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(71) Applicant: THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA [US/US]; 3600 Civic Center Boulevard, 9th Floor, Philadelphia, Pennsylvania 19104 (US).

(72) Inventors: THAISS, Christoph A.; 2413 Naudain Street, Philadelphia, Pennsylvania 19146 (US). BETLEY, John Nicholas; 120 Cynwyd Road, Bala Cynwyd, Pennsylvania 19004 (US).

(74) Agent: O'BRIEN, Kevin et al.; Saul Ewing Arnstein & Lehr LLP, 1500 Market Street, 38th Floor, Centre Square West, Philadelphia, Pennsylvania 19102 (US).

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(54) Title: COMPOSITIONS AND METHODS FOR STIMULATING AND/OR ENHANCING EXERCISE MOTIVATION AND/OR PHYSICAL PERFORMANCE

(57) Abstract: The present disclosure relates, in part, to compositions comprising Cannabinoid Receptor Type 1 (CB1) agonists, and methods of use thereof for stimulating and/or enhancing exercise motivation and/or physical performance in a subject. The present disclosure further relates to plasmids comprising at least one gene which encodes a protein that promotes fatty acid amide (FAA) biosynthesis, engineered bacterial strains comprising the same, probiotic formulations thereof, and methods of using the same for stimulating and/or enhancing exercise motivation and/or physical performance in a subject.



## TITLE OF THE INVENTION

Compositions and Methods for Stimulating and/or Enhancing Exercise Motivation and/or Physical Performance

5                   STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR  
DEVELOPMENT

This invention was made with government support under AG067492, DK119130 and DK115578 awarded by the National Institutes of Health. The government has certain rights in the invention.

## 10                   CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Patent Application No. 63/386,712, filed December 9, 2022, which is incorporated herein by reference in its entirety.

## 15                   SEQUENCE LISTING

The XML filed named "046483-7406WO1 - Sequence Listing.xml" created on December 7, 2023, comprising 43.8 Kbytes, is hereby incorporated herein by reference in its entirety.

## 20                   BACKGROUND

Exercise is possibly the single most important and accessible lifestyle component that protects from a large range of diseases, including cardiovascular, metabolic, inflammatory, neoplastic, and neurodegenerative diseases. Despite these beneficial effects of exercise, modern societies are characterized by an ever more sedentary lifestyle. It is estimated that the average  
25   daily physical activity is lower than ever before during human evolution, and there is a direct causal relationship between the decrease in physical activity and the surge in metabolic and neurological diseases worldwide.

Thus, there is a need in the art for compositions and/or methods for stimulating and/or motivating exercise in a subject. The present disclosure addresses this need.

## 30                   BRIEF SUMMARY

In one aspect, the present disclosure provides a method of stimulating and/or enhancing exercise motivation and/or physical performance in a subject, the method comprising administering to the subject at least one dietary supplement composition of the present disclosure.

5 In another aspect, the present disclosure provides a method of stimulating and/or enhancing exercise motivation and/or physical performance in a subject, the method comprising administering to the subject at least one pharmaceutical composition of the present disclosure.

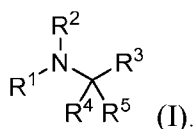
In another aspect, the present disclosure provides a method of stimulating and/or enhancing exercise motivation and/or physical performance in a subject, the method comprising  
10 administering to the subject at least one engineered bacterial strain comprising the plasmid of the present disclosure.

In another aspect, the present disclosure provides a method of stimulating and/or enhancing exercise motivation and/or physical performance in a subject, the method comprising administering to the subject at least one probiotic composition of the present disclosure.

15 In another aspect, the present disclosure provides a method of stimulating and/or enhancing exercise motivation and/or physical performance in a subject, the method comprising administering to the subject at least one probiotic composition comprising at least one bacterial strain selected from the group consisting of *Coprococcus* bacterium and *Eubacterium* bacterium.

In another aspect, the present disclosure provides a method of stimulating and/or  
20 enhancing exercise motivation and/or physical performance in a subject, the method comprising administering to the subject at least one Cannabinoid Receptor Type 1 (CB1) agonist.

In certain embodiments, the CB1 agonist is a fatty acid amide. In certain embodiments, the fatty acid amid is a compound of Formula (I), or a salt, solvate, stereoisomer, or isotopologue thereof, wherein R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>, R<sup>4</sup>, and R<sup>5</sup> are defined elsewhere herein.



25 In one aspect, the present disclosure provides a plasmid comprising at least one gene which encodes a protein that promotes fatty acid amide (FAA) biosynthesis which when inserted into a bacterium stimulates and/or enhances exercise motivation and/or physical performance in a subject.

In one aspect, the present disclosure provides an engineered bacterial strain comprising at least one plasmid of the present disclosure.

In another aspect, the present disclosure provides an engineered bacterial strain comprising at least one plasmid comprising at least one gene which encodes a protein that promotes fatty acid amide (FAA) biosynthesis.

In another aspect, the present disclosure provides a pharmaceutical composition comprising at least one fatty acid amide (FAA) selected from the group consisting of *N*-stearoyl serotonin, *N*-arachidonoyl dopamine, *N*-oleoyl dopamine, *N*-palmitoyl dopamine, *N*-stearoyl dopamine, *N*-stearoyl taurine, *N*-arachidonoyl ethanolamide, *N*-palmitoyl ethanolamide, and *N*-oleoyl ethanolamide, and at least one pharmaceutically acceptable carrier.

In certain embodiments, the present disclosure provides a pharmaceutical composition comprising at least two fatty acid amide (FAAs) selected from the group consisting of *N*-stearoyl serotonin, *N*-arachidonoyl dopamine, *N*-oleoyl dopamine, *N*-palmitoyl dopamine, *N*-stearoyl dopamine, *N*-stearoyl taurine, *N*-arachidonoyl ethanolamide, *N*-palmitoyl ethanolamide, and *N*-oleoyl ethanolamide, and at least one pharmaceutically acceptable carrier.

In certain embodiments, the present disclosure provides a pharmaceutical composition comprising at least three fatty acid amide (FAAs) selected from the group consisting of *N*-stearoyl serotonin, *N*-arachidonoyl dopamine, *N*-oleoyl dopamine, *N*-palmitoyl dopamine, *N*-stearoyl dopamine, *N*-stearoyl taurine, *N*-arachidonoyl ethanolamide, *N*-palmitoyl ethanolamide, and *N*-oleoyl ethanolamide, and at least one pharmaceutically acceptable carrier.

In certain embodiments, the present disclosure provides a pharmaceutical composition comprising at least four fatty acid amide (FAAs) selected from the group consisting of *N*-stearoyl serotonin, *N*-arachidonoyl dopamine, *N*-oleoyl dopamine, *N*-palmitoyl dopamine, *N*-stearoyl dopamine, *N*-stearoyl taurine, *N*-arachidonoyl ethanolamide, *N*-palmitoyl ethanolamide, and *N*-oleoyl ethanolamide, and at least one pharmaceutically acceptable carrier.

In certain embodiments, the present disclosure provides a pharmaceutical composition comprising at least five fatty acid amide (FAAs) selected from the group consisting of *N*-stearoyl serotonin, *N*-arachidonoyl dopamine, *N*-oleoyl dopamine, *N*-palmitoyl dopamine, *N*-stearoyl dopamine, *N*-stearoyl taurine, *N*-arachidonoyl ethanolamide, *N*-palmitoyl ethanolamide, and *N*-oleoyl ethanolamide, and at least one pharmaceutically acceptable carrier.

## BRIEF DESCRIPTION OF THE FIGURES

The drawings illustrate generally, by way of example, but not by way of limitation, various embodiments of the present application.

FIGs. 1A-1L: The impact of the intestinal microbiome on exercise performance in genetically and metagenomically diverse mice. FIG. 1A: schematic of study cohort consisting of diversity-outbred mice derived from 8 founder strains. The cohort was profiled for genome, microbiome, metabolome, and energy metabolism and assessed for exercise performance. FIGs. 1B-1C: diversity of performance parameters on treadmill (FIG. 1B, n=106) and running wheels (FIG. 1C, n=93). FIG. 1D: heritability ( $h^2$ ) calculated for distance, time, and energy spent on treadmills. FIG. 1E: algorithm-predicted versus measured treadmill distance based on a model including all assessed non-genetic features. FIG. 1F: explained variability for “metagroups” of non-genetic variables used for prediction. FIG. 1G: algorithm-predicted versus measured treadmill distance based on a model including only features from 16S rDNA sequencing. FIG. 1H: schematic of interventions to test for microbiome causality in DO mice. FIGs. 1I-1K: Wheel (FIGs. 1I-1J) and treadmill (FIG. 1K) running of DO mice treated with antibiotics (Abx). FIG. 1L: Correlation of treadmill running by donors and recipients of microbiome transfers from DO mice to germ-free mice. Error bars indicate means  $\pm$  SEM. \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

FIGs. 2A-2N: Exercise, genome, microbiome and metabolism in diversity-outbred mice. FIG. 2A: ranking of diversity outbred (DO) mice by time spent on treadmill until exhaustion. FIG. 2B: wheel turn recording of DO mice over two consecutive days. FIG. 2C: kinship matrix of DO mice. FIG. 2D: GWAS for time spent on treadmill during endurance exercise of DO mice. FIG. 2E: classification of serum metabolomes of DO mice. FIG. 2F: taxonomies based on 16S rDNA sequencing of DO mice. FIG. 2G-2N: recording traces (FIG. 2G, FIG. 2I, FIG. 2K, and FIG. 2M) and quantification (FIG. 2H, FIG. 2J, FIG. 2L, and FIG. 2N) of horizontal movement (FIGs. 2G-2H), respiratory exchange ratio (FIGs. 2I-2J), energy expenditure (FIGs. 2K-2L), and food intake (FIGs. 2M-2N) of DO mice.

FIGs. 3A-3O: Prediction of exercise performance in diversity-outbred mice. FIGs. 3A-3G: algorithm-predicted versus measured treadmill time (FIG. 3A, FIG. 3C, FIG. 3F), energy (FIG. 3B, FIG. 3D, FIG. 3G), and distance (FIG. 3E) based on a model including all assessed non-genetic features (FIGs. 3A-3B), microbiota features (FIG. 3C-3D), and serum metabolome (FIG. 3E-3G). FIG. 3H-3K: correlation of food intake (FIG. 3H), energy expenditure (FIG. 3I),

spontaneous locomotion (FIG. 3J), and respiratory exchange ratio (FIG. 3K) with treadmill distance. FIG. 3L: ranked plot of treadmill distance of DO mice, with and without antibiotic (Abx) treatment. FIGs. 3M-3O: Kaplan-Meier plots for distance (FIG. 3M) and time (FIG. 3N) to exhaustion, and time quantification (FIG. 3O) for treadmill exercise of DO mice, with and without Abx treatment. Error bars indicate means  $\pm$  SEM. \*\*  $p < 0.01$ .

FIGs. 4A-4L: Members of the microbiota contributing to exercise performance. FIG. 4A: Kaplan-Meier plot of distance on treadmill of broad-spectrum antibiotics (Abx)-treated mice and controls. FIG. 4B: averaged hourly distance of voluntary wheel activity of Abx mice and controls. Inset shows representative recording traces. FIG. 4C: Kaplan-Meier plot of distance on treadmill of germfree (GF) mice and controls. FIG. 4D: averaged hourly distance of voluntary wheel activity of GF mice and controls. Inset shows representative recording traces. FIGs. 4E-4F: Kaplan-Meier plot (FIG. 4E) and quantification (FIG. 4F) of distance on treadmill of mice treated with the indicated antibiotics. FIGs. 4G-4H: averaged hourly distance (FIG. 4G) and quantification (FIG. 4H) of voluntary wheel activity of mice treated with the indicated antibiotics. Inset shows representative recording traces. FIG. 4I: Kaplan-Meier plot of distance on treadmill of GF mice colonized with microbiome samples from conventional (SPF), neomycin-treated (Neo) or ampicillin-treated (Amp) mice. FIG. 4J: SHAP-value ranking of all microbiota features contributing to prediction of exercise performance in DO mice. FIGs. 4K-4L: Kaplan-Meier plot (FIG. 4K) and quantification (FIG. 4L) of distance on treadmill of SPF mice, GF mice, and GF mice mono-colonized with the indicated bacterial species. Error bars indicate means  $\pm$  SEM. n.s. not significant, \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*\*  $p < 0.0001$ .

FIGs. 5A-5W The microbiome impact on exercise performance. FIGs. 5A-5D: quantifications (FIG. 5A and FIGs. 5C-5D) and Kaplan-Meier plot (FIG. 5B) of treadmill distance (FIG. 5A), time (FIGs. 5B-5C) and energy (FIG. 5D) of antibiotics (Abx)-treated female mice. FIG. 5E: wheel running quantification of Abx-treated mice. FIGs. 5F-5I: quantifications (FIG. 5F and FIGs. 5H-5I) and Kaplan-Meier plot (FIG. 5G) of treadmill distance (FIG. 5F), time (FIGs. 5G-5H) and energy (FIG. 5I) of germ-free (GF) mice. FIG. 5J: wheel running quantification of GF mice. FIGs. 5K-5O: Kaplan-Meier plots (FIG. 5K and FIG. 5M) and quantifications (FIG. 5L and FIGs. 5N-5O) of treadmill distance (FIGs. 5K-5L), time (FIGs. 5M-5N) and energy (FIG. 5O) of Abx-treated male mice. FIGs. 5P-5S: Kaplan-Meier plots (FIG. 5P and FIG. 5R) and quantifications (FIG. 5Q and FIG. 5S) of distance (FIGs. 5P-5Q) and time

(FIGs. 5R-5S) on treadmill of Abx-treated mice or mice after Abx cessation (ex-Abx). FIGs. 5T-5Q: Kaplan-Meier plots (FIG. 5T and FIG. 5V) and quantifications (FIG. 5U and FIG. 5W) of distance (FIGs. 5T-5U) and time (FIG. 5V and FIG. 5W) on treadmill of GF mice and conventionalized (ex-GF) mice. Error bars indicate means  $\pm$  SEM. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , \*\*\*\*  $p < 0.0001$ .

FIGs. 6A-6W: Taxonomic analysis of microbiome features associated with exercise performance. FIGs. 6A-6B: recording (FIG. 6A) and quantification (FIG. 6B) of free horizontal movement measured in metabolic cages for two consecutive days in Abx-treated mice and controls. FIGs. 6C-6E: open field locomotion (FIG. 6C), distance quantification (FIG. 6D) and velocity quantification (FIG. 6E) of Abx-treated mice and controls. FIGs. 6F-6I: Kaplan-Meier plots (FIG. 6F and FIG. 6H) and quantifications (FIG. 6G and FIG. 6I) of distance (FIGs. 6F-6G) and time (FIGs. 6H-6I) on treadmill of mice treated with either absorbable (Abs) or non-absorbable (Non-abs) antibiotics, or a broad-spectrum mixture (Abx). FIGs. 6J-6L: body weight (FIG. 6J), Kaplan-Meier plot (FIG. 6K) and quantification (FIG. 6L) of time on treadmill of mice treated with the indicated different antibiotics. FIGs. 6M-6P: quantifications (FIG. 6M and FIGs. 6O-6P) and Kaplan-Meier plot (FIG. 6N) of treadmill distance (FIG. 6M), time (FIGs. 6N-6O), and energy (FIG. 6P) of GF mice colonized with microbiome samples from conventional (SPF), neomycin-treated (Neo) or ampicillin-treated (Amp) mice. FIGs. 6Q-6S: phylum-level taxonomic microbiome composition (FIG. 6Q) and relative abundance of Erysipelotrichaceae (FIGs. 6R-6S) of neomycin- and ampicillin-treated mice (FIGs. 6Q-6R) and GF mice receiving their microbiome samples (FIG. 6S). FIGs. 6T-6W: bacterial load (FIG. 6T), taxonomic composition (FIG. 6U), treadmill distance, Kaplan-Meier plot (FIG. 6V) and quantification (FIG. 6W) of SPF mice, GF mice, and GF mice mono-colonized with the indicated bacterial species. Error bars indicate means  $\pm$  SEM. \*n.s. not significant,  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*\*  $p < 0.0001$ .

FIGs. 7A-7S: The impact of the microbiome on muscle physiology. FIGs. 7A-7D: weight of soleus (FIG. 7A), gastrocnemius (FIG. 7A), tibialis anterior (FIG. 7C), and extensor digitorum longus (EDL) muscles in Abx-treated mice and controls. FIGs. 7E-7F: grip strength of Abx-treated mice and controls before (FIG. 7E) and after (FIG. 7F) exercise. FIGs. 7G-7J: maximum twitch (FIG. 7G), and tetanic force (FIG. 7H), specific twitch force (FIG. 7I), and specific tetanic force (FIG. 7J) of EDL muscle from Abx-treated mice and controls. FIGs. 7K-7N: decrease in power (FIG. 7K) and specific muscle force (FIG. 7L) over time, force recovery quantification

(FIG. 7M) and force recovery over time (FIG. 7N) of EDL muscle from Abx-treated mice and controls. FIG. 7O: EDL muscle cross-sectional area in Abx-treated mice and controls. FIGs. 7P-7Q: quantification of oxidative phosphorylation and fatty acid oxidation of isolated mitochondria (FIG. 7P) and whole cell lysates (FIG. 7Q) from EDL muscle obtained from either Abx-treated mice or controls. FIGs. 7R-7S: PCA plot (FIG. 7R) and heatmap of selected genes (FIG. 7S) from EDL transcriptomes obtained from Abx-treated mice and controls. NMJ, neuro-muscular junction. Error bars indicate means  $\pm$  SEM. \*\*  $p < 0.01$ .

FIGs. 8A-8D: single-nucleus sequencing of the striatum. FIG. 8A: UMAP clustering of all cell types identified in the striatum as well as feature plots for each cluster-identifying marker. FIG. 8B: UMAP of neuronal subcluster showing expression of *Drd1* and *Drd2*. FIGs. 8C-8D: UMAP plots (FIG. 8C) and quantification (FIG. 8D) of *Fos* expression in striatal neurons from control and Abx treated mice before and during exercise.

FIGs. 9A-9R: The microbiome impacts exercise-induced dopamine responses in the striatum. FIG. 9A: UMAP plot of neurons from single-nucleus RNA-seq of the striata of control and Abx-treated mice before and during exercise. FIGs. 9B-9C: UMAP plots (FIG. 9B) and quantification (FIG. 9C) of *Arc* expression in striatal neurons from control and Abx-treated mice before and during exercise. FIG. 9D: dopamine levels in the brains of Abx-treated mice and controls, at steady-state, after exhaustion from endurance exercise, and after 60 minutes of timed exercise. FIGs. 9E-9H: schematic (FIG. 9E), recording traces (FIG. 9F), quantification (FIG. 9G), and mean signals (FIG. 9H) of fibre photometry recording of a dopamine sensor injected into the nucleus accumbens. Mice were recorded at the end of the exercise protocol. FIGs. 9I-9J: correlation of striatal dopamine concentration and distance ran in voluntary exercise by DO mice (FIG. 9I) and germ-free recipients of microbiota samples from DO mice (FIG. 9J). FIGs. 9K-9N: averaged hourly distance (FIG. 9K and FIG. 9M) and quantification (FIG. 9L and FIG. 9N) of voluntary wheel activity of Abx-treated mice injected with sulpiride (FIG. 9K and FIG. 9L), or bromocriptine (FIGs. 9M-9N). Insets show representative recording traces. FIG. 9O: expression of *Maoa* in the striatum before and after exercise in control and Abx-treated mice. FIG. 9P: dopamine levels in the brain of Abx-treated and control mice, both steady state and post-exercise, injected i.p. with vehicle or the MAO inhibitor pargyline. FIGs. 9Q-9R: averaged hourly distance (FIG. 9Q) and quantification (FIG. 9R) of voluntary wheel activity of Abx-treated mice injected with the MAO inhibitor pargyline. Inset shows representative recording

traces. Error bars indicate means  $\pm$  SEM. n.s. not significant, \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , \*\*\*\*  $p < 0.0001$ .

FIGs. 10A-10M: The role of microbiome-mediated dopamine responses in exercise performance. FIG. 10A: schematic depicting *in vivo* fibre photometry of dopamine sensor fluorescence in the nucleus accumbens of mice during treadmill running. FIGs. 10B-10D: fibre photometry recording of dopamine dynamics at the end of exercise in the ventral striatum (FIG. 10B), the dorsal striatum (FIG. 10C), and the ventral striatum after different durations of exercise. FIGs. 10E-10F: glutamate (FIG. 10E) and acetylcholine (FIG. 10F) levels in the brain of Abx-treated mice and controls, at steady-state and after endurance exercise. FIG. 10G: post-exercise dopamine levels in brain tissue of GF mice, and GF mice colonized with stool from SPF, neomycin- or ampicillin-treated mice. FIG. 10H-10K: Kaplan-Meier plots (FIG. 10H and FIG. 10J) and quantifications (FIG. 10I and FIG. 10K) of distance (FIGs. 10H-10I) and time (FIGs. 10J-10K) on treadmill of Abx-treated Slc6a3<sup>hM4Di</sup> mice, with or without CNO treatment. FIGs. 10L-10M: averaged hourly distance (FIG. 10L) and quantification (FIG. 10M) of voluntary wheel activity of Abx-treated Slc6a3<sup>hM4Di</sup> mice, with or without CNO treatment. Inset shows representative recording traces. Error bars indicate means  $\pm$  SEM. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , \*\*\*\*  $p < 0.0001$ .

FIGs. 11A-11M: Mechanisms of microbiome-mediated control of striatal dopamine responses. FIGs. 11A-11E: schematic (FIG. 11A), recording traces (FIG. 11B), quantification (FIG. 11C), mean signals (FIG. 11D), and maximum signals (FIG. 11E) of fibre photometry recording from the VTA of Slc6a3-Cre mice injected with a GCamp6-expressing virus. Mice were recorded at the end of the exercise protocol. FIGs. 11F-11I: Kaplan-Meier plots (FIG. 11F and FIG. 11H) and quantifications (FIG. 11G and FIG. 11I) of distance (FIGs. 11F-11G) and time (FIGs. 11H-11I) on treadmill of Abx-treated mice, with or without leptin injection i.p. or into the VTA. FIGs. 11J-11M: Kaplan-Meier plots (FIG. 11J and FIG. 11L) and quantifications (FIG. 11K and FIG. 11M) of distance (FIGs. 11J-11K) and time (FIGs. 11L-11M) on treadmill of Abx-treated mice, with or without pargyline treatment. All results are representative of two independent experiments. Error bars indicate means  $\pm$  SEM. \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , \*\*\*\*  $p < 0.0001$ .

FIGs. 12A-12L: Circulating versus neuronal factors as mediators of the microbiome impact on exercise. FIG. 12A: heatmap of differentially abundant serum metabolites between

Abx-treated mice and controls. FIG. 12B: correlation of fold change of serum metabolite abundance between Abx-treated mice and controls with the correlation of the same metabolites with treadmill distance in the DO cohort. FIGs. 12C-12H: Kaplan-Meier plots (FIG. 12C, FIG. 12E, FIG. 12F, and FIG. 12G) and quantifications (FIG. 12D, FIG. 12F, and FIG. 12H) of distance (FIGs. 12E-12F) and time (FIGs. 12C-12D and FIGs. 12G-12H) on treadmill of *Trpv1<sup>DTA</sup>* mice (FIGs. 12C-12D) and CNO-injected of *Trpv1<sup>hM4Di</sup>* mice. FIGs. 12I-12J: averaged hourly distance (FIG. 12I) and quantification (FIG. 12J) of voluntary wheel activity of Abx-treated mice, with or without capsaicin treatment. Inset shows representative recording traces. FIGs. 12K-12L: Kaplan-Meier plot (FIG. 12K) and quantifications (FIG. 12L) time on treadmill of Abx-treated mice, with or without capsaicin treatment. Error bars indicate means  $\pm$  SEM. \* p < 0.05, \*\* p < 0.01, \*\*\*\* p < 0.0001.

FIGs. 13A-13P: The microbiome influences exercise performance via afferent sensory neurons. FIGs. 13A-13B: averaged hourly distance (FIG. 13A) and quantification (FIG. 13B) of voluntary wheel activity of wild-type (WT) and *Trpv1<sup>DTA</sup>* mice, with and without Abx treatment. Inset shows representative recording traces. FIGs. 13C-13D: Kaplan-Meier plot (FIG. 13C) and quantification (FIG. 13D) of distance on treadmill of controls, Abx-treated mice and *Trpv1<sup>DTA</sup>* mice. FIGs. 13E-13F: Kaplan-Meier plot (FIG. 13E) and quantification (FIG. 13F) of treadmill distance of Abx-treated mice and controls, with or without capsaicin treatment. FIG. 13G: expression of Arc in the dorsal root ganglia of sedentary and post-exercise mice, with or without Abx treatment. FIG. 13H: expression of Maa in the striatum of post-exercise Abx-treated and control mice, with or without capsaicin treatment. FIG. 13I: dopamine levels in the brain of control mice, Abx-treated mice, and *Trpv1<sup>DTA</sup>* mice, with or without capsaicin treatment. FIGs. 13J-13L: recording traces (FIG. 13J), quantification (FIG. 13K), and maximal signals (FIG. 13L) of post-exercise Abx-treated mice and controls, with or without capsaicin treatment. FIGs. 13M-13N: averaged hourly distance (FIG. 13M) and quantification (FIG. 13N) of voluntary wheel activity of Abx-treated mice and controls, with or without capsaicin and sulpiride treatment. FIGs. 13O-13P: averaged hourly distance (FIG. 13O) and quantification (FIG. 13P) of voluntary wheel activity of Abx-treated and *Trpv1<sup>DTA</sup>* mice, with or without bromocriptine treatment. Insets show representative recording traces. Error bars indicate means  $\pm$  SEM. \* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001, \*\*\*\* p < 0.0001.

FIGs. 14A-14I: The impact of exercise and the microbiome on sensory neuron activity.

FIG. 14A: representative RNAScope images of dorsal root and nodose ganglia before and after exercise, with and without Abx treatment. FIG. 14B: number of cFos<sup>+</sup> cells in dorsal root ganglia before and after exercise. FIG. 14C: proportion of cFos<sup>+</sup> cells in dorsal root ganglia from exercised mice that are TRPV1<sup>+</sup> and TRPV1<sup>-</sup>. FIG. 14D-14G: number of TRPV1<sup>+</sup> (FIG. 14D and FIG. 14F) and cFos<sup>+</sup> TRPV1<sup>+</sup> (FIG. 14E and FIG. 14G) cells in the dorsal root ganglia (FIGs. 14D-14E) and nodose ganglia (FIGs. 14F-14G) in exercised Abx-treated mice and controls. FIGs. 14H-14I: expression of *Fos* (FIG. 14H) and *Homer1* (FIG. 14I) in the dorsal root ganglia of sedentary and post-exercise mice, with or without Abx treatment. Error bars indicate means  $\pm$  SEM. \* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001, \*\*\*\* p < 0.0001.

FIGs. 15A-15N: Contribution of spinal and vagal afferents to exercise performance.

FIGs. 15A-15D: averaged hourly distance (FIG. 15A and FIG. 15C) and quantifications (FIG. 15B and FIG. 15D) of voluntary wheel activity of mice receiving CCK-SAP injection into the nodose ganglia (FIGs. 15A-15B) and mice with surgical resection of the celiac/superior mesenteric ganglion (CSMG) (FIGs. 15C-15D). Insets show representative recording traces. FIGs. 15E-15J: Kaplan-Meier plots (FIG. 15E, FIG. 15G, and FIG. 15J) and quantifications (FIG. 15F and FIGs. 15H-15I) of distance (FIGs. 15E-15F), time (FIGs. 15G-15H), and energy (FIGs. 15I-15J) spent on treadmills by mice with surgical resection of the CSMG. FIGs. 15K-15L: expression of *Maoa* (FIG. 15K) and dopamine levels in the striatum (FIG. 15L) of mice with surgical resection of the CSMG after exercise. FIG. 15M: mean dopamine indicator signal in post-exercise Abx-treated mice, with or without capsaicin treatment. FIG. 15N: correlation of striatal dopamine levels with distance in running wheels of *Trpv1*<sup>DTA</sup> mice, Abx-treated mice, and controls, with or without capsaicin treatment. Error bars indicate means  $\pm$  SEM. \* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001.

FIGs. 16A-16O: Intestinal endocannabinoids drive exercise performance. FIG. 16A: recording traces of calcium imaging of DRG neurons exposed to stool filtrates from Abx-treated mice and controls. Arrow indicates treatment time. FIGs. 16B-16C: quantification (FIG. 16B) and correlation with treadmill distance (FIG. 16C) of calcium imaging of DRG neurons exposed to stool filtrates from mice treated with different Abx. Arrow indicates treatment time. FIG. 16D: heatmap of metabolites detected in caecal contents from mice treated with different Abx. FIG. 16E: quantifications of calcium imaging of DRG neurons exposed to individual metabolites.

FIG. 16F: normalized abundance of oleoylethanolamide (OEA) in caecal contents from mice treated with different Abx. FIG. 16G: correlation between OEA abundance and wheel running in DO mice. FIGs. 16H-16O: averaged hourly distance (FIG. 16H, FIG. 16J, FIG. 16L, and FIG. 16N) and quantification (FIG. 16I, FIG. 16K, FIG. 16M, and FIG. 16O) of voluntary wheel activity of Abx mice and controls, fed a fatty acid amide (FAA)-supplemented or control diet (FIG. 16H, FIG. 16I, FIG. 16L, and FIG. 16M), with or without treatment with the peripheral CB1 antagonist AM6545 (FIGs. 16J-16O) and the dopamine receptor agonist bromocriptine (FIGs. 16N-16O). Insets show representative recording traces. Error bars indicate means  $\pm$  SEM. n.s. not significant, \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , \*\*\*\*  $p < 0.0001$ .

FIGs. 17A-17K: DRG neuron stimulation by intestinal metabolites correlates with exercise performance. FIG. 17A: quantification of calcium imaging of DRG neurons exposed to stool filtrates from Abx-treated mice and controls. FIG. 17B-17C: recording traces (FIG. 17B) and quantification (FIG. 17C) of calcium imaging of DRG neurons exposed to stool filtrates from GF mice and controls. Arrow indicates treatment time. FIGs. 17D-17G: recording traces (FIG. 17D), quantification (FIG. 17E), correlation with wheel running (FIG. 17F), and correlation with post-exercise dopamine levels in the striatum of calcium imaging of DRG neurons exposed to stool filtrates from DO mice. Arrow indicates treatment time. FIGs. 17H-17I: recording traces of calcium imaging of DRG neurons exposed to stool filtrates from mice treated with different Abx (FIG. 17H) and to individual metabolites (FIG. 17I). Arrow indicates treatment time. FIG. 17J-17K: recording traces (FIG. 17J) and quantification (FIG. 17K) of calcium imaging of DRG neurons exposed to oleoylethanolamide (OEA) or capsaicin. Error bars indicate means  $\pm$  SEM. \*\*\*  $p < 0.001$ , \*\*\*\*  $p < 0.0001$ .

FIGs. 18A-18Q: Dietary supplementation of fatty acid amides enhances exercise performance. FIGs. 18A-18F: averaged recording traces (FIG. 18A, FIG. 18C, and FIG. 18E) and quantifications (FIG. 18B, FIG. 18D, and FIG. 18F) of calcium imaging of DRG neurons exposed to individual metabolites (FIGs. 18A-18B), a fatty acid amide (FAA)-supplemented diet (FIGs. 18C-18D), or stool extracts from mice fed a FAA-supplemented diet (FIGs. 18E-18F). FIGs. 18G-18L: post-exercise expression of *Arc* and *Fos* in DRGs (FIGs. 18G-18H), post-exercise expression of *Maoa* and dopamine levels in the striatum (FIGs. 18I-18J), and Kaplan-Meier plot and quantification of distance on treadmill (FIGs. 18K-18L) by Abx-treated mice and control, fed a FAA-supplemented or control diet. FIGs. 18M-18O: wheel running of Abx-treated

DO mice, fed a FAA-supplemented or control diet. FIGs. 18P-18Q: schematic (FIGs. 18P) and striatal dopamine levels (FIG. 18Q) of Abx-treated mice receiving gastric infusion of FAA, with or without treadmill exercise. Error bars indicate means  $\pm$  SEM. \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , \*\*\*\*  $p < 0.0001$ .

5            FIGs. 19A-19K: Microbiome engineering to enhance fatty acid amide production and exercise performance. FIG. 19A: OEA levels in GF mice and GF mice mono-colonized with *Coprococcus eutactus*. FIG. 19B: schematic of generation of *Escherichia coli* expressing the *ereA-T* gene cluster from *Eubacterium rectale*. FIG. 19C: OEA levels in GF mice and GF mice mono-colonized with either *E. coli*<sup>*ereA-T*</sup> or the empty vector-containing strain *E. coli*<sup>*WT*</sup>. FIGs. 10    19D-19E: averaged recording traces (FIG. 19D) and quantification (FIG. 19E) of calcium imaging of DRG neurons exposed to in stool extracts from GF mice and GF mice monocolonized with either *E. coli*<sup>*ereA-T*</sup> or *E. coli*<sup>*WT*</sup>. FIGs. 19F-19G: averaged hourly distance (FIG. 19F) and quantification (FIG. 19G) of voluntary wheel activity of GF mice and GF mice monocolonized with either *E. coli*<sup>*ereA-T*</sup> or *E. coli*<sup>*WT*</sup>. Inset shows representative recording traces. 15    FIGs. 19H-19K: Kaplan-Meier plots (FIG. 19H and FIG. 19J) and quantifications (FIG. 19I and FIG. 19K) of distance (FIGs. 19H-19I) and time (FIGs. 19J-19K) on treadmills by GF mice and GF mice mono-colonized with either *E. coli*<sup>*ereA-T*</sup> or *E. coli*<sup>*WT*</sup>. Error bars indicate means  $\pm$  SEM. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*\*  $p < 0.0001$ .

FIGs. 20A-20Q: Stimulation of endocannabinoid receptors drives exercise performance. 20    FIGs. 20A-20B: UMAP plot of all cell types identified in DRGs36 (FIG. 20A) and expression of *Trpv1* and *Cnr1* (FIG. 20B). FIGs. 20C-20E: expression of Arc (FIG. 20C) and Fos (FIG. 20D) in the dorsal root ganglia, and dopamine levels in the striatum (FIG. 20E) of exercised *Cnr1*-deficient mice and controls. FIGs. 20F-20G: averaged hourly distance (FIG. 20F) and quantification (FIG. 20G) of voluntary wheel activity of *Cnr1*-deficient mice and controls. Inset 25    shows representative recording traces. FIGs. 20H-20I: Kaplan-Meier plot (FIG. 20H) and quantification (FIG. 20I) of distance on treadmills by *Cnr1*-deficient mice and controls. FIGs. 20J-20M: averaged hourly distance (FIG. 20J and FIG. 20L) and quantifications (FIG. 20K and FIG. 20M) of voluntary wheel activity of Abx-treated mice and controls, with or without treatment with the CB1 inhibitor O-2050 (FIGs. 20J-20K) or the CB1 agonist CP55,940 (FIGs. 30    20L-20M). Insets show representative recording traces. FIGs. 20N-20Q: Kaplan-Meier plots (FIG. 20N and FIG. 20P) and quantifications (FIG. 20O and FIG. 20Q) of distance (FIGs. 20N-

200) and time (FIGs. 20P-20Q) on treadmills by Abx-treated mice and controls, with or without treatment with the peripheral CB1 inhibitor AM6545. Error bars indicate means  $\pm$  SEM. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , \*\*\*\*  $p < 0.0001$ .

FIGs. 21A-21J: Peripheral endocannabinoid signalling drives exercise physiology. FIGs. 21A-21E: *Fos* expression in DRGs (FIG. 21A), Kaplan-Meier plots (FIG. 21B and FIG. 21D) and quantifications (FIG. 21C and FIG. 21E) of distance (FIGs. 21B-21C) and time (FIGs. 21D-21E) on treadmills by GF mice and GF mice mono-colonized with *E. coli*<sup>ereA-T</sup>, with and without AM6545 treatment. FIGs. 21F-21G: expression of *Maoa* (FIG. 21F) and dopamine levels in the striatum (FIG. 21G) of AM6545-treated mice after treadmill exercise. FIG. 21H: schematic of pathway model linking the intestinal microbiome to exercise performance. FIGs. 21I-21J: latency to paw withdrawal on a hot plate by Abx-treated mice and controls before and after exercise (FIG. 21I) and after exercise, with and without AM6545 treatment (FIG. 21J). Error bars indicate means  $\pm$  SEM. \*  $p < 0.05$ , \*\*\*\*  $p < 0.0001$ .

## DETAILED DESCRIPTION OF THE INVENTION

Reference will now be made in detail to certain embodiments of the disclosed subject matter, examples of which are illustrated in part in the accompanying drawings. While the disclosed subject matter will be described in conjunction with the enumerated claims, it will be understood that the exemplified subject matter is not intended to limit the claims to the disclosed subject matter.

Throughout this document, values expressed in a range format should be interpreted in a flexible manner to include not only the numerical values explicitly recited as the limits of the range, but also to include all the individual numerical values or sub-ranges encompassed within that range as if each numerical value and sub-range is explicitly recited. For example, a range of "about 0.1% to about 5%" or "about 0.1% to 5%" should be interpreted to include not just about 0.1% to about 5%, but also the individual values (*e.g.*, 1%, 2%, 3%, and 4%) and the sub-ranges (*e.g.*, 0.1% to 0.5%, 1.1% to 2.2%, 3.3% to 4.4%) within the indicated range. The statement "about X to Y" has the same meaning as "about X to about Y," unless indicated otherwise. Likewise, the statement "about X, Y, or about Z" has the same meaning as "about X, about Y, or about Z," unless indicated otherwise.

In this document, the terms "a," "an," or "the" are used to include one or more than one unless the context clearly dictates otherwise. The term "or" is used to refer to a nonexclusive "or" unless otherwise indicated. The statement "at least one of A and B" or "at least one of A or B" has the same meaning as "A, B, or A and B." In addition, it is to be understood that the phraseology or terminology employed herein, and not otherwise defined, is for the purpose of description only and not of limitation. Any use of section headings is intended to aid reading of the document and is not to be interpreted as limiting; information that is relevant to a section heading may occur within or outside of that particular section. All publications, patents, and patent documents referred to in this document are incorporated by reference herein in their entirety, as though individually incorporated by reference.

In the methods described herein, the acts can be carried out in any order, except when a temporal or operational sequence is explicitly recited. Furthermore, specified acts can be carried out concurrently unless explicit claim language recites that they be carried out separately. For example, a claimed act of doing X and a claimed act of doing Y can be conducted simultaneously within a single operation, and the resulting process will fall within the literal scope of the claimed process.

## Description

Exercise exerts a wide range of beneficial effects for healthy physiology, and the sedentary lifestyle of modern societies is associated with several major diseases. However, the mechanisms regulating an individual's motivation to engage in physical activity remain incompletely understood. An important factor stimulating the engagement in both competitive and recreational exercise is the motivating pleasure derived from prolonged physical activity, which is triggered by exercise-induced neurochemical changes in the brain.

The present disclosure relates, in part, to the discovery of a gut-brain connection in mice that enhances exercise performance by augmenting dopamine signaling during physical activity. As described herein, it has been found that microbiome-dependent production of endocannabinoid metabolites in the gut stimulates the activity of TRPV1-expressing sensory neurons and thereby elevates dopamine levels in the ventral striatum during exercise. Stimulation of this pathway (*i.e.*, interoceptive stimulation) improves running performance, while microbiome

depletion, peripheral endocannabinoid receptor inhibition, ablation of spinal afferent neurons, or dopamine blockade abrogate exercise capacity.

These findings indicate that the rewarding properties of exercise are influenced by gut derived interoceptive circuits and provide a microbiome-dependent explanation for inter-individual variability in exercise performance. The data provided herein provide support for the use of interoceptomimetic molecules that stimulate the transmission of gut-derived signals to the brain to enhance the motivation for exercise.

### Definitions

The term "about" as used herein can allow for a degree of variability in a value or range, for example, within 10%, within 5%, or within 1% of a stated value or of a stated limit of a range, and includes the exact stated value or range.

The term "alkenyl" as used herein refers to straight and branched chain and cyclic alkyl groups as defined herein, except that at least one double bond exists between two carbon atoms. Thus, alkenyl groups have from 2 to 40 carbon atoms, or 2 to about 20 carbon atoms, or 2 to 12 carbon atoms or, in some embodiments, from 2 to 8 carbon atoms. Examples include, but are not limited to vinyl,  $-\text{CH}=\text{CCH}_2$ ,  $-\text{CH}=\text{CH}(\text{CH}_3)$ ,  $-\text{CH}=\text{C}(\text{CH}_3)_2$ ,  $-\text{C}(\text{CH}_3)=\text{CH}_2$ ,  $-\text{C}(\text{CH}_3)=\text{CH}(\text{CH}_3)$ ,  $-\text{C}(\text{CH}_2\text{CH}_3)=\text{CH}_2$ , cyclohexenyl, cyclopentenyl, cyclohexadienyl, butadienyl, pentadienyl, and hexadienyl among others.

The term "alkoxy" as used herein refers to an oxygen atom connected to an alkyl group, including a cycloalkyl group, as are defined herein. Examples of linear alkoxy groups include but are not limited to methoxy, ethoxy, propoxy, butoxy, pentyloxy, hexyloxy, and the like. Examples of branched alkoxy include but are not limited to isopropoxy, sec-butoxy, tert-butoxy, isopentyloxy, isohexyloxy, and the like. Examples of cyclic alkoxy include but are not limited to cyclopropyloxy, cyclobutyloxy, cyclopentyloxy, cyclohexyloxy, and the like. An alkoxy group can include about 1 to about 12, about 1 to about 20, or about 1 to about 40 carbon atoms bonded to the oxygen atom, and can further include double or triple bonds, and can also include heteroatoms. For example, an allyloxy group or a methoxyethoxy group is also an alkoxy group within the meaning herein, as is a methylenedioxy group in a context where two adjacent atoms of a structure are substituted therewith.

The term "alkyl" as used herein refers to straight chain and branched alkyl groups and

cycloalkyl groups having from 1 to 40 carbon atoms, 1 to about 20 carbon atoms, 1 to 12 carbons or, in some embodiments, from 1 to 8 carbon atoms. Examples of straight chain alkyl groups include those with from 1 to 8 carbon atoms such as methyl, ethyl, n-propyl, n-butyl, n-pentyl, n-hexyl, n-heptyl, and n-octyl groups. Examples of branched alkyl groups include, but are not limited to, isopropyl, iso-butyl, sec-butyl, t-butyl, neopentyl, isopentyl, and 2,2-dimethylpropyl groups. As used herein, the term "alkyl" encompasses n-alkyl, isoalkyl, and anteisoalkyl groups as well as other branched chain forms of alkyl. Representative substituted alkyl groups can be substituted one or more times with any of the groups listed herein, for example, amino, hydroxy, cyano, carboxy, nitro, thio, alkoxy, and halogen groups.

The term "amine" as used herein refers to primary, secondary, and tertiary amines having, *e.g.*, the formula  $N(\text{group})_3$  wherein each group can independently be H or non-H, such as alkyl, aryl, and the like. Amines include but are not limited to  $R-NH_2$ , for example, alkylamines, arylamines, alkylarylamines;  $R_2NH$  wherein each R is independently selected, such as dialkylamines, diarylamines, aralkylamines, heterocyclylamines and the like; and  $R_3N$  wherein each R is independently selected, such as trialkylamines, dialkylarylamines, alkyl diarylamines, triarylamines, and the like. The term "amine" also includes ammonium ions as used herein.

The term "amino acid" as used herein refers to an organic compound comprising both a carboxylic acid group and an amine group. The term "amino acid" includes both "natural" and "unnatural" or "non-natural" amino acids. Additionally, the term amino acid includes O-alkylated or N-alkylated amino acids, as well as amino acids having nitrogen or oxygen-containing side chains (*e.g.*, Lys, Orn, or Ser) in which the nitrogen or oxygen atom has been acylated or alkylated. Amino acids may be pure L or D isomers or mixtures of L and D isomers, including racemic mixtures.

The term "amino group" as used herein refers to a substituent of the form  $-NH_2$ ,  $-NHR$ ,  $-NR_2$ ,  $-NR_3^+$ , wherein each R is independently selected, and protonated forms of each, except for  $-NR_3^+$ , which cannot be protonated. Accordingly, any compound substituted with an amino group can be viewed as an amine. An "amino group" within the meaning herein can be a primary, secondary, tertiary, or quaternary amino group. An "alkylamino" group includes a monoalkylamino, dialkylamino, and trialkylamino group.

The term "aryl" as used herein refers to cyclic aromatic hydrocarbon groups that do not contain heteroatoms in the ring. Thus aryl groups include, but are not limited to, phenyl,

azulenyl, heptalenyl, biphenyl, indacenyl, fluorenyl, phenanthrenyl, triphenylenyl, pyrenyl, naphthacenyl, chrysenyl, biphenylenyl, anthracenyl, and naphthyl groups. In some embodiments, aryl groups contain about 6 to about 14 carbons in the ring portions of the groups. Aryl groups can be unsubstituted or substituted, as defined herein. Representative substituted  
5 aryl groups can be mono-substituted or substituted more than once, such as, but not limited to, a phenyl group substituted at any one or more of 2-, 3-, 4-, 5-, or 6-positions of the phenyl ring, or a naphthyl group substituted at any one or more of 2- to 8-positions thereof.

The term "cycloalkyl" as used herein refers to cyclic alkyl groups such as, but not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and cyclooctyl groups. In  
10 some embodiments, the cycloalkyl group can have 3 to about 8-12 ring members, whereas in other embodiments the number of ring carbon atoms range from 3 to 4, 5, 6, or 7. Cycloalkyl groups further include polycyclic cycloalkyl groups such as, but not limited to, norbornyl, adamantyl, bornyl, camphenyl, isocamphenyl, and carenyl groups, and fused rings such as, but not limited to, decalinyl, and the like. Cycloalkyl groups also include rings that are substituted  
15 with straight or branched chain alkyl groups as defined herein. Representative substituted cycloalkyl groups can be mono-substituted or substituted more than once, such as, but not limited to, 2,2-, 2,3-, 2,4- 2,5- or 2,6-disubstituted cyclohexyl groups or mono-, di- or tri-substituted norbornyl or cycloheptyl groups, which can be substituted with, for example, amino, hydroxy, cyano, carboxy, nitro, thio, alkoxy, and halogen groups. The term "cycloalkenyl" alone  
20 or in combination denotes a cyclic alkenyl group.

The term "dietary supplement" as used herein refers to a product intended to supplement or complement a subject's diet, the product comprising one or more substances with a nutritional and/or physiological effect on a subject. The dietary supplement can be a partial nutritional composition, which does not contain all the essential macro- and micronutrients and hence may  
25 not be used to replace one or more daily meals and/or may not be used as the sole source of nutrition of a subject. The dietary supplement may be a liquid, powder, gel, paste, solid, concentrate, suspension or ready-to-use formulation. The dietary supplement may further comprise one or more additional ingredients, including vitamins, minerals, herbs or other botanicals, amino acids, and substances such as enzymes, organ tissues, glandulars, and  
30 metabolites.

The term "edible base" as used herein refers to any edible material, hard or soft,

including vary degrees of hardness or softness. Examples of suitable bases and/or substrates include, but are not limited to, inulin, starch, modified starches, xanthan gum, carboxymethyl cellulose, methyl cellulose, hydroxypropylmethyl cellulose, konjac, chitosan, tragacanth, karaya, ghatti, larch, carageenan, alginate, chemically modified alginate, agar, guar, locust bean, psyllium, tara, gellan, curdlan, pullan, gum arabic, gelatin, pectin, and combinations thereof. In certain embodiments, the edible base is intended to include any edible food, such as fish, chicken, vegetables, legumes, fruits, meats, and the like.

As used herein, the terms "effective amount," "pharmaceutically effective amount" and "therapeutically effective amount" refer to a nontoxic but sufficient amount of an agent to provide the desired biological result. That result may be reduction and/or alleviation of the signs, symptoms, or causes of a disease, or any other desired alteration of a biological system. An appropriate therapeutic amount in any individual case may be determined by one of ordinary skill in the art using routine experimentation.

The term "engineered bacteria" or "engineered bacterium" as used herein refers to a modified bacterium or bacterial population that includes one or more gene sequences (*e.g.*, nucleic acid sequences) or activities distinct from a nucleic acid sequence or activity present in the bacteria utilized as a starting point for modification and/or alteration (*e.g.*, host bacteria and/or wild type counterpart). In certain embodiments, an engineered bacteria includes a heterologous polynucleotide.

The term "exercise" as used herein refers, in its broad sense, to putting oneself into action or to tiring effort for the sake of developing and maintaining physical fitness, including but not limited to aerobic exercise, anaerobic exercise, and non-traditional exercises, such as for example, dance, physical therapy.

The terms "halo," "halogen," or "halide" group, as used herein, by themselves or as part of another substituent, mean, unless otherwise stated, a fluorine, chlorine, bromine, or iodine atom.

The term "heteroaryl" as used herein refers to aromatic ring compounds containing 5 or more ring members, of which, one or more is a heteroatom such as, but not limited to, N, O, and S; for instance, heteroaryl rings can have 5 to about 8-12 ring members. A heteroaryl group is a variety of a heterocyclyl group that possesses an aromatic electronic structure. A heteroaryl group designated as a C<sub>2</sub>-heteroaryl can be a 5-ring with two carbon atoms and three

heteroatoms, a 6-ring with two carbon atoms and four heteroatoms and so forth. Likewise a C<sub>4</sub>-heteroaryl can be a 5-ring with one heteroatom, a 6-ring with two heteroatoms, and so forth. The number of carbon atoms plus the number of heteroatoms sums up to equal the total number of ring atoms. Heteroaryl groups include, but are not limited to, groups such as pyrrolyl, pyrazolyl, triazolyl, tetrazolyl, oxazolyl, isoxazolyl, thiazolyl, pyridinyl, thiophenyl, benzothiophenyl, benzofuranyl, indolyl, azaindolyl, indazolyl, benzimidazolyl, azabenzimidazolyl, benzoxazolyl, benzothiazolyl, benzothiadiazolyl, imidazopyridinyl, isoxazolopyridinyl, thianaphthalenyl, purinyl, xanthinyl, adeninyl, guaninyl, quinolinyl, isoquinolinyl, tetrahydroquinolinyl, quinoxalinyl, and quinazolinyl groups. Heteroaryl groups can be unsubstituted, or can be substituted with groups as is discussed herein. Representative substituted heteroaryl groups can be substituted one or more times with groups such as those listed herein.

Additional examples of aryl and heteroaryl groups include but are not limited to phenyl, biphenyl, indenyl, naphthyl (1-naphthyl, 2-naphthyl), N-hydroxytetrazolyl, N-hydroxytriazolyl, N-hydroxyimidazolyl, anthracenyl (1-anthracenyl, 2-anthracenyl, 3-anthracenyl), thiophenyl (2-thienyl, 3-thienyl), furyl (2-furyl, 3-furyl), indolyl, oxadiazolyl, isoxazolyl, quinazolinyl, fluorenyl, xanthenyl, isoindanyl, benzhydryl, acridinyl, thiazolyl, pyrrolyl (2-pyrrolyl), pyrazolyl (3-pyrazolyl), imidazolyl (1-imidazolyl, 2-imidazolyl, 4-imidazolyl, 5-imidazolyl), triazolyl (1,2,3-triazol-1-yl, 1,2,3-triazol-2-yl, 1,2,3-triazol-4-yl, 1,2,4-triazol-3-yl), oxazolyl (2-oxazolyl, 4-oxazolyl, 5-oxazolyl), thiazolyl (2-thiazolyl, 4-thiazolyl, 5-thiazolyl), pyridyl (2-pyridyl, 3-pyridyl, 4-pyridyl), pyrimidinyl (2-pyrimidinyl, 4-pyrimidinyl, 5-pyrimidinyl, 6-pyrimidinyl), pyrazinyl, pyridazinyl (3-pyridazinyl, 4-pyridazinyl, 5-pyridazinyl), quinolyl (2-quinolyl, 3-quinolyl, 4-quinolyl, 5-quinolyl, 6-quinolyl, 7-quinolyl, 8-quinolyl), isoquinolyl (1-isoquinolyl, 3-isoquinolyl, 4-isoquinolyl, 5-isoquinolyl, 6-isoquinolyl, 7-isoquinolyl, 8-isoquinolyl), benzo[b]furanyl (2-benzo[b]furanyl, 3-benzo[b]furanyl, 4-benzo[b]furanyl, 5-benzo[b]furanyl, 6-benzo[b]furanyl, 7-benzo[b]furanyl), 2,3-dihydro-benzo[b]furanyl (2-(2,3-dihydro-benzo[b]furanyl), 3-(2,3-dihydro-benzo[b]furanyl), 4-(2,3-dihydro-benzo[b]furanyl), 5-(2,3-dihydro-benzo[b]furanyl), 6-(2,3-dihydro-benzo[b]furanyl), 7-(2,3-dihydro-benzo[b]furanyl), benzo[b]thiophenyl (2-benzo[b]thiophenyl, 3-benzo[b]thiophenyl, 4-benzo[b]thiophenyl, 5-benzo[b]thiophenyl, 6-benzo[b]thiophenyl, 7-benzo[b]thiophenyl), 2,3-dihydro-benzo[b]thiophenyl, (2-(2,3-dihydro-benzo[b]thiophenyl), 3-(2,3-dihydro-benzo[b]thiophenyl), 4-(2,3-dihydro-benzo[b]thiophenyl), 5-(2,3-dihydro-benzo[b]thiophenyl),

6-(2,3-dihydro-benzo[b]thiophenyl), 7-(2,3-dihydro-benzo[b]thiophenyl), indolyl (1-indolyl, 2-indolyl, 3-indolyl, 4-indolyl, 5-indolyl, 6-indolyl, 7-indolyl), indazole (1-indazolyl, 3-indazolyl, 4-indazolyl, 5-indazolyl, 6-indazolyl, 7-indazolyl), benzimidazolyl (1-benzimidazolyl, 2-benzimidazolyl, 4-benzimidazolyl, 5-benzimidazolyl, 6-benzimidazolyl, 7-benzimidazolyl, 8-benzimidazolyl), benzoxazolyl (1-benzoxazolyl, 2-benzoxazolyl), benzothiazolyl (1-benzothiazolyl, 2-benzothiazolyl, 4-benzothiazolyl, 5-benzothiazolyl, 6-benzothiazolyl, 7-benzothiazolyl), carbazolyl (1-carbazolyl, 2-carbazolyl, 3-carbazolyl, 4-carbazolyl), 5H-dibenz[b,f]azepine (5H-dibenz[b,f]azepine-1-yl, 5H-dibenz[b,f]azepine-2-yl, 5H-dibenz[b,f]azepine-3-yl, 5H-dibenz[b,f]azepine-4-yl, 5H-dibenz[b,f]azepine-5-yl), 10,11-dihydro-5H-dibenz[b,f]azepine (10,11-dihydro-5H-dibenz[b,f]azepine-1-yl, 10,11-dihydro-5H-dibenz[b,f]azepine-2-yl, 10,11-dihydro-5H-dibenz[b,f]azepine-3-yl, 10,11-dihydro-5H-dibenz[b,f]azepine-4-yl, 10,11-dihydro-5H-dibenz[b,f]azepine-5-yl), and the like.

The term "heterocycloalkyl" as used herein refers to an aliphatic, partially unsaturated or fully saturated, 3- to 14-membered ring system, including single rings of 3 to 8 atoms and bi- and tricyclic ring systems where at least one of the carbon atoms of the ring is replaced with a heteroatom such as, but not limited to, nitrogen, oxygen, sulfur, or phosphorus. A heterocycloalkyl can include one to four heteroatoms independently selected from oxygen, nitrogen, and sulfur, wherein a nitrogen and sulfur heteroatom optionally can be oxidized and a nitrogen heteroatom can be optionally substituted. Representative heterocycloalkyl groups include, but are not limited, to the following exemplary groups: pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperidinyl, piperazinyl, oxazolidinyl, isoxazolidinyl, morpholinyl, thiazolidinyl, isothiazolidinyl, and tetrahydrofuryl. The term heterocycloalkyl group can also be a C<sub>2</sub> heterocycloalkyl, C<sub>2</sub>-C<sub>3</sub> heterocycloalkyl, C<sub>2</sub>-C<sub>4</sub> heterocycloalkyl, C<sub>2</sub>-C<sub>5</sub> heterocycloalkyl, C<sub>2</sub>-C<sub>6</sub> heterocycloalkyl, C<sub>2</sub>-C<sub>7</sub> heterocycloalkyl, C<sub>2</sub>-C<sub>8</sub> heterocycloalkyl, C<sub>2</sub>-C<sub>9</sub> heterocycloalkyl, C<sub>2</sub>-C<sub>10</sub> heterocycloalkyl, C<sub>2</sub>-C<sub>11</sub> heterocycloalkyl, and the like, up to and including a C<sub>2</sub>-C<sub>14</sub> heterocycloalkyl. For example, a C<sub>2</sub> heterocycloalkyl comprises a group which has two carbon atoms and at least one heteroatom, including, but not limited to, aziridinyl, diazetidinyl, oxiranyl, thiiranyl, and the like. Alternatively, for example, a C<sub>5</sub> heterocycloalkyl comprises a group which has five carbon atoms and at least one heteroatom, including, but not limited to, piperidinyl, tetrahydropyranyl, tetrahydrothiopyranyl, diazepanyl,

and the like. It is understood that a heterocycloalkyl group may be bound either through a heteroatom in the ring, where chemically possible, or one of carbons comprising the heterocycloalkyl ring. The heterocycloalkyl group can be substituted or unsubstituted.

The term "hydrocarbon" or "hydrocarbyl" as used herein refers to a molecule or functional group that includes carbon and hydrogen atoms. The term can also refer to a molecule or functional group that normally includes both carbon and hydrogen atoms but wherein all the hydrogen atoms are substituted with other functional groups.

The term "independently selected from" as used herein refers to referenced groups being the same, different, or a mixture thereof, unless the context clearly indicates otherwise. Thus, under this definition, the phrase " $X^1$ ,  $X^2$ , and  $X^3$  are independently selected from noble gases" would include the scenario where, for example,  $X^1$ ,  $X^2$ , and  $X^3$  are all the same, where  $X^1$ ,  $X^2$ , and  $X^3$  are all different, where  $X^1$  and  $X^2$  are the same but  $X^3$  is different, and other analogous permutations.

The term "metabolite" as used herein refers to a derivative of a compound that is formed when the compound is metabolized. Non-limiting examples include decarboxylation of serine to provide ethanolamine, and the biosynthesis of taurine from cysteine.

The term "metabolized" as used herein refers to the sum of the processes by which a particular substance is converted by an organism, including but not limited to hydrolysis reactions and enzyme-catalyzed reactions. In some embodiments, the enzyme produces a structurally modified compound. For example, cytochrome P450 catalyzes various oxidation and reduction reactions which chemically modify substrates. More information on metabolism can be found in *The Pharmacologic Basis of Therapeutics*, 9th Edition, McGraw-Hill (1996).

The term "motivation" as used herein refers to an internal drive to engage in reward seeking behavior (*e.g.*, dopamine response to physical exercise and/or exertion).

The term "natural amino acid" and equivalent expressions refer to L-amino acids commonly found in naturally occurring proteins. Examples of natural amino acids include, without limitation, alanine (Ala), cysteine (Cys), aspartic acid (Asp), glutamic acid (Glu), phenylalanine (Phe), glycine (Gly), histidine (His), isoleucine (Ile), lysine (Lys), leucine (Leu), methionine (Met), asparagine (Asn), proline (Pro), glutamine (Gln), arginine (Arg), serine (Ser), threonine (Thr), valine (Val), tryptophan (Trp), tyrosine (Tyr),  $\beta$ -alanine ( $\beta$ -ALA), and  $\gamma$ -aminobutyric acid (GABA).

The terms "patient," "subject," or "individual" are used interchangeably herein, and refer to any animal, or cells thereof whether in vitro or in situ, amenable to the methods described herein. In a non-limiting embodiment, the patient, subject or individual is a human.

As used herein, the term "pharmaceutically acceptable" refers to a material, such as a carrier or diluent, which does not abrogate the biological activity or properties of the compound, and is relatively non-toxic, *i.e.*, the material may be administered to an individual without causing undesirable biological effects or interacting in a deleterious manner with any of the components of the composition in which it is contained.

As used herein, the language "pharmaceutically acceptable salt" refers to a salt of the administered compounds prepared from pharmaceutically acceptable non-toxic acids or bases, including inorganic acids or bases, organic acids or bases, solvates, hydrates, or clathrates thereof.

Suitable pharmaceutically acceptable acid addition salts may be prepared from an inorganic acid or from an organic acid. Examples of inorganic acids include hydrochloric, hydrobromic, hydriodic, nitric, carbonic, sulfuric (including sulfate and hydrogen sulfate), and phosphoric acids (including hydrogen phosphate and dihydrogen phosphate). Appropriate organic acids may be selected from aliphatic, cycloaliphatic, aromatic, araliphatic, heterocyclic, carboxylic and sulfonic classes of organic acids, examples of which include formic, acetic, propionic, succinic, glycolic, gluconic, lactic, malic, tartaric, citric, ascorbic, glucuronic, maleic, malonic, saccharin, fumaric, pyruvic, aspartic, glutamic, benzoic, anthranilic, 4-hydroxybenzoic, phenylacetic, mandelic, embonic (pamoic), methanesulfonic, ethanesulfonic, benzenesulfonic, pantothenic, trifluoromethanesulfonic, 2-hydroxyethanesulfonic, p-toluenesulfonic, sulfanilic, cyclohexylaminosulfonic, stearic, alginic,  $\beta$ -hydroxybutyric, salicylic, galactaric and galacturonic acid.

Suitable pharmaceutically acceptable base addition salts of compounds described herein include, for example, ammonium salts, metallic salts including alkali metal, alkaline earth metal and transition metal salts such as, for example, calcium, magnesium, potassium, sodium and zinc salts. Pharmaceutically acceptable base addition salts also include organic salts made from basic amines such as, for example, N,N'-dibenzylethylene-diamine, chlorprocaine, choline, diethanolamine, ethylenediamine, meglumine (N-methylglucamine) and procaine. All of these salts may be prepared from the corresponding compound by reacting, for example, the

appropriate acid or base with the compound.

