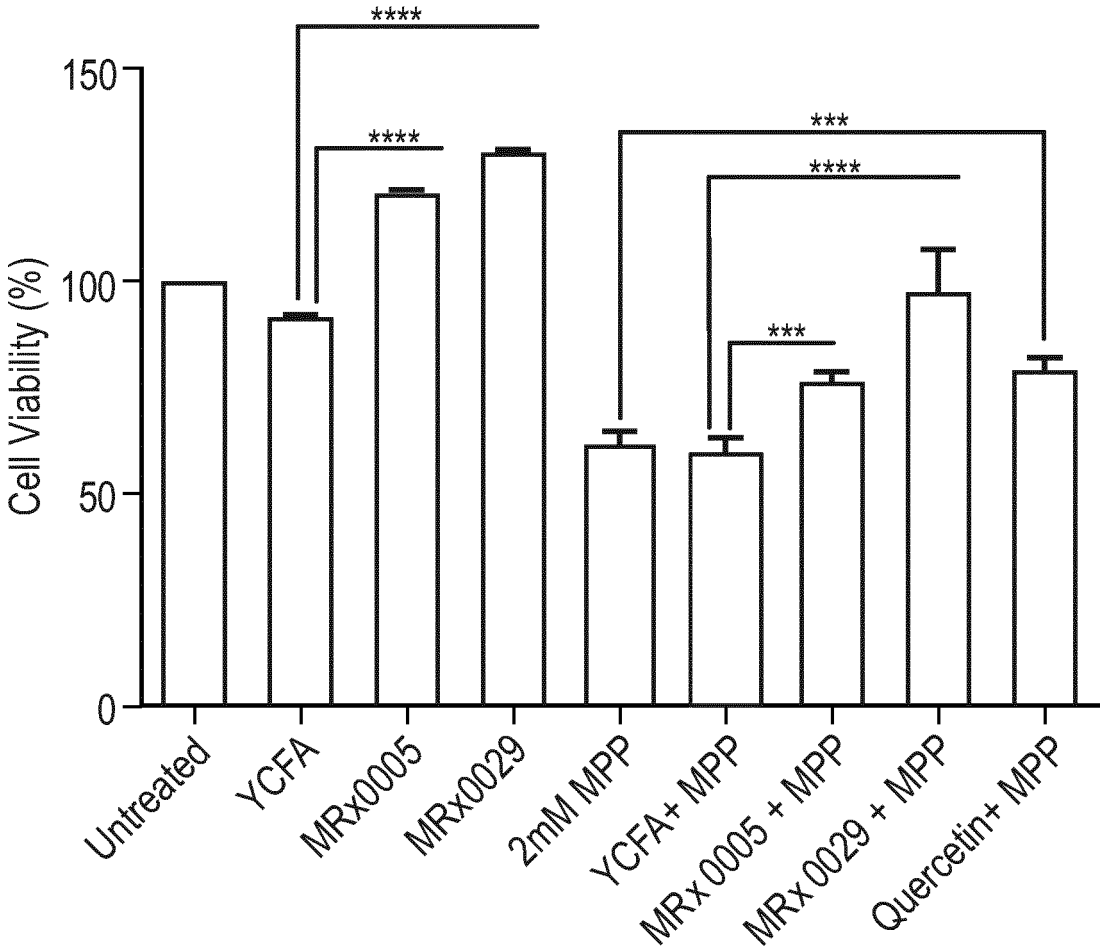


FIG. 12 Production of neurotransmitters in the brain

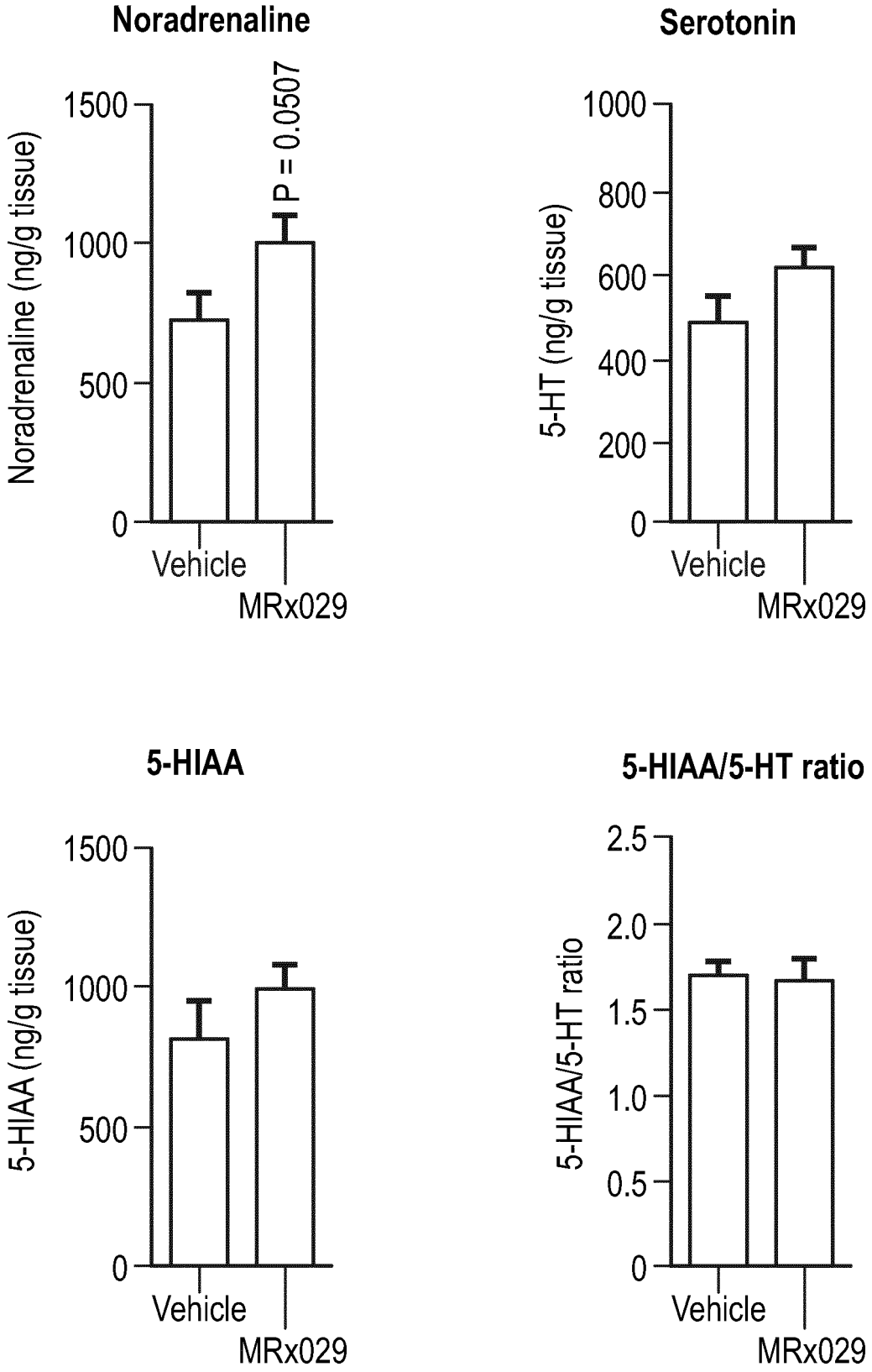


FIG. 13 Bacterial metabolites in the supernatant

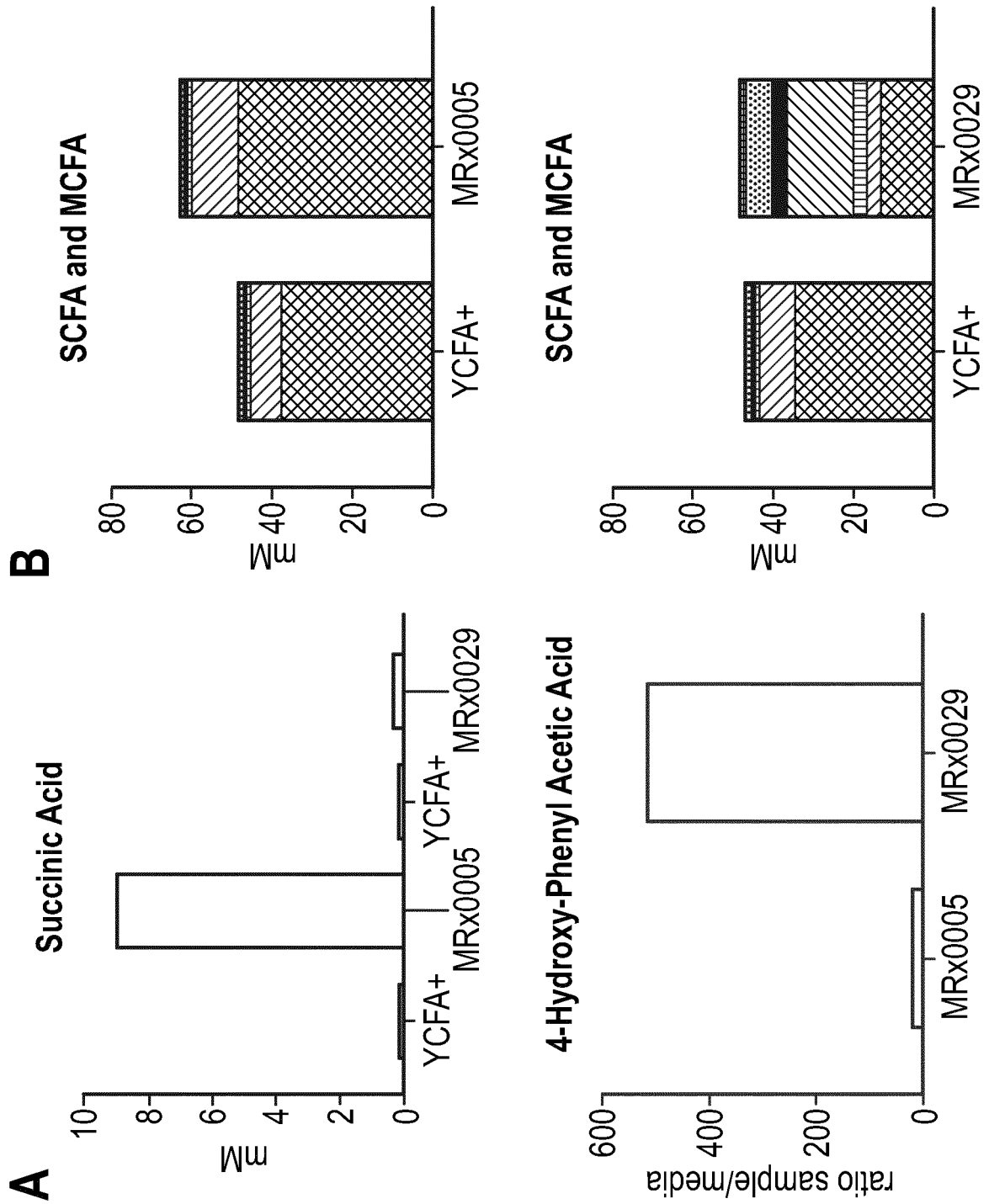
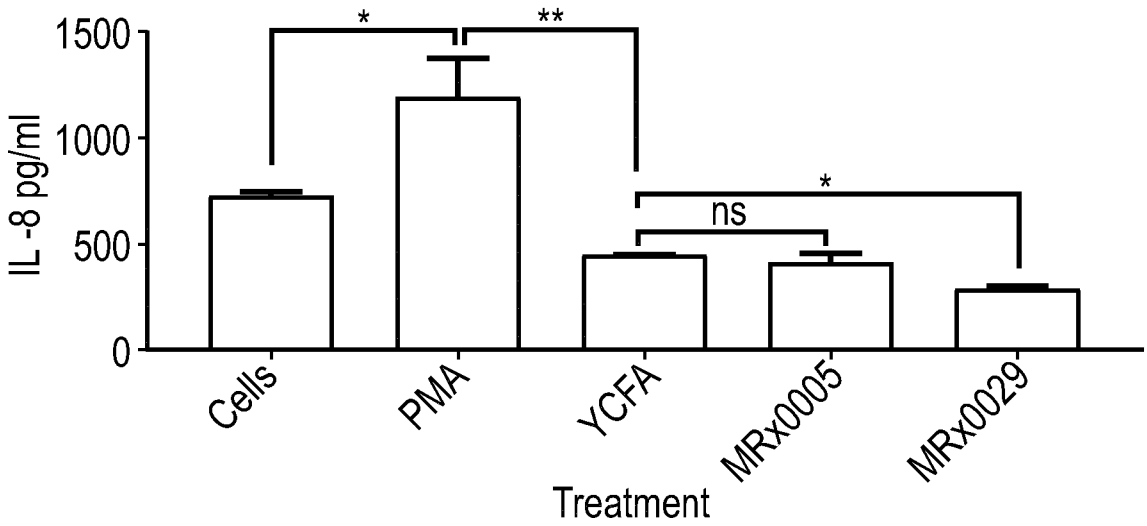


FIG. 14A


1500
1000
500
0
IL -8 pg/ml
*
**
*
ns
Cells
PMA
YCFA
MRx0005
MRx0029
Treatment

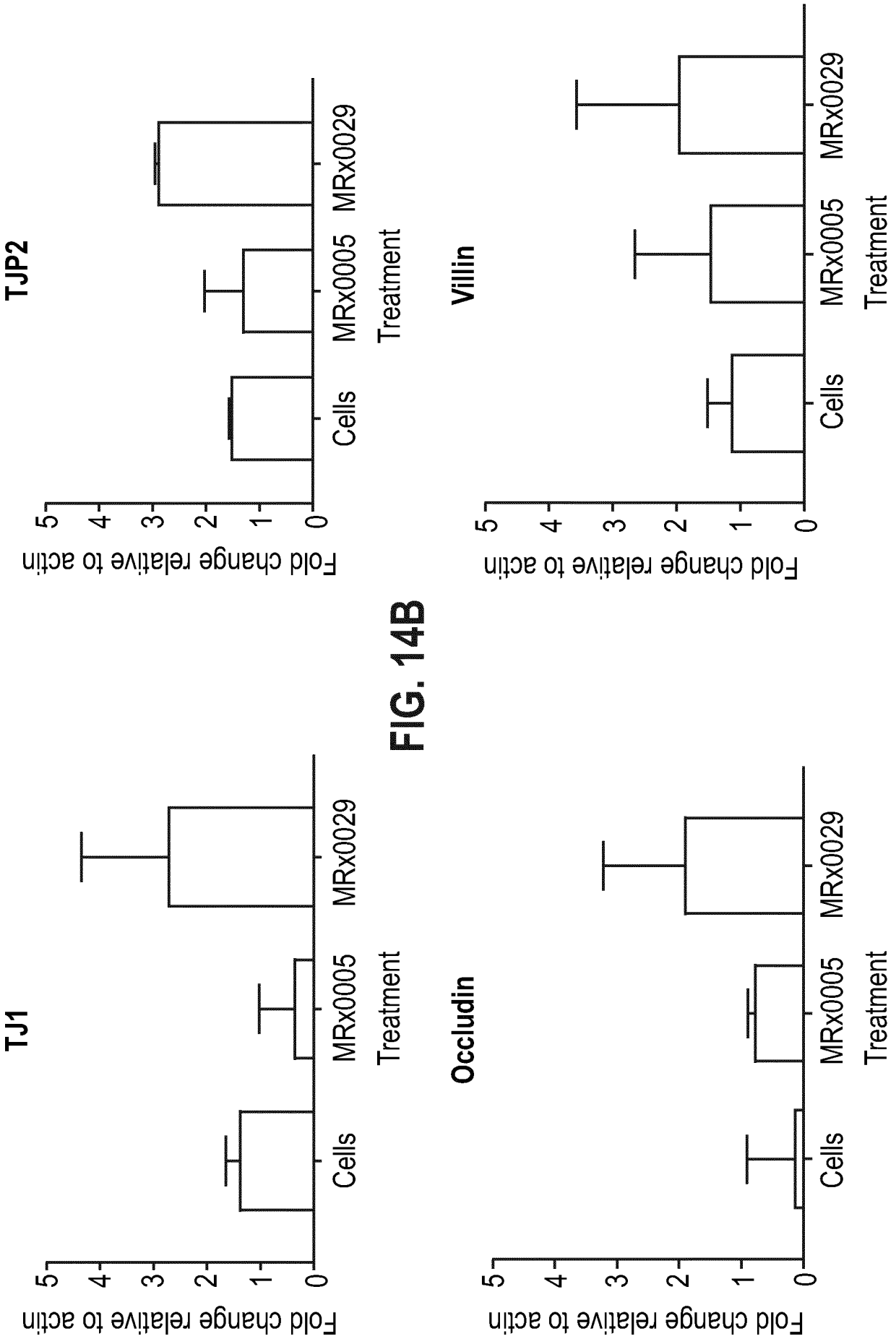

TJP2
Villin
TJ1
Occludin
Fold change relative to actin
0
1
2
3
4
5
Cells
MRx0005
MRx0029
Treatment

FIG. 14B

FIG. 14C

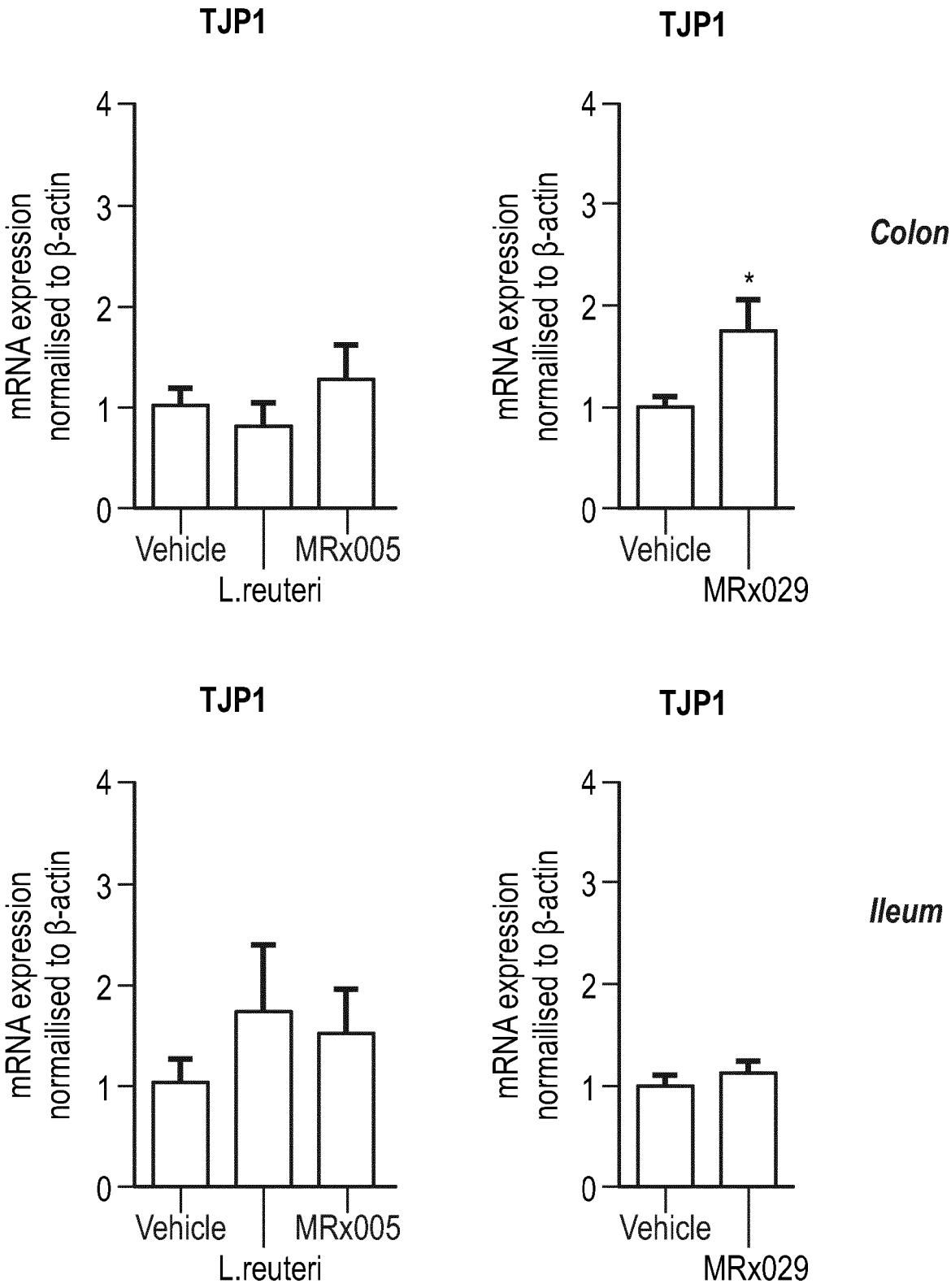

TJP1
mRNA expression normalised to β-actin
4
3
2
1
0
Vehicle
MRx005
L.reuteri
TJP1
mRNA expression normalised to β-actin
*
Vehicle
MRx029
Colon
TJP1
mRNA expression normalised to β-actin
Vehicle
MRx005
L.reuteri
TJP1
mRNA expression normalised to β-actin
Vehicle
MRx029
Ileum

