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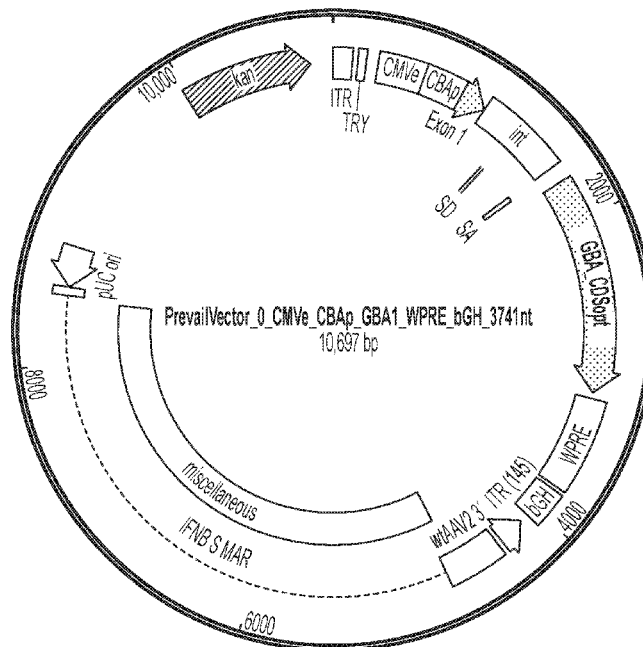


FIG. 1

(57) Abstract: The disclosure relates, in some aspects, to compositions and methods for treatment of diseases associated with aberrant lysosomal function, for example Parkinson's disease and Gaucher disease. In some embodiments, the disclosure provides expression constructs comprising a transgene encoding beta-Glucocerebrosidase (GBA) or a portion thereof, Lysosomal Membrane Protein 2 (LIMP2), Prosaposin, or any combination of the foregoing. In some embodiments, the disclosure provides methods of Parkinson's disease by administering such expression constructs to a subject in need thereof.



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GENE THERAPIES FOR LYSOSOMAL DISORDERS

RELATED APPLICATIONS

This Application claims the benefit under 35 U.S.C. 119(e) of the filing date of U.S.

5 Provisional Application Serial Number 62/567,296, filed October 3, 2017, entitled “GENE THERAPIES FOR LYSOSOMAL DISORDERS”, the entire contents of which are incorporated herein by reference.

BACKGROUND

Gaucher disease is a rare inborn error of glycosphingolipid metabolism due to
10 deficiency of lysosomal acid β -glucocerebrosidase (Gcase, “GBA”). Patients suffer from non-CNS symptoms and findings including hepatosplenomegaly, bone marrow insufficiency leading to pancytopenia, lung disorders and fibrosis, and bone defects. In addition, a significant number of patients suffer from neurological manifestations, including defective saccadic eye movements and gaze, seizures, cognitive deficits, developmental delay, and movement disorders including
15 Parkinson’s disease.

Several therapeutics exist that address the peripheral disease and the principal clinical manifestations in hematopoietic bone marrow and viscera, including enzyme replacement therapies, chaperone-like small molecule drugs that bind to defective Gcase and improve stability, and substrate reduction therapy that block the production of substrates that accumulate
20 in Gaucher disease, leading to symptoms and pathology. However, other aspects of Gaucher disease and appear refractory to treatment.

SUMMARY

In addition to Gaucher disease patients (who possess mutations in both chromosomal
25 alleles of *GBA1* gene), patients with mutations in only one allele of *GBA1* are at highly increased risk of Parkinson’s disease (PD). The severity of PD symptoms- which include gait difficulty, a tremor at rest, rigidity, and often depression, sleep difficulties, and cognitive decline - correlate with the degree of enzyme activity reduction. Thus, Gaucher disease patients have the most severe course, whereas patient with a single mild mutation in *GBA1* typically have a
30 more benign course. Mutation carriers are also at high risk of other PD-related disorders, including Lewy Body Dementia, characterized by executive dysfunction, psychosis, and a PD-like movement disorder, and multi-system atrophy, with characteristic motor and cognitive impairments. No therapies exist that alter the inexorable course of these disorders.

Deficits in enzymes such as Gcase (*e.g.*, the gene product of *GBA1* gene), as well as common variants in many genes implicated in lysosome function or trafficking of macromolecules to the lysosome (*e.g.*, Lysosomal Membrane Protein 1 (LIMP), also referred to as SCARB2), have been associated with increased PD risk. The disclosure is based, in part, on expression constructs (*e.g.*, vectors) encoding Gcase (or a portion thereof), prosaposin (or a portion thereof), LIMP2 (or a portion thereof), or a combination of Gcase (or a portion thereof) and one or more additional gene products from PD-associated genes (*e.g.*, LIMP2, Prosaposin, and/or α -Synuclein (α -Syn)). In some embodiments, combinations of gene products described herein act together (*e.g.*, synergistically) to reduce one or more signs and symptoms of PD when expressed in a subject.

Accordingly, in some aspects, the disclosure provides an isolated nucleic acid comprising an expression construct encoding a Gcase (*e.g.*, the gene product of *GBA1* gene). In some embodiments, the isolated nucleic acid comprises a Gcase-encoding sequence that has been codon optimized (*e.g.*, codon optimized for expression in mammalian cells, for example human cells). In some embodiments, the nucleic acid sequence encoding the Gcase encodes a protein comprising an amino acid sequence as set forth in SEQ ID NO: 14 (*e.g.*, as set forth in NCBI Reference Sequence NP_000148.2). In some embodiments, the isolated nucleic acid comprises the sequence set forth in SEQ ID NO: 15. In some embodiments the expression construct comprises adeno-associated virus (AAV) inverted terminal repeats (ITRs), for example AAV ITRs flanking the nucleic acid sequence encoding the Gcase.

In some aspects, the disclosure provides an isolated nucleic acid comprising an expression construct encoding Prosaposin (*e.g.*, the gene product of *PSAP* gene). In some embodiments, the isolated nucleic acid comprises a prosaposin-encoding sequence that has been codon optimized (*e.g.*, codon optimized for expression in mammalian cells, for example human cells). In some embodiments, the nucleic acid sequence encoding the prosaposin encodes a protein comprising an amino acid sequence as set forth in SEQ ID NO: 16 (*e.g.*, as set forth in NCBI Reference Sequence NP_002769.1). In some embodiments, the isolated nucleic acid comprises the sequence set forth in SEQ ID NO: 17. In some embodiments the expression construct comprises adeno-associated virus (AAV) inverted terminal repeats (ITRs), for example AAV ITRs flanking the nucleic acid sequence encoding the prosaposin.

In some aspects, the disclosure provides an isolated nucleic acid comprising an expression construct encoding LIMP2/SCARB2 (*e.g.*, the gene product of *SCARB2* gene). In some embodiments, the isolated nucleic acid comprises a SCARB2-encoding sequence that has been codon optimized (*e.g.*, codon optimized for expression in mammalian cells, for example

human cells). In some embodiments, the nucleic acid sequence encoding the LIMP2/SCARB2 encodes a protein comprising an amino acid sequence as set forth in SEQ ID NO: 18 (*e.g.*, as set forth in NCBI Reference Sequence NP_005497.1). In some embodiments, the isolated nucleic acid comprises the sequence set forth in SEQ ID NO: 29. In some embodiments the expression
5 construct comprises adeno-associated virus (AAV) inverted terminal repeats (ITRs), for example AAV ITRs flanking the nucleic acid sequence encoding the SCARB2.

In some aspects, the disclosure provides an isolated nucleic acid comprising an expression construct encoding a first gene product and a second gene product, wherein each gene product independently is selected from the gene products, or portions thereof, set forth in
10 Table 1.

In some embodiments, a first gene product or a second gene product is a Gcase protein, or a portion thereof. In some embodiments, a first gene product or a second gene product is LIMP2 or a portion thereof, or Prosaposin or a portion thereof. In some embodiments, the first gene product is a Gcase protein, and the second gene product is LIMP2 or a portion thereof, or
15 Prosaposin or a portion thereof.

In some embodiments, an expression construct further encodes an interfering nucleic acid (*e.g.*, shRNA, miRNA, dsRNA, *etc.*). In some embodiments, an interfering nucleic acid inhibits expression of α -Synuclein (α -Synuclein). In some embodiments, an interfering nucleic acid that targets α -Synuclein comprises a sequence set forth in any one of SEQ ID NOs: 20-25.

20 In some embodiments, an interfering nucleic acid that targets α -Synuclein binds to (*e.g.*, hybridizes with) a sequence set forth in any one of SEQ ID NO: 20-25.

In some embodiments, an expression construct further comprises one or more promoters. In some embodiments, a promoter is a chicken-beta actin (CBA) promoter, a CAG promoter, a CD68 promoter, or a JeT promoter. In some embodiments, a promoter is a RNA pol II promoter
25 (*e.g.*, or an RNA pol III promoter (*e.g.*, U6, *etc.*).

In some embodiments, an expression construct further comprises an internal ribosomal entry site (IRES). In some embodiments, an IRES is located between a first gene product and a second gene product.

In some embodiments, an expression construct further comprises a self-cleaving peptide
30 coding sequence. In some embodiments, a self-cleaving peptide is a T2A peptide.

In some embodiments, an expression construct comprises two adeno-associated virus (AAV) inverted terminal repeat (ITR) sequences. In some embodiments, ITR sequences flank a first gene product and a second gene product (*e.g.*, are arranged as follows from 5'-end to 3'-end: ITR-first gene product-second gene product-ITR). In some embodiments, one of the ITR

sequences of an isolated nucleic acid lacks a functional terminal resolution site (trs). For example, in some embodiments, one of the ITRs is a Δ ITR.

The disclosure relates, in some aspects, to rAAV vectors comprising an ITR having a modified “D” region (*e.g.*, a D sequence that is modified relative to wild-type AAV2 ITR, SEQ ID NO: 29). In some embodiments, the ITR having the modified D region is the 5’ ITR of the rAAV vector. In some embodiments, a modified “D” region comprises an “S” sequence, for example as set forth in SEQ ID NO: 26. In some embodiments, the ITR having the modified “D” region is the 3’ ITR of the rAAV vector. In some embodiments, a modified “D” region comprises a 3’ ITR in which the “D” region is positioned at the 3’ end of the ITR (*e.g.*, on the outside or terminal end of the ITR relative to the transgene insert of the vector). In some embodiments, a modified “D” region comprises a sequence as set forth in SEQ ID NO: 26 or 27.

In some embodiments, an isolated nucleic acid (*e.g.*, an rAAV vector) comprises a TRY region. In some embodiments, a TRY region comprises the sequence set forth in SEQ ID NO: 28.

In some embodiments, an isolated nucleic acid described by the disclosure comprises or consists of the sequence set forth in any one of SEQ ID NOs: 1 to 13, 15, 17, and 19. In some embodiments, an isolated nucleic acid described by the disclosure encodes a peptide comprising or consisting of the sequence set forth in any one of SEQ ID NOs: 14, 16, and 18.

In some aspects, the disclosure provides a vector comprising an isolated nucleic acid as described by the disclosure. In some embodiments, a vector is a plasmid, or a viral vector. In some embodiments, a viral vector is a recombinant AAV (rAAV) vector. In some embodiments, an rAAV vector is single-stranded (*e.g.*, single-stranded DNA).

In some aspects, the disclosure provides a host cell comprising an isolated nucleic acid as described by the disclosure or a vector as described by the disclosure.

In some aspects, the disclosure provides a recombinant adeno-associated virus (rAAV) comprising a capsid protein and an isolated nucleic acid or a vector as described by the disclosure.

In some embodiments, a capsid protein is capable of crossing the blood-brain barrier, for example an AAV9 capsid protein or an AAVrh.10 capsid protein. In some embodiments, an rAAV transduces neuronal cells and non-neuronal cells of the central nervous system (CNS).

In some aspects, the disclosure provides a method for treating a subject having or suspected of having Parkinson’s disease, the method comprising administering to the subject a composition (*e.g.*, a composition comprising an isolated nucleic acid or a vector or a rAAV) as described by the disclosure.

In some embodiments, administration comprises direct injection to the CNS of a subject. In some embodiments, direct injection is intracerebral injection, intraparenchymal injection, intrathecal injection, intra-cisterna magna injection, or any combination thereof. In some embodiments, direct injection to the CNS of a subject comprises convection enhanced delivery (CED).

In some embodiments, administration comprises peripheral injection. In some embodiments, peripheral injection is intravenous injection.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic depicting one embodiment of a plasmid comprising an rAAV vector that includes an expression construct encoding Gcase (*e.g.*, *GBA1* or a portion thereof).

FIG. 2 is a schematic depicting one embodiment of a plasmid comprising an rAAV vector that includes an expression construct encoding Gcase (*e.g.*, *GBA1* or a portion thereof) and LIMP2 (SCARB2) or a portion thereof. The coding sequences of Gcase and LIMP2 are separated by an internal ribosomal entry site (IRES).

FIG. 3 is a schematic depicting one embodiment of a plasmid comprising an rAAV vector that includes an expression construct encoding Gcase (*e.g.*, *GBA1* or a portion thereof) and LIMP2 (SCARB2) or a portion thereof. Expression of the coding sequences of Gcase and LIMP2 are each driven by a separate promoter.

FIG. 4 is a schematic depicting one embodiment of a plasmid comprising an rAAV vector that includes an expression construct encoding Gcase (*e.g.*, *GBA1* or a portion thereof), LIMP2 (SCARB2) or a portion thereof, and an interfering RNA for α -Syn.

FIG. 5 is a schematic depicting one embodiment of a plasmid comprising an rAAV vector that includes an expression construct encoding Gcase (*e.g.*, *GBA1* or a portion thereof), Prosaposin (*e.g.*, *PSAP* or a portion thereof), and an interfering RNA for α -Syn.

FIG. 6 is a schematic depicting one embodiment of a plasmid comprising an rAAV vector that includes an expression construct encoding Gcase (*e.g.*, *GBA1* or a portion thereof) and Prosaposin (*e.g.*, *PSAP* or a portion thereof). The coding sequences of Gcase and Prosaposin are separated by an internal ribosomal entry site (IRES).

FIG. 7 is a schematic depicting one embodiment of an rAAV vector that includes an expression construct encoding a Gcase (*e.g.*, *GBA1* or a portion thereof). In this embodiment, the vector comprises a CBA promoter element (CBA), consisting of four parts: the CMV enhancer (CMVe), CBA promoter (CBAP), Exon 1, and intron (int) to constitutively express the codon optimized coding sequence of human *GBA1*. The 3' region also contains a WPRE

regulatory element followed by a bGH polyA tail. Three transcriptional regulatory activation sites are included at the 5' end of the promoter region: TATA, RBS, and YY1. The flanking ITRs allow for the correct packaging of the intervening sequences. Two variants of the 5' ITR sequence (inset box) were evaluated; these have several nucleotide differences within the 20-nucleotide “D” region of wild-type AAV2 ITR. In some embodiments, an rAAV vector contains the “D” domain nucleotide sequence shown on the top line. In some embodiments, an rAAV vector comprises a mutant “D” domain (*e.g.*, an “S” domain, with the nucleotide changes shown on the bottom line).

FIG. 8 is a schematic depicting one embodiment of a plasmid encoding the rAAV vector described in FIG. 7.

FIG. 9 shows representative data for delivery of an rAAV comprising a transgene encoding a Gcase (*e.g.*, *GBA1* or a portion thereof) in a CBE mouse model of Parkinson's disease. Daily IP delivery of PBS vehicle, 25 mg/kg CBE, 37.5 mg/kg CBE, or 50 mg/kg CBE (left to right) initiated at P8. Survival (top left) was checked two times a day and weight (top right) was checked daily. All groups started with $n = 8$. Behavior was assessed by total distance traveled in Open Field (bottom left) at P23 and latency to fall on Rotarod (bottom middle) at P24. Levels of the GCase substrates were analyzed in the cortex of mice in the PBS and 25 mg/kg CBE treatment groups both with (Day 3) and without (Day 1) CBE withdrawal. Aggregate GluSph and GalSph levels (bottom right) are shown as pmol per mg wet weight of the tissue. Means are presented. Error bars are SEM. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, nominal p-values for treatment groups by linear regression.

FIG. 10 is a schematic depicting one embodiment of a study design for maximal rAAV dose in a CBE mouse model. Briefly, rAAV was delivered by ICV injection at P3, and daily CBE treatment was initiated at P8. Behavior was assessed in the Open Field and Rotarod assays at P24-25 and substrate levels were measured at P36 and P38.

FIG. 11 shows representative data for in-life assessment of maximal rAAV dose in a CBE mouse model. At P3, mice were treated with either excipient or 8.8×10^9 vg rAAV via ICV delivery. Daily IP delivery of either PBS or 25 mg/kg CBE was initiated at P8. At the end of the study, half the mice were sacrificed one day after their last CBE dose at P36 (Day 1) while the remaining half went through 3 days of CBE withdrawal before sacrifice at P38 (Day3). All treatment groups (excipient + PBS $n = 8$, rAAV + PBS $n = 7$, excipient + CBE $n = 8$, and rAAV + CBE $n = 9$) were weighed daily (top left), and the weight at P36 was analyzed (top right). Behavior was assessed by total distance traveled in Open Field at P23 (bottom left) and latency to fall on Rotarod at P24 (bottom right), evaluated for each animal as the median across 3 trials.

Due to lethality, $n = 7$ for the excipient + CBE group for the behavioral assays, while $n=8$ for all other groups. Means across animals are presented. Error bars are SEM. $*p<0.05$; $***p<0.001$, nominal p-values for treatment groups by linear regression in the CBE-treated animals.

FIG. 12 shows representative data for biochemical assessment of maximal rAAV dose in a CBE mouse model. The cortex of all treatment groups (excipient + PBS $n = 8$, rAAV + PBS $n = 7$, excipient + CBE $n = 7$, and rAAV + CBE $n = 9$) was used to measure GCase activity (top left), GluSph levels (top right), GluCer levels (bottom left), and vector genomes (bottom right) in the groups before (Day 1) or after (Day 3) CBE withdrawal. Biodistribution is shown as vector genomes per 1 μg of genomic DNA. Means are presented. Error bars are SEM.

($*$) $p<0.1$; ($**$) $p<0.01$; ($***$) $p<0.001$, nominal p-values for treatment groups by linear regression in the CBE-treated animals, with collection days and gender corrected for as covariates.

FIG. 13 shows representative data for behavioral and biochemical correlations in a CBE mouse model after administration of excipient + PBS, excipient + CBE, and rAAV + CBE treatment groups. Across treatment groups, performance on Rotarod was negatively correlated with GluCer accumulation (A, $p=0.0012$ by linear regression), and GluSph accumulation was negatively correlated with increased GCase activity (B, $p=0.0086$ by linear regression).

FIG. 14 shows representative data for biodistribution of GBA1 rAAV in a CBE mouse model. Presence of vector genomes was assessed in the liver, spleen, kidney, and gonads for all treatment groups (excipient + PBS $n = 8$, rAAV + PBS $n = 7$, excipient + CBE $n = 7$, and rAAV + CBE $n = 9$). Biodistribution is shown as vector genomes per 1 μg of genomic DNA. Vector genome presence was quantified by quantitative PCR using a vector reference standard curve; genomic DNA concentration was evaluated by A260 optical density measurement. Means are presented. Error bars are SEM. $*p<0.05$; $**p<0.01$; $***p<0.001$, nominal p-values for treatment groups by linear regression in the CBE-treated animals, with collection days and gender corrected for as covariates.

FIG. 15 shows representative data for in-life assessment of rAAV dose ranging in a CBE mouse model. Mice received excipient or one of three different doses of GBA1 rAAV by ICV delivery at P3: $3.2\text{e}9$ vg, $1.0\text{e}10$ vg, or $3.2\text{e}10$ vg. At P8, daily IP treatment of 25 mg/kg CBE was initiated. Mice that received excipient and CBE or excipient and PBS served as controls. All treatment groups started with $n = 10$ (5M/5F) per group. All mice were sacrificed one day after their final CBE dose (P38-P40). All treatment groups were weighed daily, and their weight was analyzed at P36. Motor performance was assessed by latency to fall on Rotarod at P24 and latency to traverse the Tapered Beam at P30. Due to early lethality, the number of mice participating in the behavioral assays was: excipient + PBS $n = 10$, excipient + CBE $n = 9$, and

3.2e9 vg rAAV + CBE n = 6, 1.0e10 vg rAAV + CBE n = 10, 3.2e10 vg rAAV + CBE n = 7. Means are presented. Error bars are SEM; * p<0.05; **p<0.01 for nominal p-values by linear regression in the CBE-treated groups, with gender corrected for as a covariate.

FIG. 16 shows representative data for biochemical assessment of rAAV dose ranging in a CBE mouse model. The cortex of all treatment groups (excipient + PBS n = 10, excipient + CBE n = 9, and 3.2e9 vg rAAV + CBE n = 6, 1.0e10 vg rAAV + CBE n = 10, 3.2e10 vg rAAV + CBE n = 7) was used to measure GCase activity, GluSph levels, GluCer levels, and vector genomes. GCase activity is shown as ng of GCase per mg of total protein. GluSph and GluCer levels are shown as pmol per mg wet weight of the tissue. Biodistribution is shown as vector genomes per 1 µg of genomic DNA. Vector genome presence was quantified by quantitative PCR using a vector reference standard curve; genomic DNA concentration was evaluated by A260 optical density measurement. Vector genome presence was also measured in the liver (E). Means are presented. Error bars are SEM. **p<0.01; ***p<0.001 for nominal p-values by linear regression in the CBE-treated groups, with gender corrected for as a covariate.

FIG. 17 shows representative data for tapered beam analysis in maximal dose GBA1 rAAV in a genetic mouse model. Motor performance of the treatment groups (WT + excipient, n = 5), 4L/PS-NA + excipient (n = 6), and 4L/PS-NA + rAAV (n = 5)) was assayed by Beam Walk 4 weeks post rAAV administration. The total slips and active time are shown as total over 5 trials on different beams. Speed and slips per speed are shown as the average over 5 trials on different beams. Means are presented. Error bars are SEM.