As used herein, the term "pharmaceutically acceptable carrier" or "pharmaceutically acceptable excipient" means a pharmaceutically acceptable material, composition or carrier, such as a liquid or solid filler, stabilizer, dispersing agent, suspending agent, diluent, excipient, thickening agent, solvent or encapsulating material, involved in carrying or transporting a compound described herein within or to the patient such that it may perform its intended function. Typically, such compounds are carried or transported from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation, including the compound(s) described herein, and not injurious to the patient. Some examples of materials that may serve as pharmaceutically acceptable carriers include: sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; surface active agents; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; phosphate buffer solutions; and other non-toxic compatible substances employed in pharmaceutical formulations. As used herein, "pharmaceutically acceptable carrier" also includes any and all coatings, antibacterial and antifungal agents, and absorption delaying agents, and the like that are compatible with the activity of the compound(s) described herein, and are physiologically acceptable to the patient. Supplementary active compounds may also be incorporated into the compositions. The "pharmaceutically acceptable carrier" may further include a pharmaceutically acceptable salt of the compound(s) described herein. Other additional ingredients that may be included in the pharmaceutical compositions used with the methods or compounds described herein are known in the art and described, for example in Remington's Pharmaceutical Sciences (Genaro, Ed., Mack Publishing Co., 1985, Easton, PA), which is incorporated herein by reference.

The term "physical performance" as used herein refers to endurance (*e.g.*, cardiovascular endurance), muscle mass, muscle function, muscle strength, and/or body composition of an

individual. The term “enhanced physical performance” as used herein, refers to a measurable improvement and/or enhancement in at least one of body composition, endurance, muscle mass, muscle function, and muscle strength of an individual. In a non-limiting embodiment, enhancement in physical performance may include improved cardiovascular endurance (*e.g.*, capacity for aerobic exercise). In further non-limiting embodiments, with respect to body composition, an enhancement in physical performance may include an increased ratio of lean body mass (*i.e.*, ratio of muscle mass to fat mass) in a subject.

The term “precursor” or “biosynthetic precursor” as used herein refers to an agent or compound which, when provided to, brought into contact with, or included in the culture medium of a microorganism, serves to enhance or increase biosynthesis of the desired product.

The term “plasmid” or “vector” as used herein refers to a genetic construct that is composed of genetic material (*i.e.*, nucleic acids). Typically, a plasmid or a vector contains an origin of replication that is functional in bacterial host cells (*e.g.*, *Escherichia coli*), and selectable markers for detecting bacterial host cells including the plasmid. Plasmids and vectors may include one or more genetic elements as described herein arranged such that an inserted coding sequence can be transcribed and translated in a suitable expression cells. In addition, the plasmid or vector may include one or more nucleic acid segments, genes, promoters, enhancers, activators, multiple cloning regions, or any combination thereof, including segments that are obtained from or derived from one or more natural and/or artificial sources.

The term “prebiotic” as used herein refers to any non-digestible substance that stimulates the growth and/or activity of bacteria in the digestive system.

The term “probiotic” as used herein refers to a microbial cell population or preparation, or a component of a cell population or preparation, which when administered to a subject in an effective amount promotes a health benefit (*e.g.*, stimulation and/or motivation to exercise and/or enhanced exercise performance) in the subject.

The term “unnatural amino acid” refers to any derivative of a natural amino acid including D forms, and  $\alpha$ - and  $\beta$ -amino acid derivatives. The terms “unnatural amino acid” and “non-natural amino acid” are used interchangeably herein and are meant to include the same moieties. It is noted that certain amino acids, *e.g.*, hydroxyproline, that are classified as a non-natural amino acid herein, may be found in nature within a certain organism or a particular protein. Amino acids with many different protecting groups appropriate for immediate use in the

solid phase synthesis of peptides are commercially available. In addition to the twenty most common naturally occurring amino acids, the following examples of non-natural amino acids and amino acid derivatives may be used according to the invention (common abbreviations in parentheses): 2-aminoadipic acid (Aad), 3-aminoadipic acid ( $\beta$ -Aad), 2-aminobutyric acid (2-Abu),  $\alpha,\beta$ -dehydro-2-aminobutyric acid (8-AU), 1-aminocyclopropane-1-carboxylic acid (ACPC), aminoisobutyric acid (Aib), 3-aminoisobutyric acid ( $\beta$ -Aib), 2-amino-thiazoline-4-carboxylic acid, 5-aminovaleric acid (5-Ava), 6-aminoheptanoic acid (6-Ahx), 2-aminoheptanoic acid (Ahe), 8-aminooctanoic acid (8-Aoc), 11-aminoundecanoic acid (11-Aun), 12-aminododecanoic acid (12-Ado), 2-aminobenzoic acid (2-Abz), 3-aminobenzoic acid (3-Abz), 4-aminobenzoic acid (4-Abz), 4-amino-3-hydroxy-6-methylheptanoic acid (Statine, Sta), aminooxyacetic acid (Aoa), 2-aminotetraline-2-carboxylic acid (ATC), 4-amino-5-cyclohexyl-3-hydroxypentanoic acid (ACHPA), para-aminophenylalanine (4-NH<sub>2</sub>-Phe), 2-aminopimelic acid (Apm), biphenylalanine (Bip), para-bromophenylalanine (4-Br-Phe), ortho-chlorophenylalanine (2-Cl-Phe), meta-chlorophenylalanine (3-Cl-Phe), para-chlorophenylalanine (4-Cl-Phe), meta-chlorotyrosine (3-Cl-Tyr), para-benzoylphenylalanine (Bpa), tert-butylglycine (TLG), cyclohexylalanine (Cha), cyclohexylglycine (Chg), desmosine (Des), 2,2-diaminopimelic acid (Dpm), 2,3-diaminopropionic acid (Dpr), 2,4-diaminobutyric acid (Dbu), 3,4-dichlorophenylalanine (3,4-Cl<sub>2</sub>-Phe), 3,4-difluorophenylalanine (3,4-F<sub>2</sub>-Phe), 3,5-diiodotyrosine (3,5-I<sub>2</sub>-Tyr), N-ethylglycine (EtGly), N-ethylasparagine (EtAsn), ortho-fluorophenylalanine (2-F-Phe), meta-fluorophenylalanine (3-F-Phe), para-fluorophenylalanine (4-F-Phe), meta-fluorotyrosine (3-F-Tyr), homoserine (Hse), homophenylalanine (Hfe), homotyrosine (Htyr), hydroxylysine (Hyl), allo-hydroxylysine (aHyl), 5-hydroxytryptophan (5-OH-Trp), 3- or 4-hydroxyproline (3- or 4-Hyp), para-iodophenylalanine (4-I-Phe), 3-iodotyrosine (3-I-Tyr), indoline-2-carboxylic acid (Idc), isodesmosine (Ide), allo-isoleucine (a-Ile), isonipecotic acid (Inp), N-methylisoleucine (Melle), N-methyllysine (MeLys), meta-methyltyrosine (3-Me-Tyr), N-methylvaline (MeVal), 1-naphthylalanine (1-NaI), 2-naphthylalanine (2-NaI), para-nitrophenylalanine (4-NO<sub>2</sub>-Phe), 3-nitrotyrosine (3-NO<sub>2</sub>-Tyr), norleucine (Nle), norvaline (Nva), ornithine (Orn), ortho-phosphotyrosine (H<sub>2</sub>PO<sub>3</sub>-Tyr), octahydroindole-2-carboxylic acid (Oic), penicillamine (Pen), pentafluorophenylalanine (F<sub>5</sub>-Phe), phenylglycine (Phg), pipecolic acid (Pip), propargylglycine (Pra), pyroglutamic acid (PGLU), sarcosine (Sar),

tetrahydroisoquinoline-3-carboxylic acid (Tic), thienylalanine, and thiazolidine-4-carboxylic acid (thioprolin, Th).

The term "substantially" as used herein refers to a majority of, or mostly, as in at least about 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9%, 99.99%, or at least about 99.999% or more, or 100%. The term "substantially free of" as used herein can mean having none or having a trivial amount of, such that the amount of material present does not affect the material properties of the composition including the material, such that the composition is about 0 wt% to about 5 wt% of the material, or about 0 wt% to about 1 wt%, or about 5 wt% or less, or less than, equal to, or greater than about 4.5 wt%, 4, 3.5, 3, 2.5, 2, 1.5, 1, 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1, 0.01, or about 0.001 wt% or less. The term "substantially free of" can mean having a trivial amount of, such that a composition is about 0 wt% to about 5 wt% of the material, or about 0 wt% to about 1 wt%, or about 5 wt% or less, or less than, equal to, or greater than about 4.5 wt%, 4, 3.5, 3, 2.5, 2, 1.5, 1, 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1, 0.01, or about 0.001 wt% or less, or about 0 wt%.

The term "substituted" as used herein in conjunction with a molecule or an organic group as defined herein refers to the state in which one or more hydrogen atoms contained therein are replaced by one or more non-hydrogen atoms. The term "functional group" or "substituent" as used herein refers to a group that can be or is substituted onto a molecule or onto an organic group. Examples of substituents or functional groups include, but are not limited to, a halogen (*e.g.*, F, Cl, Br, and I); an oxygen atom in groups such as hydroxy groups, alkoxy groups, aryloxy groups, aralkyloxy groups, oxo(carbonyl) groups, carboxyl groups including carboxylic acids, carboxylates, and carboxylate esters; a sulfur atom in groups such as thiol groups, alkyl and aryl sulfide groups, sulfoxide groups, sulfone groups, sulfonyl groups, and sulfonamide groups; a nitrogen atom in groups such as amines, hydroxyamines, nitriles, nitro groups, N-oxides, hydrazides, azides, and enamines; and other heteroatoms in various other groups. Non-limiting examples of substituents that can be bonded to a substituted carbon (or other) atom include F, Cl, Br, I, OR, OC(O)N(R)<sub>2</sub>, CN, NO, NO<sub>2</sub>, ONO<sub>2</sub>, azido, CF<sub>3</sub>, OCF<sub>3</sub>, R, O (oxo), S (thiono), C(O), S(O), methylenedioxy, ethylenedioxy, N(R)<sub>2</sub>, SR, SOR, SO<sub>2</sub>R, SO<sub>2</sub>N(R)<sub>2</sub>, SO<sub>3</sub>R, C(O)R, C(O)C(O)R, C(O)CH<sub>2</sub>C(O)R, C(S)R, C(O)OR, OC(O)R, C(O)N(R)<sub>2</sub>, OC(O)N(R)<sub>2</sub>, C(S)N(R)<sub>2</sub>, (CH<sub>2</sub>)<sub>0-2</sub>N(R)C(O)R, (CH<sub>2</sub>)<sub>0-2</sub>N(R)N(R)<sub>2</sub>, N(R)N(R)C(O)R, N(R)N(R)C(O)OR, N(R)N(R)CON(R)<sub>2</sub>, N(R)SO<sub>2</sub>R, N(R)SO<sub>2</sub>N(R)<sub>2</sub>, N(R)C(O)OR, N(R)C(O)R, N(R)C(S)R,

N(R)C(O)N(R)<sub>2</sub>, N(R)C(S)N(R)<sub>2</sub>, N(COR)COR, N(OR)R, C(=NH)N(R)<sub>2</sub>, C(O)N(OR)R, and C(=NOR)R, wherein R can be hydrogen or a carbon-based moiety; for example, R can be hydrogen, (C<sub>1</sub>-C<sub>100</sub>) hydrocarbyl, alkyl, acyl, cycloalkyl, aryl, aralkyl, heterocyclyl, heteroaryl, or heteroarylalkyl; or wherein two R groups bonded to a nitrogen atom or to adjacent nitrogen atoms can together with the nitrogen atom or atoms form a heterocyclyl.

## Methods

In one aspect, the present disclosure provides a method of stimulating and/or enhancing exercise motivation and/or physical performance in a subject, the method comprising administering to the subject at least one dietary supplement composition of the present disclosure.

In another aspect, the present disclosure provides a method of stimulating and/or enhancing exercise motivation and/or physical performance in a subject, the method comprising administering to the subject at least one pharmaceutical composition of the present disclosure.

In another aspect, the present disclosure provides a method of stimulating and/or enhancing exercise motivation and/or physical performance in a subject, the method comprising administering to the subject at least one engineered bacterial strain comprising the plasmid of the present disclosure.

In another aspect, the present disclosure provides a method of stimulating and/or enhancing exercise motivation and/or physical performance in a subject, the method comprising administering to the subject at least one probiotic composition of the present disclosure.

In another aspect, the present disclosure provides a method of stimulating and/or enhancing exercise motivation and/or physical performance in a subject, the method comprising administering to the subject at least one probiotic composition comprising at least one bacterial strain selected from the group consisting of *Coprococcus* bacterium and *Eubacterium* bacterium.

In another aspect, the present disclosure provides a method of stimulating and/or enhancing exercise motivation and/or physical performance in a subject, the method comprising administering to the subject at least one Cannabinoid Receptor Type 1 (CB1) agonist.

In certain embodiments, the method comprises oral administration.

In certain embodiments, the CB1 agonist is a fatty acid amide. In certain embodiments, the fatty acid amide comprises a fatty acyl moiety covalently bonded to a naturally occurring primary amine.

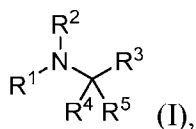
In certain embodiments, the fatty acyl moiety is selected from the group consisting of a saturated fatty acyl moiety and an unsaturated fatty acyl moiety. In certain embodiments, the saturated fatty acyl moiety is selected from the group consisting of stearoyl, palmitoyl, capryloyl, caproyl, lauroyl, myristoyl, arachidoyl, behenoyl, lignoceroyl, and cerotoyl. In certain embodiments, the unsaturated fatty acyl moiety is selected from the group consisting of arachidonoyl, oleoyl, myristoleoyl, palmitoleoyl, sapienoyl, elaidoyl, vaccenoyl, linoleoyl, linoelaidoyl,  $\alpha$ -linolenoyl, eicosapentaenoyl, erucoyl, and docosahexaenoyl.

In certain embodiments, the naturally occurring amine is selected from the group consisting of a neurotransmitter and an amino acid, or a metabolite and/or biosynthetic precursor thereof.

In certain embodiments, the neurotransmitter is selected from the group consisting of serotonin, dopamine, glutamate,  $\gamma$ -aminobutyric acid, acetylcholine, norepinephrine, and epinephrine.

In certain embodiments, naturally occurring amine is a metabolite and/or biosynthetic precursor of an amino acid. In certain embodiments, the metabolite and/or biosynthetic precursor of an amino acid is selected from the group consisting of ethanolamine and taurine.

In certain embodiments, the fatty acid amid is a compound of Formula (I), or a salt, solvate, stereoisomer, or isotopologue thereof:



wherein:

$\text{R}^1$  is selected from the group consisting of  $\text{C}(=\text{O})$ (optionally substituted  $\text{C}_1$ - $\text{C}_{25}$  alkyl) and  $\text{C}(=\text{O})$ (optionally substituted  $\text{C}_2$ - $\text{C}_{25}$  alkenyl);

$\text{R}^2$  is selected from the group consisting of H and optionally substituted  $\text{C}_1$ - $\text{C}_6$  alkyl;

$\text{R}^3$  is  $\text{C}_1$ - $\text{C}_6$  alkyl substituted with at least one substituent selected from the group consisting of optionally substituted  $\text{C}_6$ - $\text{C}_{10}$  aryl, optionally substituted  $\text{C}_2$ - $\text{C}_{10}$  heteroaryl, halogen,  $\text{OR}^A$ ,  $\text{N}(\text{R}^A)(\text{R}^B)$ ,  $\text{C}(=\text{O})\text{OR}^A$ ,  $\text{C}(=\text{O})\text{N}(\text{R}^A)(\text{R}^B)$ ,  $\text{S}(=\text{O})_2\text{OR}^A$ ,  $\text{P}(=\text{O})(\text{OR}^A)(\text{OR}^B)$ ,

$N(R^A)C(=NR^B)N(R^C)(R^D)$ ,  $OC(=O)R^A$ ,  $N(R^A)C(=O)R^B$ ,  $N(R^A)C(=O)OR^B$ ,  $OS(=O)_2R^A$ ,  $N(R^A)S(=O)_2R^A$ , and  $OP(=O)(OR^A)(OR^B)$ ;

$R^4$  and  $R^5$  are each independently from the group consisting of H, optionally substituted C<sub>1</sub>-C<sub>6</sub> alkyl;

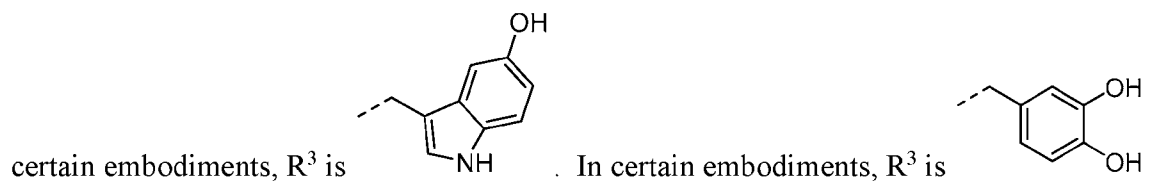
5  $R^A$ ,  $R^B$ ,  $R^C$ , and  $R^D$ , if present, are each independently selected from the group consisting of H, optionally substituted C<sub>1</sub>-C<sub>6</sub> alkyl, optionally substituted C<sub>2</sub>-C<sub>6</sub> heteroalkyl, optionally substituted C<sub>3</sub>-C<sub>8</sub> cycloalkyl, optionally substituted C<sub>2</sub>-C<sub>8</sub> heterocycloalkyl, optionally substituted C<sub>2</sub>-C<sub>6</sub> alkenyl, optionally substituted C<sub>6</sub>-C<sub>10</sub> aryl, and optionally substituted C<sub>2</sub>-C<sub>10</sub> heteroaryl.

10 In certain embodiments, each occurrence of optionally substituted C<sub>1</sub>-C<sub>6</sub> alkyl, optionally substituted C<sub>1</sub>-C<sub>25</sub> alkyl, optionally substituted C<sub>2</sub>-C<sub>6</sub> alkenyl, optionally substituted C<sub>2</sub>-C<sub>25</sub> alkenyl, optionally substituted C<sub>3</sub>-C<sub>8</sub> cycloalkyl, optionally substituted C<sub>2</sub>-C<sub>8</sub> heterocycloalkyl, and optionally substituted C<sub>2</sub>-C<sub>6</sub> heteroalkyl, optionally substituted C<sub>6</sub>-C<sub>10</sub> aryl, and optionally substituted C<sub>2</sub>-C<sub>10</sub> heteroaryl is independently optionally substituted with at least one substituent  
 15 selected from the group consisting of optionally substituted C<sub>1</sub>-C<sub>6</sub> alkyl, optionally substituted C<sub>2</sub>-C<sub>6</sub> alkenyl, optionally substituted C<sub>3</sub>-C<sub>8</sub> cycloalkyl, optionally substituted C<sub>2</sub>-C<sub>8</sub> heterocycloalkyl, optionally substituted C<sub>2</sub>-C<sub>6</sub> heteroalkyl, C<sub>6</sub>-C<sub>10</sub> aryl, halogen, NO<sub>2</sub>, CN, OR<sup>a</sup>, N(R<sup>a</sup>)(R<sup>b</sup>), C(=O)OR<sup>a</sup>, C(=O)N(R<sup>a</sup>)(R<sup>b</sup>), S(=O)<sub>2</sub>OR<sup>a</sup>, P(=O)(OR<sup>a</sup>)(OR<sup>b</sup>), OC(=O)R<sup>a</sup>, N(R<sup>a</sup>)C(=O)R<sup>a</sup>, OS(=O)<sub>2</sub>R<sup>a</sup>, N(R<sup>a</sup>)S(=O)<sub>2</sub>R<sup>a</sup>, OP(=O)(OR<sup>a</sup>)(OR<sup>b</sup>), wherein R<sup>a</sup> and R<sup>b</sup> are each  
 20 independently selected from the group consisting of H, C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>2</sub>-C<sub>6</sub> heteroalkyl, C<sub>3</sub>-C<sub>8</sub> cycloalkyl, C<sub>2</sub>-C<sub>8</sub> heterocycloalkyl, C<sub>2</sub>-C<sub>6</sub> alkenyl, C<sub>6</sub>-C<sub>10</sub> aryl, and C<sub>2</sub>-C<sub>10</sub> heteroaryl.

In certain embodiments, R<sup>1</sup> is selected from the group consisting of stearoyl, palmitoyl, capryloyl, caproyl, lauroyl, myristoyl, arachidoyl, behenoyl, lignoceroyl, cerotoyl, arachidonoyl, oleoyl, myristoleoyl, palmitoleoyl, sapienoyl, elaidoyl, vaccenoyl, linoleoyl, linoelaidoyl, α-  
 25 linolenoyl, eicosapentaenoyl, erucoyl, and docosahexaenoyl.

In certain embodiments, R<sup>2</sup> is H.

In certain embodiments, R<sup>3</sup> is CH<sub>2</sub>OH. In certain embodiments, R<sup>3</sup> is CH<sub>2</sub>S(=O)<sub>2</sub>OH. In



In certain embodiments, R<sup>4</sup> is H.

In certain embodiments, R<sup>5</sup> is H.

In certain embodiments, fatty acid amide is at least one selected from the group consisting of *N*-stearoyl serotonin, *N*-arachidonoyl dopamine, *N*-oleoyl dopamine, *N*-palmitoyl dopamine, *N*-stearoyl dopamine, *N*-stearoyl taurine, *N*-arachidonoyl ethanolamide, *N*-palmitoyl ethanolamide, and *N*-oleoyl ethanolamide.

In certain embodiments, the subject is administered at least two fatty acid amides selected from the group consisting of *N*-stearoyl serotonin, *N*-arachidonoyl dopamine, *N*-oleoyl dopamine, *N*-palmitoyl dopamine, *N*-stearoyl dopamine, *N*-stearoyl taurine, *N*-arachidonoyl ethanolamide, *N*-palmitoyl ethanolamide, and *N*-oleoyl ethanolamide.

In certain embodiments, the subject is administered at least three fatty acid amides selected from the group consisting of *N*-stearoyl serotonin, *N*-arachidonoyl dopamine, *N*-oleoyl dopamine, *N*-palmitoyl dopamine, *N*-stearoyl dopamine, *N*-stearoyl taurine, *N*-arachidonoyl ethanolamide, *N*-palmitoyl ethanolamide, and *N*-oleoyl ethanolamide.

In certain embodiments, the subject is administered at least four fatty acid amides selected from the group consisting of *N*-stearoyl serotonin, *N*-arachidonoyl dopamine, *N*-oleoyl dopamine, *N*-palmitoyl dopamine, *N*-stearoyl dopamine, *N*-stearoyl taurine, *N*-arachidonoyl ethanolamide, *N*-palmitoyl ethanolamide, and *N*-oleoyl ethanolamide.

In certain embodiments, the subject is administered at least five fatty acid amides selected from the group consisting of *N*-stearoyl serotonin, *N*-arachidonoyl dopamine, *N*-oleoyl dopamine, *N*-palmitoyl dopamine, *N*-stearoyl dopamine, *N*-stearoyl taurine, *N*-arachidonoyl ethanolamide, *N*-palmitoyl ethanolamide, and *N*-oleoyl ethanolamide.

In certain embodiments, the subject is administered *N*-palmitoyl dopamine, *N*-arachidonoyl dopamine, *N*-oleoyl dopamine, *N*-oleoyl ethanolamide, and *N*-palmitoyl ethanolamide.

In certain embodiments, the subject is administered at least one probiotic composition comprising at least one bacterial strain selected from the group consisting of *Coprococcus* bacterium and *Eubacterium* bacterium. In certain embodiments, the *Coprococcus* bacterium is *Coprococcus eutactus*. In certain embodiments, the *Eubacterium* is *Eubacterium rectale*.

In certain embodiments, enhanced striatal dopamine levels are observed in the subject after physical exercise as compared to striatal dopamine levels in the same subject after physical

exercise without administration of the CB1 agonist, optionally wherein the CB1 agonist is a fatty acid amide.

In certain embodiments, enhanced striatal dopamine levels are observed in the subject after physical exercise as compared to striatal dopamine levels in the same subject after physical exercise without administration of the dietary supplement composition of the present disclosure.

In certain embodiments, enhanced striatal dopamine levels are observed in the subject after physical exercise as compared to striatal dopamine levels in the same subject after physical exercise without administration of the pharmaceutical composition of the present disclosure.

In certain embodiments, enhanced striatal dopamine levels are observed in the subject after physical exercise as compared to striatal dopamine levels in the same subject after physical exercise without administration of the engineered bacterial strain comprising the plasmid of the present disclosure.

In certain embodiments, enhanced striatal dopamine levels are observed in the subject after physical exercise as compared to striatal dopamine levels in the same subject after physical exercise without administration of the probiotic composition of the present disclosure.

In certain embodiments, enhanced striatal dopamine levels are observed in the subject after physical exercise as compared to striatal dopamine levels in the same subject after physical exercise without administration of the probiotic composition comprising at least one bacterial strain selected from the group consisting of *Coproccoccus* bacterium and *Eubacterium* bacterium.

In certain embodiments, the subject exercises more frequently after administration of the CB1 agonist than before administration of the CB1 agonist, optionally wherein the CB1 agonist is a fatty acid amide.

In certain embodiments, the subject exercises more frequently after administration of the dietary supplement composition of the present disclosure than before administration of the dietary supplement composition of the present disclosure.

In certain embodiments, the subject exercises more frequently after administration of the pharmaceutical composition of the present disclosure than before administration of the pharmaceutical composition of the present disclosure.

In certain embodiments, the subject exercises more frequently after administration of the engineered bacterial strain than before administration of the engineered bacterial strain.

In certain embodiments, the subject exercises more frequently after administration of the probiotic composition of the present disclosure than before administration of the probiotic composition of the present disclosure.

5 In certain embodiments, the subject exercises more frequently after administration of the probiotic composition of the present disclosure than before administration of the probiotic composition comprising at least one bacterial strain selected from the group consisting of *Coprococcus* bacterium and *Eubacterium* bacterium.

10 In certain embodiments, the subject exercises for a longer period of time after administration of the CB1 agonist than before administration of the CB1 agonist, optionally wherein the CB1 agonist is a fatty acid amide.

In certain embodiments, the subject exercises for a longer period of time after administration of the dietary supplement composition of the present disclosure than before administration of the dietary supplement composition of the present disclosure.

15 In certain embodiments, the subject exercises for a longer period of time after administration of the pharmaceutical composition of the present disclosure than before administration of the pharmaceutical composition of the present disclosure.

In certain embodiments, the subject exercises for a longer period of time after administration of the engineered bacterial strain than before administration of the engineered bacterial strain.

20 In certain embodiments, the subject exercises for a longer period of time after administration of the probiotic composition of the present disclosure than before administration of the probiotic composition of the present disclosure.

25 In certain embodiments, the subject exercises for a longer period of time after administration of the probiotic composition of the present disclosure than before administration of the probiotic composition comprising at least one bacterial strain selected from the group consisting of *Coprococcus* bacterium and *Eubacterium* bacterium.

In certain embodiments, the subject has increased cardiovascular endurance and/or physical performance after administration of the CB1 agonist as compared to before administration of the CB1 agonist, optionally wherein the CB1 agonist is a fatty acid amide.

30 In certain embodiments, the subject has increased cardiovascular endurance and/or physical performance after administration of the dietary supplement composition of the present

disclosure as compared to before administration of the dietary supplement composition of the present disclosure.

In certain embodiments, the subject has increased cardiovascular endurance and/or physical performance after administration of the pharmaceutical composition of the present disclosure as compared to before administration of the pharmaceutical composition of the present disclosure.

In certain embodiments, the subject has increased cardiovascular endurance and/or physical performance after administration of the engineered bacterial strain as compared to before administration of the engineered bacterial strain.

In certain embodiments, the subject has increased cardiovascular endurance and/or physical performance after administration of the probiotic composition of the present disclosure as compared to before administration of the probiotic composition of the present disclosure.

In certain embodiments, the subject has increased cardiovascular endurance and/or physical performance after administration of the probiotic composition of the present disclosure as compared to before administration of the probiotic composition comprising at least one bacterial strain selected from the group consisting of *Coproccoccus* bacterium and *Eubacterium* bacterium.

### Plasmids

In one aspect, the present disclosure provides a plasmid comprising at least one gene which encodes a protein that promotes fatty acid amide (FAA) biosynthesis which when inserted into a bacterium stimulates and/or enhances exercise motivation and/or physical performance in a subject.

In certain embodiments, the protein is an enzyme. In certain embodiments, the enzyme is at least one selected from the group consisting of EreS (SEQ ID NO:22), EreT (SEQ ID NO:23), EreA (SEQ ID NO:24), and EreC (SEQ ID NO:25).

In certain embodiments, the plasmid comprises at least one gene selected from the group consisting of *ereS* (SEQ ID NO:26), *ereT* (SEQ ID NO:27), *ereA* (SEQ ID NO:28), and *ereC* (SEQ ID NO:29).

In certain embodiments, the plasmid comprises each of *ereS* (SEQ ID NO:26), *ereT* (SEQ ID NO:27), *ereA* (SEQ ID NO:28), and *ereC* (SEQ ID NO:29).

In certain embodiments, the plasmid comprises a polynucleotide, wherein at least a portion of the polynucleotide shares at least 85% sequence homology with at least one polynucleotide sequence selected from the group consisting of SEQ ID NO:30 and SEQ ID NO:31. In certain embodiments, the plasmid comprises a polynucleotide, wherein at least a portion of the polynucleotide shares at least 90% sequence homology with at least one polynucleotide sequence selected from the group consisting of SEQ ID NO:30 and SEQ ID NO:31. In certain embodiments, the plasmid comprises a polynucleotide, wherein at least a portion of the polynucleotide shares at least 95% sequence homology with at least one polynucleotide sequence selected from the group consisting of SEQ ID NO:30 and SEQ ID NO:31. In certain embodiments, the plasmid comprises a polynucleotide, wherein at least a portion of the polynucleotide shares at least 96% sequence homology with at least one polynucleotide sequence selected from the group consisting of SEQ ID NO:30 and SEQ ID NO:31. In certain embodiments, the plasmid comprises a polynucleotide, wherein at least a portion of the polynucleotide shares at least 97% sequence homology with at least one polynucleotide sequence selected from the group consisting of SEQ ID NO:30 and SEQ ID NO:31. In certain embodiments, the plasmid comprises a polynucleotide, wherein at least a portion of the polynucleotide shares at least 98% sequence homology with at least one polynucleotide sequence selected from the group consisting of SEQ ID NO:30 and SEQ ID NO:31. In certain embodiments, the plasmid comprises a polynucleotide, wherein at least a portion of the polynucleotide shares at least 99% sequence homology with at least one polynucleotide sequence selected from the group consisting of SEQ ID NO:30 and SEQ ID NO:31. In certain embodiments, the plasmid comprises a polynucleotide, wherein at least a portion of the polynucleotide shares at least 100% sequence homology with at least one polynucleotide sequence selected from the group consisting of SEQ ID NO:30 and SEQ ID NO:31. In certain embodiments, the plasmid comprises a polynucleotide, wherein the polynucleotide comprises a concatenation of SEQ ID NO:30 and SEQ ID NO:31.

### **Engineered Bacterial Strains**

In one aspect, the present disclosure provides an engineered bacterial strain comprising at least one plasmid of the present disclosure.

In another aspect, the present disclosure provides an engineered bacterial strain comprising at least one plasmid comprising at least one gene which encodes a protein that promotes fatty acid amide (FAA) biosynthesis.

In certain embodiments, the protein is an enzyme. In certain embodiments, the enzyme is at least one selected from the group consisting of EreS, EreT, EreA, and EreC.

In certain embodiments, the plasmid comprises at least one gene selected from the group consisting of *ereS*, *ereT*, *ereA*, and *ereC*. In certain embodiments, the plasmid comprises each of *ereS*, *ereT*, *ereA*, and *ereC*.

In certain embodiments, the bacterial strain is *Escherichia coli*. In certain embodiments, the *E. coli* strain is a BAP1 *E. coli* strain.

## Compositions

### *Dietary Supplement Compositions*

In one aspect, the present disclosure provides a dietary supplement composition comprising at least one fatty acid amide (FAA) selected from the group consisting of *N*-stearoyl serotonin, *N*-arachidonoyl dopamine, *N*-oleoyl dopamine, *N*-palmitoyl dopamine, *N*-stearoyl dopamine, *N*-stearoyl taurine, *N*-arachidonoyl ethanolamide, *N*-palmitoyl ethanolamide, and *N*-oleoyl ethanolamide.

In certain embodiments, the present disclosure provides a dietary supplement composition comprising at least two fatty acid amides (FAAs) selected from the group consisting of *N*-stearoyl serotonin, *N*-arachidonoyl dopamine, *N*-oleoyl dopamine, *N*-palmitoyl dopamine, *N*-stearoyl dopamine, *N*-stearoyl taurine, *N*-arachidonoyl ethanolamide, *N*-palmitoyl ethanolamide, and *N*-oleoyl ethanolamide.

In certain embodiments, the present disclosure provides a dietary supplement composition comprising at least three fatty acid amides (FAAs) selected from the group consisting of *N*-stearoyl serotonin, *N*-arachidonoyl dopamine, *N*-oleoyl dopamine, *N*-palmitoyl dopamine, *N*-stearoyl dopamine, *N*-stearoyl taurine, *N*-arachidonoyl ethanolamide, *N*-palmitoyl ethanolamide, and *N*-oleoyl ethanolamide.

In certain embodiments, the present disclosure provides a dietary supplement composition comprising at least four fatty acid amides (FAAs) selected from the group consisting of *N*-stearoyl serotonin, *N*-arachidonoyl dopamine, *N*-oleoyl dopamine, *N*-palmitoyl

dopamine, *N*-stearoyl dopamine, *N*-stearoyl taurine, *N*-arachidonoyl ethanolamide, *N*-palmitoyl ethanolamide, and *N*-oleoyl ethanolamide.

In certain embodiments, the present disclosure provides a dietary supplement composition comprising at least five fatty acid amides (FAAs) selected from the group consisting of *N*-stearoyl serotonin, *N*-arachidonoyl dopamine, *N*-oleoyl dopamine, *N*-palmitoyl dopamine, *N*-stearoyl dopamine, *N*-stearoyl taurine, *N*-arachidonoyl ethanolamide, *N*-palmitoyl ethanolamide, and *N*-oleoyl ethanolamide.

In certain embodiments, the dietary supplement composition further comprises at least one solid, liquid, or semi-solid edible base substance.

In certain embodiments, the composition comprises *N*-palmitoyl dopamine, *N*-arachidonoyl dopamine, *N*-oleoyl dopamine, *N*-oleoyl ethanolamide, and palmitoyl ethanolamide.

In certain embodiments, each FAA component may comprise about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or about 99% or more of the composition by weight.

In certain embodiments, the composition has a ratio of FAAs of about 1:1:1:1 by weight.

In certain embodiments, the composition is suitable for oral administration.

In another aspect, the present disclosure provides a probiotic composition comprising the engineered bacterial strain of the present disclosure and at least one pharmaceutically acceptable carrier and/or at least one base substance.

### *Pharmaceutical Compositions*

In another aspect, the present disclosure provides a pharmaceutical composition comprising:

- (a) at least one fatty acid amide (FAA) selected from the group consisting of *N*-stearoyl serotonin, *N*-arachidonoyl dopamine, *N*-oleoyl dopamine, *N*-palmitoyl dopamine, *N*-stearoyl dopamine, *N*-stearoyl taurine, *N*-arachidonoyl ethanolamide, *N*-palmitoyl ethanolamide, and *N*-oleoyl ethanolamide; and

- (b) at least one pharmaceutically acceptable carrier.

In certain embodiments, the present disclosure provides a pharmaceutical composition comprising:

- (a) at least two fatty acid amide (FAAs) selected from the group consisting of *N*-stearoyl serotonin, *N*-arachidonoyl dopamine, *N*-oleoyl dopamine, *N*-palmitoyl dopamine, *N*-stearoyl dopamine, *N*-stearoyl taurine, *N*-arachidonoyl ethanolamide, *N*-palmitoyl ethanolamide, and *N*-oleoyl ethanolamide; and

- (b) at least one pharmaceutically acceptable carrier.

In certain embodiments, the present disclosure provides a pharmaceutical composition comprising:

- (a) at least three fatty acid amide (FAAs) selected from the group consisting of *N*-stearoyl serotonin, *N*-arachidonoyl dopamine, *N*-oleoyl dopamine, *N*-palmitoyl dopamine, *N*-stearoyl dopamine, *N*-stearoyl taurine, *N*-arachidonoyl ethanolamide, *N*-palmitoyl ethanolamide, and *N*-oleoyl ethanolamide; and

- (b) at least one pharmaceutically acceptable carrier.

In certain embodiments, the present disclosure provides a pharmaceutical composition comprising:

- (a) at least four fatty acid amide (FAAs) selected from the group consisting of *N*-stearoyl serotonin, *N*-arachidonoyl dopamine, *N*-oleoyl dopamine, *N*-palmitoyl dopamine, *N*-stearoyl dopamine, *N*-stearoyl taurine, *N*-arachidonoyl ethanolamide, *N*-palmitoyl ethanolamide, and *N*-oleoyl ethanolamide; and

- (b) at least one pharmaceutically acceptable carrier.

In certain embodiments, the present disclosure provides a pharmaceutical composition comprising:

- (a) at least five fatty acid amide (FAAs) selected from the group consisting of *N*-stearoyl serotonin, *N*-arachidonoyl dopamine, *N*-oleoyl dopamine, *N*-palmitoyl dopamine, *N*-stearoyl dopamine, *N*-stearoyl taurine, *N*-arachidonoyl ethanolamide, *N*-palmitoyl ethanolamide, and *N*-oleoyl ethanolamide; and

- (b) at least one pharmaceutically acceptable carrier.

In certain embodiments, the composition comprises *N*-palmitoyl dopamine, *N*-arachidonoyl dopamine, *N*-oleoyl dopamine, *N*-oleoyl ethanolamide, and palmitoyl ethanolamide.

In certain embodiments, each FAA component may comprise about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or about 99% or more of the composition by weight.

In certain embodiments, the composition has a ratio of FAAs of about 1:1:1:1 by weight.

In certain embodiments, the composition is suitable for oral administration.

### **Dietary Supplement and/or Probiotic Formulations**

Described herein, in part, are formulations for the delivery of a probiotic and/or dietary supplement. In certain embodiments, the formulations described herein comprise edible compositions that deliver one or more active agents. The compositions described herein are configured to release one or more active ingredients into the GI system of an individual who ingests the composition, wherein the active ingredients are selected to exert either local (*i.e.* local to the GI tract) or systemic effects (*e.g.*, interoceptive stimulation) or both on the individual. As used herein an individual may comprise a human or animal.

The compositions described herein include a base substance that carries or contains the active ingredients of the formulation. A base substance may comprise a solid, liquid, or semi-solid edible substance depending on the particular embodiment of the composition. For example, some embodiments of the compositions as described herein comprise a confection such as, for example, a gummy containing one or more active ingredients. In such embodiments, the gummy comprises a base substance that contains the active ingredients of the composition.

In general, a base substance as described herein is configured to provide a number of qualities to the composition including, for example, enhanced palatability of the active ingredients by providing, for example, a satisfying taste or texture to the composition.

Additionally, in some embodiments, a base substance as described herein provides a delivery vehicle for an active ingredient by, for example, encapsulating a micro-organism in a protective

environment (where the micro-organism comprises the active ingredient). For example, a base substance as described herein, in some embodiments, encapsulates a micro-organism in an oxygen free or essentially oxygen free environment.

In some embodiments, the composition of the base substance itself is configured to control the mechanism of release or timing of release of the active ingredient. For example, a base substance that is resistant to digestion by stomach acid will release active ingredients further along the GI tract than a base substance that is digested by stomach acid. In this way, targeted delivery of specific probiotics to specific targets in the GI tract is achievable. For example, probiotics for reducing or preventing *H. pylori* colonization of the stomach is best delivered via a base substance that is digested quickly in the upper GI tract, while, for example, probiotics for the treatment of Inflammatory Bowel Syndrome are best delivered to the colon via a base substance that takes a relatively longer time to digest. The mechanism of digestion of the base unit is further controlled in some embodiments by the addition of a coating of the entire composition.

In some of the embodiments of the probiotic compositions described herein, a base substance comprises a colloid or a hydrocolloid. In some of these embodiments, a hydrocolloid is a gum. In some of these embodiments, a hydrocolloid is a gelatin. In some of these embodiments a hydrocolloid is a starch. In some of these embodiments, a hydrocolloid is a pectin. In some of these embodiments, a hydrocolloid is a gel. In general, a hydrocolloid is formed by mixing a powdered or dehydrated base substance with water. In some embodiments, the mixture of water and powdered base substance is heated to form the hydrocolloid. In some embodiments, the mixture of water and powdered base substance is heated under pressure to form the hydrocolloid. In general, the concentration of the powdered substance relative to the water in the mixture will determine the consistency of the hydrocolloid. As such, in some embodiments, to obtain a more solid consistency, water is intentionally evaporated from the hydrocolloid, thus increasing the relative concentration of the powdered base substance. Similarly, water may be added to a hydrocolloid to decrease the relative concentration of the powdered base substance and thus cause the hydrocolloid to become relatively more liquid or semi-solid. In some embodiments wherein the compositions as described herein comprise a hydrocolloid as the base substances, the base substance is poured into a mold and formed into a gummy.

In some embodiments of the compositions described herein, the base unit is a soft chew having, for example, a fondant-like texture and consistency. Exemplary components of a soft chew base unit include one or more of a sweetener in the form of, for example, a syrup, glycerin, and gelatin.

5 In some embodiments of the compositions described herein, additives provide or enhance flavoring, texture, and/or coloring. Exemplary flavorants include salts, acids, and sweeteners. Acids such as, for example, citric acid, in addition to providing flavor, provide texture by promoting the gelling of hydrocolloids in embodiments that include hydrocolloids. In some  
10 embodiments, citric acid is further combined with one or more of lactic acid, malic acid and ascorbic acid. In some embodiments, the base substance in the composition comprises 5% by weight of an acid. In some embodiments, the base substance in the composition comprises 4% by weight of an acid. In some embodiments, the base substance in the composition comprises 3% by weight of an acid. In some embodiments, the base substance in the composition comprises 2% by  
15 weight of an acid. In some embodiments, the base substance in the composition comprises 1% by weight of an acid. In some embodiments, the base substance in the composition comprises no acid. Sweeteners such as, for example, sucrose, fructose, glucose or other sugars, invert sugar, or syrup. In some embodiments, a sweetener comprises an artificial sweetener. In some  
20 embodiments, sweetener is incorporated into the base substance in the composition and/or sweetener is layered or coated onto one or more surfaces of the hydrocolloid. In some embodiments, the base substance in the composition comprises about 85%-95% carbohydrates by weight. In some embodiments, the base substance in the composition comprises about 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or about 99% carbohydrates by weight.

In a preferred embodiment of the compositions described herein, the composition  
25 includes one or more active ingredients.

Exemplary active ingredients suitable for use in the compositions described herein include prebiotics, probiotics, vitamins, minerals, and other dietary supplement nutrients (*e.g.*, oils, amino acids).

Exemplary prebiotics suitable for use in the compositions described herein include  
30 fructooligosaccharide, galactooligosaccharide, lactulose, beta-glucan, inulin, pectin and digestion

resistant starch. In some embodiments of the compositions described herein, the compositions comprise one or more prebiotics.

Exemplary probiotics suitable for use in the compositions described herein include strains from the following genera: *Lactobacillus*, *Bifidobacterium*, *Saccharomyces*, *Enterococcus*,  
5 *Streptococcus*, *Pediococcus*, *Leuconostoc*, *Bacillus*, *Escherichia coli*. Microorganisms from these genera in particular have been demonstrated to provide health benefits when ingested. See, for example, *S. Fijan*. Microorganisms with Claimed Probiotic Properties: An Overview of Recent Literature. Int J Environ Res Public Health. 2014 May; 11(5): 4745-4767. Non-limiting examples of specific micro-organisms suitable for use in the compositions described herein  
10 include *Lactobacillus acidophilus*, *L. casei*, *L. paracasei*, *L. rhamnosus*, *L. delbrueckii subsp. bulgaricus*, *L. brevis*, *L. johnsonii*, *L. plantarum*, *L. fermentum*, *Bifidobacterium infantis*, *B. adolescentis*, *B. animalis subsp animalis*, *B. animalis subsp lactis*, *B. bifidum*, *B. longum*, *B. breve*, *B. infantis*, *Saccharomyces bayanus*, *S. cerevisiae*, *S. boulardii*, *Lactococcus lactis subsp. Lactis*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *S. thermophiles*, vancomycin-  
15 resistant *Enterococcus faecium*, *B. subtilis*, *B. coagulans*, *B. subtilis*, *B. cereus*, *B. indicus*, *B. licheniformis*, and *Escherichia coli*. In some embodiments of the compositions described herein, the compositions comprise one or more types (e.g. one or more species or one or more genera) of probiotics.

Exemplary vitamins suitable for use with the compositions described herein include  
20 Vitamin A, Vitamin B1, Vitamin B2, Vitamin B3 (Niacin), Vitamin B5 (Pantothenic Acid), Vitamin B6 (Pyridoxine), Vitamin B7 (Biotin), Vitamin B9 (Folate), Vitamin B12, Vitamin C, Vitamin D, Vitamin E, Vitamin K, and Choline. In some embodiments of the compositions described herein, the compositions comprise one or more vitamins.

Exemplary minerals suitable for use with the compositions described herein include  
25 Potassium, Chloride, Sodium, Calcium, Phosphorus, Magnesium, Iron, Zinc, Manganese, Copper, Iodine, Chromium, Molybdenum, Selenium, and Cobalt.

Exemplary dietary supplement nutrients include omega fatty acids, fish oil, amino acids, green tea extract and other botanical extracts.

The compositions described herein comprise mixtures in some embodiments. In some  
30 embodiments, a composition comprises a homogenous mixture of one or more active ingredients with a base substance, wherein in different embodiments, the base substance respectively

comprises a solid, a liquid, or a semi-solid. For example, in some embodiments, a base substance comprising a gummy includes one or more active ingredients mixed within it (i.e. within the substance of the gummy itself). In some embodiments, a composition comprises a homogenous mixture of two or more active ingredients with one another but neither of which forms a  
5 homogenous mixture with the base substance. For example, in some embodiments, a base substance entirely encapsulates a mixture of one or more active ingredients such as, for example, a gummy containing a liquid center which contains a homogenous mixture of two or more active ingredients.

In some embodiments, a composition as described herein is sealed or covered with a  
10 coating. In some embodiments, the coating comprises a sugar-based coating. In some embodiments, the coating comprises an edible wax.

#### **Administration/Dosage/Formulations**

The regimen of administration may affect what constitutes an effective amount. Further,  
15 several divided dosages, as well as staggered dosages may be administered daily or sequentially. Further, the dosages of the therapeutic formulations may be proportionally increased or decreased as indicated by the exigencies of the therapeutic situation.

Administration of the compositions described herein to a patient, preferably a mammal, more preferably a human, may be carried out using known procedures, at dosages and for  
20 periods of time effective to treat the achieved the therapeutic effect. An effective amount of the therapeutic compound necessary to achieve a therapeutic effect may vary according to factors such as the age, sex, and weight of the patient. Dosage regimens may be adjusted to provide the optimum therapeutic effect. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic  
25 situation. A non-limiting example of an effective dose range for a therapeutic compound described herein is from about 1 and 5,000 mg/kg of body weight/per day. One of ordinary skill in the art would be able to study the relevant factors and make the determination regarding the effective amount of the therapeutic compound without undue experimentation.

Actual dosage levels of the active ingredients in the pharmaceutical compositions  
30 described herein may be varied so as to obtain an amount of the active ingredient that is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of

administration, without being toxic to the patient.

In particular, the selected dosage level depends upon a variety of factors including the activity of the particular compound employed, the time of administration, the rate of excretion of the compound, the duration of the treatment, other drugs, compounds or materials used in combination with the compound, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

A medical doctor, *e.g.*, physician or veterinarian, having ordinary skill in the art may readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could start doses of the compounds described herein employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved.

In particular embodiments, it is especially advantageous to formulate the compound in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the patients to be treated; each unit containing a predetermined quantity of therapeutic compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical vehicle. The dosage unit forms of the compound(s) described herein are dictated by and directly dependent on (a) the unique characteristics of the therapeutic compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding/formulating such a therapeutic compound.

In certain embodiments, the compositions described herein are formulated using one or more pharmaceutically acceptable excipients or carriers. In certain embodiments, the pharmaceutical compositions described herein comprise a therapeutically effective amount of a compound described herein and a pharmaceutically acceptable carrier.

The carrier may be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity may be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms may be achieved by various antibacterial and antifungal agents, for example,

parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it is preferable to include isotonic agents, for example, sugars, sodium chloride, or polyalcohols such as mannitol and sorbitol, in the composition. Prolonged absorption of the injectable compositions may be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate or gelatin.

In certain embodiments, the compositions described herein are administered to the patient in dosages that range from one to five times per day or more. In other embodiments, the compositions described herein are administered to the patient in range of dosages that include, but are not limited to, once every day, every two, days, every three days to once a week, and once every two weeks. It is readily apparent to one skilled in the art that the frequency of administration of the various combination compositions described herein varies from individual to individual depending on many factors including, but not limited to, age, gender, overall health, and other factors. Thus, administration of the compounds and compositions described herein should not be construed to be limited to any particular dosage regime and the precise dosage and composition to be administered to any patient is determined by the attending physician taking all other factors about the patient into account.

The compound(s) described herein for administration may be in the range of from about 1 µg to about 10,000 mg, about 20 µg to about 9,500 mg, about 40 µg to about 9,000 mg, about 75 µg to about 8,500 mg, about 150 µg to about 7,500 mg, about 200 µg to about 7,000 mg, about 350 µg to about 6,000 mg, about 500 µg to about 5,000 mg, about 750 µg to about 4,000 mg, about 1 mg to about 3,000 mg, about 10 mg to about 2,500 mg, about 20 mg to about 2,000 mg, about 25 mg to about 1,500 mg, about 30 mg to about 1,000 mg, about 40 mg to about 900 mg, about 50 mg to about 800 mg, about 60 mg to about 750 mg, about 70 mg to about 600 mg, about 80 mg to about 500 mg, and any and all whole or partial increments therebetween.

In some embodiments, the dose of a compound described herein is from about 1 mg and about 2,500 mg. In some embodiments, a dose of a compound described herein used in compositions described herein is less than about 10,000 mg, or less than about 8,000 mg, or less than about 6,000 mg, or less than about 5,000 mg, or less than about 3,000 mg, or less than about 2,000 mg, or less than about 1,000 mg, or less than about 500 mg, or less than about 200 mg, or less than about 50 mg. Similarly, in some embodiments, a dose of a second compound as described herein is less than about 1,000 mg, or less than about 800 mg, or less than about 600

mg, or less than about 500 mg, or less than about 400 mg, or less than about 300 mg, or less than about 200 mg, or less than about 100 mg, or less than about 50 mg, or less than about 40 mg, or less than about 30 mg, or less than about 25 mg, or less than about 20 mg, or less than about 15 mg, or less than about 10 mg, or less than about 5 mg, or less than about 2 mg, or less than about 1 mg, or less than about 0.5 mg, and any and all whole or partial increments thereof.

In certain embodiments, a composition as described herein is a packaged pharmaceutical composition comprising a container holding a therapeutically effective amount of a compound described herein, alone or in combination with a second pharmaceutical agent; and instructions for using the compound.

Formulations may be employed in admixtures with conventional excipients, *i.e.*, pharmaceutically acceptable organic or inorganic carrier substances suitable for oral, parenteral, nasal, intravenous, subcutaneous, enteral, or any other suitable mode of administration, known to the art. The pharmaceutical preparations may be sterilized and if desired mixed with auxiliary agents, *e.g.*, lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure buffers, coloring, flavoring and/or aromatic substances and the like. They may also be combined where desired with other active agents, *e.g.*, other analgesic agents.

Suitable compositions and dosage forms include, for example, tablets, capsules, caplets, pills, gel caps, troches, dispersions, suspensions, solutions, syrups, granules, beads, transdermal patches, gels, powders, pellets, magmas, lozenges, creams, pastes, plasters, lotions, discs, suppositories, liquid sprays for nasal or oral administration, dry powder or aerosolized formulations for inhalation, compositions and formulations for intravesical administration and the like. It should be understood that the formulations and compositions described herein are not limited to the particular formulations and compositions that are described herein.

#### *Oral Administration*

For oral application, particularly suitable are tablets, dragees, liquids, drops, suppositories, or capsules, caplets and gelcaps. The compositions intended for oral use may be prepared according to any method known in the art and such compositions may contain one or more agents selected from the group consisting of inert, non-toxic pharmaceutically excipients that are suitable for the manufacture of tablets. Such excipients include, for example an inert diluent such as lactose; granulating and disintegrating agents such as cornstarch; binding agents

such as starch; and lubricating agents such as magnesium stearate. The tablets may be uncoated or they may be coated by known techniques for elegance or to delay the release of the active ingredients. Formulations for oral use may also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert diluent.

For oral administration, the compound(s) described herein can be in the form of tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (*e.g.*, polyvinylpyrrolidone, hydroxypropylcellulose or hydroxypropyl methylcellulose); fillers (*e.g.*, cornstarch, lactose, microcrystalline cellulose or calcium phosphate); lubricants (*e.g.*, magnesium stearate, talc, or silica); disintegrates (*e.g.*, sodium starch glycollate); or wetting agents (*e.g.*, sodium lauryl sulphate). If desired, the tablets may be coated using suitable methods and coating materials such as OPADRY™ film coating systems available from Colorcon, West Point, Pa. (*e.g.*, OPADRY™ OY Type, OYC Type, Organic Enteric OY-P Type, Aqueous Enteric OY-A Type, OY-PM Type and OPADRY™ White, 32K18400). Liquid preparation for oral administration may be in the form of solutions, syrups or suspensions. The liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (*e.g.*, sorbitol syrup, methyl cellulose or hydrogenated edible fats); emulsifying agent (*e.g.*, lecithin or acacia); non-aqueous vehicles (*e.g.*, almond oil, oily esters or ethyl alcohol); and preservatives (*e.g.*, methyl or propyl p-hydroxy benzoates or sorbic acid).

## Dosing

The therapeutically effective amount or dose of a compound described herein depends on the age, sex and weight of the subject and/or the current medical condition(s) of the subject. The skilled artisan is able to determine appropriate dosages depending on these and other factors.

A suitable dose of a compound described herein can be in the range of from about 0.01 mg to about 5,000 mg per day, such as from about 0.1 mg to about 1,000 mg, for example, from about 1 mg to about 500 mg, such as about 5 mg to about 250 mg per day. The dose may be administered in a single dosage or in multiple dosages, for example from 1 to 4 or more times per day. When multiple dosages are used, the amount of each dosage may be the same or different. For example, a dose of 1 mg per day may be administered as two 0.5 mg doses, with about a 12-hour interval between doses.

It is understood that the amount of compound dosed per day may be administered, in non-limiting examples, every day, every other day, every 2 days, every 3 days, every 4 days, or every 5 days. For example, with every other day administration, a 5 mg per day dose may be initiated on Monday with a first subsequent 5 mg per day dose administered on Wednesday, a second  
5 subsequent 5 mg per day dose administered on Friday, and so on.

Once the desired benefits have been achieved, a maintenance dose is administered if necessary. Subsequently, the dosage or the frequency of administration, or both, is reduced to a level at which the improvements are maintained. In certain embodiments, subjects require intermittent treatment on a long-term basis so as to maintain the intended therapeutic effect.

10 The compounds described herein can be formulated in unit dosage form. The term "unit dosage form" refers to physically discrete units suitable as unitary dosage for patients undergoing treatment, with each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, optionally in association with a suitable pharmaceutical carrier. The unit dosage form may be for a single daily dose or one of multiple daily doses (*e.g.*,  
15 about 1 to 4 or more times per day). When multiple daily doses are used, the unit dosage form may be the same or different for each dose.

It is to be understood that wherever values and ranges are provided herein, all values and ranges encompassed by these values and ranges, are meant to be encompassed within the scope of the present disclosure. Moreover, all values that fall within these ranges, as well as the upper  
20 or lower limits of a range of values, are also contemplated by the present application.

## EXAMPLES

Various embodiments of the present application can be better understood by reference to the following Examples which are offered by way of illustration. The scope of the present  
25 application is not limited to the Examples given herein.

## Materials and Methods

No statistical methods were used to predetermine sample size. The experiments were not randomized, and investigators were not blinded to allocation during experiments and outcome  
30 assessment.

## Mice

Animals were obtained from The Jackson Laboratory. Mice were kept under strict light-dark cycles, with lights being turned on at 7 am and turned off at 7 pm. Both male and female mice were included in the Diversity-Outbred cohort. Both male and female mice were used. A specific comparison between male and female mice is provided herein.

The following mouse strains were purchased from The Jackson Laboratory: C57L/6J (Stock No. 000664), *B6.SJL-Slc6a3<sup>tm1.1(cre)Bkmn</sup>/J* (Stock No. 006660), *B6.129-Trpv1<sup>tm1(cre)Bbm</sup>/J* (Stock No. 017769), *B6.129P2-Gt(ROSA)26Sor<sup>tm1(DTA)Lky</sup>/J* (Stock No. 009669), *B6.129-Gt(ROSA)26Sor<sup>tm1(CAG-CHRM4\*, -mCitrine)Ute</sup>/J* (Stock No. 026219), diversity outbred J:DO (Stock No. 009376). *Cnr1*-deficient mice were kindly provided by the NIH. All purchased mice were kept in the local animal facility for three weeks upon delivery and prior to experimental procedures.

For antibiotic treatment, mice were given a combination of neomycin (1 g/l), ampicillin (1 g/l), vancomycin (0.5 g/l), metronidazole (0.5 g/l), imipenem (0.5 g/l), and ciprofloxacin (0.2 g/l) in their drinking water for 3 weeks. Stool samples were collected fresh for each individual mouse into 2 ml Eppendorf tubes and immediately snap frozen in liquid nitrogen before storage at -80 °C until DNA extraction. For antibiotic treatment with only absorbable antibiotics, mice were given a combination of metronidazole, ciprofloxacin, and ampicillin; for non-absorbable antibiotic treatment, mice were given a combination of neomycin, vancomycin, and imipenem.

In the antibiotic cessation experiment mice were treated with broad spectrum antibiotics for 2 weeks, and upon successful bacterial depletion mice were given back free access to drinking water for 4 weeks. Mice were tested for endurance exercise performance after full microbial re-colonization, which was verified by 16S qPCR.

For germ-free experiments, C57BL/6 mice were born and raised in isolators under sterile conditions. Sterility was ensured by periodic microbiological checks. For microbiota transplantation experiments, 3-5 stool pellets from each donor group were collected, transferred to an anaerobic chamber, homogenized in 1.5 ml of PBS/- and filtered through 70 µm filters. 250 µL of filtrates were gavaged into germ-free recipient mice that were housed in sterile isocages upon colonization. Exercise tests were performed two weeks after microbial colonization.

## Endurance exercise

To assess the maximal performance of each mouse, a forced endurance exercise experiment was performed using treadmills with increasing speed and inclination (Exer-6; Columbus Instruments). The experiments were conducted under constant supervision. The protocol was started with an adjustment period, where each mouse was placed into a treadmill lane (lanes 1 to 6) for 10 minutes. At this stage, the speed was 0 m/min and incline 0°. After 10 minutes, the treadmill started accelerating by 0.1 m/min to a final speed of 5 m/min, and the mice ran at this speed for 20 minutes with 0° incline. The treadmills then accelerated every 20 minutes by another 2.5 m/min, and every hour the inclination was increased 534 by 5°. The maximum possible speed was 25 m/min, and maximum inclination was 15°. Mice that failed to continue the protocol fell onto a metal grid at the end of treadmill belt and were encouraged to resume running with a mild electric shock. Every second spent on the metal grid was counted. A mouse was considered exhausted once it accumulated 50 visits (50 s) to the metal grid. The distance and time to exhaustion were recorded.

#### *Voluntary wheel exercise*

Voluntary exercise was performed in single housed cages with free access to a running wheel (Coulbourn Instruments, Cat. No. ACT-551-FIL-MS-SS). Prior to experiment and data acquisition, mice were given 3 days of adjustment to the single-housed setup and wheel presence. Data were acquired every 15 seconds, 1 minute, or 15 minutes from each mouse by a Panlab recording system and software. In experiments where pharmacological modulators were administered, the baseline for each mouse was recorded for 3 consecutive days, and then based on the experiment, mice were i.p. injected daily between 5-7 pm with pharmacological modulators or vehicle, and data were acquired for another 7 days following specific treatments below. For exercise under gnotobiotic conditions, exercise equipment was introduced into germ-free isolators.

#### *Gastric Catheter Implantation*

Mice were treated with subcutaneous analgesics meloxicam (5 mg/kg), bupivacaine (2 mg/kg), and buprenorphine (1 mg/kg). Mice were anesthetized in an incubation chamber with isoflurane (3%), transferred to a sterile drape, and received continuous isoflurane (1-2%) administered through a nose cone. An intrascapular incision and an abdominal incision were

made, and the catheter was directed subdermal to the abdomen. A midline abdominal incision through the muscle was made and the stomach was isolated. Catheter tubing (7-mm length, Braintree Scientific, MRE-033) with an epoxy ball (Devcon Clear Epoxy Adhesive, 92926, Lowers) was inserted through a puncture hole in the fundus of the stomach and secured with surgical mesh (5-mm diameter piece, Bard, 0112660). Surgical mesh secured the other end of the catheter at the intrascapular incision and a metal cap sealed the exposed tubing. Mice were fed moistened chow and daily body weight was monitored until pre-surgical weight was regained. The gastric catheter was periodically flushed with water to prevent blockage. Mice were given at least 2 weeks to recovery from surgery prior to starting experiments.

#### *Central and Gastric Infusions*

Catheters were connected to tubing and syringes regulated by an infusion pump (Harvard Apparatus, 703007). Gastric infusions were performed at a rate of 0.1 ml/min until a total volume of 0.2 ml was infused. Sterile water was used to flushed catheters before and after experiments to prevent clogging. Central infusions were microinjected with a Hamilton syringe attached to an internal cannula cut 4.7 mm below the pedestal (Plastics One) using the microliter syringe pump. Central infusions were performed at a rate of 0.0024 ml/min until a total volume of 100 nL was infused.