FIG. 14D

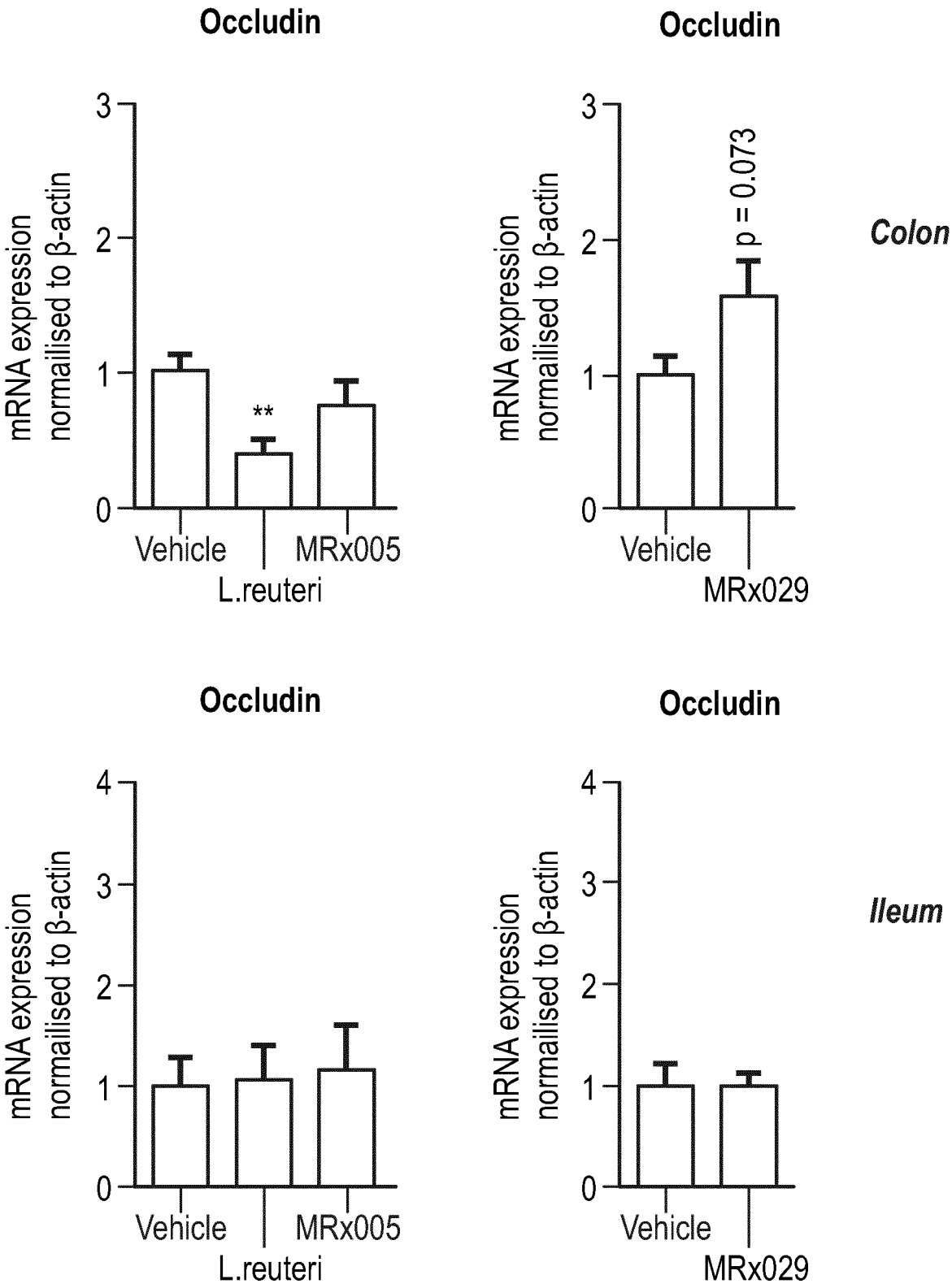
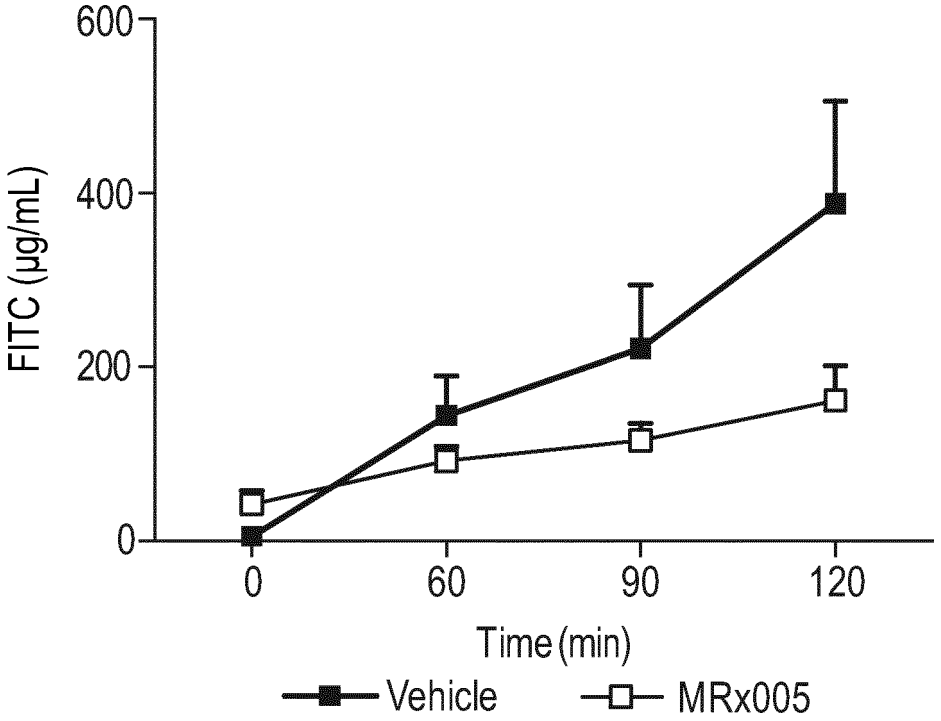

Occludin
mRNA expression normalised to β-actin
3
2
1
0
**
Vehicle
MRx005
L.reuteri
Occludin
mRNA expression normalised to β-actin
p = 0.073
Vehicle
MRx029
Colon
Occludin
mRNA expression normalised to β-actin
4
3
2
1
0
Vehicle
MRx005
L.reuteri
Occludin
mRNA expression normalised to β-actin
Vehicle
MRx029
Ileum

FIG. 14E Permeability in the Ileum



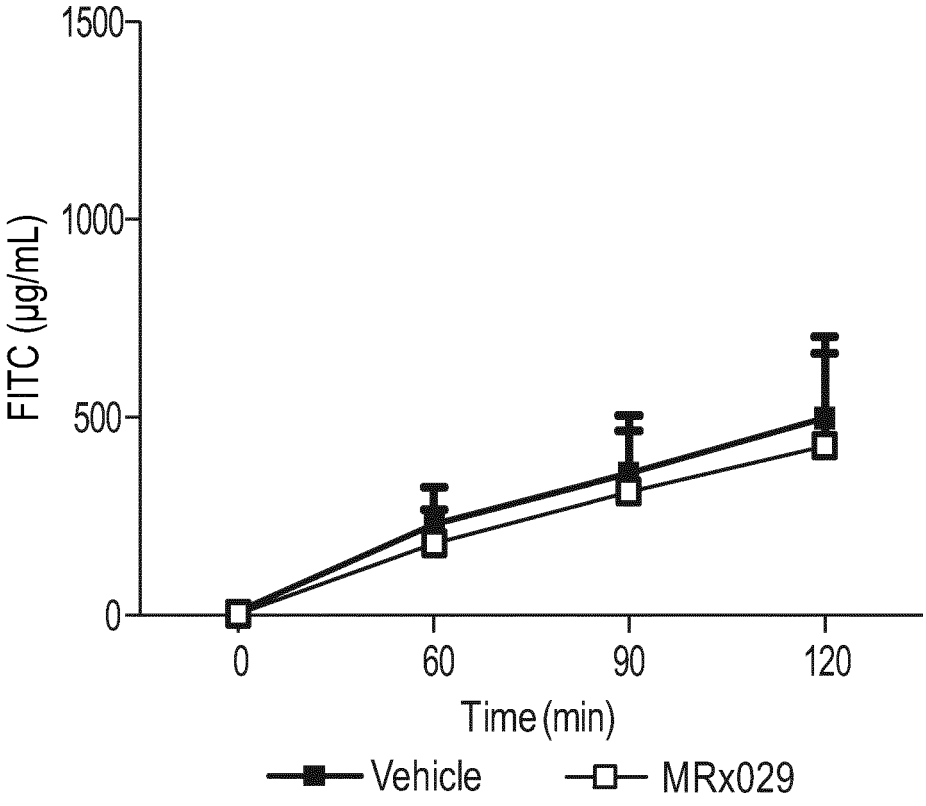
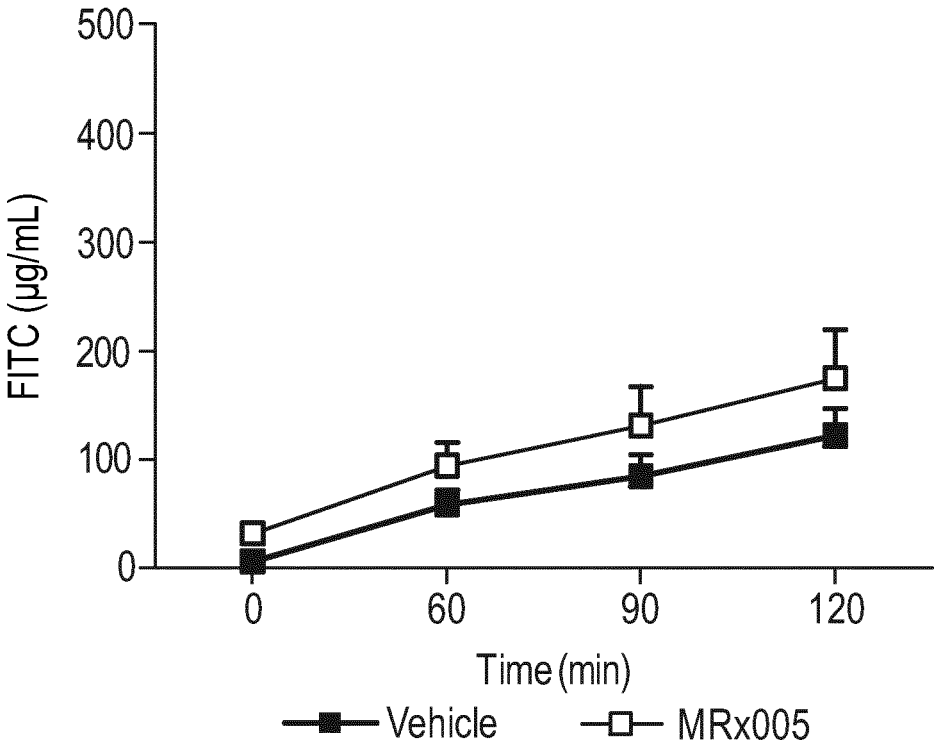
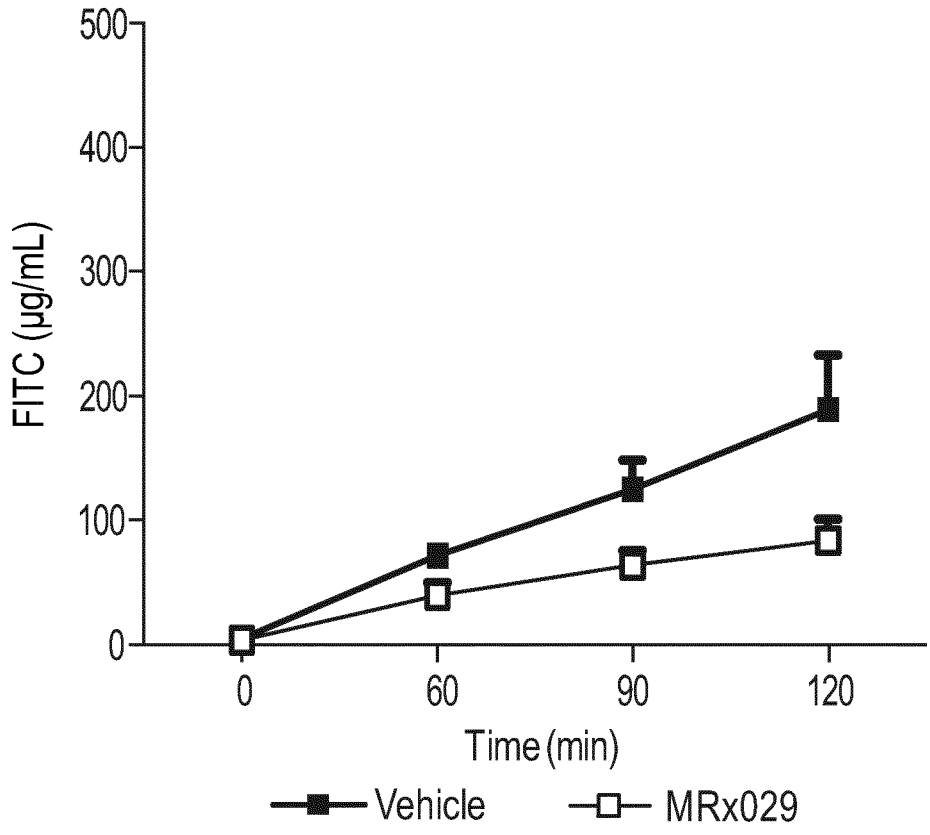


FIG. 14F Permeability in the colon





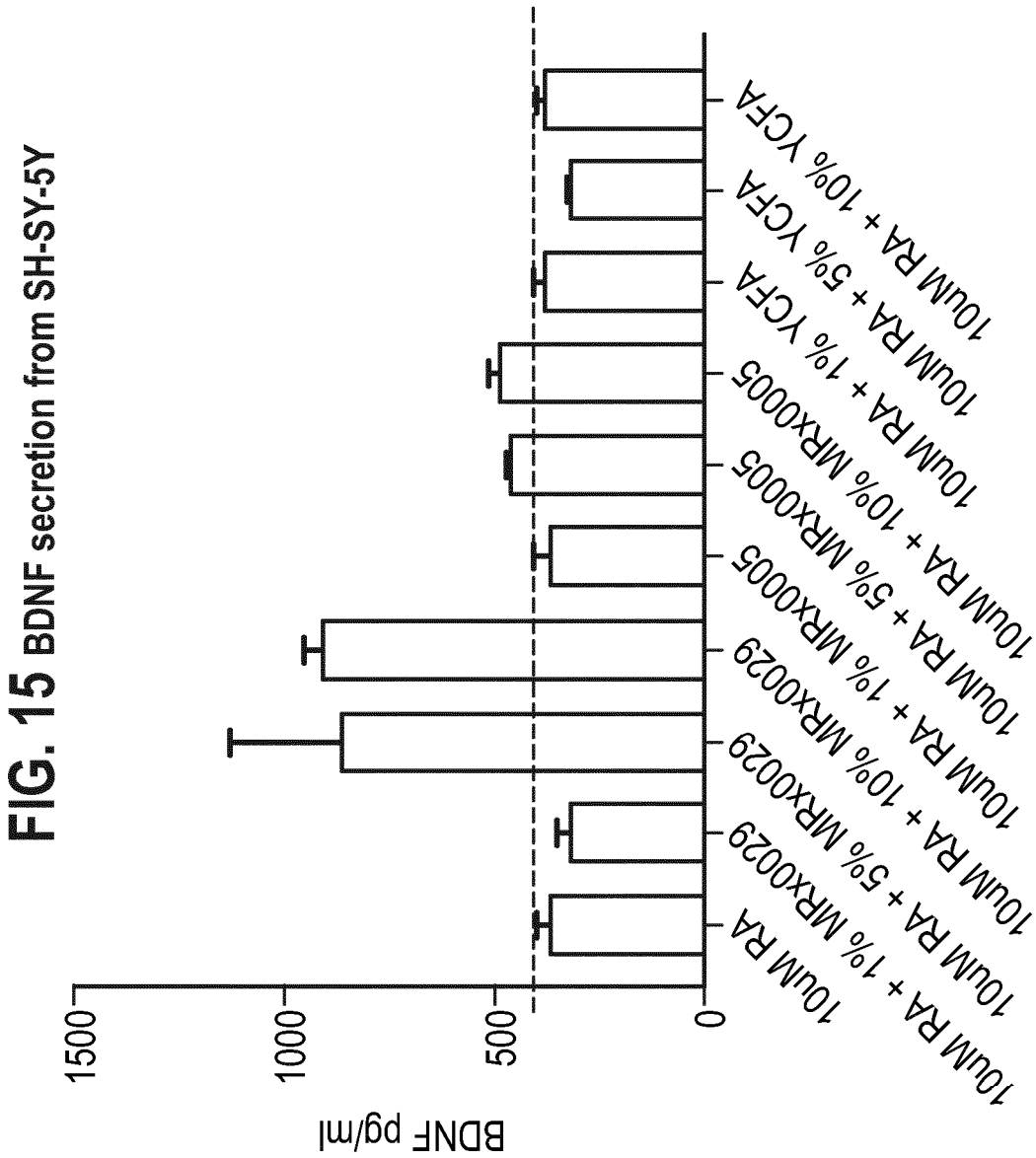


FIG. 15 BDNF secretion from SH-SY-5Y
BDNF pg/ml
1500
1000
500
0
10UM RA
10UM RA + 1% MRX029
10UM RA + 5% MRX029
10UM RA + 10% MRX029
10UM RA + 1% MRX005
10UM RA + 5% MRX005
10UM RA + 10% MRX005
10UM RA + 1% YCFA
10UM RA + 5% YCFA
10UM RA + 10% YCFA