FIG. 18 shows representative data for *in vitro* expression of rAAV constructs encoding *GBA1* in combination with Prosaposin (*PSAP*), *SCARB2*, and/or one or more inhibitory nucleic acids. Data indicate transfection of HEK293 cells with each construct resulted in overexpression of the transgenes of interest relative to GFP-transfected cells.

FIG. 19 is a schematic depicting an rAAV vectors comprising a “D” region located on the “outside” of the ITR (e.g., proximal to the terminus of the ITR relative to the transgene insert or expression construct) (top) and a wild-type rAAV vectors having ITRs on the “inside” of the vector (e.g., proximal to the transgene insert of the vector).

FIG. 20 shows data for transduction of HEK293 cells using rAAVs having ITRs with wild-type (circles) or alternative (e.g., “outside”; squares) placement of the “D” sequence. The rAAVs having ITRs placed on the “outside” were able to transduce cells as efficiently as rAAVs having wild-type ITRs.

DETAILED DESCRIPTION

The disclosure is based, in part, on compositions and methods for expression of combinations of PD-associated gene products in a subject. A gene product can be a protein, a fragment (*e.g.*, portion) of a protein, an interfering nucleic acid that inhibits a PD-associated gene, *etc.* In some embodiments, a gene product is a protein or a protein fragment encoded by a PD-associated gene. In some embodiments, a gene product is an interfering nucleic acid (*e.g.*, shRNA, siRNA, miRNA, amiRNA, *etc.*) that inhibits a PD-associated gene.

A PD-associated gene refers to a gene encoding a gene product that is genetically, biochemically or functionally associated with PD. For example, individuals having mutations in the *GBA1* gene (which encodes the protein Gcase), have been observed to have an increased risk of developing PD compared to individuals that do not have a mutation in *GBA1*. In another example, PD is associated with accumulation of protein aggregates comprising α -Synuclein (α -Syn) protein; accordingly, *SCNA* (which encodes α -Syn) is a PD-associated gene. In some embodiments, an expression cassette described herein encodes a wild-type or non-mutant form of a PD-associated gene (or coding sequence thereof). Examples of PD-associated genes are listed in Table 1.

Table 1: Examples of PD-associated genes

Name	Gene	Function	NCBI Accession No.
Lysosome membrane protein 2	<i>SCARB2/LIMP2</i>	lysosomal receptor for glucosylceramidase (GBA targeting)	NP_005497.1 (Isoform 1), NP_001191184.1 (Isoform 2)
Prosaposin	<i>PSAP</i>	precursor for saposins A, B, C, and D, which localize to the lysosomal compartment and facilitate the catabolism of glycosphingolipids with short oligosaccharide groups	AAH01503.1, AAH07612.1, AAH04275.1, AAA60303.1
beta-Glucocerebrosidase	<i>GBA1</i>	cleaves the beta-glucosidic linkage of glucocerebroside	NP_001005742.1 (Isoform 1), NP_001165282.1 (Isoform 2), NP_001165283.1 (Isoform 3)

Isolated nucleic acids and vectors

An isolated nucleic acid may be DNA or RNA. The disclosure provides, in some aspects, an isolated nucleic acid comprising an expression construct encoding a Gcase (*e.g.*, the gene product of *GBA1* gene) or a portion thereof. Gcase, also referred to as β -glucocerebrosidase or GBA, refers to a lysosomal protein that cleaves the beta-glucosidic linkage of the chemical glucocerebroside, an intermediate in glycolipid metabolism. In humans, Gcase is encoded by the *GBA1* gene, located on chromosome 1. In some embodiments, *GBA1* encodes a peptide that is represented by NCBI Reference Sequence NCBI Reference Sequence NP_000148.2 (SEQ ID NO: 14). In some embodiments, the isolated nucleic acid comprises a Gcase-encoding sequence that has been codon optimized (*e.g.*, codon optimized for expression in mammalian cells, for example human cells), such as the sequence set forth in SEQ ID NO: 15.

In some aspects, the disclosure provides an isolated nucleic acid comprising an expression construct encoding Prosaposin (*e.g.*, the gene product of *PSAP* gene). Prosaposin is a precursor glycoprotein for sphingolipid activator proteins (saposins) A, B, C, and D, which facilitate the catabolism of glycosphingolipids with short oligosaccharide groups. In humans, the *PSAP* gene is located on chromosome 10. In some embodiments, *PSAP* encodes a peptide that is represented by NCBI Reference Sequence NP_002769.1 (*e.g.*, SEQ ID NO: 16). In some embodiments, the isolated nucleic acid comprises a prosaposin-encoding sequence that has been codon optimized (*e.g.*, codon optimized for expression in mammalian cells, for example human cells), such as the sequence set forth in SEQ ID NO: 17.

Aspects of the disclosure relate to an isolated nucleic acid comprising an expression construct encoding LIMP2/SCARB2 (*e.g.*, the gene product of *SCARB2* gene). SCARB2 refers to a membrane protein that regulates lysosomal and endosomal transport within a cell. In humans, *SCARB2* gene is located on chromosome 4. In some embodiments, the *SCARB2* gene encodes a peptide that is represented by NCBI Reference Sequence NP_005497.1 (SEQ ID NO: 18). In some embodiments, the isolated nucleic acid comprises the sequence set forth in SEQ ID NO: 19. In some embodiments the isolated nucleic acid comprises a SCARB2-encoding sequence that has been codon optimized.

In some aspects, the disclosure provides an isolated nucleic acid comprising an expression construct encoding a first gene product and a second gene product, wherein each gene product independently is selected from the gene products, or portions thereof, set forth in Table 1.

In some embodiments, a gene product is encoded by a coding portion (*e.g.*, a cDNA) of a naturally occurring gene. In some embodiments, a first gene product is a protein (or a fragment

thereof) encoded by the *GBA1* gene. In some embodiments, a gene product is a protein (or a fragment thereof) encoded by the *SCARB2/LIMP2* gene and/or the *PSAP* gene. However, the skilled artisan recognizes that the order of expression of a first gene product (*e.g.*, Gcase) and a second gene product (*e.g.*, LIMP2) can generally be reversed (*e.g.*, LIMP2 is the first gene product and Gcase is the second gene product). In some embodiments, a gene product is a fragment (*e.g.*, portion) of a gene listed in Table 1. A protein fragment may comprise about 50%, about 60%, about 70%, about 80% about 90% or about 99% of a protein encoded by the genes listed in Table 1. In some embodiments, a protein fragment comprises between 50% and 99.9% (*e.g.*, any value between 50% and 99.9%) of a protein encoded by a gene listed in Table 1.

In some embodiments, an expression construct is monocistronic (*e.g.*, the expression construct encodes a single fusion protein comprising a first gene product and a second gene product). In some embodiments, an expression construct is polycistronic (*e.g.*, the expression construct encodes two distinct gene products, for example two different proteins or protein fragments).

A polycistronic expression vector may comprise a one or more (*e.g.*, 1, 2, 3, 4, 5, or more) promoters. Any suitable promoter can be used, for example, a constitutive promoter, an inducible promoter, an endogenous promoter, a tissue-specific promoter (*e.g.*, a CNS- specific promoter), *etc.* In some embodiments, a promoter is a chicken beta-actin promoter (CBA promoter), a CAG promoter (for example as described by Alexopoulou et al. (2008) *BMC Cell Biol.* 9:2; doi: 10.1186/1471-2121-9-2), a CD68 promoter, or a JeT promoter (for example as described by Tornøe et al. (2002) *Gene* 297(1-2):21-32). In some embodiments, a promoter is operably-linked to a nucleic acid sequence encoding a first gene product, a second gene product, or a first gene product and a second gene product. In some embodiments, an expression cassette comprises one or more additional regulatory sequences, including but not limited to transcription factor binding sequences, intron splice sites, poly(A) addition sites, enhancer sequences, repressor binding sites, or any combination of the foregoing.

In some embodiments, a nucleic acid sequence encoding a first gene product and a nucleic acid sequence encoding a second gene product are separated by a nucleic acid sequence encoding an internal ribosomal entry site (IRES). Examples of IRES sites are described, for example, by Mokrejs et al. (2006) *Nucleic Acids Res.* 34(Database issue):D125-30. In some embodiments, a nucleic acid sequence encoding a first gene product and a nucleic acid sequence encoding a second gene product are separated by a nucleic acid sequence encoding a self-cleaving peptide. Examples of self-cleaving peptides include but are not limited to T2A, P2A,

E2A, F2A, BmCPV 2A, and BmIFV 2A, and those described by Liu et al. (2017) *Sci Rep.* 7: 2193. In some embodiments, the self-cleaving peptide is a T2A peptide.

Pathologically, disorders such as PD and Gaucher disease are associated with accumulation of protein aggregates composed largely of α -Synuclein (α -Syn) protein.

5 Accordingly, in some embodiments, isolated nucleic acids described herein comprise an inhibitory nucleic acid that reduces or prevents expression of α -Syn protein. A sequence encoding an inhibitory nucleic acid may be placed in an untranslated region (*e.g.*, intron, 5'UTR, 3'UTR, *etc.*) of the expression vector.

In some embodiments, an inhibitory nucleic acid is positioned in an intron of an
10 expression construct, for example in an intron upstream of the sequence encoding a first gene product. An inhibitory nucleic acid can be a double stranded RNA (dsRNA), siRNA, micro RNA (miRNA), artificial miRNA (amiRNA), or an RNA aptamer. Generally, an inhibitory nucleic acid binds to (*e.g.*, hybridizes with) between about 6 and about 30 (*e.g.*, any integer between 6 and 30, inclusive) contiguous nucleotides of a target RNA (*e.g.*, mRNA). In some
15 embodiments, the inhibitory nucleic acid molecule is an miRNA or an amiRNA, for example an miRNA that targets *SNCA* (the gene encoding α -Syn protein). In some embodiments, the miRNA does not comprise any mismatches with the region of *SNCA* mRNA to which it hybridizes (*e.g.*, the miRNA is "perfected"). In some embodiments, the inhibitory nucleic acid is an shRNA (*e.g.*, an shRNA targeting *SNCA*).

20 An isolated nucleic acid as described herein may exist on its own, or as part of a vector. Generally, a vector can be a plasmid, cosmid, phagemid, bacterial artificial chromosome (BAC), or a viral vector (*e.g.*, adenoviral vector, adeno-associated virus (AAV) vector, retroviral vector, baculoviral vector, *etc.*). In some embodiments, the vector is a plasmid (*e.g.*, a plasmid comprising an isolated nucleic acid as described herein). In some embodiments, the vector is a
25 recombinant AAV (rAAV) vector. In some embodiments, an rAAV vector is single-stranded (*e.g.*, single-stranded DNA). In some embodiments, a vector is a Baculovirus vector (*e.g.*, an *Autographa californica* nuclear polyhedrosis (AcNPV) vector).

Typically an rAAV vector (*e.g.*, rAAV genome) comprises a transgene (*e.g.*, an expression construct comprising one or more of each of the following: promoter, intron,
30 enhancer sequence, protein coding sequence, inhibitory RNA coding sequence, polyA tail sequence, *etc.*) flanked by two AAV inverted terminal repeat (ITR) sequences. In some embodiments the transgene of an rAAV vector comprises an isolated nucleic acid as described by the disclosure. In some embodiments, each of the two ITR sequences of an rAAV vector is a full-length ITR (*e.g.*, approximately 145 bp in length, and containing functional *Rep* binding site

(RBS) and terminal resolution site (trs)). In some embodiments, one of the ITRs of an rAAV vector is truncated (*e.g.*, shortened or not full-length). In some embodiments, a truncated ITR lacks a functional terminal resolution site (trs) and is used for production of self-complementary AAV vectors (scAAV vectors). In some embodiments, a truncated ITR is a Δ ITR, for example
5 as described by McCarty et al. (2003) *Gene Ther.* 10(26):2112-8.

Aspects of the disclosure relate to isolated nucleic acids (*e.g.*, rAAV vectors) comprising an ITR having one or more modifications (*e.g.*, nucleic acid additions, deletions, substitutions, *etc.*) relative to a wild-type AAV ITR, for example relative to wild-type AAV2 ITR (*e.g.*, SEQ ID NO: 29). The structure of wild-type AAV2 ITR is shown in FIG. 19. Generally, a wild-type
10 ITR comprises a 125 nucleotide region that self-anneals to form a palindromic double-stranded T-shaped, hairpin structure consisting of two cross arms (formed by sequences referred to as B/B' and C/C', respectively), a longer stem region (formed by sequences A/A'), and a single-stranded terminal region referred to as the "D" region. (FIG. 19). Generally, the "D" region of an ITR is positioned between the stem region formed by the A/A' sequences and the insert
15 containing the transgene of the rAAV vector (*e.g.*, positioned on the "inside" of the ITR relative to the terminus of the ITR or proximal to the transgene insert or expression construct of the rAAV vector). In some embodiments, a "D" region comprises the sequence set forth in SEQ ID NO: 27. The "D" region has been observed to play an important role in encapsidation of rAAV vectors by capsid proteins, for example as disclosed by Ling et al. (2015) *J Mol Genet Med* 9(3).

20 The disclosure is based, in part, on the surprising discovery that rAAV vectors comprising a "D" region located on the "outside" of the ITR (*e.g.*, proximal to the terminus of the ITR relative to the transgene insert or expression construct) are efficiently encapsidated by AAV capsid proteins than rAAV vectors having ITRs with unmodified (*e.g.*, wild-type) ITRs. In some embodiments, rAAV vectors having a modified "D" sequence (*e.g.*, a "D" sequence in
25 the "outside" position) have reduced toxicity relative to rAAV vectors having wild-type ITR sequences.

In some embodiments, a modified "D" sequence comprises at least one nucleotide substitution relative to a wild-type "D" sequence (*e.g.*, SEQ ID NO: 27). A modified "D" sequence may have at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10 nucleotide substitutions
30 relative to a wild-type "D" sequence (*e.g.*, SEQ ID NO: 27). In some embodiments, a modified "D" sequence comprises at least 10, 11, 12, 13, 14, 15, 16, 17, 18, or 19 nucleic acid substitutions relative to a wild-type "D" sequence (*e.g.*, SEQ ID NO: 27). In some embodiments, a modified "D" sequence is between about 10% and about 99% (*e.g.*, 10%, 15%, 20%, 25%, 30%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99%)

identical to a wild-type “D” sequence (*e.g.*, SEQ ID NO: 27). In some embodiments, a modified “D” sequence comprises the sequence set forth in SEQ ID NO: 26, also referred to as an “S” sequence as described in Wang et al. (1995) *J Mol Biol* 250(5):573-80.

An isolated nucleic acid or rAAV vector as described by the disclosure may further comprise a “TRY” sequence, for example as set forth in SEQ ID NO: 28 or as described in Francois, et al. The Cellular TATA Binding Protein Is Required for Rep-Dependent Replication of a Minimal Adeno-Associated Virus Type 2 p5 Element. *J Virol*. 2005. In some embodiments, a TRY sequence is positioned between an ITR (*e.g.*, a 5’ ITR) and an expression construct (*e.g.*, a transgene-encoding insert) of an isolated nucleic acid or rAAV vector.

In some aspects, the disclosure relates to Baculovirus vectors comprising an isolated nucleic acid or rAAV vector as described by the disclosure. In some embodiments, the Baculovirus vector is an *Autographa californica* nuclear polyhedrosis (AcNPV) vector, for example as described by Urabe et al. (2002) *Hum Gene Ther* 13(16):1935-43 and Smith et al. (2009) *Mol Ther* 17(11):1888-1896.

In some aspects, the disclosure provides a host cell comprising an isolated nucleic acid or vector as described herein. A host cell can be a prokaryotic cell or a eukaryotic cell. For example, a host cell can be a mammalian cell, bacterial cell, yeast cell, insect cell, *etc.* In some embodiments, a host cell is a mammalian cell, for example a HEK293T cell. In some embodiments, a host cell is a bacterial cell, for example an *E. coli* cell.

rAAVs

In some aspects, the disclosure relates to recombinant AAVs (rAAVs) comprising a transgene that encodes a nucleic acid as described herein (*e.g.*, an rAAV vector as described herein). The term “rAAVs” generally refers to viral particles comprising an rAAV vector encapsidated by one or more AAV capsid proteins. An rAAV described by the disclosure may comprise a capsid protein having a serotype selected from AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, and AAV10. In some embodiments, an rAAV comprises a capsid protein from a non-human host, for example a rhesus AAV capsid protein such as AAVrh.10, AAVrh.39, *etc.* In some embodiments, an rAAV described by the disclosure comprises a capsid protein that is a variant of a wild-type capsid protein, such as a capsid protein variant that includes at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10 (*e.g.*, 15, 20, 25, 50, 100, *etc.*) amino acid substitutions (*e.g.*, mutations) relative to the wild-type AAV capsid protein from which it is derived.

In some embodiments, rAAVs described by the disclosure readily spread through the CNS, particularly when introduced into the CSF space or directly into the brain parenchyma. Accordingly, in some embodiments, rAAVs described by the disclosure comprise a capsid protein that is capable of crossing the blood-brain barrier (BBB). For example, in some
5 embodiments, an rAAV comprises a capsid protein having an AAV9 or AAVrh.10 serotype. Production of rAAVs is described, for example, by Samulski et al. (1989) *J Virol.* 63(9):3822-8 and Wright (2009) *Hum Gene Ther.* 20(7): 698–706.

In some embodiments, an rAAV as described by the disclosure (*e.g.*, comprising a recombinant rAAV genome encapsidated by AAV capsid proteins to form an rAAV capsid
10 particle) is produced in a Baculovirus vector expression system (BEVS). Production of rAAVs using BEVS are described, for example by Urabe et al. (2002) *Hum Gene Ther* 13(16):1935-43, Smith et al. (2009) *Mol Ther* 17(11):1888-1896, U.S. Patent No. 8,945,918, U.S. Patent No. 9,879,282, and International PCT Publication WO 2017/184879. However, an rAAV can be produced using any suitable method (*e.g.*, using recombinant rep and cap genes).

Pharmaceutical Compositions

In some aspects, the disclosure provides pharmaceutical compositions comprising an isolated nucleic acid or rAAV as described herein and a pharmaceutically acceptable carrier. As used herein, the term “pharmaceutically acceptable” refers to a material, such as a carrier or
20 diluent, which does not abrogate the biological activity or properties of the compound, and is relatively non-toxic, *e.g.*, 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 term “pharmaceutically acceptable carrier” means a pharmaceutically
25 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 useful within the invention within or to the patient such that it may perform its intended function. Additional ingredients that may be included in the pharmaceutical compositions used in the practice of the invention are known in
30 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.

Compositions (*e.g.*, pharmaceutical compositions) provided herein can be administered by any route, including enteral (*e.g.*, oral), parenteral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, subcutaneous, intraventricular, transdermal, interdermal, rectal,

intravaginal, intraperitoneal, topical (as by powders, ointments, creams, and/or drops), mucosal, nasal, bucal, sublingual; by intratracheal instillation, bronchial instillation, and/or inhalation; and/or as an oral spray, nasal spray, and/or aerosol. Specifically contemplated routes are oral administration, intravenous administration (*e.g.*, systemic intravenous injection), regional administration via blood and/or lymph supply, and/or direct administration to an affected site. In general, the most appropriate route of administration will depend upon a variety of factors including the nature of the agent (*e.g.*, its stability in the environment of the gastrointestinal tract), and/or the condition of the subject (*e.g.*, whether the subject is able to tolerate oral administration). In certain embodiments, the compound or pharmaceutical composition described herein is suitable for topical administration to the eye of a subject.

Methods

The disclosure is based, in part, on compositions for expression of combinations of PD-associated gene products in a subject that act together (*e.g.*, synergistically) to treat Parkinson's disease. As used herein "treat" or "treating" refers to (a) preventing or delaying onset of Parkinson's disease; (b) reducing severity of Parkinson's disease; (c) reducing or preventing development of symptoms characteristic of Parkinson's disease; (d) and/or preventing worsening of symptoms characteristic of Parkinson's disease. Symptoms of Parkinson's disease include, for example, motor dysfunction (*e.g.*, shaking, rigidity, slowness of movement, difficulty with walking), cognitive dysfunction (*e.g.*, dementia, depression, anxiety), emotional and behavioral dysfunction.

Accordingly, in some aspects, the disclosure provides a method for treating a subject having or suspected of having Parkinson's disease, the method comprising administering to the subject a composition (*e.g.*, a composition comprising an isolated nucleic acid or a vector or a rAAV) as described by the disclosure.

In some embodiments, a composition is administered directly to the CNS of the subject, for example by direct injection into the brain and/or spinal cord of the subject. Examples of CNS-direct administration modalities include but are not limited to intracerebral injection, intraventricular injection, intracisternal injection, intraparenchymal injection, intrathecal injection, and any combination of the foregoing. In some embodiments, direct injection into the CNS of a subject results in transgene expression (*e.g.*, expression of the first gene product, second gene product, and if applicable, third gene product) in the midbrain, striatum and/or cerebral cortex of the subject. In some embodiments, direct injection into the CNS results in

transgene expression (*e.g.*, expression of the first gene product, second gene product, and if applicable, third gene product) in the spinal cord and/or CSF of the subject.

In some embodiments, direct injection to the CNS of a subject comprises convection enhanced delivery (CED). Convection enhanced delivery is a therapeutic strategy that involves surgical exposure of the brain and placement of a small-diameter catheter directly into a target area of the brain, followed by infusion of a therapeutic agent (*e.g.*, a composition or rAAV as described herein) directly to the brain of the subject. CED is described, for example by Debinski et al. (2009) *Expert Rev Neurother.* 9(10):1519-27.