#### *Celiac-Superior Mesenteric Ganglionectomy*

To determine splanchnic contributions to gut-brain sensing, splanchnic afferents underwent a gangliectomy at the pre-vertebral celiac and superior mesenteric ganglia. Mice were anesthetized and treaded with analgesia, and an abdominal midline incision was made through skin and muscle. The celiac and superior mesenteric arteries were exposed by targeting the aorta at the arterial branching point medial to the left kidney. At the intersection of the three blood vessels, celiac and superior mesenteric ganglia and the surrounding tissue were gently teased away until the area between was cleared of nerve and lymphatic tissue. In sham animals, the area was exposed, and a small tear was made in the lymphatic tissue, but the ganglia were left intact. Mice were given at least 1 week to recover and regain pre-surgical weight.

#### *CCK-SAP treatment*

Mice were anesthetized with isoflurane (2-5%). After hair removal and disinfection, an incision was made in the neck area, and the sternohyoid and sternomastoid muscle connective tissue blunt dissected and moved aside to expose the carotid artery. The vagus nerve was gently separated from the after and followed to reach the nodose ganglion as it enters the foramen. The glass micropipette was advanced into the ganglion, and CCK-SAP (Cat. No. IT-31, Advanced Targeting Systems, CA) blank-SAP (Cat. No. IT-21) were injected (125 ng in 0.5  $\mu$ l per ganglion). The procedure was repeated on the other side, and the incision closed using a nylon suture. Mice will be monitored until fully awake when they will be placed back in-home cage. Analgesics (5 mg/kg Loxicom, SC) was administered once a day for the next two days.

#### *Stereotaxic Surgery, Viral Injections and Fibre Optic Implantations*

Viral injections were performed as previously described. Briefly, mice were pre-treated with subcutaneous injections of Meloxicam (5 mg/kg, Norbrook Laboratories, 29-040-11) and bupivacaine (2 mg/kg, Moore Medical, 52683). Mice were anesthetized with isoflurane (3%, Clipper, 0010250) and placed into a stereotaxic frame (Stoelting, 51725D) with continuous isoflurane (1-2%). For ventral tegmental area (VTA) fiber photometry, 200  $\mu$ L of AAV1-Syn-Flex-GCaMP6f-WPRE-SV40 was injected unilaterally at the following coordinates: bregma -3.2 mm, midline  $\pm$  1.2 mm, and skull -4.4 mm at a 10° angle from vertical in the medial/lateral axis. For nucleus accumbens (NAc) and dorsal striatum (DS) fibre photometry, 200  $\mu$ L of AAV9-hSyn-GRAB\_DA1h was injected unilaterally at the following coordinates: NAc: bregma +1.0 mm, midline  $\pm$  1.2 mm, and skull -4.1-4.0 mm; DS: bregma +1.0 mm, midline  $\pm$  1.3 mm, and skull -2.7 mm. A ferrule-capped optical fibre (400- $\mu$ m core, NA 0.48, Doric, MF2.5, 400/430-0.48) was implanted 0.2 mm above the injection site and secured to the skull with low auto-fluorescent cements: Metabond cement (Parkell, S380) and dental cement (Lang Dental Manufacturing, Ortho-jet BCA Liquid, B1306 and Jet Tooth Shade Powder, 143069). For central infusions, a unilateral 26-gauge guide cannula cut 3 mm below the pedestal (Plastics One) was implanted above the VTA at the following coordinates: bregma -3.2 mm, midline  $\pm$  1.2 mm, and skull -2.6 mm at a 10° angle from vertical in the medial/lateral axis. The cannula was secured to the skull with bone screws and dental cement and capped to prevent clogging. Mice were given at least 2 weeks for recovery and viral expression before experiments were performed.

The following rAAV vectors were used: AAV1-Syn-Flex-GCaMP6f-WPRE-SV40 (Addgene #100833, titer:  $4.216 \times 10^{13}$  GC/ml) and AAV9-hSyn-GRAB\_DA1h (Addgene #113050  $2.4 \times 10^{13}$  GC/ml). Syn, Synapsin promoter. hSyn, human Synapsin 1 promoter. SV40, sequence motif promoting polyadenylation and termination. Flex, Cre-dependent flip-excision switch. WPRE, woodchuck hepatitis virus response element. GCaMP, genetically encoded calcium indicator resulting from a fusion of GFP, M13 and Calmodulin. GRAB, G protein-coupled receptor activation-based dopamine sensor. DA1h, dopamine 1 high affinity.

Fibre photometry. GCaMP6 was imaged using dual-wavelength fibre photometry (FP) as previously described in the literature. GRAB DA1m was imaged using identical parameters except the 405-nm wavelength was not used. Briefly, for dual wavelength FP, two excitation wavelengths (470 nm and 405 nm) were modulated at 211 and 566 Hz to avoid contamination from overhead lights and crosstalk between excitation lights. Excitation illumination was generated through fibre-coupled LEDs (Thorlabs, M470F3 and M405F1) and modulated by a real-time amplifier (Tuc 615 ker-Davis Technologies, RZ5P or RZ10x). Excitation lights were filtered and combined by a fluorescence mini cube (Doric, FMC4\_IE(400-410)\_E(460-490)\_F(500-550)\_S). The combined excitation light was delivered through a 400  $\mu$ m core, 0.48 NA, low-fluorescence optical fibre (Doric, MFP\_400/430/1100-0.48\_1.5\_FCM-MF) to an implanted fibre (Doric, MFC\_400/430-0.66\_6.5mm\_MF2.5\_FLT) secured using a clamp (Thorlabs, ADAF2). LED power was set between 20 and 40  $\mu$ W emitted from the fibre tip to minimize bleaching. GCaMP6f emission fluorescence was collected through the mini cube and focused onto a femtowatt photoreceiver (Newport, Model 2151, gain set to DC LOW). Fluorescence was sampled at 1017 Hz and demodulated by the processor. LEDs were externally controlled by Synapse (Tucker-Davis Technology), and synchronized cameras (Ailipu Technology, ELP-USB100W05MT-DL36) were used to video-record mice during experiments.

Photometry during exercise. A motorized treadmill (Exer-6; Columbus Instruments) was used for exercise experiments. All mice were habituated to the treadmill for 3 days prior to exercise. Habituation day 1: 3 h rest on treadmill; Habituation day 2: 2 h rest on treadmill followed by 15 min at 6 m/min, 5 min at 8 m/min, and 5 min at 10 m/min; Habituation day 3: 1 h rest on treadmill followed by 15 min at 10 m/min, 5 min at 8 m/min, and 15 min at 10 m/min. Mice were placed on the treadmill for a 1 h acclimation period prior to recordings. Following acclimation, recording began while mice were still stationary, and running began 5 min later.

Mice ran for 10 min at 10 m/min. The treadmill was stopped, and recording continued for 15 min following the run. Recordings were aligned to the end of exercise to plot traces, calculate average  $\Delta F/F$ , and calculate the maximum and mean  $\Delta F/F$  after exercise. Mice underwent one or two baseline recordings separated by at least 24 h. They were then treated daily with 200  $\mu\text{L}$  antibiotics via oral gavage for 7 days, followed by another photometry session on the treadmill. In a subset of mice, 200  $\mu\text{L}$  of 2  $\mu\text{M}$  capsaicin was then administered i.p. for 4 days. During these 4 days, antibiotics were given in the drinking water. A third photometry session was performed immediately following last capsaicin injection on the fourth day.

Fibre Photometry Analysis. Data were exported to MATLAB (MathWorks) from Synapse using a script provided by Tucker-Davis Technology. Custom MATLAB scripts were used to independently normalize the demodulated 470-nm and 405-nm signals.  $\Delta F/F$  was calculated  $(F - F_{\text{baseline}})/F_{\text{baseline}}$ , with  $F_{\text{baseline}}$  being the median of the 300 s before the stimulus. Data were down-sampled to 1 Hz. Subsequent plotting and analysis were performed in MATLAB and Prism 8 (GraphPad). Mean  $\Delta F/F$  was calculated by integrating  $\Delta F/F$  over a 5 min period after the end of exercise in the ventral striatum or over a 2 min period after the end of exercise in the VTA and then dividing by the integration time. Maximum  $\Delta F/F$  were calculated by taking the maximum  $\Delta F/F$  value for each mouse over a 2 min period after the end of exercise.

#### *Dietary supplementation*

Fatty acid amides were supplemented into a normal chow diet (Teklad, NIH-07 Open formula mouse/rat diet (meal), Cat. No. 7022M). The mix contained 50 mg of each of the following metabolites: Palmitoyl-dopamine (PALDA, Cayman Chemical Company, Cat. No. 10007697), N-Arachidonoyl dopamine (NADA, Cayman Chemical Company, Cat. No. 90057), N-Oleoyl dopamine (NODA, Cayman Chemical Company, Cat. No. 10115), N-oleoyl ethanolamide (OEA, Cayman Chemical Company, Cat. No. 90265), and Palmitoyl ethanolamide (PEA, Cayman Chemical Company, Cat. No. 90350). The mix was added into 250 g diet powder and supplemented with 250 ml of sterile water. A homogenous dietary mixture was distributed to singly housed mice and fed for the duration of 5 days. Control diet mice were fed the same diet (7022M) without metabolites being supplemented.

#### *Hot plate test*

A hot plate was preheated to 56 °C for 15 minutes prior to the experiment. Mice were placed on the plate after completion of an exercise protocol. Latency was recorded as a time until a mouse starts licking its paws.

#### 5 *Open field test*

Mice were handled one day prior to testing and acclimated to the room for 30 minutes before testing. Mice were placed into a 40 x 40 cm box and allowed to explore for 15 minutes. Movement was recorded during the last 5 minutes of the trial with an iPhone 11 and analysed using EthoVision software version 15 (Noldus).

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#### *Metabolic cage measurement*

Food intake and other metabolic parameters were measured using CLAMS cages (Columbus Instrument), which consist of a combination of sensitive feeding sensors for automated measurement, a photobeam-based activity monitoring system that records ambulatory movements, including rearing and climbing, as well as sensors for the detection of oxygen and carbon dioxide consumption in each cage. All parameters were measured continuously and simultaneously. Mice were trained singly-housed in identical cages prior to data acquisition.

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#### *Pharmacological modulators*

In experiments using pharmacological modulators of dopamine receptor activity, Bromocriptine (Sigma Aldrich, Cat. No. 1076501-150MG) was administered daily for 5 days by i.p. injection at a concentration of 5 mg/kg body weight. Sulpiride (Sigma Aldrich, Cat. No. S8010-25G) was administered daily by i.p. injection at a concentration of 15 mg/kg body weight for 1 week. Experiments involving mice with a knock-in chemogenetic human muscarinic receptor 4 (hM4Di, Jackson laboratory, stock No: 026219) were injected daily i.p. with the DREADD agonist clozapine N-oxide hydrochloride (CNO, Cayman Chemical Company, Cat. No. 25780-25) at a concentration of 1 mg/kg body weight. In experiments targeting CB1, mice were injected daily i.p. with CB1 agonist CP55940 (Sigma Aldrich, Cat. No. C1112-10MG) at a concentration of 1 mg/kg for 1 week, or CB1 antagonist, O-2050 (Neta Scientific, Cat. No. C760X52) at concentration of 10 mg/kg body weight. In experiments where the peripheral CB1

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antagonist AM6545 was used, mice were daily injected i.p. at concentration 3 mg/kg body weight.

The MAO inhibitor pargyline hydrochloride (Sigma Aldrich, Cat. No. P8013-1G) was injected i.p. every other day for 8 days at a concentration of 150 mg/kg body weight. For specific TRPV1 stimulation, capsaicin (Sigma Aldrich, Cat. No. M2028-250MG) was injected i.p. daily with 200 µl of 2 µM concentration for 1 week. Leptin (Novus Biological, Cat. No. NBP2-76247-1 mg) was centrally injected into the VTA 5 minutes prior exercise at total quantity of 200 ng. Systemic leptin injection was administered i.p. at concentration 1 mg/kg body weight prior to exercise.

#### *Dorsal root ganglion extraction, culture and calcium imaging*

Dorsal root ganglia were extracted and cultured as previously described in the literature. The lumbar DRGs from mouse were collected into Neurobasal-A medium (Thermofisher, Cat. No. 10888022). DRGs were dissociated in 1 mg/ml collagenase 1A (Gibco, Cat. No. 17100017) for 1 h at 37 °C in serum-free media containing Advanced DMEM (Corning, Cat. No. MT10013CV), pyruvate solution (Corning, Cat. No. MT25000CI), and HEPES (Thermofisher, Cat. No. 15-630-080) buffered saline (Gibco, Cat. No. 694 10010049). DRGs were washed and triturated with glass Pasteur pipettes 60 times and centrifuged 500 x g at 4 °C for 5 minutes. The cell pellet was resuspended into culture media (10% FBS (Corning, Cat. No. MT35-010-CV), Neurobasal-A medium, B27 supplement (Gibco, Cat.No. 17504044), 50 ng/ml nerve growth factor (NGF) (Gibco, Cat. No. 549 13-290-010), and penicillin/streptomycin (Thermofisher, Cat. No. 15140122) and subsequently seeded onto poly-L-lysine (Sigma Aldrich, Cat. No. P4707-50ML) coated (at least 2h at 37°C, 5% CO<sub>2</sub>) 96-well plates and cultured overnight at 37 °C in a 5% CO<sub>2</sub> incubator.

For calcium imaging, the DRGs were washed with fresh serum-free culture media and loaded with 1 µM Fura-2-AM (Thermofisher, Cat. No. F-1221) for 1 h at 37 °C. The cells were then washed into modified extracellular Ringer's solution containing 145 mM NaCl (Sigma Aldrich, Cat. No. S7653-250G), 4.7 mM KCl (Sigma Aldrich, Cat. No. P9333-500G), 3.4 mM CaCl<sub>2</sub> (Sigma Aldrich, Cat. No. C5670-100G), 1.2 mM KH<sub>2</sub>PO<sub>4</sub> (Sigma Aldrich, 705 Cat. No. P5655-100G), 1.2 mM MgSO<sub>4</sub> (Sigma Aldrich, Cat. No. M2643-500G), 1 mM MgCl<sub>2</sub> (Sigma Aldrich, Cat. No. M4880-100G), 10 mM glucose (Sigma Aldrich, Cat. No.

G7021-100G), and 10 mM HEPES. Baseline was measured prior to addition of 50 µl of either 2 µM Capsaicin, 50 mM N-oleoyl ethanolamine (OEA), or filtered stool extracts in HBSS-/- (ThermoFisher, Cat. No. 14175079), which were directly applied onto neurons. Imaging was conducted immediately in a plate reader at room temperature with wavelengths set to 340, 380, and 510 nm.

#### *DRG metabolite candidate screening*

Metabolites were prepared in concentrations of 500 µM - 5 mM, unless otherwise stated, in PBS or DMSO. The following metabolites were used for dorsal root ganglia stimulation:

10 Sodium propionate, sodium acetate, sodium butyrate, ethanol (1%), indol-3-butyric acid (short chain fatty acids and conjugates). L-isoleucine, glycine, L-methionine, L-leucine, L-ornithine, L-alanine, L-valine, L-lysine, L-aspartate, L-glutamine, creatine, trimethyl amine, dimethyl amine, choline, acetylcholine (amino acids, amines, peptides). Oleic acid, palmitic acid, linoleic acid, quinolinic acid, arachidonic acid (fatty acids). Dipalmitin, dihexanoin, 1,2-dipalmitoyl-sn-glycerol-3-phosphoethanolamine, 1,2-dipalmitoyl-sn-glycerol, 1-oleoyl-3-arachidonoyl-glycerol, 1,2-dipalmitoyl-sn-glycerol-3-phosphatidylcholine, 6-glycero-phospho-N-oleoyl-ethanolamine, 2-arachidonoyl glycerol, 1-palmitoyl-3-linolenoyl-glycerol, 1,2-dipalmitoyl-sn-glycerol-3-phosphate (mono-/diacyl-glycerol conjugates). N-stearoyl serotonin, N-arachidonoyl dopamine, N-oleoyl dopamine, N-palmitoyl-dopamine, N-stearoyl-aurine, N-arachidonoyl ethanolamide, N-palmitoyl ethanolamide, N-oleoyl ethanolamide (fatty acid amide conjugates). Capsaicin (2 µM) was used as positive control, DMSO (5%) and PBST (pH 7.2) were used as respective negative controls.

#### *RNA Scope and confocal microscopy*

25 Probes: RNAScope® Probe-Mm-Trpv1-C2 (Cat No. 313331-C2). RNAScope® Probe-Mm-Fos (Cat No. 316921). Pre-treatment reagent (Cat. No. 3223100) including Cat. No. 322381, and Cat. No. 322000; Fluorescent detection reagents (Cat. No. 323110), TSA Buffer (Cat. No. 322809) and Wash buffer reagents (Cat. No. 310091) were used.

30 First, lumbar dorsal root ganglia and nodose ganglia were fixed in 4% PFA/PBS for 2 h and then switched to 20% sucrose/PBS solution overnight. Ganglia were mounted in OCT and sectioned at a thickness of 16 µm by cryostat. Slides were stored at -80 °C until further

processing. Slides were processed based on manufacturer's protocol (Fixed-frozen tissue sample preparation and pretreatment) with the following modifications. In brief, slides were washed with PBST for 5 minutes and then baked for 5 minutes at 37 °C. Tissues were then dehydrated, dried and treated with hydrogen peroxide. Next, Protease IV was used for 20 minutes at room temperature. Slides were then washed with preheated washing buffer for 10 minutes. Preheated probes Trpv1-C2 and cFos-C1 were prepared according to the manufacturer's instructions. TSA-Plus Cyanine 5 was used for cFos and TSA Plus Cyanine 3 was used for Trpv1-C2 probes at final dilution 1:1150. Finally, probes were hybridized, and signal developed based on the manufacturer's instructions. Slides were then imaged on a Zeiss lsm710 confocal microscope at 10x and 20x magnification.

#### *Muscle ex-vivo physiology*

Muscle physiological analysis was performed on isolated EDL muscles with an Aurora Mouse 1200A System and analysed by Dynamic Muscle Control v.5.415 software. To assess *ex vivo* muscle physiology, a previously published protocol was used. In brief, EDL muscles were dissected and placed into constantly oxygenated Ringer's extracellular solution containing 100 mM NaCl, 4.7 mM KCl, 3.4 mM CaCl<sub>2</sub>, 1.2 mM KH<sub>2</sub>PO<sub>4</sub>, 1.2 mM MgSO<sub>4</sub>, 25 mM HEPES, 5.5 mM glucose at 24 °C, pH was adjusted to 7.4-7.5.

The twitch stimulation protocol applied a single stimulus lasting 0.2 ms. Muscle length was adjusted to obtain the maximal twitch response and this length was measured and recorded as optimal length (L<sub>0</sub>) and used as reference for upcoming power normalization. Maximal tetanic force generation was achieved by repeated stimuli at a frequency of 120 Hz for 500 ms. To ensure muscle recovery prior next stimulus a 5-minute break was allowed. For muscle fatigue induction the muscle was stimulated every second for duration of 8 min using 40-Hz pulses lasting 330 ms. Following the muscle fatigue protocol, a burst protocol of 50 maximal tetanic contractions (120 Hz for 500 ms) was applied.

At this point muscle fatigue is complete, and almost no power can be generated. The recovery protocol started immediately after the last burst contraction. A maximal tetanic stimulation (120 Hz for 500 ms) was applied every 5 min for 30 min, and the force recovered was expressed as the percentage of the regained and starting power. Muscle cross-sectional area (CSA) was calculated as a division of EDL muscle mass by the product of the muscle density

coefficient ( $1.06 \text{ g/cm}^3$ ), muscle baseline L0, and the fibre length coefficient (0.45 specific for EDL). Specific force generated by muscle was determined by normalizing maximum tetanic force to CSA.

## 5 *Dopamine ELISA*

Striata were collected in 2 ml tubes and snap frozen in liquid nitrogen and stored in  $-80^\circ\text{C}$ . Brains were homogenized in PBS $^{-/-}$ , centrifuged at  $5,000 \times g$  for 10 minutes at  $+4^\circ\text{C}$ , and 50  $\mu\text{l}$  of supernatant was used for quantification of dopamine in brain tissue following manufacturer instruction (BioVision, K4219-100).

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## *Amplex fluorometry assays*

One brain hemisphere was collected in 2 ml tubes and snap frozen in liquid nitrogen for storage at  $-80^\circ\text{C}$ . Brains were homogenized in PBS $^{-/-}$ , centrifuged at  $5,000 \times g$  for 10 minutes at  $+4^\circ\text{C}$ , and 50  $\mu\text{l}$  of supernatant was mixed with 50  $\mu\text{l}$  of sample dilution buffer, and then the quantification of total acetylcholine and glutamic acid were performed according to manufacturer protocols (Amplex<sup>TM</sup> Acetylcholine/Acetylcholinesterase Assay Kit, Cat. No. A12217; and Amplex<sup>TM</sup> Red Glutamic Acid/Glutamate Oxidase Assay Kit, Cat. No. A12221).

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## *Bacterial gene cluster cloning*

The ere bacterial gene cluster from *Eubacterium rectale* containing the four biosynthetic genes *ereS*, *ereT*, *ereA*, *ereC* (lipid carrier, acyl carrier/thiolation, adenylation and condensation domain) was selected for the heterologous production of fatty acid amides (FAAs). The enzymes encoded in the gene cluster were annotated as follows: (EreS): SCP-2 (sterol carrier protein-2), sterol transfer family protein, (EreT): ACP, acyl carrier protein, (EreA): Long-chain acyl-CoA synthetases (AMP-forming), and (EreC): Non-ribosomal peptide synthetase. The DNA sequences of the biosynthetic genes *ereA* and *ereS* from *Eubacterium rectale* were codon optimized, synthesized and cloned into pMK-RQ and pOA-RQ by GeneArt (ThermoFisher Scientific), yielding pMK-RQ-*ereA* and pOA-RQ-*ereS*. The *ereA* gene was PCR amplified with the primer pair: 5'-CCCATATGCTGTTTCATACCATTCCGGATATTC-3' (SEQ ID NO:1) and 5'-CCCTCGAGTTAGCTGCGTTTGATTTTACGG-3' (SEQ ID NO:2) (NdeI and XhoI restriction sites are underlined) using pMK-RQ-*ereA* as a template. The PCR product fragment

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digested with *NdeI* and *XhoI* and ligated into *NdeI* and *XhoI* cloning sites of *pCDF-tacI/tacI* by T4 DNA ligation to generate *pCDF-tacI/tacI-ereA*. The *ereC* gene was PCR amplified with the primer pair: 5'-GGAATTCCATGAAGACGAGAAAGGGACAC-3' (SEQ ID NO:3) and 5'-CGAGCTCCTACACTAATTTAATGATTTTCGCC-3' (SEQ ID NO:4) (*EcoRI* and *SacI* restriction sites are underlined) using genomic DNA of *Eubacterium rectale* as a template. The PCR product fragment digested with *EcoRI* and *SacI* and ligated into *EcoRI* and *SacI* cloning sites of *pCDF-tacI/tacI-ereA* by T4 DNA ligation to generate *pCDF-tacI/tacI-ereAereC*. The *ereS* gene was PCR amplified with the primer pair: 5'-GGAATTCCTCATGACCTATGCCGATATGTTC-3' (SEQ ID NO:5) and 5'-CGAGCTCTTAGGCTTTTTTGCTATCGATGATCT G-3' (SEQ ID NO:6) (*EcoRI* and *SacI* restriction sites are underlined) using *pOARQ-ereS* as a template. The PCR product fragment digested with *EcoRI* and *SacI* and ligated into *EcoRI* and *SacI* cloning sites of *pCOLA-tacI/tacI* by T4 DNA ligation to generate *pCOLA-tacI/tacI-ereS*. The *ereT* gene was PCR amplified with the primer pair: 5'-CCCATATGTTTGATGAATTAGTGGAAATTATC-3' (SEQ ID NO:7) and 5'-CCCTCGAGTTATGCGTCTTTTAATGTCTCAAG-3' (SEQ ID NO:8) (*NdeI* and *XhoI* restriction sites are underlined) using genomic DNA of *E. rectale* as a template. The PCR product fragment digested with *NdeI* and *XhoI* and ligated into *NdeI* and *XhoI* cloning sites of *pCOLA-tacI/tacI-ereS* by T4 DNA ligation to generate *pCOLA-tacI/tacI-ereS-ereT*. The resulting constructs were introduced into *E. coli BAP1* which harbours a promiscuous phosphopantetheinyl transferase required for carrier protein activation.

#### *Bacterial cultures and inoculations*

*Coprococcus eutactus* (Holdeman and Moore, Cat. No. 27759), and *Eubacterium rectale* (Hauduroy et al., Cat. No. 33656) were purchased from ATCC and grown according to ATCC protocols for 24 to 48 hours at 37 °C under anaerobic conditions. *Ruminococcus gnavus* and *Blautia producta* were grown in ATCC media (supplemented with hemin, L-cystein, vitamin K1) under anaerobic gas chamber at 37 °C for 24 to 48 hours. *Escherichia coli* WT and *Escherichia coli EreA-T* knock-in mutant were grown in LB-media (Thermofisher scientific, Cat. No. DF0446-17-3) under facultative anaerobic conditions at 37 °C overnight. Fatty acid amide production in *E. coli EreA-T* strain was induced by supplementation of 10 mM of IPTG (Isopropyl-β-D-thiogalactopyranoside) (Cat. No. I56000-5.0, Research products international) in

the drinking water for 5 days. Bacteria cultures were spun down for 3 minutes at 2000 x g. Media were decanted and pellets resuspended. 250 µL per mouse were gavaged. After 5 days the colonization was confirmed by 16S RT-qPCR and 16S sequencing.

#### 5 *DNA stool extraction, 16S rRNA library preparation and microbiota analysis*

Stool collected into 2 ml tubes was snap frozen and stored in -80 °C until DNA extraction using the DNeasy PowerSoil Kit (Qiagen, Cat. No. 12888-100) according to the manufacturer's instruction. DNA was amplified by using the KAPA HiFi HotStart ReadyMix (Roche, KK2602) and 16S specific primers spanning the variable region 1 and 2 (V1/V2) of the 16S rRNA gene  
 10 were generated by using the following barcoded primers: Fwd i27-  
 TATGGTAATTGTAGAGTTTGATCCTGGCTCAG (SEQ ID NO:9), Rev 338-  
 XXXXXXXXXXXXXTGCTGCCTCCCGTAGGAGT (SEQ ID NO:10), where each X represents  
 a barcode base. After PCR amplification, the library was sequenced using 500 bp paired-end  
 sequencing (Illumina MiSeq, Cat. No. MS-102-2003). The paired-end reads were processed  
 15 using the QIIME 2 pipeline. Briefly, fastq files and a sample mapping file including the unique  
 barcode sequence corresponding to each sample were used as inputs for analysis. Paired-end  
 reads were split according to the unique barcodes, and then taxonomical classification was  
 performed using the Greengenes database with 97% OTU identity. Rarefaction was used to  
 equalize the number of reads per sample and to exclude samples with insufficient reads.

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#### *Quantification of 16S amplicons by qPCR*

The 16S variable regions were targeted by combination of 4 unique forward primers and 1 common reverse primer. Fwd1: 5'-CNACGCGAAGAACCTTANC-3' (SEQ ID NO:11),  
 Fwd2:5'-ATACGCGARGAACCTTACC-3' (SEQ ID NO:12), Fwd3: 5'-  
 25 CTAACCGANGAACCTYACC-3' (SEQ ID NO:13), Fwd4: 5'-  
 CAACGCGMARAACTTACC-3' (SEQ ID NO:14), 115-1046 Rev: 5'-  
 CGACRRCCATGCANACCT-3' (SEQ ID NO:15). DNA was amplified using Luna Universal  
 qPCR Master Mix (New England Biolabs, Cat. No. M3003E) using following protocol: 12.5 µl  
 of mix was combined with 0.5 µl each forward and 2 µl of reverse primer, 4.5 µl of undiluted  
 30 DNA was added. *E. coli* K12 WT strain was used as an internal standard for absolute bacterial  
 quantification.

*Single nucleus RNA-sequencing*

For single nucleus RNAseq (SNuc-Seq), striatum was dissected from antibiotics-treated and SPF mice both at steady state and after exercise. Nuclei isolation was performed as previously described. Sorted nuclei were then immediately encapsulated into droplets, libraries prepared using the 10X Genomics platform and single end sequencing was performed on NextSeq 550 instrument using 75-cycle kit. BCL files were demultiplexed, aligned to mouse mm10 genome, filtered, and UMI counted using CellRanger software (10X Genomics), and downstream analysis performed with Seurat v4. Data was filtered to remove cells with high mitochondrial reads (>5%), low gene detection (<200), and high gene detection (>4,000). In total, 44,100 nuclei passed these filtration parameters. Normalization was performed with SCTransform (Hafemeister and Satija, 2019) and integrated. Subsequently, cells were clustered using Louvain clustering (resolution = 0.5), and UMAP was used for visualization. Clusters were annotated as has previously been described for the striatum. Expression levels for each cluster and condition were visualized using the VlnPlot(), DotPlot(), and FeaturePlot() function in Seurat package. Single-cell datasets of mouse DRG was obtained from GEO (Accession number: GSE139088)<sup>36</sup>. Data was analysed with Seurat v. Normalization was performed with SCTransform<sup>56</sup>, clustered, and UMAP was used for visualization. Clusters were annotated and reproduced as described<sup>36</sup>. Expression levels for each cluster was visualized using the VlnPlot(), DotPlot(), and FeaturePlot() function in Seurat.

*Transcriptional profiling by bulk RNA-seq*

Libraries were prepared using the illumina TruSeq stranded mRNA kit (Cat. no. 20020595) with IDT for illumina TruSeq Unique dual indexes (Cat. no. 20022371) according to the manufacturer's instructions. Quality and quantity control of RNA and libraries were performed using Agilent 4200 TapeStation and Qubit 4, respectively. Libraries were sequenced on an Illumina NextSeq 550 to produce 75-base pair single end reads with an average sequencing depth of 7 million reads per sample. Raw reads were mapped to the mouse reference transcriptome (Ensembl; Mus musculus version 67) using Kallisto version 0.46.0. Subsequent analysis was carried out using the statistical computing environment R version 3.6.1 in RStudio version 1.2.5019 and Bioconductor version 3.8. Briefly, transcript quantification data were

summarized to genes using the tximport package and normalized using the trimmed mean of M values (TMM) method in edgeR. Genes with  $<1$  CPM in  $n+1$  of the samples, where  $n$  is the size of the smallest group of replicates, were filtered out. Differentially expressed genes were identified with linear modelling using limma ( $FDR \leq 0.05$ ; absolute  $\log FC \geq 1$ ) after correcting for multiple testing using Benjamini-Hochberg.

#### *Quantitative real-time PCR*

RNA was isolated using phenol-chloroform extractions, and cDNA was prepared by using 2000 ng/10  $\mu$ l of RNA input using High-Capacity cDNA Reverse Transcription kit (Applied Biosystems, Cat. No. 43-688-13). Quantitative real time PCR was performed by using Luna Universal qPCR Master Mix (New England Biolabs, Cat. No. M3003E). 4  $\mu$ l of cDNA were mixed with 5  $\mu$ l Luna Master Mix, and 1  $\mu$ l of primer mix was added. Amplification was set up by manufacturer protocol. Sequences for primers used to assess gene expression: Fos Fwd (5'-CAGCCTTTCCTACTACCATTC-3') (SEQ ID NO:16), Rev (5'-ACAGATCTGCGCAAAGTCC-3') (SEQ ID NO:17), Arc Fwd (5'-ACAGAGGATGAGACTGAGGCAC-3') (SEQ ID NO:18), Rev (5'-TATTCAGGCTGGGTCCTGTAC-3') (SEQ ID NO:19), and as a housekeeping gene Gapdh was used with sequence: Gapdh Fwd (5'-GGGTGTGAACACGAGAAATATG-3') (SEQ ID NO:20), Rev (5'-TGTGAGGGAGATGCTCAGTGTTG-3') (SEQ ID NO:21). To further verify gene expression, quantitative real time PCR using TaqMan Fast Advanced Master Mix was performed, (Applied Biosystems, Cat. No. 44-445-57), with Taq man probes for Maoa (ThermoFisher, Cat. No. Mm00558004\_m1), Fos (ThermoFisher, Mm00487425\_m1), Arc (ThermoFisher, Mm01204954\_g1), Homer1 (ThermoFisher, Cat. No. Mm00516275\_m1) and Gapdh (ThermoFisher, Cat. No. Mm99999915\_g1) as housekeeping gene. For assessing relative gene expression by calculating:  $2^{-(Ct\ Gapdh - Ct\ gene)}$ . In both protocols Applied Biosystems (Quantstudio 6) thermal cycler was used, and cycling condition was set following the manufacturer's instruction.

#### *Untargeted metabolomics*

1 ml of ice-cold 80% cold methanol containing 0.1% formic acid was added to 2 ml screw-top vials containing 25 or 50 mg of caecal contents and a mixture of 0.1 mm and 1 mm

zirconium oxide beads (tubes lacking caecal contents were used to generate method blanks). Samples were homogenized twice for 30 seconds in a Precellys 24 homogenizer at 9,000 rpm, allowing samples to cool on ice between cycles. Samples were centrifuged at 4 °C and >21,000 x g for >10 minutes to clarify supernatants. Clarified supernatants were transferred to new 1.5 ml microfuge tubes and dried via speedvac. Dried samples were stored at -80 °C until analysis. For analysis, samples were resuspended in 10% acetonitrile containing 1 µM chlopropamide to a concentration of 0.25 mg/µl, followed by vortexing and sonication until completely dissolved. Resuspended samples were centrifuged at 4 °C and >21,000 x g for >10 minutes to remove residual insoluble material. 5 µL of clarified supernatant was then diluted to a final concentration of 12.5 µg/µl (1:20) into 10% acetonitrile containing 1 µM chlopropamide. Samples were centrifuged at 4 °C and >21,000 x g for >10 minutes before transferring to MS vials for analysis. Additionally, a portion of each extract was also pooled to generate a quality control sample. Samples were randomized and 1 µL was injected for analysis using separate ddMS2 positive and negative ESI modes (Thermo Fisher Orbitrap Fusion Lumos) using both HILIC and RP chromatography methods. A minimum of three QC and blank injections were performed across each analysis to control for analytical drift, as well as background signal, respectively. Data were mined using MS-DIAL and export to Excel for further analysis.

#### *Relative quantification of OEA in caecal content*

Metal beads (0.5 cm) were added into 15 ml tubes with a caecum content. Tubes were vortexed for 5 minutes until the contents were fully homogeneous. Next, we added 1 ml UPW and samples were vortexed for 2 minutes, then 1 ml of chloroform added and the solution vortexed for another 2 minutes. After separation, the chloroform layer was collected and samples evaporated. Dry samples were stored in -20 °C until HPLC-MS analysis.

High Resolution High Performance Liquid Chromatography Mass Spectrometry (HPLC-MS) was used to identify and relatively quantify N-Oleoylethanolamide (OEA). For the identification pure OEA was used as reference. The extracts were dissolved in a ACN:MeOH (7:1)-mixture, centrifuged and the supernatant utilized for LC-MS measurements. A volume of 5 µL was injected into a Dionex Ultimate 3000 HPLC coupled to a Bruker Impact<sup>TM</sup> II ESI-Q-OTOF instrument and set to positive ionization mode. Separation was performed on a C18 [Waters, ACQUITY UPLC BEH column, 50 mm x 2.1 mm x 1.7 µm] column with acetonitrile

[0.1% formic acid] and H<sub>2</sub>O [0.1% formic acid] as solvent. The flow rate was 0.4 ml/min with the following gradient: 0-2 min 5 % ACN, 2-14 min 5-95 % ACN, 14-15 95 % ACN, 15-16 min 5 % ACN. The mass range was measured from 100-1200 m/z. Data analysis was performed with the software DataAnalysis 4.3 [Bruker]. For relative quantification, the peak area of the OEA signals were compared.

#### *HPLC-MS/MS for endocannabinoid measurements in serum*

Endocannabinoids were extracted from plasma, based on a previously described protocol. Briefly, 50 µL of plasma was added to 0.5 ml of ice-cold methanol/Tris buffer [50 mmol/l, pH 8] containing internal standards. To each mixture, 0.5 ml of Tris buffer [50 mmol/l, pH 8] and 1.5 ml of (2:1) methanol–chloroform mixture was added. The mixture was centrifuged for 2 minutes at 500 g, and the chloroform phase was transferred to a glass tube. The extraction was repeated twice. The combined extract was dried under nitrogen stream and the dried residue was reconstituted in chloroform. Further, 2 ml of acetone was added to all samples and the solution was centrifuged at 16,000 x g for 5 minutes. The clear supernatant was collected, dried and reconstituted in methanol. Endocannabinoids were analysed by LC-MS/MS. Mobile phase A consisted of water/B (95/5) with 0.1% formic acid and mobile phase B consisted of (95/5) acetonitrile and methanol with 0.1% formic acid. A linear gradient was run as follows: 0 min 30% B; 5 min 50% B; 15 min 100% B; 17 min 100% B; 17.5 min 30% B; 20.5 min 30% B at a flow rate of 0.25 ml/min with a total run time of 20.5 min. MS/MS analysis was performed using Waters Xevo TQ-S instrument equipped with electrospray ionization. Separation of the AEA and 2-AG was accomplished using a Waters 930 Acquity UPLC BEH column (2.1 x 150 mm) at 20 °C. Positive electrospray ionization data were acquired using multiple reactions monitoring (MRM). The mass transitions, collision energy, cone used were: AEA (348.2/287.3, 14, 30), AEA d4 (352.1/287.3, 14, 30), 2-AG:(379.4/287.2, 14, 30); 2-AG d5: (384.4/287.4, 14, 30).

#### *Shapley computational models*

Shapley values were calculated for the variables collected from DO cohort to determine additive feature contribution to the variation in treadmill endurance performance using Shap package for R. Each feature contribution to phenotype variability was assessed separately, as well as in combination in groups with respect to the variable origin (metabolomics, 16S, etc.).

### *Genetic analysis*

DO mice specimens were collected, DNA was extracted, and Illumina genotyping libraries sequenced by Neogen Corporation. Data obtained from genotype-sequencing of the DO cohort were analysed using the broman, qtl2, qtlcharts, ggplot2, mclust, DOQTL, kableExtra, knitr, fst, dplyr, cluster, tidyr, optparse, rhdf5 packages for R. In brief, the data were first prepared into array form using geneseeek2qtl2.R, and geneseeek2intensity.R and genoprobs were calculated using qtl2. Genoprobs array was then analysed as outlined in:

[https://kbroman.org/qtl2/assets/vignettes/do\\_diagnostics.html](https://kbroman.org/qtl2/assets/vignettes/do_diagnostics.html). Covariates such as sex, DO

generation were used in qtl2 analysis. All duplicate pairs were removed. Next, genotyping error LOD scores were calculated, and apparent genotyping error. There were no samples detected with genotype error >1%. The marker missing data and marker genotype frequencies were assessed. Markers with higher rates of missing genotypes (miss >10% of markers) showing higher error rates were removed from following analysis. These markers were mostly

monomorphic, low frequency markers. Markers with error rate >5% and samples lacking >5% of genotypes were also removed. Next, the founder proportions were plotted for all markers over all chromosomes. Kinship was assessed using calc\_kinship(), genome was scanned by using scan1perm() and LOD peaks found using find\_peaks(). QTL effect was estimated by using scan1coef(), scan1blup(), plot\_coefCC(), and GWAS estimation for each phenotype found end plotted by using scan1snps(). Heritability was estimated by est\_herit() function of qtl2 package from kinship matrix.

### *Statistical analysis*

Data are expressed as mean  $\pm$  SEM. Bonferroni-adjusted p-values <0.05 were considered significant. The Benjamini-Hochberg procedure was used to control the false discovery rate, with a cut-off of  $q < 0.05$ . Results from animal experiments are representative of at least two independent repetitions. Two-group comparisons were done using Mann-Whitney U-test. Comparisons between multiple groups were done using ANOVA with post-hoc test. Survival curve comparisons for p-values assessment were analysed using Log-rank Mantel-Cox test. P-values for linear regressions were calculated using Pearson's correlation.

**Example 1: Identification of metagenomic contributions to exercise performance**

To identify new regulators of exercise performance, a cohort of diversity outbred (DO) mice whose genomes were derived from eight defined inter-crossed genetic backgrounds were used (FIG. 1A). This cohort was deeply profiled by SNP genotyping, serum metabolomics, 16S rDNA sequencing of stool samples, and multi-parameter metabolic analysis (FIG. 1A), resulting in over 10,500 collected data points per mouse and close to 2.1 million data points in total. Subsequently, the mice underwent exercise profiling by either voluntary running in wheels or endurance running on treadmills (FIG. 1A). All mice were untrained prior to exercise. The performance data revealed marked variability in both treadmill and wheel running, with over 10-fold differences between the best- and worst-performing animals (FIGs. 1B-1C and FIGs. 2A-2B).

The genetic contribution to this high variability was first assessed in exercise performance. To this end, the mice were genotyped, a genetic kinship matrix was constructed (FIG. 2C), genome-wide association studies were performed for each treadmill parameter (FIG. 2D), and narrow-sense heritability was calculated. For each endpoint, heritability was rather low (below 0.2, FIG. 1D), consistent with previous studies and suggesting that the genetic contribution to inter-individual variability in exercise capacity was minor.

Next, non-genetic parameters were assessed for their relative contribution to exercise performance. Serum metabolomes, intestinal microbiome composition, and metabolic parameters varied widely across the animal cohort (FIGs. 2E-2N). A machine-learning approach was then used to unbiasedly identify variables with strong predictive contribution to exercise performance, again using treadmill parameters as the endpoint. A gradient boosting decision trees algorithm was trained and feature contributions were analyzed in the resulting model using SHAP (SHapley Additive exPlanation), a game theoretic approach which interprets the output of machine learning models by estimating the contribution (Shapley value) of each feature. The overall model achieved high accuracy of prediction for all treadmill parameters (FIG. 1E and FIGs. 3A-3B), suggesting a strong cumulative effect of all assessed variables on exercise performance. To identify features driving these predictions, all variables were categorized into “metagroups” and their relative contribution was determined (FIG. 1F). Notably, prediction based solely on 16S rDNA sequencing results achieved accuracies that were almost as high as those achieved with the serum metabolome or all measured parameters combined, despite

entailing a much smaller number of variables within the microbiome metagroup (FIG. 1G and FIGs. 3C-3G), while major metabolic and behavioral features did not predict endurance capacity (FIG. 1F and FIGs. 3H-3K).

Recent reports have highlighted a potential role for the intestinal microbiome in influencing exercise performance, but its overall significance and underlying mechanisms remain unclear. To determine how much of the inter-individual variability in exercise performance of DO mice was driven by the microbiome, loss-of-function (microbiome depletion) and gain-of-function (microbiome transplantation) experiments were performed (FIG. 1H). Microbiota ablation with broad-spectrum antibiotics diminished exercise performance on both running wheels (FIGs. 1I-1J and FIG. 3L) and treadmills (FIG. 1K and FIGs. 3M-3O). Transfer of microbiome samples from DO mice to germ-free mice led to a strong correlation between running wheel performances of donors and recipients (FIG. 1L). These results suggest a strong functional contribution of the microbiome to exercise capacity in DO mice.

To identify taxonomic elements of the microbiome that drive its contribution to physical performance, isogenic mice on the C57BL/6J background were used, which allowed variation of only microbiome parameters, while keeping all genetic and other environmental factors constant. First, the causality for the microbiome in multiple orthogonal approaches was tested. As with DO mice, microbiome ablation in C57BL/6 mice by broad-spectrum antibiotics reduced both treadmill and running wheel performance by about 50% (FIGs. 4A-4B and FIGs. 5A-5E). To ascertain that these observations were not due to potentially toxic side effects of oral antibiotics, exercise tests under sterile conditions with germ-free mice were performed, which showed a similarly reduced performance (FIGs. 4C-4D and FIGs. 5F-5J). The microbiome dependency of optimal exercise performance was observed in both male and female mice (FIGs. 5A-5E, FIG. 5K, and FIG. 5O). Importantly, cessation of antibiotic treatment as well as conventionalization of germ-free mice fully restored the performance (FIGs. 5P-5W), indicating that the impact of microbiome ablation on exercise is acute and reversible. This detrimental effect of microbiome depletion was specific to exercise, and did not affect movement in general, since neither home cage nor open field locomotion was impaired in antibiotics-treated mice (FIGs. 6A-6E).

To narrow down possible members of the microbiome involved in the phenotype, the spectrum of antibiotic combinations that were administered was reduced. Treatment with either only absorbable or only non-absorbable antibiotics similarly reduced treadmill running (FIGs.

6F-6I), further excluding possible systemic side effects. When each antibiotic was individually applied, which did not affect body weight (FIG. 6J), it was noted that neomycin-treated mice were not impaired in their exercise capabilities, while other antibiotics reduced performance in both the treadmill and wheel settings (FIGs. 4E-4H and FIGs. 6K-6L). Consistently, germ-free mice reconstituted with faecal microbiome samples from neomycin treated donors (which showed normal running ability) reached treadmill parameters of those reconstituted with samples from fully colonized conventional animals, while germ-free recipients of microbiome samples from ampicillin-treated donors (which showed diminished exercise performance) performed worse (FIG. 4I and FIGs. 6M-6P). These results suggest that certain taxonomic elements of the microbiome are both necessary and sufficient to regulate exercise performance.

To identify such taxonomic elements, 16S rDNA sequencing of mice treated with neomycin and ampicillin (FIG. 6Q) were performed, and a comparative analysis of the results alongside those obtained from the DO cohort was performed. All variables from the microbiome “metagroup” (FIG. 1F) were first ranked by their mean absolute SHAP values, which nominated several members of the *Erysipelotrichaceae* and *Lachnospiraceae* families as possible drivers of exercise performance (FIG. 4J). This was in line with *Erysipelotrichaceae* being resistant to neomycin but sensitive to ampicillin and thus positively correlated with running activity after microbiome transfer to germ-free mice (FIGs. 6R-6S). The causality of individual species was subsequently tested by performing mono-colonization experiments in gnotobiotic mice. Germ-free mice were colonized with a member of the *Erysipelotrichaceae* family contributing to the prediction of exercise performance (*Eubacterium rectale*), a member of the *Lachnospiraceae* family likewise involved in the prediction of exercise (*Coproccoccus eutactus*), a member of the *Lachnospiraceae* family with no predictive value for exercise (*Blautia producta*) and a taxonomically distinct member of the microbiota with high predictive power (*Ruminococcus gnavus*). We verified successful mono-colonization by 16S qPCR and sequencing (FIGs. 6T-6U) and then exercised the recipient mice on treadmills. *Eubacterium* and *Coproccoccus* enhanced exercise performance compared to germ-free mice, reaching levels that are comparable to conventionally colonized mice, while *Blautia* and *Ruminococcus* did not (FIGs. 4K-4L and FIGs. 6V-6W). Together, these data demonstrate the causative role of the microbiome in regulating exercise performance and highlight certain members of the *Erysipelotrichaceae* and *Lachnospiraceae* families as possible taxonomic contributors.

**Example 2: Evaluation of microbiome impact on exercise-induced striatal dopamine signaling**

Next, the mechanisms by which the microbiome influences exercise performance were evaluated. Based on previous reports, it was hypothesized that an intact microbiome was required for optimal muscle function. Indeed, a reduction in the mass of the soleus, gastrocnemius and tibialis anterior muscles in antibiotics-treated mice was observed (FIGs. 7A-7C). In contrast, the extensor digitorum longus was not affected by microbiome depletion (FIG. 7D). Grip strength was unaffected by exercise and the microbiome (FIGs. 7E-7F). Furthermore, no difference was observed in twitch or tetanic muscle forces in *ex vivo* tests, nor in muscle recovery after stimulation (FIGs. 7G-7N), indicating that overall muscle function was intact despite microbiome ablation. Likewise, muscle fibre size was normal, and neither isolated mitochondria nor whole cell lysates from the muscles of antibiotics-treated mice showed any defects in oxygen consumption (FIGs. 7O-7Q). In addition, the extensor digitorum longus transcriptome was comparable between antibiotics-treated mice and controls, and no differences were observed in the expression of genes implicated in mitochondrial metabolism, energy homeostasis, sensory motor function, or neuro-muscular junction (FIGs. 7R-7S). These results suggest that the pronounced effect of the gut microbiome on voluntary and endurance exercise may not be mediated by changes in muscle physiology.

A major contributor to exercise performance apart from musculoskeletal function is the motivational state. A brain region critically involved in motivated behaviour as well as the initiation of physical activity is the striatum. To explore the impact of the microbiome on the striatal response to exercise, single-nucleus RNA-sequencing of the striatum of antibiotics-treated mice and controls was performed before and after treadmill exercise. A total of 44,100 cells were sequenced and cell types were identified based on canonical markers (FIG. 8A). Next, the neuronal population was examined and the major groups of medium spiny neurons expressing the dopamine receptors D1 and D2 were identified (FIG. 9A and FIG. 8B). It was noted that the expression of immediate-early genes, such as *Arc* and *Fos*, was strongly elevated by exercise; however, this elevation was dampened in the absence of the microbiome (FIGs. 9B-9C and FIGs. 8C-8D). These data suggest that intestinal microbial colonization is important for exercise-triggered activation of the striatum.

Given that striatal medium spiny neurons are controlled by dopamine, which is a critical regulator of the drive for physical activity, striatal dopamine levels in microbiome-depleted mice were next measured. Expectedly, striatal dopamine levels were increased after exercise. Remarkably, this elevation was blunted in microbiome depleted mice (FIG. 9D), indicating that intestinal microbial colonization is required for efficient post-exercise dopamine release. This was not due to the reduced exercise capacity of microbiome-deficient mice, since the dopamine response to exercise was likewise decreased when the exercise protocol was timed and terminated before the first animal became exhausted (FIG. 9D). Real-time measurements of dopamine levels, using a fluorescent dopamine sensor (FIG. 9E and FIG. 10A), demonstrated a rapid and sustained upregulation of dopamine in the ventral and dorsal striatum following different durations of exercise (FIGs. 10B-10D). Strikingly, this dopamine response was nearly eliminated in antibiotics-treated animals (FIGs. 9G-9H), further emphasizing the importance of the microbiome for striatal dopamine responses to physical activity. It is important to note that this impaired striatal dopamine response was only observed in the exercised state, whereas basal levels of dopamine were unaffected by the microbiome (FIG. 9D), consistent with intact basal locomotion in antibiotics-treated mice (FIGs. 6A-6E). Other major striatal neurotransmitters, such as glutamate and acetylcholine, were neither affected by exercise nor the microbiome (FIGs. 10E-10F).

Several experiments were subsequently performed to evaluate whether the exercise-triggered dopamine response was mechanistically involved in the performance-stimulating effect of the microbiome. First evidence came from the finding that post-exercise striatal dopamine levels correlated with wheel running in DO mice (FIG. 9J). Second, germ-free mice receiving exercise-enhancing microbiome transplantations, either from top performing DO mice or from conventionally colonized C57BL/6 mice, also exhibited elevations in dopamine levels, while germ-free controls or those mice receiving fecal grafts from low-performing DO mice or ampicillin-treated donors showed a reduced dopamine response (FIG. 9J and FIG. 10G). Third, inhibition of dopamine signaling, either by the pharmacological dopamine receptor antagonist sulpiride or by chemogenetic inhibition of dopaminergic neurons in the ventral tegmental area (VTA), phenocopied the effect of microbiome depletion (FIGs. 9K-9L and FIGs. 10H-10M). Furthermore, activation of dopamine signalling by the pharmacological dopamine receptor agonist bromocriptine restored the exercise performance of antibiotics-treated mice (FIGs. 9M-

9N). These experiments suggest that striatal dopamine signalling is an important mediator of the microbiome impact on exercise activity.

Based on these findings, the mechanisms whereby microbiome ablation impaired exercise-induced dopamine levels in the striatum were explored. An involvement of dopaminergic cells in the VTA was hypothesized, since these neurons are primarily responsible for the release of dopamine in the striatum. Accordingly, neural activity dynamics were monitored using calcium imaging of VTA dopaminergic neurons during exercise, and the influence of antibiotic treatment on VTA neuron activity was evaluated (FIG. 11A). However, no differences in calcium flux were observed (FIGs. 11B-11E), making it unlikely that the impact of the microbiome on dopamine levels and physical performance was mediated by differences in VTA neuron activity. Consistently, administration of leptin, which has been shown to inhibit the rewarding effects of exercise via the VTA, had an additive effect with microbiome ablation with respect to exercise suppression (FIGs. 11F-11I), further arguing that the microbiome did not directly affect the VTA. Next, the possibility that dopamine levels might be post-synaptically influenced by differential turnover in the striatum was considered. Dopamine degradation is primarily regulated by the enzyme monoamine oxidase (MAO). Remarkably, striatal expression of *Maoa* was suppressed in exercising mice, which did not occur in the absence of the gut microbiome (FIG. 9O). It was thus hypothesized that sustained MAO levels are responsible for the blunted dopamine response to exercise in microbiome-depleted mice, and that blockade of MAO would normalize dopamine signaling. Indeed, pharmacological MAO inhibition with pargyline enhanced exercise induced dopamine levels and restored exercise performance in antibiotics-treated mice, on both running wheels and treadmills (FIGs. 9P-9R and FIGs. 11J-11M). These results suggest that intestinal microbial colonization enhances exercise-induced dopamine signaling in the striatum by limiting MAO-driven dopamine turnover, rather than by increasing dopaminergic input from the VTA. Altogether, the experiments described herein indicate that the impact of the microbiome on exercise performance is mediated by the modulation of exercise induced dopamine levels in the brain.

### **Example 3: Demonstration of exercise regulation by afferent sensory neurons**

Having established striatal dopamine as an important element in microbiome-mediated regulation of exercise performance, it was next asked how intestinal microbial colonization

impacts midbrain dopamine levels. Signal transduction from the intestinal lumen to the brain can generally occur by two possible pathways: either through circulating factors, such as microbial metabolites, or through afferent neuronal circuits. Humoral factors were first evaluated, given that markedly altered serum metabolite profiles in antibiotics treated mice were observed (FIG. 12A). However, the correlations between microbiome-dependent and exercise-associated metabolites in the DO cohort were weak, and comparative analysis did not yield any strong candidate humoral mediator (FIG. 12B).

Gut-innervating afferent sensory neurons were subsequently evaluated. Denervated *Trpv1<sup>DTA</sup>* mice were used, which lack a large fraction of spinal and vagal afferent neurons that express the vanilloid receptor TRPV1. Notably, *Trpv1<sup>DTA</sup>* mice were impaired in their exercise capabilities in a similar manner as microbiome-depleted mice on both running wheels and treadmills, and antibiotic treatment did not further modulate their exercise performance (FIGs. 13A-13D and FIGs. 12C-12D). A similar impairment of exercise performance was observed after chemogenetic silencing of TRPV1-expressing neurons (FIGs. 12E-12H). Activation of TRPV1<sup>+</sup> afferent neurons with low doses of the TRPV1 agonist capsaicin restored wheel and treadmill running in antibiotics-treated mice (FIGs. 13E-13F and FIGs. 12I-12L).

Given that *TRPV1* is broadly expressed by sensory neurons, it was subsequently sought to determine whether spinal or vagal afferents were the primary mediator of the microbiome impact on exercise. To this end, dorsal root ganglia (DRGs) and nodose ganglia (NGs) from exercising mice were examined. It was noted that treadmill running induced neuronal activity, as indicated by cFos, in *TRPV1<sup>+</sup>* neurons (FIGs. 14A-14C). Antibiotic treatment reduced exercise-induced neuronal activation in DRGs, but not NGs (FIG. 13G and FIGs. 14D-14I), suggesting that the spinal pathway was more sensitive to microbiome-derived signals. Consistently, ablation of gut-innervating vagal afferents by NG injection of the cholecystokinin-conjugated neurotoxin saporin (CCKSAP) did not affect running capacity (FIGs. 15A-15B), whereas severing of gut-innervating spinal afferent fibers by surgical removal of the celiac/superior mesenteric ganglion (CSMG) reduced exercise performance similar to microbiome ablation (FIGs. 15C-15J).

To explore whether stimulation of sensory neurons affected the MAO-dopamine pathway, *Maoa* transcripts and post-exercise dopamine levels in the striatum were measured. It was found that capsaicin treatment restored both *Maoa* transcripts and normal dopamine levels in microbiome-depleted mice, while *Trpv1<sup>DTA</sup>* mice showed post-exercise dopamine levels that

were as low as in mice lacking an intact microbiome (FIGs. 13H-13I). Similarly, CSMG removal elevated *Maoa* expression and suppressed dopamine levels (FIGs. 15K-15L). Real-time recording of a dopamine sensor in the nucleus accumbens of the ventral striatum confirmed that post-exercise striatal dopamine levels were rescued by peripheral administration of capsaicin (FIGs. 13J-13L and FIG. 15M). To determine whether the exercise-stimulating effect of capsaicin functionally depended on dopamine signaling, striatal dopamine levels with exercise performance on the level of individual animals were first compared, and high degree of correlation was found (FIG. 15N). Next, an experiment was performed in which microbiome-depleted and control mice were co-treated with capsaicin and sulpiride, to inhibit dopamine signaling while stimulating afferent neurons. Importantly, sulpiride treatment blocked the exercise-enhancing effect of capsaicin (FIGs. 13M-13N), indicating that dopamine signaling was indeed required. Furthermore, dopamine receptor stimulation with bromocriptine bypassed the requirement for the microbiome and afferent sensory neurons and fully restored wheel running in *Trpv1<sup>DTA</sup>* and in antibiotics treated mice. Collectively, these findings demonstrate that the impact of the microbiome on dopamine levels in the striatum and exercise performance requires TRPV1+ afferent neurons.

#### **Example 4: Demonstration of exercise regulation by intestinal metabolites**

Possible mechanisms by which the microbiome shapes the activity of gut-innervating afferent sensory nerves to influence exercise performance were next evaluated. To simulate sensing of luminal components by afferent neurons, isolated DRG neurons were exposed *in vitro* to stool extracts from antibiotics-treated mice, germ-free mice, and conventionally colonized controls and recorded their activity by calcium imaging. The stimulatory potential was reduced in extracts from microbiome-deficient mice (FIG. 16A and FIG. 17A), suggesting that a luminal component may be sensed by DRG neurons. Similarly, luminal content from germ-free mice showed reduced DRG neuron stimulation compared to colonized controls, which was reversible upon conventionalization of germ-free mice (FIGs. 17B-17C). To explore whether DRG neuron stimulation by microbiome products was associated with striatal dopamine levels and exercise performance, stool extracts from the DO cohort were used and tested for DRG neuron-stimulatory capacity. A wide range in calcium signal was observed, which correlated with both activity-induced dopamine release in the striatum and exercise performance (FIGs. 17D-17G).

Next, the identification of specific components of the intestinal lumen that may constitute microbiome-dependent DRG neuron stimuli was sought. To this end, a strategy of differential antibiotic treatment was applied again. DRG neurons were stimulated with stool extracts from mice on different antibiotics and observed that the calcium signal strongly correlated with the animals' exercise performance (FIGs. 16B-16C and FIG. 17H). Untargeted metabolomics were performed, and the results were correlated with exercise parameters (FIG. 16D). Candidate molecules were screened for their ability to activate calcium signalling in DRG neurons (FIG. 16E and FIG. 17I). Interestingly, among the most potent metabolites were numerous fatty acid amides (FAAs), such as N-oleoylethanolamide (OEA), whose DRG-stimulatory activity was comparable to that of capsaicin (FIG. 16E and FIGs. 17J-17K). OEA levels were depleted by those antibiotics that impaired exercise performance and correlated with wheel running in DO mice (FIGs. 16F-16G), suggesting that FAA metabolites like OEA may stimulate sensory neurons to promote physical activity.

To determine whether FAAs are sufficient to restore exercise capacity in the absence of the microbiome, a dietary supplementation approach was pursued. Five FAAs with DRG-stimulatory activity (FIGs. 18A-18B) and supplemented them to a rodent diet (FIGs. 18C-18D). When fed to antibiotics treated mice, this FAA diet recovered the ability of stool extracts to induce neuronal calcium signalling (FIGs. 18E-18F). Strikingly, it also restored DRG activity, striatal *Maoa* expression, dopamine levels, and exercise performance in the absence of the microbiome (FIGs. 16H-16I and FIGs. 18G-18L). It likewise enhanced running capacity in microbiome-ablated DO mice (FIGs. 18M-18O). Given that OEA has been shown to stimulate dopamine release in the context of dietary reward, it was next questioned whether gastric infusion of FAAs was sufficient to recapitulate the effects of exercise on dopamine levels in the striatum. In sedentary mice, a dopamine response to FAA infusion was not observed. However, upon treadmill exercise, intestinal FAAs potentiated the dopamine release induced by physical activity (FIGs. 18P-18Q). These results suggest that intestinal FAAs enhance the motivation and/or reward for exercise.

Next, it was explored whether FAA production by the microbiome can account for its performance-stimulating effect. Elevated OEA levels in germ-free mice mono-colonized with exercise-enhancing bacteria suggested that microbiota production of FAAs could stimulate running performance (FIG. 19A). Interestingly, certain commensal microorganisms, including

*Eubacterium* from the *Erysipelotrichaceae* family and *Coproccoccus* from the *Lachnospiraceae* family, have recently been found to contain a biosynthetic gene cluster responsible for FAA biosynthesis. To test whether the presence of this gene cluster in the microbiome was sufficient to enhance exercise, an *Escherichia coli* strain was generated containing the essential genes of the cluster and colonized germ-free mice with *E. coli* strains that either harboured the FAA biosynthetic gene cluster or the empty vector (FIG. 19B). It was first verified that the gene cluster endowed *E. coli* with the ability to produce FAAs like OEA *in vivo* and to stimulate calcium signaling in DRG neurons (FIGs. 19C-19E). The mono-colonized mice were next exercised to determine the impact of the engineered strain on physical activity. Remarkably, while the empty vector control strain did not improve the performance of germ-free mice, the FAA-producing strain enhanced running on both wheels and treadmills (FIGs. 19F-19K). These findings highlight that a microbial gene cluster enabling the production of a metabolite family can influence host exercise capacity.