FIG. 16A Total ROS production

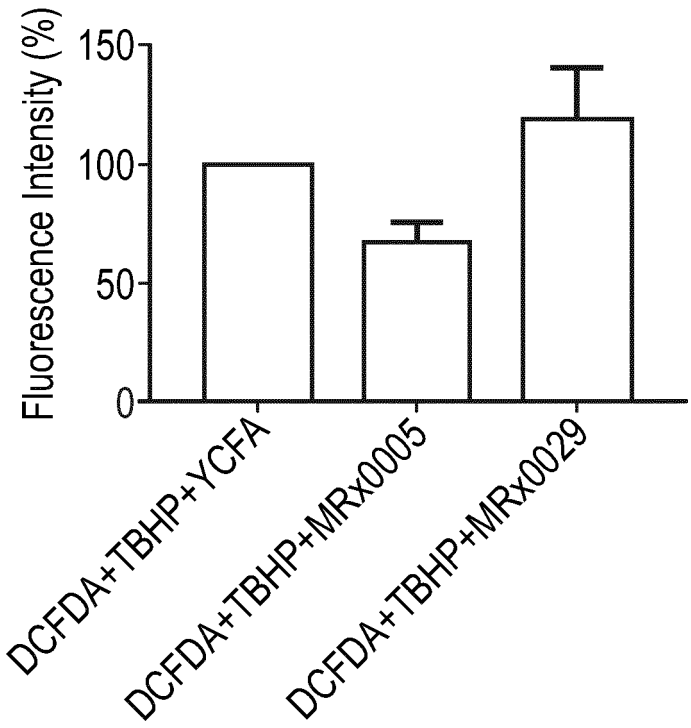
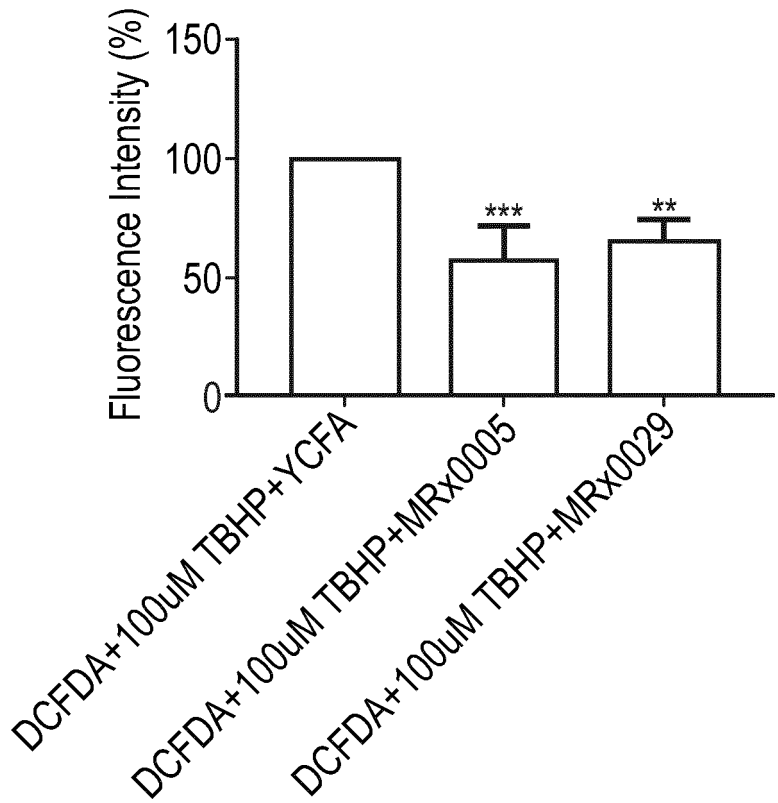


FIG. 16B Total ROS production



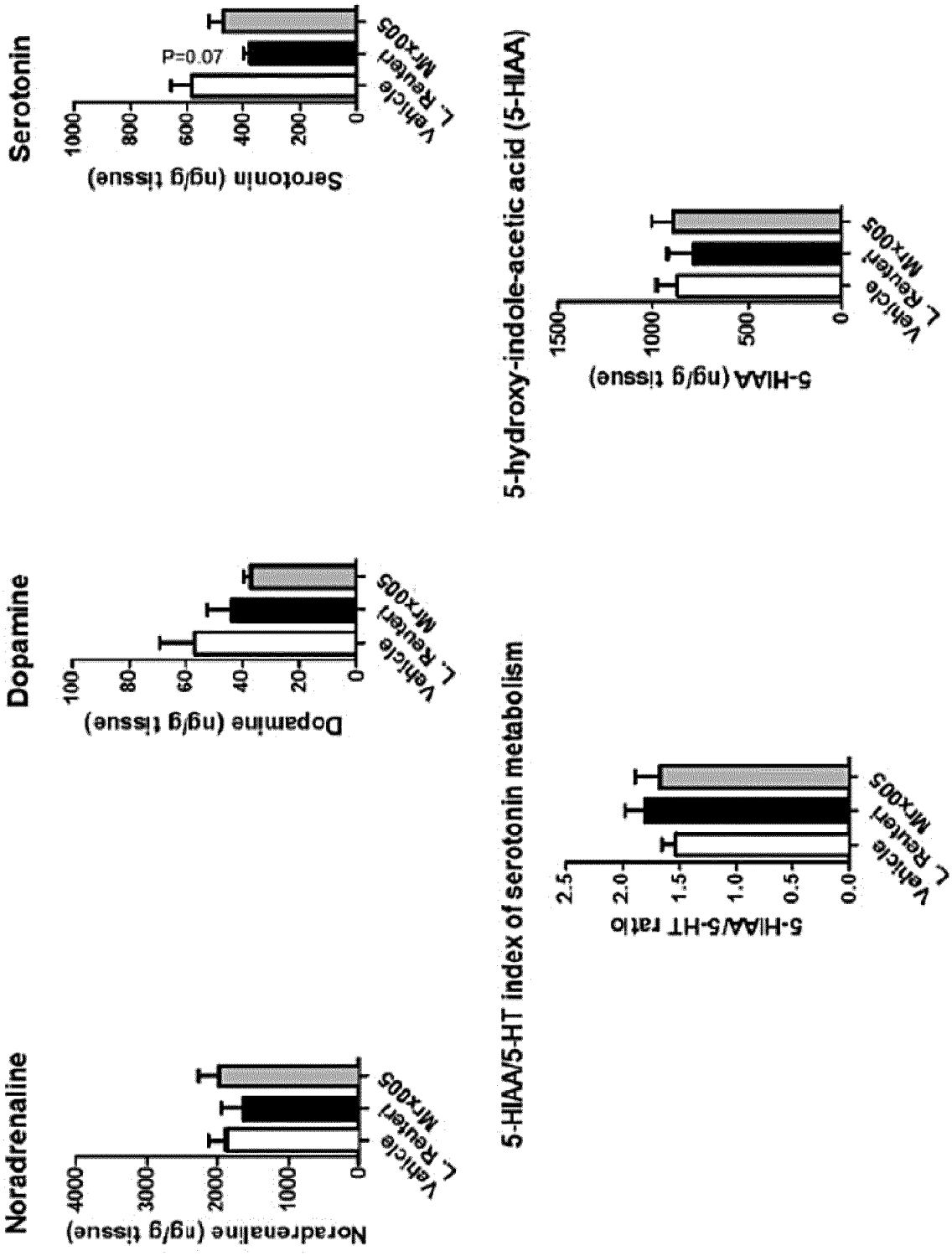
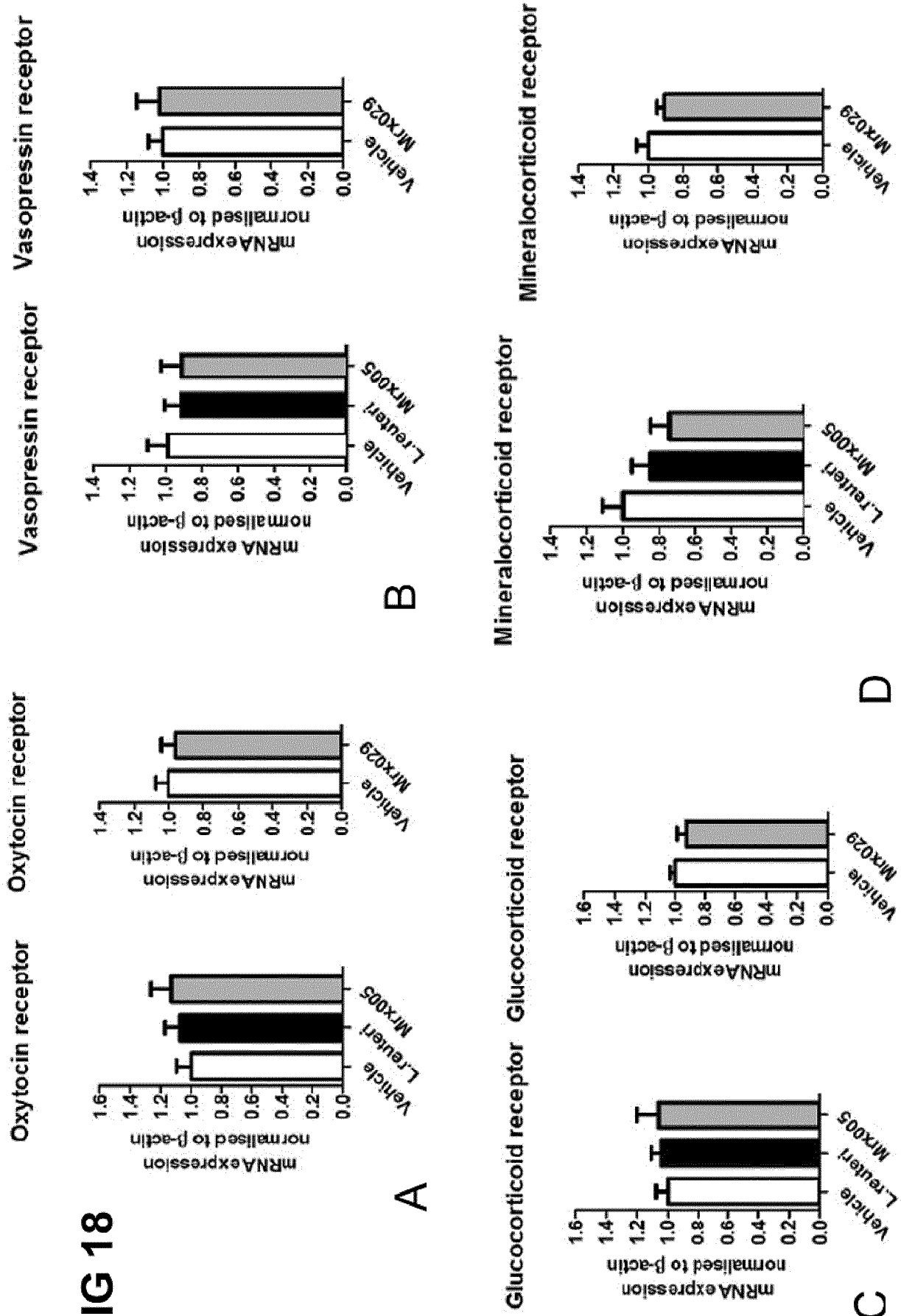

Noradrenaline
Noradrenaline (ng/g tissue)
4000
3000
2000
1000
0
Vehicle
L. Reuteri
Mrx005
Dopamine
Dopamine (ng/g tissue)
100
80
60
40
20
0
Vehicle
L. Reuteri
Mrx005
Serotonin
Serotonin (ng/g tissue)
1000
800
600
400
200
0
P=0.07
Vehicle
L. Reuteri
Mrx005
5-HIAA/5-HT index of serotonin metabolism
5-HIAA/5-HT ratio
2.5
2.0
1.5
1.0
0.5
0.0
Vehicle
L. Reuteri
Mrx005
5-hydroxy-indole-acetic acid (5-HIAA)
5-HIAA (ng/g tissue)
1500
1000
500
0
Vehicle
L. Reuteri
Mrx005

FIG 17

FIG 18


Oxytocin receptor
Oxytocin receptor
Vasopressin receptor
Vasopressin receptor
Glucocorticoid receptor
Glucocorticoid receptor
Mineralocorticoid receptor
Mineralocorticoid receptor
mRNA expression normalised to β-actin
Vehicle
L.reuteri
MRx005
MRx029
A
B
C
D

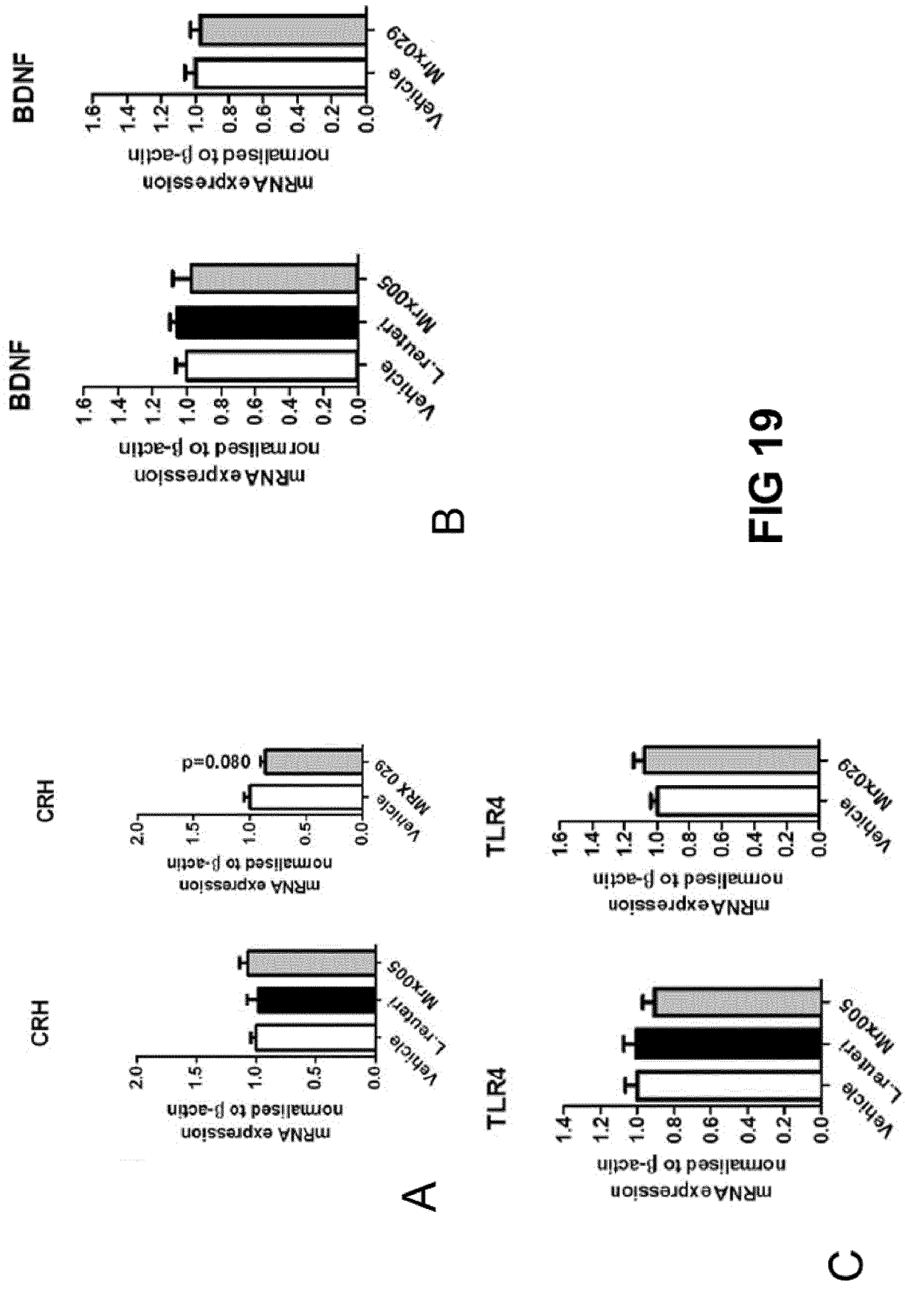

A
CRH
mRNA expression normalised to β-actin
2.0
1.5
1.0
0.5
0.0
Vehicle
L.reuteri
Mrx005
CRH
mRNA expression normalised to β-actin
p=0.080
Vehicle
MRX 029
B
BDNF
mRNA expression normalised to β-actin
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Vehicle
L.reuteri
Mrx005
BDNF
mRNA expression normalised to β-actin
Vehicle
Mrx029
C
TLR4
mRNA expression normalised to β-actin
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Vehicle
L.reuteri
Mrx005
TLR4
mRNA expression normalised to β-actin
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Vehicle
Mrx029

FIG 19

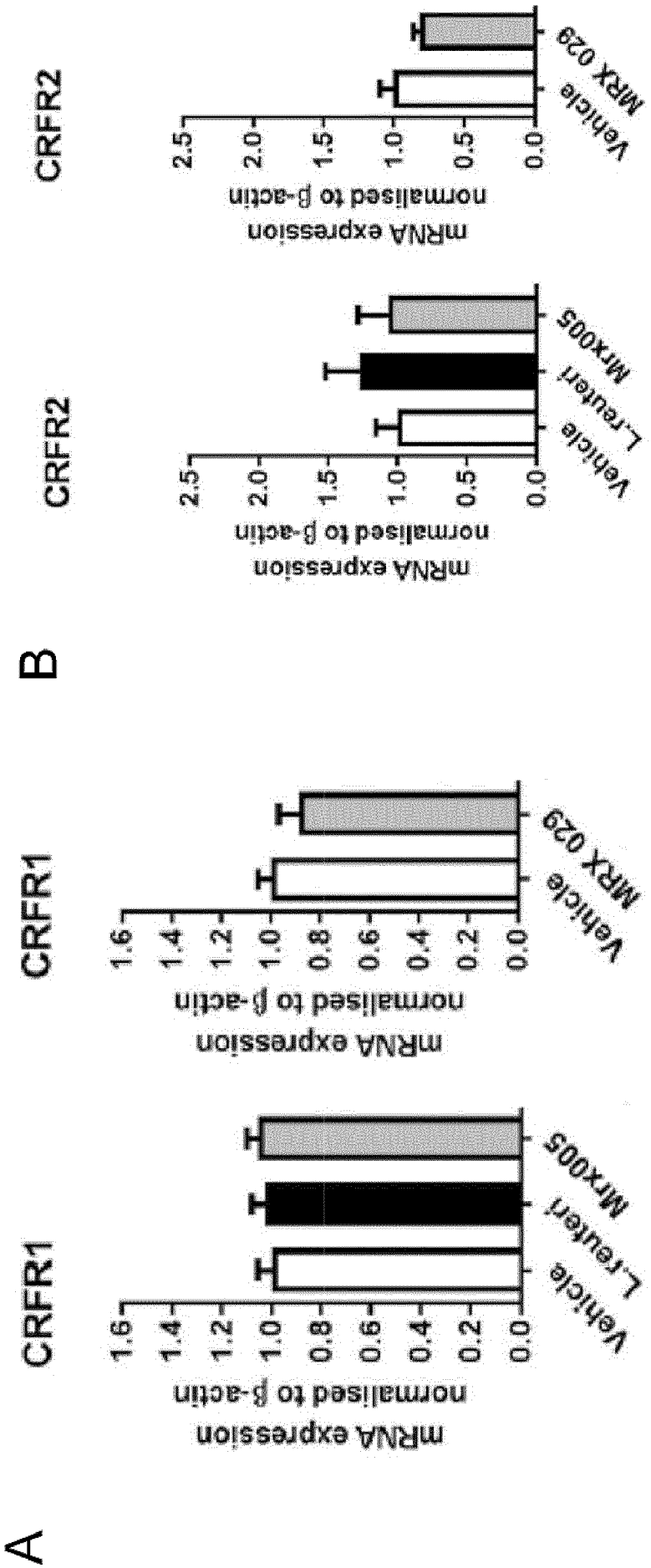

A
CRFR1
mRNA expression
normalised to β-actin
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Vehicle
L_feuter
Mrx005
CRFR1
mRNA expression
normalised to β-actin
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Vehicle
MRX 029
B
CRFR2
mRNA expression
normalised to β-actin
2.5
2.0
1.5
1.0
0.5
0.0
Vehicle
L_feuter
Mrx005
CRFR2
mRNA expression
normalised to β-actin
2.5
2.0
1.5
1.0
0.5
0.0
Vehicle
MRX 029