In some embodiments, a composition is administered peripherally to a subject, for example by peripheral injection. Examples of peripheral injection include subcutaneous injection, intravenous injection, intra-arterial injection, intraperitoneal injection, or any combination of the foregoing. In some embodiments, the peripheral injection is intra-arterial injection, for example injection into the carotid artery of a subject.

In some embodiments, a composition (*e.g.*, a composition comprising an isolated nucleic acid or a vector or a rAAV) as described by the disclosure is administered both peripherally and directly to the CNS of a subject. For example, in some embodiments, a subject is administered a composition by intra-arterial injection (*e.g.*, injection into the carotid artery) and by intraparenchymal injection (*e.g.*, intraparenchymal injection by CED). In some embodiments, the direct injection to the CNS and the peripheral injection are simultaneous (*e.g.*, happen at the same time). In some embodiments, the direct injection occurs prior (*e.g.*, between 1 minute and 1 week, or more before) to the peripheral injection. In some embodiments, the direct injection occurs after (*e.g.*, between 1 minute and 1 week, or more after) the peripheral injection.

The amount of composition (*e.g.*, a composition comprising an isolated nucleic acid or a vector or a rAAV) as described by the disclosure administered to a subject will vary depending on the administration method. For example, in some embodiments, a rAAV as described herein is administered to a subject at a titer between about 10^9 Genome copies (GC)/kg and about 10^{14} GC/kg (*e.g.*, about 10^9 GC/kg, about 10^{10} GC/kg, about 10^{11} GC/kg, about 10^{12} GC/kg, about 10^{12} GC/kg, or about 10^{14} GC/kg). In some embodiments, a subject is administered a high titer (*e.g.*, $>10^{12}$ Genome Copies GC/kg of an rAAV) by injection to the CSF space, or by intraparenchymal injection.

A composition (*e.g.*, a composition comprising an isolated nucleic acid or a vector or a rAAV) as described by the disclosure can be administered to a subject once or multiple times (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, or more) times. In some embodiments, a composition is administered to a subject continuously (*e.g.*, chronically), for example via an infusion pump.

EXAMPLES

Example 1: rAAV vectors

AAV vectors are generated using cells, such as HEK293 cells for triple-plasmid transfection. The ITR sequences flank an expression construct comprising a promoter/enhancer element for each transgene of interest, a 3' polyA signal, and posttranslational signals such as the WPRE element. Multiple gene products can be expressed simultaneously such as *GBA1* and *LIMP2* and/or Prosaposin, by fusion of the protein sequences; or using a 2A peptide linker, such as T2A or P2A, which leads 2 peptide fragments with added amino acids due to prevention of the creation of a peptide bond; or using an IRES element; or by expression with 2 separate expression cassettes. The presence of a short intronic sequence that is efficiently spliced, upstream of the expressed gene, can improve expression levels. shRNAs and other regulatory RNAs can potentially be included within these sequences. Examples of plasmids comprising rAAV vectors described by the disclosure are shown in FIGs. 1-6 and in Table 2 below.

Table 2

Name	Promoter 1	shRNA	CDS1	PolyA 1	Bicistronic element	Promoter 2	CDS2	PolyA2	Length between ITRs
CMVe_CBAP_GBA1_WPRE_bGH	CBA		GBA1	WPRE-bGH					3741
LT1s_JetLong_mRNAi aSYn_SCARB2-T2A-GBA1_bGH	JetLong	aSyn	SCARB2	bGH	T2A		GBA1		4215
LI1_JetLong_SCARB2-IRES-GBA1_bGH	JetLong		SCARB2	bGH	IRES		GBA1		4399
FP1_JetLong_GBA1_bGH_JetLong_SCARB2_SV40L	JetLong		GBA1	bGH		JetLong	SCARB2	SV40L	4464
PrevailVector_LT2s_JetLong_mRNAi aSYn_P SAP-T2A-GBA1_bGH_4353nt	JetLong	aSyn	PSAP	bGH	T2A	-	GBA1	-	4353
PrevailVector_LI2_JetLong_PSAP_IRES_GBA1_SyntheticpolyA_433	JetLong	-	PSAP	Synthetic pA	IRES	-	GBA1	-	4337

7nt									
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Example 2: Cell based assays of viral transduction into GBA-deficient cells

Cells deficient in *GBA1* are obtained, for example as fibroblasts from GD patients, monocytes, or hES cells, or patient-derived induced pluripotent stem cells (iPSCs). These cells accumulate substrates such as glucosylceramide and glucosylsphingosine (GluCer and GluSph). Treatment of wild-type or mutant cultured cell lines with Gcase inhibitors, such as CBE, is also be used to obtain GBA deficient cells.

Using such cell models, lysosomal defects are quantified in terms of accumulation of protein aggregates, such as of α -Synuclein with an antibody for this protein or phospho- α Syn, followed by imaging using fluorescent microscopy. Imaging for lysosomal abnormalities by ICC for protein markers such as LAMP1, LAMP2, LIMP1, LIMP2, or using dyes such as LysoTracker, or by uptake through the endocytic compartment of fluorescent dextran or other markers is also performed. Imaging for autophagy marker accumulation due to defective fusion with the lysosome, such as for LC3, can also be performed. Western blotting and/or ELISA is used to quantify abnormal accumulation of these markers. Also, the accumulation of glycolipid substrates and products of GBA1 is measured using standard approaches.

Therapeutic endpoints (*e.g.*, reduction of PD-associated pathology) are measured in the context of expression of transduction of the AAV vectors, to confirm and quantify activity and function. Gcase can is also quantified using protein ELISA measures, or by standard Gcase activity assays.

Example 3: In vivo assays using mutant mice

This example describes *in vivo* assays of AAV vectors using mutant mice. *In vivo* studies of AAV vectors as above in mutant mice are performed using assays described, for example, by Liou *et al.* (2006) *J. Biol. Chem.* 281(7): 4242–4253, Sun *et al.* (2005) *J. Lipid Res.* 46:2102–2113, and Farfel-Becker *et al.* (2011) *Dis. Model Mech.* 4(6):746–752.

The intrathecal or intraventricular delivery of vehicle control and AAV vectors (*e.g.*, at a dose of 2×10^{11} vg/mouse) are performed using concentrated AAV stocks, for example at an injection volume between 5–10 μ L. Intraparenchymal delivery by convection enhanced delivery is performed.

Treatment is initiated either before onset of symptoms, or subsequent to onset. Endpoints measured are the accumulation of substrate in the CNS and CSF, accumulation of

Gcase enzyme by ELISA and of enzyme activity, motor and cognitive endpoints, lysosomal dysfunction, and accumulation of α -Synuclein monomers, protofibrils or fibrils.

Example 4: Chemical models of disease

5 This example describes *in vivo* assays of AAV vectors using a chemically-induced mouse model of Gaucher disease (*e.g.*, the CBE mouse model). *In vivo* studies of these AAV vectors are performed in a chemically-induced mouse model of Gaucher disease, for example as described by Vardi *et al.* (2016) *J Pathol.* 239(4):496-509.

10 Intrathecal or intraventricular delivery of vehicle control and AAV vectors (*e.g.*, at a dose of 2×10^{11} vg/mouse) are performed using concentrated AAV stocks, for example with injection volume between 5–10 μ L. Intraparenchymal delivery by convection enhanced delivery is performed. Peripheral delivery is achieved by tail vein injection.

Treatment is initiated either before onset of symptoms, or subsequent to onset. Endpoints measured are the accumulation of substrate in the CNS and CSF, accumulation of
15 Gcase enzyme by ELISA and of enzyme activity, motor and cognitive endpoints, lysosomal dysfunction, and accumulation of α -Synuclein monomers, protofibrils or fibrils.

Example 5: Clinical trials in PD, LBD, Gaucher disease patients

20 In some embodiments, patients having certain forms of Gaucher disease (*e.g.*, GD1) have an increased risk of developing Parkinson's disease (PD) or Lewy body dementia (LBD). This Example describes clinical trials to assess the safety and efficacy of rAAVs as described by the disclosure, in patients having Gaucher disease, PD and/or LBD.

Clinical trials of such vectors for treatment of Gaucher disease, PD and/or LBD are performed using a study design similar to that described in Grabowski *et al.* (1995) *Ann. Intern.*
25 *Med.* 122(1):33-39.

Example 6: Treatment of peripheral disease

30 In some embodiments, patients having certain forms of Gaucher disease exhibit symptoms of peripheral neuropathy, for example as described in Biegstraaten *et al.* (2010) *Brain* 133(10):2909–2919.

This example describes *in vivo* assays of AAV vectors as described herein for treatment of peripheral neuropathy associated with Gaucher disease (*e.g.*, Type 1 Gaucher disease). Briefly, Type 1 Gaucher disease patients identified as having signs or symptoms of peripheral neuropathy are administered a rAAV as described by the disclosure. In some embodiments, the

peripheral neuropathic signs and symptoms of the subject are monitored, for example using methods described in Biegstraaten *et al.*, after administration of the rAAV.

Levels of transduced gene products as described by the disclosure present in patients (*e.g.*, in serum of a patient, in peripheral tissue (*e.g.*, liver tissue, spleen tissue, *etc.*)) of a patient are assayed, for example by Western blot analysis, enzymatic functional assays, or imaging studies.

Example 7: Treatment of CNS forms

This example describes *in vivo* assays of rAAVs as described herein for treatment of CNS forms of Gaucher disease. Briefly, Gaucher disease patients identified as having a CNS form of Gaucher disease (*e.g.*, Type 2 or Type 3 Gaucher disease) are administered a rAAV as described by the disclosure. Levels of transduced gene products as described by the disclosure present in the CNS of patients (*e.g.*, in serum of the CNS of a patient, in cerebrospinal fluid (CSF) of a patient, or in CNS tissue of a patient) are assayed, for example by Western blot analysis, enzymatic functional assays, or imaging studies.

Example 8: Gene therapy of Parkinson's Disease in subjects having mutations in GBA1

This example describes administration of a recombinant adeno-associated virus (rAAV) encoding *GBA1* to a subject having Parkinson's disease characterized by a mutation in *GBA1* gene.

The rAAV vector insert contains the CBA promoter element (CBA), consisting of four parts: the CMV enhancer (CMVe), CBA promoter (CBAP), Exon 1, and intron (int) to constitutively express the codon optimized coding sequence (CDS) of human GBA1 (maroon). The 3' region also contains a Woodchuck hepatitis virus Posttranscriptional Regulatory Element (WPRE) posttranscriptional regulatory element followed by a bovine Growth Hormone polyA signal (bGH polyA) tail. The flanking ITRs allow for the correct packaging of the intervening sequences. Two variants of the 5' ITR sequence (FIG. 7, inset box, bottom sequence) were evaluated; these variants have several nucleotide differences within the 20-nucleotide "D" region of the ITR, which is believed to impact the efficiency of packaging and expression. The rAAV product contains the "D" domain nucleotide sequence shown in FIG. 7 (inset box, top sequence). A variant vector, harbors a mutant "D" domain (termed an "S" domain herein, with the nucleotide changes shown by shading), performed similarly in preclinical studies. The backbone contains the gene to confer resistance to kanamycin as well as a stuffer sequence to

prevent reverse packaging. A schematic depicting the rAAV vector is shown in FIG. 8. The rAAV vector is packaged into an rAAV using AAV9 serotype capsid proteins.

GBA1-rAAV is administered to a subject as a single dose via a fluoroscopy guided sub-occipital injection into the cisterna magna (intracisternal magna; ICM). One embodiment of a dosing regimen study is as follows:

A single dose of rAAV is administered to patients (N=12) at one of two dose levels (3e13 vg (low dose); 1e14 vg (high dose), *etc.*) which are determined based on the results of nonclinical pharmacology and toxicology studies.

Initial studies were conducted in a chemical mouse model involving daily delivery of conduritol-b-epoxide (CBE), an inhibitor of GCase to assess the efficacy and safety of the rAAV vector and a variant rAAV S-variant construct (as described further below). Additionally, initial studies were performed in a genetic mouse model, which carries a homozygous *GBA1* mutation and is partially deficient in saposins (4L/PS-NA). Additional dose-ranging studies in mice and nonhuman primates (NHPs) are conducted to further evaluate vector safety and efficacy.

Two slightly different versions of the 5' inverted terminal repeat (ITR) in the AAV backbone were tested to assess manufacturability and transgene expression (FIG. 7). The 20 bp "D" domain within the 145 bp 5' ITR is thought to be necessary for optimal viral vector production, but mutations within the "D" domain have also been reported to increase transgene expression in some cases. Thus, in addition to the viral vector, which harbors an intact "D" domain, a second vector form with a mutant D domain (termed an "S" domain herein) was also evaluated. Both rAAV and variant rAAV express the same transgene. While both vectors produced virus that was efficacious in vivo as detailed below, the rAAV which contains a wild-type "D" domain, was selected for further development.

To establish the CBE model of GCase deficiency, juvenile mice were dosed with CBE, a specific inhibitor of GCase. Mice were given CBE by IP injection daily, starting at postnatal day 8 (P8). Three different CBE doses (25 mg/kg, 37.5 mg/kg, 50 mg/kg) and PBS were tested to establish a model that exhibits a behavioral phenotype (FIG. 9). Higher doses of CBE led to lethality in a dose-dependent manner. All mice treated with 50 mg/kg CBE died by P23, and 5 of the 8 mice treated with 37.5 mg/kg CBE died by P27. There was no lethality in mice treated with 25 mg/kg CBE. Whereas CBE-injected mice showed no general motor deficits in the open field assay (traveling the same distance and at the same velocity as mice given PBS), CBE-treated mice exhibited a motor coordination and balance deficit as measured by the rotarod assay.

Mice surviving to the end of the study were sacrificed on the day after their last CBE dose (P27, “Day 1”) or after three days of CBE withdrawal (P29, “Day 3”). Lipid analysis was performed on the cortex of mice given 25 mg/kg CBE to evaluate the accumulation of GCase substrates in both the Day 1 and Day 3 cohorts. GluSph and GalSph levels (measured in
5 aggregate in this example) were significantly accumulated in the CBE-treated mice compared to PBS-treated controls, consistent with GCase insufficiency.

Based on the study described above, the 25 mg/kg CBE dose was selected since it produced behavioral deficits without impacting survival. To achieve widespread GBA1 distribution throughout the brain and transgene expression during CBE treatment, rAAV or
10 excipient was delivered by intracerebroventricular (ICV) injection at postnatal day 3 (P3) followed by daily IP CBE or PBS treatment initiated at P8 (FIG. 10).

CBE-treated mice that received rAAV performed statistically significantly better on the rotarod than those that received excipient (FIG. 11). Mice in the variant vector treatment group did not differ from excipient treated mice in terms of other behavioral measures, such as the total
15 distance traveled during testing (FIG. 11).

At the completion of the in-life study, half of the mice were sacrificed the day after the last CBE dose (P36, “Day 1”) or after three days of CBE withdrawal (P38, “Day 3”) for biochemical analysis (FIG. 12). Using a fluorometric enzyme assay performed in biological triplicate, GCase activity was assessed in the cortex. GCase activity was increased in mice that
20 were treated with GBA1 rAAV, while CBE treatment reduced GCase activity. Additionally, mice that received both CBE and GBA1-rAAV had GCase activity levels that were similar to the PBS-treated group, indicating that delivery of rAAV is able to overcome the inhibition of GCase activity induced by CBE treatment. Lipid analysis was performed on the motor cortex of the mice to examine levels of the substrates GluCer and GluSph. Both lipids accumulated in the
25 brains of mice given CBE, and rAAV treatment significantly reduced substrate accumulation.

Lipid levels were negatively correlated with both GCase activity and performance on the Rotarod across treatment groups. The increased GCase activity after rAAV administration was associated with substrate reduction and enhanced motor function (FIG. 13). As shown in FIG. 14, preliminary biodistribution was assessed by vector genome presence, as measured by qPCR
30 (with >100 vector genomes per 1 µg genomic DNA defined as positive). Mice that received GBA1-rAAV, both with and without CBE, were positive for rAAV vector genomes in the cortex, indicating that ICV delivery results in rAAV delivery to the cortex. Additionally, vector genomes were detected in the liver, few in spleen, and none in the heart, kidney or gonads. For

all measures, there was no statistically significant difference between the Day 1 and Day 3 groups.

A larger study in the CBE model further explored efficacious doses of GBA1-rAAV in the CBE model. Using the 25 mg/kg CBE dose model, excipient or GBA1-rAAV was delivered via ICV at P3, and daily IP PBS or CBE treatment initiated at P8. Given the similarity between the groups with and without CBE withdrawal observed in the previous studies, all mice were sacrificed one day after the final CBE dose (P38-40). The effect of three different rAAV doses was assessed, resulting in the following five groups, with 10 mice (5M/5F) per group:

Excipient ICV + PBS IP

Excipient ICV + 25 mg/kg CBE IP

3.2e9 vg (2.13e10 vg/g brain) rAAV ICV + 25 mg/kg CBE IP

1.0e10 vg (6.67e10 vg/g brain) rAAV ICV + 25 mg/kg CBE IP

3.2e10 vg (2.13e11 vg/g brain) rAAV ICV + 25 mg/kg CBE IP.

The highest dose of rAAV rescued the CBE treatment-related failure to gain weight at P37. Additionally, this dose resulted in a statistically significant increase in performance on the rotarod and tapered beam compared to the Excipient + CBE treated group (FIG. 15). Lethality was observed in several groups, including both excipient-treated and rAAV-treated groups (Excipient + PBS: 0; Excipient + 25 mg/kg CBE: 1; 3.2e9 vg rAAV + 25 mg/kg CBE: 4; 1.0e10 vg rAAV + 25 mg/kg CBE: 0; 3.2e10 vg rAAV + 25 mg/kg CBE: 3).

At the completion of the in-life study, mice were sacrificed for biochemical analysis (FIG. 16). GCase activity in the cortex was assessed in biological triplicates by a fluorometric assay. CBE-treated mice showed reduced GCase activity whereas mice that received a high rAAV dose showed a statistically significant increase in GCase activity compared to CBE treatment. CBE-treated mice also had accumulation of GluCer and GluSph, both of which were rescued by administering a high dose of rAAV.

In addition to the established chemical CBE model, GBA1-rAAV is also evaluated in the 4L/PS-NA genetic model, which is homozygous for the V394L GD mutation in Gba1 and is also partially deficient in saposins, which affect GCase localization and activity. These mice exhibit motor strength, coordination, and balance deficits, as evidenced by their performance in the beam walk, rotarod, and wire hang assays. Typically the lifespan of these mice is less than 22 weeks. In an initial study, 3 μ l of maximal titer virus was delivered by ICV at P23, with a final dose of 2.4e10 vg (6.0e10 vg/g brain). With 6 mice per group, the treatment groups were:

WT + Excipient ICV

4L/PS-NA + Excipient ICV

4L/PS-NA + 2.4e10 vg (6.0e10 vg/g brain) rAAV ICV

Motor performance by the beam walk test was assessed 4 weeks post-rAAV delivery.

5 The group of mutant mice that received GBA1-rAAV showed a trend towards fewer total slips and fewer slips per speed when compared to mutant mice treated with excipient, restoring motor function to near WT levels (FIG. 17). Since the motor phenotypes become more severe as these mice age, their performance on this and other behavioral tests is assessed at later time points. At the completion of the in-life study, lipid levels, GCase activity, and biodistribution are assessed
10 in these mice.

Additional lower doses of rAAV are currently being tested using the CBE model, corresponding to 0.03x, 0.1x, and 1x the proposed phase 1 high clinical dose. Each group includes 10 mice (5M/5F) per group:

Excipient ICV

15 Excipient ICV + 25 mg/kg CBE IP

3.2e8 vg (2.13e9 vg/g brain) rAAV ICV + 25 mg/kg CBE IP

1.0e9 vg (6.67e9 vg/g brain) rAAV ICV + 25 mg/kg CBE IP

1.0e10 vg (6.67e10 vg/g brain) rAAV ICV + 25 mg/kg CBE IP.

20 In addition to motor phenotypes, lipid levels and GCase activity are assessed in the cortex. Time course of treatments and analyses are also performed.

A larger dose ranging study was initiated to evaluate efficacy and safety data. 10 4L/PS-NA mice (5M/5F per group) were injected with 10 µl of rAAV. Using an allometric brain weight calculation, the doses correlate to 0.15x, 1.5x, 4.4x, and 14.5x the proposed phase 1 high
25 clinical dose. The injection groups consist of:

WT + Excipient ICV

4L/PS-NA + Excipient ICV

4L/PS-NA + 4.3e9 vg (1.1e10 vg/g brain) rAAV ICV

4L/PS-NA + 4.3e10 vg (1.1e11 vg/g/ brain) rAAV ICV

30 4L/PS-NA + 1.3e11 vg (3.2e11 vg/g brain) rAAV ICV

4L/PS-NA + 4.3e11 vg (1.1e12 vg/g brain) rAAV ICV.

A summary of nonclinical studies in the CBE model are shown in Table 3 below.

Table 3: Summary of Results in CBE Mouse Model

Test Material	Study Number	Dose Cohort	Behavioral Changes			Lipids	Enzyme	BD	
			Rotarod	Tapered Beam	Open Field			Brain	Liver
GBA1-rAAV	PRV-2018-005 Dose-ranging rAAV in CBE Model	3.2e9 vg (2.13e10 vg/g brain)	NS	NS	NS	NS	NS	+	-
		1.10e10 vg (6.67e10 vg/g brain)	T	NS	NS	T/S	NS	+	+
		2.3e10vg (2.13e11 vg/g brain)	S	S	NS	S	S	+	+
variant GBA1-rAAV	PRV-2018-005 Dose-ranging rAAV in CBE Model	8.8e9 vg (5.9e10 vg/g brain)	S	N/A	NS	S	S	+	+

Note that positive biodistribution is defined as >100 vg/1 µg genomic DNA.

Abbreviations: BD = biodistribution; NS = nonsignificant; T = trend; S = significant; N/A = not applicable; + = positive; - = negative.