The mechanisms by which intestinal FAA may stimulate sensory neurons and their impact on exercise motivation were next explored. Several FAA metabolites serve as agonists of the endocannabinoid receptor CB1, which is expressed by TRPV1<sup>+</sup> DRG neurons (FIGs. 20A-20B). These observations thus raised the possibility that stimulation of CB1 by FAAs may drive sensory neuron activity, striatal dopamine release, and exercise performance. Consistent with this hypothesis, CB1-deficient mice showed diminished DRG neuron activity in response to exercise, reduced striatal dopamine, and impaired exercise capacity (FIGs. 20C-20I), in line with previous findings. Similarly, the CB1 receptor antagonist O-2050 inhibited wheel running, while the CB1 receptor agonist C55,940 enhanced performance (FIGs. 20J-20M). To disentangle the impact of peripheral CB1 signaling from endocannabinoid effects on the brain, the peripherally restricted CB1 inhibitor AM654546 was used, which phenocopied the effects of CB1 deficiency and resulted in a similar exercise impairment as microbiome ablation (FIGs. 16J-16K and FIGs. 20N-20Q). Importantly, the impact of intestinal interventions that enhance exercise, such as dietary supplementation of FAAs and mono-colonization with FAA-producing *E. coli*, was abolished by co-treatment with AM6545, indicating that these interventions act through peripheral CB1 signaling (FIGs. 16L-16M and FIGs. 21A-21E). Without wishing to be bound by theory, the impact of peripheral CB1 stimulation on exercise may be mediated by dopamine signaling in the striatum. Indeed, AM6545 treatment elevated *Maoa* expression and blunted the exercise-induced

surge of dopamine (FIGs. 21F-21G). Furthermore, AM6545-treated mice regained full exercise capacity when receiving dopamine receptor stimulation with bromocriptine (FIGs. 16N-16O), demonstrating that the impact of peripheral CB1 signaling on exercise was mediated by dopamine.

Thus, the present disclosure demonstrates that the gut microbiome contributes to the generation of intestinal fatty acid amides, including endocannabinoids, and that such metabolites trigger CB1-expressing TRPV1<sup>+</sup> sensory neurons, which send an exercise-induced afferent signal to the brain and promote the downregulation of MAO expression in the striatum. This downregulation of MAO contributes to higher levels of dopamine and enhanced exercise capability (FIG. 21H). In the absence of the microbiome, the afferent signal is inhibited, MAO levels are sustained, and thus the exercise induced surge in dopamine is blunted, with profound consequences for physical performance.

These findings have several important implications. First, they suggest that the neurochemical effects underlying the “runner’s high” (*i.e.*, the phenomenon of pleasure, reward, anxiolysis and analgesia that is driven by endocannabinoid release after prolonged physical activity) might be influenced by the gastrointestinal tract. Consistently, it was found that the analgesic effects of exercise are likewise dependent on intestinal microbial colonization and on peripheral CB1 signaling (FIGs. 21I-21J), indicating that the pathway discovered and described herein may regulate additional aspects of exercise physiology. Previous studies have identified an exercise-enhancing impact of the microbiome via lactate clearance and short-chain fatty acid production, indicating that several parallel pathways mediate the microbiome impact on physical performance. Evolutionarily, the regulation of exercise-induced reward and motivation circuits by gut metabolites may serve to couple nutrient availability and the state of intestinal microbial colonization to the readiness and capacity to engage in prolonged physical activity.

Second, these results expand the scope of knowledge about neuronal pathways communicating between the gut and the striatum, by demonstrating that this communication is enhanced by exercise, that the impact of exercise on the striatum is shaped by intestinal metabolites, and that exercise-induced activity of spinal sensory neurons is critically involved in the regulation of striatal dopamine signaling through the downstream regulation of MAO.

Further, the present disclosure demonstrates that mesolimbic dopamine-dependent reward and motivation circuits can be modulated via an interoceptive pathway that originates in the

gastrointestinal tract. This discovery may suggest that other behaviours that are dependent on striatal dopamine signaling could likewise be modifiable through lifestyle interventions, diet, or metabolite supplementation. It thus paves the way for the more general concept of

“interoceptomimetics” (*i.e.*, molecules that stimulate afferent sensory pathways and thereby

influence brain activity by peripheral intervention). The present disclosure addresses the need for interoceptomimetics that stimulate the motivation for exercise, and represents a powerful opportunity to counteract the detrimental health impact of a sedentary lifestyle.

### Sequence Listing

SEQ ID NO:1 ereA primer

CCCATATGCTGTTTCATACCATTCGGATATTC

SEQ ID NO:2 ereA primer

CCCTCGAGTTAGCTGCGTTTGATTTTACGG

SEQ ID NO:3 ereC primer

GGAATTCCATGAAGACGAGAAAGGGACAC

SEQ ID NO:4 ereC primer

CGAGCTCCTACACTAATTTAATGATTTCGCC

SEQ ID NO:5 ereS primer

GGAATTCCCATGACCTATGCCGATATGTTC

SEQ ID NO:6 ereS primer

CGAGCTCTTAGGCTTTTTTGCTATCGATGATCTG

SEQ ID NO:7 ereT primer

CCCATATGTTTGATGAATTAGTGGAATTATC

SEQ ID NO:8 ereT primer

CCCTCGAGTTATGCGTCTTTTAATGTCTCAAG

**SEQ ID NO:9**

TATGGTAATTGTAGAGTTTGATCCTGGCTCAG

5

**SEQ ID NO:10**

XXXXXXXXXXXXTGCTGCCTCCCGTAGGAGT, wherein each X represents a  
barcode base

10 **SEQ ID NO:11**

CNACGCGAAGAACCTTANC

**SEQ ID NO:12**

ATACGCGARGAACCTTACC

15

**SEQ ID NO:13**

CTAACCGANGAACCTYACC

**SEQ ID NO:14**

20 CAACGCGMARAACTTACC

**SEQ ID NO:15**

CGACRRCCATGCANACCT

25 **SEQ ID NO:16**

CAGCCTTTCCTACTACCATTCC

**SEQ ID NO:17**

ACAGATCTGCGCAAAAGTCC

30

**SEQ ID NO:18**

ACAGAGGATGAGACTGAGGCAC

**SEQ ID NO:19**

TATTCAGGCTGGGTCTGTGCAC

**5 SEQ ID NO:20**

GGGTGTGAACCACGAGAAATATG

**SEQ ID NO:21**

TGTGAGGGAGATGCTCAGTGTTG

10

**SEQ ID NO:22 (EreS)**MTYADMFSKVKGLFMESDVSDISEHLAFQFNITGEAEGIFYAEVKDGVLAPEPYEYFDRDAIFI  
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EAVDYLETLKDA**SEQ ID NO:24 (EreA)**

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5 YLCKIMFKGAENPELTIGEIIKLV

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TG TAGCGCAGAAACCCTGTTTAAACTGGCAGAAGGTCGTATTGATCCGATTCTGGCCTTTACCA  
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CAAAAAAGCCTAA

**15 SEQ ID NO:27 (EreT)**

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GAAGCAGTTGATTATCTGGAAACCCTGAAAGATGCCTAA

20

**SEQ ID NO:28 (EreA)**

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15 **SEQ ID NO:29 (EreC)**  
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**SEQ ID NO:30 (>pCOLA\_tacl/1-ereS(opt.)-ereT)**

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**SEQ ID NO:31 (>pCDF\_tacl/l\_ereC\_ereA(opt.))**

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 20 TTAATACGACTCACTATA

### Enumerated Embodiments

The following exemplary embodiments are provided, the numbering of which is not to be construed as designating levels of importance:

25

Embodiment 1 provides a dietary supplement composition comprising:

- (a) at least two fatty acid amides (FAAs) selected from the group consisting of *N*-stearoyl serotonin, *N*-arachidonoyl dopamine, *N*-oleoyl dopamine, *N*-palmitoyl dopamine, *N*-stearoyl dopamine, *N*-stearoyl taurine, *N*-arachidonoyl ethanolamide, *N*-palmitoyl ethanolamide, and *N*-oleoyl ethanolamide; and
- 30 (b) at least one solid, liquid, or semi-solid edible base substance.

Embodiment 2 provides the composition of Embodiment 1, wherein the composition comprises *N*-palmitoyl dopamine, *N*-arachidonoyl dopamine, *N*-oleoyl dopamine, *N*-oleoyl ethanolamide, and palmitoyl ethanolamide.

Embodiment 3 provides the composition of Embodiment 2, wherein the composition has  
5 a ratio of FAAs of about 1:1:1:1:1 by weight.

Embodiment 4 provides the composition of any one of Embodiments 1-3, wherein the composition is suitable for oral administration.

Embodiment 5 provides a pharmaceutical composition comprising:

- 10 (a) at least two fatty acid amides (FAAs) selected from the group consisting of *N*-stearoyl serotonin, *N*-arachidonoyl dopamine, *N*-oleoyl dopamine, *N*-palmitoyl dopamine, *N*-stearoyl dopamine, *N*-stearoyl taurine, *N*-arachidonoyl ethanolamide, *N*-palmitoyl ethanolamide, and *N*-oleoyl ethanolamide; and  
(b) at least one pharmaceutically acceptable carrier.

Embodiment 6 provides the pharmaceutical composition of Embodiment 5, wherein the  
15 composition comprises *N*-palmitoyl dopamine, *N*-arachidonoyl dopamine, *N*-oleoyl dopamine, *N*-oleoyl ethanolamide, and palmitoyl ethanolamide.

Embodiment 7 provides the pharmaceutical composition of Embodiment 6, wherein the composition has a ratio of FAAs of about 1:1:1:1:1 by weight.

Embodiment 8 provides the pharmaceutical composition of any one of Embodiments 5-7,  
20 wherein the composition is suitable for oral administration.

Embodiment 9 provides a plasmid comprising at least one gene which encodes a protein that promotes fatty acid amide (FAA) biosynthesis which when inserted into a bacterium stimulates and/or enhances exercise motivation and/or physical performance in a subject.

Embodiment 10 provides the plasmid of Embodiment 9, wherein the protein is an  
25 enzyme.

Embodiment 11 provides the plasmid of Embodiment 10, wherein the enzyme is at least one selected from the group consisting of EreS (SEQ ID NO:22), EreT (SEQ ID NO:23), EreA (SEQ ID NO:24), and EreC (SEQ ID NO:25).

Embodiment 12 provides the plasmid of any one of Embodiments 9-11, wherein the  
30 plasmid comprises at least one gene selected from the group consisting of *ereS* (SEQ ID NO:26), *ereT* (SEQ ID NO:27), *ereA* (SEQ ID NO:28), and *ereC* (SEQ ID NO:29).

Embodiment 13 provides the plasmid of any one of Embodiments 9-12, wherein the plasmid comprises each of *ereS* (SEQ ID NO:26), *ereT* (SEQ ID NO:27), *ereA* (SEQ ID NO:28), and *ereC* (SEQ ID NO:29).

5 Embodiment 14 provides the plasmid of any one of Embodiments 9-13, wherein plasmid comprises a polynucleotide, wherein at least of portion of the polynucleotide shares at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence homology with at least one polynucleotide sequence selected from the group consisting of SEQ ID NO:30 and SEQ ID NO:31.

10 Embodiment 15 provides a probiotic composition comprising an engineered bacterial strain comprising the plasmid of any one of Embodiments 9-14 and at least one pharmaceutically acceptable carrier and/or at least one base substance.

Embodiment 16 provides a method of stimulating and/or enhancing exercise motivation and/or physical performance in a subject, the method comprising administering to the subject at least one selected from the group consisting of:

- 15 (a) at least one Cannabinoid Receptor Type 1 (CB1) agonist;
- (b) at least one dietary supplement composition of any one of Embodiments 1-4;
- (c) at least one pharmaceutical composition of any one of Embodiments 5-8;
- (d) at least one bacterial strain comprising the plasmid of any one of Embodiments 9-14;
- (e) at least one probiotic composition of Embodiment 15;
- 20 (f) at least one probiotic composition comprising at least one bacterial strain selected from the group consisting of *Coprococcus* bacterium and *Eubacterium* bacterium.

Embodiment 17 provides the method of Embodiment 16, wherein the method comprises oral administration of (a), (b), (c), (d), (e), and/or (f).

25 Embodiment 18 provides the method of Embodiment 16 or 17, wherein the CB1 agonist is a fatty acid amide.

Embodiment 19 provides the method of Embodiment 18, wherein the fatty acid amide comprises a fatty acyl moiety covalently bonded to a naturally occurring primary amine.

30 Embodiment 20 provides the method of Embodiment 19, wherein the fatty acyl moiety is selected from the group consisting of a saturated fatty acyl moiety and an unsaturated fatty acyl moiety.

Embodiment 21 provides the method of Embodiment 20, wherein the saturated fatty acyl moiety is selected from the group consisting of stearoyl, palmitoyl, capryloyl, caproyl, lauroyl, myristoyl, arachidoyl, behenoyl, lignoceroyl, and cerotoyl.

Embodiment 22 provides the method of Embodiment 20, wherein the unsaturated fatty acyl moiety is selected from the group consisting of arachidonoyl, oleoyl, myristoleoyl, palmitoleoyl, sapienoyl, elaidoyl, vaccenoyl, linoleoyl, linoelaidoyl,  $\alpha$ -linolenoyl, eicosapentaenoyl, erucoyl, and docosahexaenoyl.

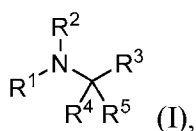
Embodiment 23 provides the method of any one of Embodiments 19-22, wherein the naturally occurring amine is selected from the group consisting of a neurotransmitter and an amino acid, or a metabolite and/or biosynthetic precursor thereof.

Embodiment 24 provides the method of Embodiment 23, wherein the neurotransmitter is selected from the group consisting of serotonin, dopamine, glutamate,  $\gamma$ -aminobutyric acid, acetylcholine, norepinephrine, and epinephrine.

Embodiment 25 provides the method of Embodiment 23, wherein naturally occurring amine is a metabolite and/or biosynthetic precursor of an amino acid.

Embodiment 26 provides the method of Embodiment 25, wherein the metabolite and/or biosynthetic precursor of an amino acid is selected from the group consisting of ethanolamine and taurine.

Embodiment 27 provides the method of Embodiment 18, wherein the fatty acid amid is a compound of Formula (I), or a salt, solvate, stereoisomer, or isotopologue thereof:



wherein:

$\text{R}^1$  is selected from the group consisting of  $\text{C}(=\text{O})$ (optionally substituted  $\text{C}_1$ - $\text{C}_{25}$  alkyl) and  $\text{C}(=\text{O})$ (optionally substituted  $\text{C}_2$ - $\text{C}_{25}$  alkenyl);

$\text{R}^2$  is selected from the group consisting of H and optionally substituted  $\text{C}_1$ - $\text{C}_6$  alkyl;

$\text{R}^3$  is  $\text{C}_1$ - $\text{C}_6$  alkyl substituted with at least one substituent selected from the group consisting of optionally substituted  $\text{C}_6$ - $\text{C}_{10}$  aryl, optionally substituted  $\text{C}_2$ - $\text{C}_{10}$  heteroaryl, halogen,  $\text{OR}^A$ ,  $\text{N}(\text{R}^A)(\text{R}^B)$ ,  $\text{C}(=\text{O})\text{OR}^A$ ,  $\text{C}(=\text{O})\text{N}(\text{R}^A)(\text{R}^B)$ ,  $\text{S}(=\text{O})_2\text{OR}^A$ ,  $\text{P}(=\text{O})(\text{OR}^A)(\text{OR}^B)$ ,

$N(R^A)C(=NR^B)N(R^C)(R^D)$ ,  $OC(=O)R^A$ ,  $N(R^A)C(=O)R^B$ ,  $N(R^A)C(=O)OR^B$ ,  $OS(=O)_2R^A$ ,  $N(R^A)S(=O)_2R^A$ , and  $OP(=O)(OR^A)(OR^B)$ ;

$R^4$  and  $R^5$  are each independently from the group consisting of H, optionally substituted C<sub>1</sub>-C<sub>6</sub> alkyl;

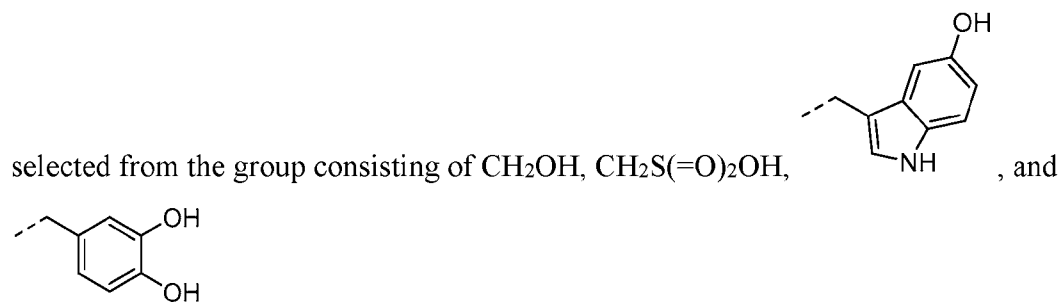
5  $R^A$ ,  $R^B$ ,  $R^C$ , and  $R^D$ , if present, are each independently selected from the group consisting of H, optionally substituted C<sub>1</sub>-C<sub>6</sub> alkyl, optionally substituted C<sub>2</sub>-C<sub>6</sub> heteroalkyl, optionally substituted C<sub>3</sub>-C<sub>8</sub> cycloalkyl, optionally substituted C<sub>2</sub>-C<sub>8</sub> heterocycloalkyl, optionally substituted C<sub>2</sub>-C<sub>6</sub> alkenyl, optionally substituted C<sub>6</sub>-C<sub>10</sub> aryl, and optionally substituted C<sub>2</sub>-C<sub>10</sub> heteroaryl.

10 Embodiment 28 provides the method of Embodiment 27, wherein each occurrence of optionally substituted C<sub>1</sub>-C<sub>6</sub> alkyl, optionally substituted C<sub>1</sub>-C<sub>25</sub> alkyl, optionally substituted C<sub>2</sub>-C<sub>6</sub> alkenyl, optionally substituted C<sub>2</sub>-C<sub>25</sub> alkenyl, optionally substituted C<sub>3</sub>-C<sub>8</sub> cycloalkyl, optionally substituted C<sub>2</sub>-C<sub>8</sub> heterocycloalkyl, and optionally substituted C<sub>2</sub>-C<sub>6</sub> heteroalkyl, optionally substituted C<sub>6</sub>-C<sub>10</sub> aryl, and optionally substituted C<sub>2</sub>-C<sub>10</sub> heteroaryl is independently  
 15 optionally substituted with at least one substituent selected from the group consisting of optionally substituted C<sub>1</sub>-C<sub>6</sub> alkyl, optionally substituted C<sub>2</sub>-C<sub>6</sub> alkenyl, optionally substituted C<sub>3</sub>-C<sub>8</sub> cycloalkyl, optionally substituted C<sub>2</sub>-C<sub>8</sub> heterocycloalkyl, optionally substituted C<sub>2</sub>-C<sub>6</sub> heteroalkyl, C<sub>6</sub>-C<sub>10</sub> aryl, halogen, NO<sub>2</sub>, CN, OR<sup>a</sup>, N(R<sup>a</sup>)(R<sup>b</sup>), C(=O)OR<sup>a</sup>, C(=O)N(R<sup>a</sup>)(R<sup>b</sup>), S(=O)<sub>2</sub>OR<sup>a</sup>, P(=O)(OR<sup>a</sup>)(OR<sup>b</sup>), OC(=O)R<sup>a</sup>, N(R<sup>a</sup>)C(=O)R<sup>a</sup>, OS(=O)<sub>2</sub>R<sup>a</sup>, N(R<sup>a</sup>)S(=O)<sub>2</sub>R<sup>a</sup>,  
 20 OP(=O)(OR<sup>a</sup>)(OR<sup>b</sup>), wherein R<sup>a</sup> and R<sup>b</sup> are each independently selected from the group consisting of H, C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>2</sub>-C<sub>6</sub> heteroalkyl, C<sub>3</sub>-C<sub>8</sub> cycloalkyl, C<sub>2</sub>-C<sub>8</sub> heterocycloalkyl, C<sub>2</sub>-C<sub>6</sub> alkenyl, C<sub>6</sub>-C<sub>10</sub> aryl, and C<sub>2</sub>-C<sub>10</sub> heteroaryl.

Embodiment 29 provides the method of Embodiment 27 or 28, wherein R<sup>1</sup> is selected from the group consisting of stearoyl, palmitoyl, capryloyl, caproyl, lauroyl, myristoyl,  
 25 arachidoyl, behenoyl, lignoceroyl, cerotoyl, arachidonoyl, oleoyl, myristoleoyl, palmitoleoyl, sapienoyl, elaidoyl, vaccenoyl, linoleoyl, linoelaidoyl, α-linolenoyl, eicosapentaenoyl, erucoyl, and docosahexaenoyl.

Embodiment 30 provides the method of any one of Embodiments 27-29, wherein R<sup>2</sup> is H.

Embodiment 31 provides the method of any one of Embodiments 27-30, wherein R<sup>3</sup> is



Embodiment 32 provides the method of any one of Embodiments 27-31, wherein R<sup>4</sup> is H.

5 Embodiment 33 provides the method of any one of Embodiments 27-32, wherein R<sup>5</sup> is H.

Embodiment 34 provides the method of any one of Embodiments 18-33, wherein the fatty acid amide is at least one selected from the group consisting of *N*-stearoyl serotonin, *N*-arachidonoyl dopamine, *N*-oleoyl dopamine, *N*-palmitoyl dopamine, *N*-stearoyl dopamine, *N*-stearoyl taurine, *N*-arachidonoyl ethanolamide, *N*-palmitoyl ethanolamide, and *N*-oleoyl  
10 ethanolamide.

Embodiment 35 provides the method of any one of Embodiments 18-34, wherein the subject is administered at least two, at least three, at least four, or at least five fatty acid amides selected from the group consisting of *N*-stearoyl serotonin, *N*-arachidonoyl dopamine, *N*-oleoyl dopamine, *N*-palmitoyl dopamine, *N*-stearoyl dopamine, *N*-stearoyl taurine, *N*-arachidonoyl  
15 ethanolamide, *N*-palmitoyl ethanolamide, and *N*-oleoyl ethanolamide.

Embodiment 36 provides the method of any one of Embodiments 16-35, wherein the subject is administered *N*-palmitoyl dopamine, *N*-arachidonoyl dopamine, *N*-oleoyl dopamine, *N*-oleoyl ethanolamide, and *N*-palmitoyl ethanolamide.

Embodiment 37 provides the method of any one of Embodiments 16-36, wherein the  
20 subject is administered at least one probiotic composition comprising at least one bacterial strain selected from the group consisting of *Coprococcus* bacterium and *Eubacterium* bacterium.

Embodiment 38 provides the method of Embodiment 37, wherein the *Coprococcus* bacterium is *Coprococcus eutactus*.

Embodiment 39 provides the method of Embodiment 37 or 38, wherein the *Eubacterium*  
25 bacterium is *Eubacterium rectale*.

Embodiment 40 provides the method of any one of Embodiments 16-39, wherein enhanced striatal dopamine levels are observed in the subject after physical exercise as compared

to striatal dopamine levels in the same subject after physical exercise without administration of (a), (b), (c), (d), (e), and/or (f).

Embodiment 41 provides the method of any one of Embodiments 16-40, wherein the subject exercises more frequently after administration of (a), (b), (c), (d), (e), and/or (f) than before administration of (a), (b), (c), (d), (e), and/or (f).

Embodiment 42 provides the method of any one of Embodiments 16-41, wherein the subject exercises for a longer period of time after administration of (a), (b), (c), (d), (e), and/or (f) than before administration of (a), (b), (c), (d), (e), and/or (f).

Embodiment 43 provides the method of any one of Embodiments 16-42, wherein the subject has increased cardiovascular endurance and/or physical performance after administration of (a), (b), (c), (d), (e), and/or (f) as compared to before administration of (a), (b), (c), (d), (e), and/or (f).

The terms and expressions employed herein are used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the embodiments of the present application. Thus, it should be understood that although the present application describes specific embodiments and optional features, modification and variation of the compositions, methods, and concepts herein disclosed may be resorted to by those of ordinary skill in the art, and that such modifications and variations are considered to be within the scope of embodiments of the present application.

## CLAIMS

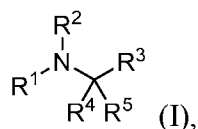
What is claimed is:

1. A dietary supplement composition comprising:
  - (a) at least two fatty acid amides (FAAs) selected from the group consisting of *N*-stearoyl serotonin, *N*-arachidonoyl dopamine, *N*-oleoyl dopamine, *N*-palmitoyl dopamine, *N*-stearoyl dopamine, *N*-stearoyl taurine, *N*-arachidonoyl ethanolamide, *N*-palmitoyl ethanolamide, and *N*-oleoyl ethanolamide; and
  - (b) at least one solid, liquid, or semi-solid edible base substance.
2. The composition of claim 1, wherein the composition comprises *N*-palmitoyl dopamine, *N*-arachidonoyl dopamine, *N*-oleoyl dopamine, *N*-oleoyl ethanolamide, and palmitoyl ethanolamide.
3. The composition of claim 2, wherein the composition has a ratio of FAAs of about 1:1:1:1:1 by weight.
4. The composition of any one of claims 1-3, wherein the composition is suitable for oral administration.
5. A pharmaceutical composition comprising:
  - (a) at least two fatty acid amides (FAAs) selected from the group consisting of *N*-stearoyl serotonin, *N*-arachidonoyl dopamine, *N*-oleoyl dopamine, *N*-palmitoyl dopamine, *N*-stearoyl dopamine, *N*-stearoyl taurine, *N*-arachidonoyl ethanolamide, *N*-palmitoyl ethanolamide, and *N*-oleoyl ethanolamide; and
  - (b) at least one pharmaceutically acceptable carrier.
6. The pharmaceutical composition of claim 5, wherein the composition comprises *N*-palmitoyl dopamine, *N*-arachidonoyl dopamine, *N*-oleoyl dopamine, *N*-oleoyl ethanolamide, and palmitoyl ethanolamide.

7. The pharmaceutical composition of claim 6, wherein the composition has a ratio of FAAs of about 1:1:1:1:1 by weight.
8. The pharmaceutical composition of any one of claims 5-7, wherein the composition is suitable for oral administration.
9. A plasmid comprising at least one gene which encodes a protein that promotes fatty acid amide (FAA) biosynthesis which when inserted into a bacterium stimulates and/or enhances exercise motivation and/or physical performance in a subject.
10. The plasmid of claim 9, wherein the protein is an enzyme.
11. The plasmid of claim 10, wherein the enzyme is at least one selected from the group consisting of EreS (SEQ ID NO:22), EreT (SEQ ID NO:23), EreA (SEQ ID NO:24), and EreC (SEQ ID NO:25).
12. The plasmid of any one of claims 9-11, wherein the plasmid comprises at least one gene selected from the group consisting of *ereS* (SEQ ID NO:26), *ereT* (SEQ ID NO:27), *ereA* (SEQ ID NO:28), and *ereC* (SEQ ID NO:29).
13. The plasmid of any one of claims 9-12, wherein the plasmid comprises each of *ereS* (SEQ ID NO:26), *ereT* (SEQ ID NO:27), *ereA* (SEQ ID NO:28), and *ereC* (SEQ ID NO:29).
14. The plasmid of any one of claims 9-13, wherein plasmid comprises a polynucleotide, wherein at least of portion of the polynucleotide shares at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence homology with at least one polynucleotide sequence selected from the group consisting of SEQ ID NO:30 and SEQ ID NO:31.
15. A probiotic composition comprising an engineered bacterial strain comprising the plasmid of any one of claims 9-14 and at least one pharmaceutically acceptable carrier and/or at least one base substance.

16. A method of stimulating and/or enhancing exercise motivation and/or physical performance in a subject, the method comprising administering to the subject at least one selected from the group consisting of:
- (a) at least one Cannabinoid Receptor Type 1 (CB1) agonist;
  - (b) at least one dietary supplement composition of any one of claims 1-4;
  - (c) at least one pharmaceutical composition of any one of claims 5-8;
  - (d) at least one bacterial strain comprising the plasmid of any one of claims 9-14;
  - (e) at least one probiotic composition of claim 15; and
  - (f) at least one probiotic composition comprising at least one bacterial strain selected from the group consisting of *Coprococcus* bacterium and *Eubacterium* bacterium.
17. The method of claim 16, wherein the method comprises oral administration of (a), (b), (c), (d), (e), and/or (f).
18. The method of claim 16 or 17, wherein the CB1 agonist is a fatty acid amide.
19. The method of claim 18, wherein the fatty acid amide comprises a fatty acyl moiety covalently bonded to a naturally occurring primary amine.
20. The method of claim 19, wherein the fatty acyl moiety is selected from the group consisting of a saturated fatty acyl moiety and an unsaturated fatty acyl moiety.
21. The method of claim 20, wherein the saturated fatty acyl moiety is selected from the group consisting of stearoyl, palmitoyl, capryloyl, caproyl, lauroyl, myristoyl, arachidoyl, behenoyl, lignoceroyl, and cerotoyl.
22. The method of claim 20, wherein the unsaturated fatty acyl moiety is selected from the group consisting of arachidonoyl, oleoyl, myristoleoyl, palmitoleoyl, sapienoyl, elaidoyl, vaccenoyl, linoleoyl, linoelaidoyl,  $\alpha$ -linolenoyl, eicosapentaenoyl, erucoyl, and docosahexaenoyl.

23. The method of any one of claims 19-22, wherein the naturally occurring amine is selected from the group consisting of a neurotransmitter and an amino acid, or a metabolite and/or biosynthetic precursor thereof.
24. The method of claim 23, wherein the neurotransmitter is selected from the group consisting of serotonin, dopamine, glutamate,  $\gamma$ -aminobutyric acid, acetylcholine, norepinephrine, and epinephrine.
25. The method of claim 23, wherein naturally occurring amine is a metabolite and/or biosynthetic precursor of an amino acid.
26. The method of claim 25, wherein the metabolite and/or biosynthetic precursor of an amino acid is selected from the group consisting of ethanolamine and taurine.
27. The method of claim 18, wherein the fatty acid amid is a compound of Formula (I), or a salt, solvate, stereoisomer, or isotopologue thereof:



wherein:

$\text{R}^1$  is selected from the group consisting of  $\text{C}(=\text{O})$ (optionally substituted  $\text{C}_1$ - $\text{C}_{25}$  alkyl) and  $\text{C}(=\text{O})$ (optionally substituted  $\text{C}_2$ - $\text{C}_{25}$  alkenyl);

$\text{R}^2$  is selected from the group consisting of H and optionally substituted  $\text{C}_1$ - $\text{C}_6$  alkyl;

$\text{R}^3$  is  $\text{C}_1$ - $\text{C}_6$  alkyl substituted with at least one substituent selected from the group consisting of optionally substituted  $\text{C}_6$ - $\text{C}_{10}$  aryl, optionally substituted  $\text{C}_2$ - $\text{C}_{10}$  heteroaryl, halogen,  $\text{OR}^A$ ,  $\text{N}(\text{R}^A)(\text{R}^B)$ ,  $\text{C}(=\text{O})\text{OR}^A$ ,  $\text{C}(=\text{O})\text{N}(\text{R}^A)(\text{R}^B)$ ,  $\text{S}(=\text{O})_2\text{OR}^A$ ,  $\text{P}(=\text{O})(\text{OR}^A)(\text{OR}^B)$ ,  $\text{N}(\text{R}^A)\text{C}(=\text{NR}^B)\text{N}(\text{R}^C)(\text{R}^D)$ ,  $\text{OC}(=\text{O})\text{R}^A$ ,  $\text{N}(\text{R}^A)\text{C}(=\text{O})\text{R}^B$ ,  $\text{N}(\text{R}^A)\text{C}(=\text{O})\text{OR}^B$ ,  $\text{OS}(=\text{O})_2\text{R}^A$ ,  $\text{N}(\text{R}^A)\text{S}(=\text{O})_2\text{R}^A$ , and  $\text{OP}(=\text{O})(\text{OR}^A)(\text{OR}^B)$ ;

$\text{R}^4$  and  $\text{R}^5$  are each independently from the group consisting of H, optionally substituted  $\text{C}_1$ - $\text{C}_6$  alkyl;

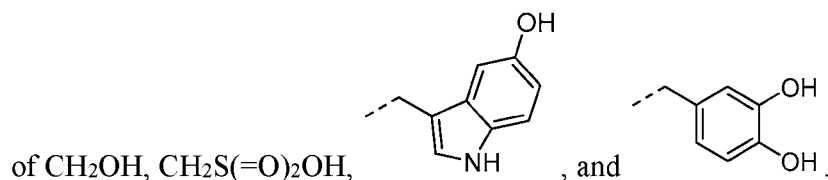
$R^A$ ,  $R^B$ ,  $R^C$ , and  $R^D$ , if present, are each independently selected from the group consisting of H, optionally substituted C<sub>1</sub>-C<sub>6</sub> alkyl, optionally substituted C<sub>2</sub>-C<sub>6</sub> heteroalkyl, optionally substituted C<sub>3</sub>-C<sub>8</sub> cycloalkyl, optionally substituted C<sub>2</sub>-C<sub>8</sub> heterocycloalkyl, optionally substituted C<sub>2</sub>-C<sub>6</sub> alkenyl, optionally substituted C<sub>6</sub>-C<sub>10</sub> aryl, and optionally substituted C<sub>2</sub>-C<sub>10</sub> heteroaryl.

28. The method of claim 27, wherein each occurrence of optionally substituted C<sub>1</sub>-C<sub>6</sub> alkyl, optionally substituted C<sub>1</sub>-C<sub>25</sub> alkyl, optionally substituted C<sub>2</sub>-C<sub>6</sub> alkenyl, optionally substituted C<sub>2</sub>-C<sub>25</sub> alkenyl, optionally substituted C<sub>3</sub>-C<sub>8</sub> cycloalkyl, optionally substituted C<sub>2</sub>-C<sub>8</sub> heterocycloalkyl, and optionally substituted C<sub>2</sub>-C<sub>6</sub> heteroalkyl, optionally substituted C<sub>6</sub>-C<sub>10</sub> aryl, and optionally substituted C<sub>2</sub>-C<sub>10</sub> heteroaryl is independently optionally substituted with at least one substituent selected from the group consisting of optionally substituted C<sub>1</sub>-C<sub>6</sub> alkyl, optionally substituted C<sub>2</sub>-C<sub>6</sub> alkenyl, optionally substituted C<sub>3</sub>-C<sub>8</sub> cycloalkyl, optionally substituted C<sub>2</sub>-C<sub>8</sub> heterocycloalkyl, optionally substituted C<sub>2</sub>-C<sub>6</sub> heteroalkyl, C<sub>6</sub>-C<sub>10</sub> aryl, halogen, NO<sub>2</sub>, CN, OR<sup>a</sup>, N(R<sup>a</sup>)(R<sup>b</sup>), C(=O)OR<sup>a</sup>, C(=O)N(R<sup>a</sup>)(R<sup>b</sup>), S(=O)<sub>2</sub>OR<sup>a</sup>, P(=O)(OR<sup>a</sup>)(OR<sup>b</sup>), OC(=O)R<sup>a</sup>, N(R<sup>a</sup>)C(=O)R<sup>a</sup>, OS(=O)<sub>2</sub>R<sup>a</sup>, N(R<sup>a</sup>)S(=O)<sub>2</sub>R<sup>a</sup>, OP(=O)(OR<sup>a</sup>)(OR<sup>b</sup>), wherein R<sup>a</sup> and R<sup>b</sup> are each independently selected from the group consisting of H, C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>2</sub>-C<sub>6</sub> heteroalkyl, C<sub>3</sub>-C<sub>8</sub> cycloalkyl, C<sub>2</sub>-C<sub>8</sub> heterocycloalkyl, C<sub>2</sub>-C<sub>6</sub> alkenyl, C<sub>6</sub>-C<sub>10</sub> aryl, and C<sub>2</sub>-C<sub>10</sub> heteroaryl.

29. The method of claim 27 or 28, wherein R<sup>1</sup> is selected from the group consisting of stearoyl, palmitoyl, capryloyl, caproyl, lauroyl, myristoyl, arachidoyl, behenoyl, lignoceroyl, cerotoyl, arachidonoyl, oleoyl, myristoleoyl, palmitoleoyl, sapienoyl, elaidoyl, vaccenoyl, linoleoyl, linoelaidoyl, α-linolenoyl, eicosapentaenoyl, erucoyl, and docosahexaenoyl.

30. The method of any one of claims 27-29, wherein R<sup>2</sup> is H.

31. The method of any one of claims 27-30, wherein R<sup>3</sup> is selected from the group consisting



32. The method of any one of claims 27-31, wherein R<sup>4</sup> is H.

33. The method of any one of claims 27-32, wherein R<sup>5</sup> is H.

34. The method of any one of claims 18-33, wherein the fatty acid amide is at least one selected from the group consisting of *N*-stearoyl serotonin, *N*-arachidonoyl dopamine, *N*-oleoyl dopamine, *N*-palmitoyl dopamine, *N*-stearoyl dopamine, *N*-stearoyl taurine, *N*-arachidonoyl ethanolamide, *N*-palmitoyl ethanolamide, and *N*-oleoyl ethanolamide.

35. The method of any one of claims 18-34, wherein the subject is administered at least two, at least three, at least four, or at least five fatty acid amides selected from the group consisting of *N*-stearoyl serotonin, *N*-arachidonoyl dopamine, *N*-oleoyl dopamine, *N*-palmitoyl dopamine, *N*-stearoyl dopamine, *N*-stearoyl taurine, *N*-arachidonoyl ethanolamide, *N*-palmitoyl ethanolamide, and *N*-oleoyl ethanolamide.

36. The method of any one of claims 16-35, wherein the subject is administered *N*-palmitoyl dopamine, *N*-arachidonoyl dopamine, *N*-oleoyl dopamine, *N*-oleoyl ethanolamide, and *N*-palmitoyl ethanolamide.

37. The method of any one of claims 16-36, wherein the subject is administered at least one probiotic composition comprising at least one bacterial strain selected from the group consisting of *Coprococcus* bacterium and *Eubacterium* bacterium.

38. The method of claim 37, wherein the *Coprococcus* bacterium is *Coprococcus eutactus*.

39. The method of claim 37 or 38, wherein the *Eubacterium* bacterium is *Eubacterium rectale*.

40. The method of any one of claims 16-39, wherein enhanced striatal dopamine levels are observed in the subject after physical exercise as compared to striatal dopamine levels in the same subject after physical exercise without administration of (a), (b), (c), (d), (e), and/or (f).

41. The method of any one of claims 16-40, wherein the subject exercises more frequently after administration of (a), (b), (c), (d), (e), and/or (f) than before administration of (a), (b), (c), (d), (e), and/or (f).

42. The method of any one of claims 16-41, wherein the subject exercises for a longer period of time after administration of (a), (b), (c), (d), (e), and/or (f) than before administration of (a), (b), (c), (d), (e), and/or (f).

43. The method of any one of claims 16-42, wherein the subject has increased cardiovascular endurance and/or physical performance after administration of (a), (b), (c), (d), (e), and/or (f) as compared to before administration of (a), (b), (c), (d), (e), and/or (f).

FIG. 1A

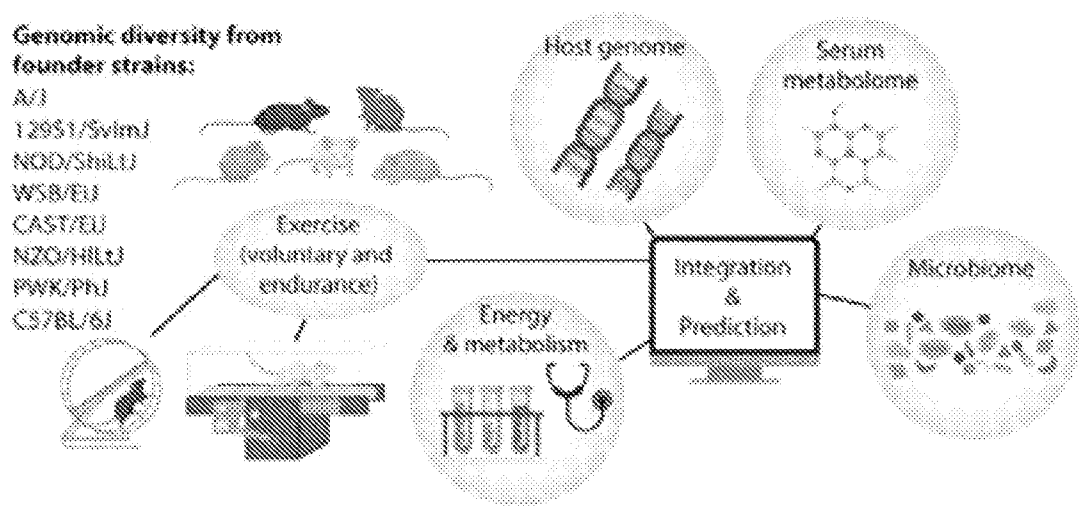


FIG. 1B

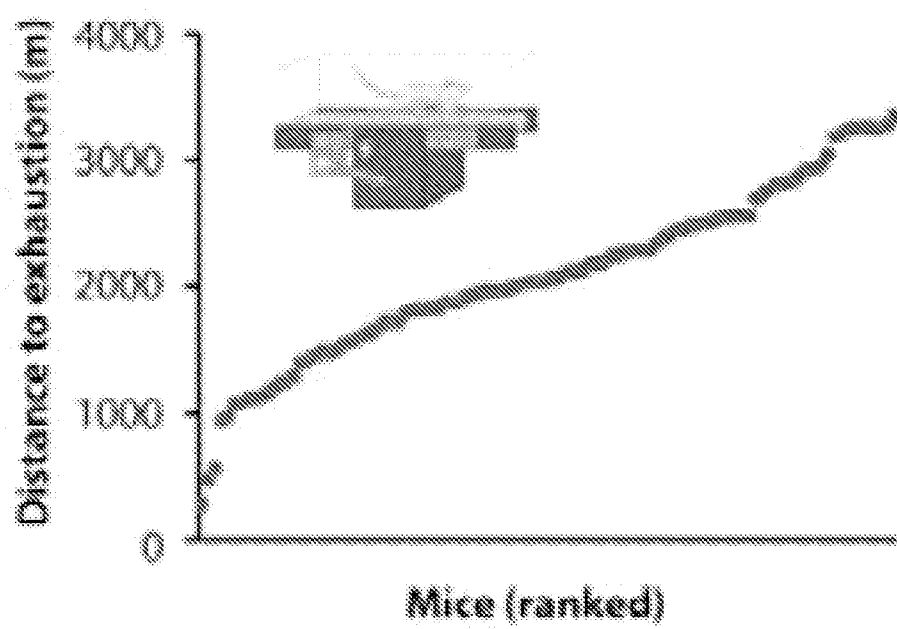


FIG. 1C

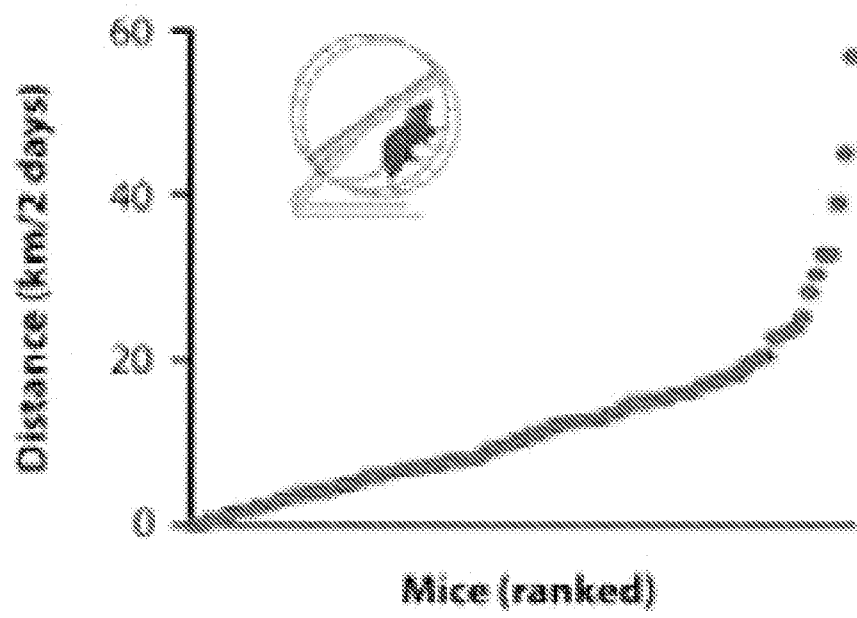


FIG. 1D

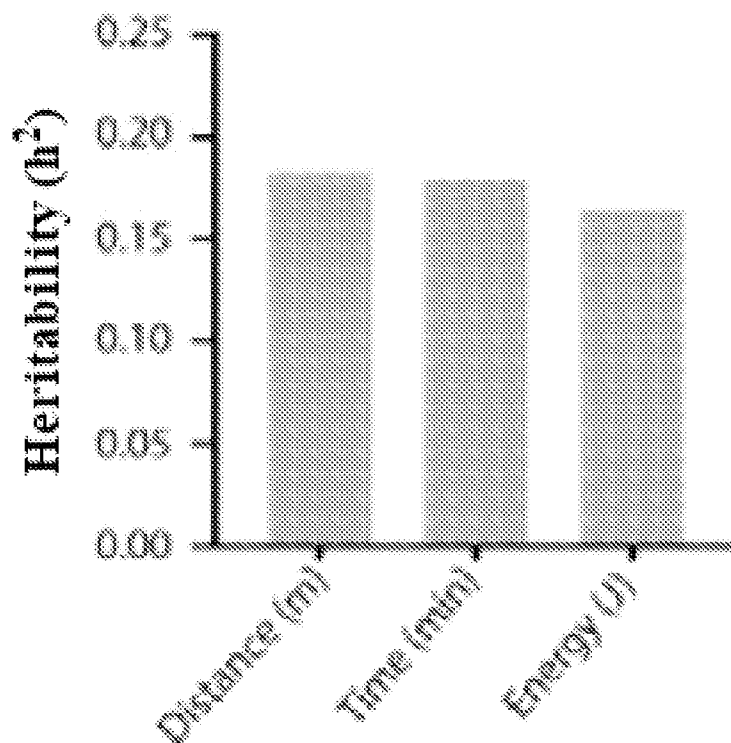


FIG. 1E

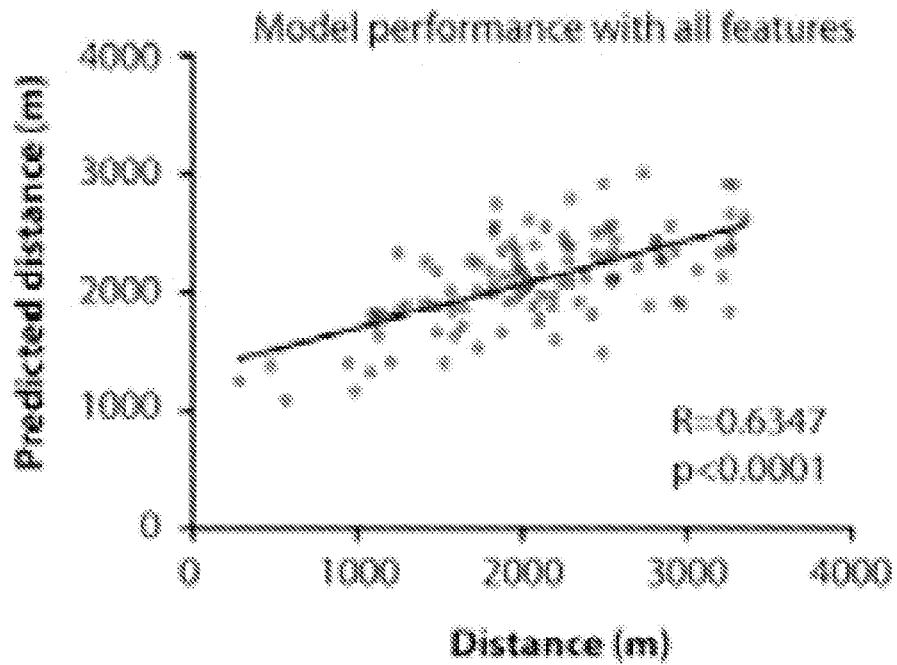


FIG. 1F

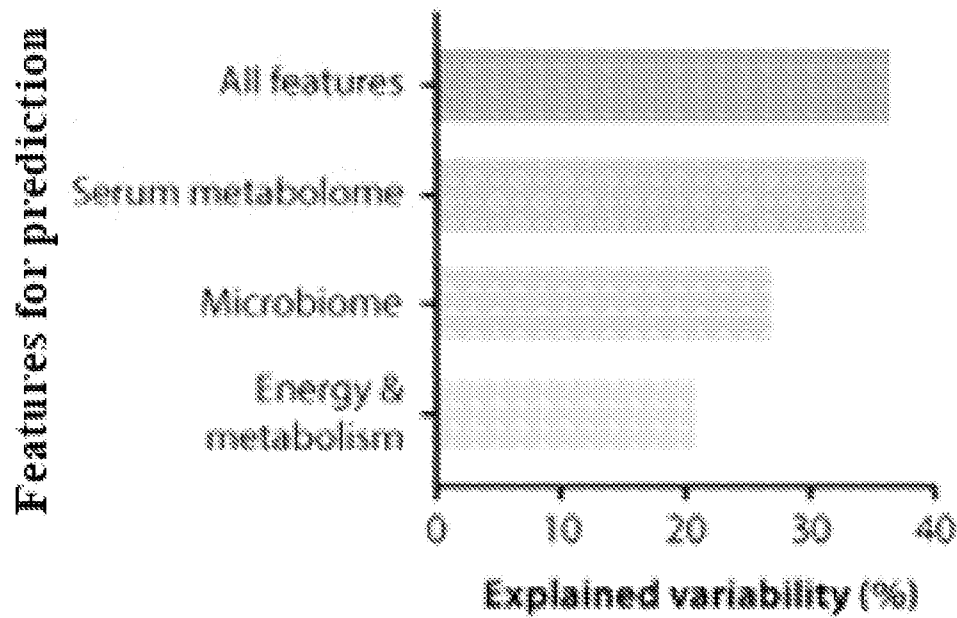


FIG. 1G

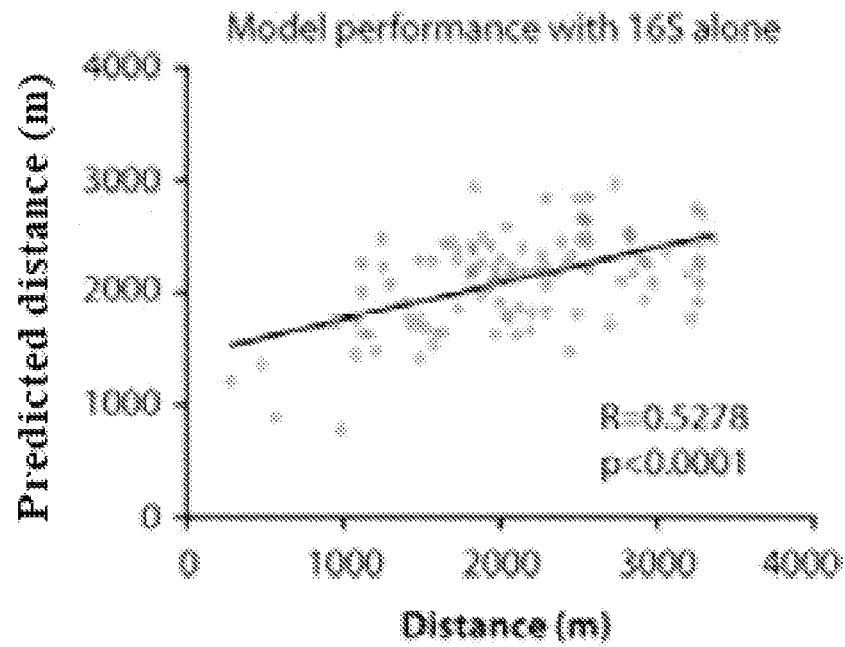


FIG. 1H

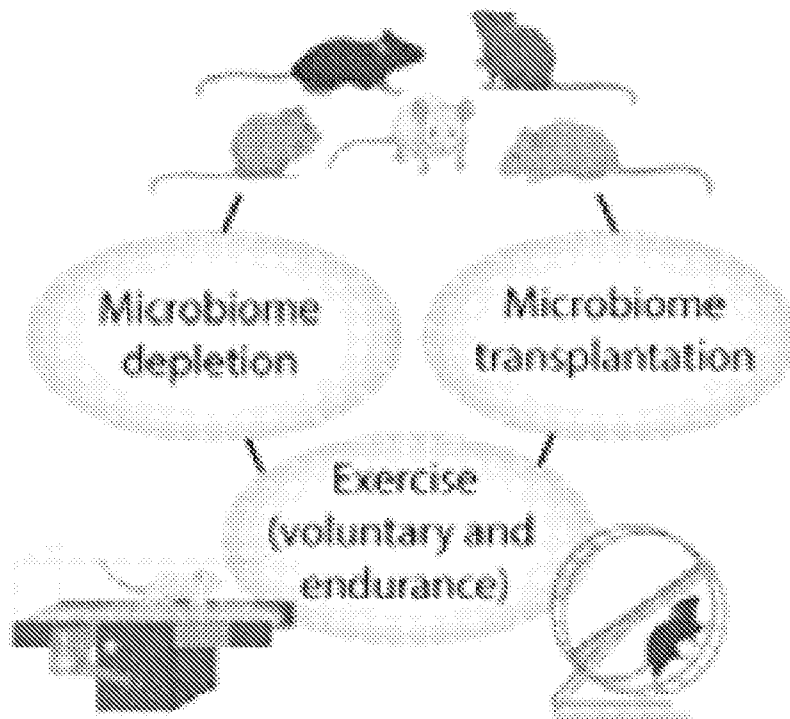


FIG. 1I

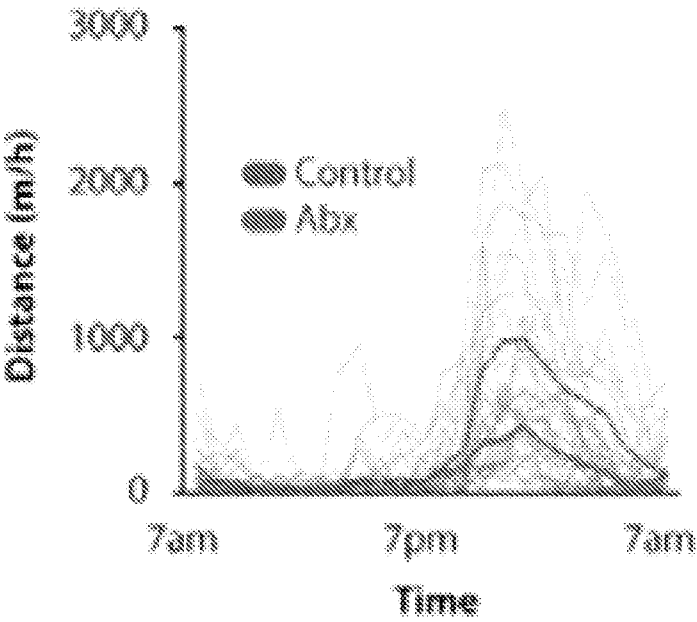


FIG. 1J

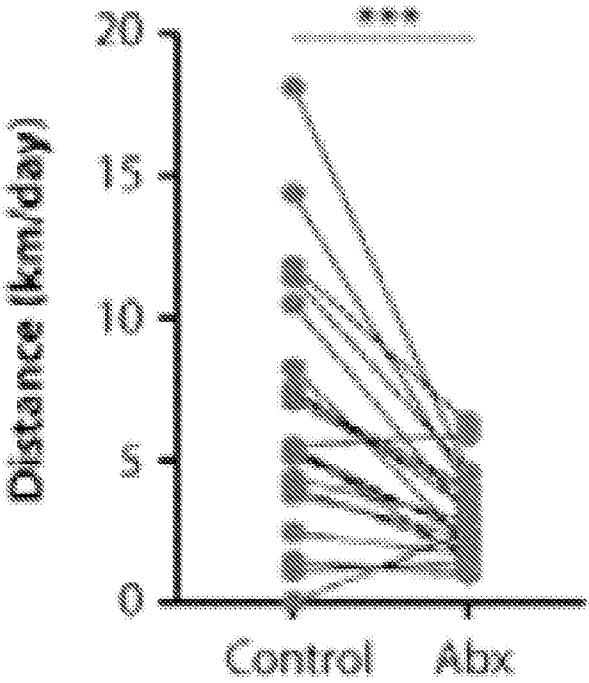


FIG. 1K

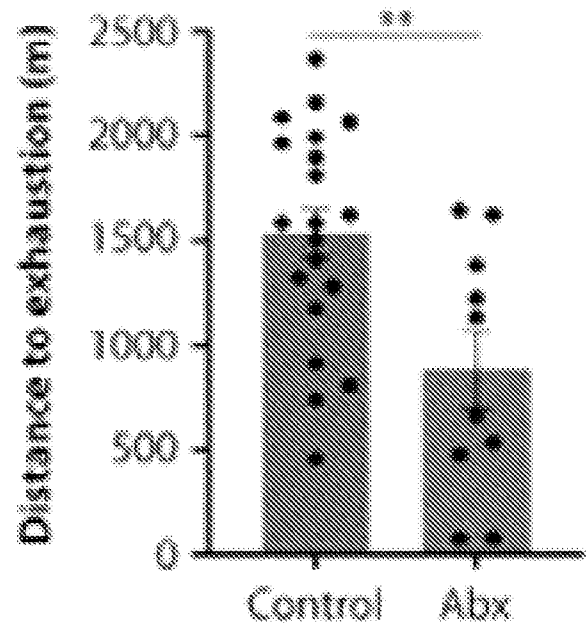


FIG. 1L

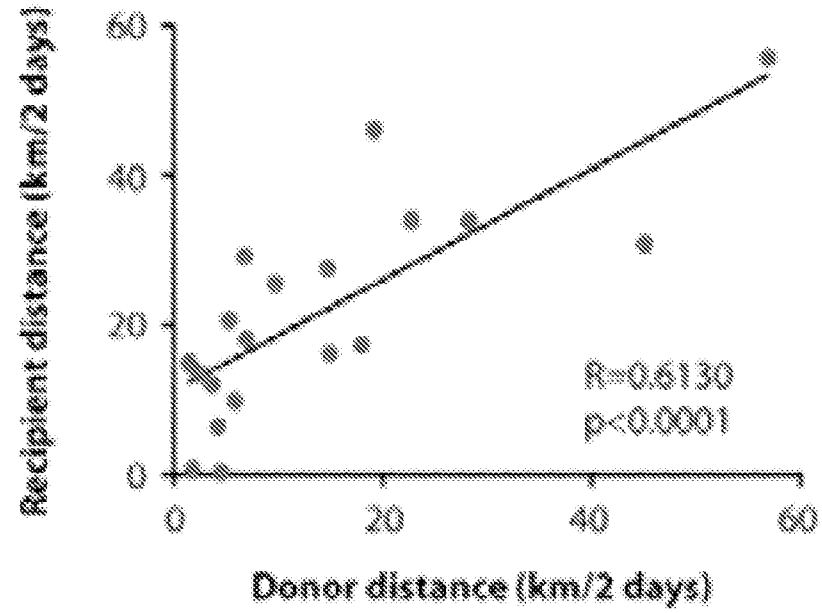


FIG. 2A

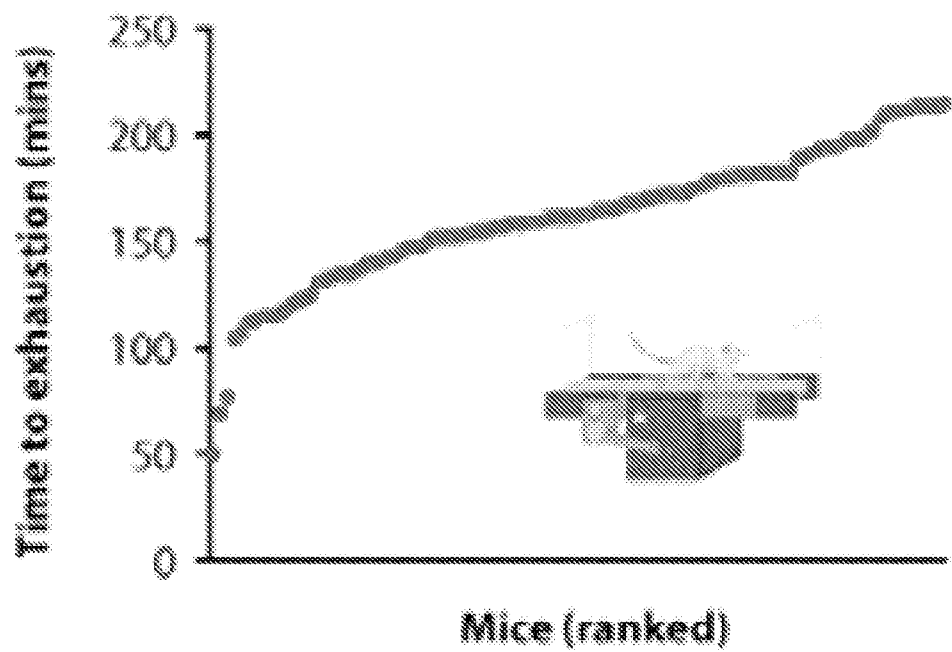


FIG. 2B

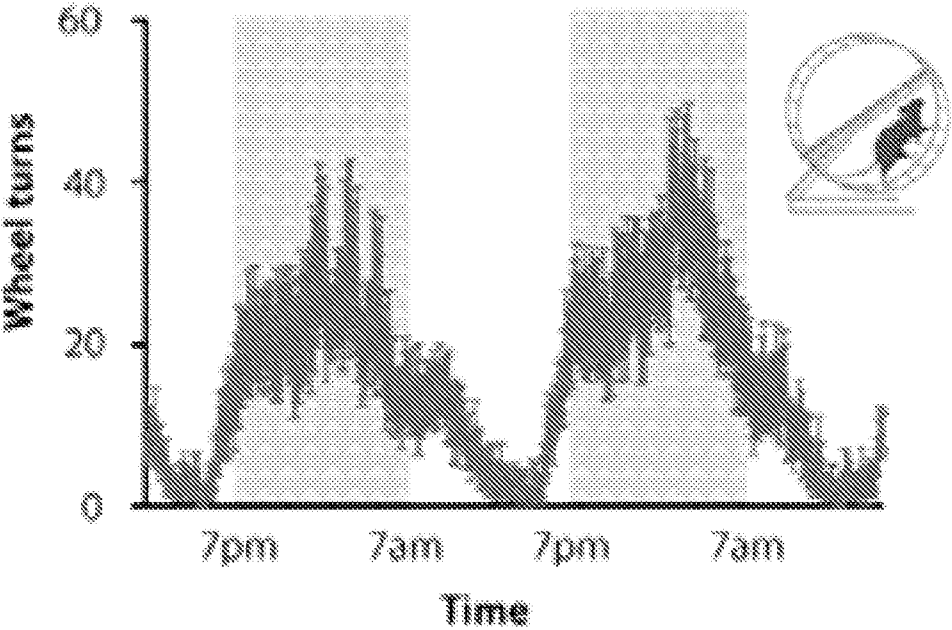
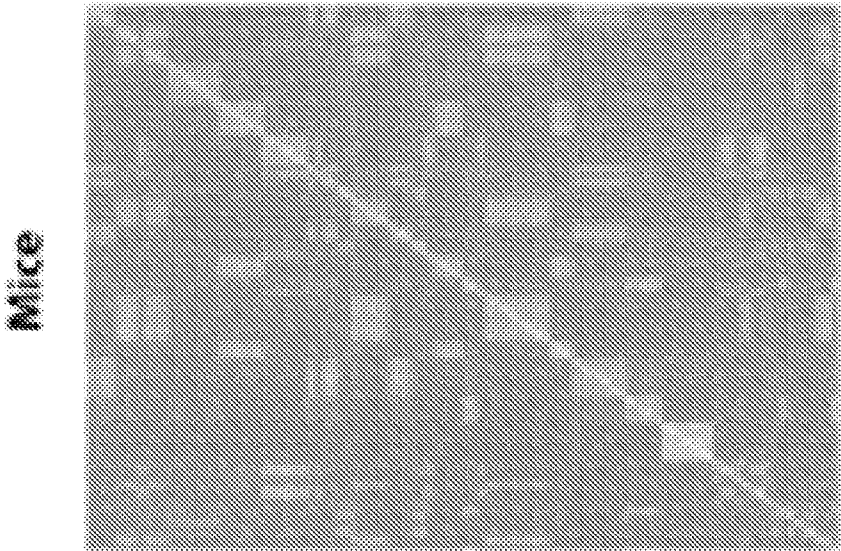