FIG 20

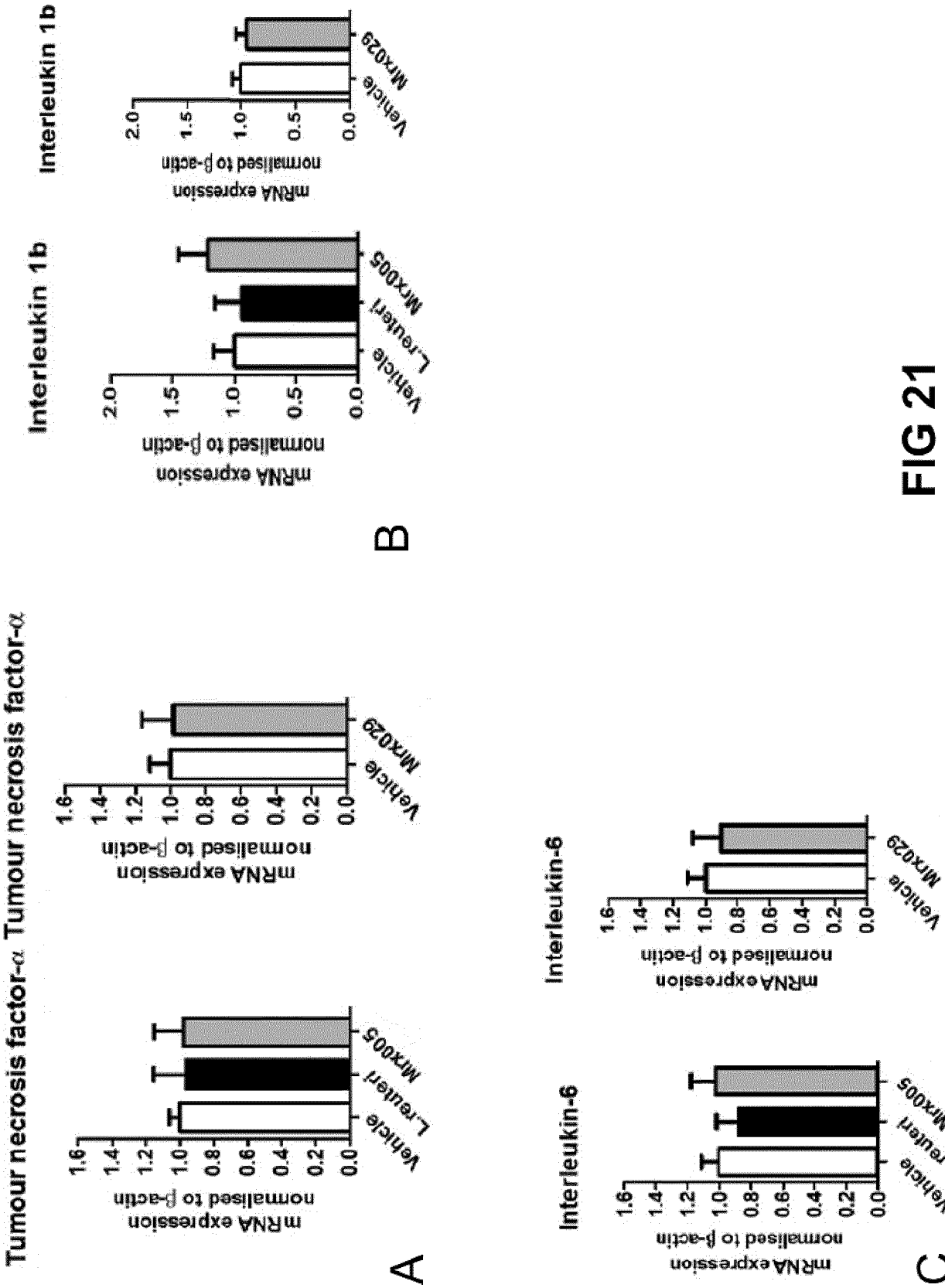

Tumour necrosis factor-α
mRNA expression normalised to β-actin
Vehicle
Mrx029
Mrx005
L-reuteri
A
Interleukin 1b
B
Interleukin-6
C

FIG 21

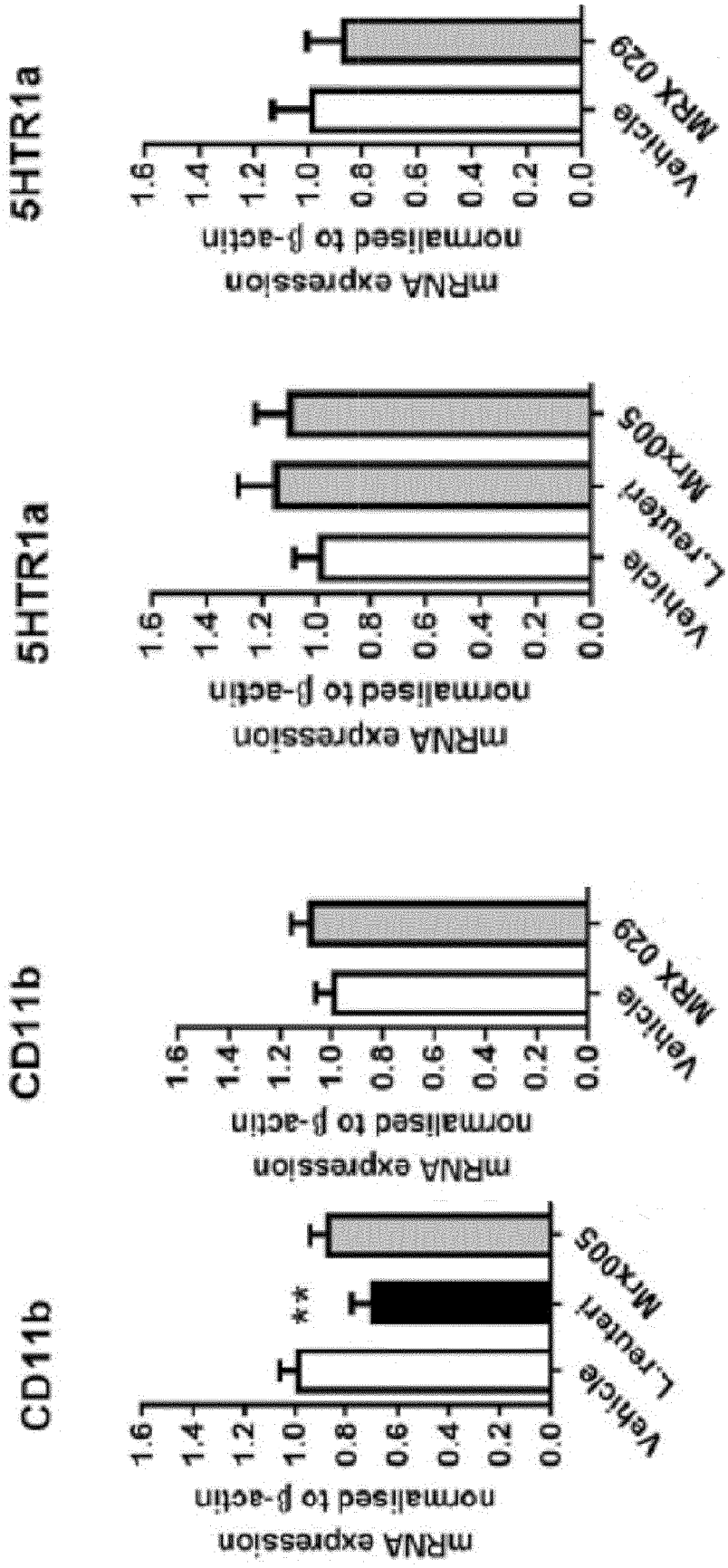

5HTR1a
mRNA expression
normalised to β-actin
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Vehicle
MRX 029
B
5HTR1a
mRNA expression
normalised to β-actin
Vehicle
L-reuteri
MRX005
CD11b
mRNA expression
normalised to β-actin
Vehicle
MRX 029
A
CD11b
mRNA expression
normalised to β-actin
**
Vehicle
L-reuteri
MRX005

FIG 22

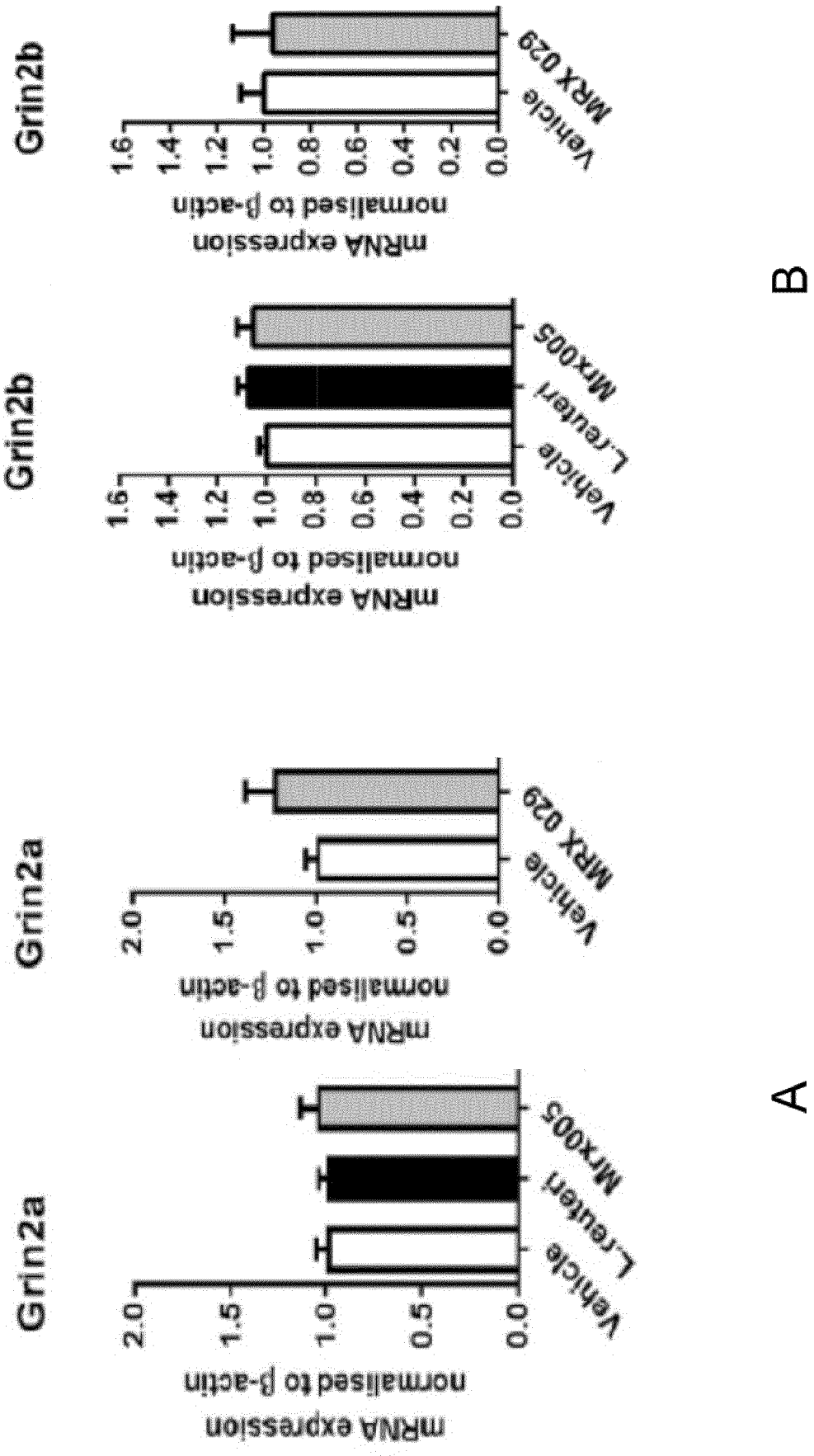

A
B
Grin2a
Grin2b
mRNA expression
normalised to β-actin
2.0
1.5
1.0
0.5
0.0
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Vehicle
L-reuteri
Mrx005
MRX 029

FIG 23

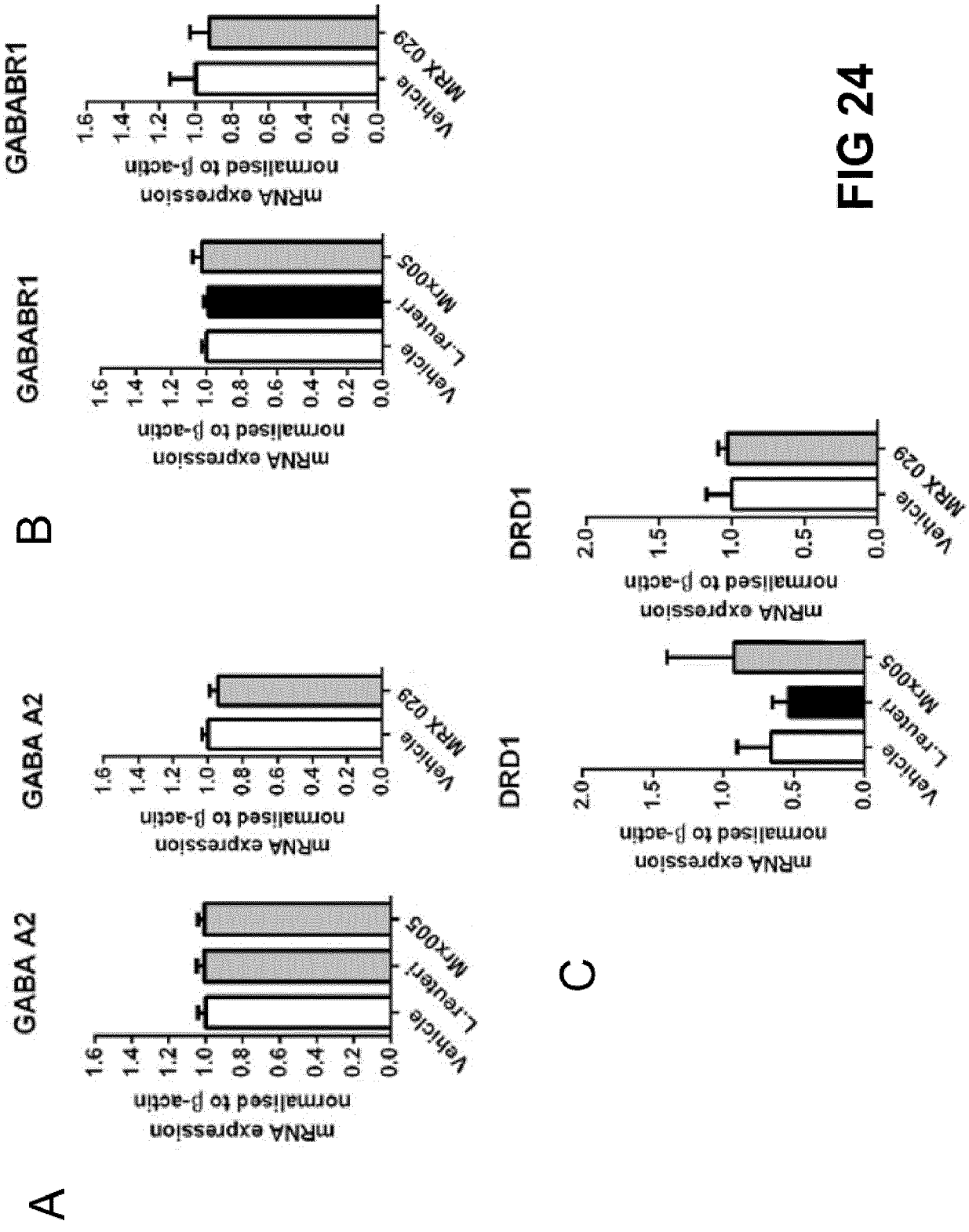

A
GABA A2
mRNA expression normalised to β-actin
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Vehicle
L-reuteri
MRX005
GABA A2
mRNA expression normalised to β-actin
Vehicle
MRX 029
B
GABABR1
mRNA expression normalised to β-actin
Vehicle
L-reuteri
MRX005
GABABR1
mRNA expression normalised to β-actin
Vehicle
MRX 029
C
DRD1
mRNA expression normalised to β-actin
2.0
1.5
1.0
0.5
0.0
Vehicle
L-reuteri
MRX005
DRD1
mRNA expression normalised to β-actin
Vehicle
MRX 029

FIG 24

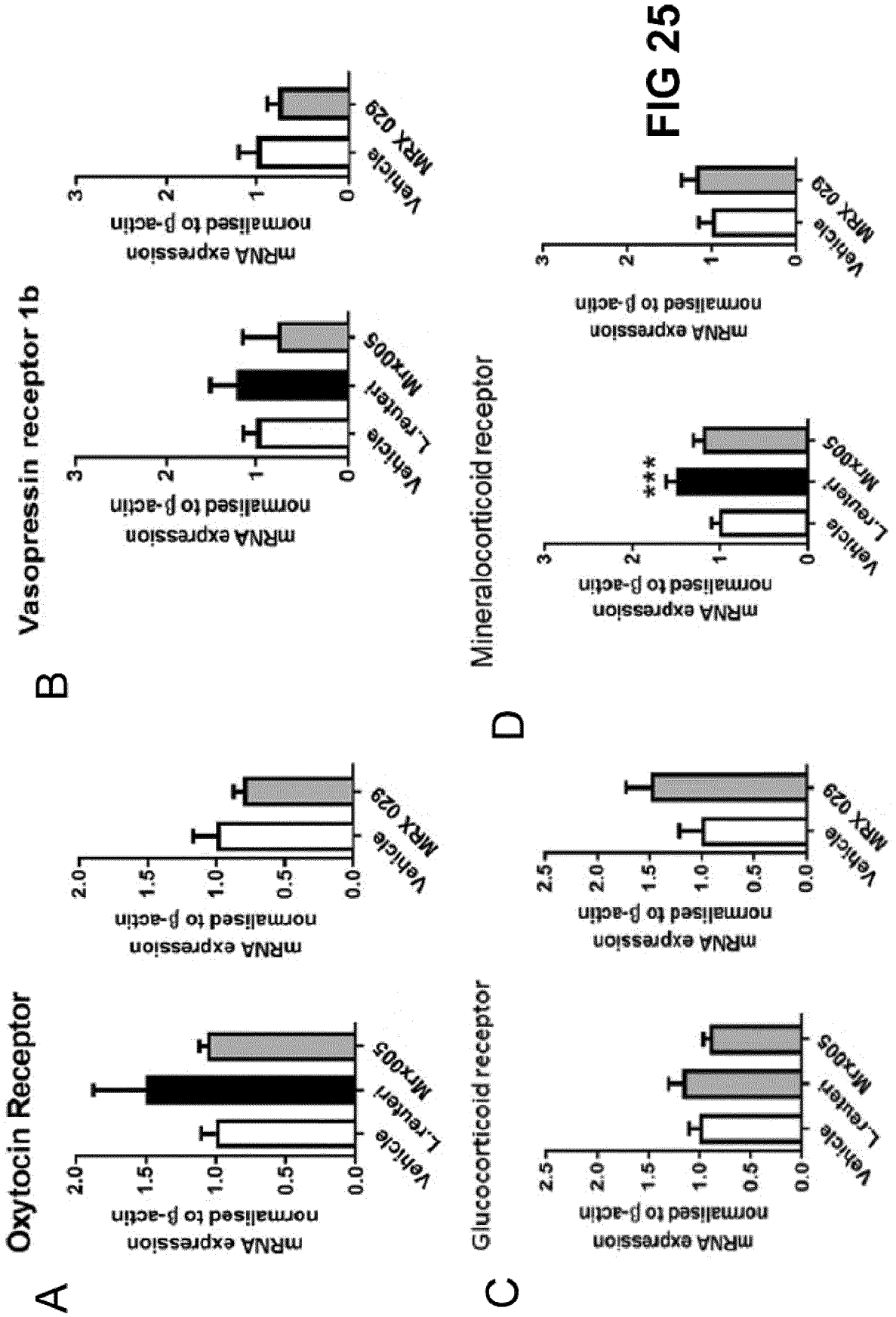
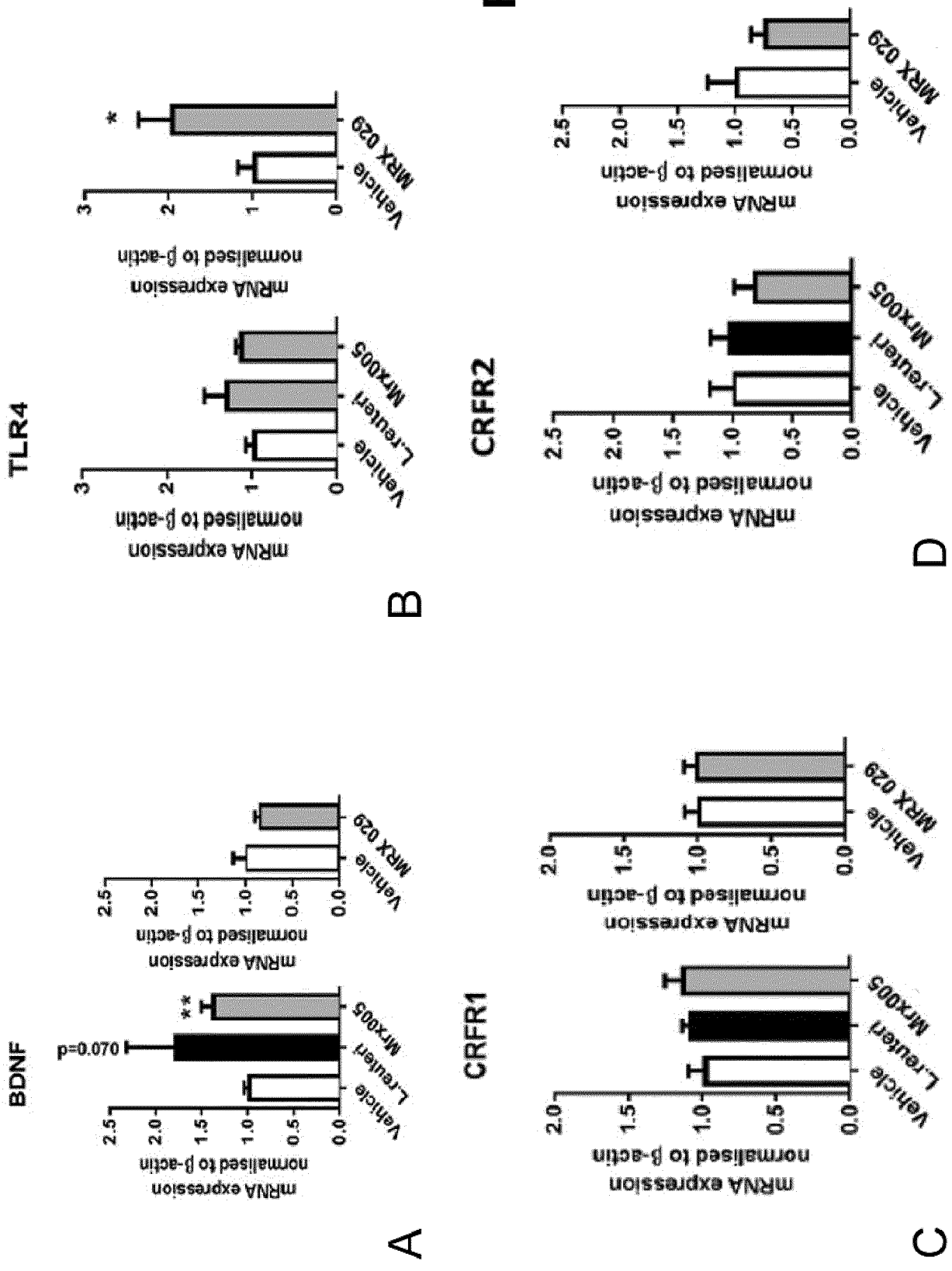