Example 9: *In vitro* analysis of rAAV vectors

A pilot study was performed to assess *in vitro* activity of rAAV vectors encoding Prosaposin (*PSAP*) and *SCARB2*, alone or in combination with *GBA1* and/or one or more inhibitory RNAs. One construct encoding PSAP and progranulin (PGRN) was also tested. Vectors tested include those shown in Table 4. “Opt” refers to a nucleic acid sequence codon optimized for expression in mammalian cells (*e.g.*, human cells). FIG. 18 shows representative data indicating that transfection of HEK293 cells with each of the constructs resulted in overexpression of the corresponding gene product compared to mock transfected cells.

Table 4

ID	Promoter	Inhibitory RNA	Promoter	Transgene
I00015	JL_intronic	SCNA	JetLong	Opt-PSAP_GBA1
I00039	-	-	JetLong	Opt-PSAP-GRN
I00046	-	-		Opt-PSAP
I00014	JetLong	SCNA	JetLong	Opt-SCARB2_GBA1

Example 10: ITR “D” sequence placement and cell transduction

The effect of placement of ITR “D” sequence on cell transduction of rAAV vectors was investigated. HEK 293 cells were transduced with Gcase-encoding rAAVs having 1) wild-type ITRs (*e.g.*, “D” sequences proximal to the transgene insert and distal to the terminus of the ITR) or 2) ITRs with the “D” sequence located on the “outside” of the vector (*e.g.*, “D” sequence located proximal to the terminus of the ITR and distal to the transgene insert), as shown in FIG. 19. Surprisingly, data indicate that rAAVs having the “D” sequence located in the “outside” position retain the ability to be packaged and transduce cells efficiently (FIG. 20).

Example 11: In vitro Toxicity Studies

Fifty (50) mice were administered GBA1-encoding rAAVs via a 4 μ l intracerebroventricular (ICV) injection on post-natal day 3. All mice received daily intraperitoneal (IP) injections of conduritol B-epoxide (CBE) or PBS, depending on treatment group, from post-natal day 8 to the end of the study. Animals were euthanized 24 hours after their last IP dose. After euthanasia, target tissues were harvested, drop fixed in chilled 4% paraformaldehyde and stored at 4°C, then sent for histopathological processing and evaluation. There were eight (8) early death animals over the course of the study, which were not sent to or analyzed.

Tissues from the forty-two (42) animals euthanized at 38-40 days were trimmed, processed, and embedded in paraffin blocks. They were then sectioned at ~5 μ m, stained with hematoxylin and eosin (H&E) and affixed to slides for evaluation.

There were no histopathologic findings or evidence of toxicity due to treatment with the rAAVs. In the mice treated with conduritol B-epoxide (CBE), there were findings in the central nervous system (CNS) that included glial scars and neuronal necrosis in the cerebral cortex, and

neuronal necrosis in the brain stem and thoracic spinal cord. High dose rAAV treatment resulted in a notable reduction in the incidence of these CNS findings, while the low and mid dose virus had a dose dependent reduction in the incidence of glial scars in the cerebral cortex, with equivocal effects on the other CNS findings.

5

EQUIVALENTS

This Application incorporates by reference the contents of the following documents in their entirety: the International PCT Application referred to by Attorney Docket Number P1094.70003WO00, filed October 3 2018; International PCT Application referred to by Attorney Docket Number P1094.70004WO00, filed October 3, 2018; Provisional Application Serial Numbers 62/567,311, filed October 3, 2017, entitled "GENE THERAPIES FOR LYSOSOMAL DISORDERS"; 62/567,319, filed October 3, 2017, entitled "GENE THERAPIES FOR LYSOSOMAL DISORDERS"; 62/567,301, filed October 3, 2018, entitled "GENE THERAPIES FOR LYSOSOMAL DISORDERS"; 62/567,310, filed October 3, 2017, entitled "GENE THERAPIES FOR LYSOSOMAL DISORDERS"; 62/567,303, filed October 3, 2017, entitled "GENE THERAPIES FOR LYSOSOMAL DISORDERS"; and 62/567,305, filed October 3, 2017, entitled "GENE THERAPIES FOR LYSOSOMAL DISORDERS".

Having thus described several aspects of at least one embodiment of this invention, it is to be appreciated that various alterations, modifications, and improvements will readily occur to those skilled in the art. Such alterations, modifications, and improvements are intended to be part of this disclosure, and are intended to be within the spirit and scope of the invention. Accordingly, the foregoing description and drawings are by way of example only.

While several embodiments of the present invention have been described and illustrated herein, those of ordinary skill in the art will readily envision a variety of other means and/or structures for performing the functions and/or obtaining the results and/or one or more of the advantages described herein, and each of such variations and/or modifications is deemed to be within the scope of the present invention. More generally, those skilled in the art will readily appreciate that all parameters, dimensions, materials, and configurations described herein are meant to be exemplary and that the actual parameters, dimensions, materials, and/or configurations will depend upon the specific application or applications for which the teachings of the present invention is/are used. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. It is, therefore, to be understood that the

foregoing embodiments are presented by way of example only and that, within the scope of the appended claims and equivalents thereto, the invention may be practiced otherwise than as specifically described and claimed. The present invention is directed to each individual feature, system, article, material, and/or method described herein. In addition, any combination of two or more such features, systems, articles, materials, and/or methods, if such features, systems, articles, materials, and/or methods are not mutually inconsistent, is included within the scope of the present invention.

The indefinite articles “a” and “an,” as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to mean “at least one.”

The phrase “and/or,” as used herein in the specification and in the claims, should be understood to mean “either or both” of the elements so conjoined, i.e., elements that are conjunctively present in some cases and disjunctively present in other cases. Other elements may optionally be present other than the elements specifically identified by the “and/or” clause, whether related or unrelated to those elements specifically identified unless clearly indicated to the contrary. Thus, as a non-limiting example, a reference to “A and/or B,” when used in conjunction with open-ended language such as “comprising” can refer, in one embodiment, to A without B (optionally including elements other than B); in another embodiment, to B without A (optionally including elements other than A); in yet another embodiment, to both A and B (optionally including other elements); *etc.*

As used herein in the specification and in the claims, “or” should be understood to have the same meaning as “and/or” as defined above. For example, when separating items in a list, “or” or “and/or” shall be interpreted as being inclusive, i.e., the inclusion of at least one, but also including more than one, of a number or list of elements, and, optionally, additional unlisted items. Only terms clearly indicated to the contrary, such as “only one of” or “exactly one of,” or, when used in the claims, “consisting of,” will refer to the inclusion of exactly one element of a number or list of elements. In general, the term “or” as used herein shall only be interpreted as indicating exclusive alternatives (i.e. “one or the other but not both”) when preceded by terms of exclusivity, such as “either,” “one of,” “only one of,” or “exactly one of.” “Consisting essentially of,” when used in the claims, shall have its ordinary meaning as used in the field of patent law.

As used herein in the specification and in the claims, the phrase “at least one,” in reference to a list of one or more elements, should be understood to mean at least one element selected from any one or more of the elements in the list of elements, but not necessarily including at least one of each and every element specifically listed within the list of elements

and not excluding any combinations of elements in the list of elements. This definition also allows that elements may optionally be present other than the elements specifically identified within the list of elements to which the phrase “at least one” refers, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, “at least one of A and B” (or, equivalently, “at least one of A or B,” or, equivalently “at least one of A and/or B”) can refer, in one embodiment, to at least one, optionally including more than one, A, with no B present (and optionally including elements other than B); in another embodiment, to at least one, optionally including more than one, B, with no A present (and optionally including elements other than A); in yet another embodiment, to at least one, optionally including more than one, A, and at least one, optionally including more than one, B (and optionally including other elements); *etc.*

In the claims, as well as in the specification above, all transitional phrases such as “comprising,” “including,” “carrying,” “having,” “containing,” “involving,” “holding,” and the like are to be understood to be open-ended, i.e., to mean including but not limited to. Only the transitional phrases “consisting of” and “consisting essentially of” shall be closed or semi-closed transitional phrases, respectively, as set forth in the United States Patent Office Manual of Patent Examining Procedures, Section 2111.03.

Use of ordinal terms such as “first,” “second,” “third,” *etc.*, in the claims to modify a claim element does not by itself connote any priority, precedence, or order of one claim element over another or the temporal order in which acts of a method are performed, but are used merely as labels to distinguish one claim element having a certain name from another element having a same name (but for use of the ordinal term) to distinguish the claim elements.

It should also be understood that, unless clearly indicated to the contrary, in any methods claimed herein that include more than one step or act, the order of the steps or acts of the method is not necessarily limited to the order in which the steps or acts of the method are recited.

SEQUENCES

In some embodiments, an expression cassette encoding one or more gene products (*e.g.*, a first, second and/or third gene product) comprises or consists of (or encodes a peptide having) a sequence set forth in any one of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25. In some embodiments, a gene product is encoded by a portion (*e.g.*, fragment) of any one of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25.

Reference to any prior art in the specification is not an acknowledgement or suggestion that this prior art forms part of the common general knowledge in any jurisdiction or that this prior art could reasonably be expected to be combined with any other piece of prior art by a skilled person in the art.

CLAIMS

What is claimed is:

1. An isolated nucleic acid comprising:
 - 5 (i) an expression construct comprising a chicken-beta actin (CBA) promoter, a CMV enhancer, Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element (WPRE), a Bovine Growth Hormone polyA signal tail, and a transgene encoding a beta-Glucocerebrosidase (Gcase) protein, wherein the transgene comprises the nucleotide sequence of SEQ ID NO: 15; and
 - 10 (ii) two adeno-associated virus (AAV) inverted terminal repeats (ITR) sequences flanking the expression construct, wherein the first ITR sequence is a 5' ITR, and the second ITR sequence is a 3' ITR.
2. The isolated nucleic acid of claim 1, wherein each of the two ITR sequences is a

15 wild-type AAV2 ITR sequence.
3. The isolated nucleic acid of claim 1, wherein each of the two ITR sequences comprises a "D" region (SEQ ID NO: 27) that is proximal to the expression construct.
- 20 4. The isolated nucleic acid of claim 1, wherein at least one of the ITR sequences comprises a "D" region (SEQ ID NO: 27) positioned on the outside of the ITR sequence relative to the expression construct.
- 25 5. The isolated nucleic acid of claim 1, wherein the ITR sequence positioned 5' relative to the expression construct comprises a "D" region (SEQ ID NO: 27) that is proximal to the expression construct, and the ITR sequence positioned 3' relative to the expression construct comprises a "D" region (SEQ ID NO: 27) positioned on the outside of the ITR sequence relative to the expression construct.
- 30 6. The isolated nucleic acid of claim 5, wherein the nucleic acid sequence of the 5' ITR is nucleotides 1 – 145 of SEQ ID NO: 1 and the nucleic acid sequence of the 3' ITR is nucleotides 3867 – 4011 of SEQ ID NO: 1.

7. The isolated nucleic acid of claim 1, further comprising a TRY region between the 5' ITR sequence and the expression construct, wherein the TRY region has the sequence set forth in SEQ ID NO: 28.

5 8. A recombinant adeno-associated virus (rAAV) vector comprising an expression construct comprising a CBA promoter, a CMV enhancer, a WPRE, a Bovine Growth Hormone polyA signal tail, and a transgene encoding a Gcase protein flanked by two AAV ITR sequences, wherein the transgene comprises the nucleotide sequence of SEQ ID NO: 15, and wherein the first ITR sequence is a 5' ITR, and the second ITR sequence is a 3' ITR.

10 9. The rAAV vector of claim 8, wherein each of the two ITR sequences is a wild-type AAV2 ITR sequence.

15 10. The rAAV vector of claim 8, wherein each of the two ITR sequences comprises a "D" region (SEQ ID NO: 27) that is proximal to the expression construct.

20 11. The rAAV vector of claim 8, wherein at least one of the ITR sequences comprises a "D" region (SEQ ID NO: 27) positioned on the outside of the ITR sequence relative to the expression construct.

25 12. The rAAV vector of claim 8, wherein the ITR sequence positioned 5' relative to the expression construct comprises a "D" region (SEQ ID NO: 27) that is proximal to the expression construct, and the ITR sequence positioned 3' relative to the expression construct comprises a "D" region (SEQ ID NO: 27) positioned on the outside of the ITR sequence relative to the expression construct.

30 13. The rAAV vector of claim 12, wherein the nucleic acid sequence of the 5' ITR is nucleotides 1 – 145 of SEQ ID NO: 1 and the nucleic acid sequence of the 3' ITR is nucleotides 3867 – 4011 of SEQ ID NO: 1.

14. The rAAV vector of claim 8, further comprising a TRY region between the 5' ITR sequence and the expression construct, wherein the TRY region has the sequence set forth in SEQ ID NO: 28.

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15. A rAAV comprising:
- (i) an AAV capsid protein; and
 - (ii) the rAAV vector of any one of claims 8-14.
- 5 16. The rAAV of claim 15, wherein the AAV capsid protein is AAV9 capsid protein.
17. A rAAV vector comprising a nucleic acid comprising, in 5' to 3' order:
- (a) a 5' AAV ITR;
 - (b) a CMV enhancer;
 - 10 (c) a CBA promoter;
 - (d) a transgene encoding a Gcase protein, wherein the transgene comprises the nucleotide sequence of SEQ ID NO: 15;
 - (e) a WPRE;
 - (f) a Bovine Growth Hormone polyA signal tail; and
 - 15 (g) a 3' AAV ITR.
18. A rAAV comprising:
- (i) an AAV capsid protein; and
 - (ii) the rAAV vector of claim 17.
- 20 19. The rAAV of claim 18, wherein the AAV capsid protein is AAV9 capsid protein.
20. A plasmid comprising the rAAV vector of any one of claims 8-14 and 17.
- 25 21. A Baculovirus vector comprising an expression construct comprising a CBA promoter, a CMV enhancer, a WPRE, a Bovine Growth Hormone polyA signal tail, and the nucleotide sequence set forth in SEQ ID NO: 15, and two AAV ITR sequences flanking the expression construct.
- 30 22. A cell comprising:
- (i) a first vector encoding one or more AAV rep proteins and/or one or more AAV cap proteins; and
 - (ii) a second vector comprising an expression construct comprising a CBA promoter, a CMV enhancer, a WPRE, a Bovine Growth Hormone polyA signal tail, and the nucleotide

sequence set forth in SEQ ID NO: 15, and two AAV ITR sequences flanking the expression construct.

23. The cell of claim 22, wherein the first vector is a plasmid and the second vector is a plasmid.

24. The cell of claim 22 or 23, wherein the cell is a mammalian cell.

25. The cell of claim 24, wherein the mammalian cell is a HEK293 cell.

26. The cell of claim 22, wherein the first vector is a Baculovirus vector and the second vector is a Baculovirus vector.

27. The cell of claim 22 or 26, wherein the cell is an insect cell.

28. A method of producing the rAAV of any one of claims 15, 16, 18, and 19, the method comprising:

(i) delivering to a cell a first vector encoding one or more AAV rep proteins and/or one or more AAV cap proteins, and a second rAAV vector comprising an expression construct comprising a CBA promoter, a CMV enhancer, a WPRE, a Bovine Growth Hormone polyA signal tail, and the nucleotide sequence of SEQ ID NO: 15, and two AAV ITR sequences flanking the expression construct;

(ii) culturing the cells under conditions allowing for packaging the rAAV; and

(iii) harvesting the cultured cells or culture medium for collection of the rAAV.

29. A method for treating a subject having or suspected of having Parkinson's disease or Gaucher disease, the method comprising administering to the subject an isolated nucleic acid of any one of claims 1-7, the rAAV vector of any one of claims 8-14 and 17, or the rAAV of any one of claims 15, 16, 18, and 19.

30. The method of claim 29, wherein the administration comprises direct injection to the CNS of the subject.

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31. The method of claim 30, wherein the direct injection is intracerebral injection, intraparenchymal injection, intrathecal injection, intra-cisterna magna injection or any combination thereof.

5 32. The method of claim 30 or 31, wherein the direct injection to the CNS of the subject comprises convection enhanced delivery (CED).

33. The method of claim 29, wherein the administration comprises peripheral injection.

10

34. The method of claim 33, wherein the peripheral injection is intravenous injection.

35. Use of an isolated nucleic acid of any one of claims 1-7, the rAAV vector of any one of claims 8-14 and 17, or the rAAV of any one of claims 15, 16, 18, and 19 in the manufacture of a medicament for treating a subject having or suspected of having Parkinson's disease or Gaucher disease.