FIG. 2C

Kinship matrix



Mice

FIG. 2D

GWAS (Treadmill time)

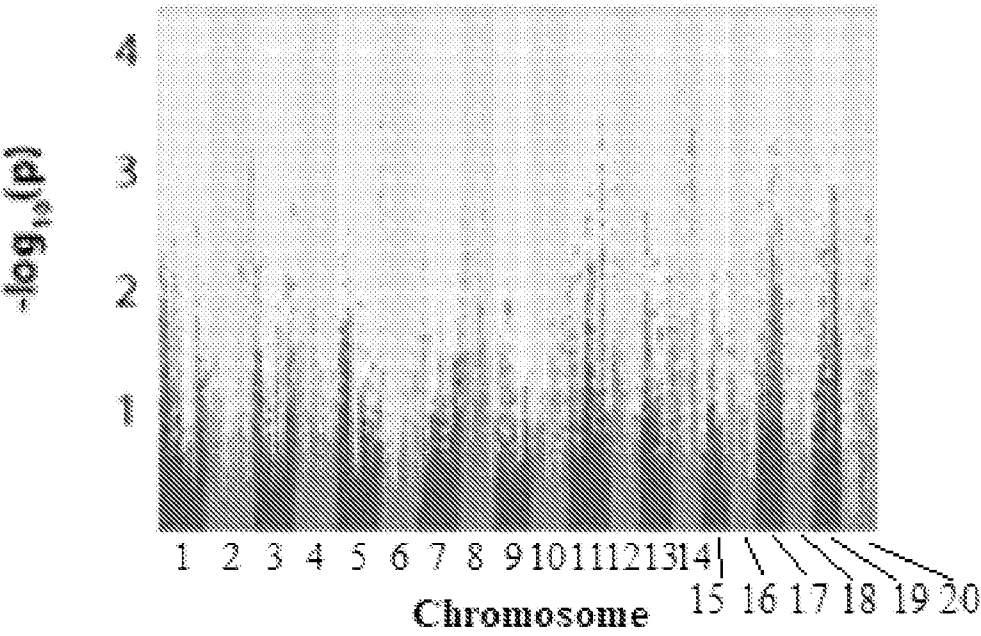


FIG. 2E

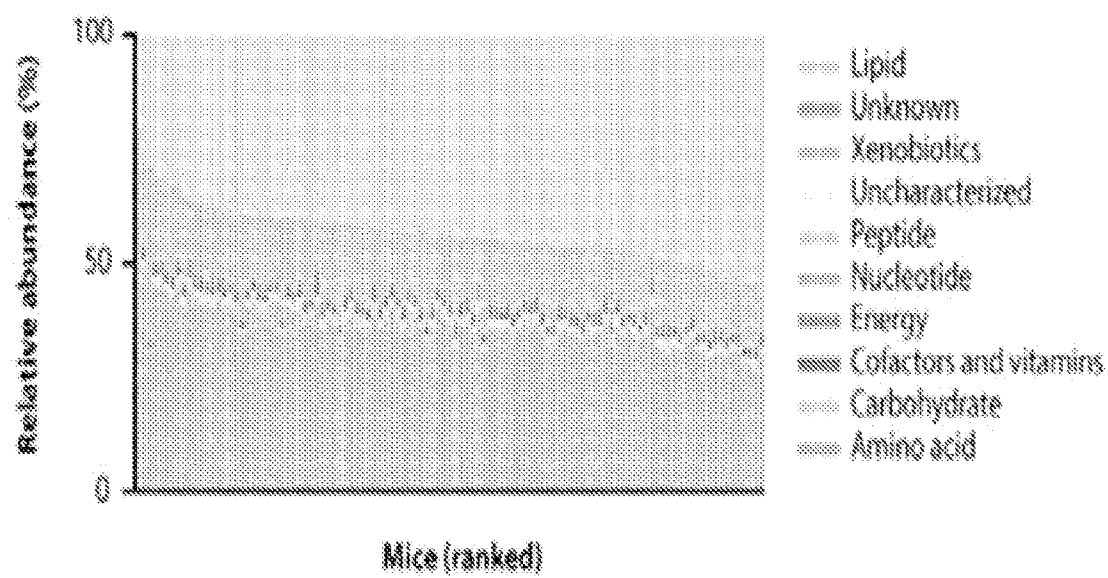


FIG. 2F

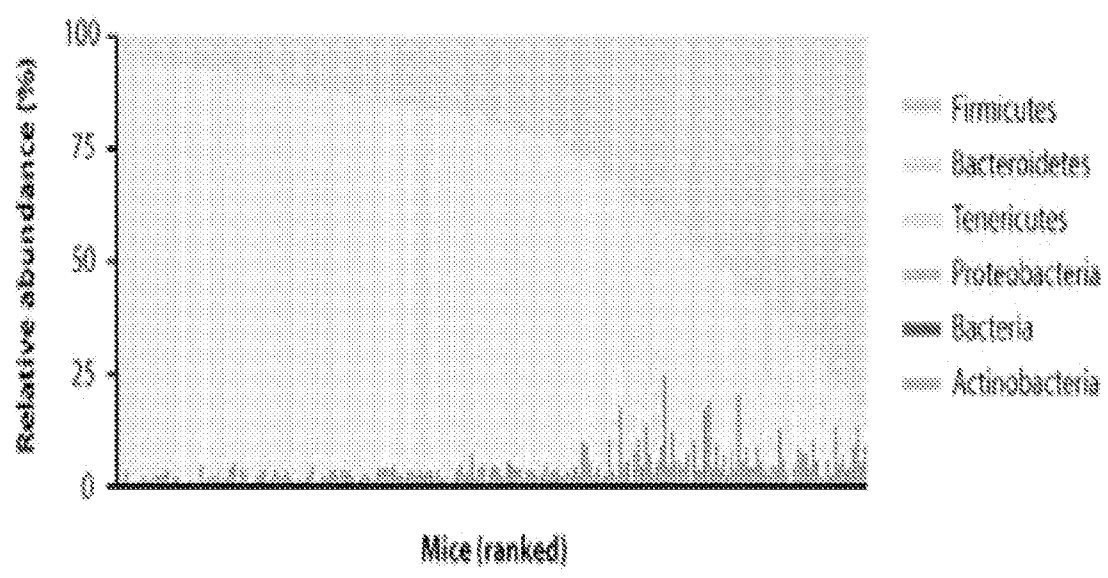


FIG. 2G

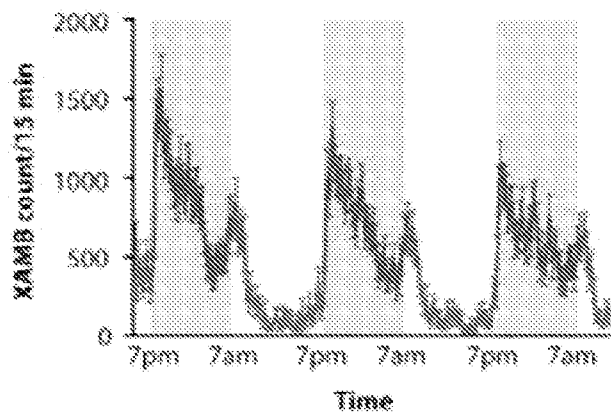


FIG. 2H

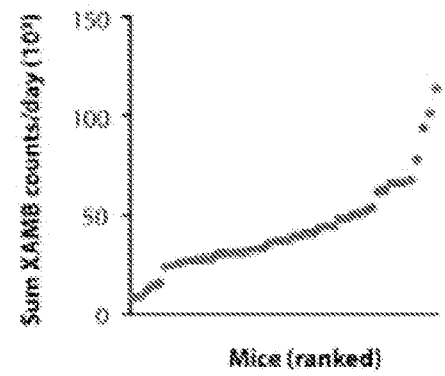


FIG. 2I

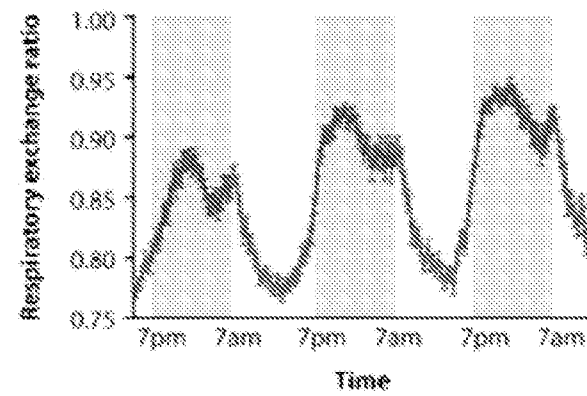


FIG. 2J

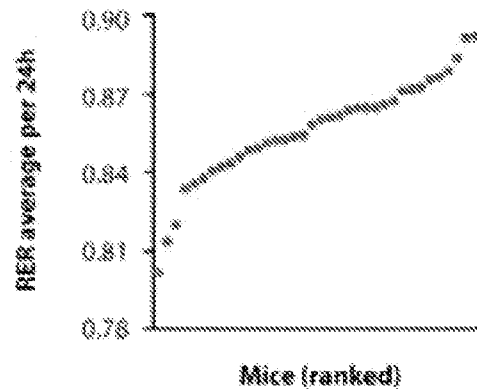


FIG. 2K

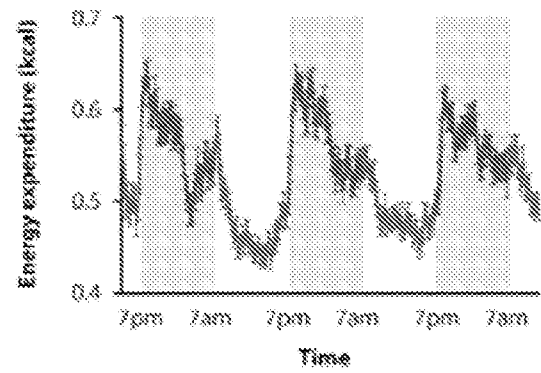


FIG. 2L

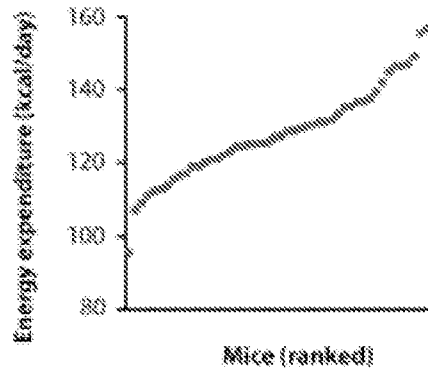


FIG. 2M

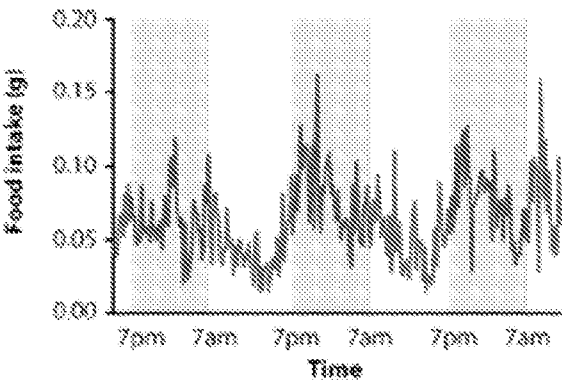


FIG. 2N

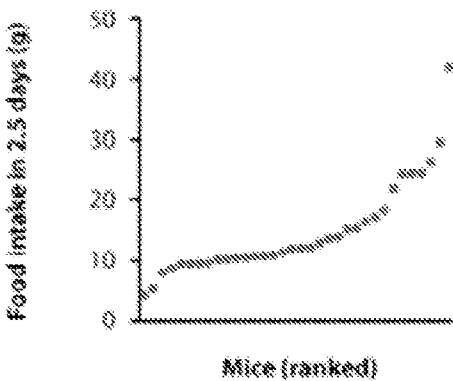


FIG. 3A

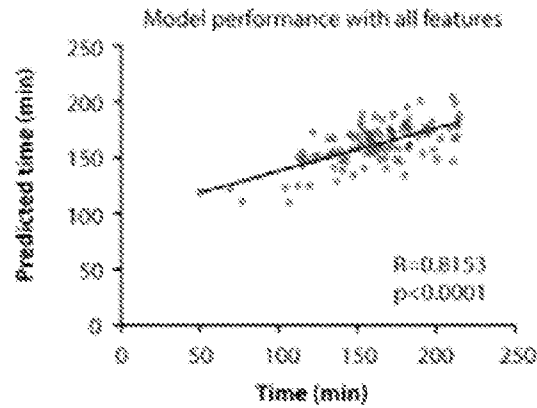


FIG. 3B

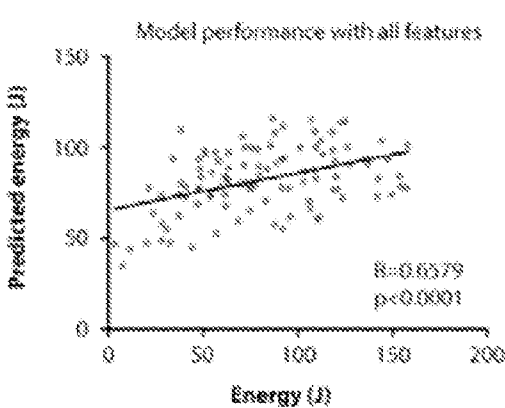


FIG. 3C

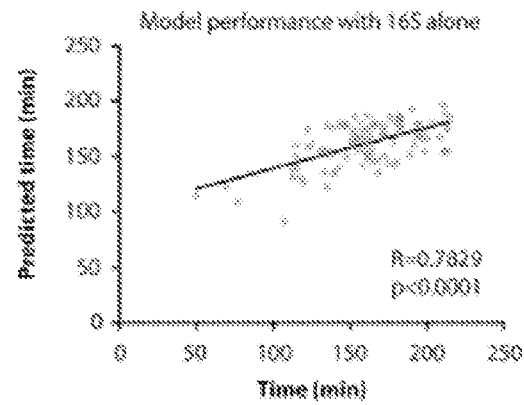


FIG. 3D

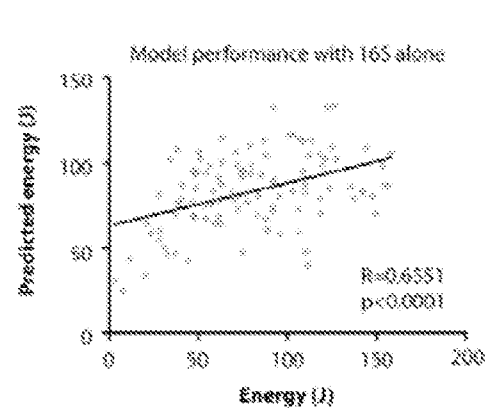


FIG. 3E

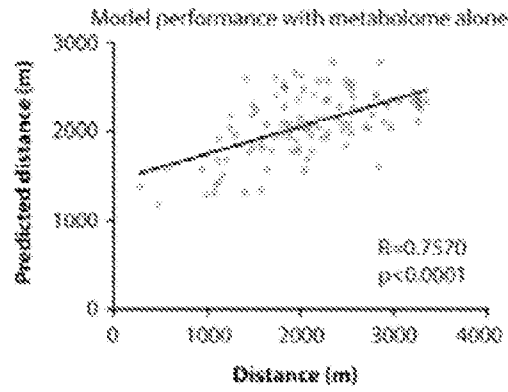


FIG. 3F

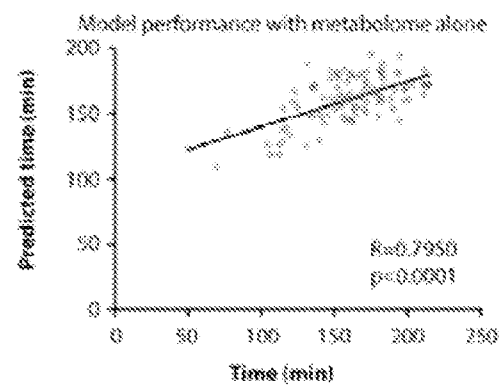


FIG. 3G

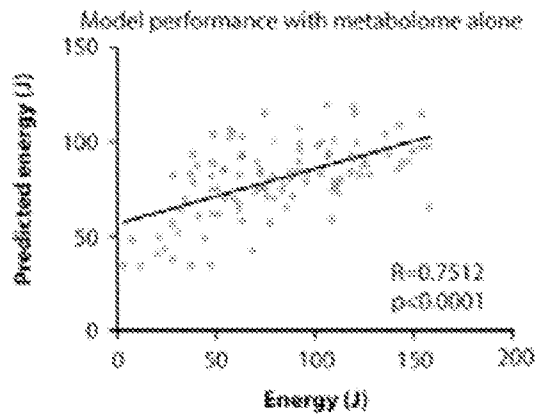


FIG. 3H

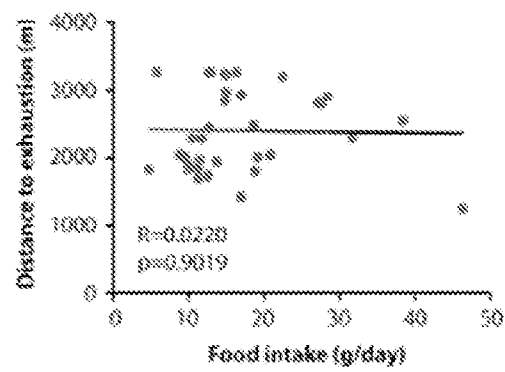


FIG. 3I

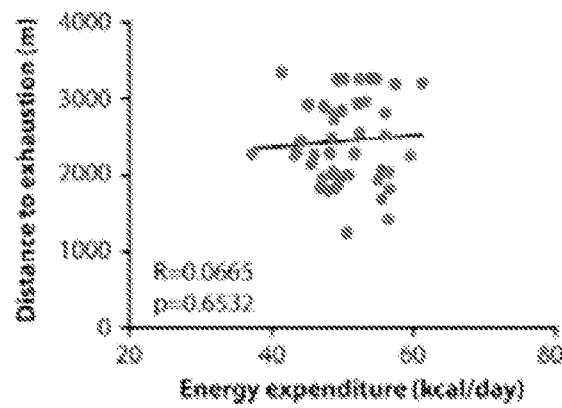


FIG. 3J

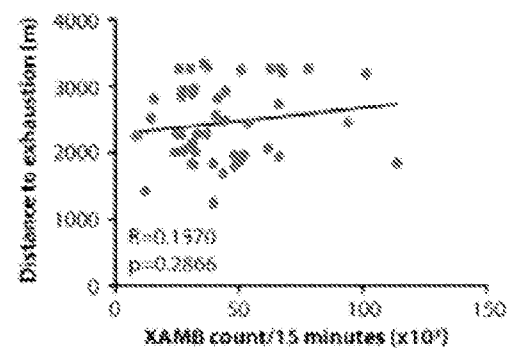


FIG. 3K

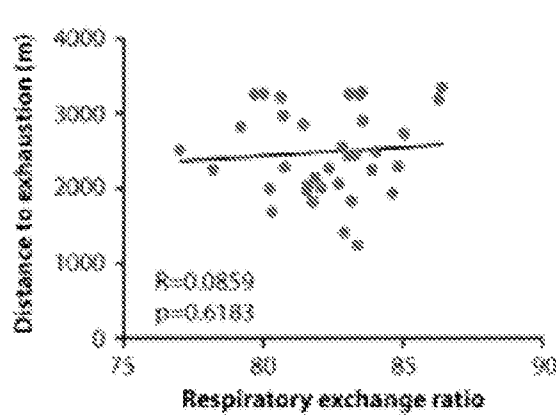


FIG. 3L

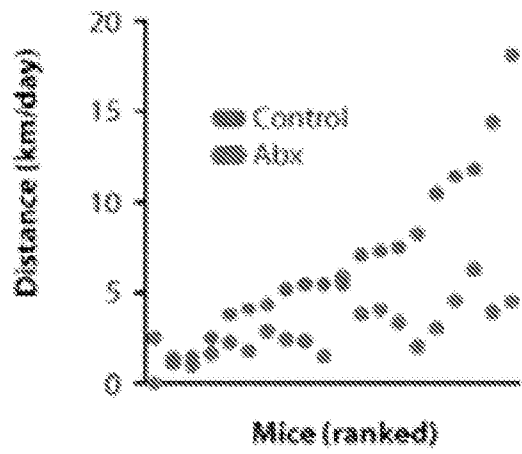


FIG. 3M

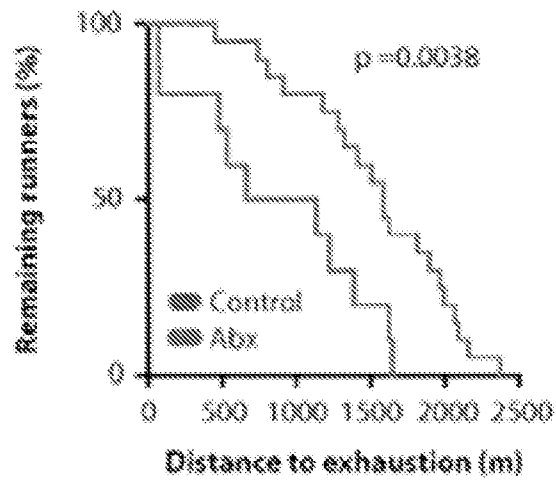


FIG. 3N

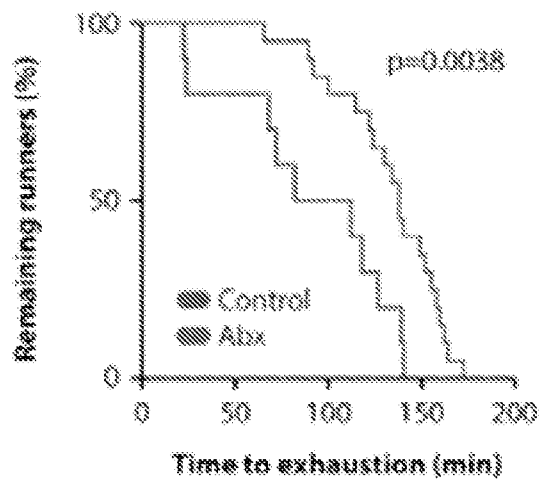


FIG. 3O

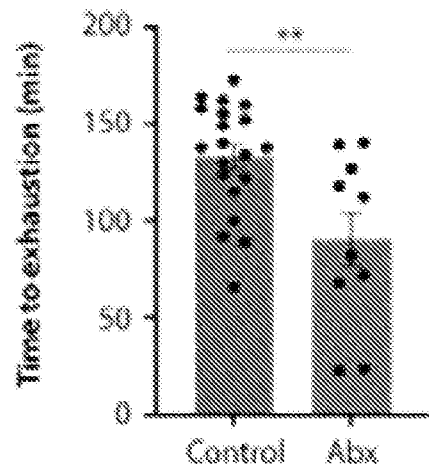


FIG. 4A

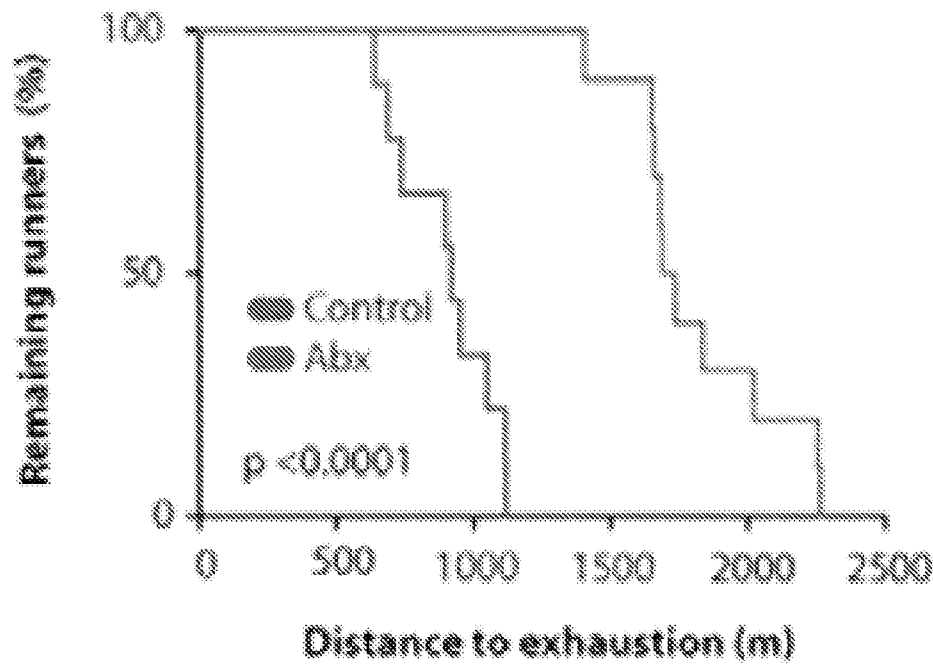


FIG. 4B

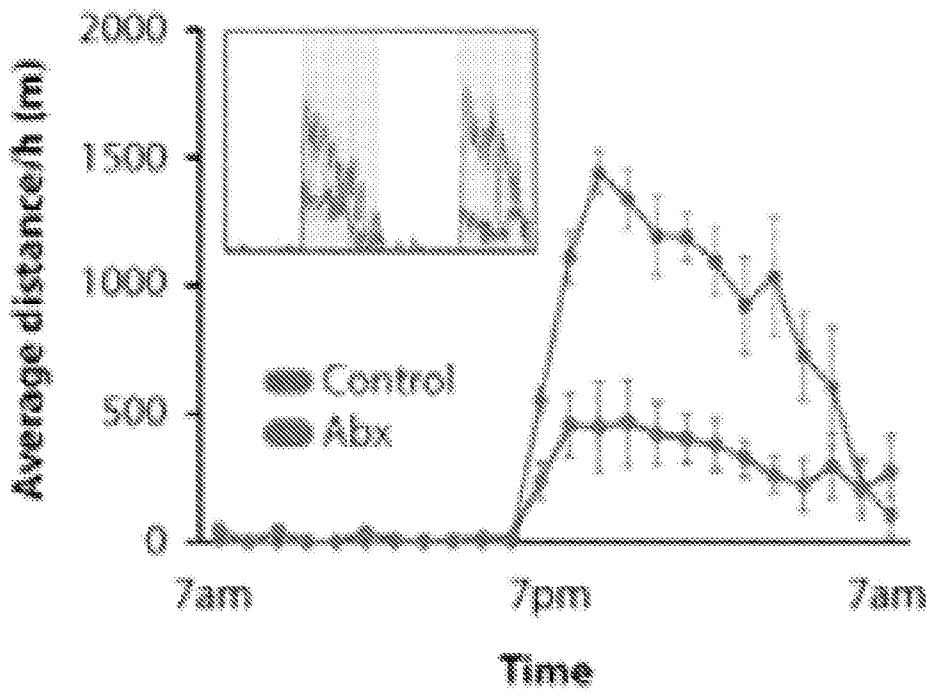


FIG. 4C

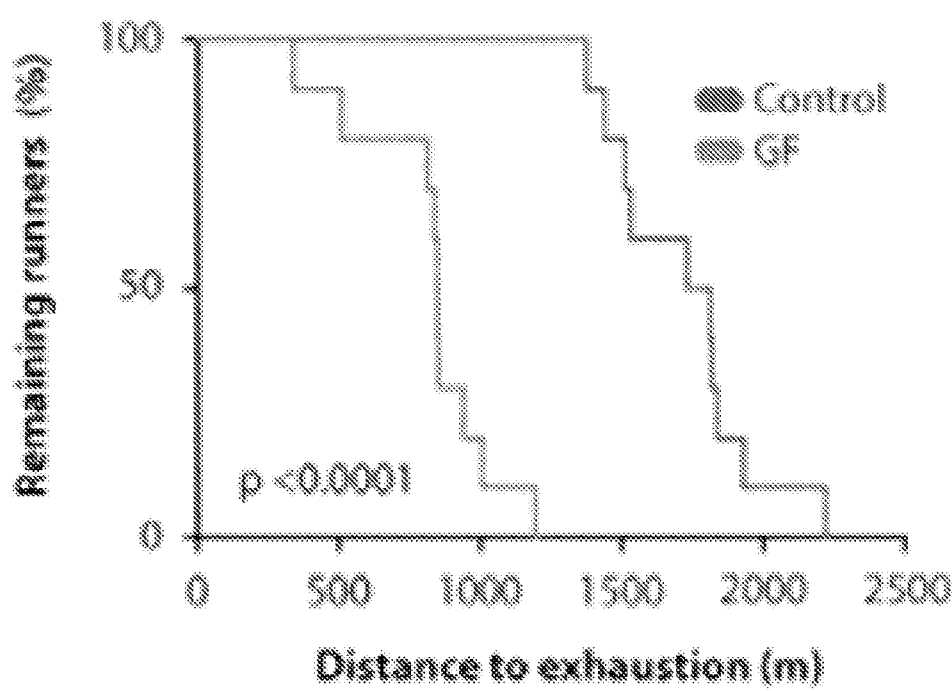


FIG. 4D

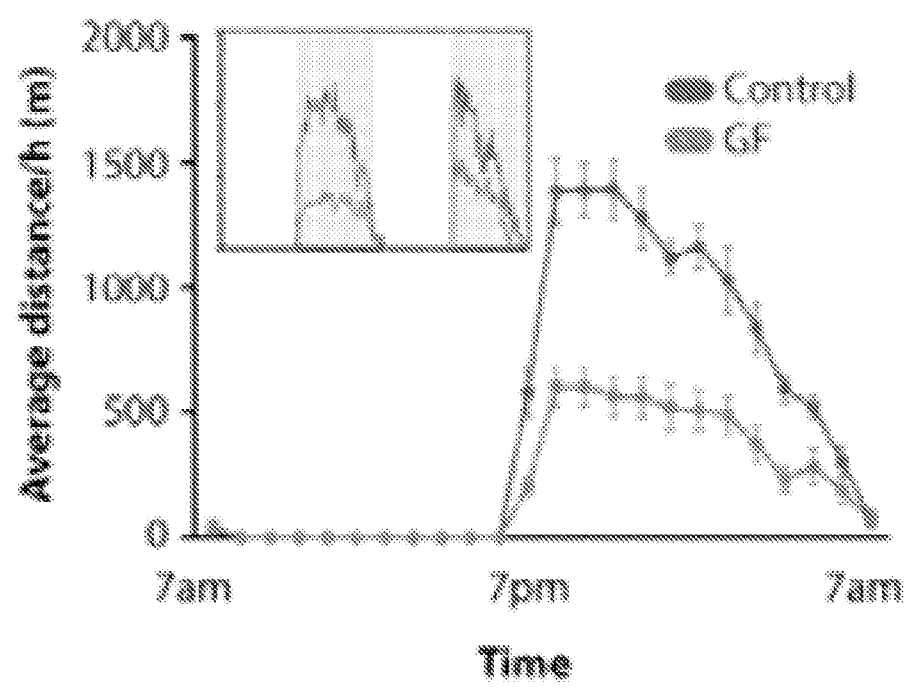


FIG. 4E

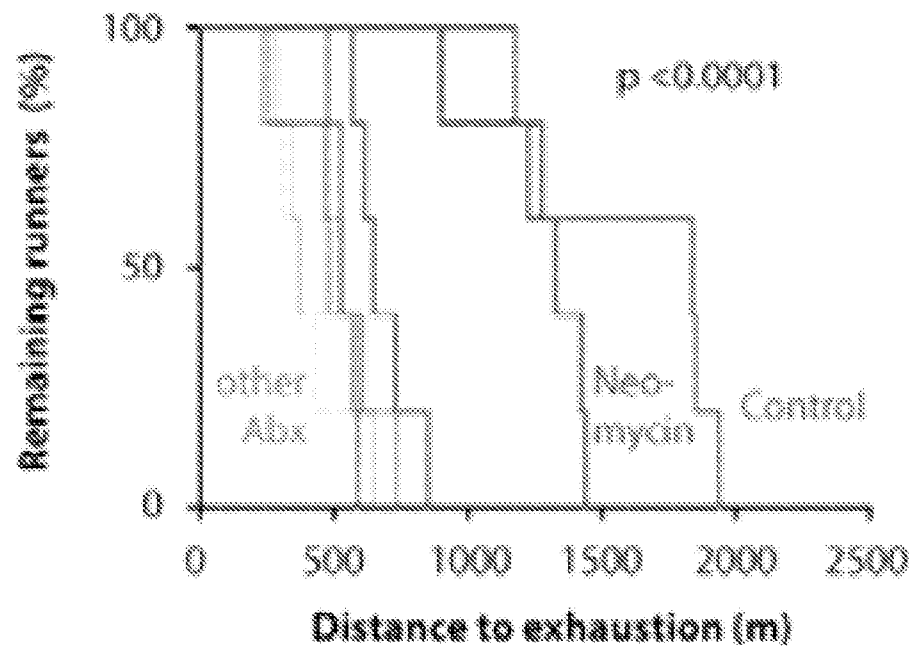


FIG. 4F

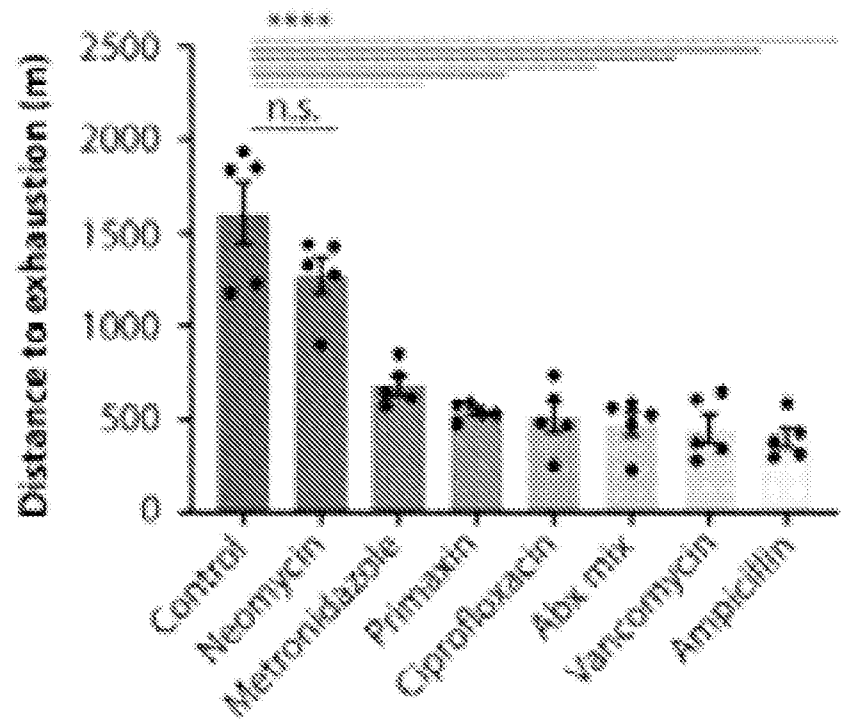


FIG. 4G

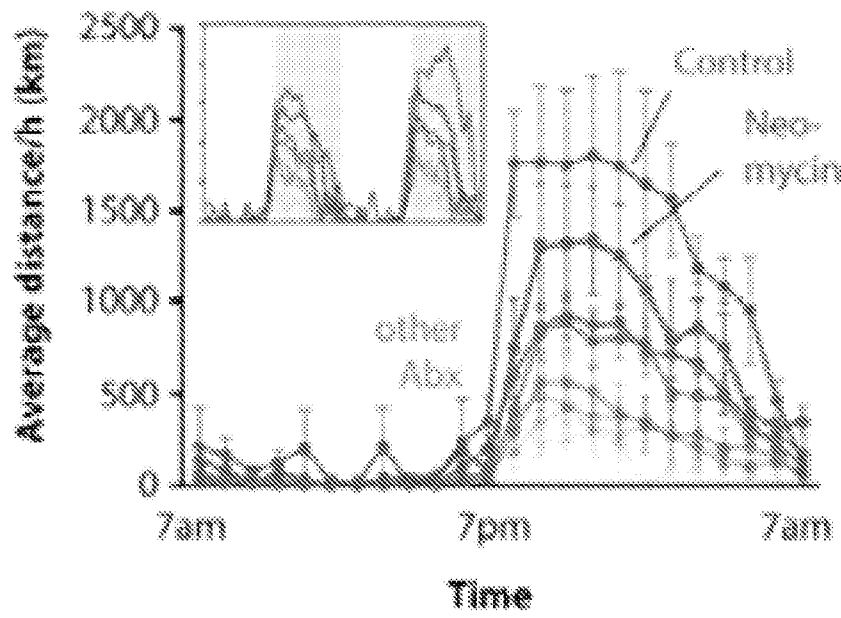


FIG. 4H

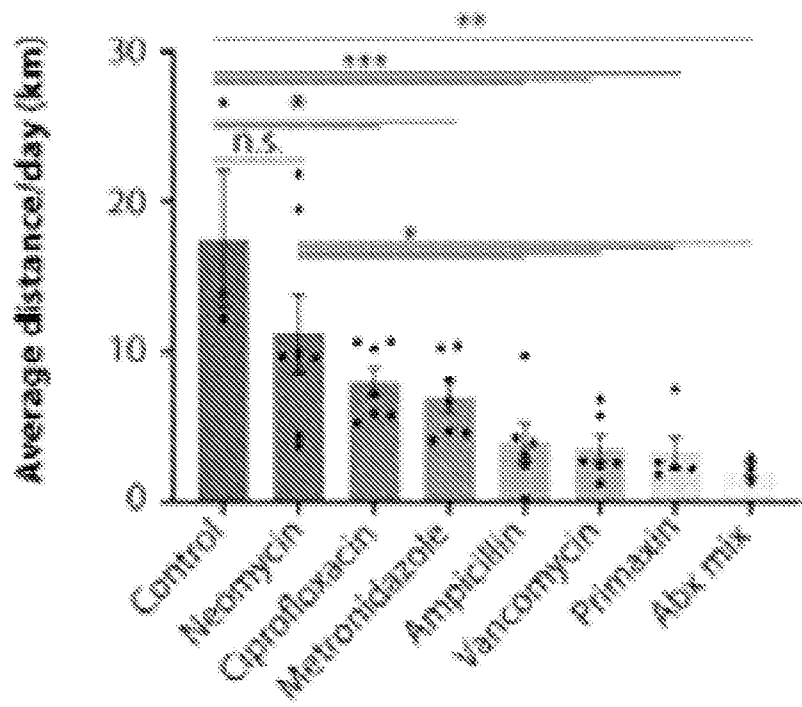


FIG. 4I

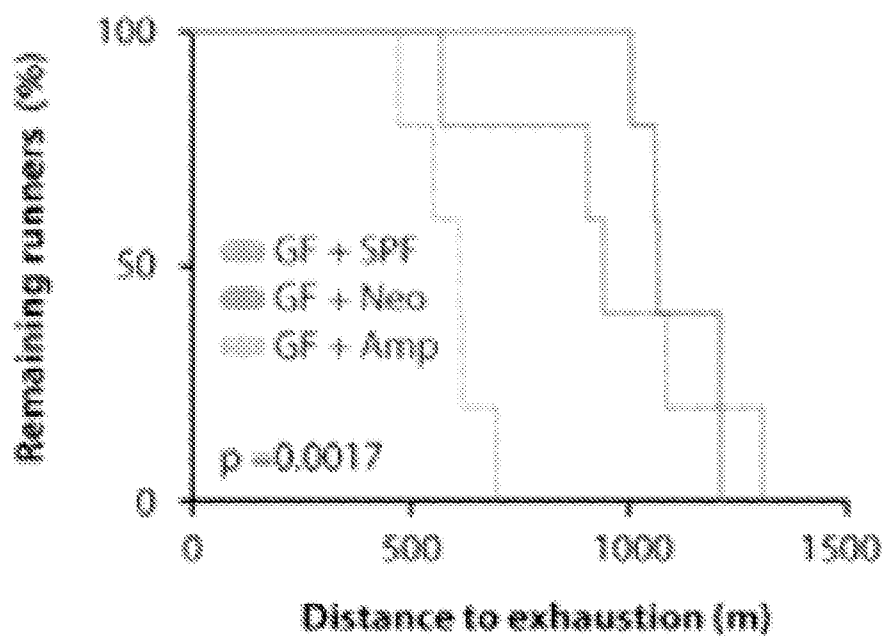


FIG. 4J

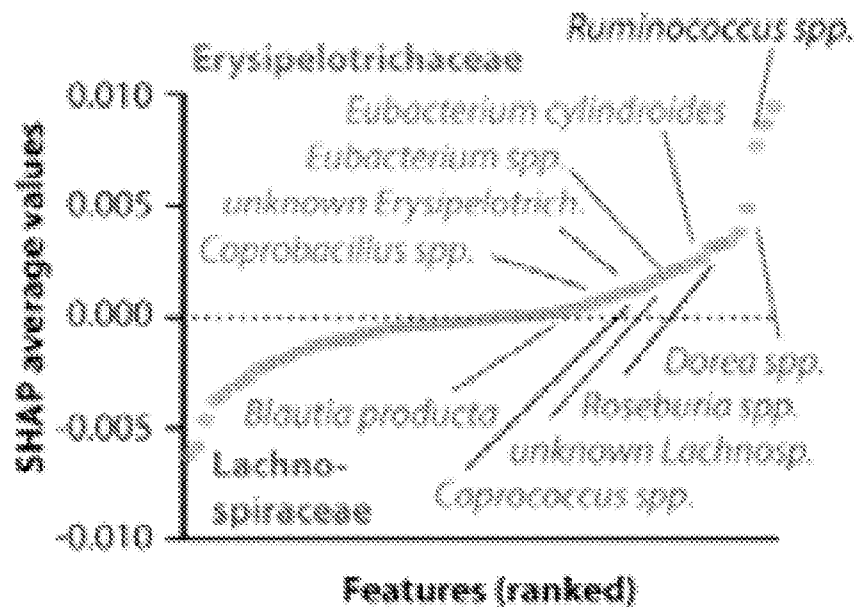


FIG. 4K

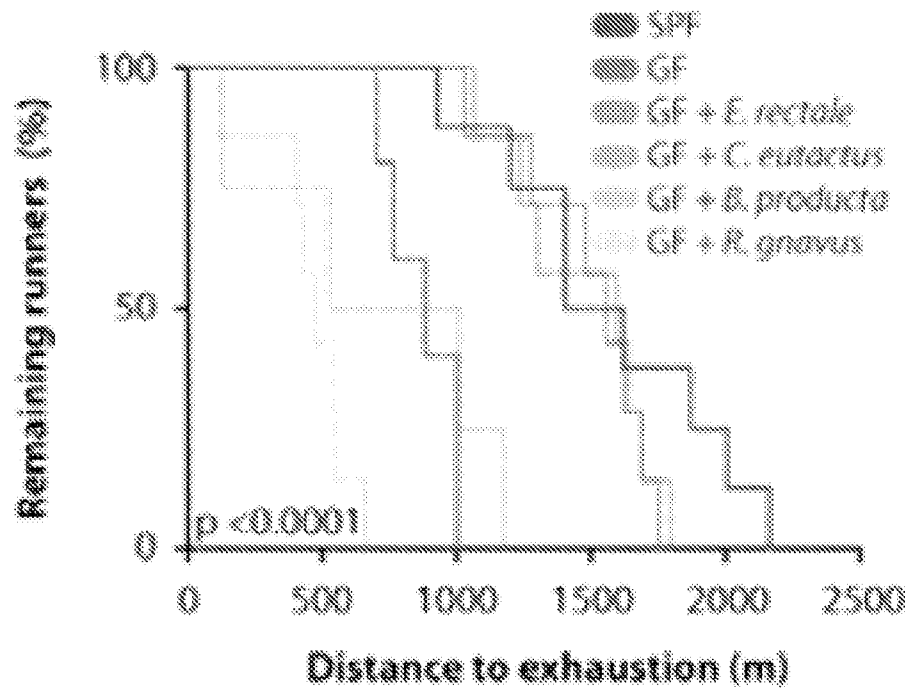


FIG. 4L

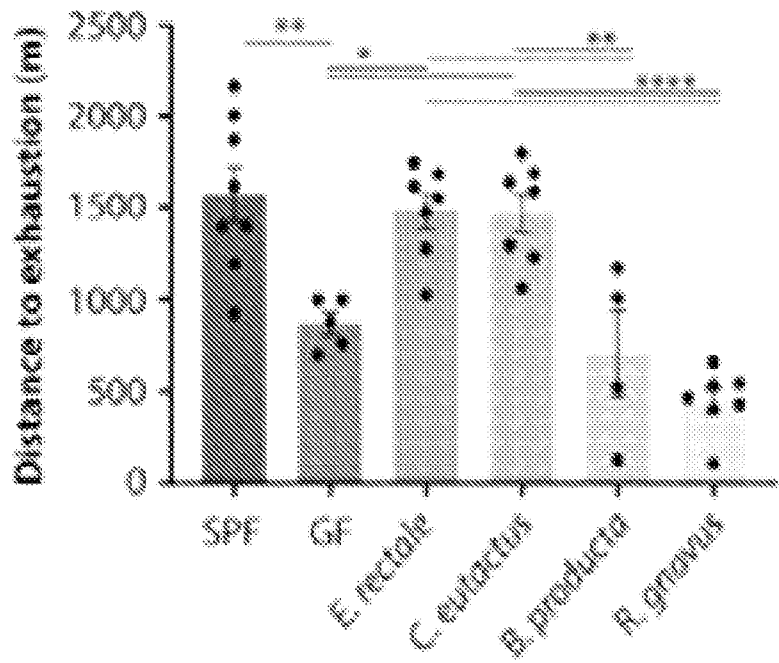


FIG. 5A

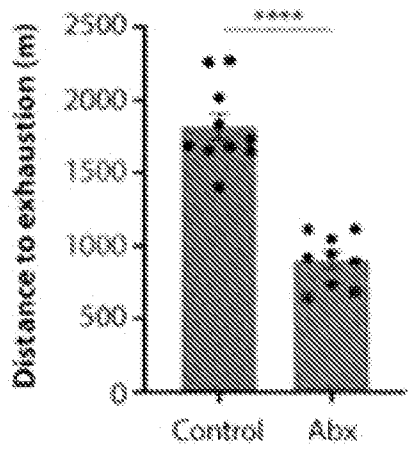


FIG. 5B

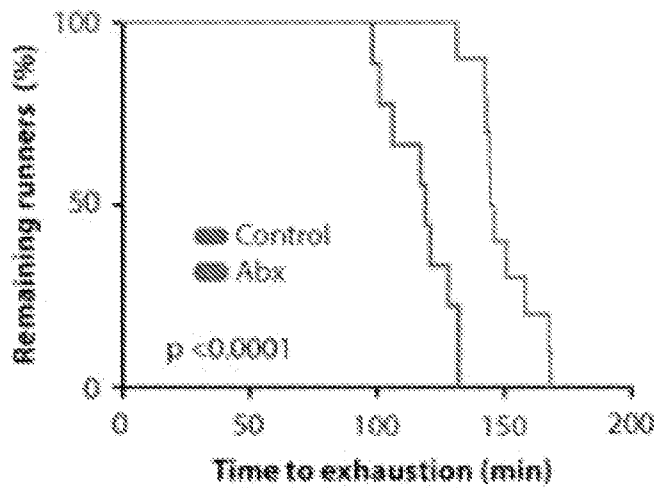


FIG. 5C

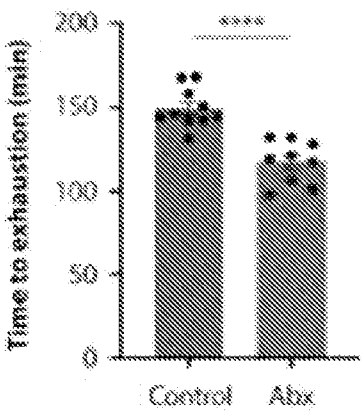


FIG. 5D

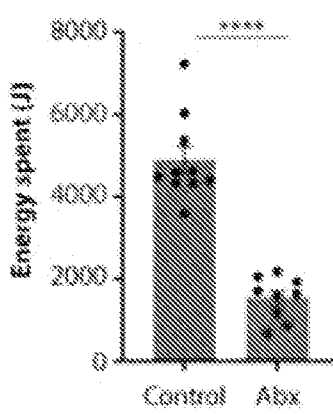


FIG. 5E

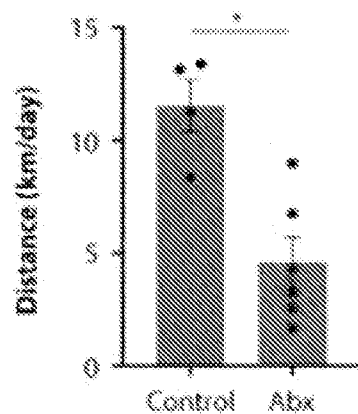


FIG. 5F

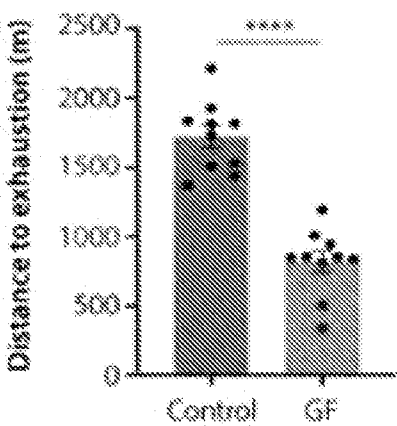


FIG. 5G

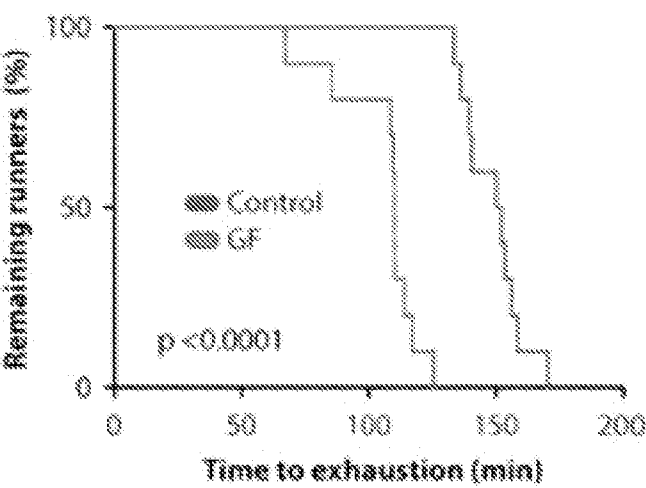


FIG. 5H

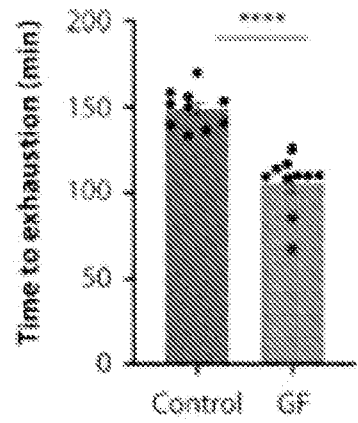


FIG. 5I

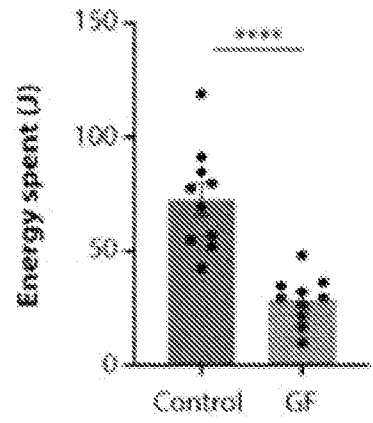


FIG. 5J

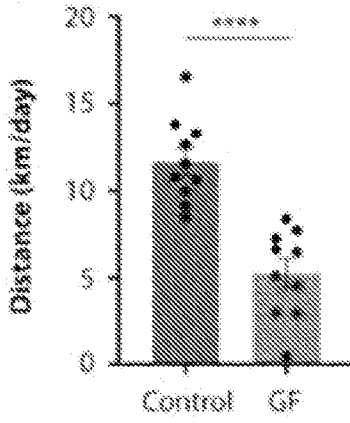


FIG. 5K

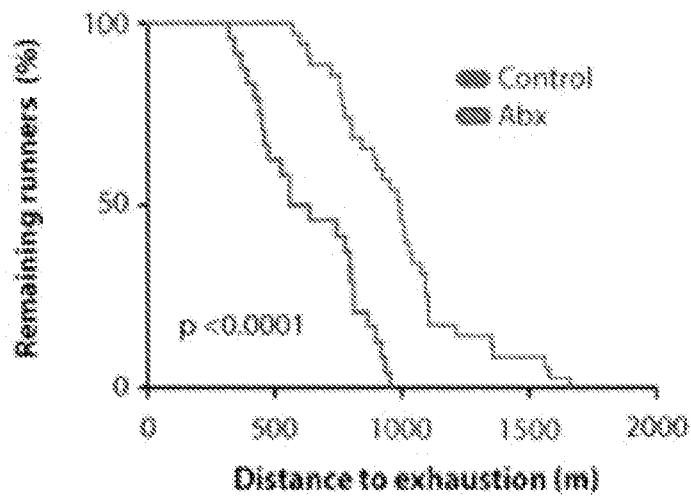


FIG. 5L

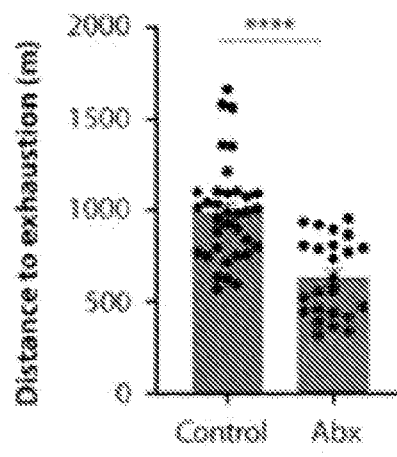


FIG. 5M

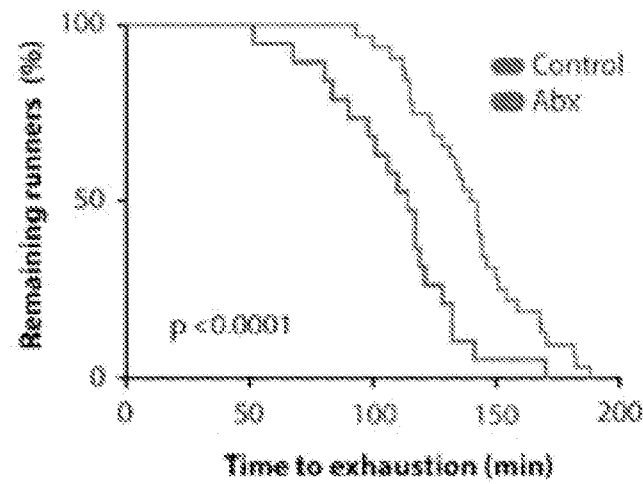


FIG. 5N

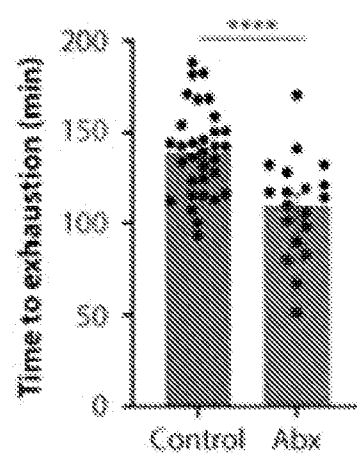


FIG. 5O

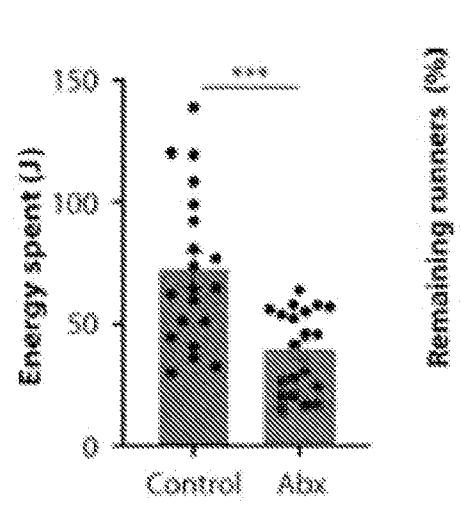


FIG. 5P

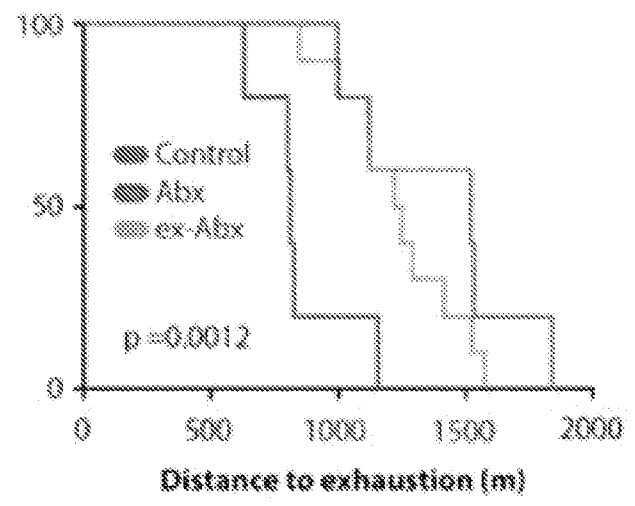


FIG. 5Q

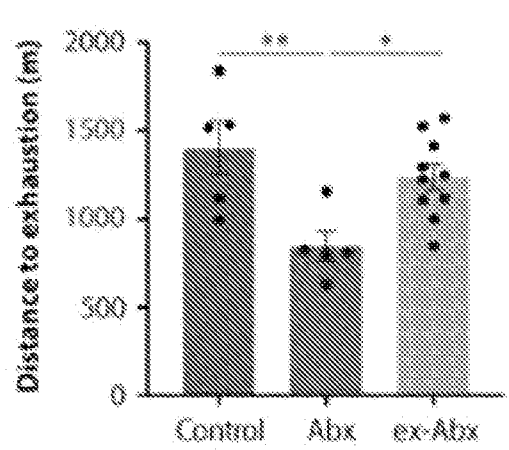


FIG. 5R

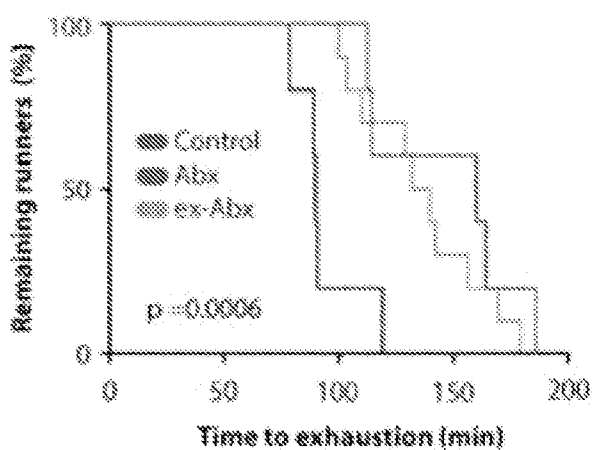


FIG. 5S

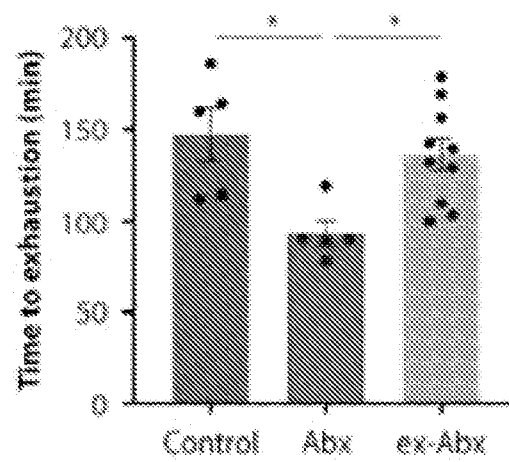


FIG. 5T

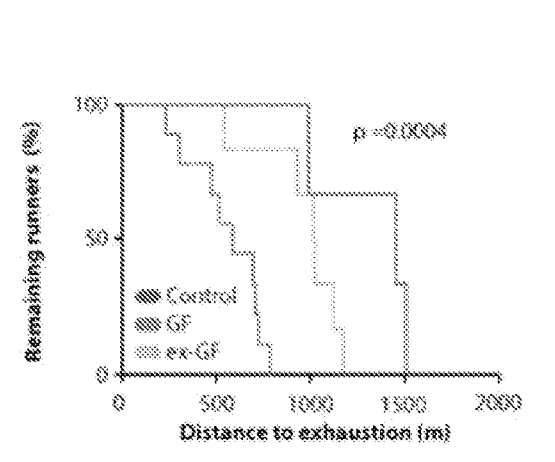


FIG. 5U

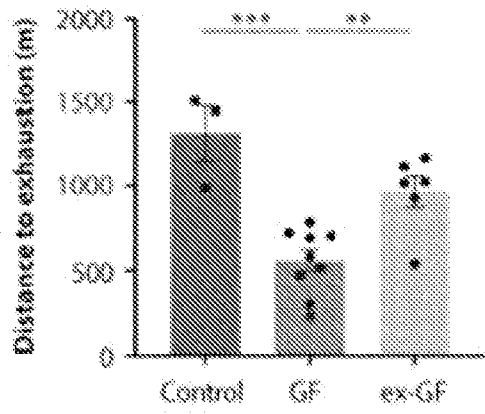


FIG. 5V

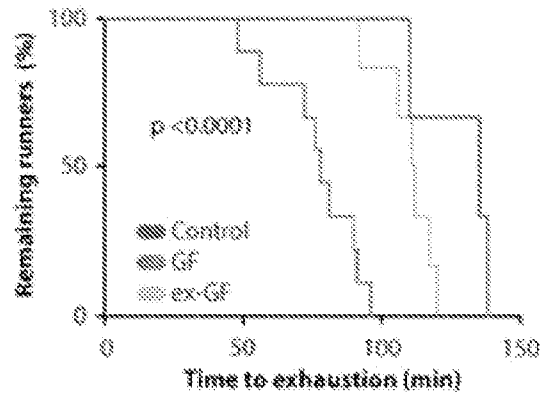


FIG. 5W

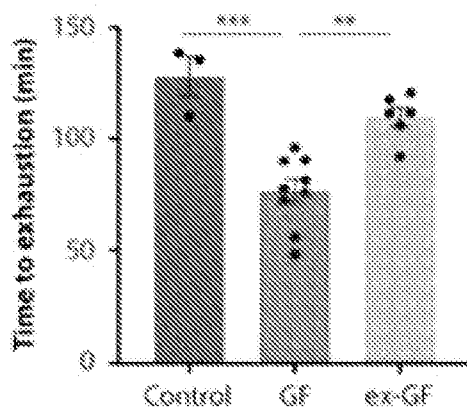


FIG. 6A

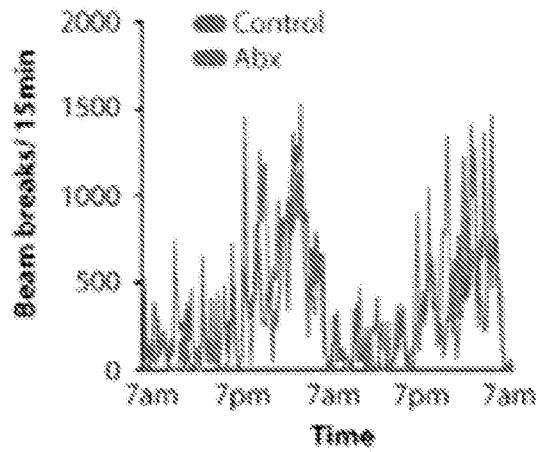


FIG. 6B

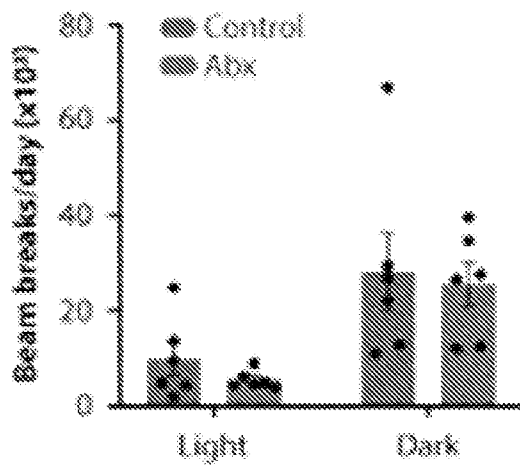


FIG. 6C

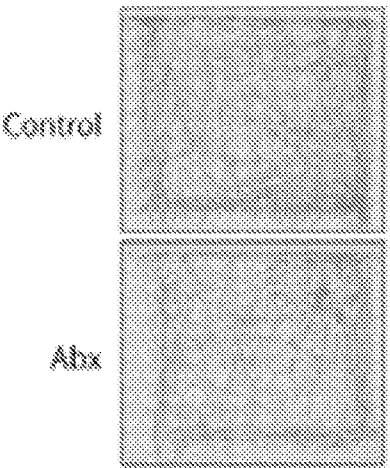


FIG. 6D

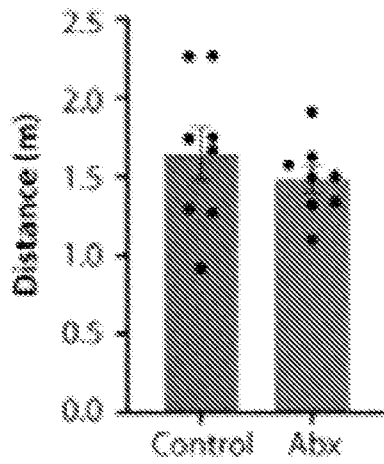


FIG. 6E

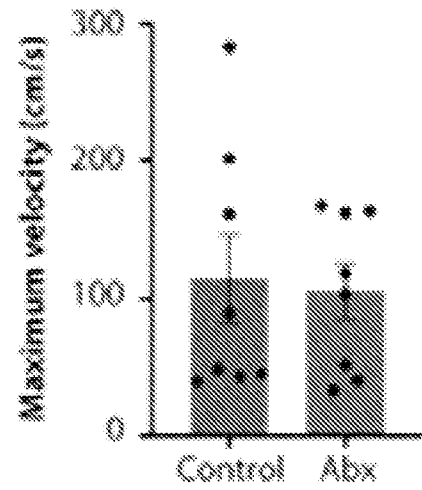


FIG. 6F

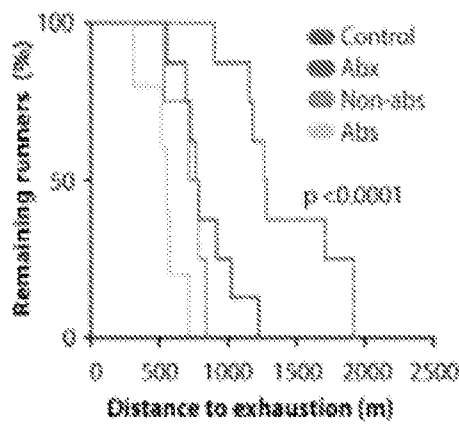


FIG. 6G

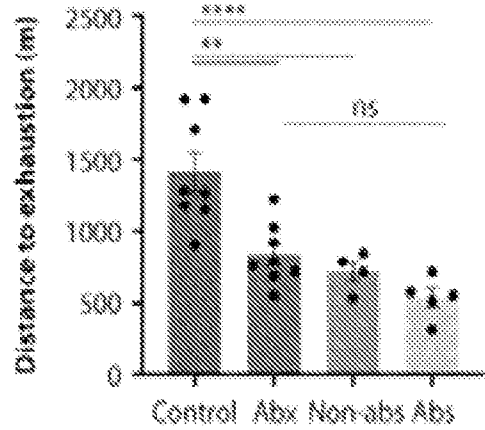


FIG. 6H

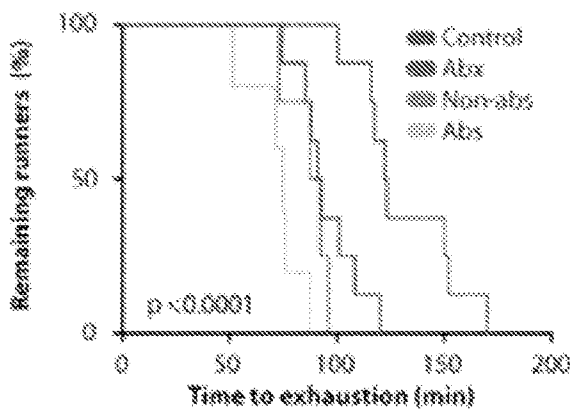


FIG. 6I

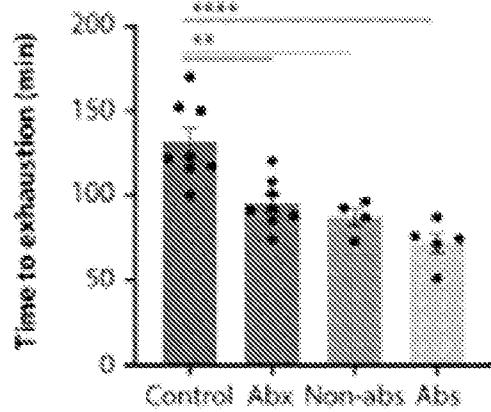


FIG. 6J

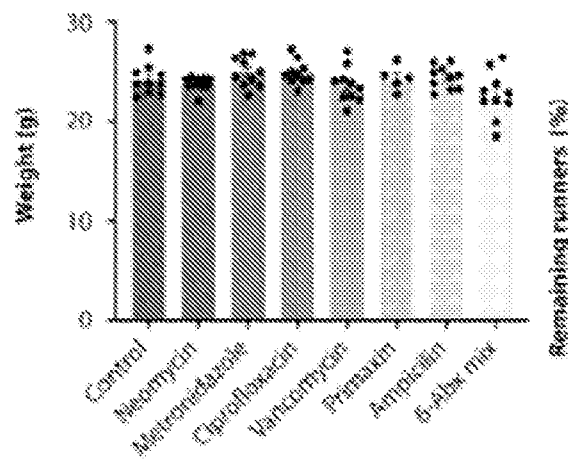


FIG. 6K

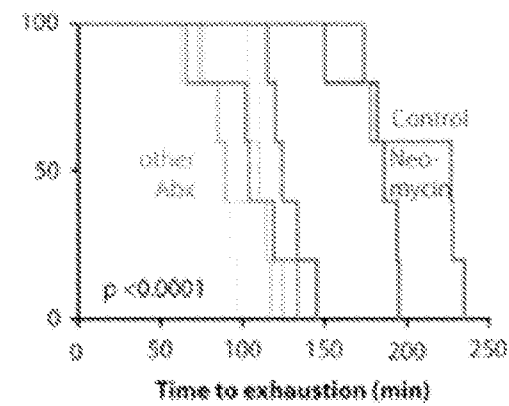


FIG. 6L

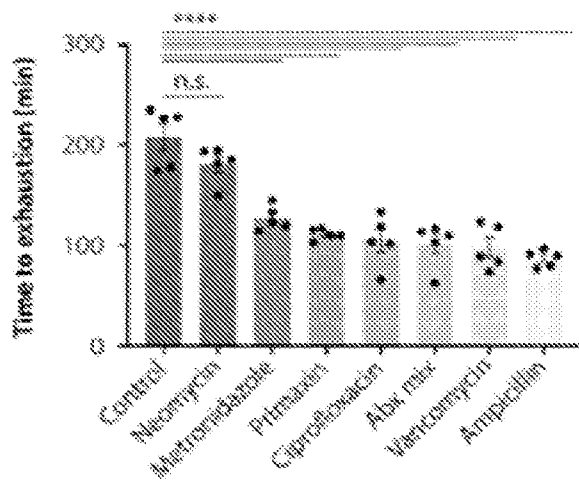


FIG. 6M

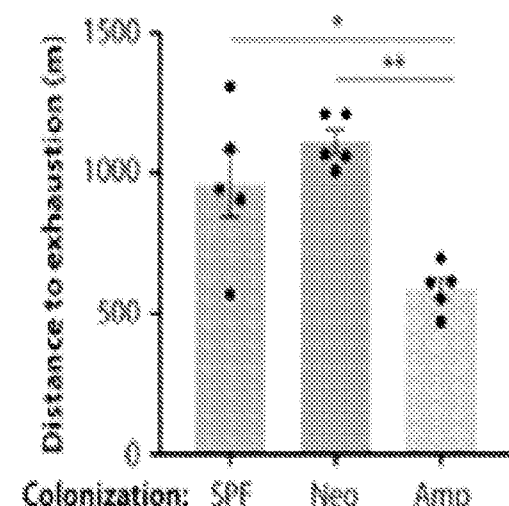


FIG. 6N

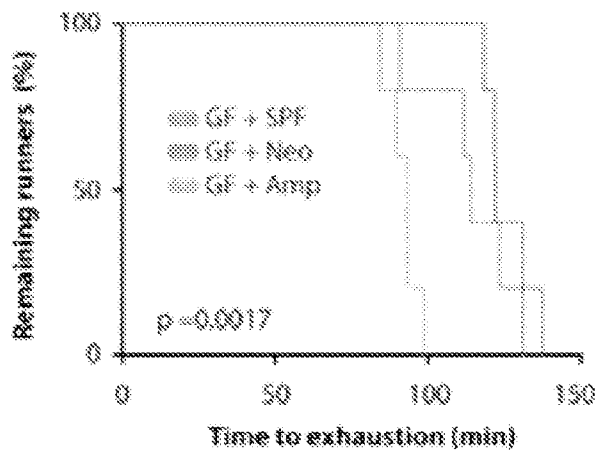


FIG. 6O

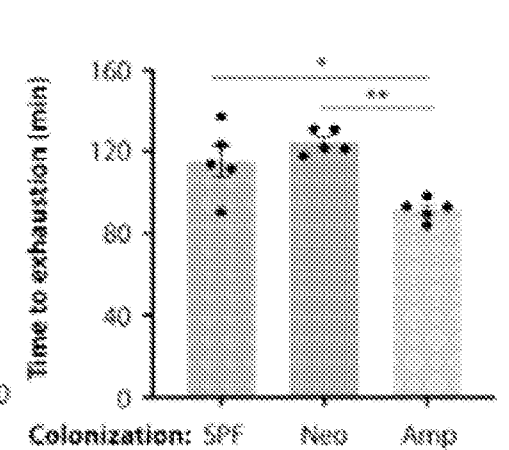


FIG. 6P

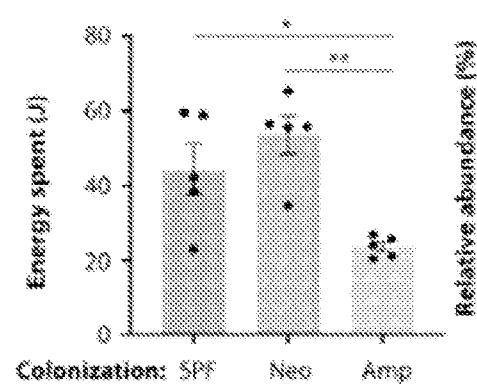


FIG. 6Q

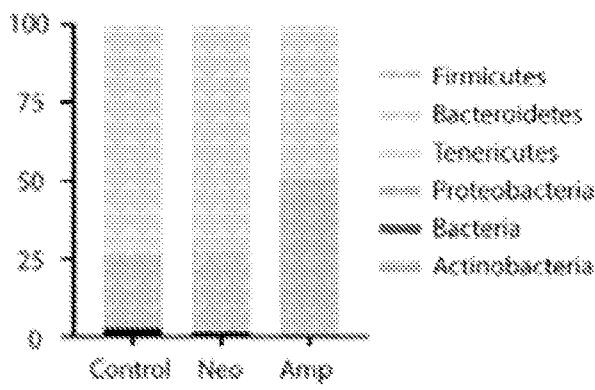


FIG. 6R

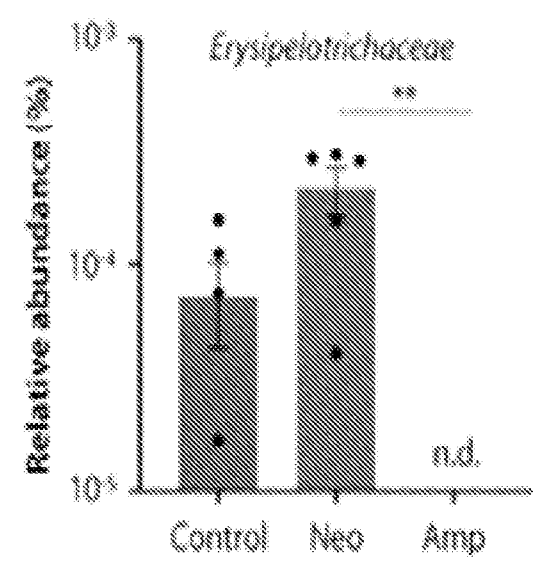


FIG. 6S

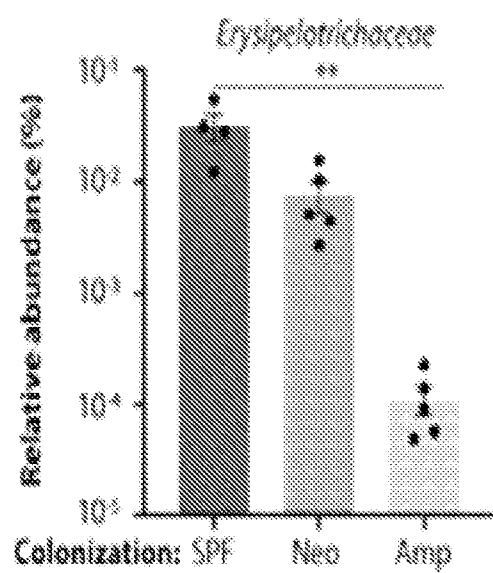


FIG. 6T

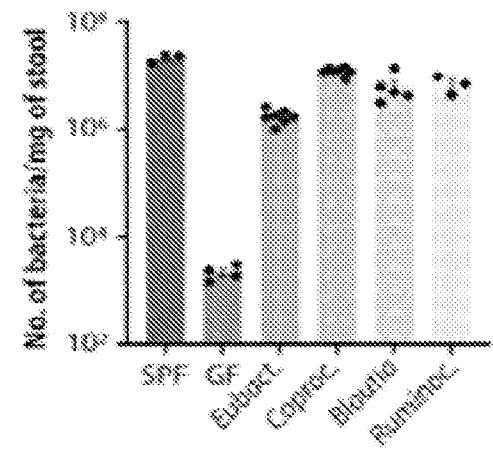


FIG. 6U

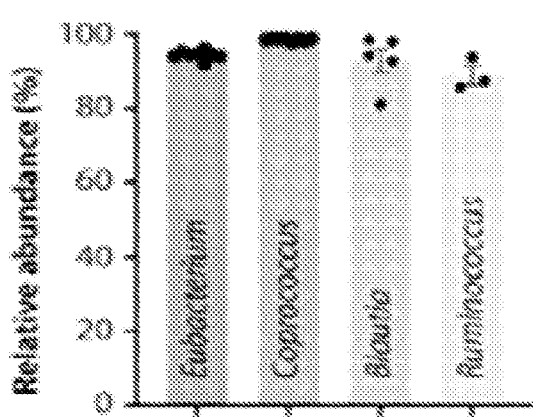


FIG. 6V

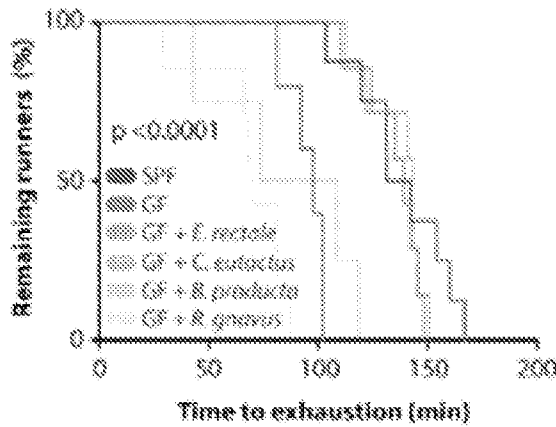


FIG. 6W

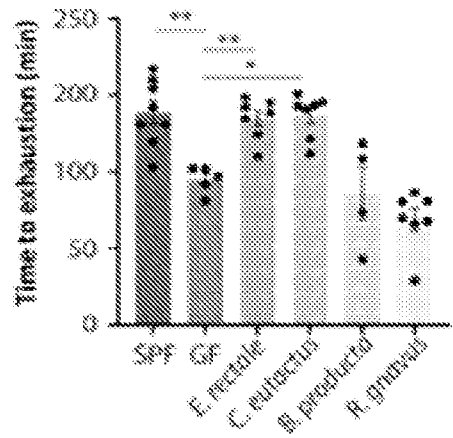


FIG. 7A

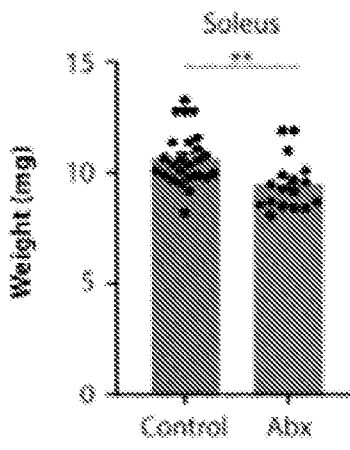


FIG. 7B

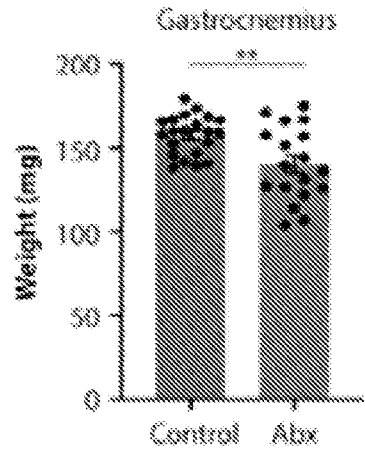


FIG. 7C

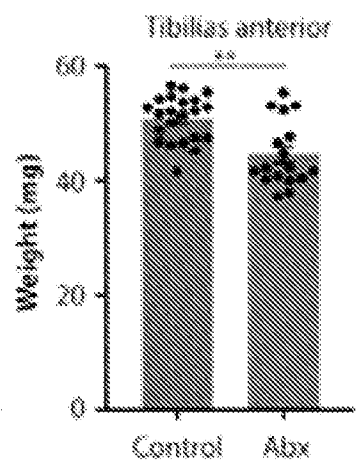


FIG. 7D

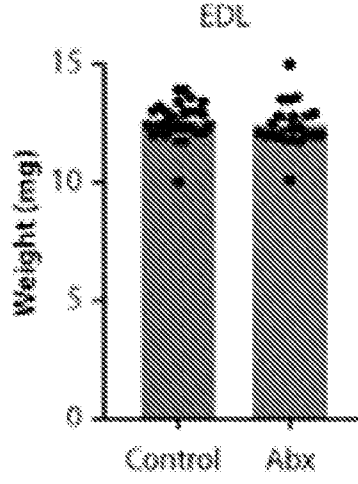


FIG. 7E

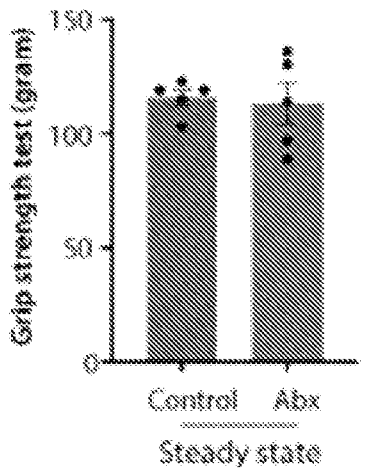


FIG. 7F

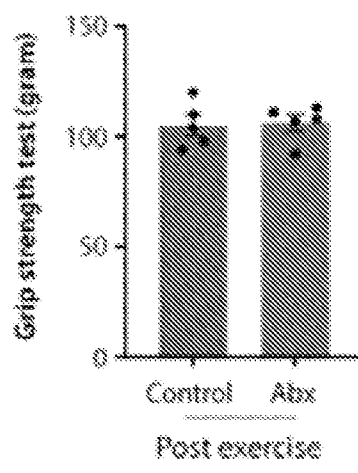


FIG. 7G

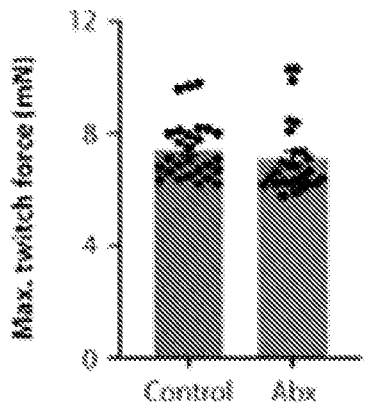


FIG. 7H

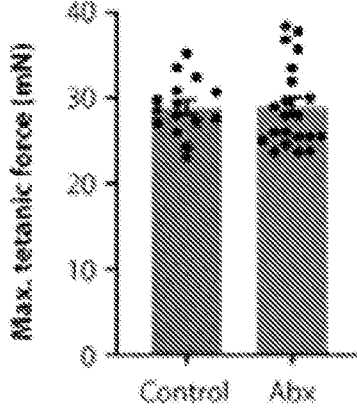


FIG. 7I

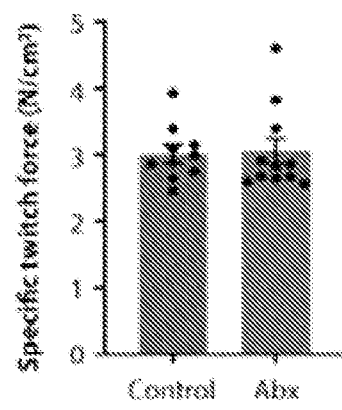


FIG. 7J

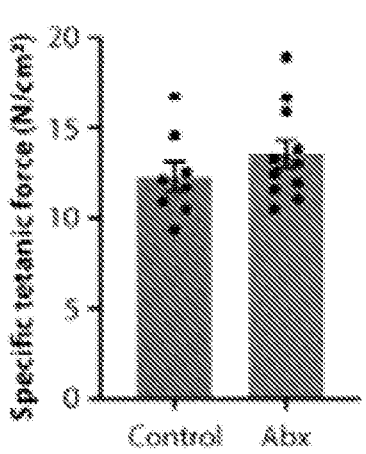


FIG. 7K

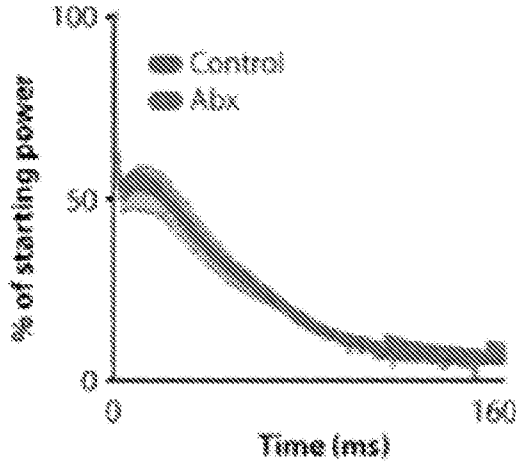


FIG. 7L

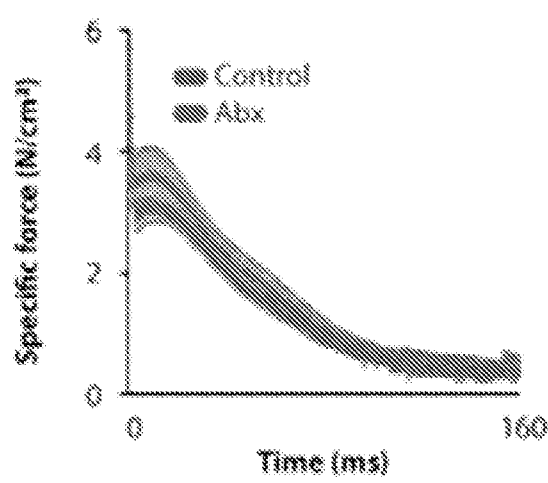


FIG. 7M

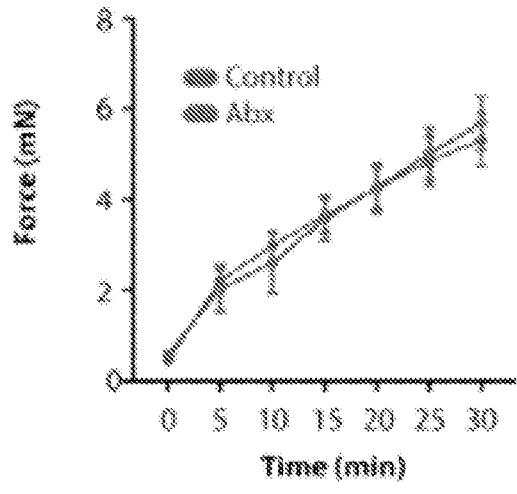


FIG. 7N

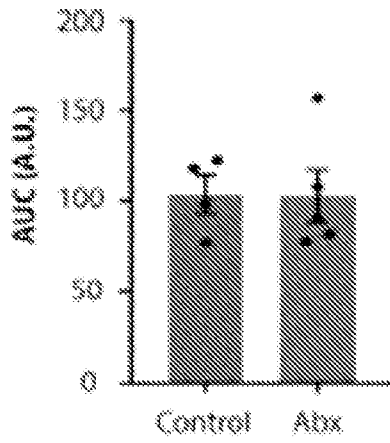


FIG. 7O

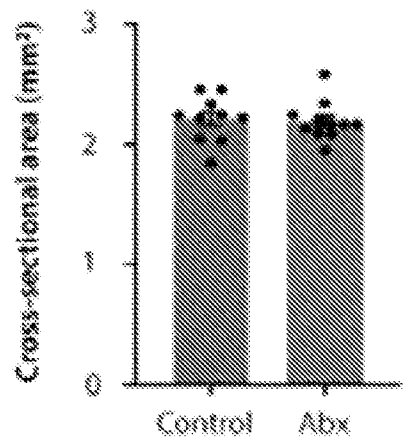


FIG. 7P

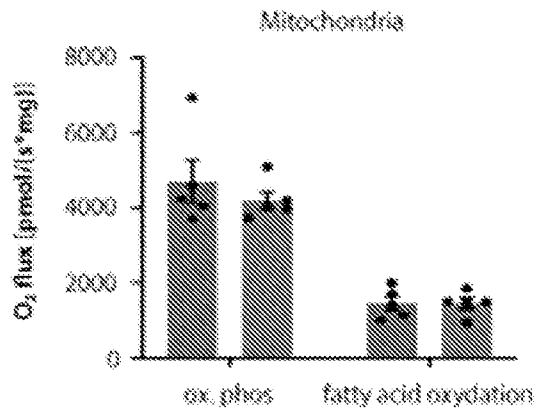


FIG. 7Q

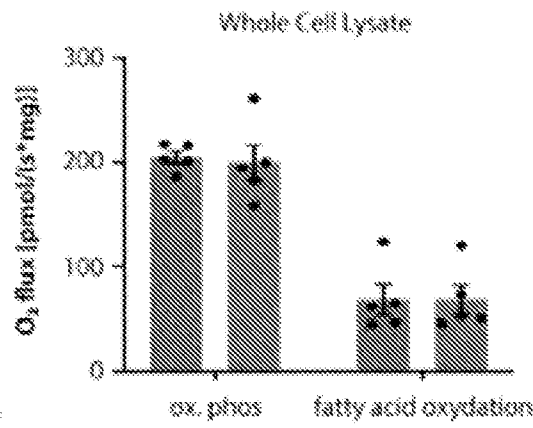


FIG. 7R

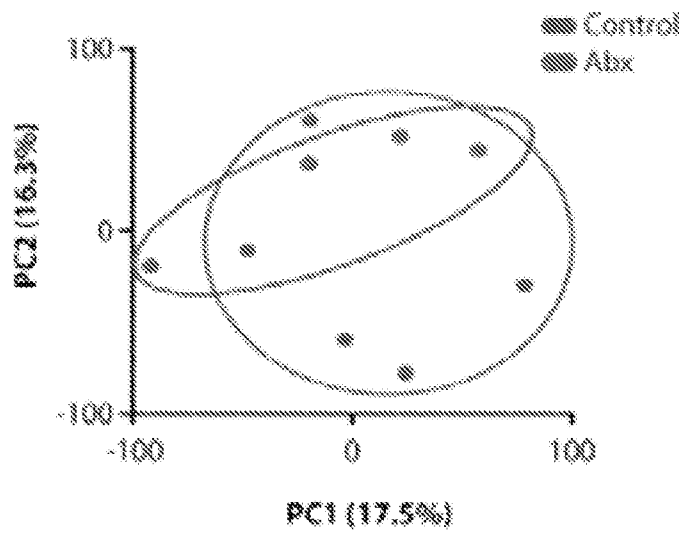


FIG. 7S

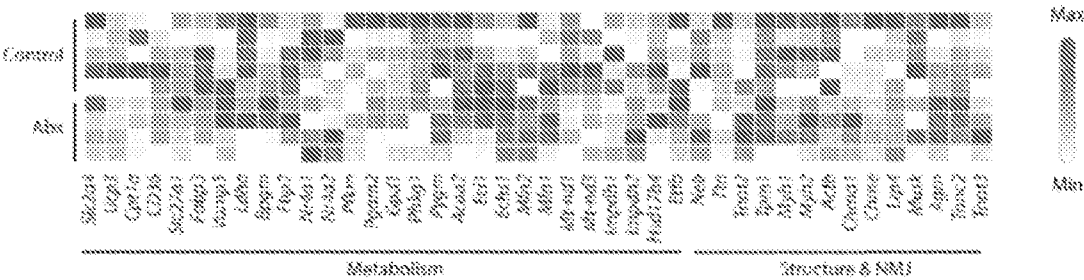


FIG. 8A

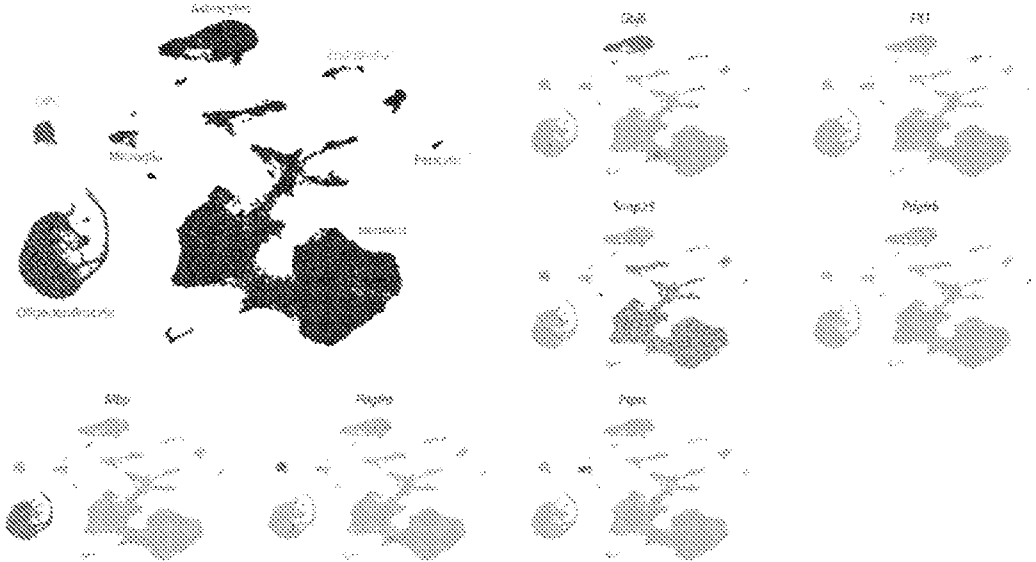


FIG. 8B

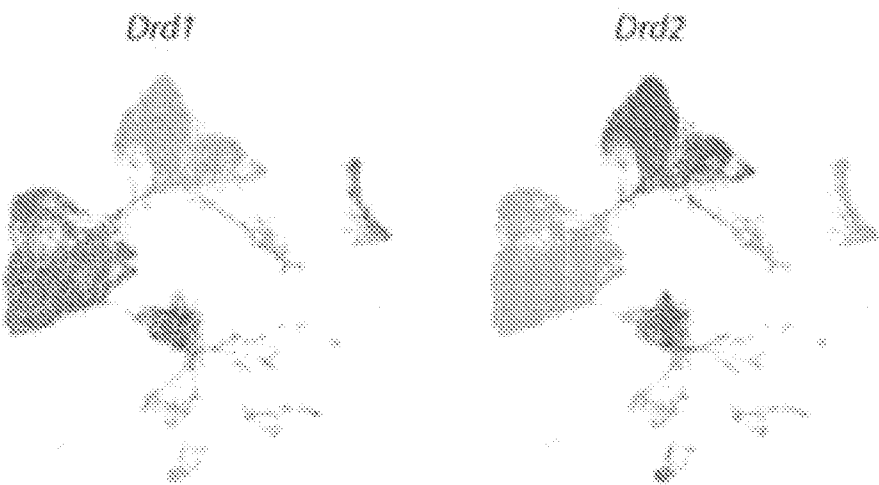


FIG. 8C

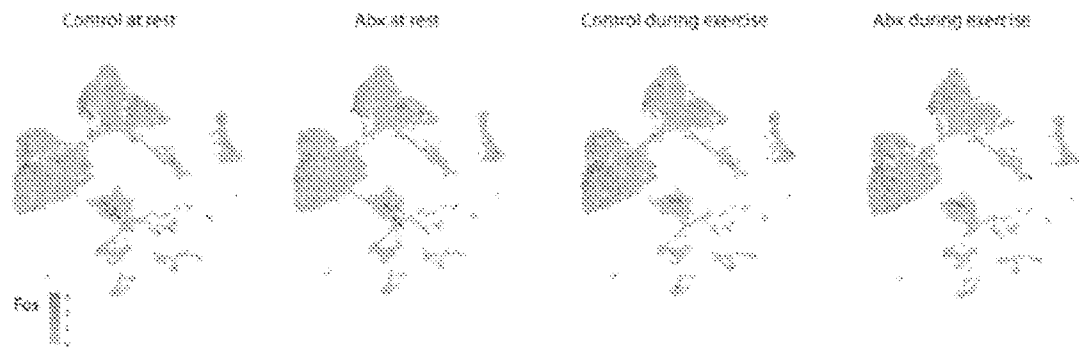


FIG. 8D

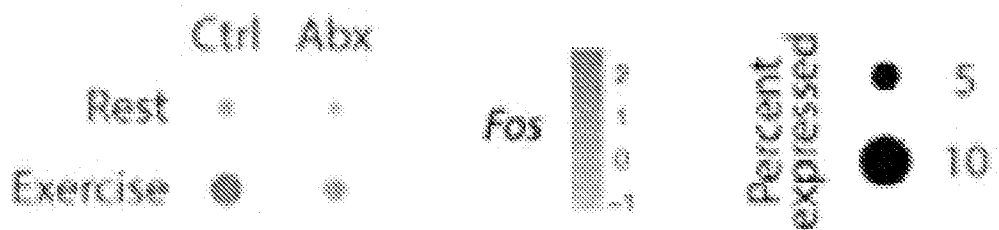


FIG. 9A

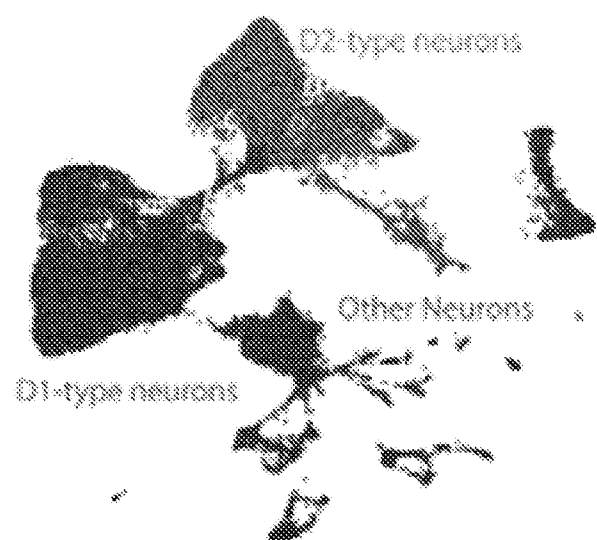


FIG. 9B