A
Oxytocin Receptor
mRNA expression
normalised to β-actin
2.0
1.5
1.0
0.5
0.0
Vehicle
L.reuteri
Mrx005
B
Vasopressin receptor 1b
mRNA expression
normalised to β-actin
3
2
1
0
Vehicle
L.reuteri
Mrx005
C
Glucocorticoid receptor
mRNA expression
normalised to β-actin
2.5
2.0
1.5
1.0
0.5
0.0
Vehicle
L.reuteri
Mrx005
D
Mineralocorticoid receptor
mRNA expression
normalised to β-actin
***
3
2
1
0
Vehicle
L.reuteri
Mrx005

FIG 25

FIG 26


A
BDNF
mRNA expression
normalised to β-actin
Vehicle
L-reuteri
MrX005
MRX 029
p=0.070
**
B
TLR4
mRNA expression
normalised to β-actin
Vehicle
L-reuteri
MrX005
MRX 029
*
C
CRFR1
mRNA expression
normalised to β-actin
Vehicle
L-reuteri
MrX005
MRX 029
D
CRFR2
mRNA expression
normalised to β-actin
Vehicle
L-reuteri
MrX005
MRX 029

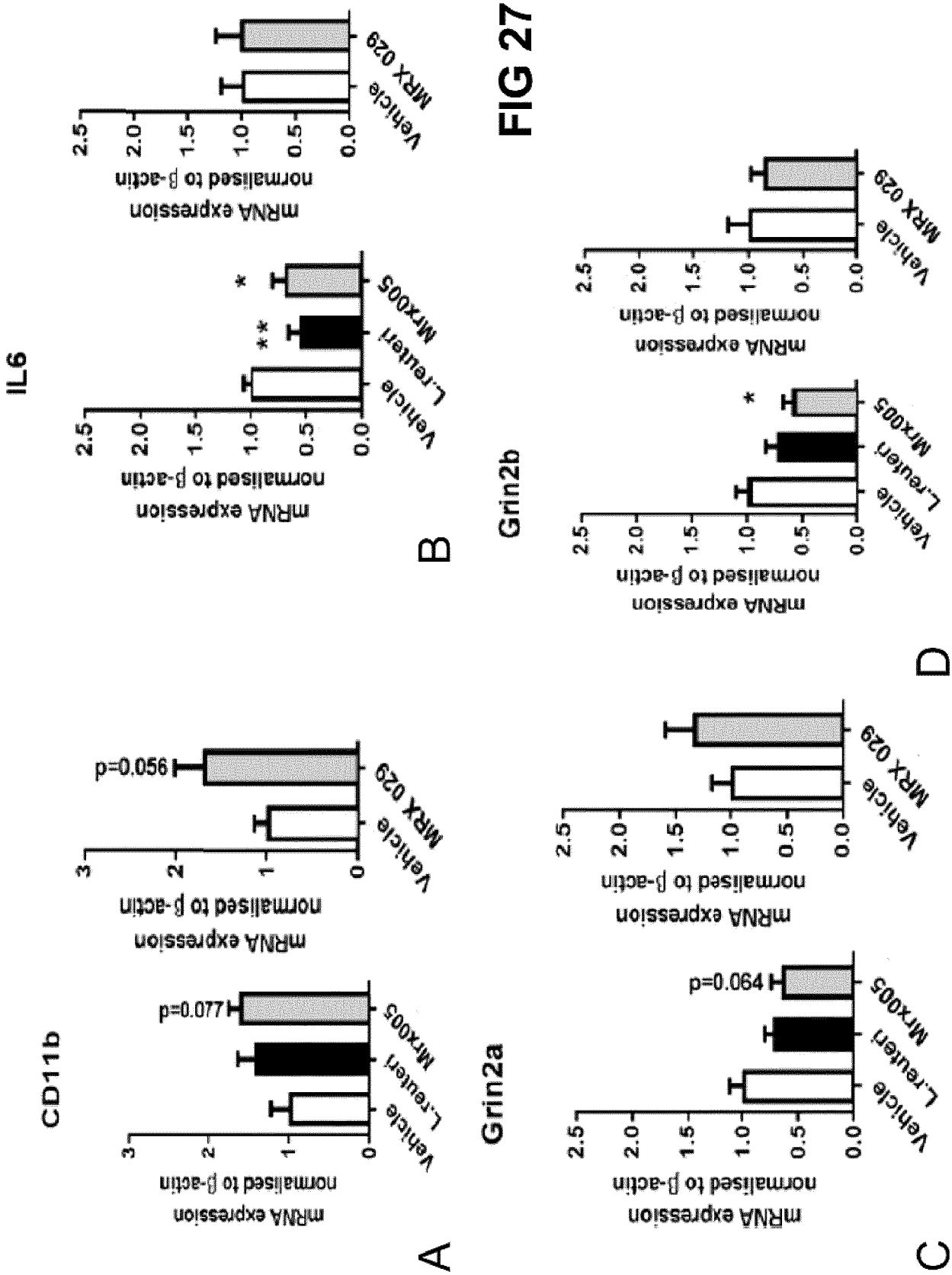
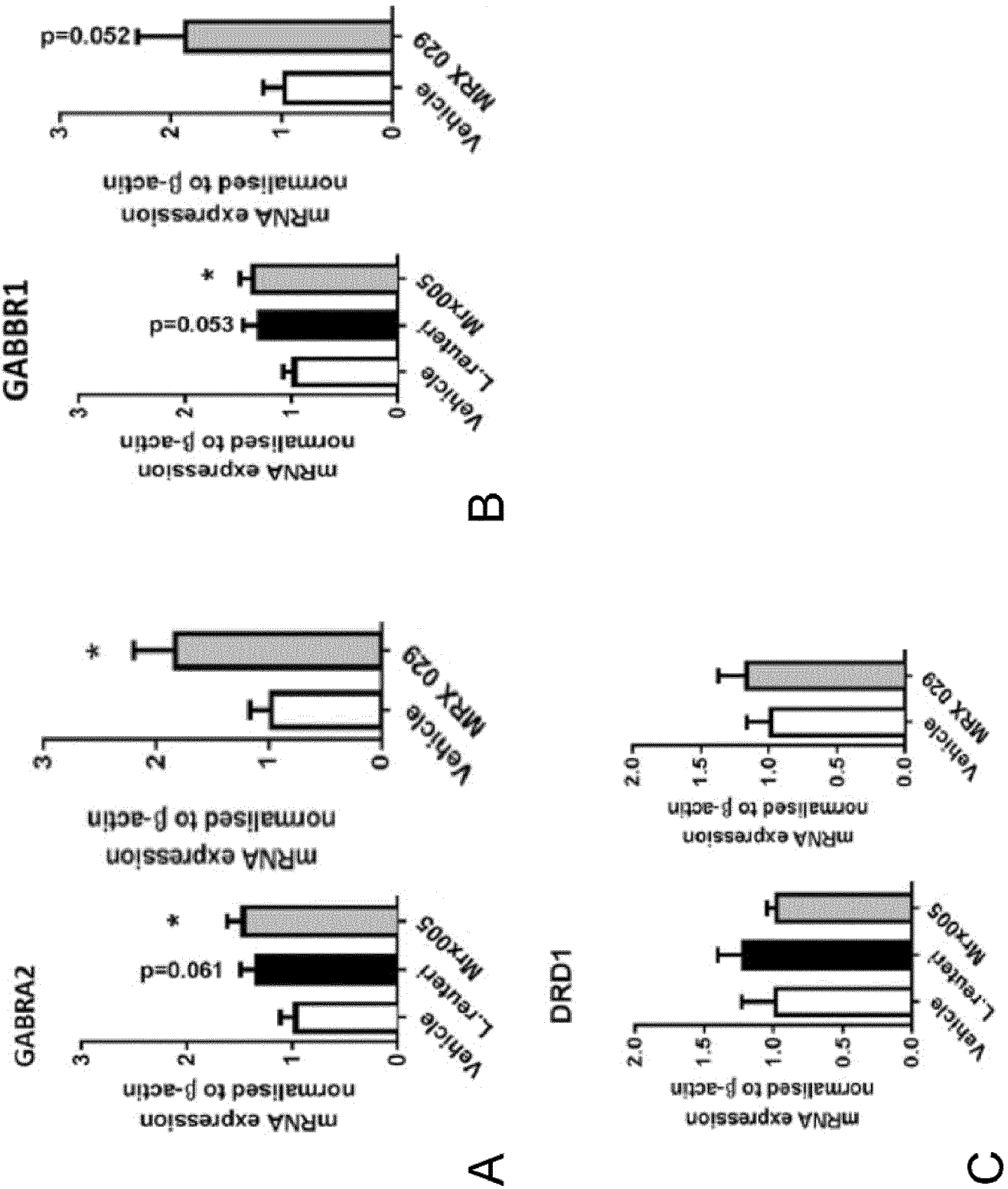

A
CD11b
mRNA expression
normalised to β-actin
Vehicle
L.reuteri
MRx005
p=0.077
B
IL6
mRNA expression
normalised to β-actin
Vehicle
L.reuteri
MRx005
*
**
C
Grin2a
mRNA expression
normalised to β-actin
Vehicle
L.reuteri
MRx005
p=0.064
D
Grin2b
mRNA expression
normalised to β-actin
Vehicle
L.reuteri
MRx005
*
Vehicle
MRX 029
p=0.056

FIG 27

FIG 28


A
GABRA2
mRNA expression
normalised to β-actin
Vehicle
MRX 029
L-reuteri
MRX005
p=0.061
*
B
GABBR1
mRNA expression
normalised to β-actin
Vehicle
MRX 029
L-reuteri
MRX005
p=0.053
p=0.052
*
C
DRD1
mRNA expression
normalised to β-actin
Vehicle
MRX 029
L-reuteri
MRX005

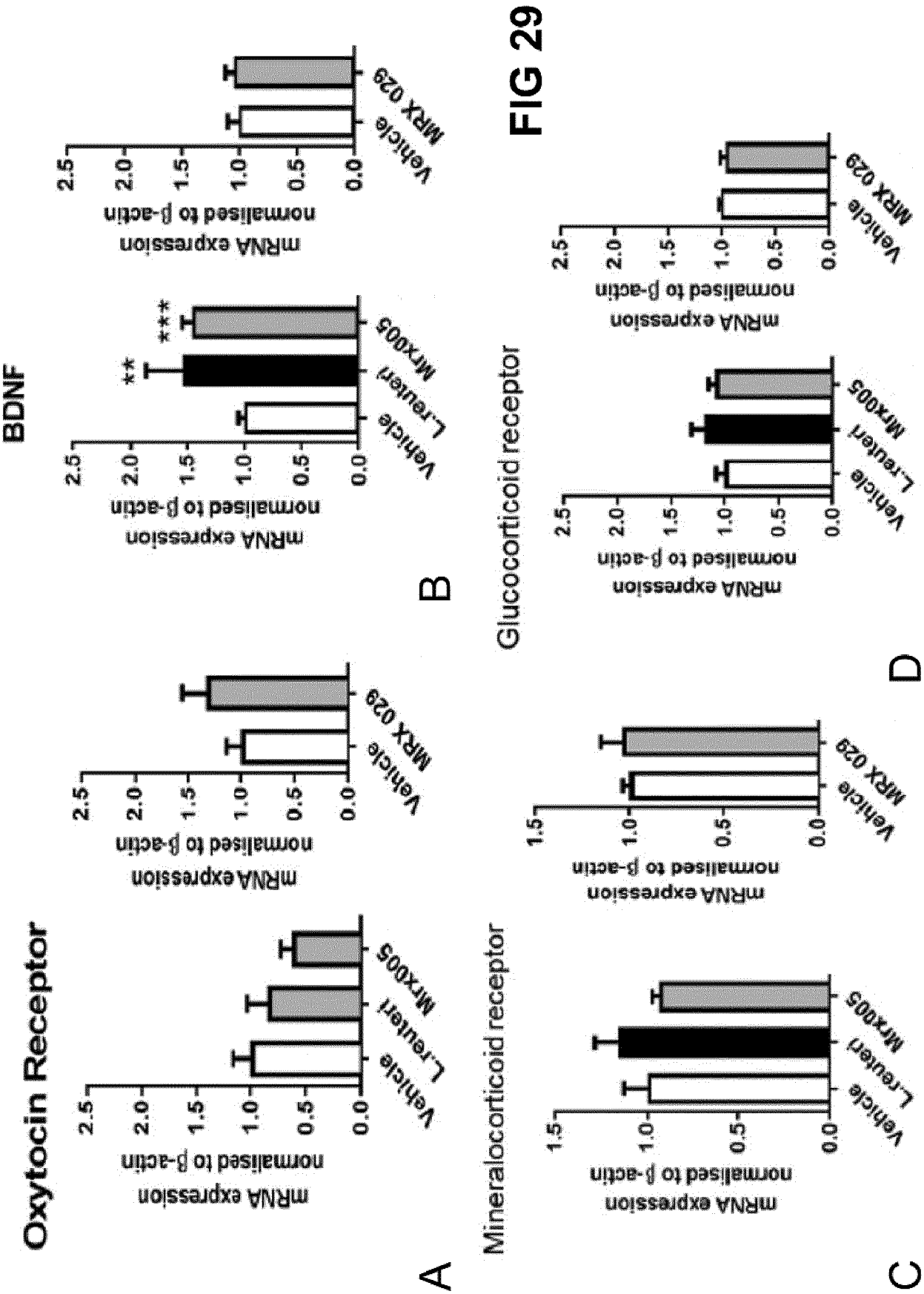

A
Oxytocin Receptor
mRNA expression
normalised to β-actin
2.5
2.0
1.5
1.0
0.5
0.0
Vehicle
L_reuteri
MrX005
B
BDNF
mRNA expression
normalised to β-actin
**
***
Vehicle
L_reuteri
MrX005
mRNA expression
normalised to β-actin
Vehicle
MRX 029
C
Mineralocorticoid receptor
mRNA expression
normalised to β-actin
1.5
1.0
0.5
0.0
Vehicle
L_reuteri
MrX005
mRNA expression
normalised to β-actin
Vehicle
MRX 029
D
Glucocorticoid receptor
mRNA expression
normalised to β-actin
Vehicle
L_reuteri
MrX005
mRNA expression
normalised to β-actin
Vehicle
MRX 029