15

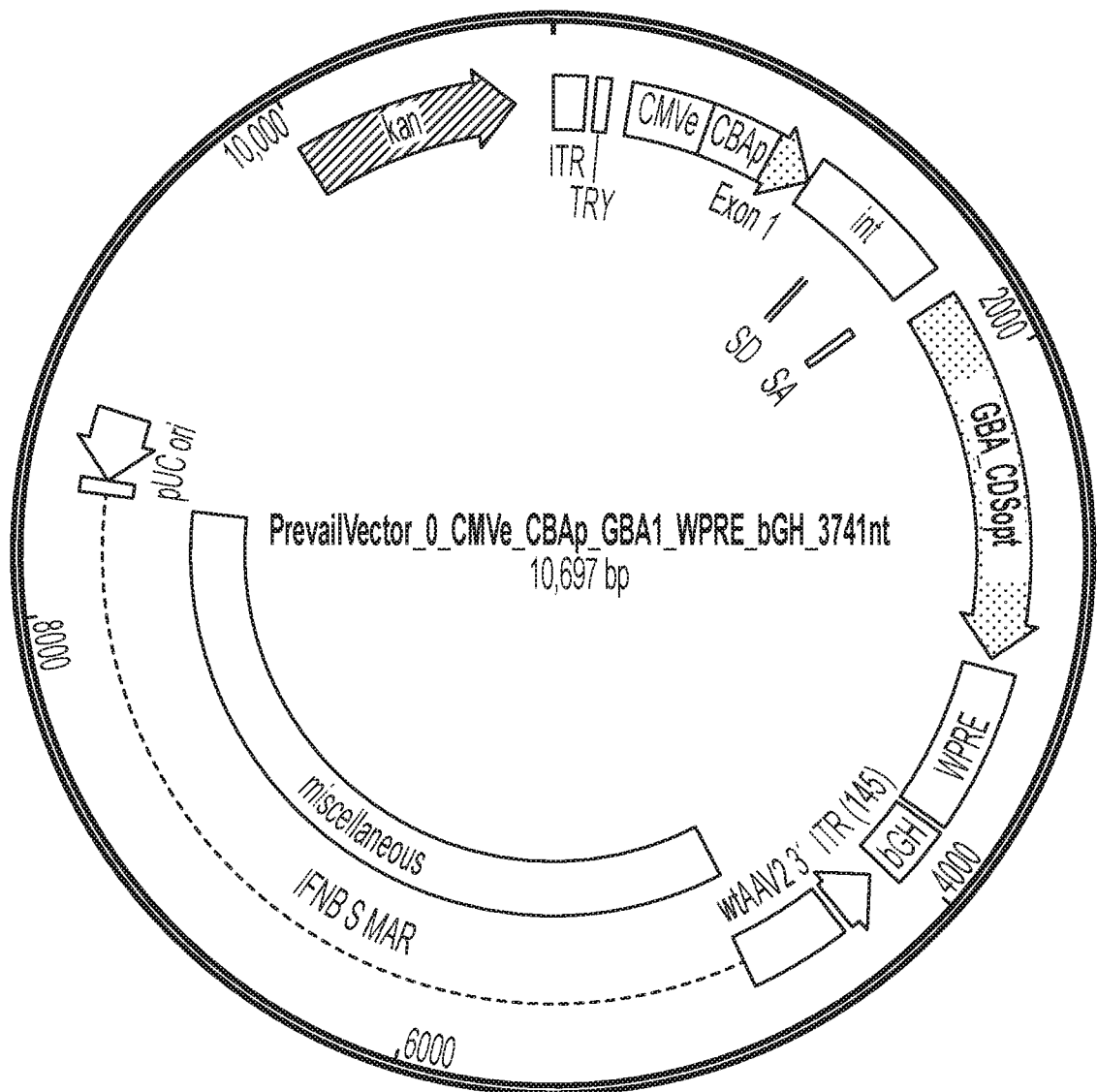


FIG. 1

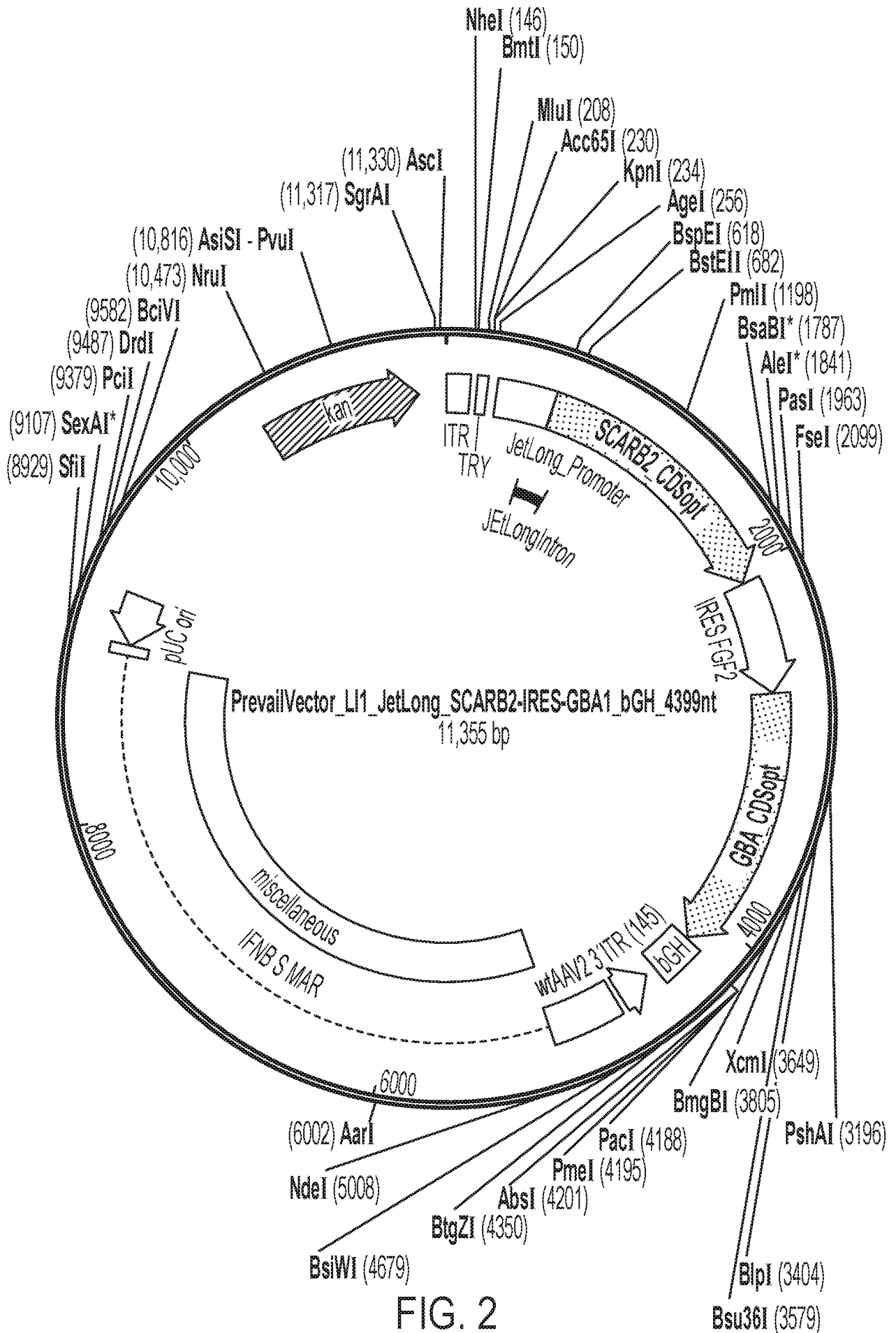
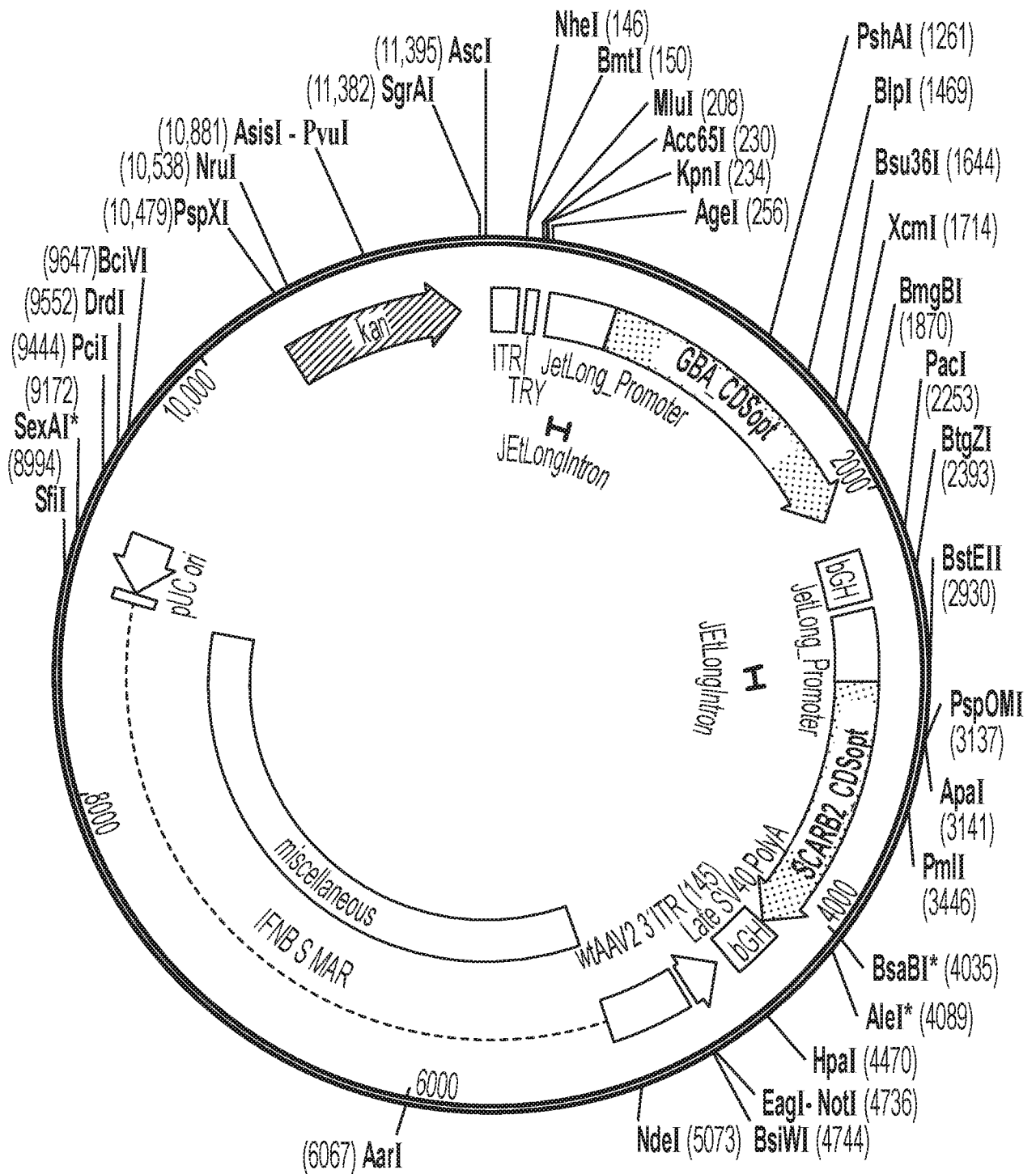


FIG. 2

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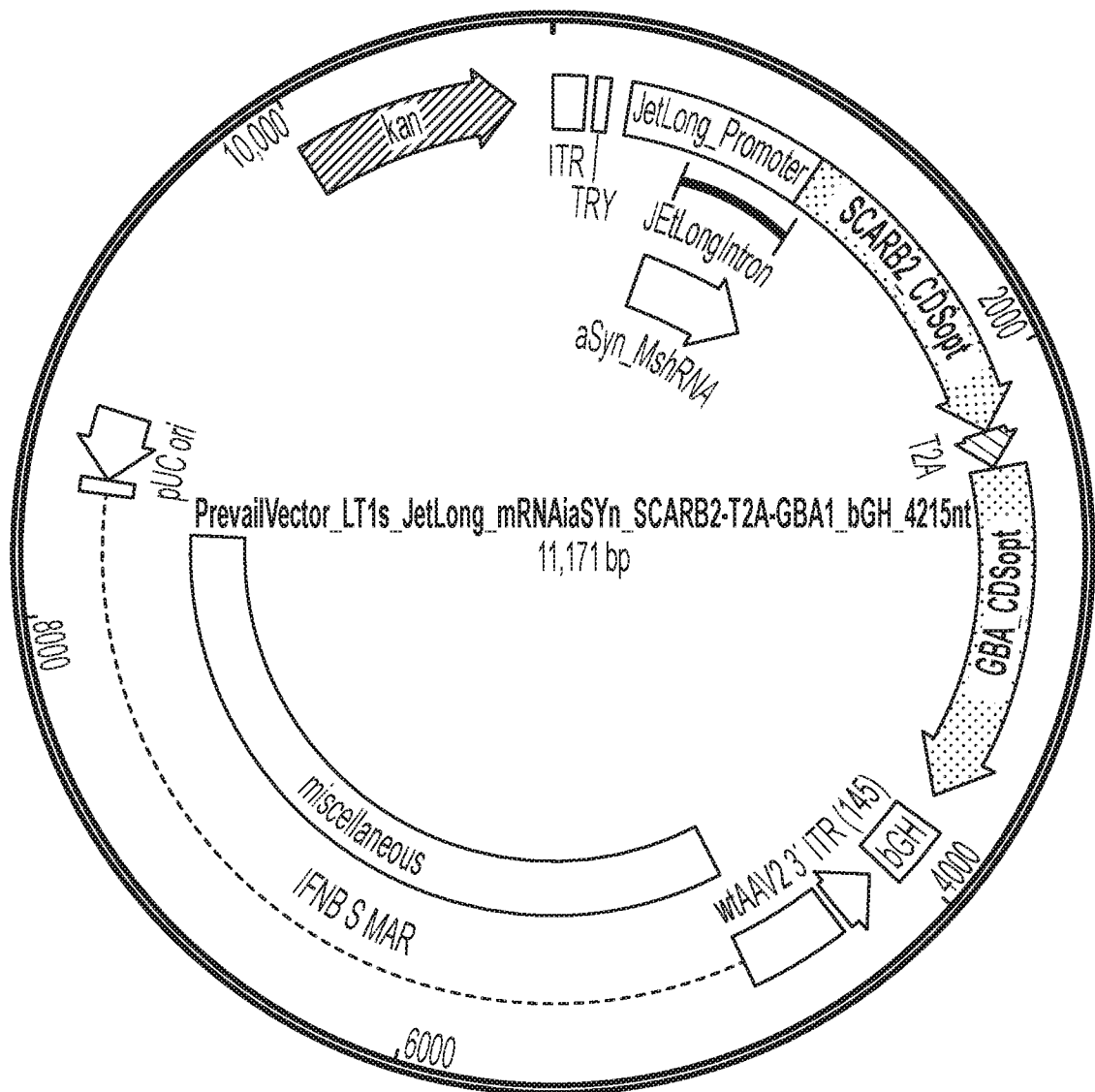


FIG. 4

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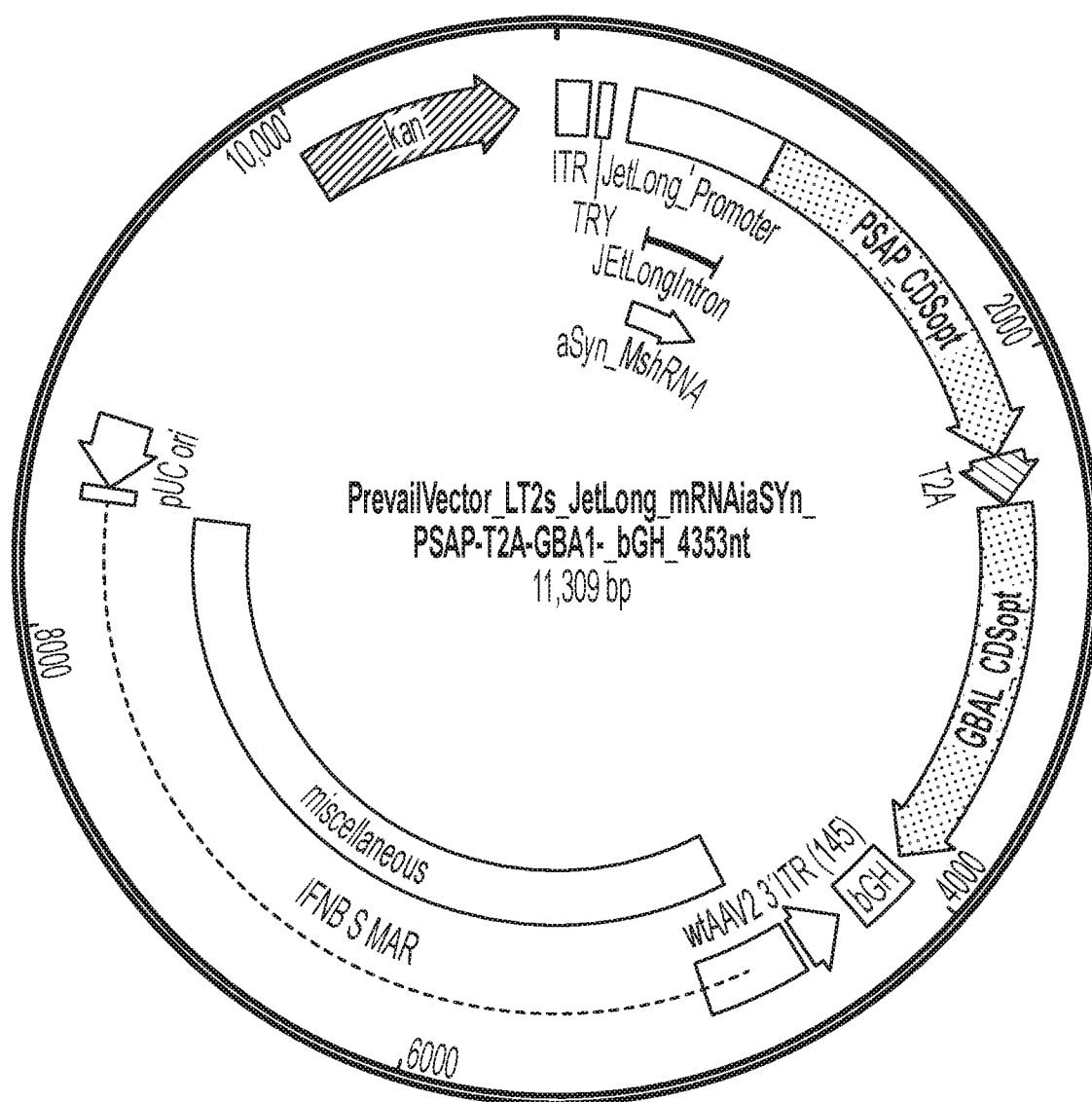


FIG. 5

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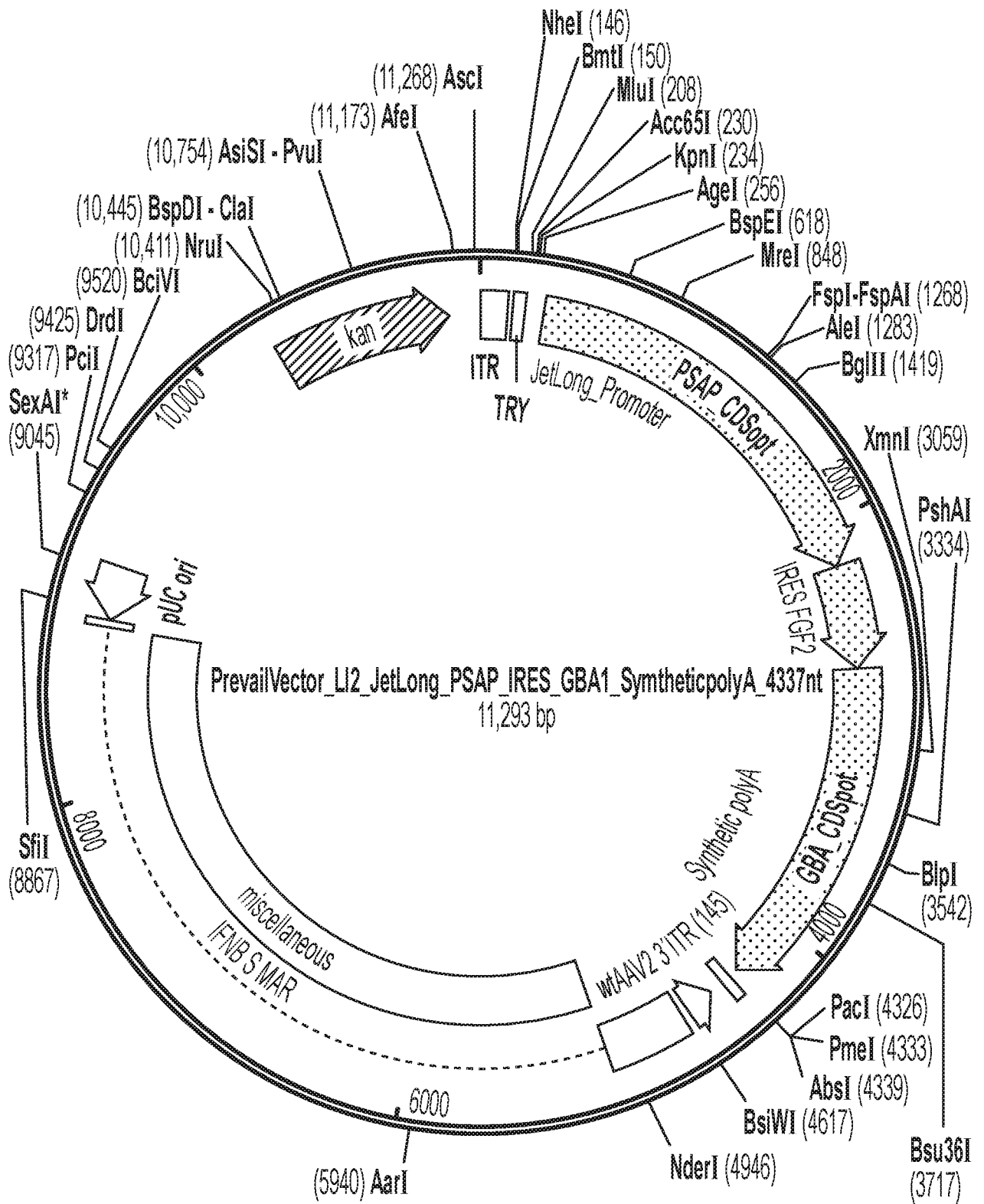


FIG. 6

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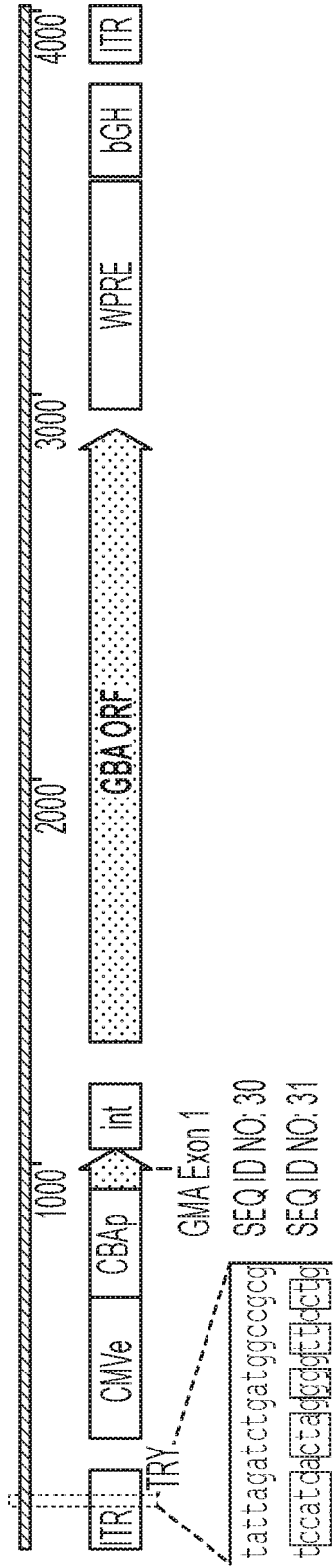


FIG. 7

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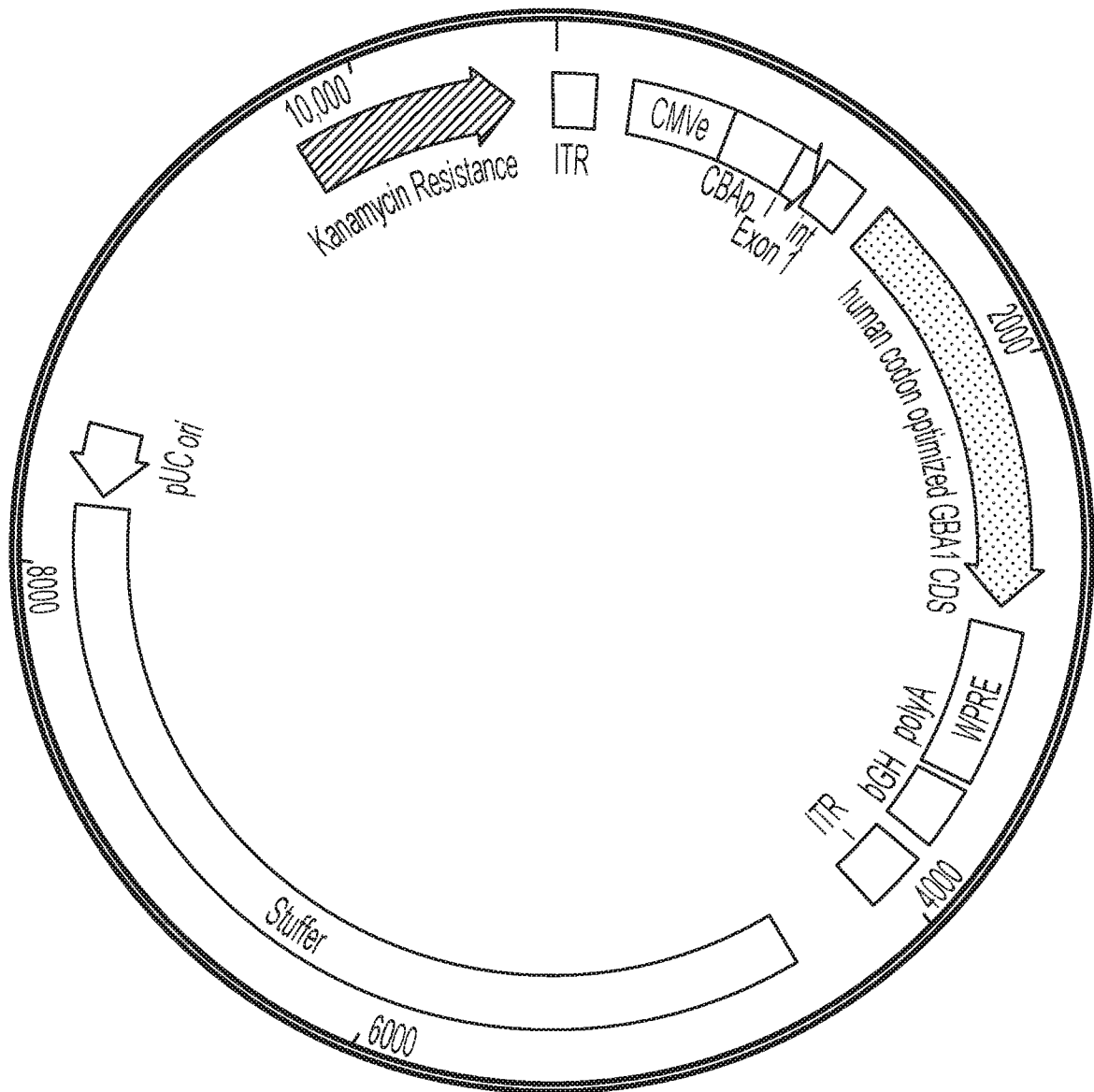