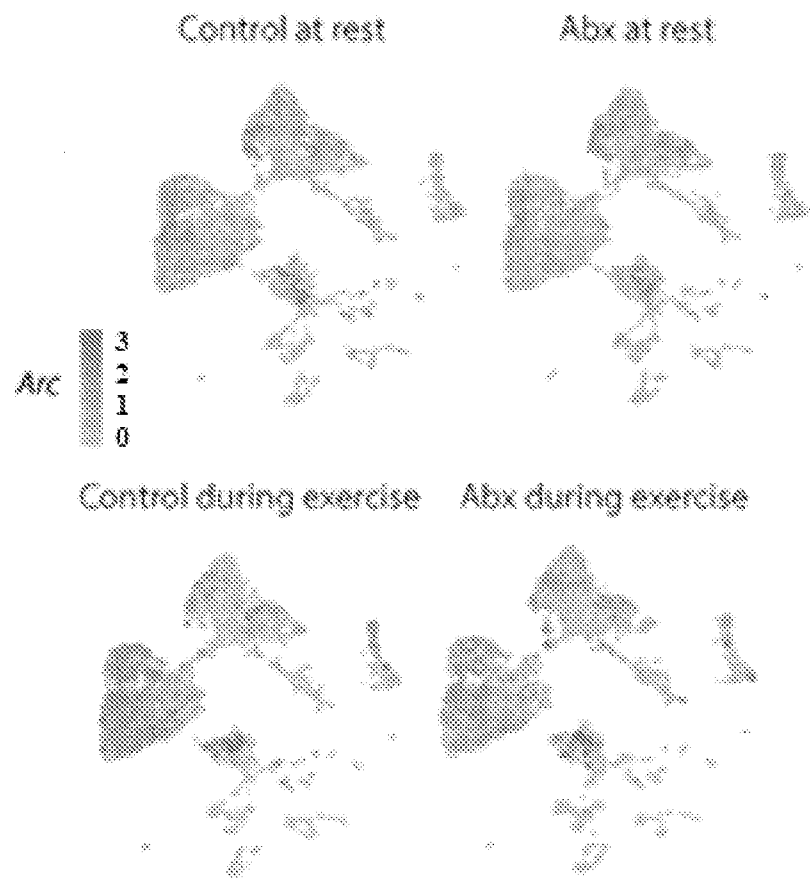


FIG. 9C

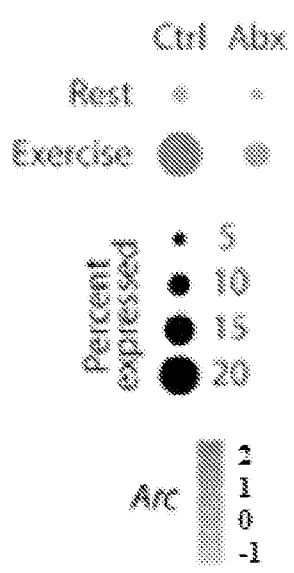


FIG. 9D

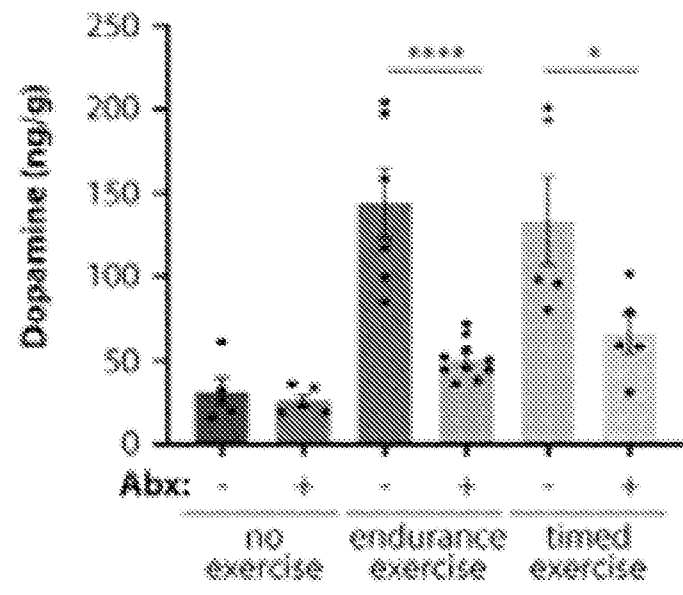


FIG. 9E

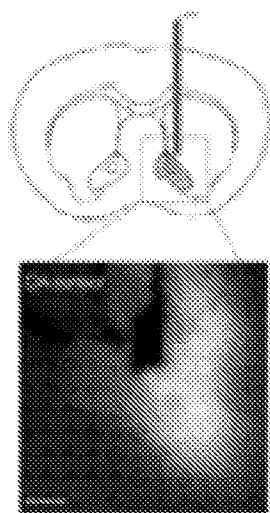


FIG. 9F

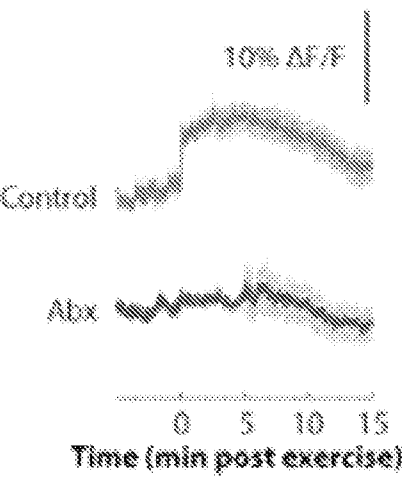


FIG. 9G

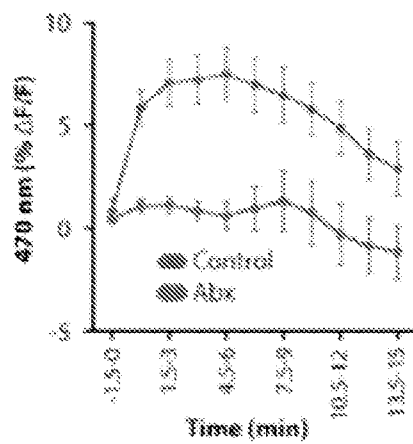


FIG. 9H

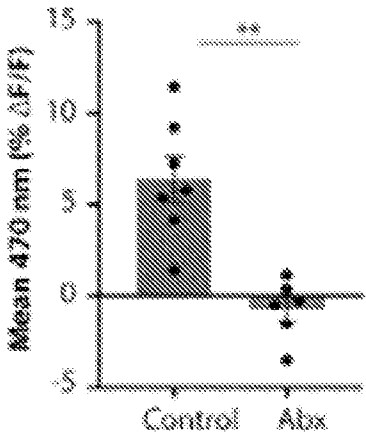


FIG. 9I

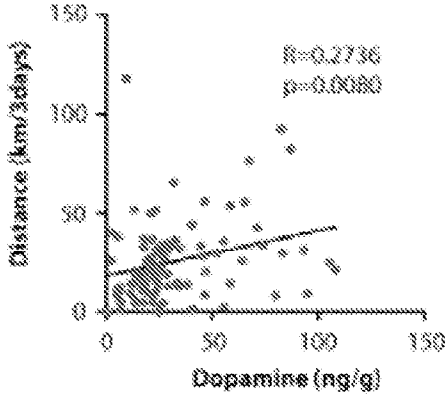


FIG. 9J

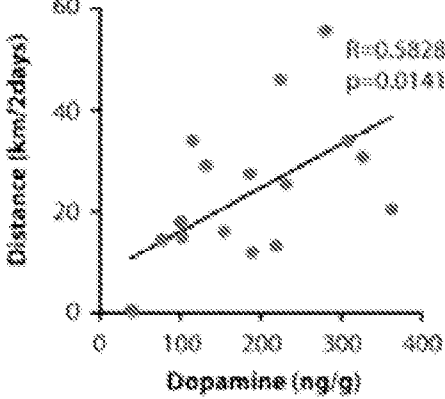


FIG. 9K

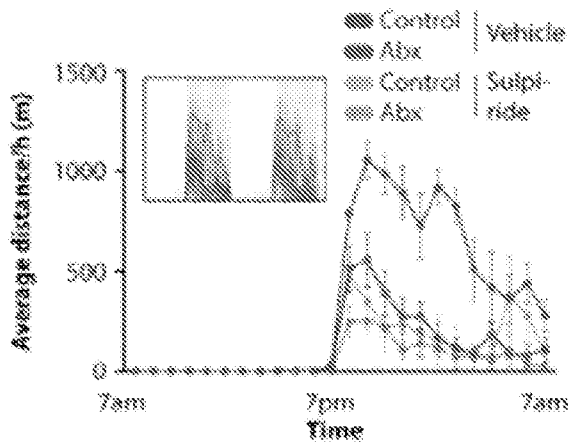


FIG. 9L

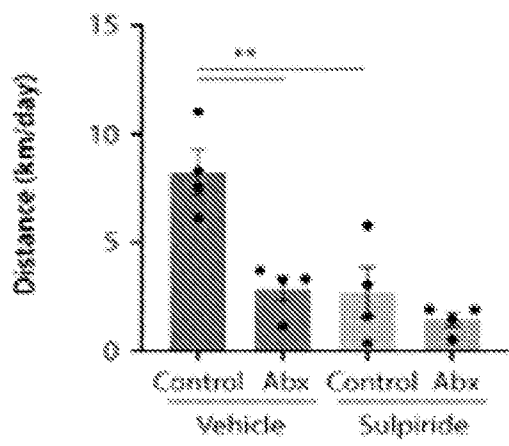


FIG. 9M

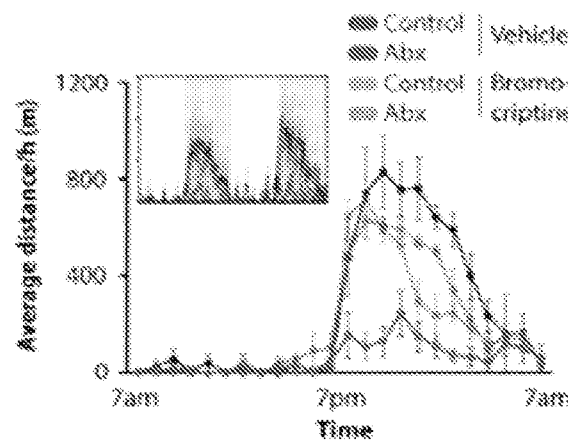


FIG. 9N

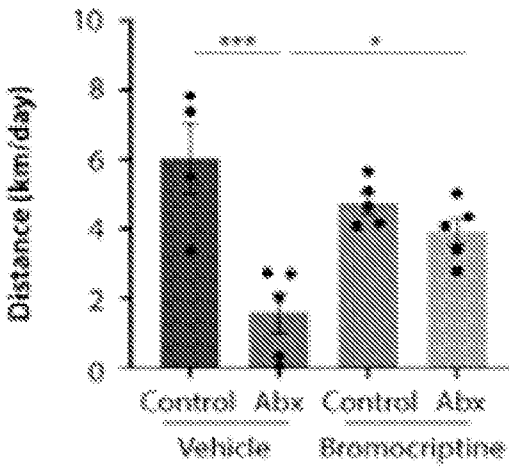


FIG. 9O

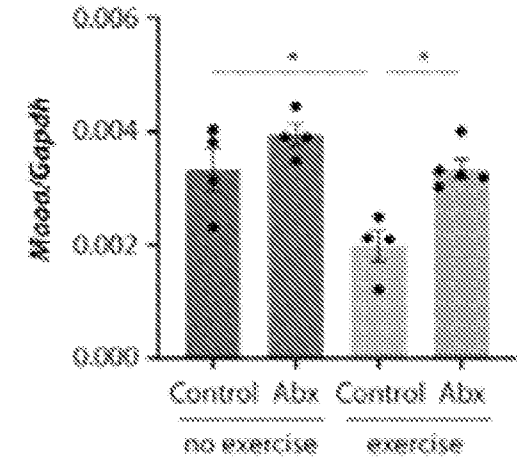


FIG. 9P

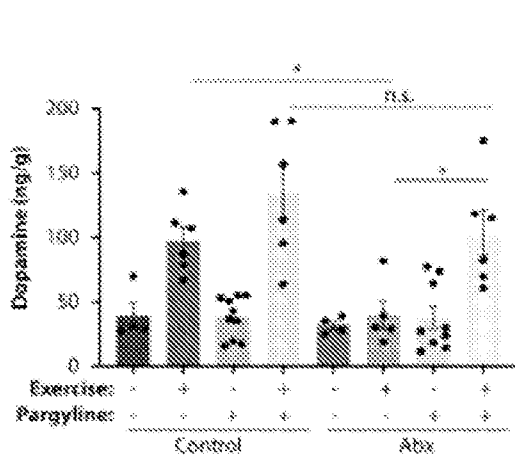


FIG. 9Q

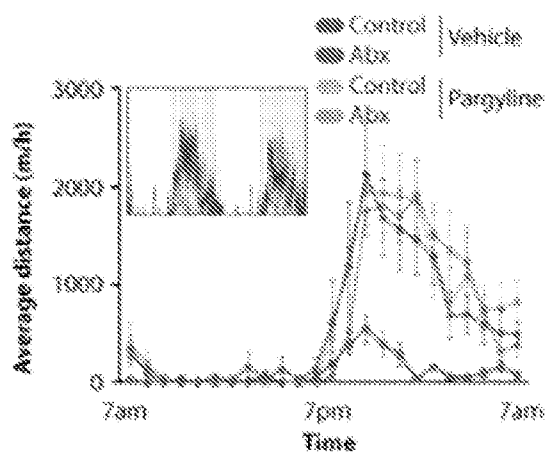


FIG. 9R

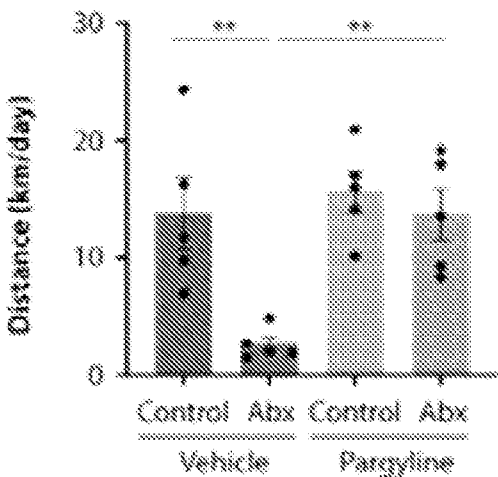


FIG. 10A

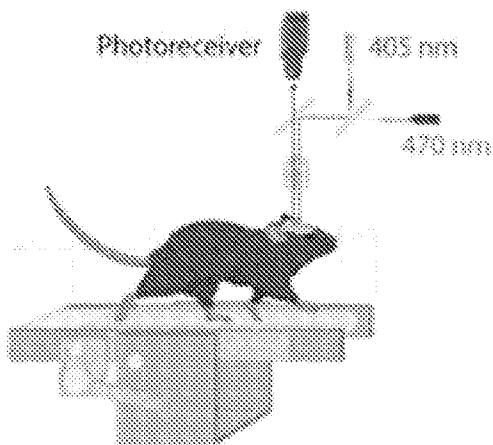


FIG. 10B

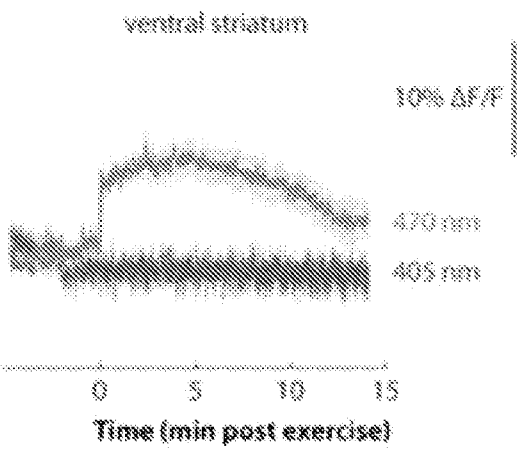


FIG. 10C

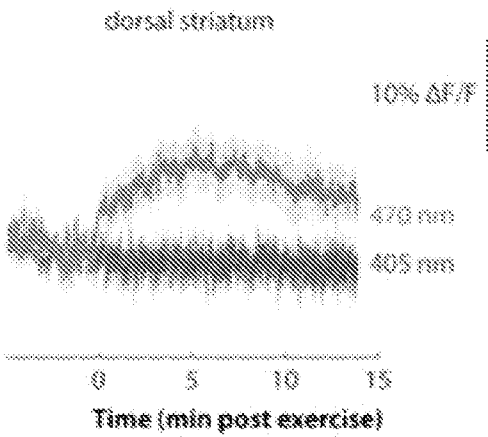


FIG. 10D

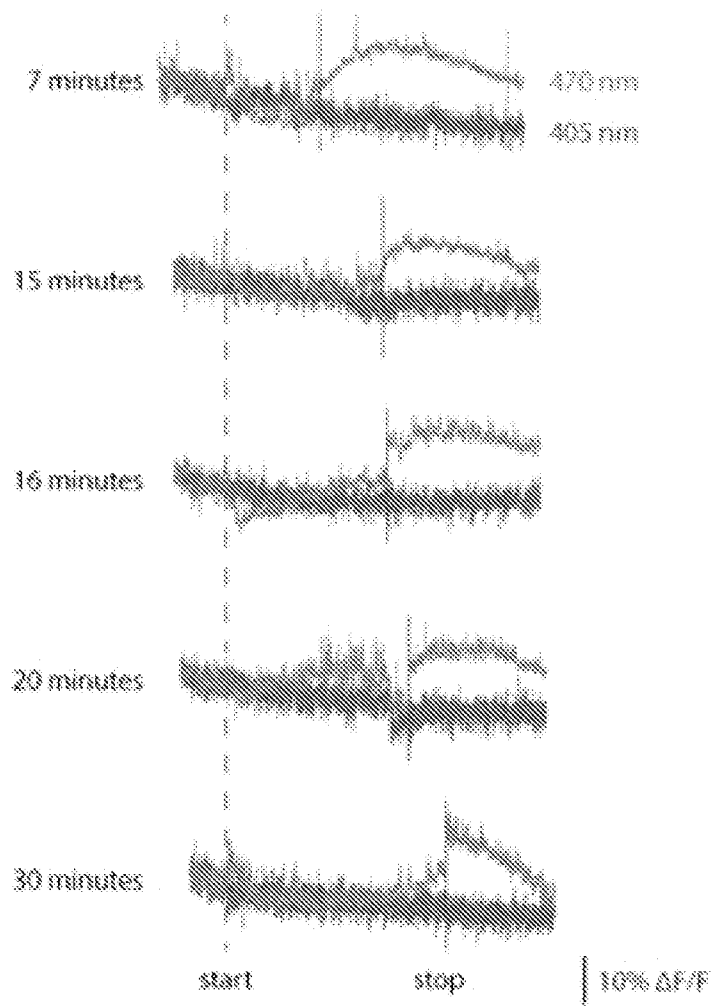


FIG. 10E

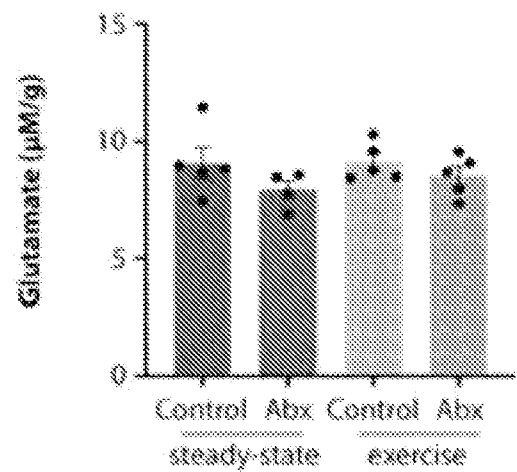


FIG. 10F

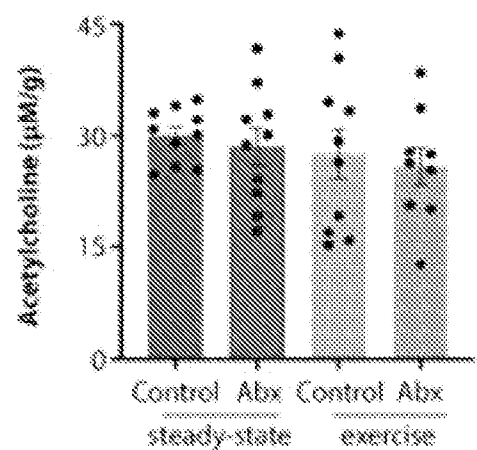


FIG. 10G

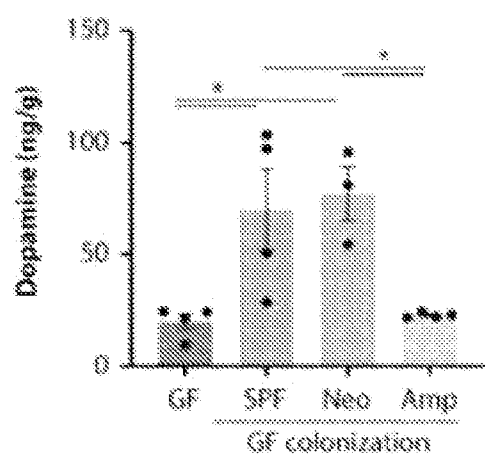


FIG. 10H

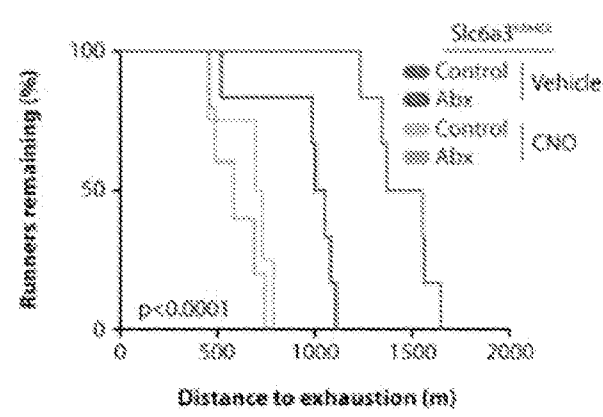


FIG. 10I

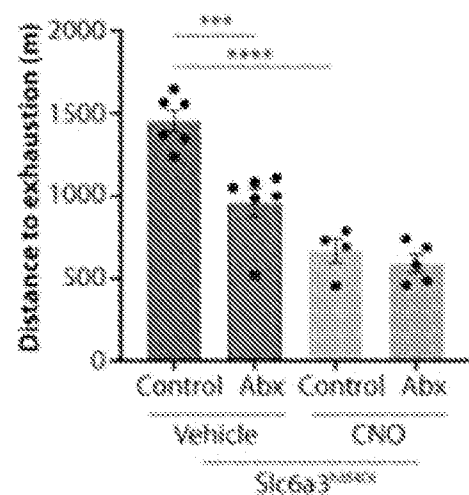


FIG. 10J

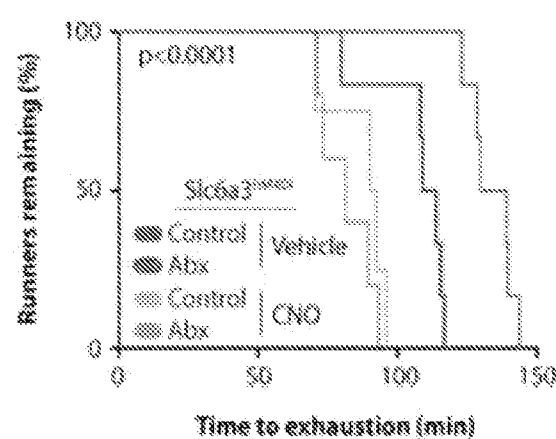


FIG. 10K

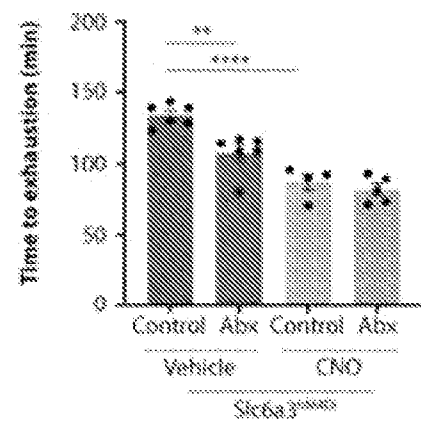


FIG. 10L

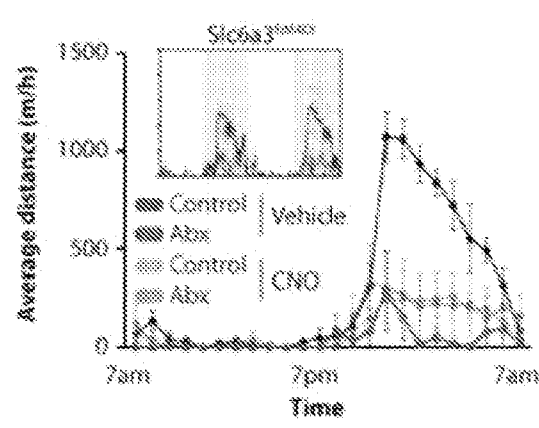


FIG. 10M

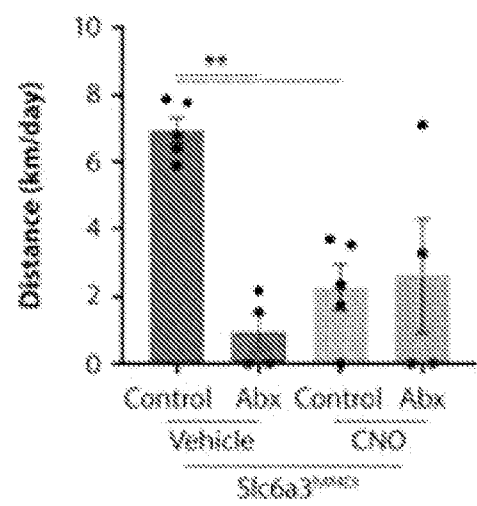


FIG. 11A



FIG. 11B

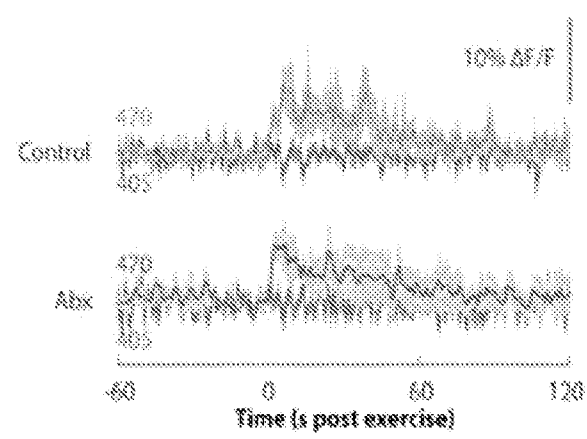


FIG. 11C

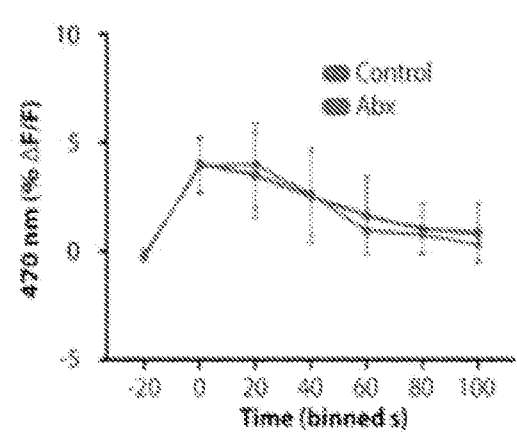


FIG. 11D

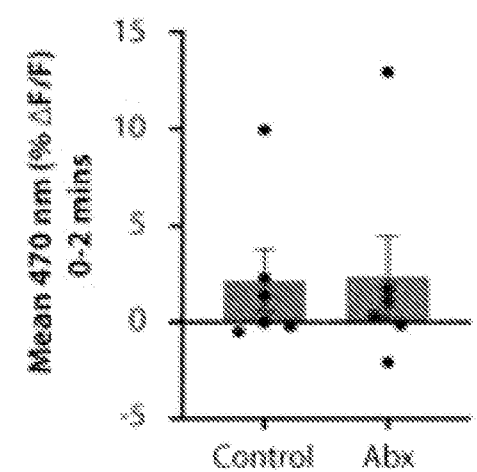


FIG. 11E

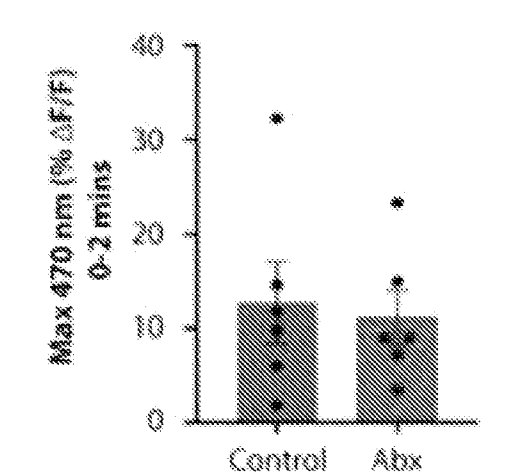


FIG. 11F

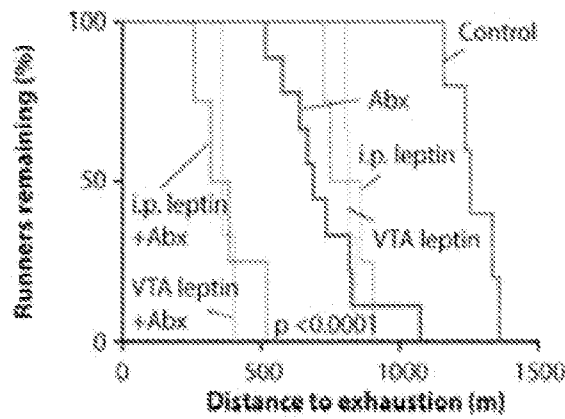


FIG. 11G

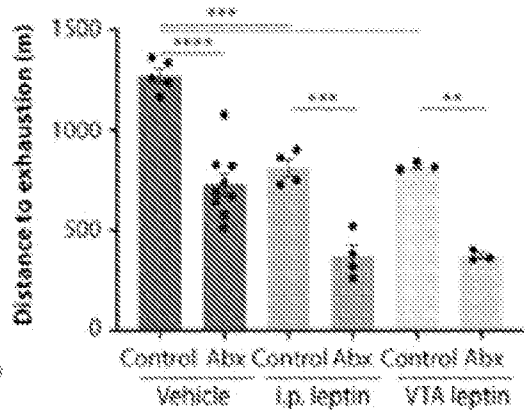


FIG. 11H

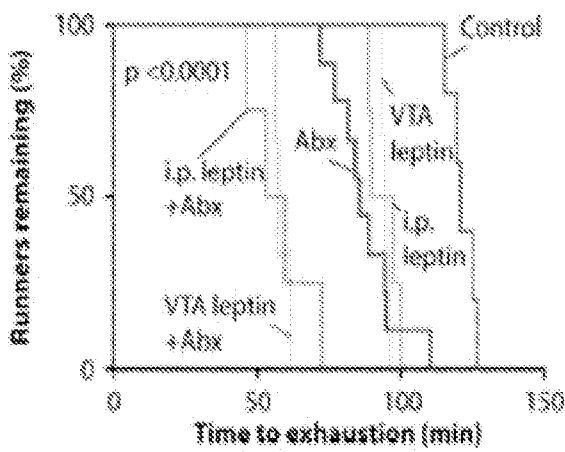


FIG. 11I

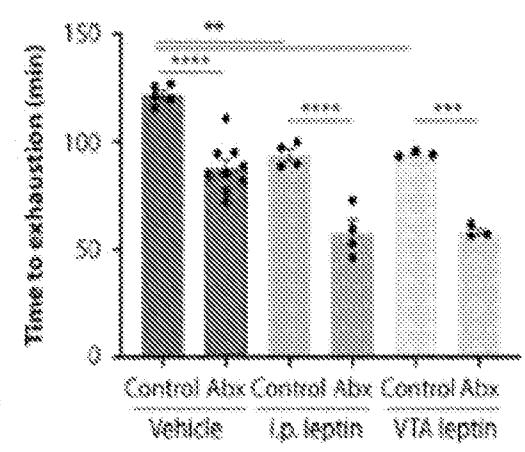


FIG. 11J

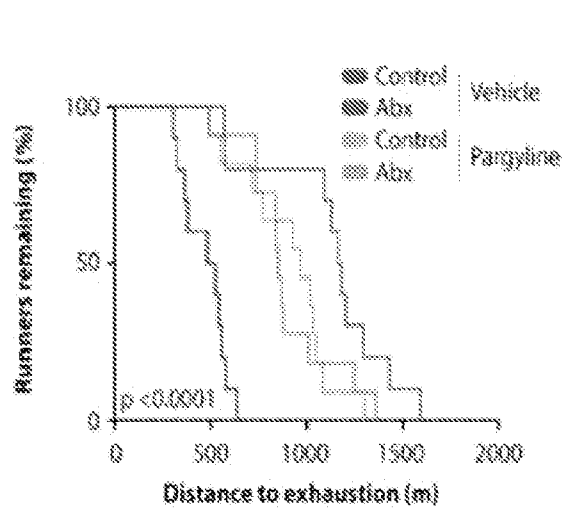


FIG. 11K

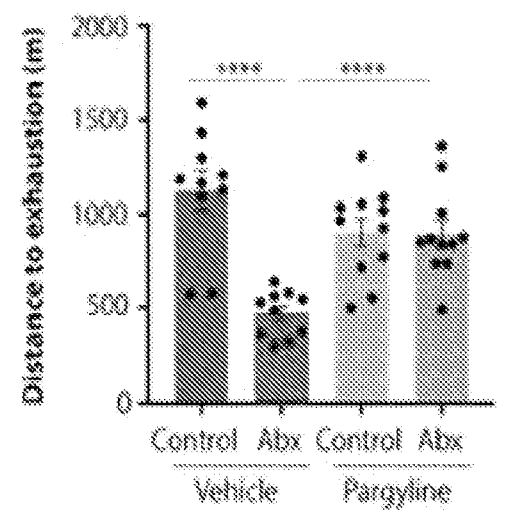


FIG. 11L

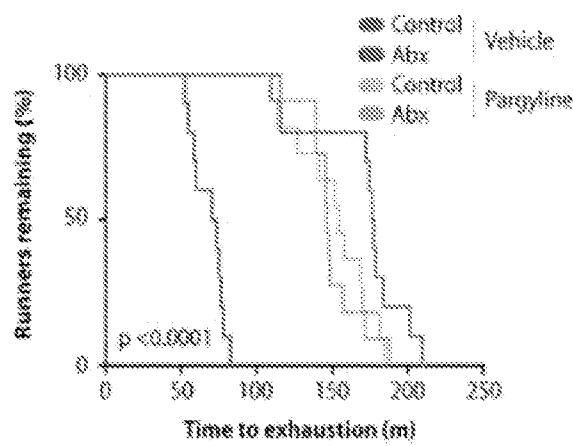


FIG. 11M

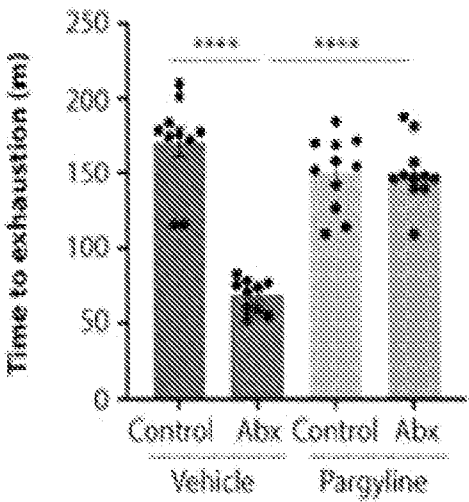


FIG. 12A

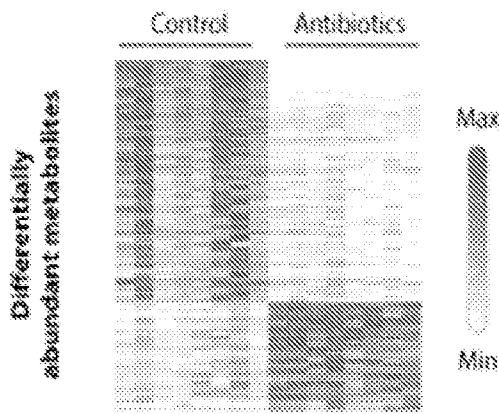


FIG. 12B

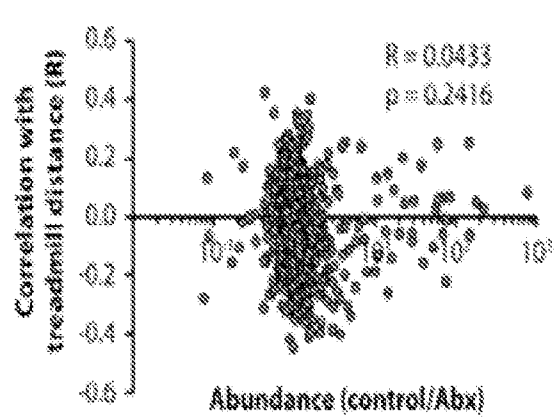


FIG. 12C

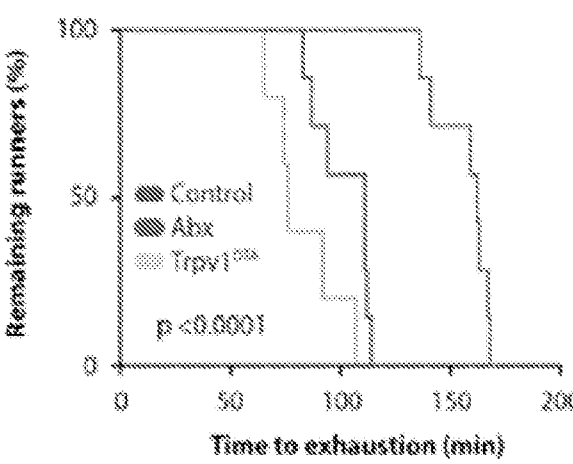


FIG. 12D

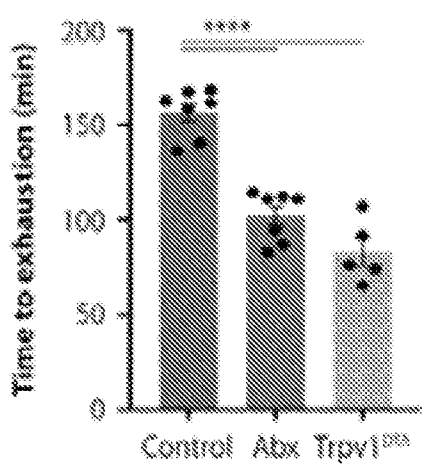


FIG. 12E

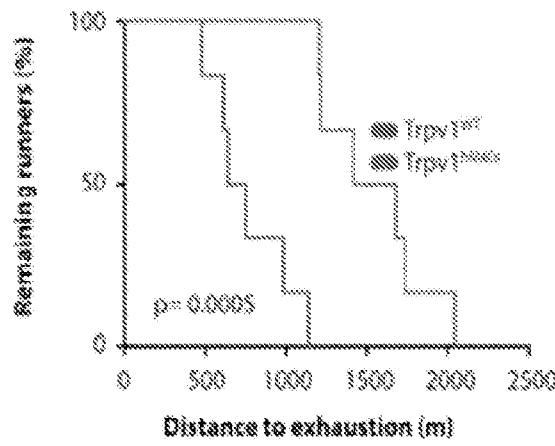


FIG. 12F

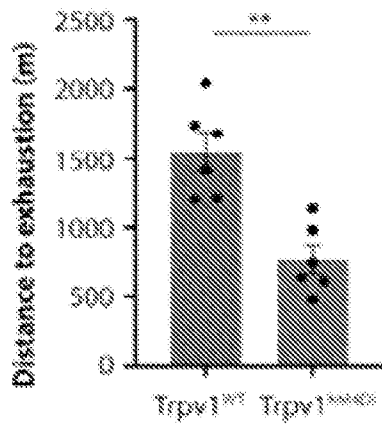


FIG. 12G

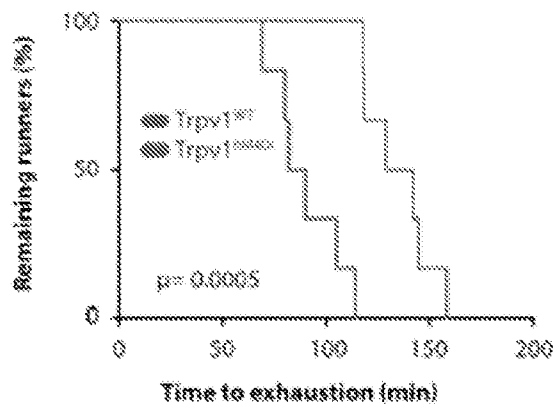


FIG. 12H

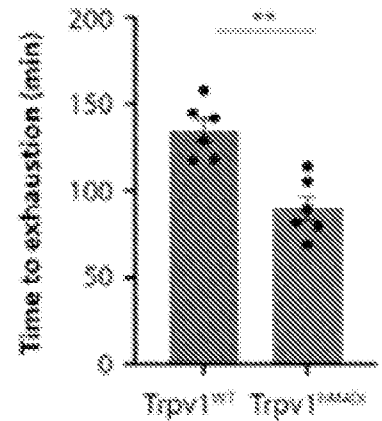


FIG. 12I

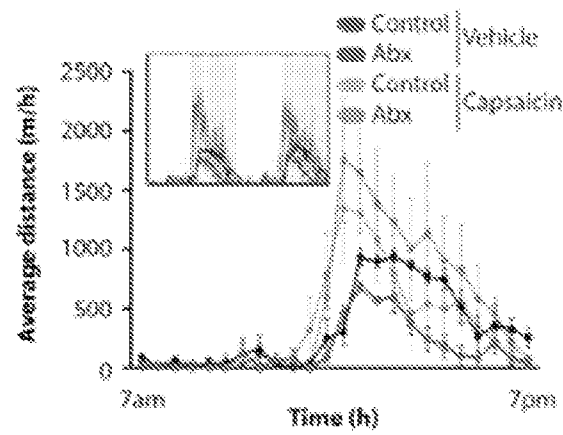


FIG. 12J

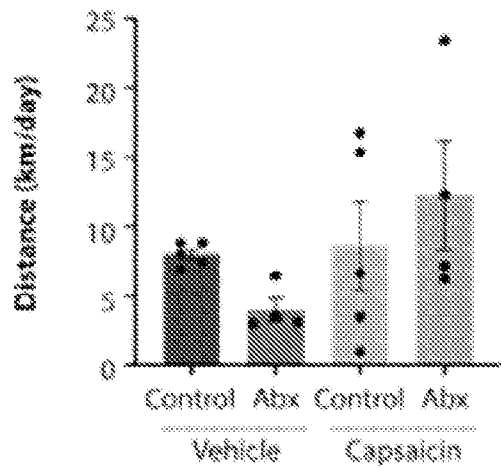


FIG. 12K

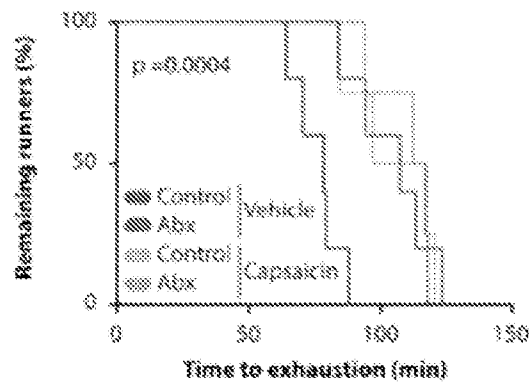


FIG. 12L

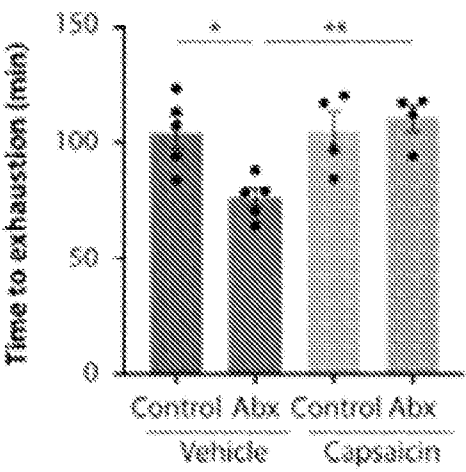


FIG. 13A

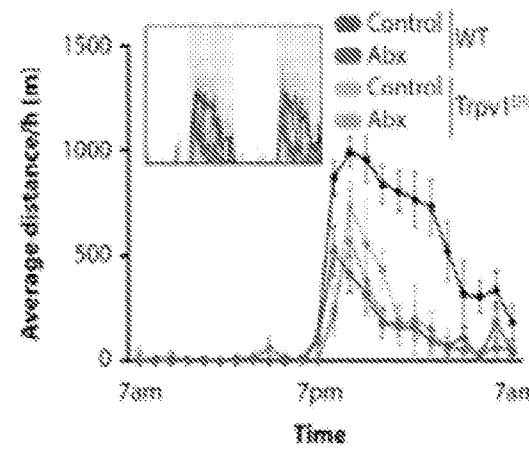


FIG. 13B

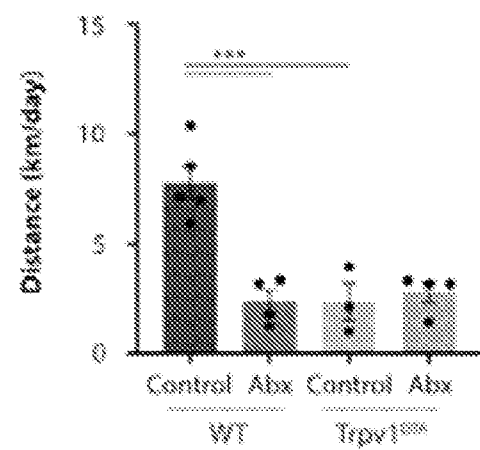


FIG. 13C

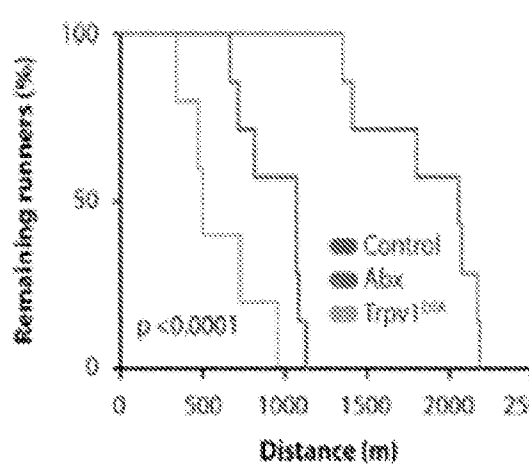


FIG. 13D

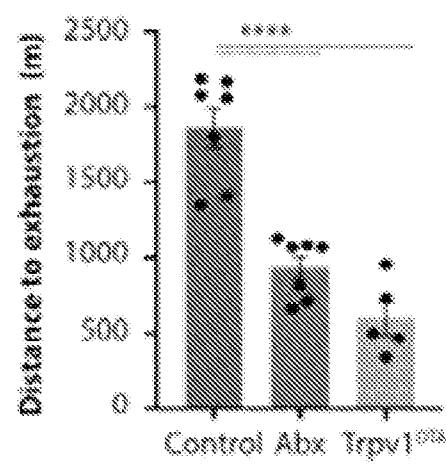


FIG. 13E

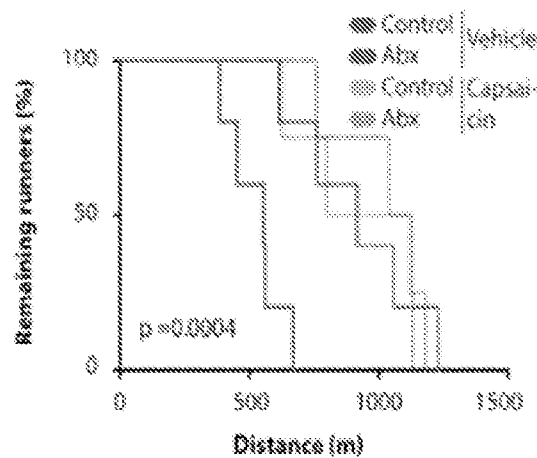


FIG. 13F

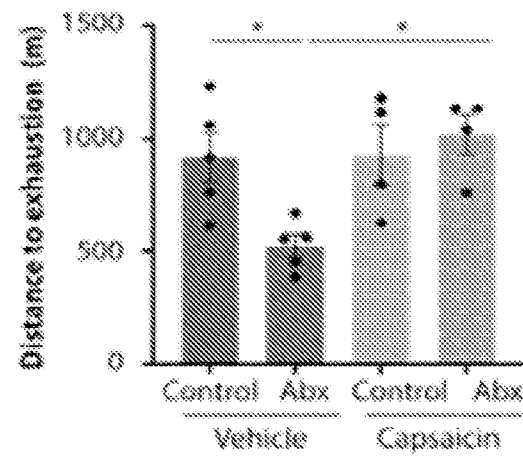


FIG. 13G

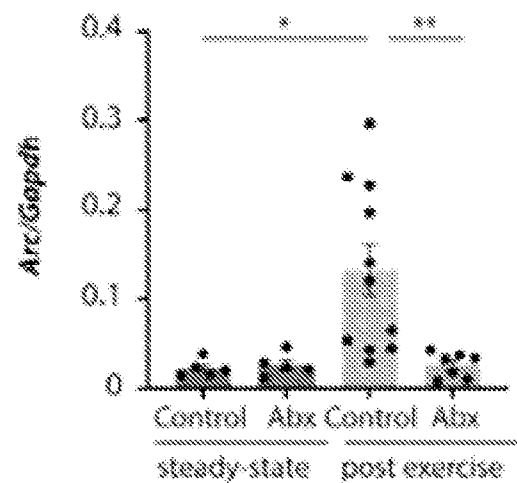


FIG. 13H

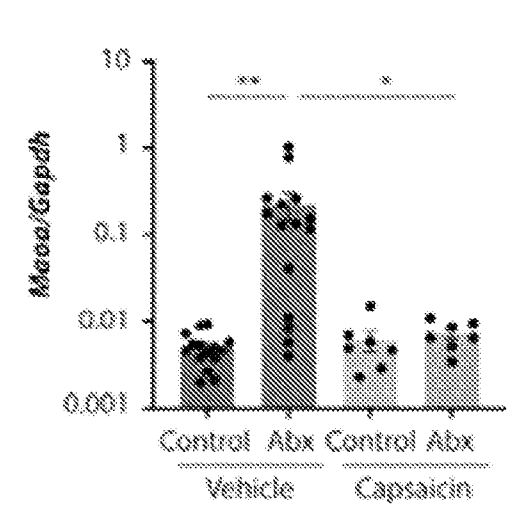


FIG. 13I

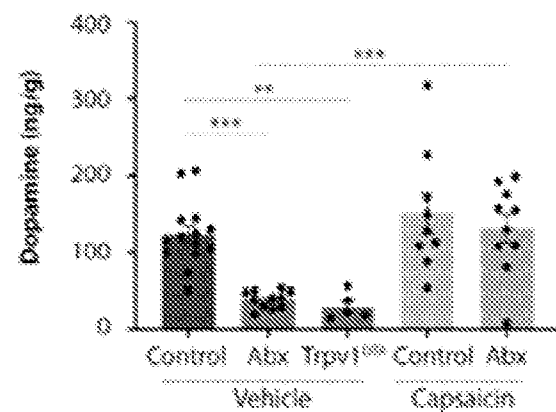


FIG. 13J

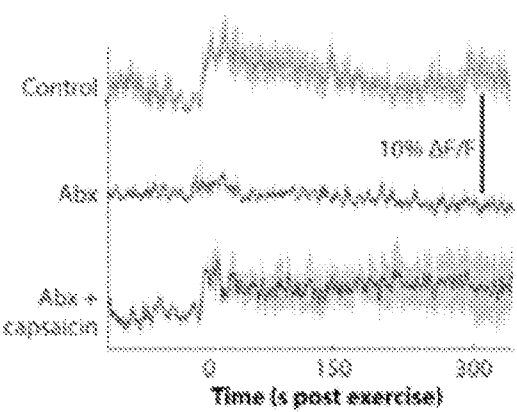


FIG. 13K

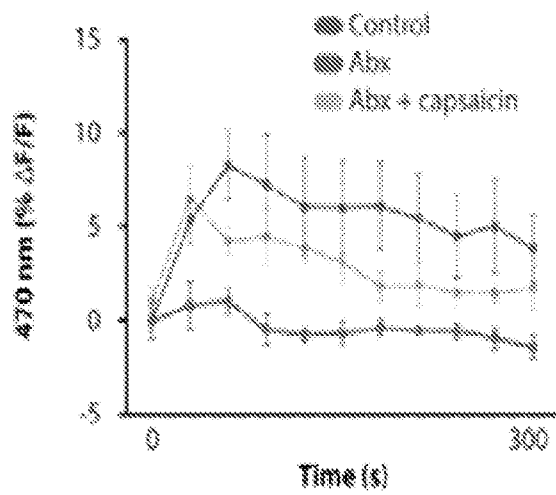


FIG. 13L

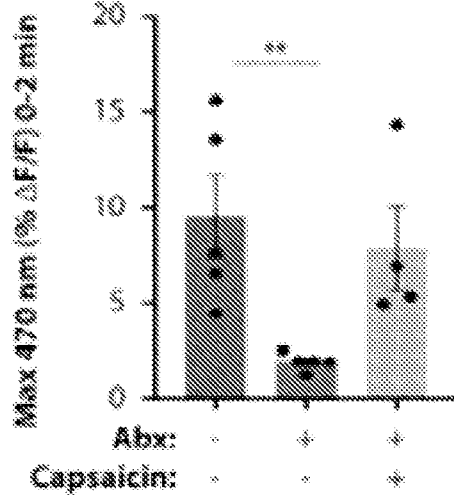


FIG. 13M

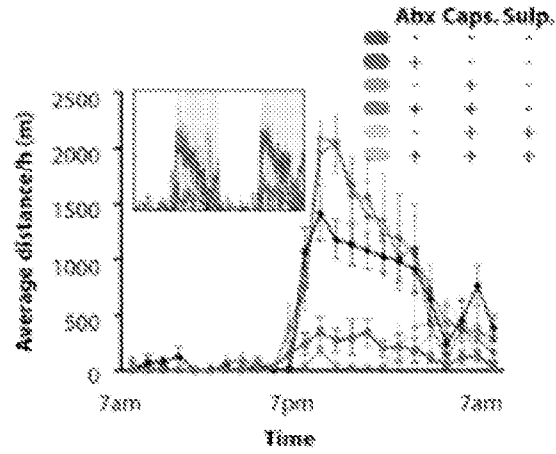


FIG. 13N

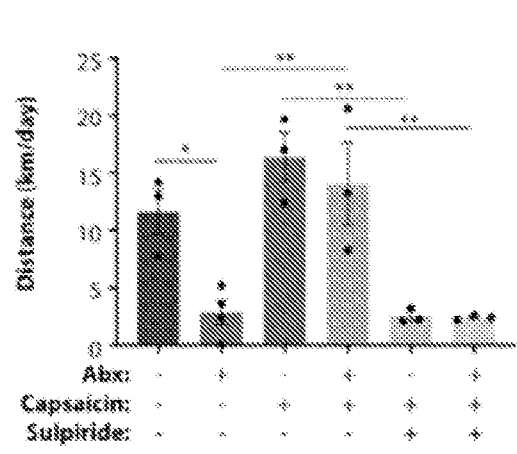


FIG. 13O

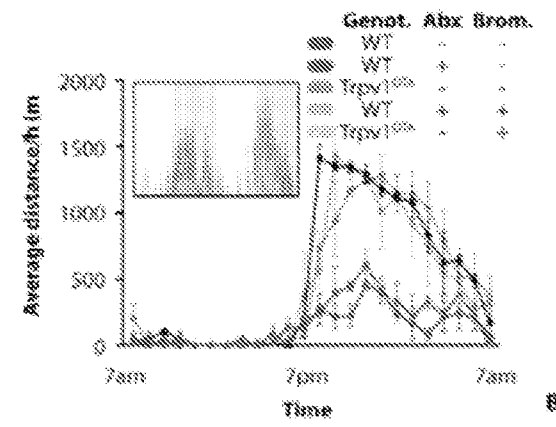


FIG. 13P

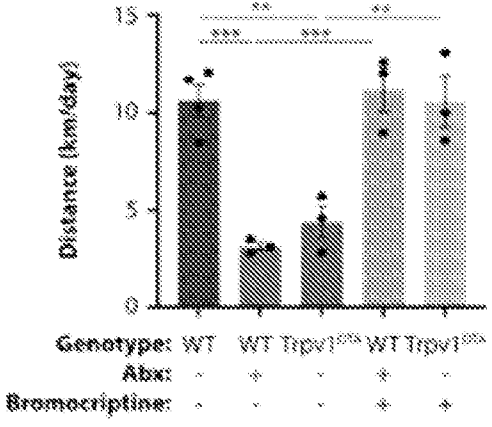


FIG. 14A

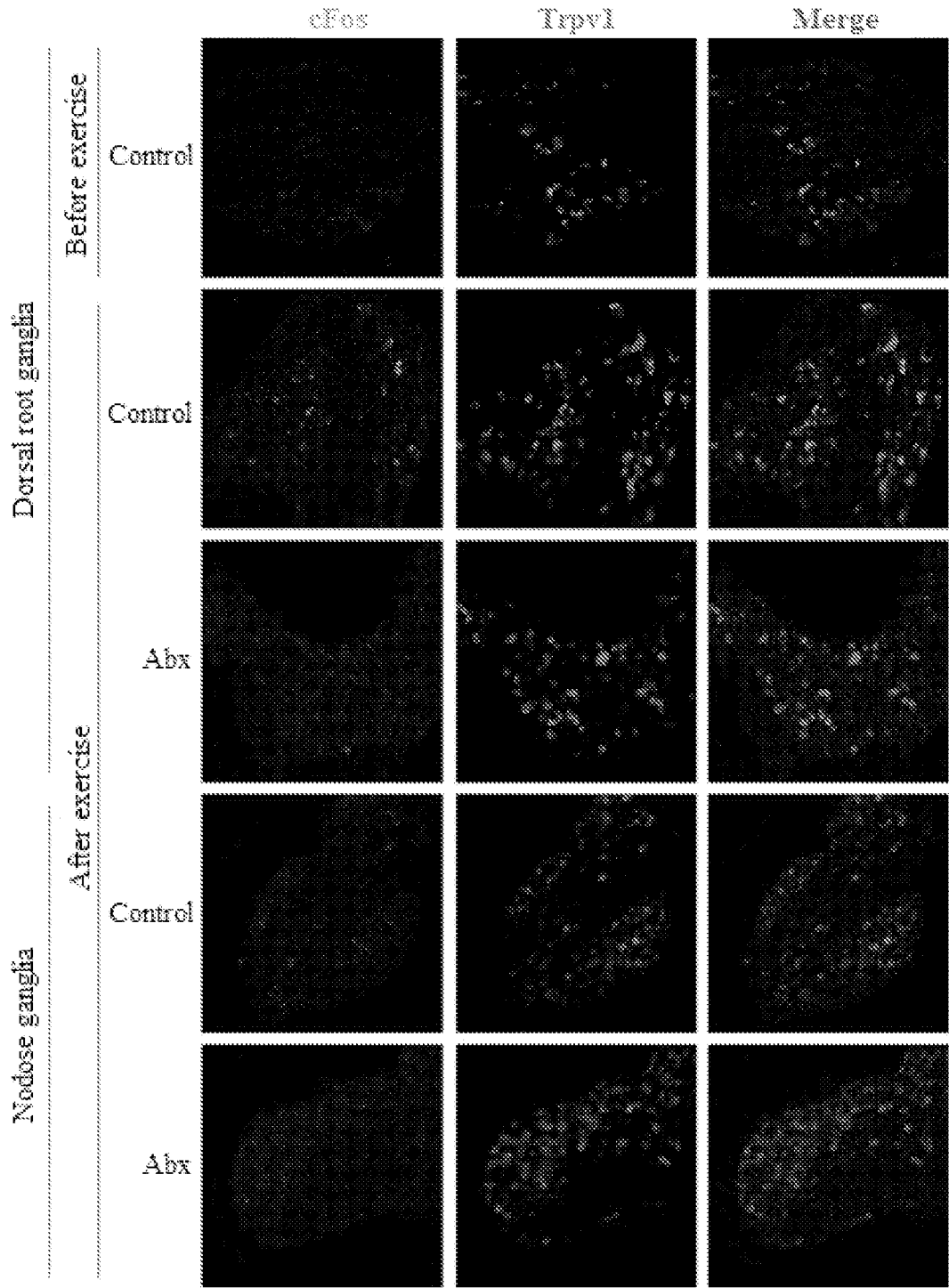


FIG. 14B

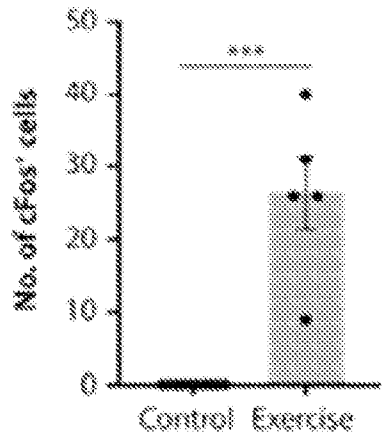


FIG. 14C

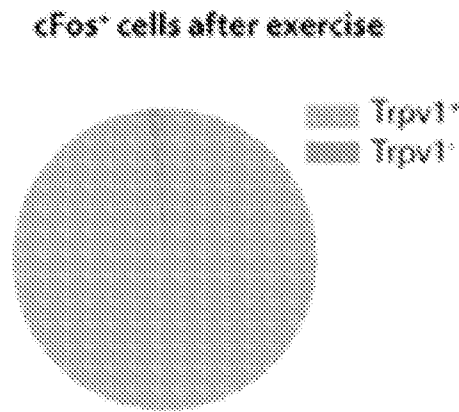


FIG. 14D

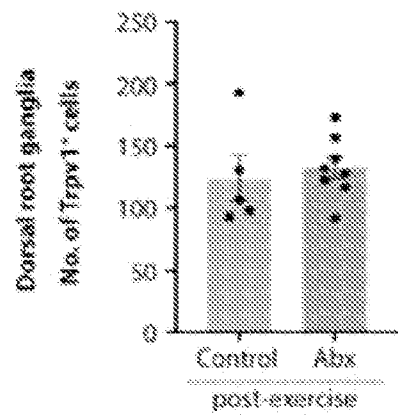


FIG. 14E

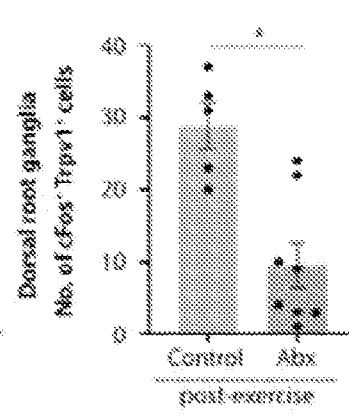


FIG. 14F

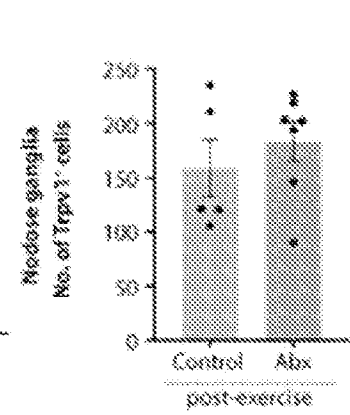


FIG. 14G

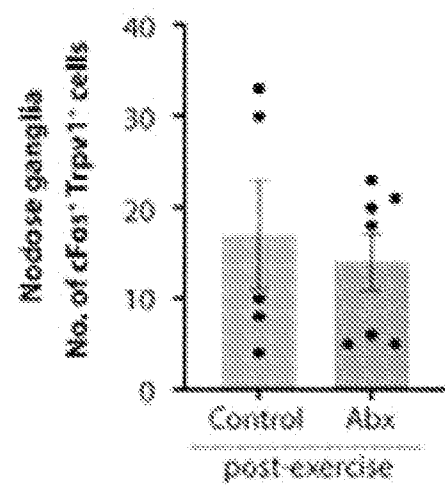


FIG. 14H

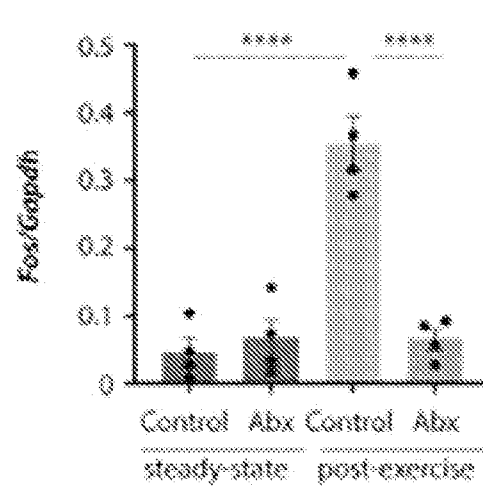


FIG. 14I

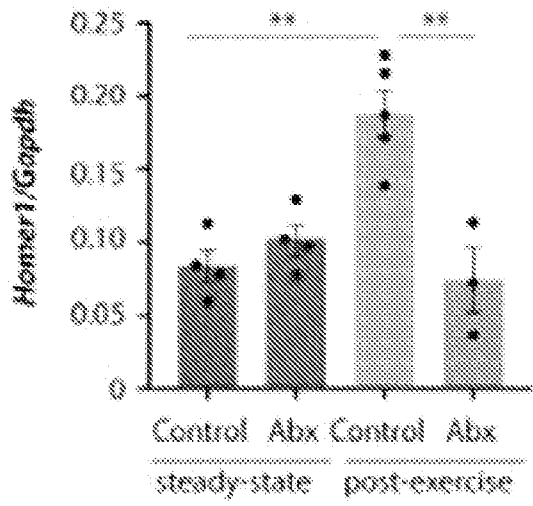


FIG. 15A

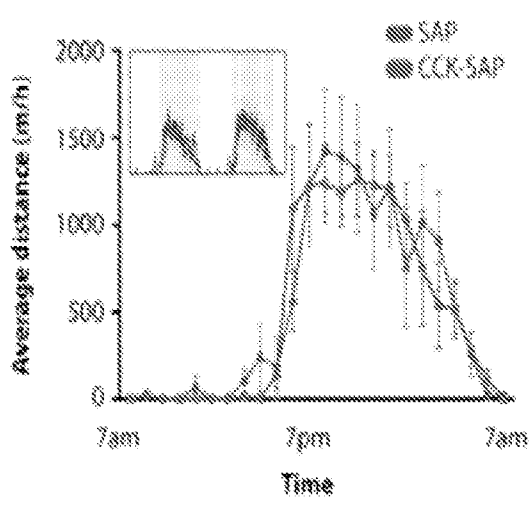


FIG. 15B

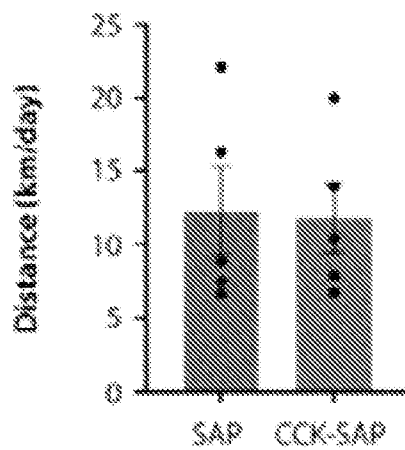


FIG. 15C

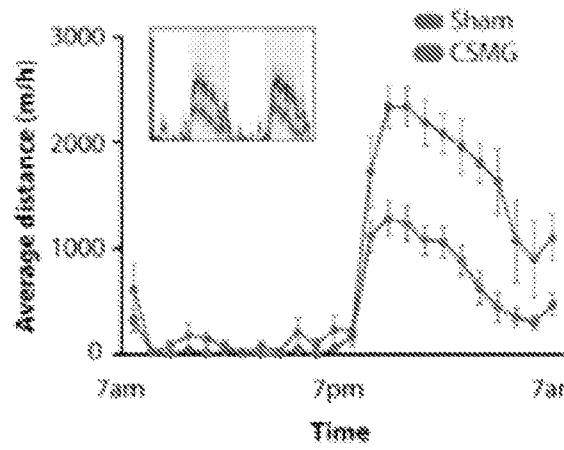


FIG. 15D

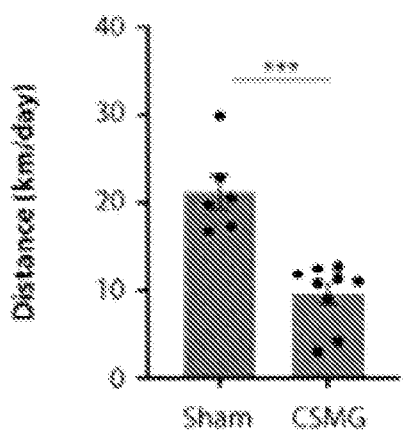


FIG. 15E

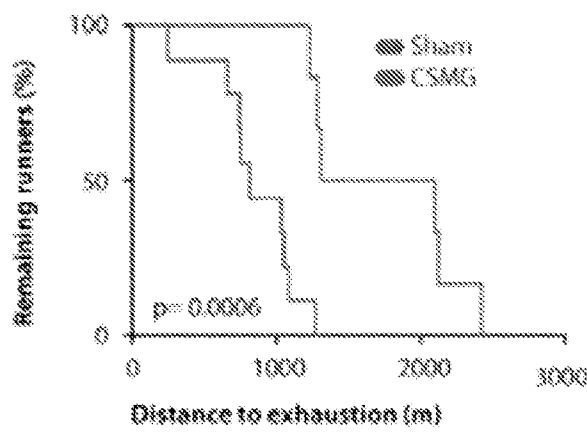


FIG. 15F

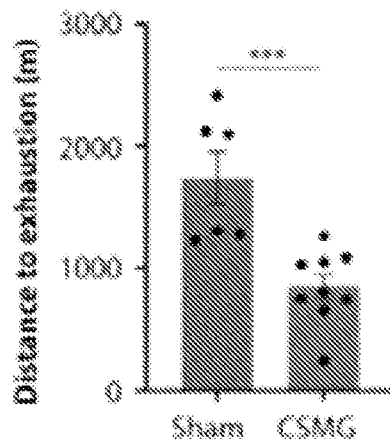


FIG. 15G

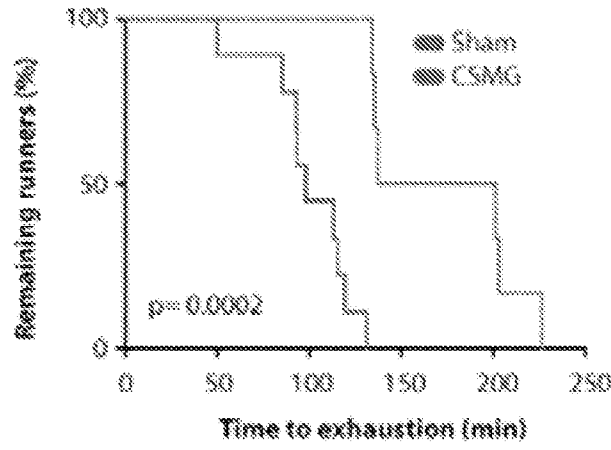


FIG. 15H

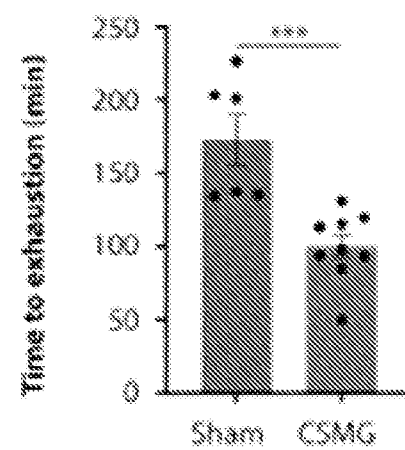


FIG. 15I

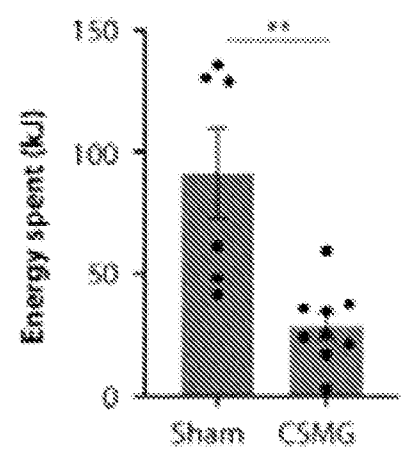


FIG. 15J

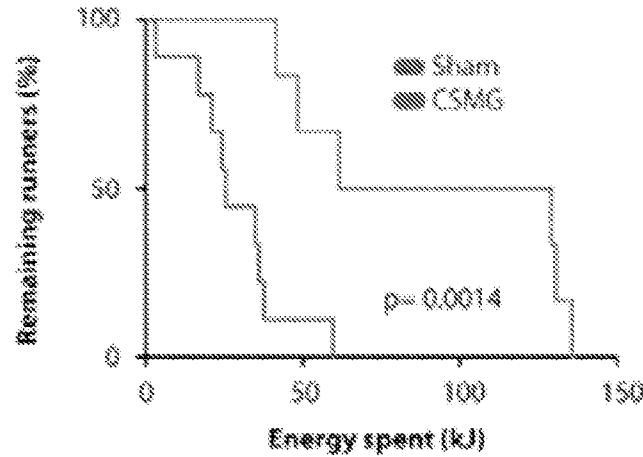


FIG. 15K

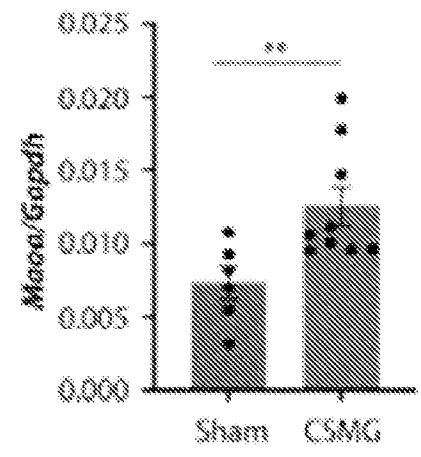


FIG. 15L

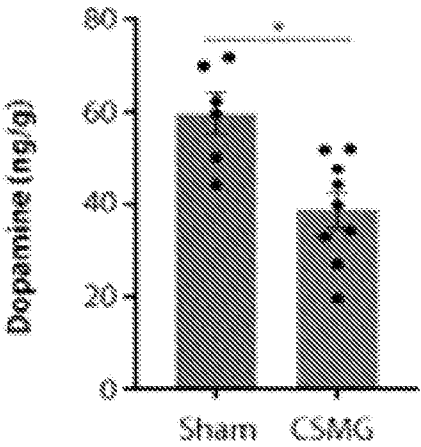


FIG. 15M

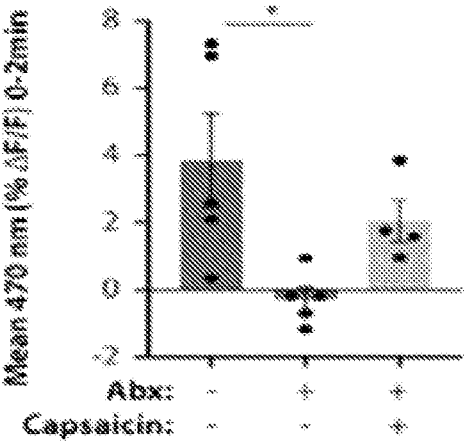


FIG. 15N

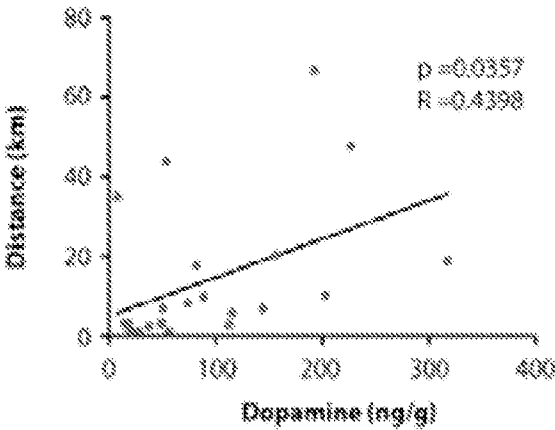


FIG. 16A

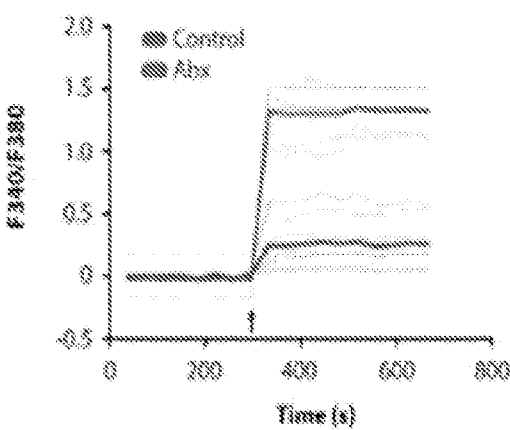


FIG. 16B

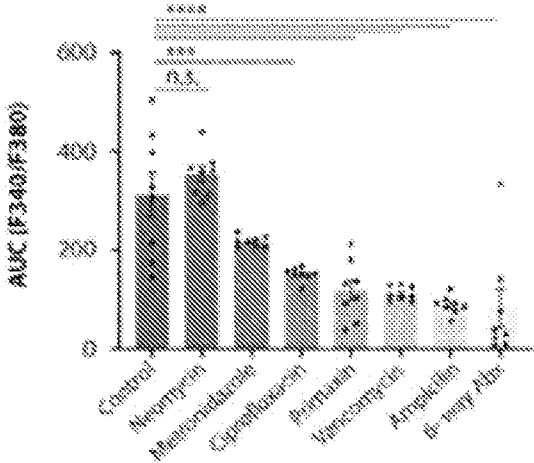


FIG. 16C

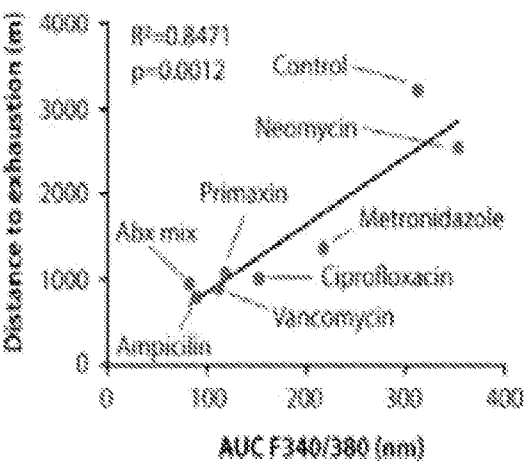


FIG. 16D

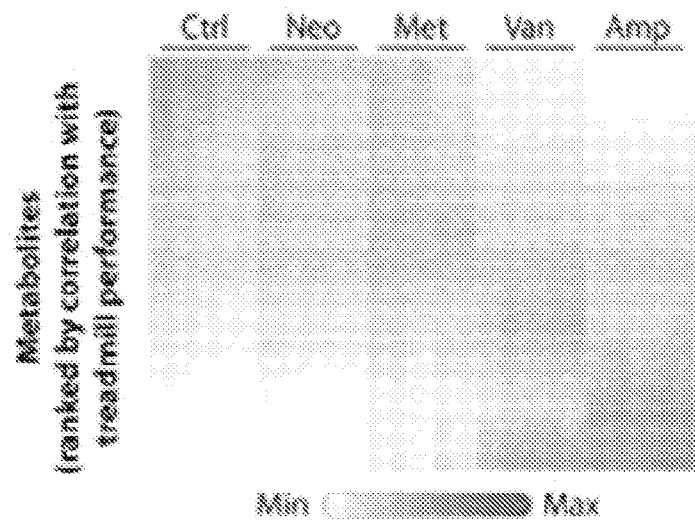


FIG. 16E

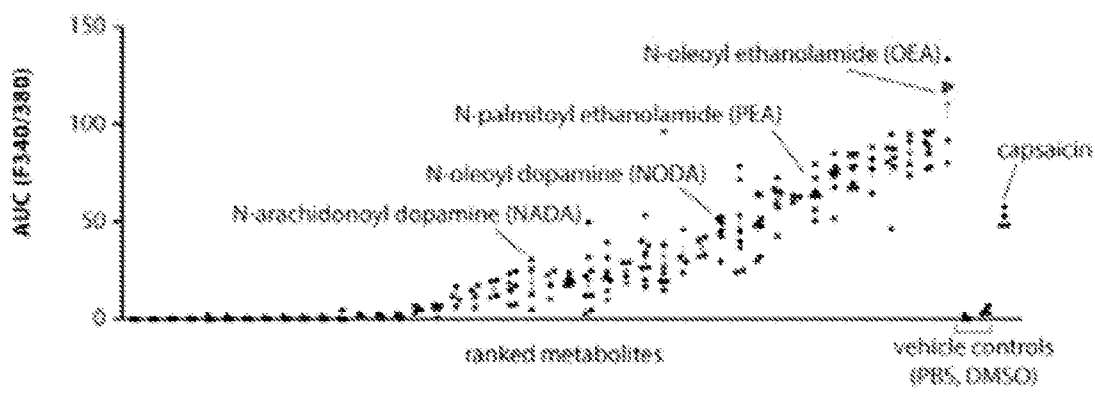


FIG. 16F

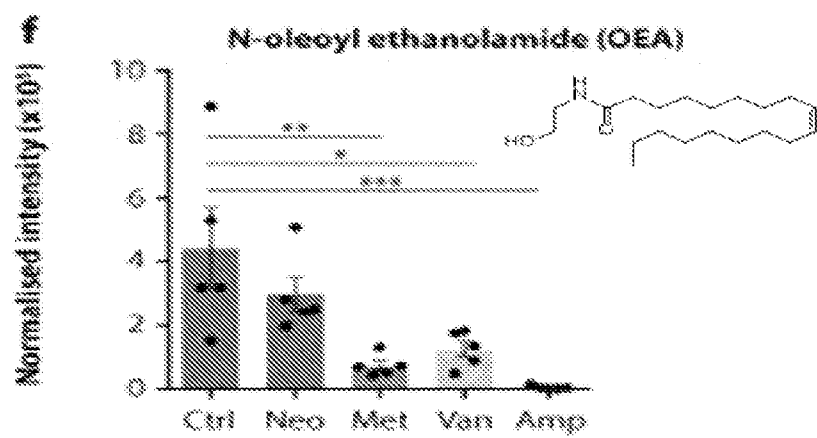


FIG. 16G

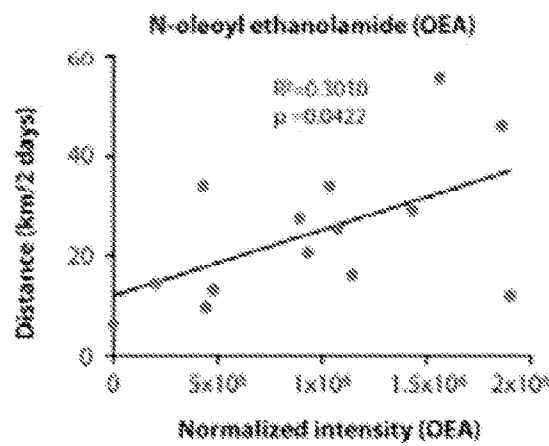


FIG. 16H

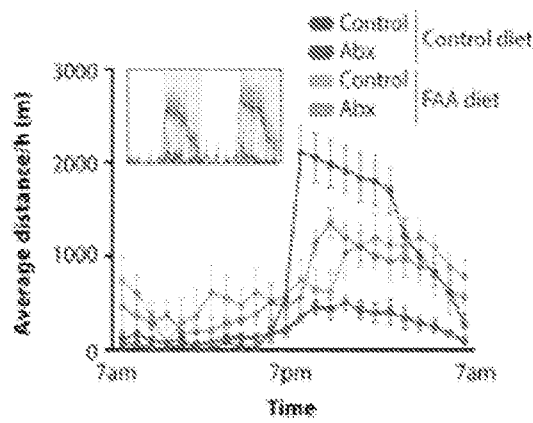


FIG. 16I

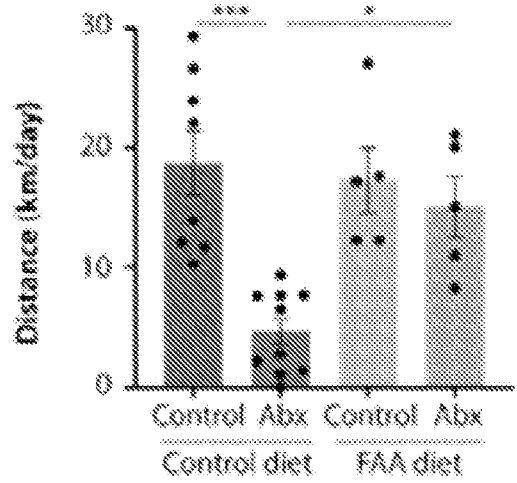


FIG. 16J

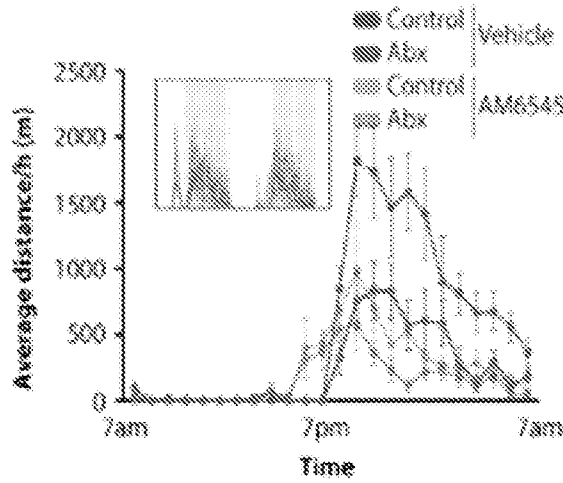


FIG. 16K

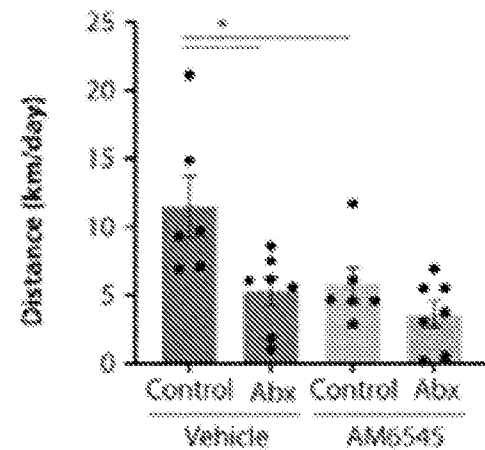


FIG. 16L

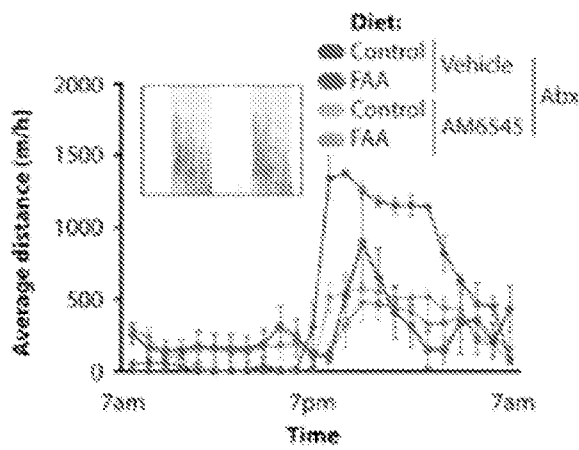


FIG. 16M

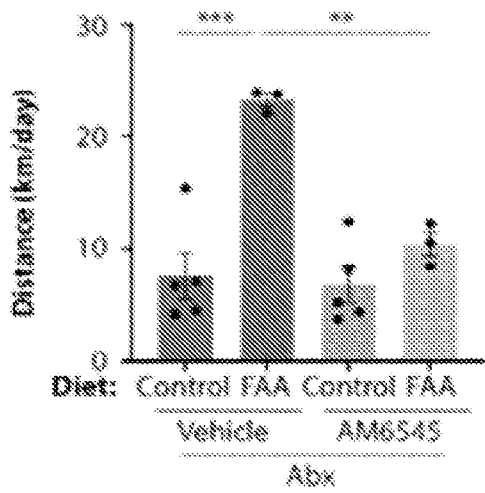


FIG. 16N

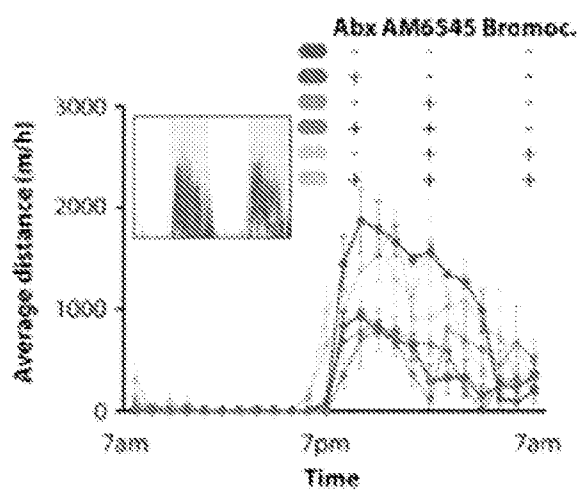


FIG. 16O

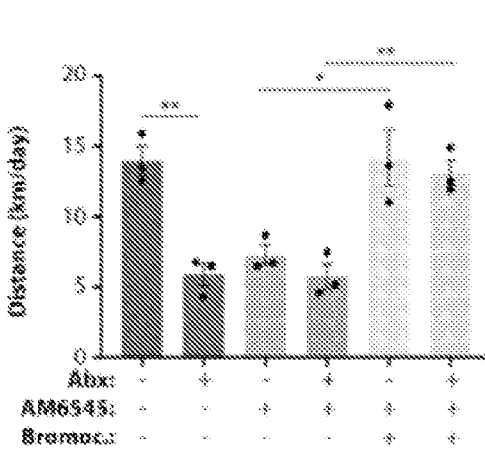