FIG 29

FIG 30

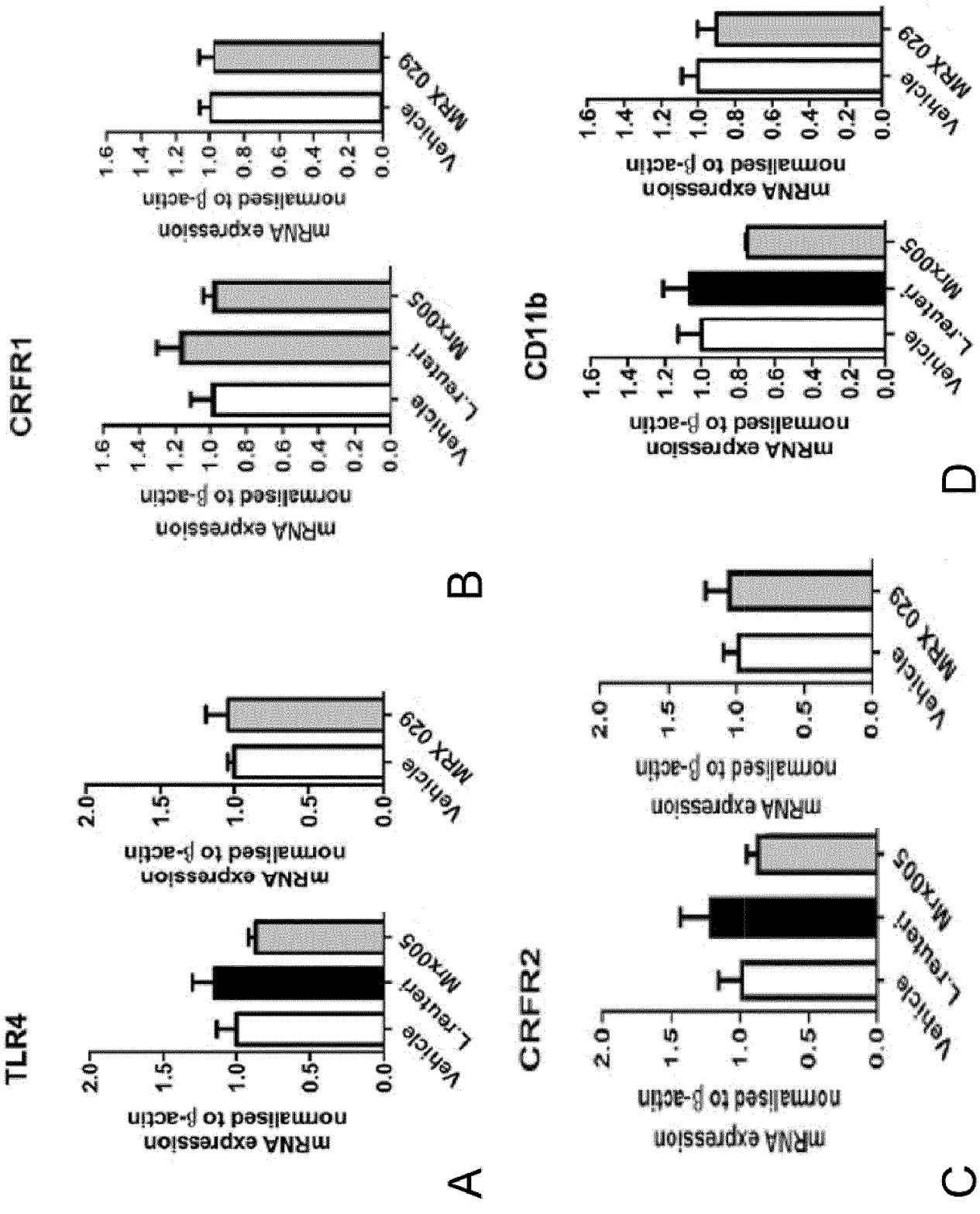

TLR4
CRFR1
CD11b
CRFR2
A
B
C
D
mRNA expression normalised to β-actin
Vehicle
L.reuteri
MrX005
MRX 029
0.0
0.5
1.0
1.5
2.0
0.2
0.4
0.6
0.8
1.2
1.4
1.6

FIG 31

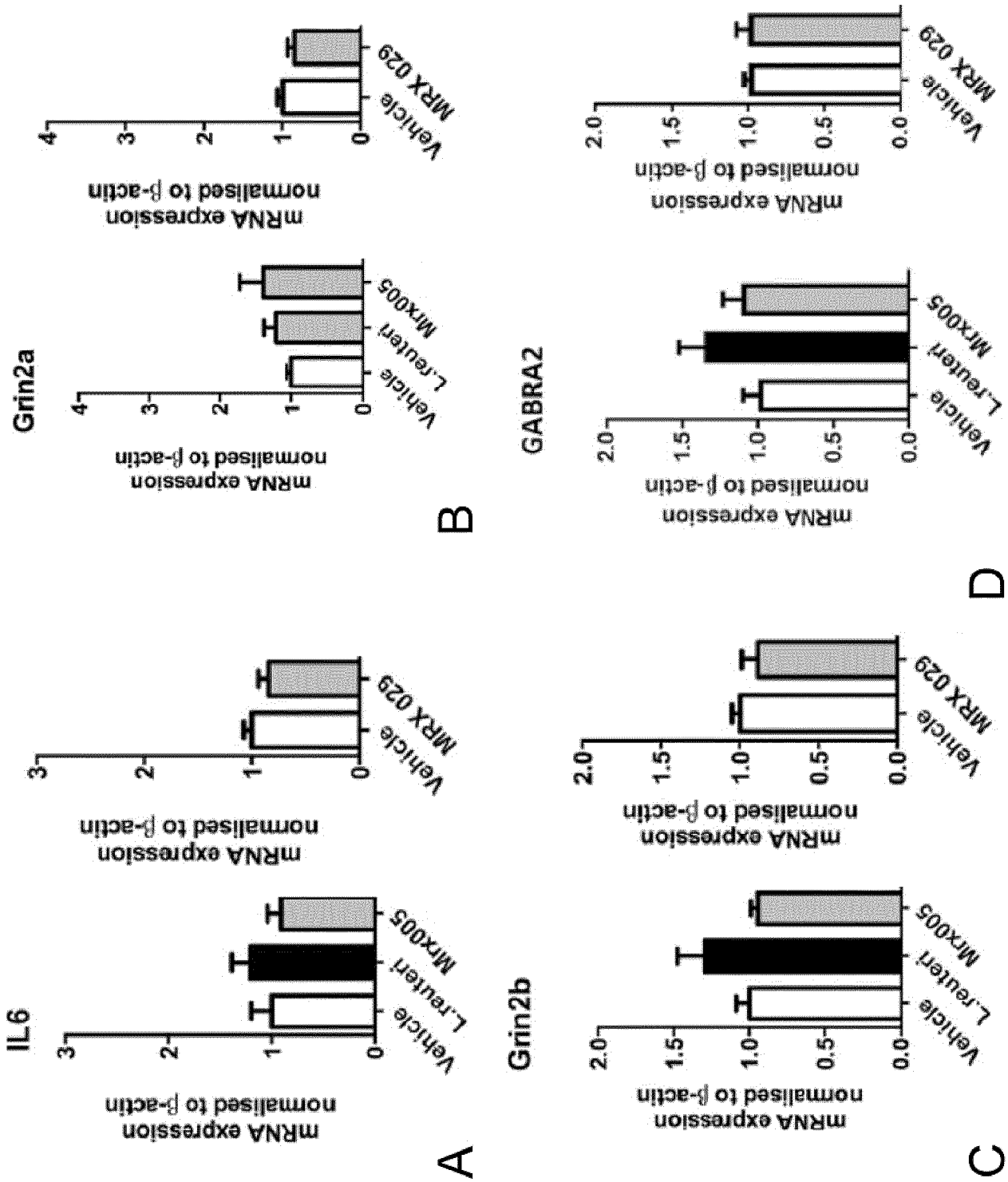

A
IL6
mRNA expression
normalised to β-actin
Vehicle
L.reuteri
MrX005
MRX 029
B
Grin2a
mRNA expression
normalised to β-actin
Vehicle
L.reuteri
MrX005
MRX 029
C
Grin2b
mRNA expression
normalised to β-actin
Vehicle
L.reuteri
MrX005
MRX 029
D
GABRA2
mRNA expression
normalised to β-actin
Vehicle
L.reuteri
MrX005
MRX 029

FIG 32

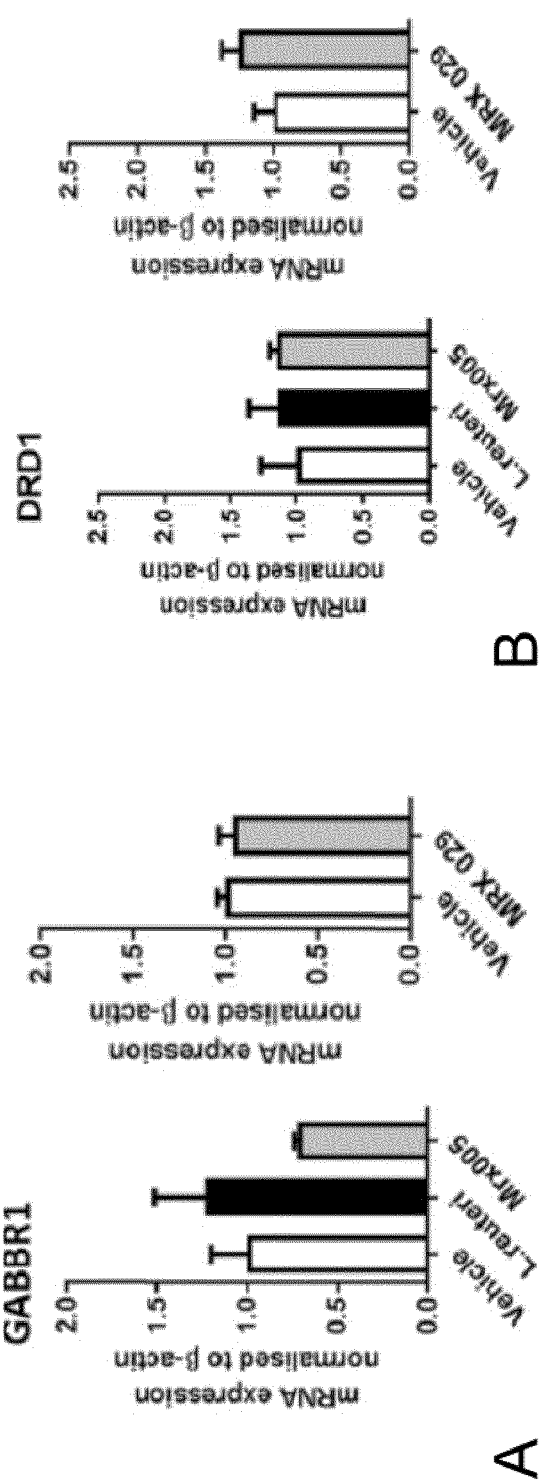

A
GABBR1
mRNA expression
normalised to β-actin
2.0
1.5
1.0
0.5
0.0
Vehicle
L.reuteri
MRx005
mRNA expression
normalised to β-actin
2.0
1.5
1.0
0.5
0.0
Vehicle
MRX 029
B
DRD1
mRNA expression
normalised to β-actin
2.5
2.0
1.5
1.0
0.5
0.0
Vehicle
L.reuteri
MRx005
mRNA expression
normalised to β-actin
2.5
2.0
1.5
1.0
0.5
0.0
Vehicle
MRX 029

FIG 33

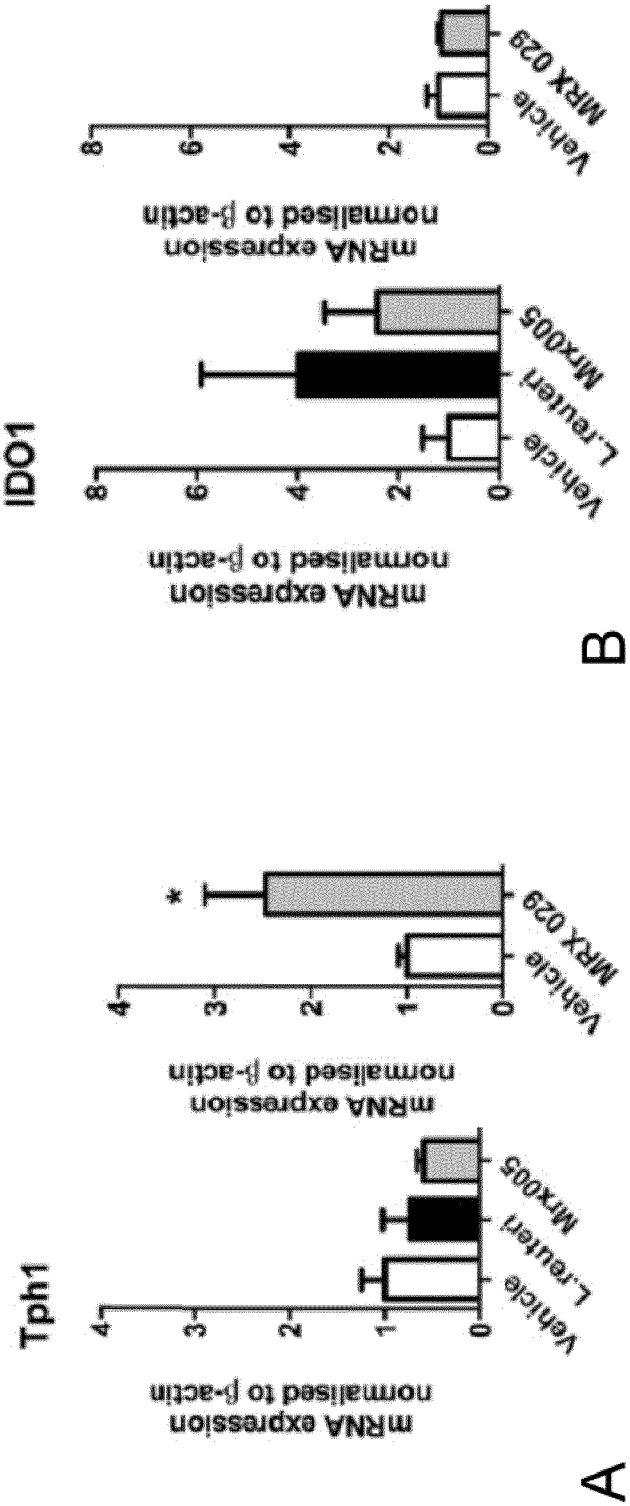
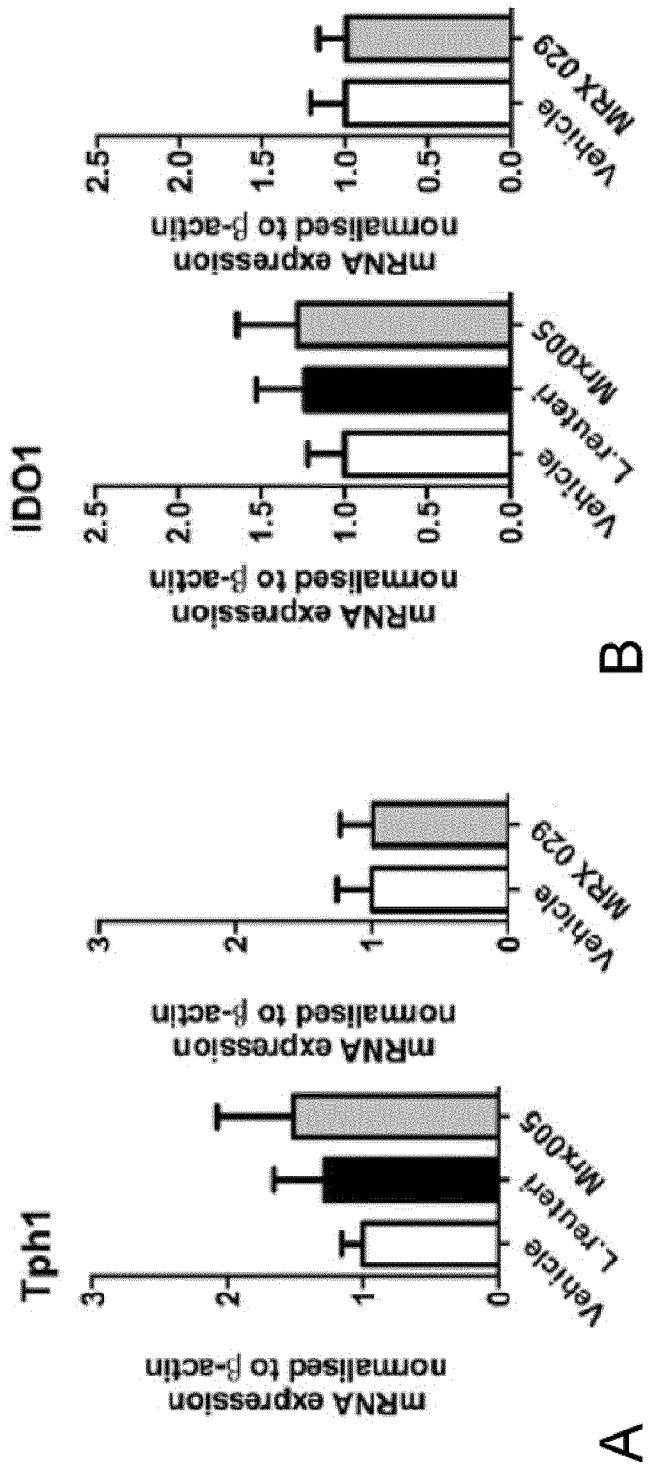

A
Tph1
mRNA expression
normalised to β-actin
4
3
2
1
0
Vehicle
L.reuteri
MRx005
mRNA expression
normalised to β-actin
4
3
2
1
0
*
Vehicle
MRX 029
B
IDO1
mRNA expression
normalised to β-actin
8
6
4
2
0
Vehicle
L.reuteri
MRx005
mRNA expression
normalised to β-actin
8
6
4
2
0
Vehicle
MRX 029

FIG 34


A
Tph1
mRNA expression
normalised to β-actin
3
2
1
0
Vehicle
L.reuteri
Mrx005
mRNA expression
normalised to β-actin
3
2
1
0
Vehicle
MRX 029
B
IDO1
mRNA expression
normalised to β-actin
2.5
2.0
1.5
1.0
0.5
0.0
Vehicle
L.reuteri
Mrx005
mRNA expression
normalised to β-actin
2.5
2.0
1.5
1.0
0.5
0.0
Vehicle
MRX 029

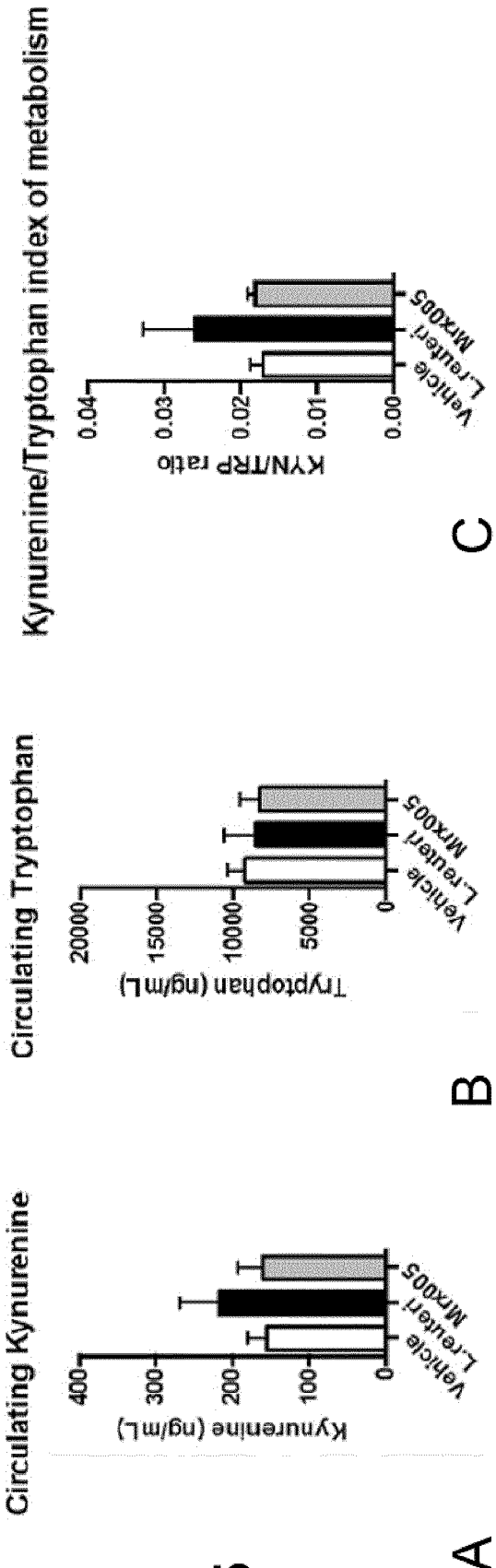

Circulating Kynurenine
Kynurenine (ng/mL)
400
300
200
100
0
Vehicle
L-reuteri
MRX005
A
Circulating Tryptophan
Tryptophan (ng/mL)
20000
15000
10000
5000
0
Vehicle
L.reuteri
MRX005
B
Kynurenine/Tryptophan index of metabolism
KYN/TRP ratio
0.04
0.03
0.02
0.01
0.00
Vehicle
L.reuteri
MRX005
C