FIG. 8

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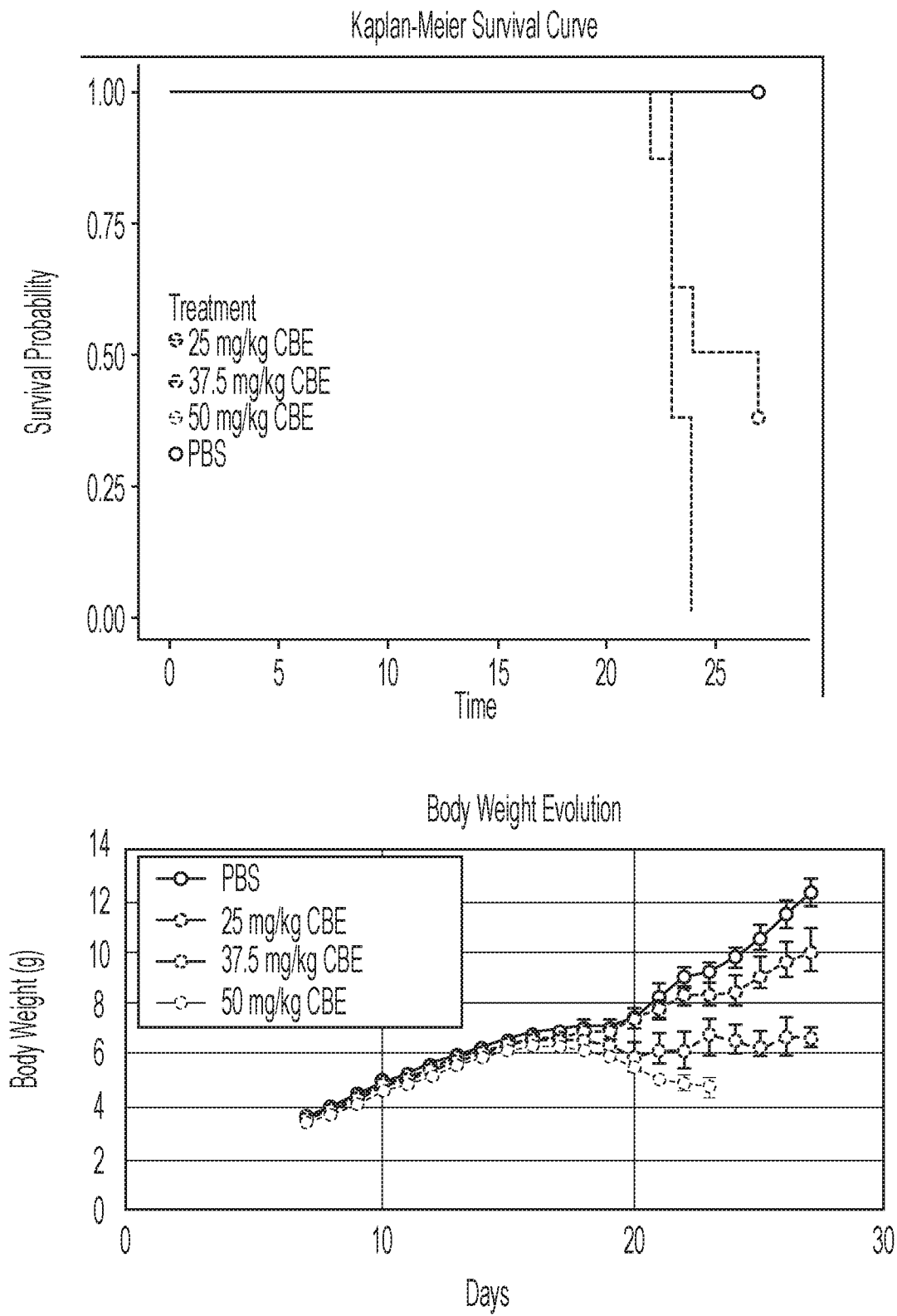


FIG. 9

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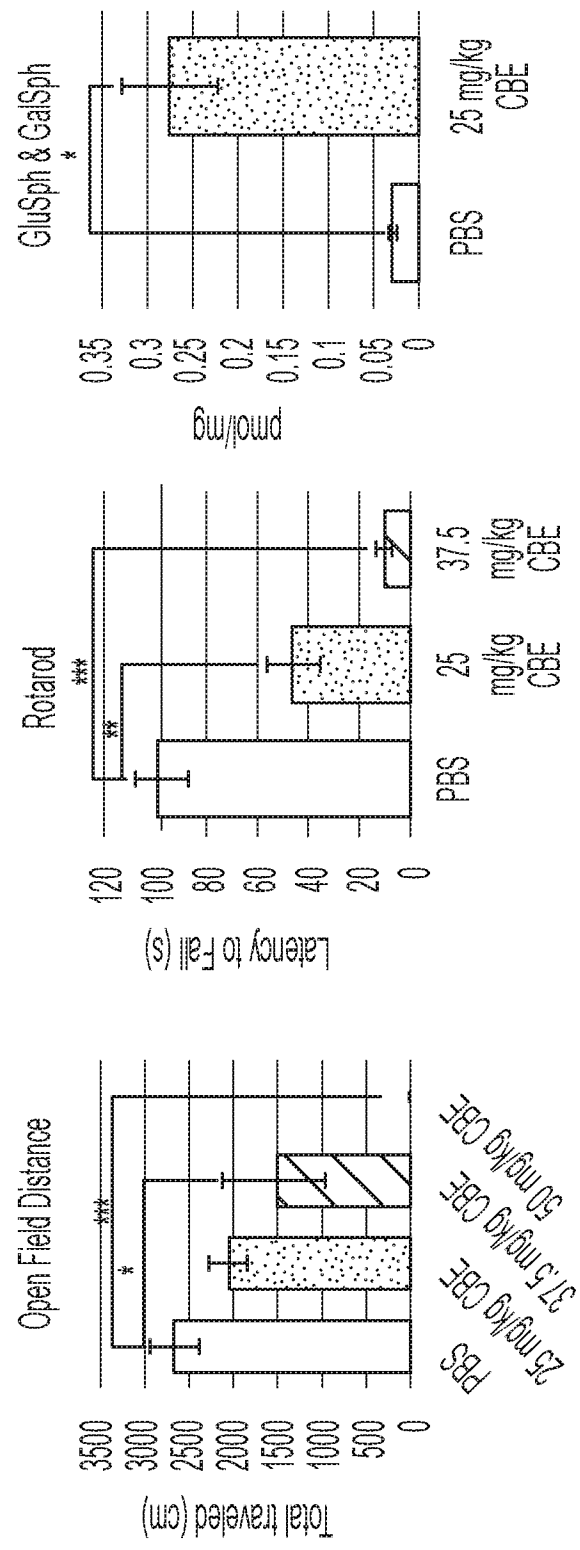


FIG. 9 cont.

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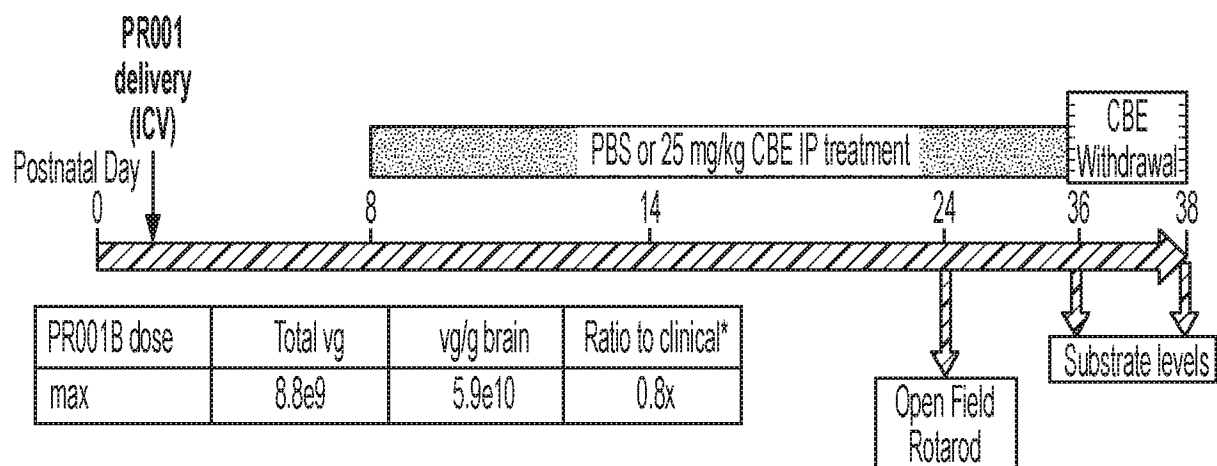


FIG. 10

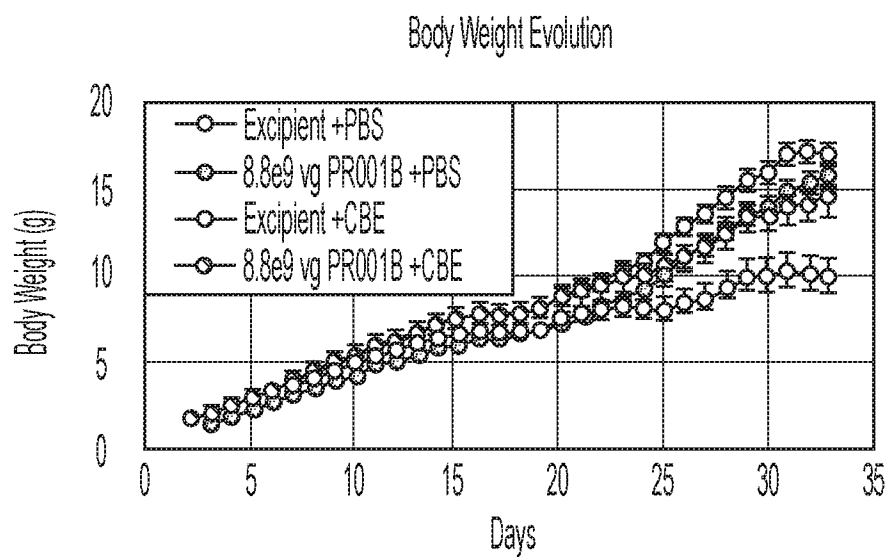


FIG. 11

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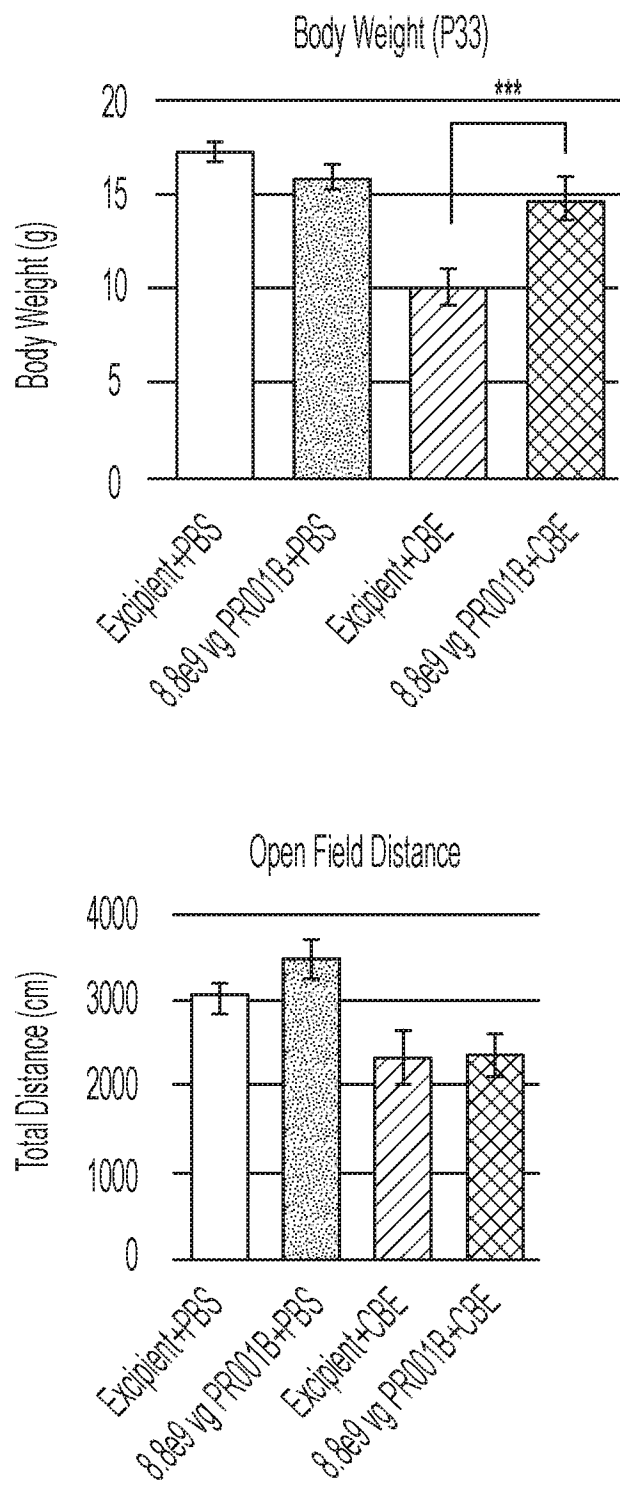


FIG. 11 cont.

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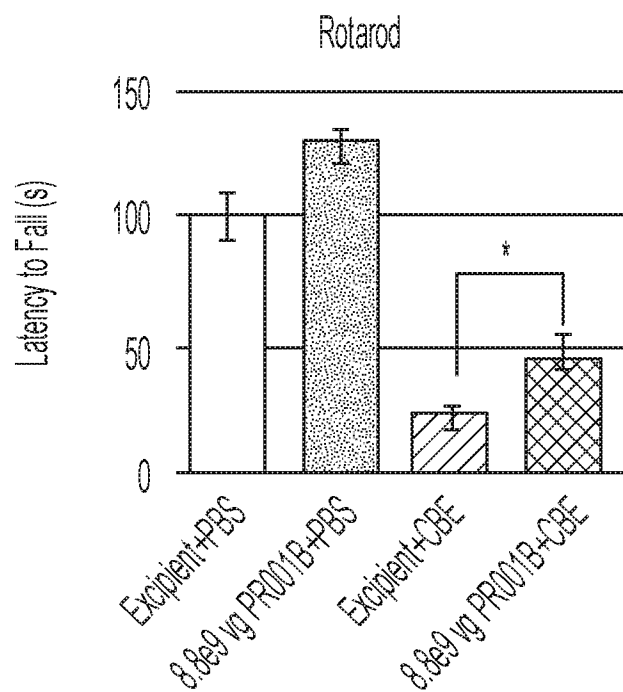


FIG. 11 cont.

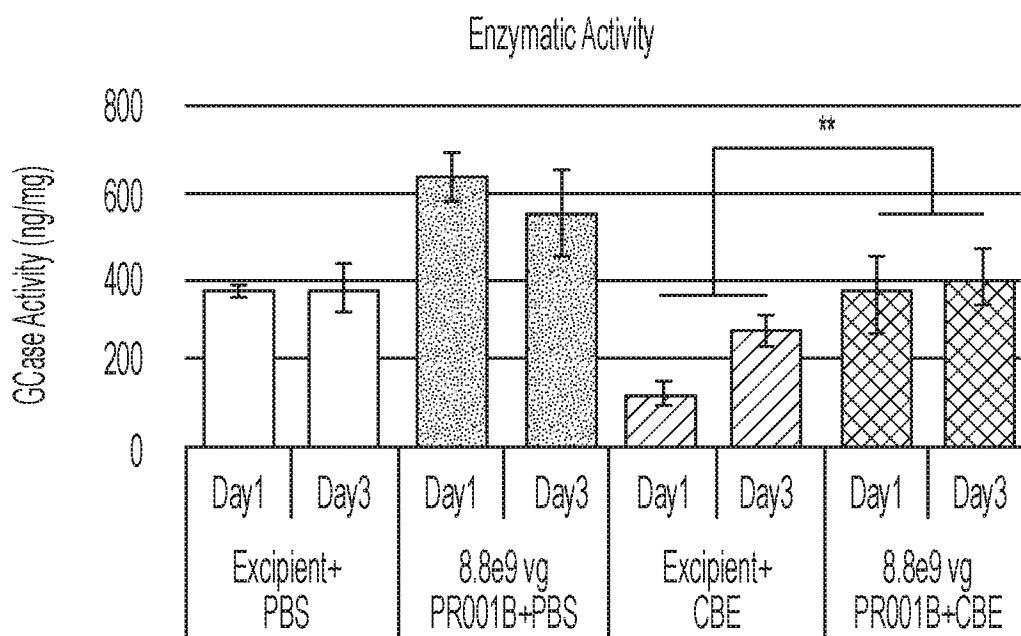


FIG. 12

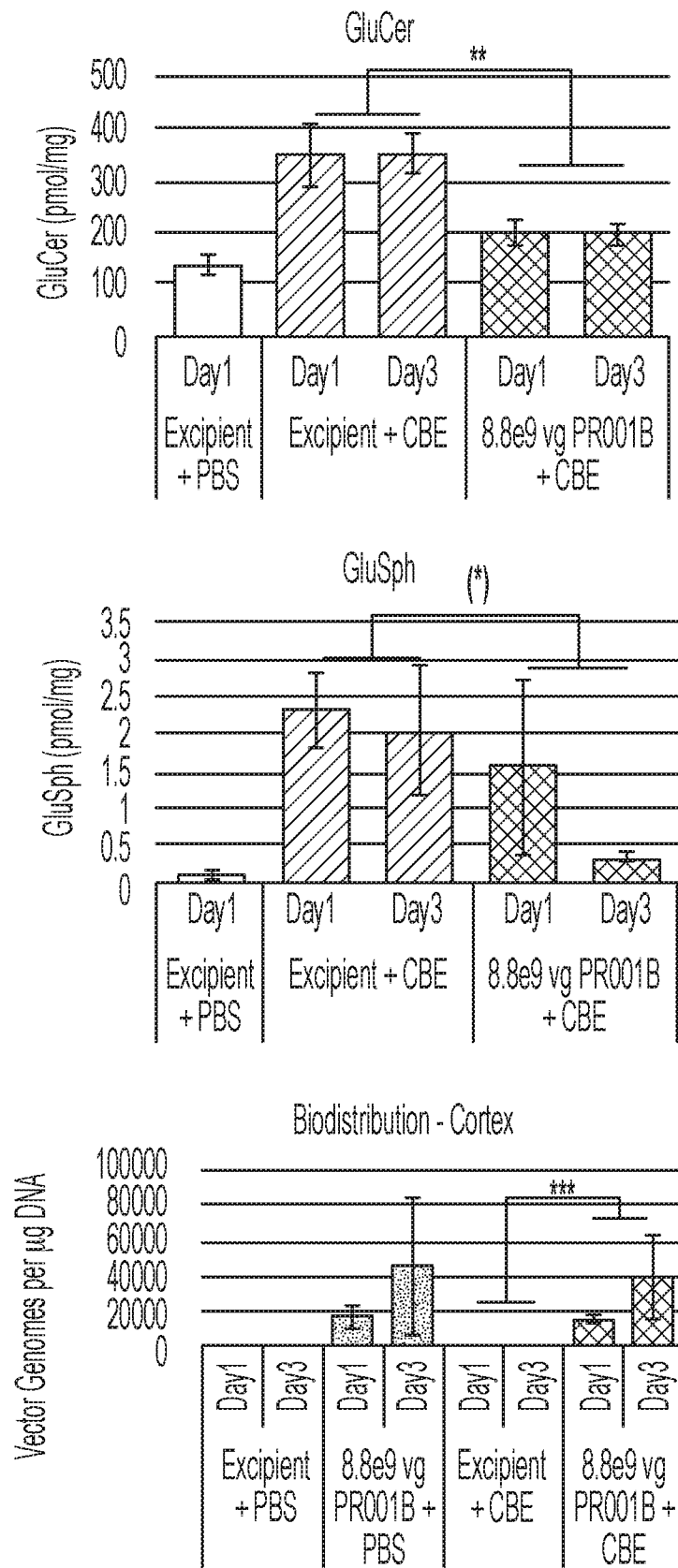


FIG. 12 cont.