FIG. 17A

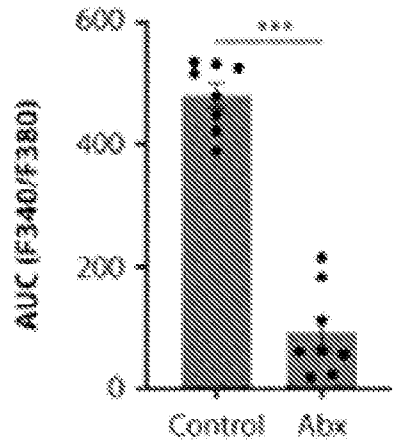


FIG. 17B

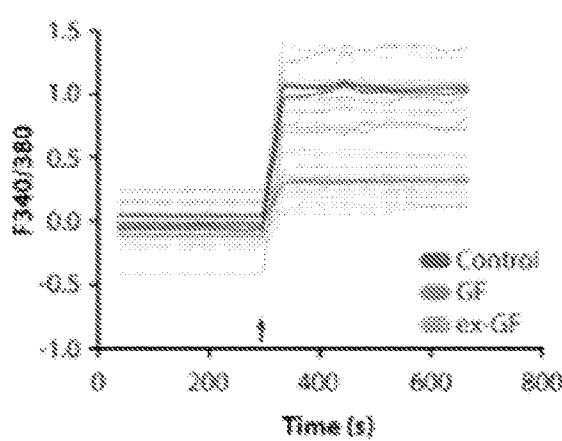


FIG. 17C

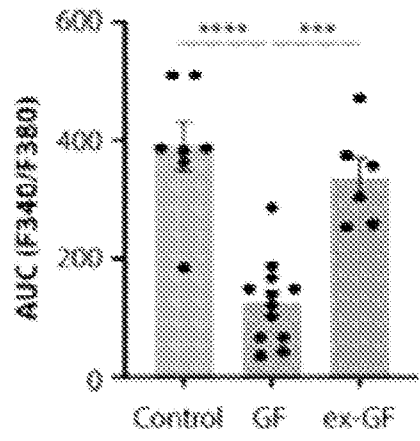


FIG. 17D

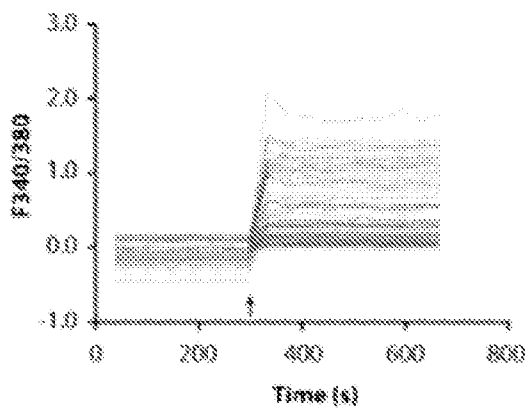


FIG. 17E

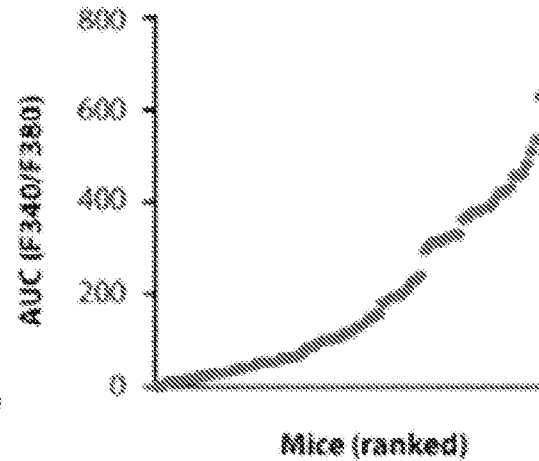


FIG. 17F

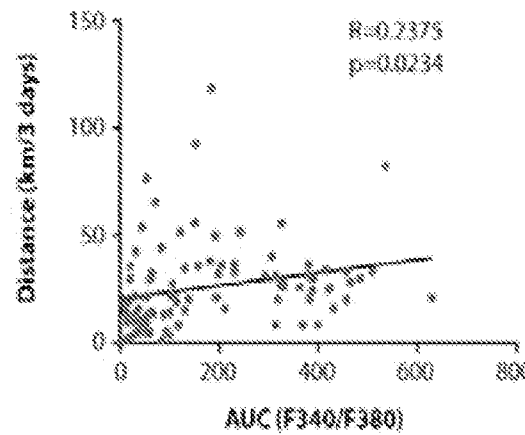


FIG. 17G

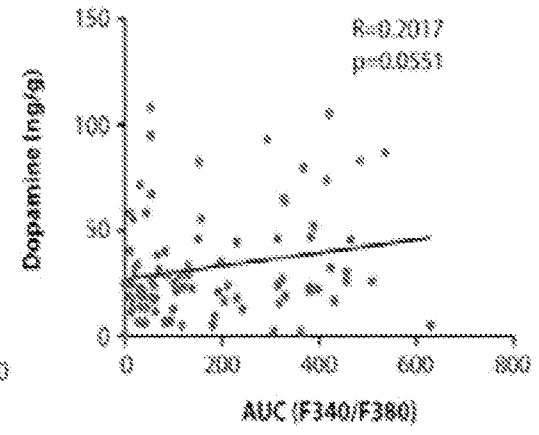


FIG. 17H

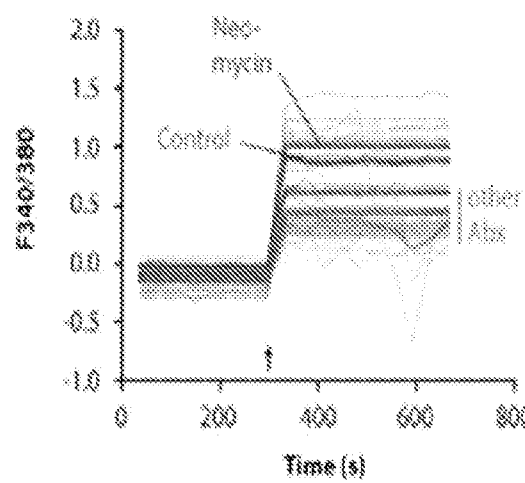


FIG. 17I

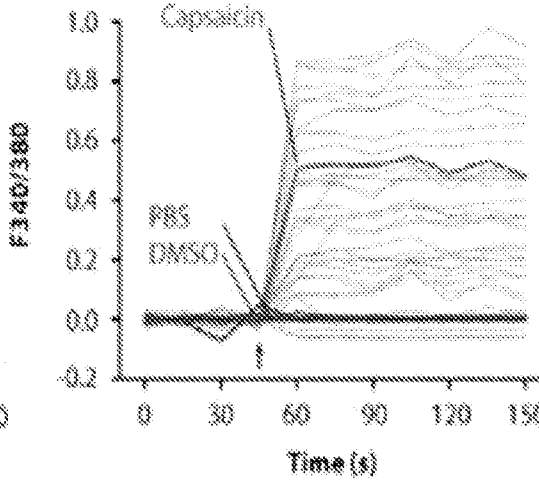


FIG. 17J

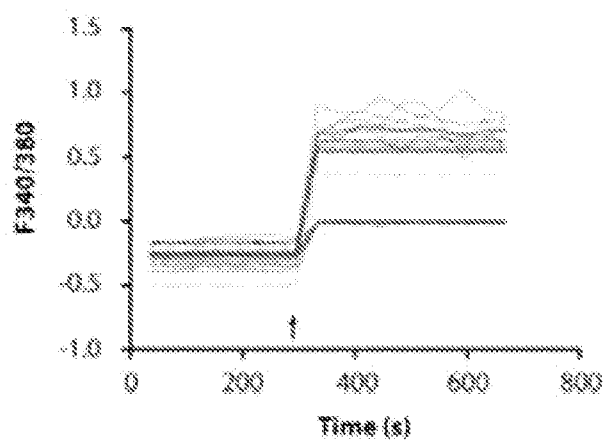


FIG. 17K

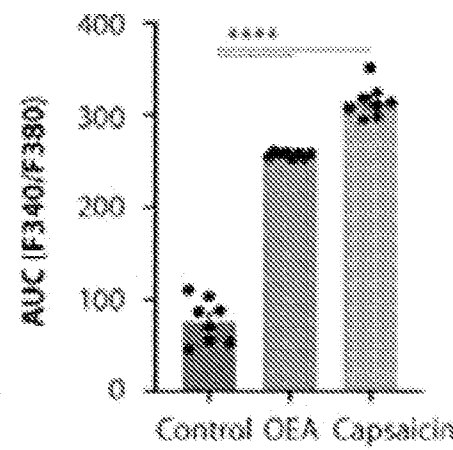


FIG. 18A

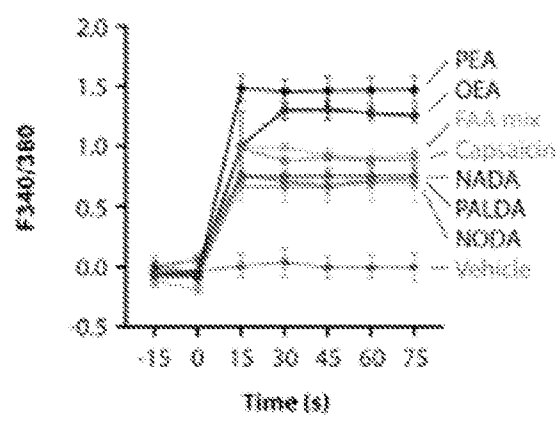


FIG. 18B

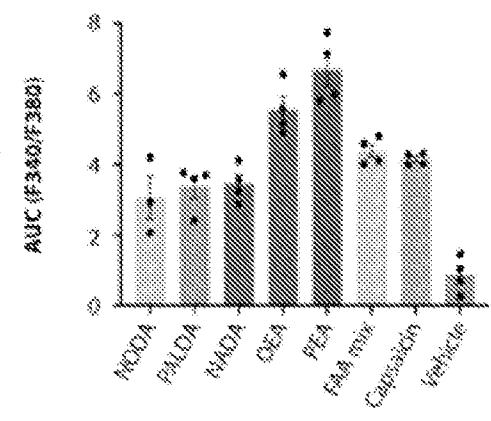


FIG. 18C

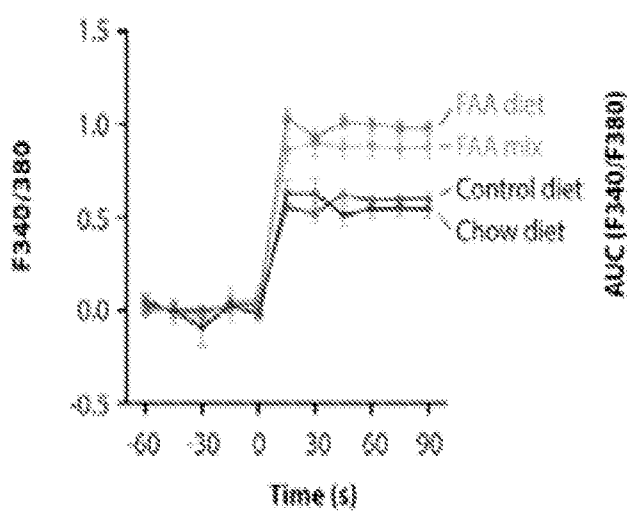


FIG. 18D

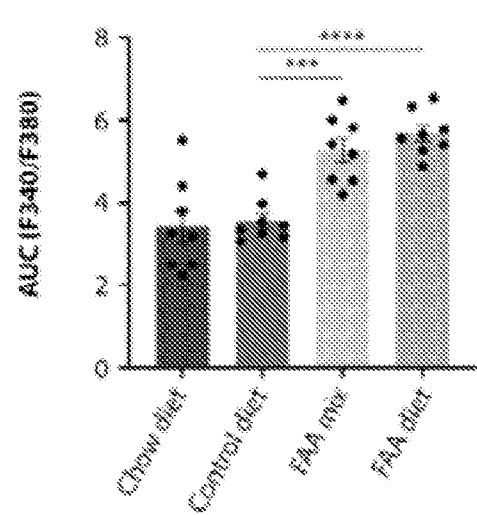


FIG. 18E

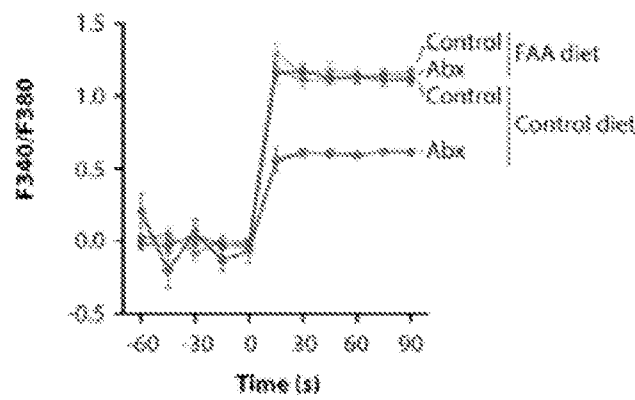


FIG. 18F

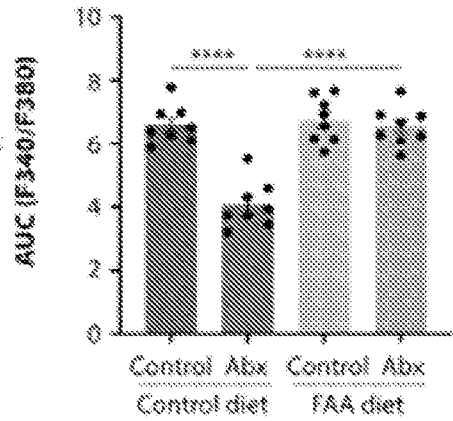


FIG. 18G

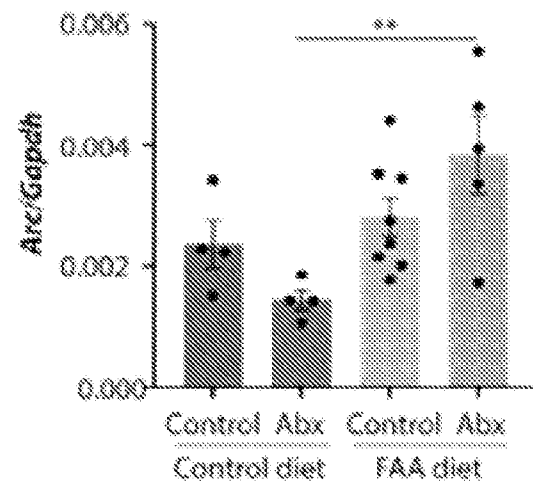


FIG. 18H

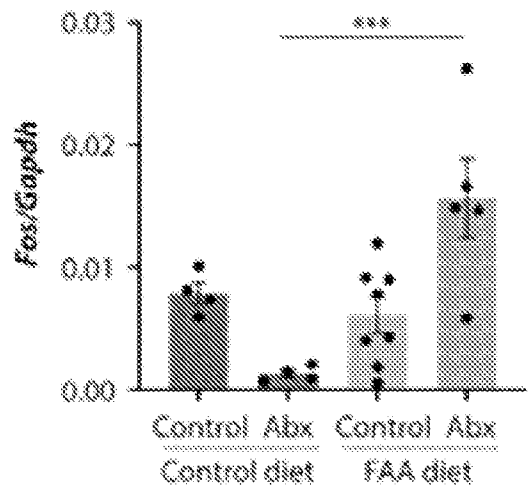


FIG. 18I

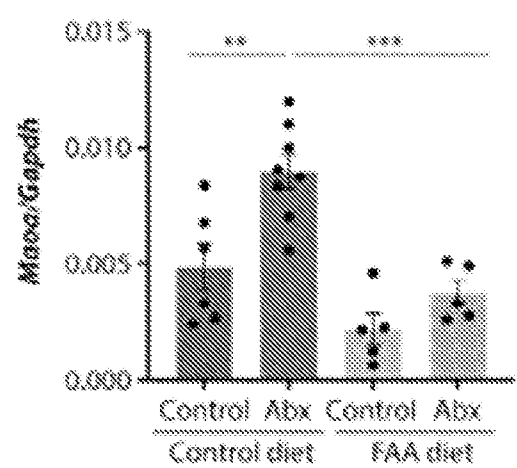


FIG. 18J

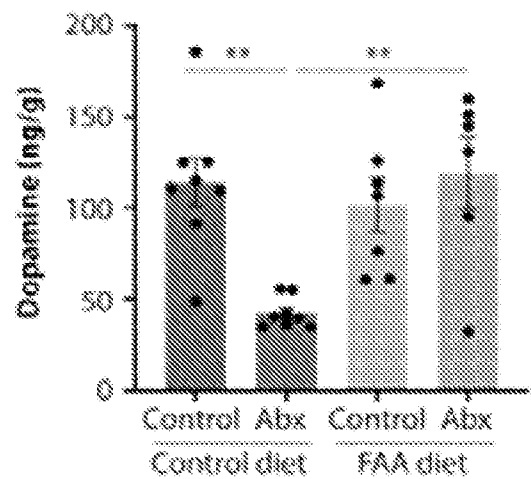


FIG. 18K

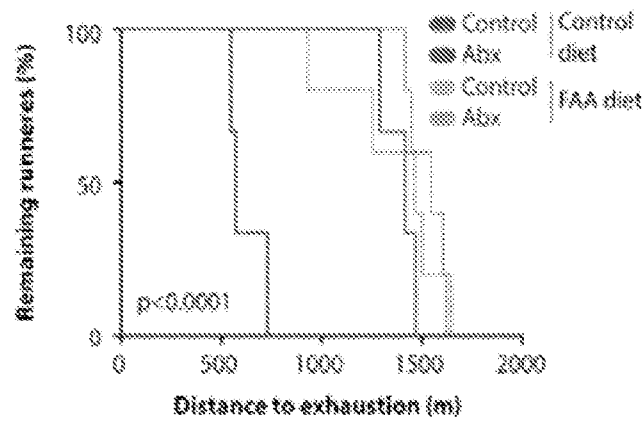


FIG. 18L

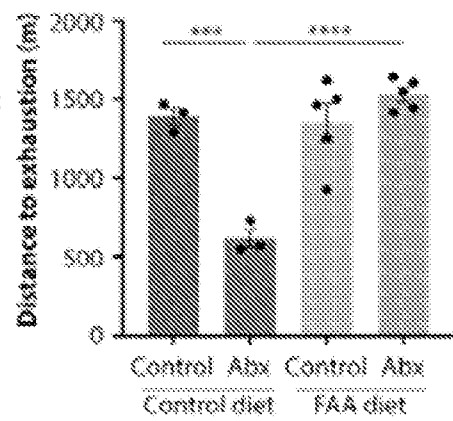


FIG. 18M

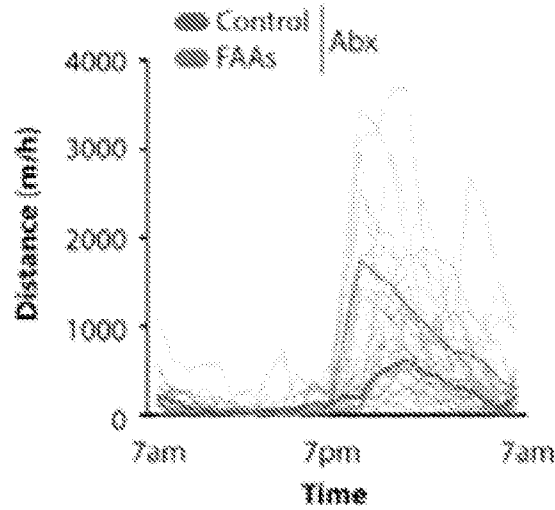


FIG. 18N

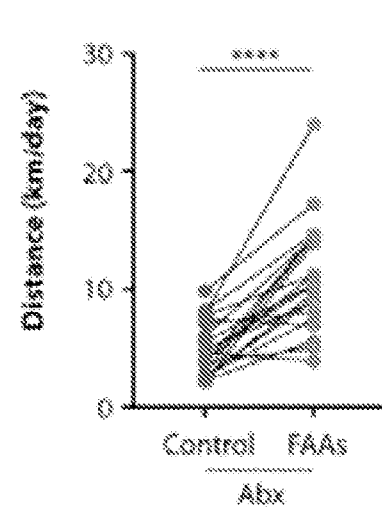


FIG. 18O

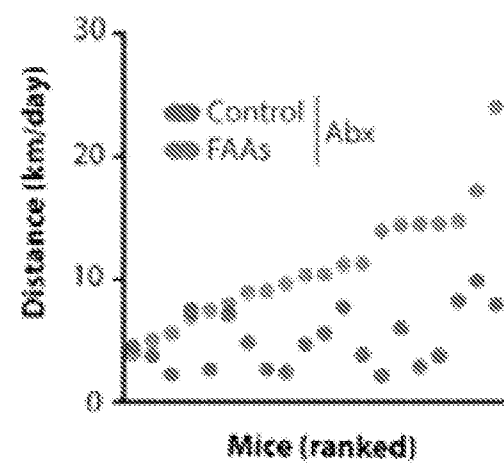


FIG. 18P

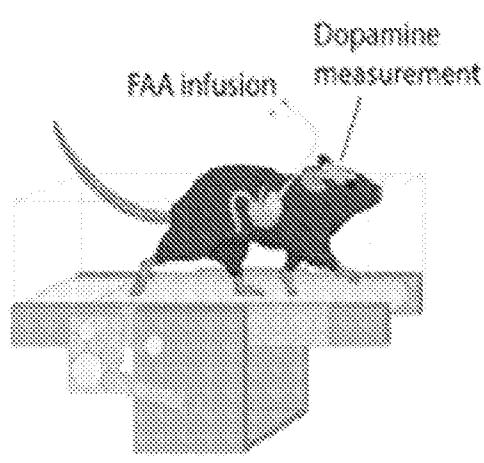


FIG. 18Q

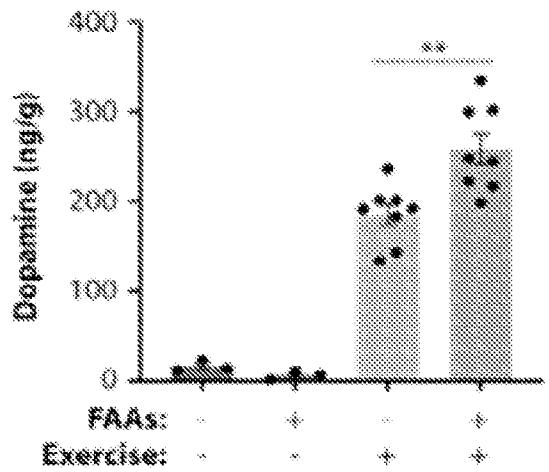


FIG. 19A

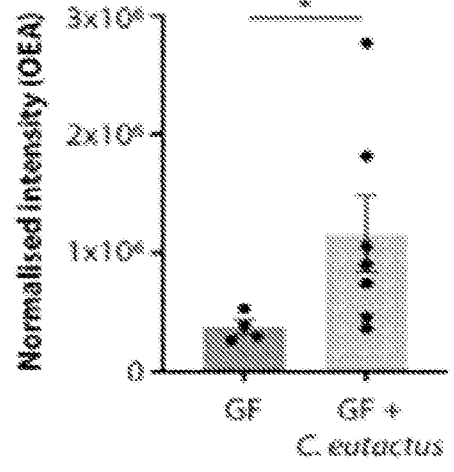


FIG. 19B

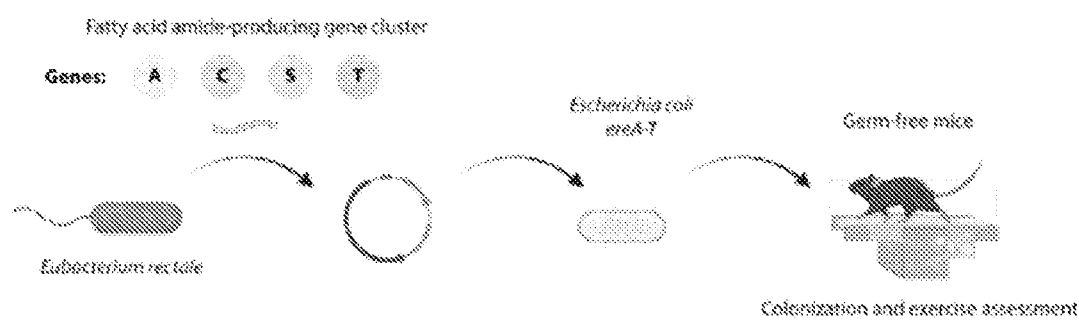


FIG. 19C

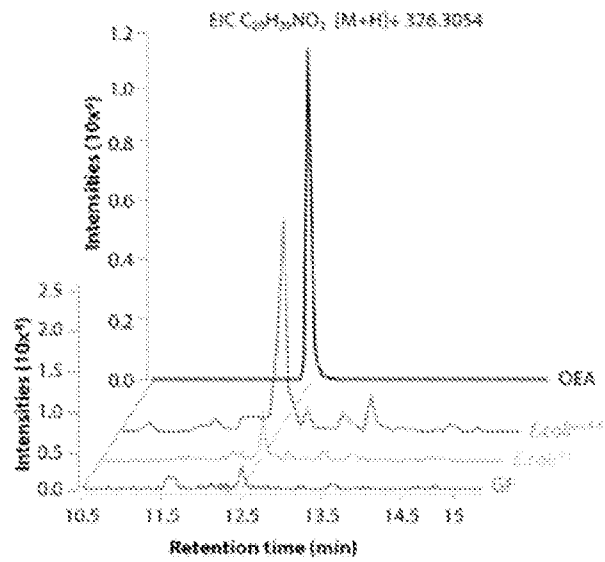


FIG. 19D

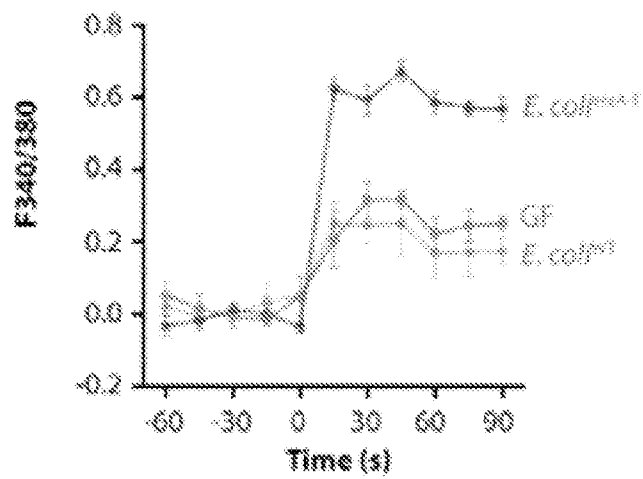


FIG. 19E

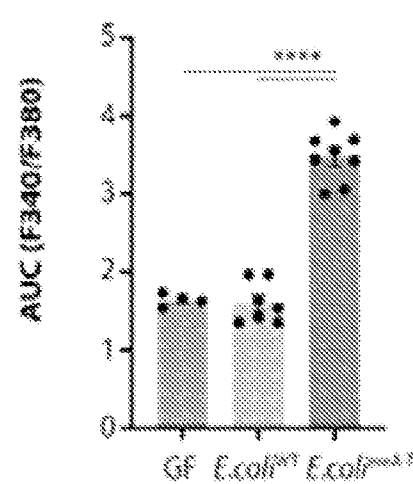


FIG. 19F

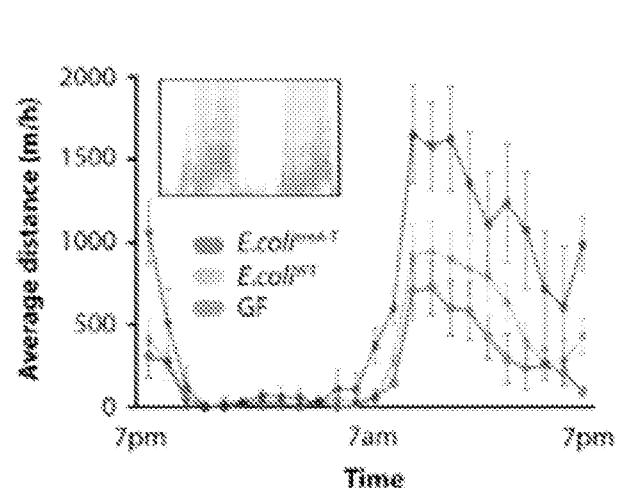


FIG. 19G

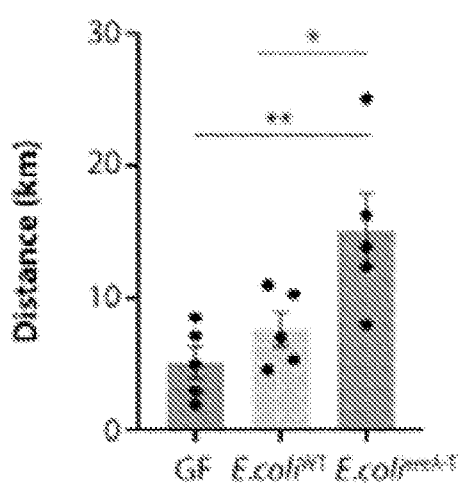


FIG. 19H

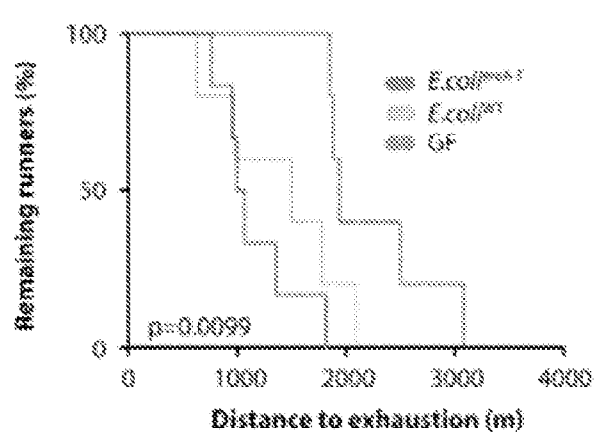


FIG. 19I

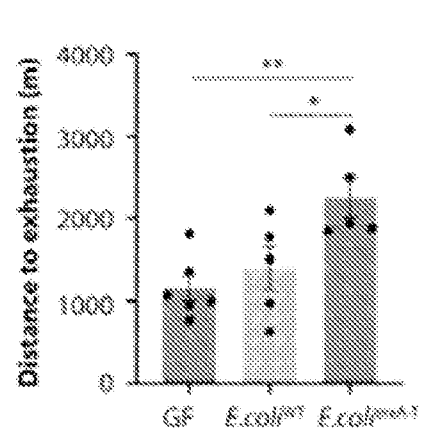


**FIG. 19J**

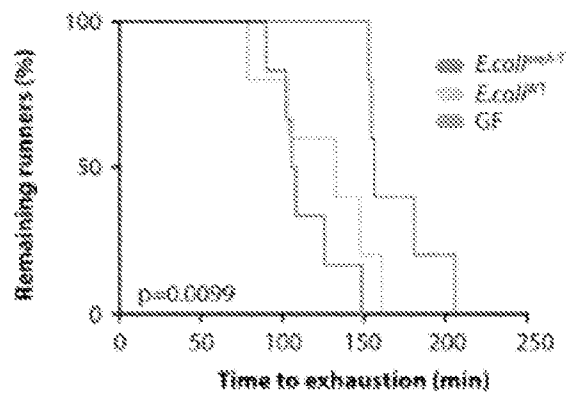


**FIG. 19K**

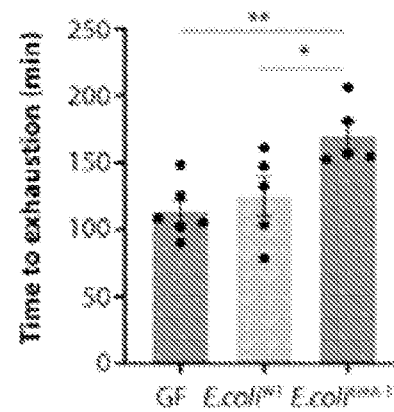


**FIG. 20A**

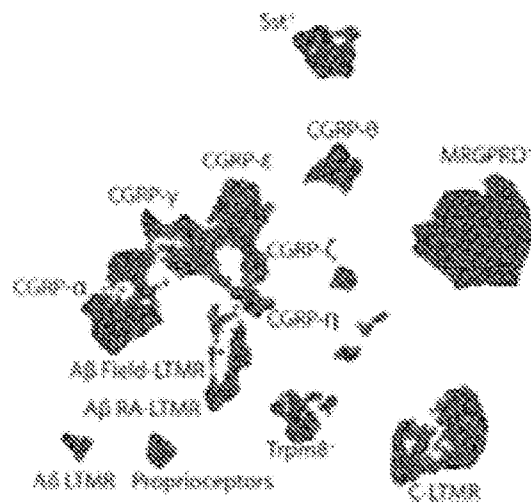


**FIG. 20B**

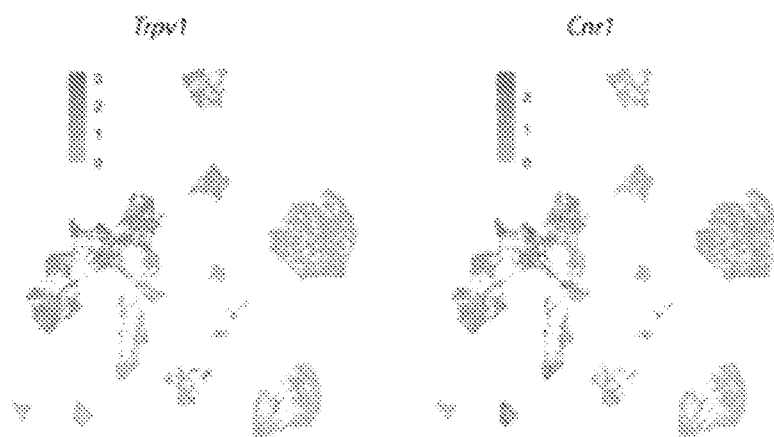


FIG. 20C

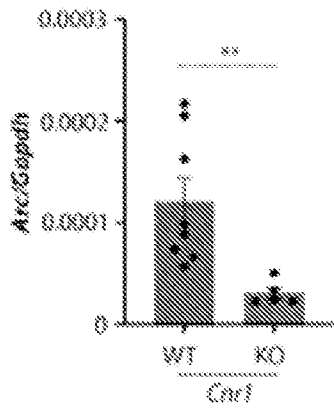


FIG. 20D

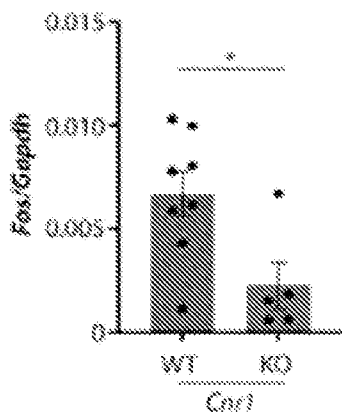


FIG. 20E

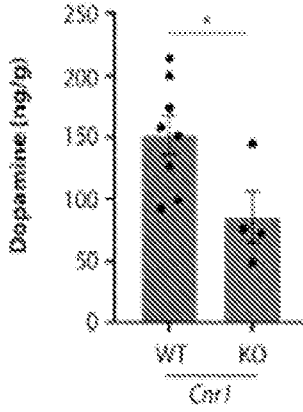


FIG. 20F

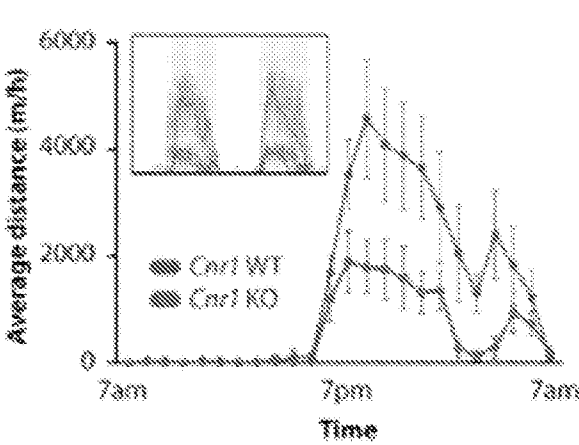


FIG. 20G

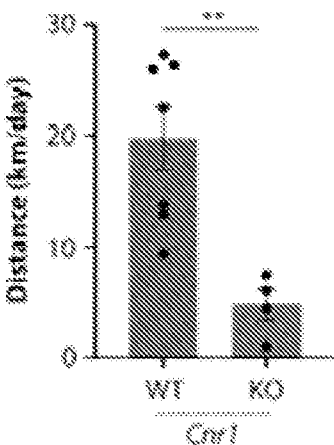


FIG. 20H

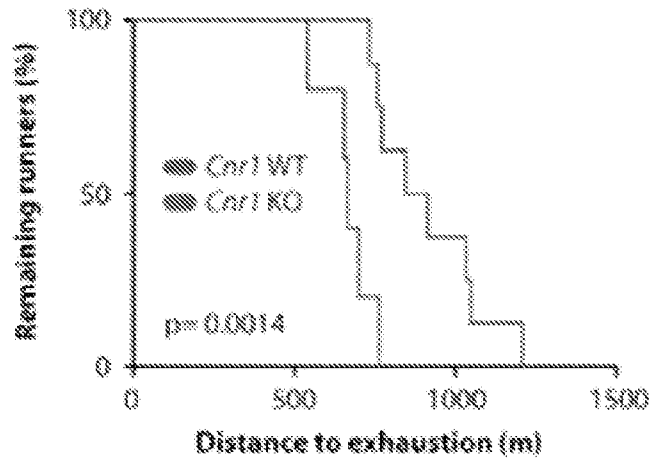


FIG. 20I

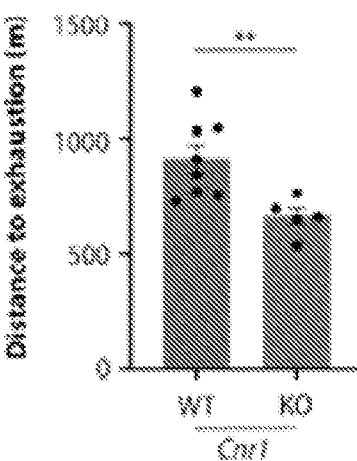


FIG. 20J

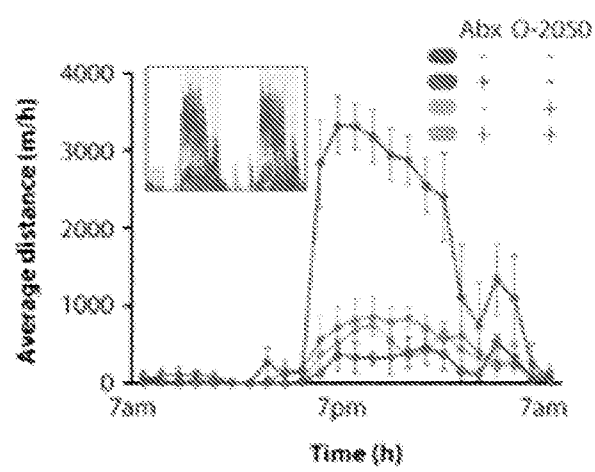


FIG. 20K

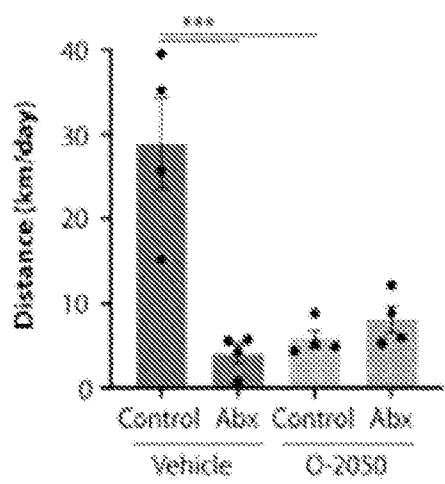


FIG. 20L

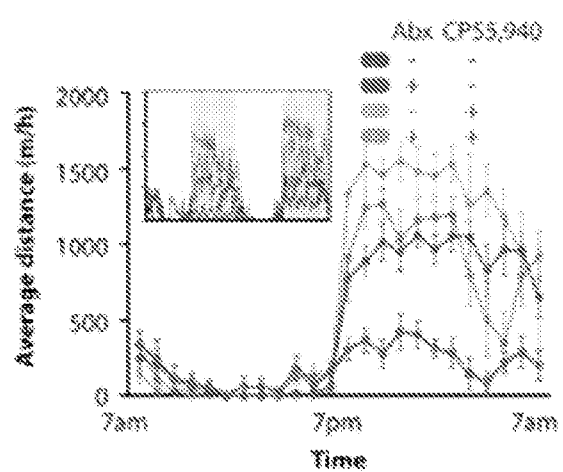


FIG. 20M

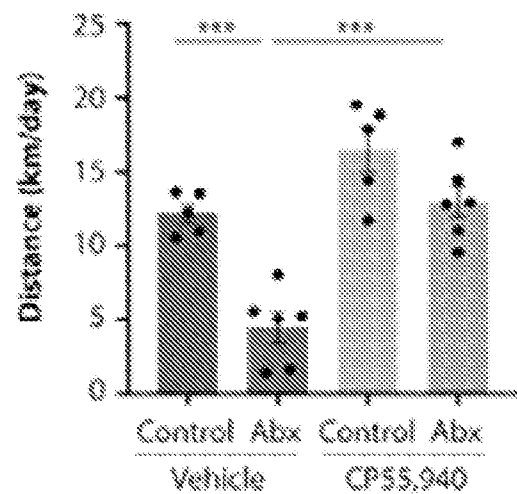


FIG. 20N

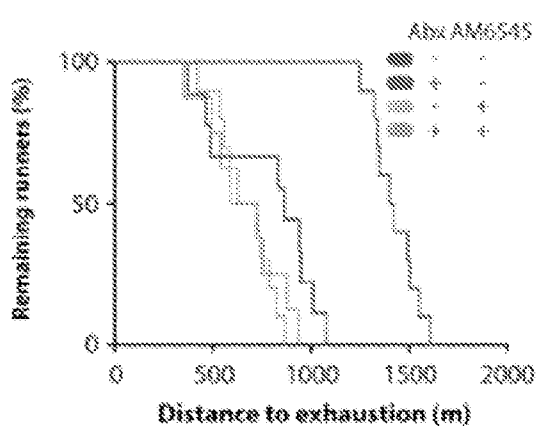


FIG. 20O

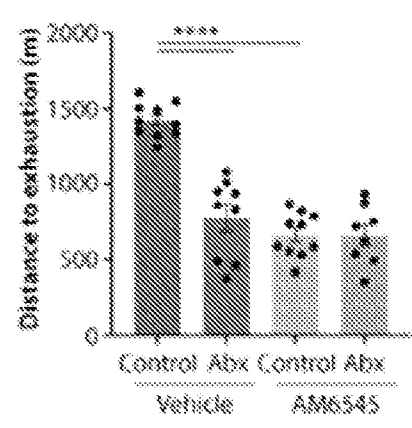


FIG. 20P

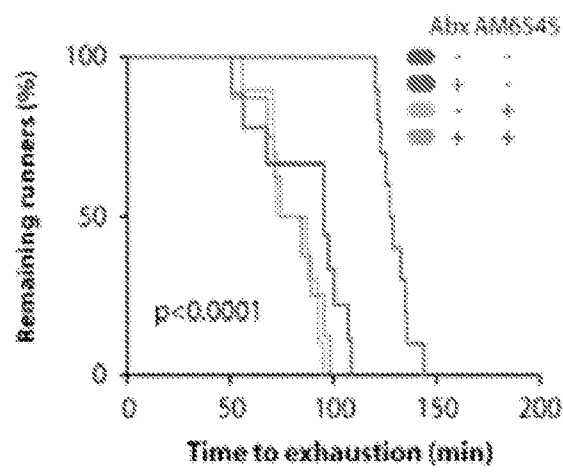


FIG. 20Q

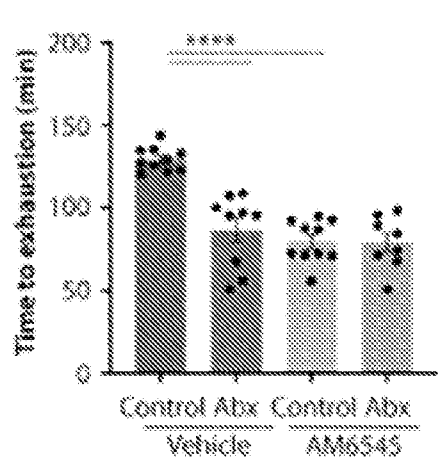


FIG. 21A

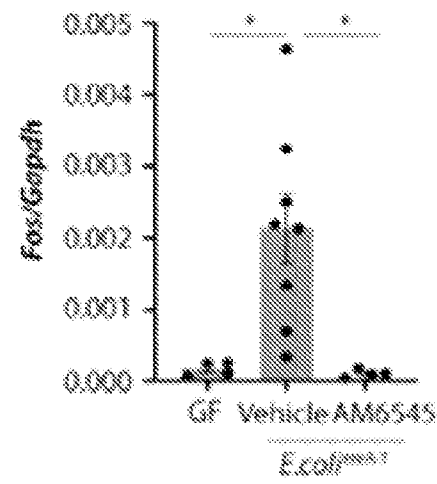


FIG. 21B

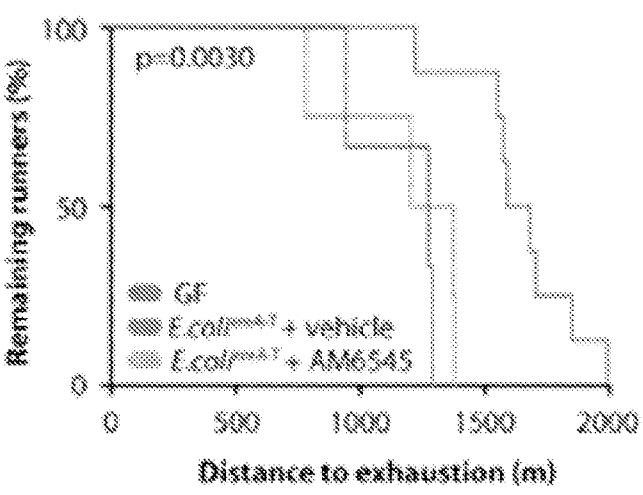


FIG. 21C

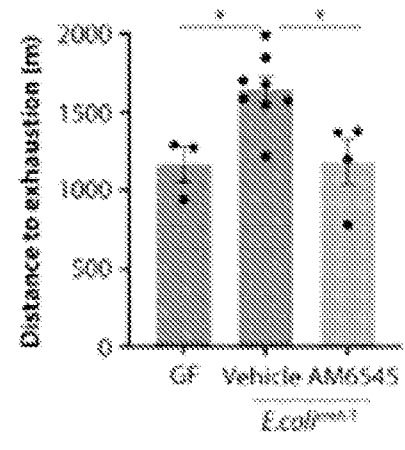


FIG. 21D

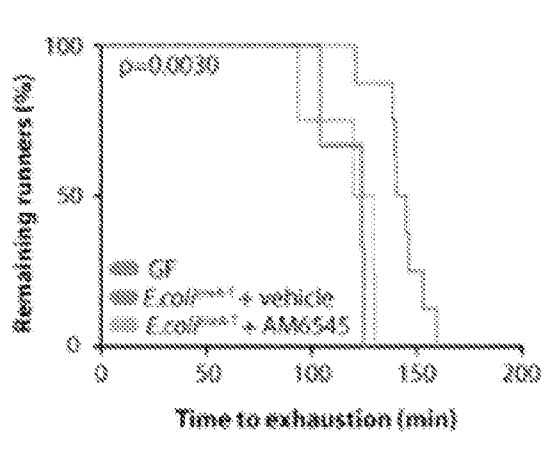


FIG. 21E

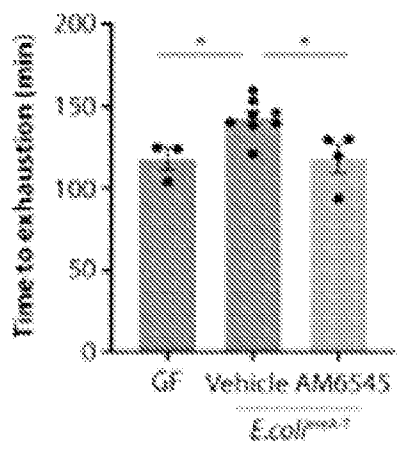


FIG. 21F

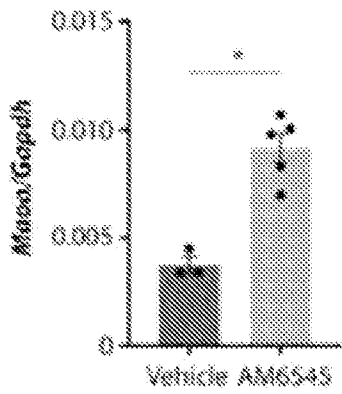


FIG. 21G

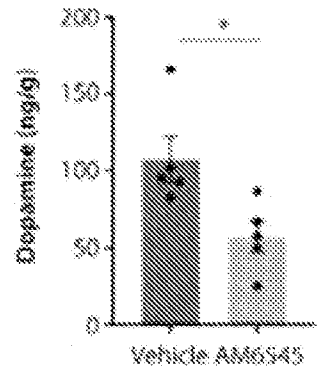


FIG. 21H

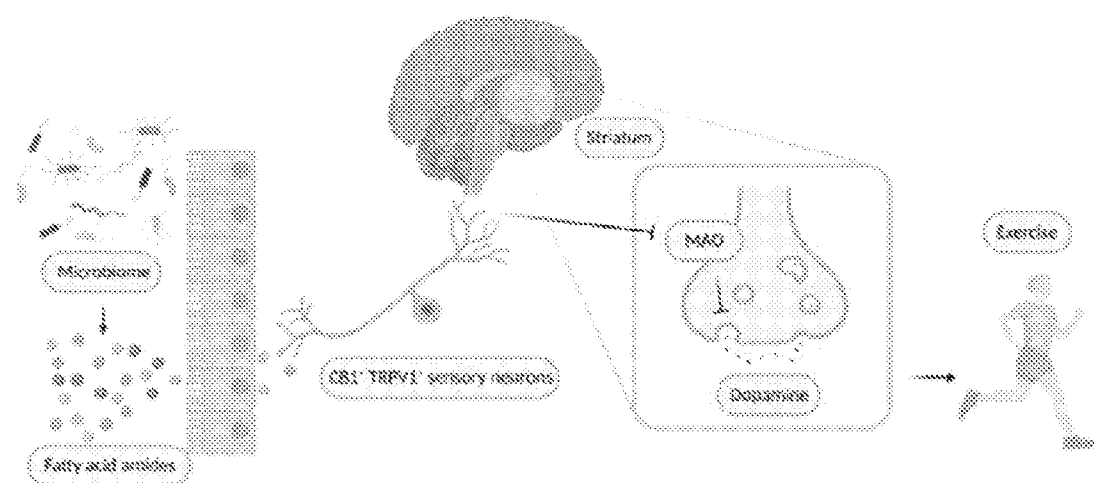


FIG. 21I

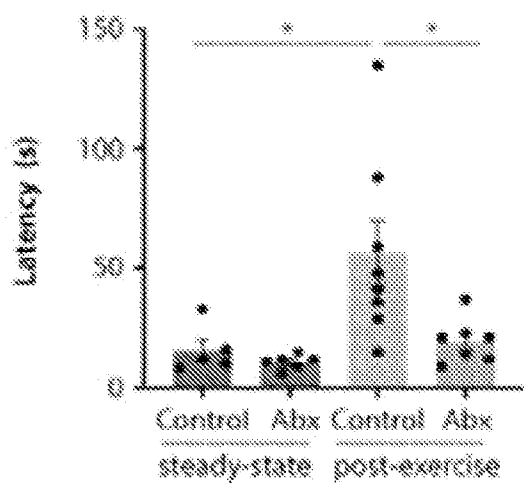
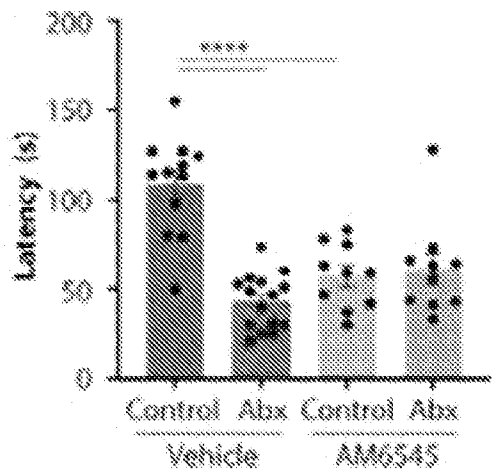


FIG. 21J



## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 23/83183

A. CLASSIFICATION OF SUBJECT MATTER  
IPC - INV. A61K 31/16, A23K 20/158 (2024.01)  
ADD. A23L 33/12 (2024.01)

CPC - INV. A61K 31/16, A23K 20/158

ADD. A23L 33/12

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
See Search History document

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                            | Relevant to claim No.      |
|-----------|-----------------------------------------------------------------------------------------------------------------------------------------------|----------------------------|
| X<br>---  | WO 2008/075978 A2 (Seperex Nutritionals Limited) 26 June 2008 (26.06.2008) pg10, ln3,5-6; pg18, ln1-2,21;                                     | 1, 4/1, 5, 8/5             |
| Y         |                                                                                                                                               | 2-3, 4/(2-3), 6-7, 8/(6-7) |
| Y         | US 2010/0130603 A1 (Takahashi) 27 May 2010 (27.05.2010) para [0024]                                                                           | 2-3, 4/(2-3), 6-7, 8/(6-7) |
| A         | US 8,323,680 B1 (Berger et al.) 4-December 2012 (04.12.2012) entire document especially abstract                                              | 1-8                        |
| A         | US 2003/0018081 A1 (Piomelli et al.) 23 January 2003 (23.01.2003) entire document                                                             | 1-8                        |
| A         | WO 2007/047575 A2 (The Board Of Trustees Of The University Of Illinois) 26 April 2007 (26.04.2007) entire document                            | 1-8                        |
| A         | US 2011/0046225 A1 (Vermont Italia Srl) 24 February 2011 (24.02.2011) entire document                                                         | 1-8                        |
| A         | Kooten et al. Fatty acid amide supplementation decreases impulsivity in young adult heavy drinkers. Physiol Behav. 2016 March 1; 155: 131-140 | 1-8                        |

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