FIG 35

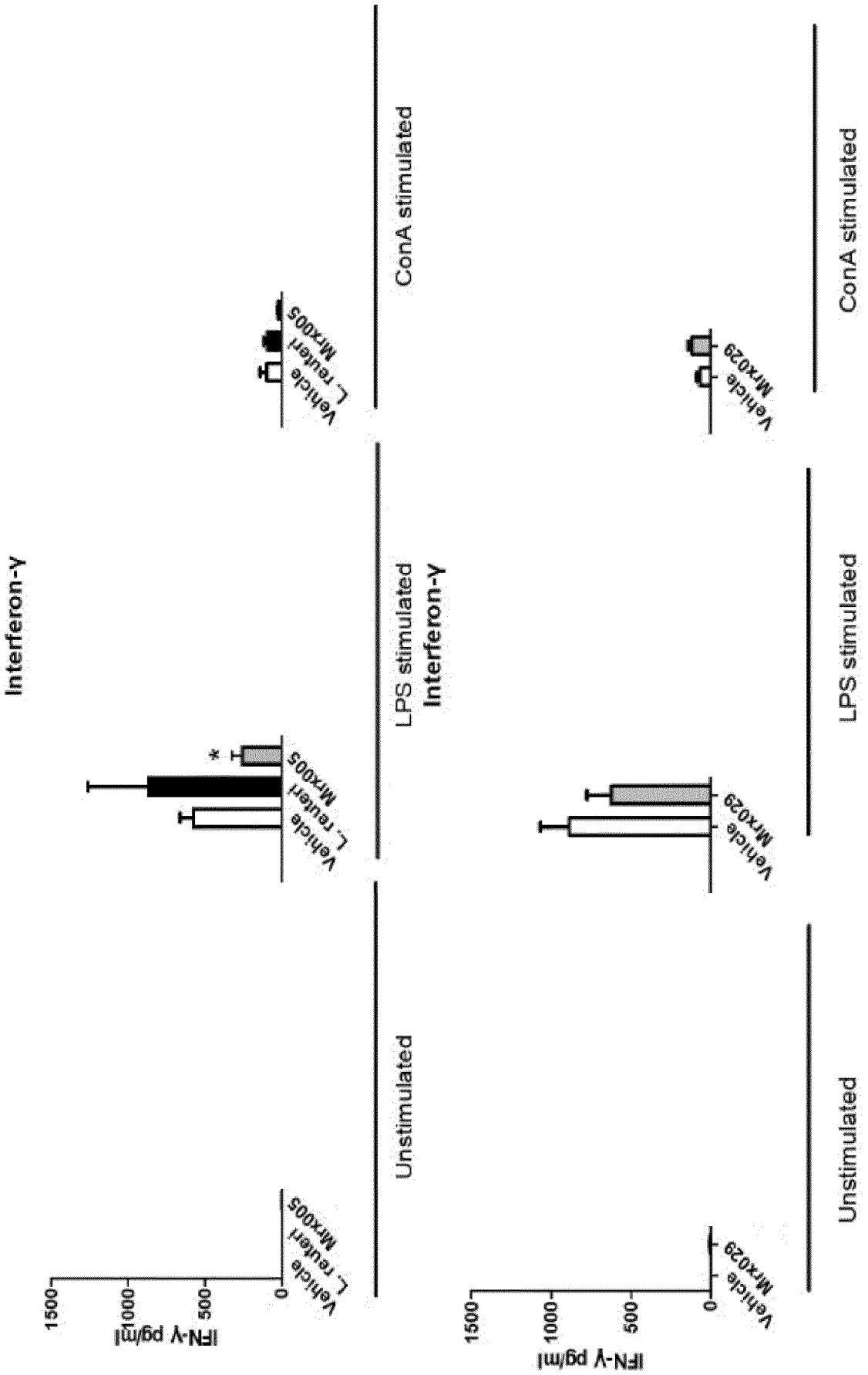

Interferon-γ
Unstimulated
LPS stimulated
ConA stimulated
IFN-γ pg/ml
1500
1000
500
0
Vehicle
L. reuteri Mrx005
Vehicle
Mrx029
*

FIG 36

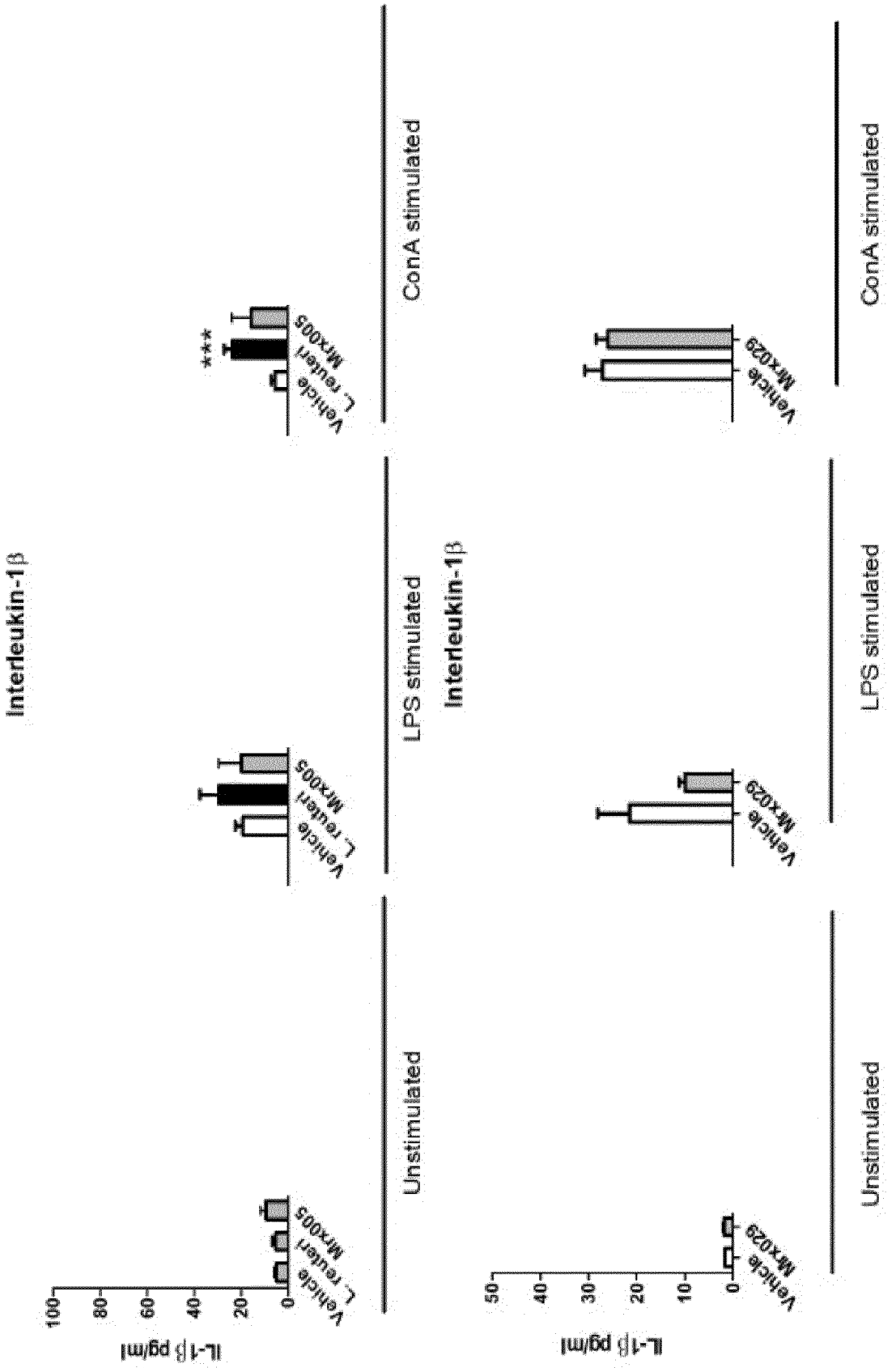
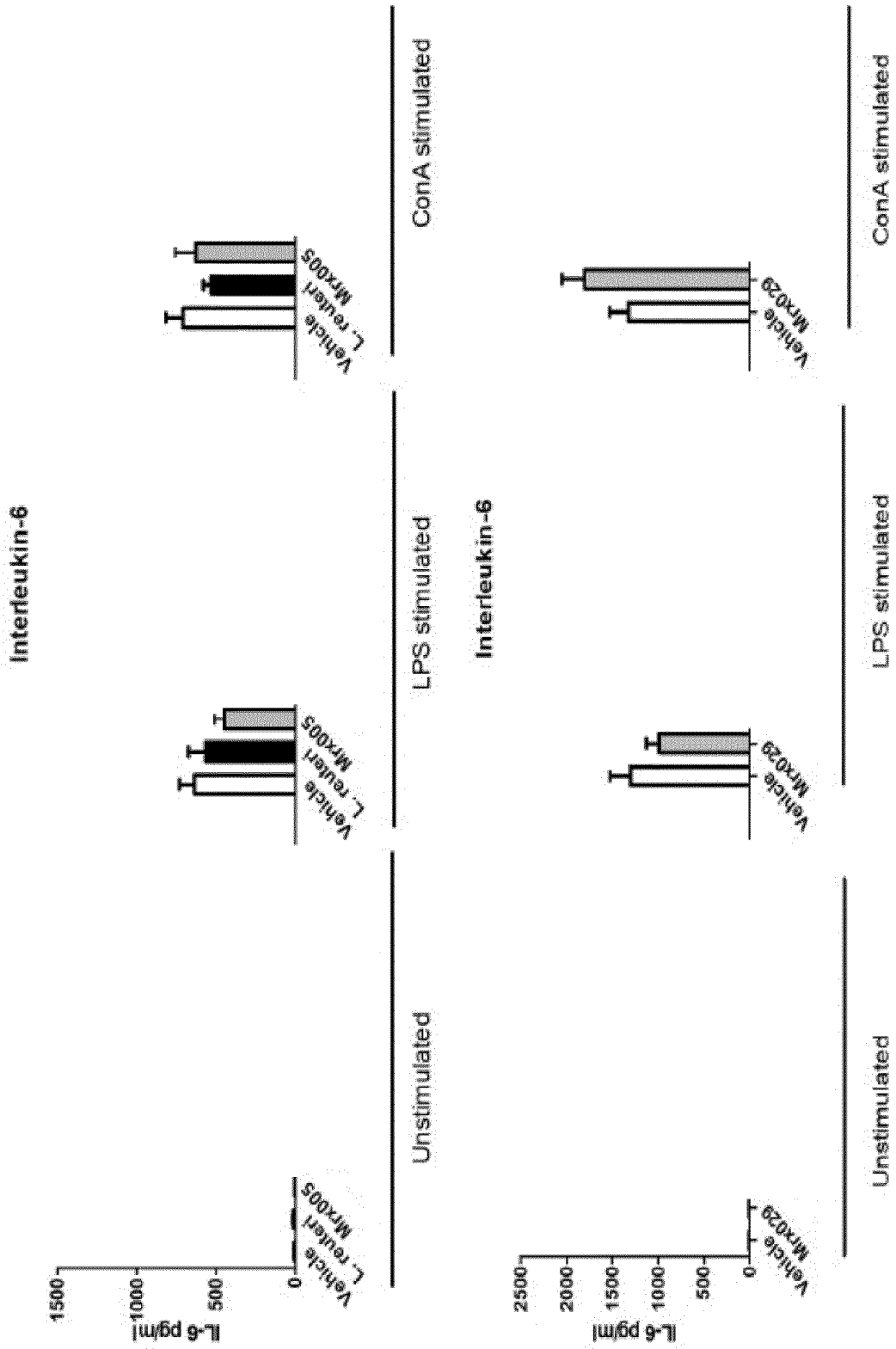

Interleukin-1β
IL-1β pg/ml
100
80
60
40
20
0
Vehicle
L. reuteri Mx005
Unstimulated
LPS stimulated
ConA stimulated
***
Interleukin-1β
IL-1β pg/ml
50
40
30
20
10
0
Vehicle
Mx029
Unstimulated
LPS stimulated
ConA stimulated

FIG 37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IL-6 pg/ml
1500
1000
500
0
Vehicle
L. reuteri
MRx005
Unstimulated
LPS stimulated
ConA stimulated
Interleukin-6
IL-6 pg/ml
2500
2000
1500
1000
500
0
Vehicle
MRx029
Unstimulated
LPS stimulated
ConA stimulated

FIG 38

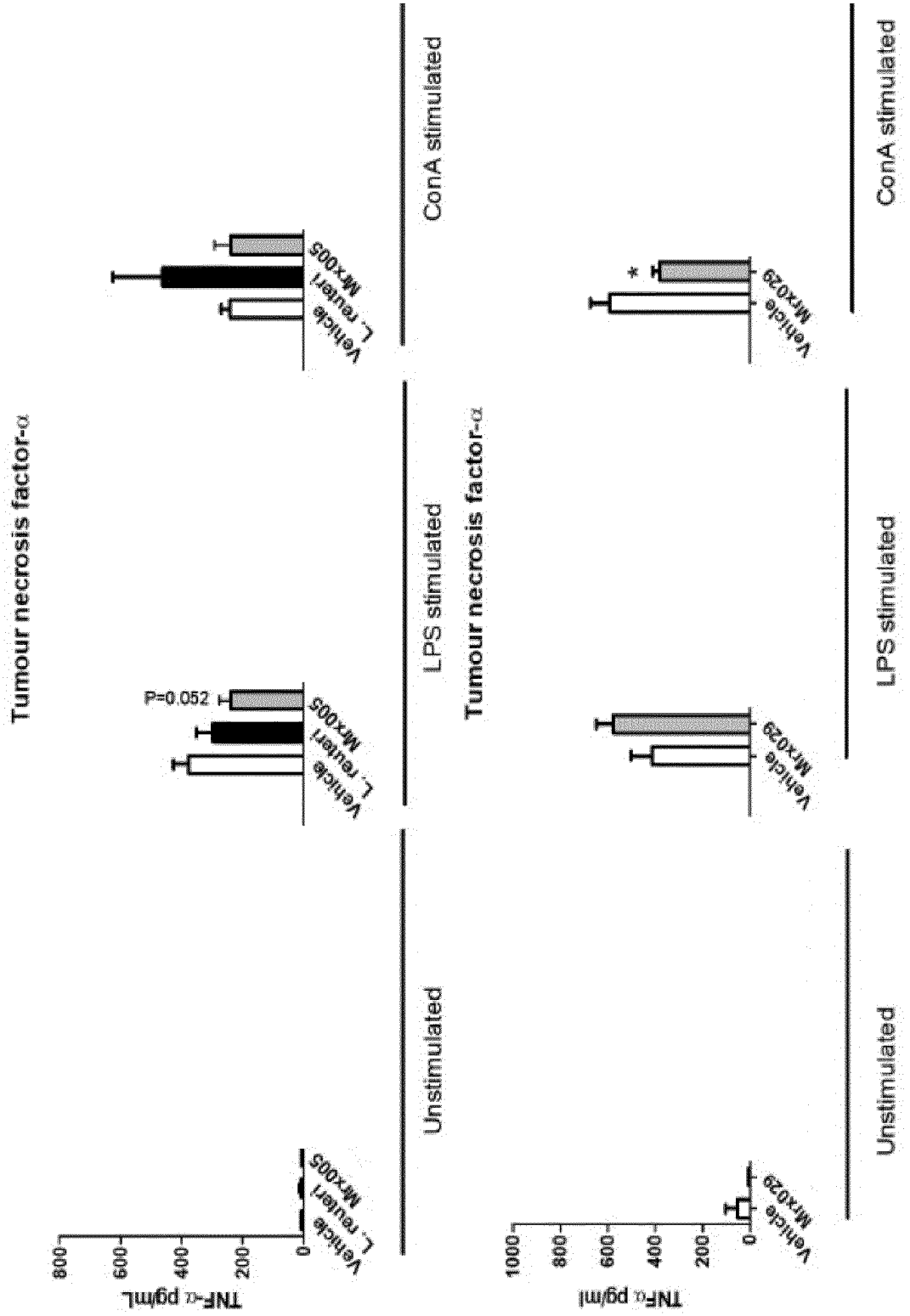

Tumour necrosis factor-α
TNF-α pg/mL
800
600
400
200
0
Vehicle
L. reuteri Mx005
P=0.052
Unstimulated
LPS stimulated
ConA stimulated
Tumour necrosis factor-α
TNFα pg/ml
1000
800
600
400
200
0
Vehicle
Mx029
*
Unstimulated
LPS stimulated
ConA stimulated

FIG 39

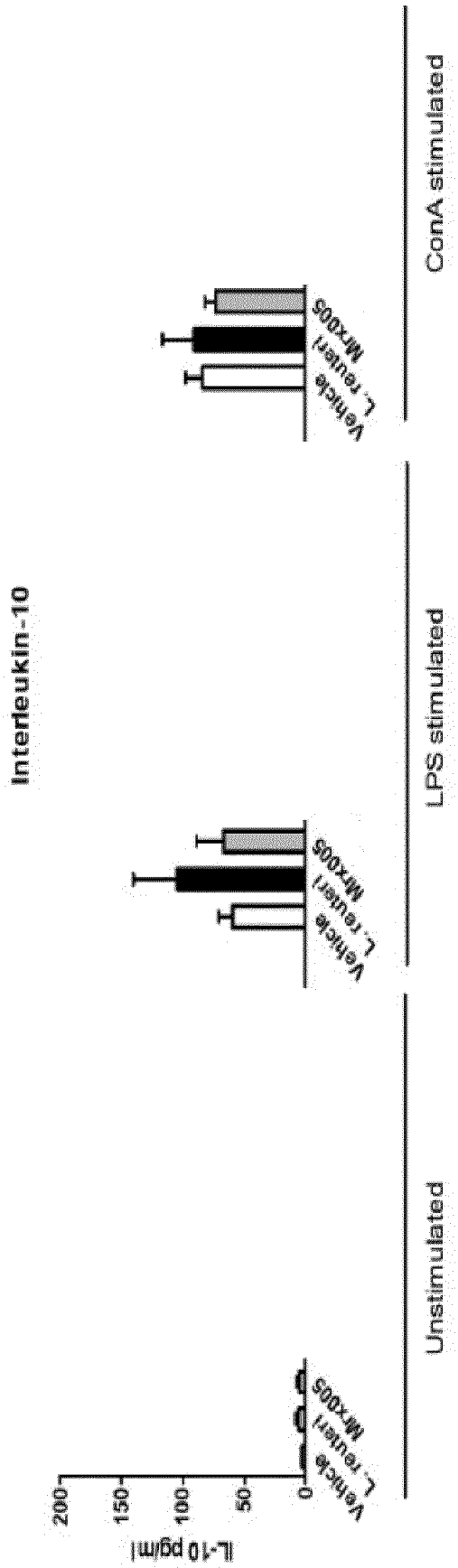

Interleukin-10
IL-10 pg/ml
200
150
100
50
0
Vehicle
L. reuteri Mx005
Unstimulated
LPS stimulated
ConA stimulated