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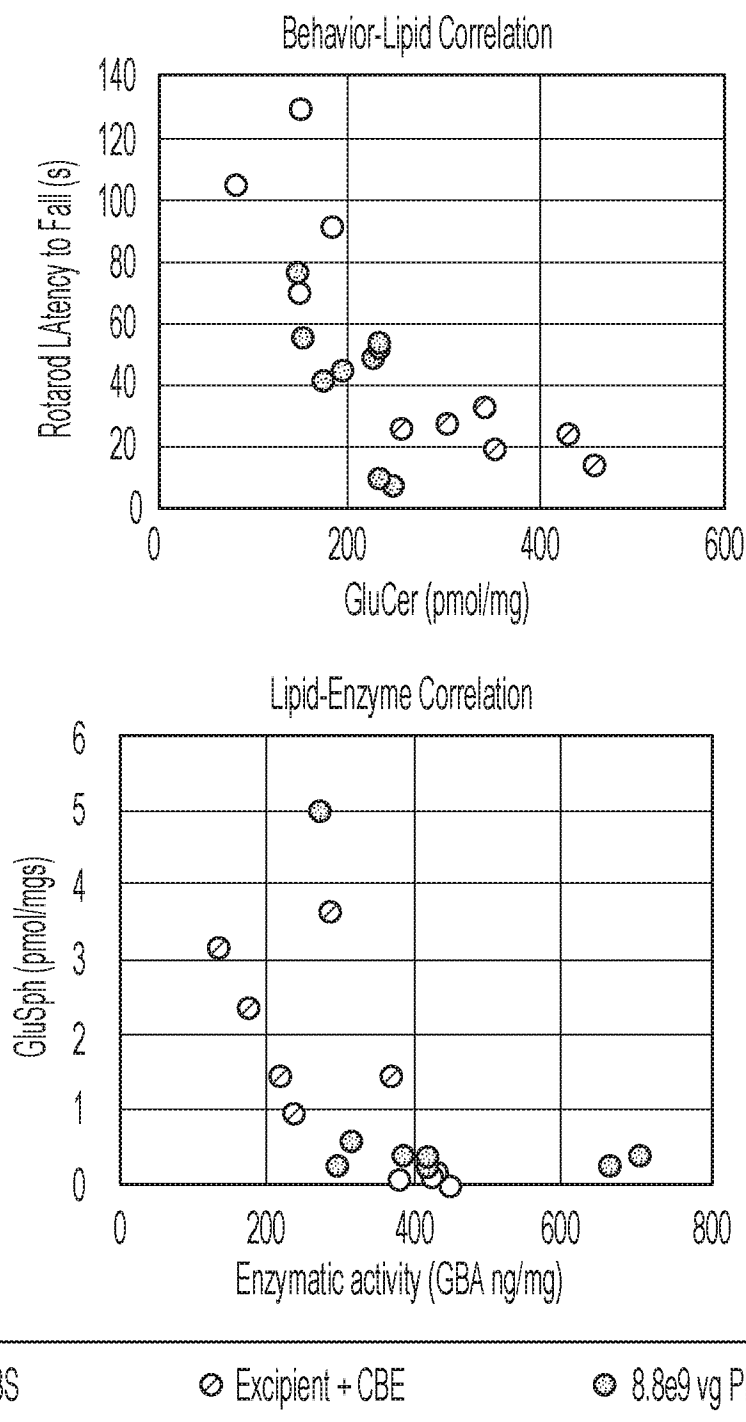


FIG. 13

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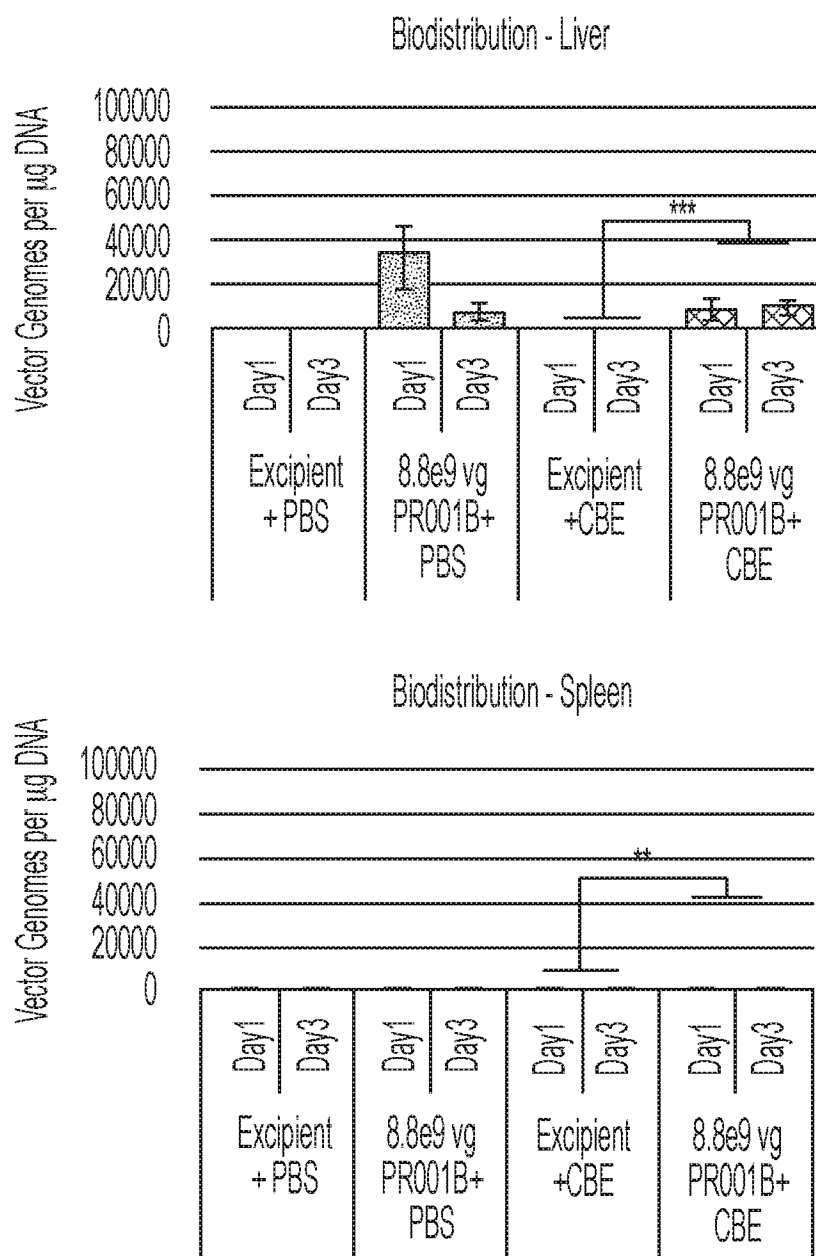


FIG. 14

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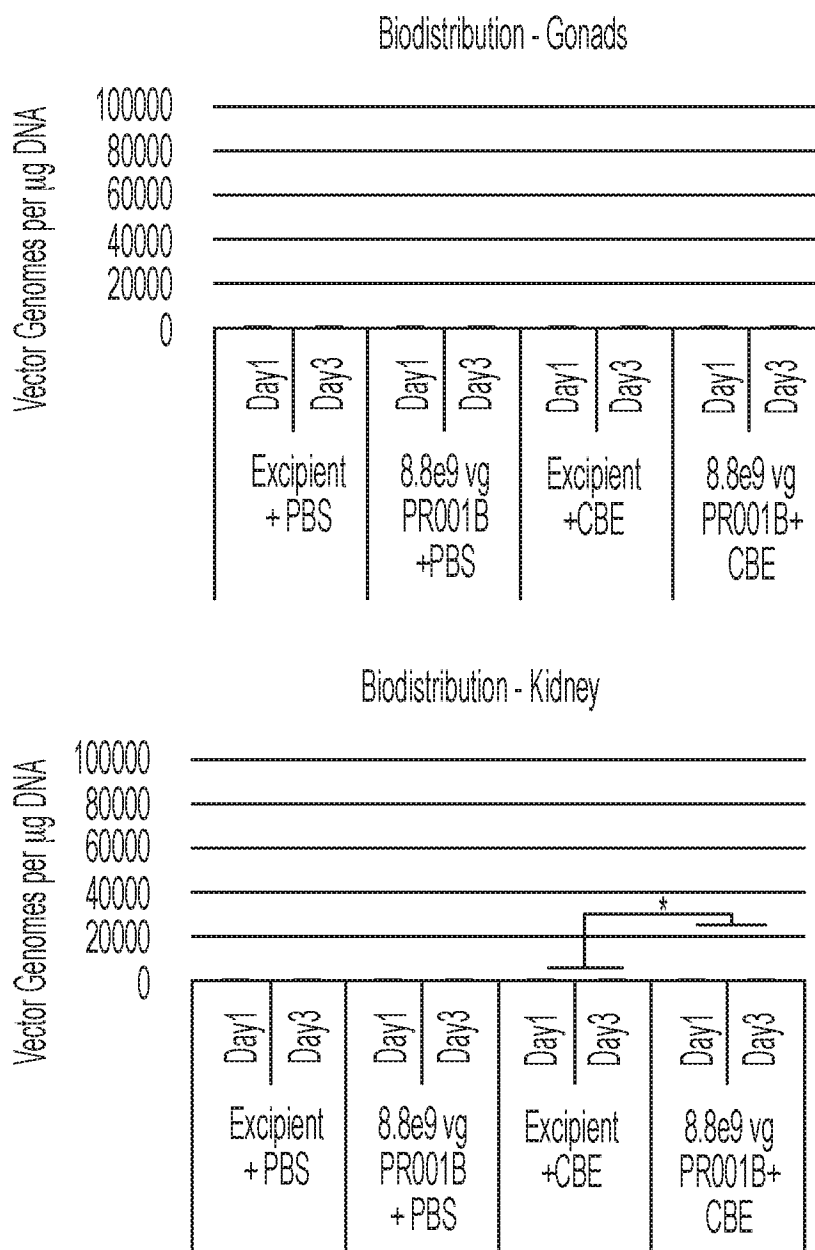


FIG. 14 cont.

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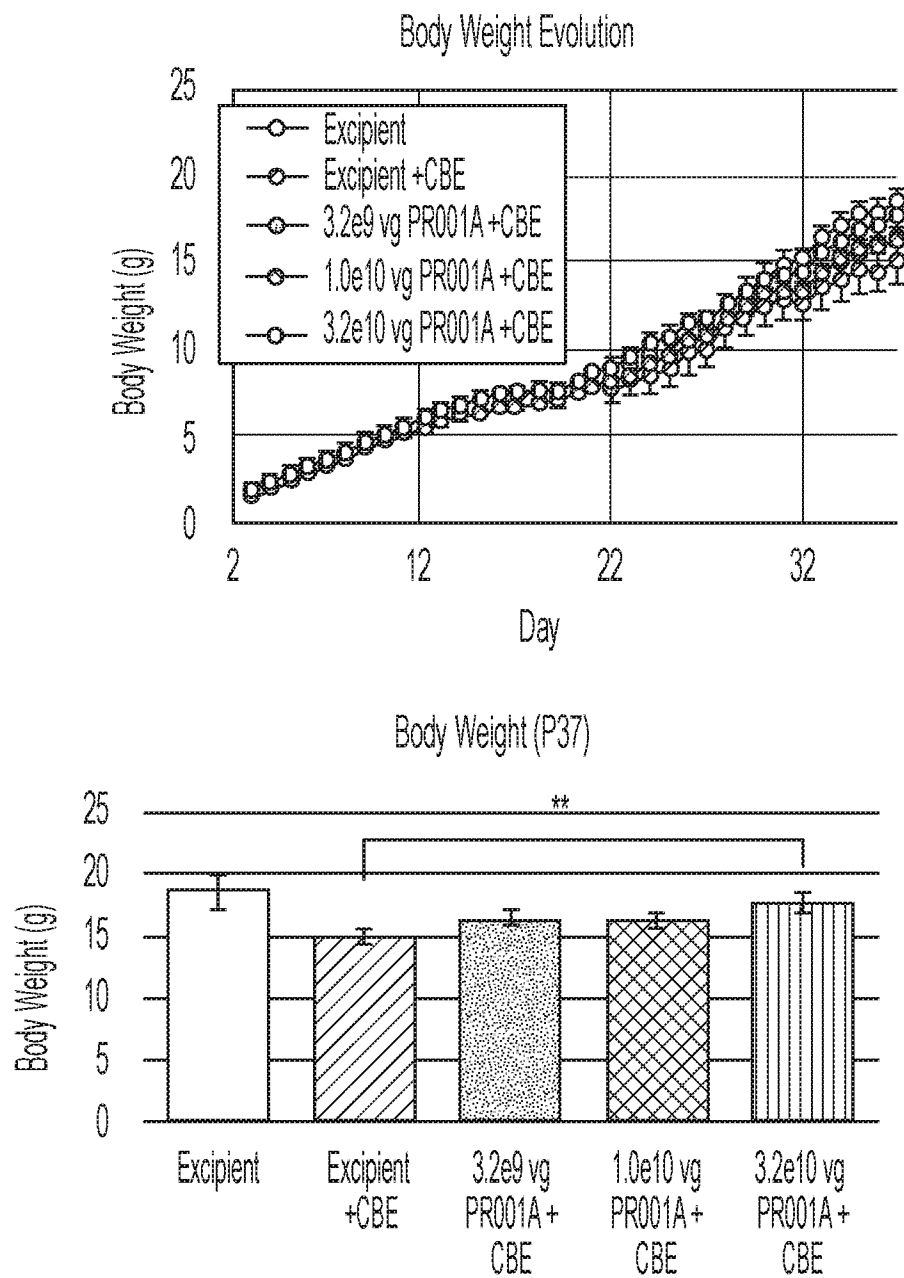


FIG. 15

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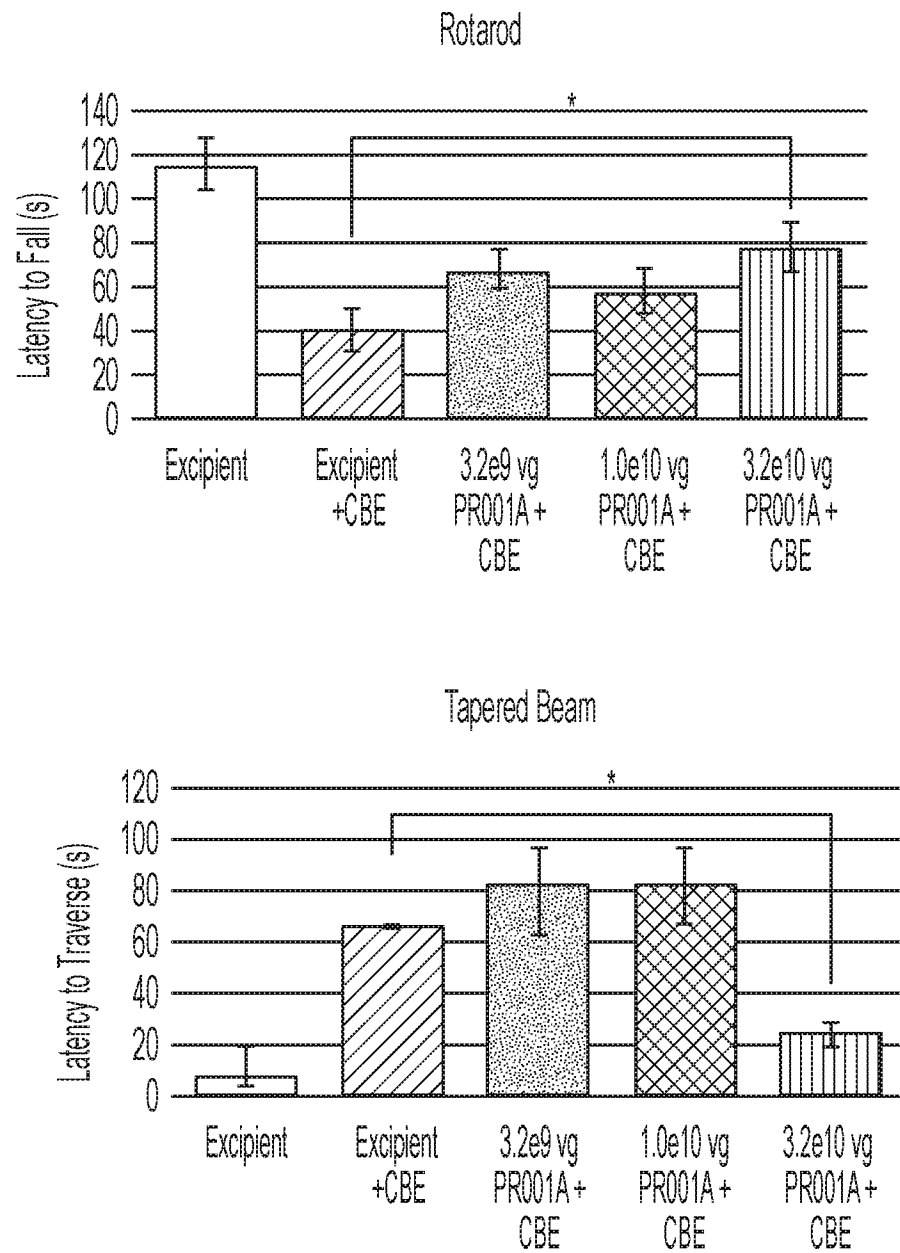


FIG. 15 cont.

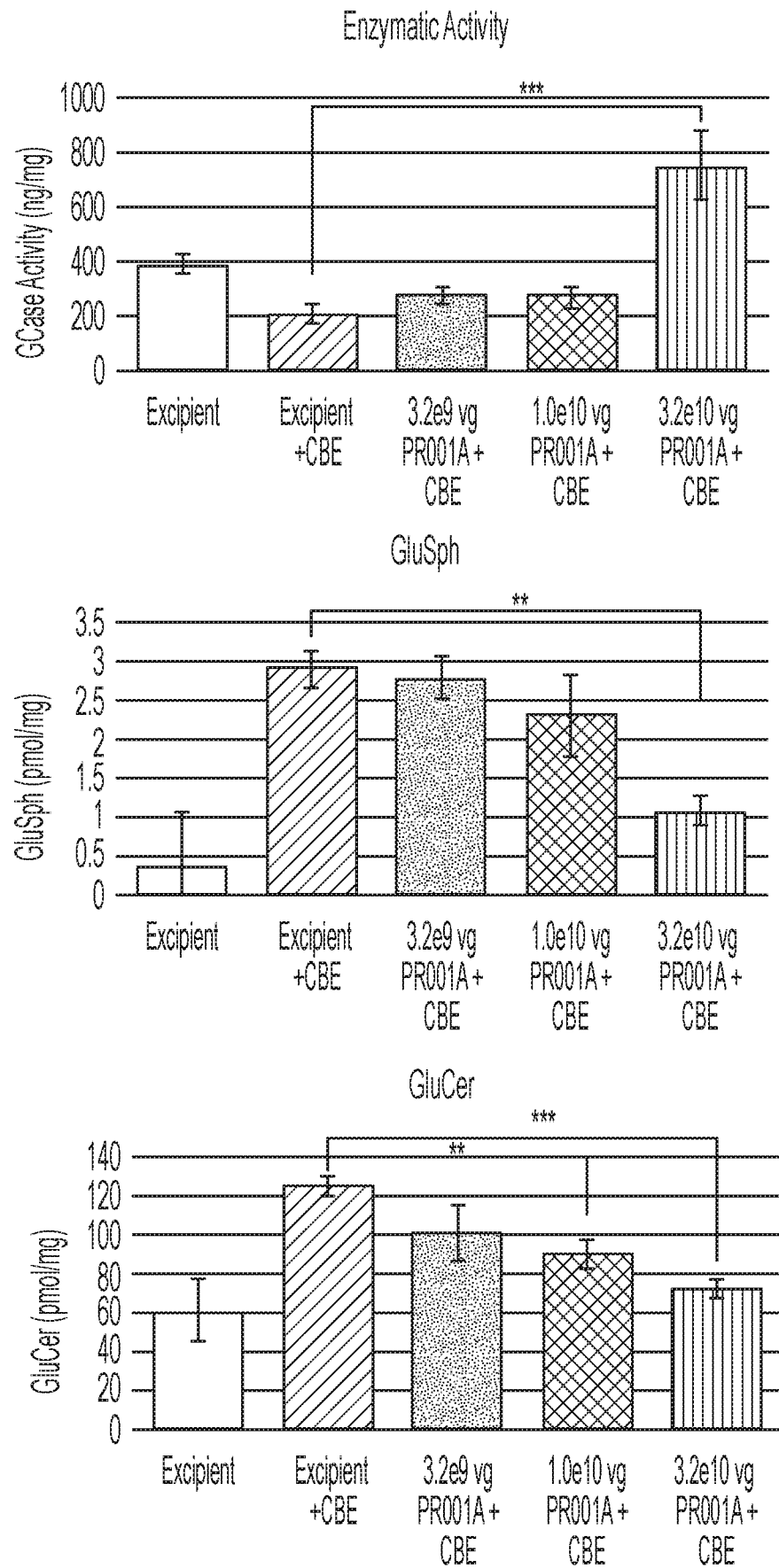


FIG. 16

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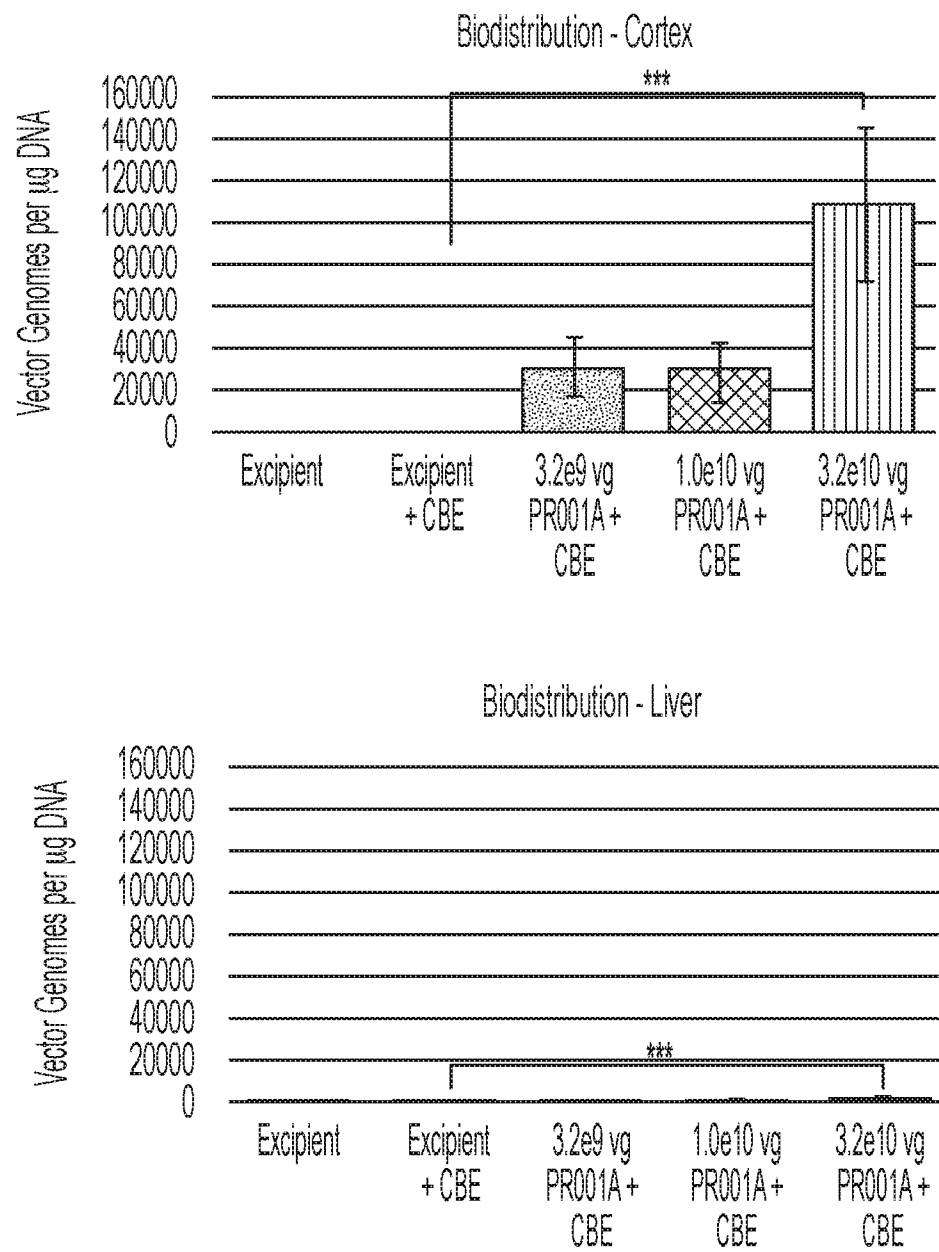


FIG. 16 cont.