|                                                                                                                                                                         |                                                                                                                                                                                                                                                  |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| * Special categories of cited documents:                                                                                                                                | "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention                                              |
| "A" document defining the general state of the art which is not considered to be of particular relevance                                                                | "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone                                                                     |
| "D" document cited by the applicant in the international application                                                                                                    | "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| "E" earlier application or patent but published on or after the international filing date                                                                               | "&" document member of the same patent family                                                                                                                                                                                                    |
| "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) |                                                                                                                                                                                                                                                  |
| "O" document referring to an oral disclosure, use, exhibition or other means                                                                                            |                                                                                                                                                                                                                                                  |
| "P" document published prior to the international filing date but later than the priority date claimed                                                                  |                                                                                                                                                                                                                                                  |

Date of the actual completion of the international search

14 February 2024

Date of mailing of the international search report

**APR 22 2024**

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents  
P.O. Box.1450, Alexandria, Virginia 22313-1450  
Facsimile No. 571-273-8300

Authorized officer

Kari Rodriguez

Telephone No. PCT Helpdesk: 571-272-4300

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 23/83183

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

a. ☒ forming part of the international application as filed.

b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13ter.1(a)),

☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 23/83183

## Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 13-43  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:  
---see supplemental box---

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:  
claims 1-8

### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 23/83183

## Box III: lack of unity

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I: Claims 1-8, drawn to a dietary supplement composition comprising: (a) at least two fatty acid amides (FAAs) selected from the group consisting of N-stearoyl serotonin, N-arachidonoyl dopamine, N-oleoyl dopamine, N-palmitoyl dopamine, N-stearoyl dopamine, N-stearoyl taurine, N-arachidonoyl ethanolamide, N-palmitoyl ethanolamide, and N-oleoyl ethanolamide; and (b) at least one solid, liquid, or semi-solid edible base substance.

Group II: Claims 9-12 drawn to a plasmid comprising at least one gene which encodes a protein that promotes fatty acid amide (FAA) biosynthesis which when inserted into a bacterium stimulates and/or enhances exercise motivation and/or physical performance in a subject.

The inventions listed as Groups I-II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

### Special Technical Feature:

Group I requires a dietary supplement composition comprising: (a) at least two fatty acid amides (FAAs) selected from the group consisting of N-stearoyl serotonin, N-arachidonoyl dopamine, N-oleoyl dopamine, N-palmitoyl dopamine, N-stearoyl dopamine, N-stearoyl taurine, N-arachidonoyl ethanolamide, N-palmitoyl ethanolamide, and N-oleoyl ethanolamide; and (b) at least one solid, liquid, or semi-solid edible base substance, not required by group II.

Group II requires a plasmid comprising at least one gene which encodes a protein that promotes fatty acid amide (FAA) biosynthesis which when inserted into a bacterium stimulates and/or enhances exercise motivation and/or physical performance in a subject, not required by group I.

### Shared Common Features:

No shared technical feature between group I and II.

Groups I-II therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

### Item 4 continued:

Claims 13-43 are determined unsearchable because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).