FIG 40

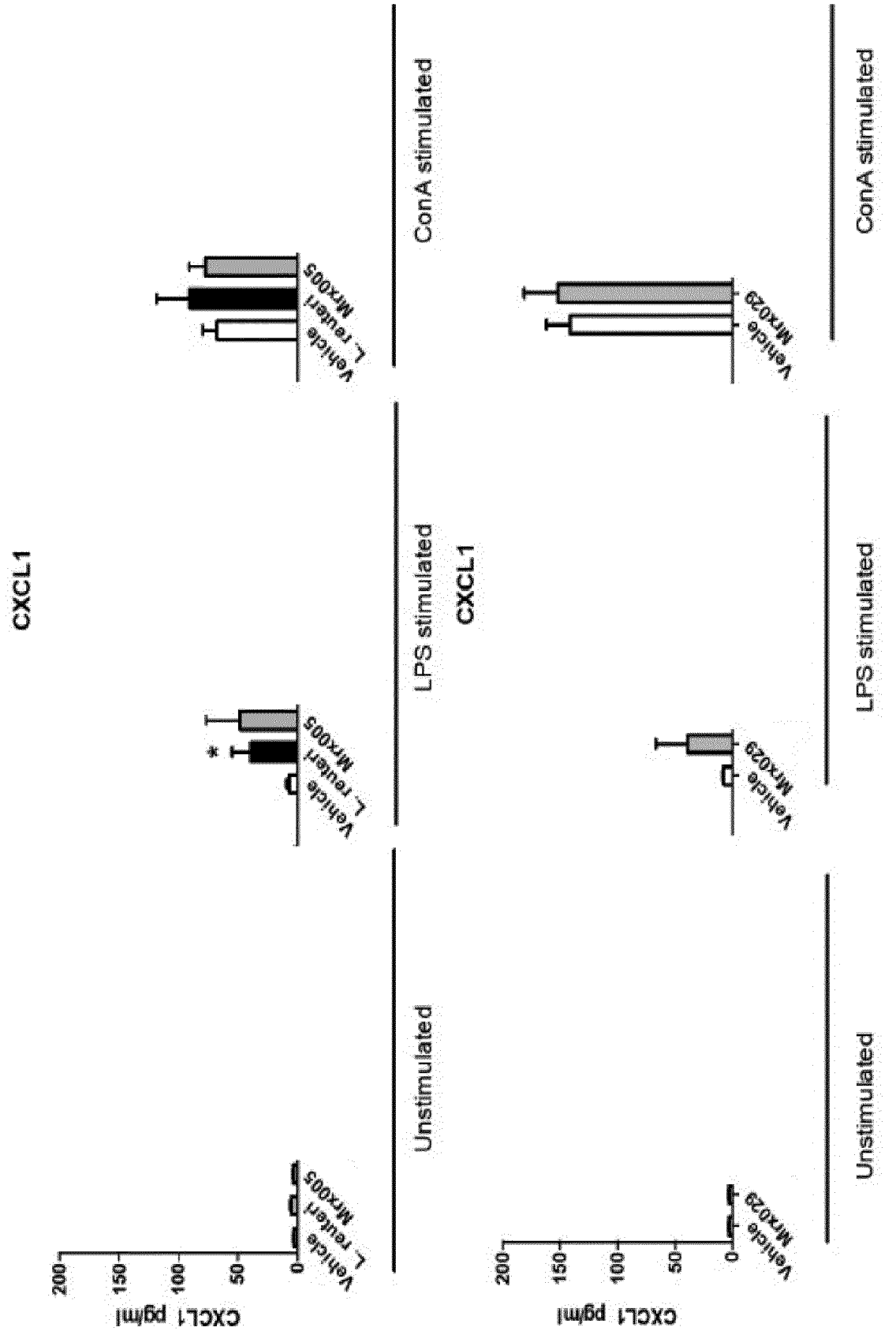

CXCL1
CXCL1 pg/ml
200
150
100
50
0
Vehicle
L. reuteri Mx005
Unstimulated
LPS stimulated
ConA stimulated
*
Vehicle
Mx029

FIG 41

FIG 42

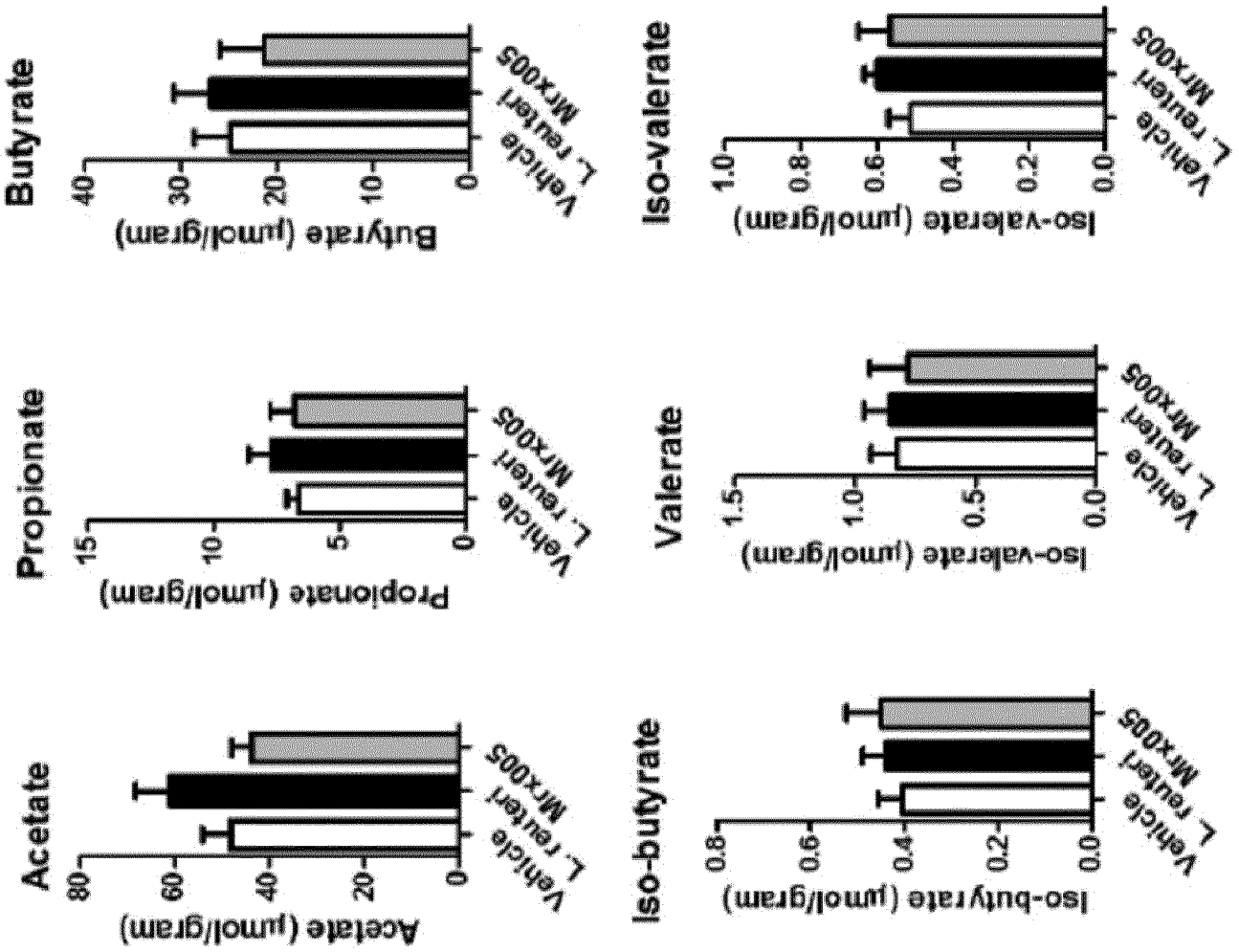
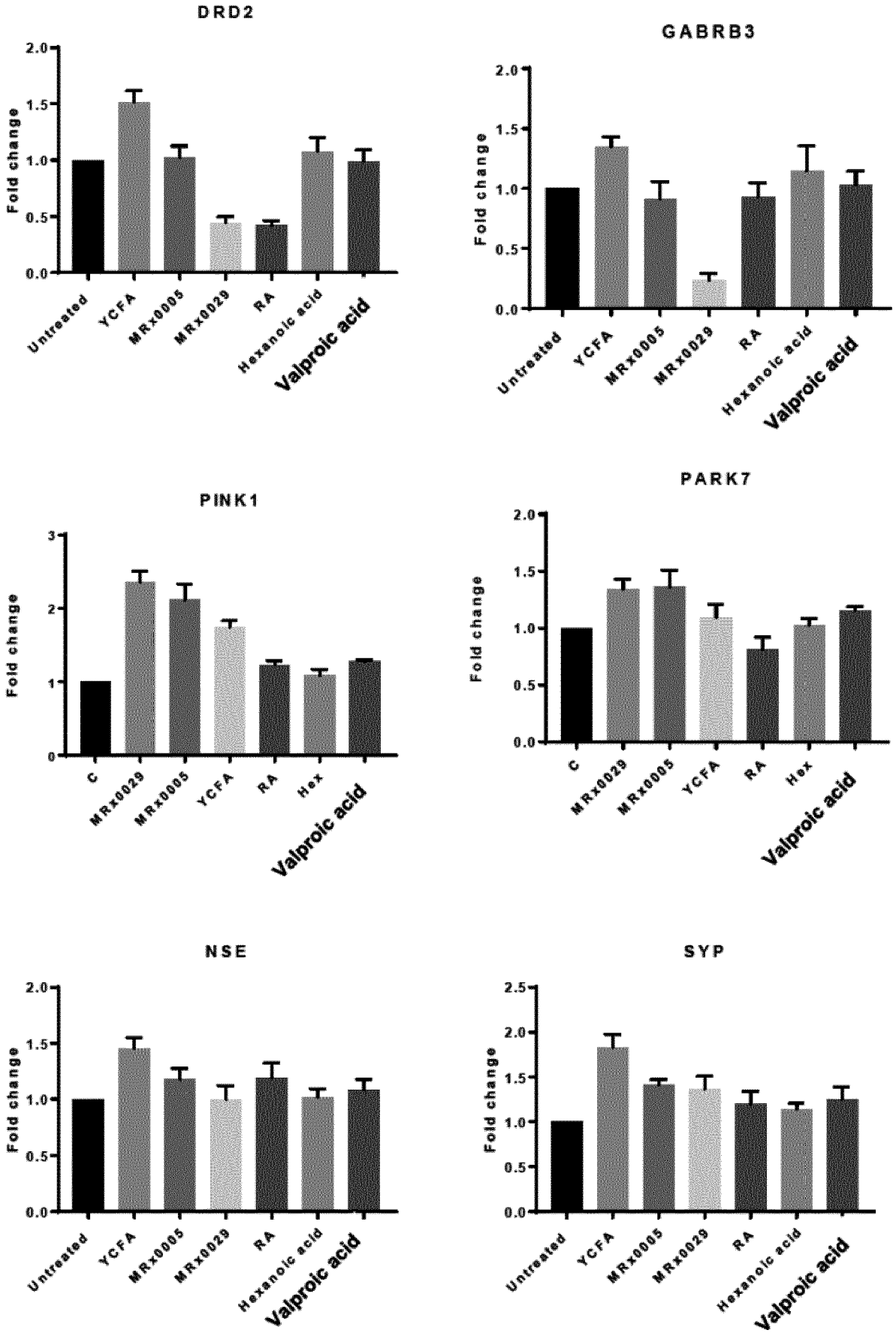

Butyrate
Butyrate (µmol/gram)
Iso-valerate
Iso-valerate (µmol/gram)
Propionate
Propionate (µmol/gram)
Valerate
Iso-valerate (µmol/gram)
Acetate
Acetate (µmol/gram)
Iso-butyrate
Iso-butyrate (µmol/gram)
Vehicle
L. reuteri Mx005

FIG 43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Fold change
2.0
1.5
1.0
0.5
0.0
Untreated
YCFA
MRx0005
MRx0029
RA
Hexanoic acid
Valproic acid
GABRB3
Fold change
2.0
1.5
1.0
0.5
0.0
Untreated
YCFA
MRx0005
MRx0029
RA
Hexanoic acid
Valproic acid
PINK1
Fold change
3
2
1
0
C
MRx0029
MRx0005
YCFA
RA
Hex
Valproic acid
PARK7
Fold change
2.0
1.5
1.0
0.5
0.0
C
MRx0029
MRx0005
YCFA
RA
Hex
Valproic acid
NSE
Fold change
2.0
1.5
1.0
0.5
0.0
Untreated
YCFA
MRx0005
MRx0029
RA
Hexanoic acid
Valproic acid
SYP
Fold change
2.5
2.0
1.5
1.0
0.5
0.0
Untreated
YCFA
MRx0005
MRx0029
RA
Hexanoic acid
Valproic acid

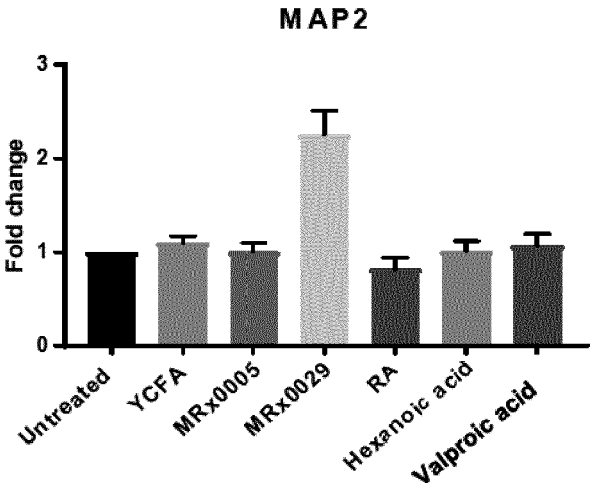


FIG 43 continued

FIG 44

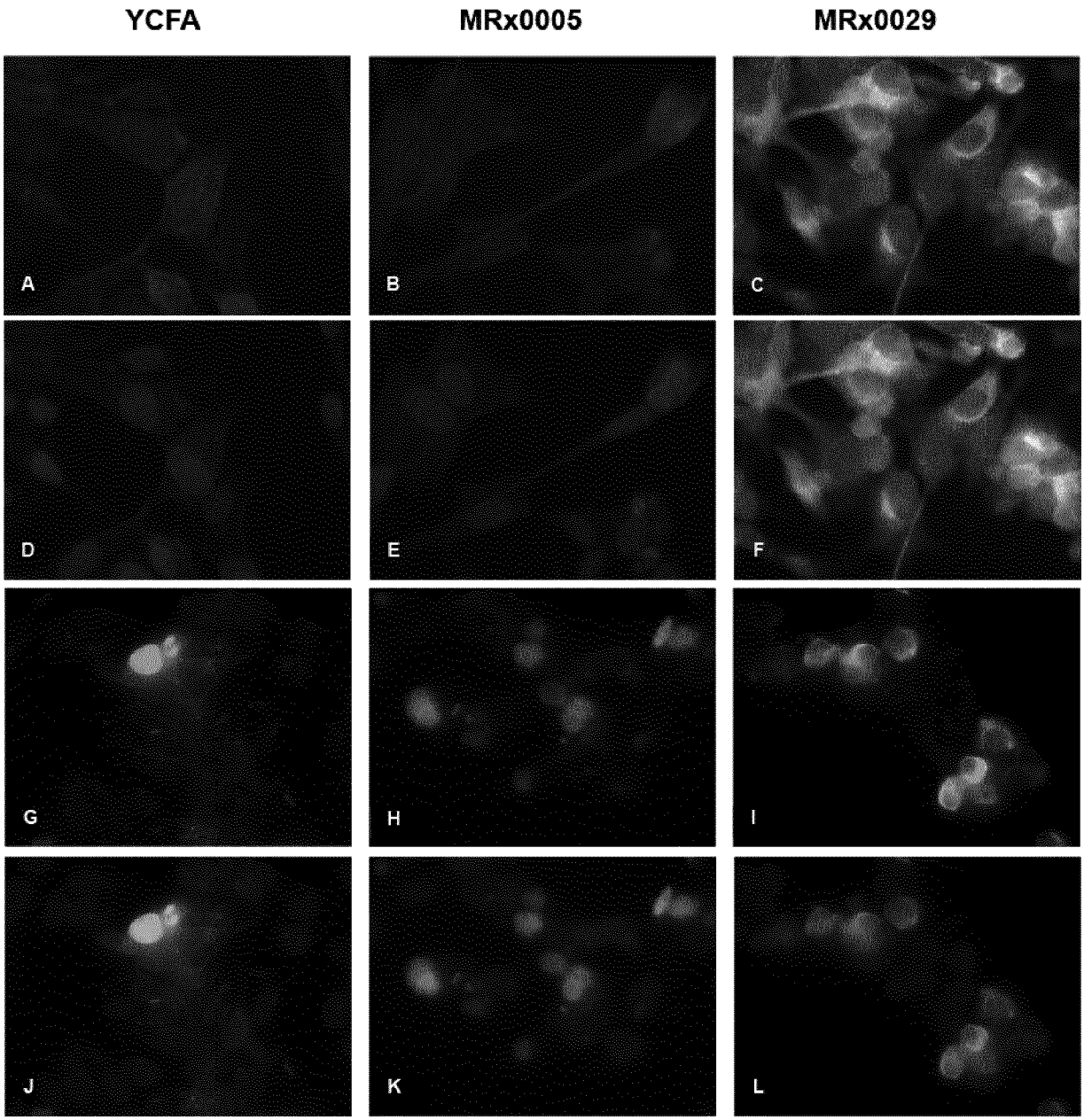
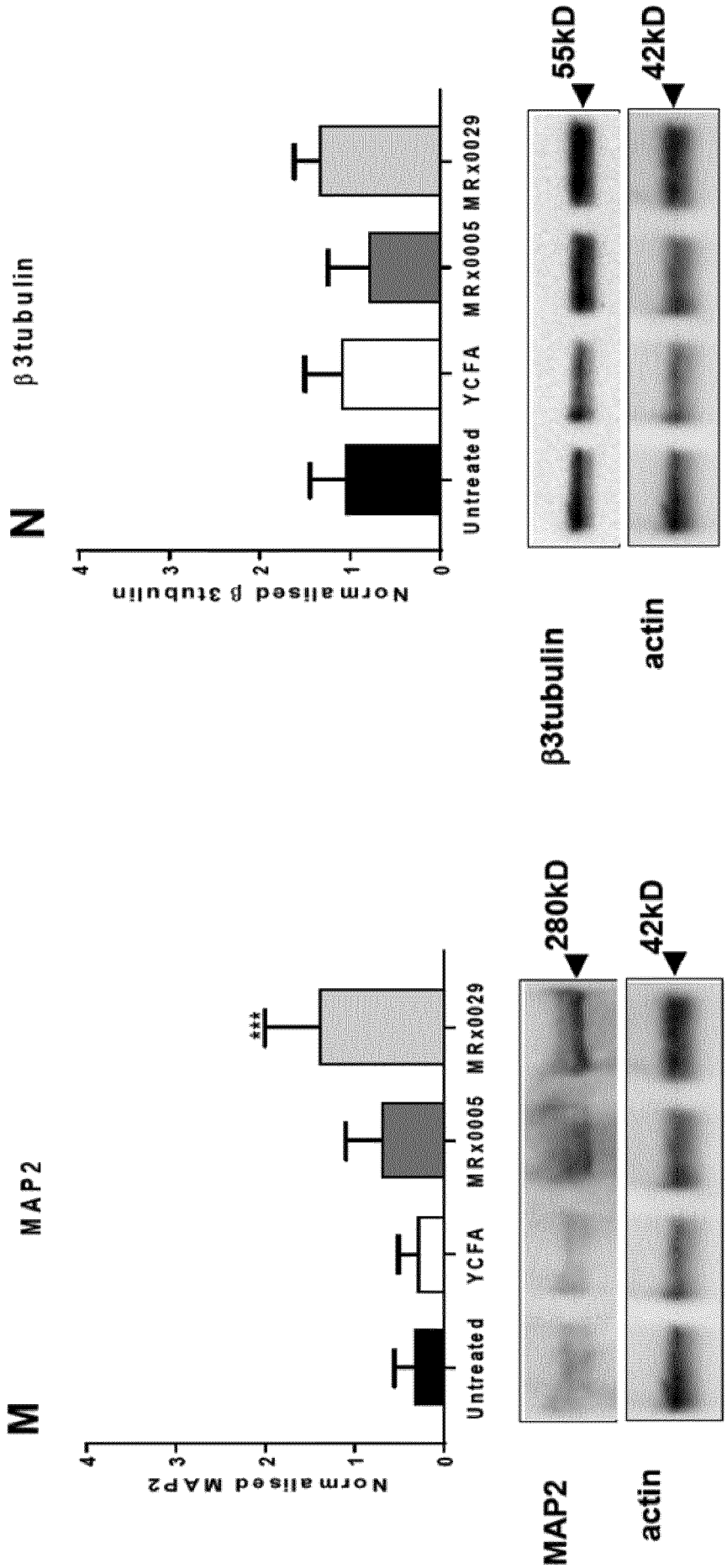


FIG 44 continued



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<212> DNA
<213> Parabacteroides merdae

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