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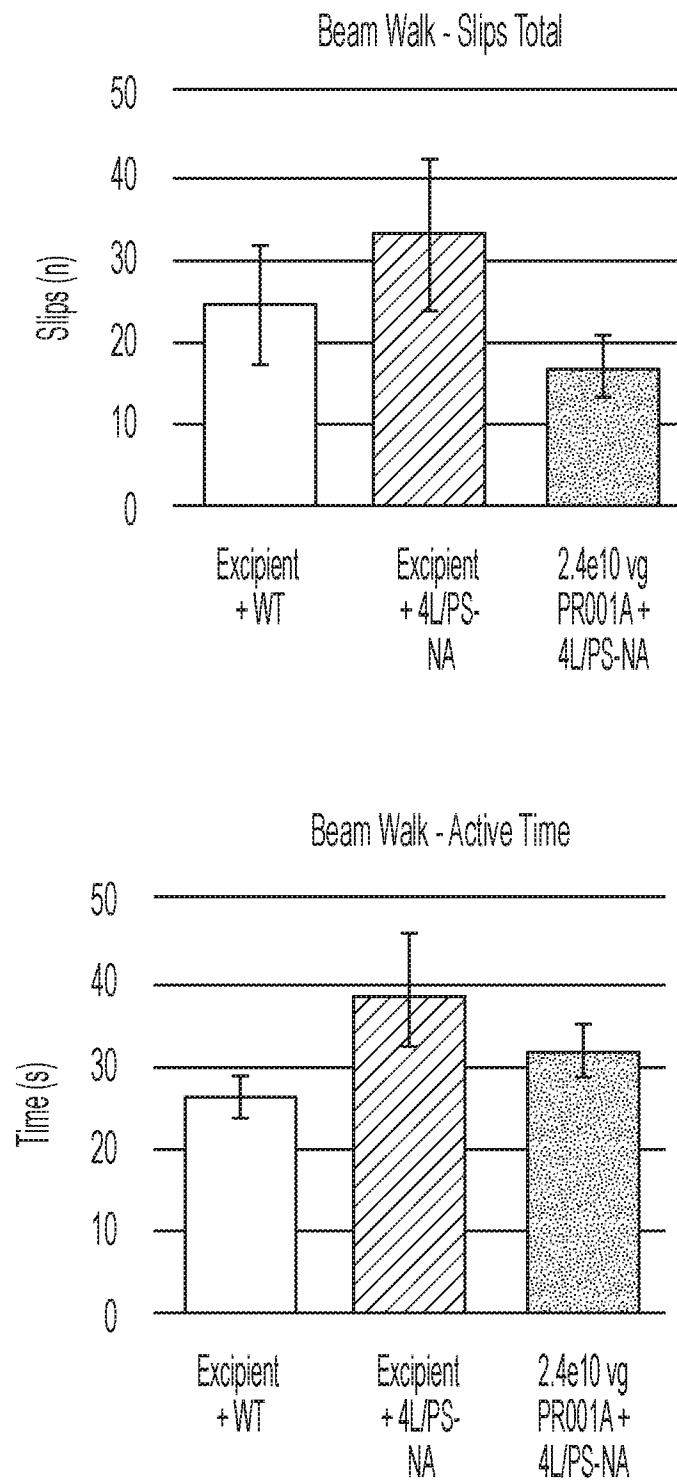


FIG. 17

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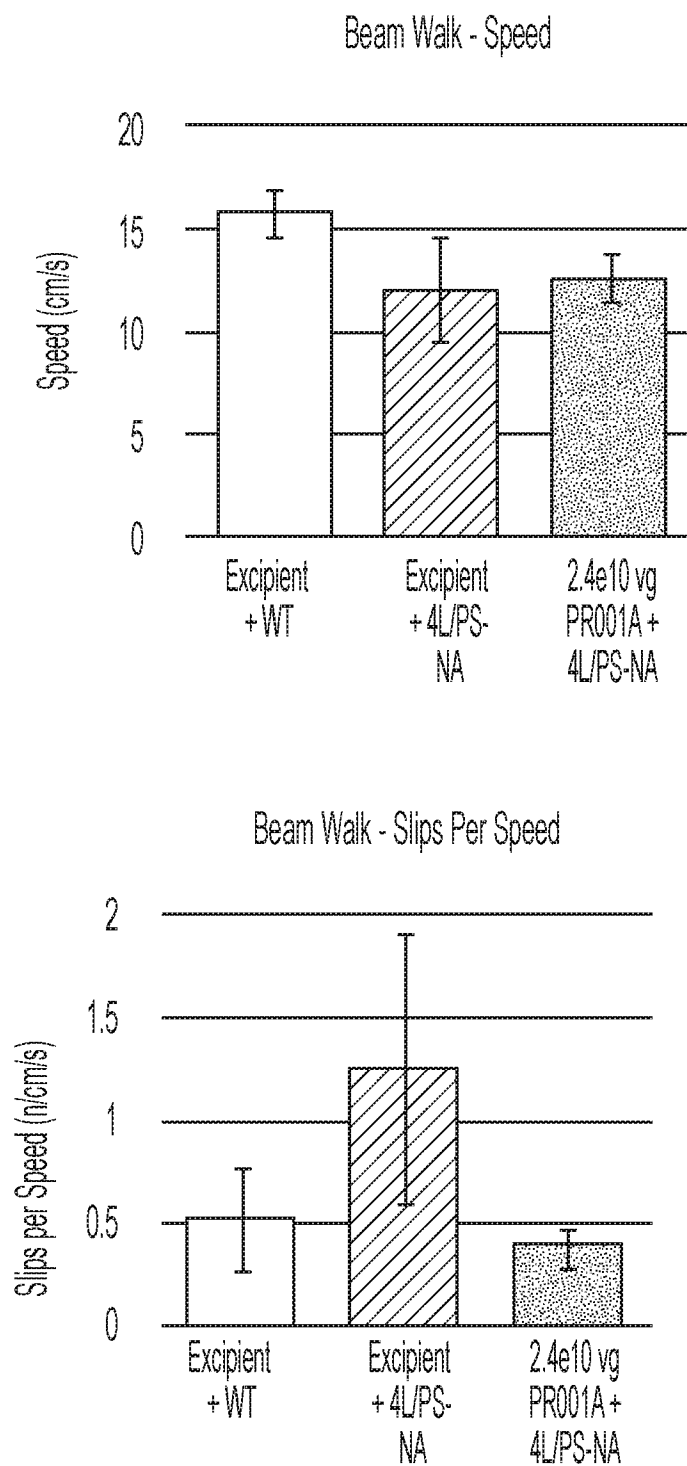


FIG. 17 cont.

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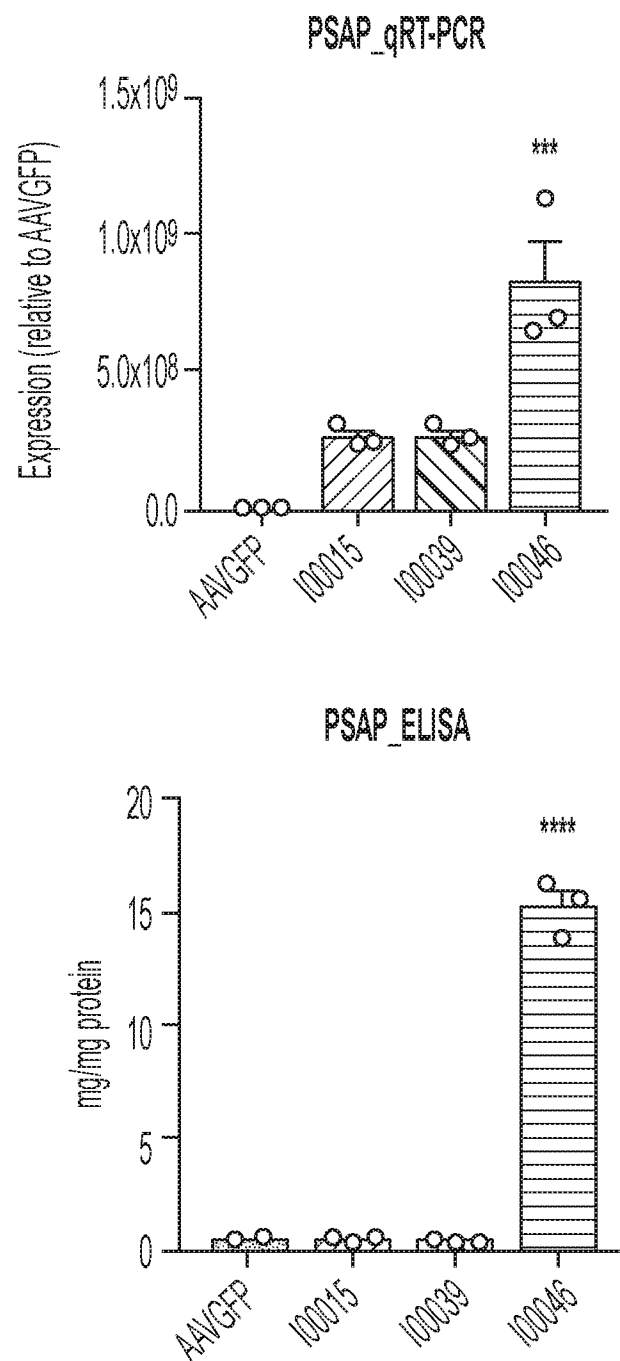


FIG. 18

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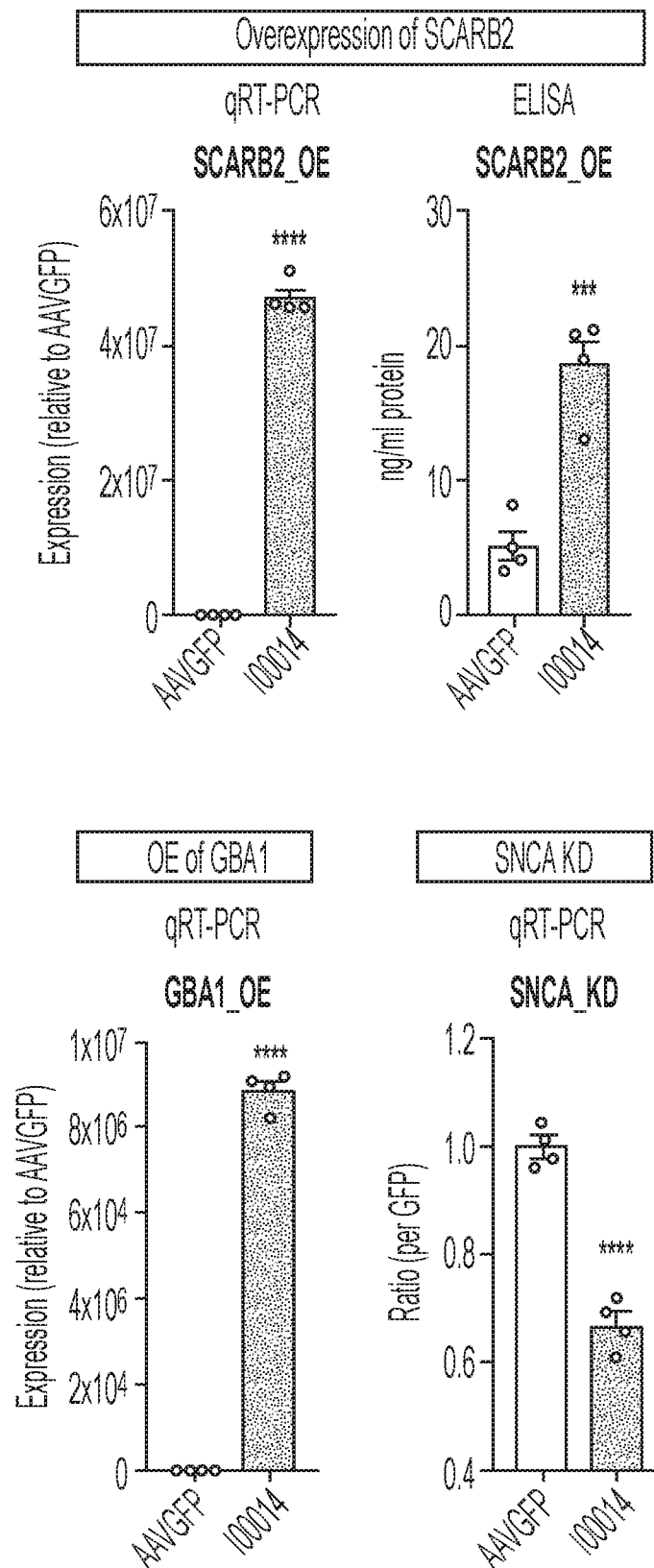


FIG. 18 cont.

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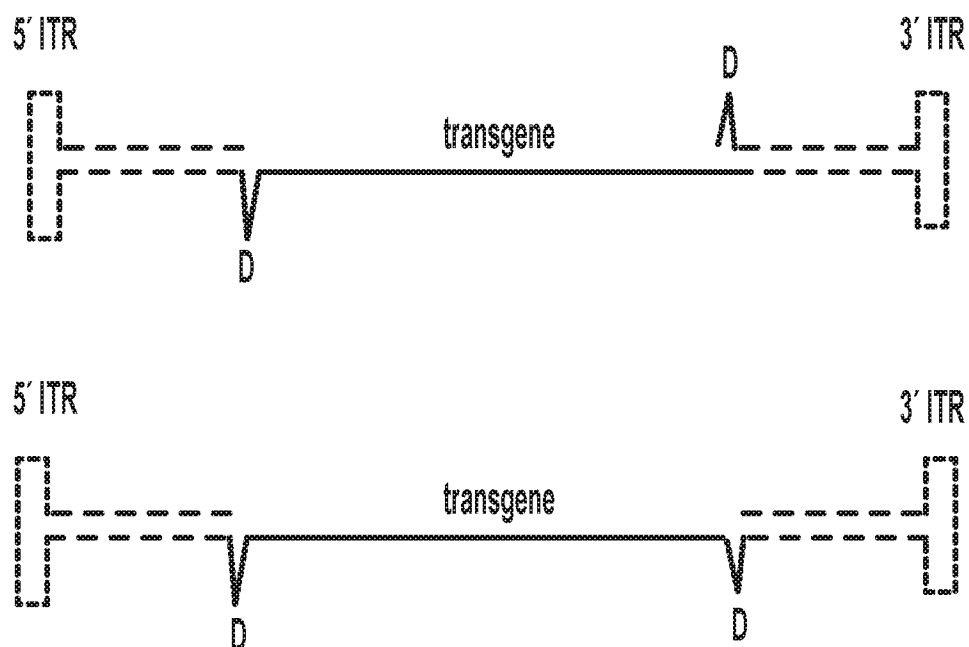


FIG. 19

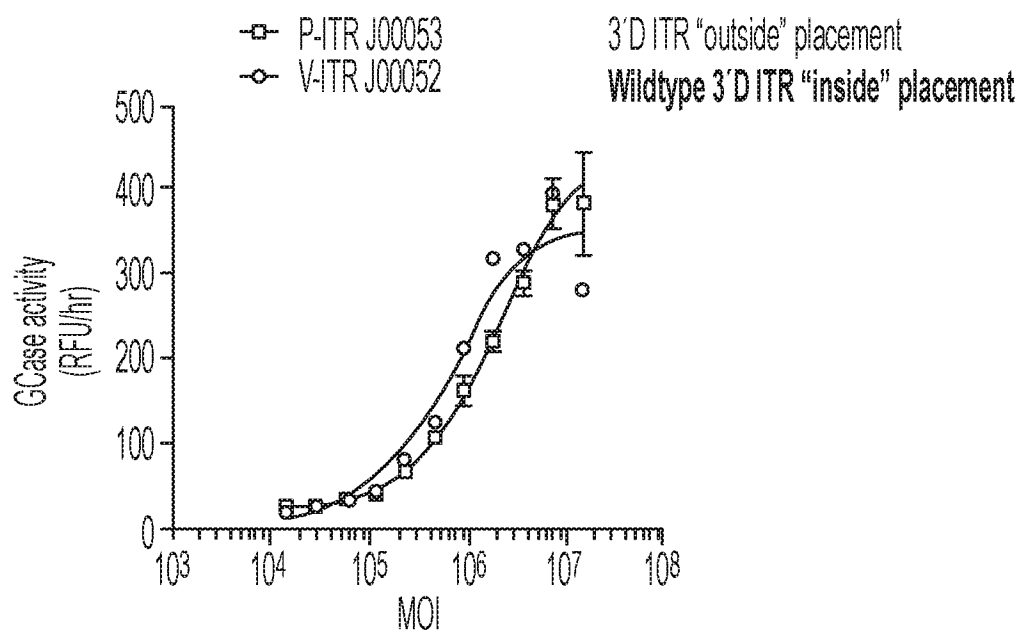


FIG. 20

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<141> Concurrently herewith
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<160> 31
<170> PatentIn version 3.5
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<220>
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<210> 14
 <211> 536
 <212> PRT
 <213> Homo sapiens

<400> 14

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Arg Val Ser Ile Met Ala Gly Ser Leu Thr Gly Leu Leu Leu Leu Gln
 20 25 30

Ala Val Ser Trp Ala Ser Gly Ala Arg Pro Cys Ile Pro Lys Ser Phe
 35 40 45

Gly Tyr Ser Ser Val Val Cys Val Cys Asn Ala Thr Tyr Cys Asp Ser
 50 55 60

Phe Asp Pro Pro Thr Phe Pro Ala Leu Gly Thr Phe Ser Arg Tyr Glu
 65 70 75 80

Ser Thr Arg Ser Gly Arg Arg Met Glu Leu Ser Met Gly Pro Ile Gln
 85 90 95

Ala Asn His Thr Gly Thr Gly Leu Leu Leu Thr Leu Gln Pro Glu Gln
 100 105 110

Lys Phe Gln Lys Val Lys Gly Phe Gly Gly Ala Met Thr Asp Ala Ala
 115 120 125

Ala Leu Asn Ile Leu Ala Leu Ser Pro Pro Ala Gln Asn Leu Leu Leu
 130 135 140

Lys Ser Tyr Phe Ser Glu Glu Gly Ile Gly Tyr Asn Ile Ile Arg Val
 145 150 155 160

Pro Met Ala Ser Cys Asp Phe Ser Ile Arg Thr Tyr Thr Tyr Ala Asp

165

170

175

Thr Pro Asp Asp Phe Gln Leu His Asn Phe Ser Leu Pro Glu Glu Asp
 180 185 190

Thr Lys Leu Lys Ile Pro Leu Ile His Arg Ala Leu Gln Leu Ala Gln
 195 200 205

Arg Pro Val Ser Leu Leu Ala Ser Pro Trp Thr Ser Pro Thr Trp Leu
 210 215 220

Lys Thr Asn Gly Ala Val Asn Gly Lys Gly Ser Leu Lys Gly Gln Pro
 225 230 235 240

Gly Asp Ile Tyr His Gln Thr Trp Ala Arg Tyr Phe Val Lys Phe Leu
 245 250 255

Asp Ala Tyr Ala Glu His Lys Leu Gln Phe Trp Ala Val Thr Ala Glu
 260 265 270

Asn Glu Pro Ser Ala Gly Leu Leu Ser Gly Tyr Pro Phe Gln Cys Leu
 275 280 285

Gly Phe Thr Pro Glu His Gln Arg Asp Phe Ile Ala Arg Asp Leu Gly
 290 295 300

Pro Thr Leu Ala Asn Ser Thr His His Asn Val Arg Leu Leu Met Leu
 305 310 315 320

Asp Asp Gln Arg Leu Leu Leu Pro His Trp Ala Lys Val Val Leu Thr
 325 330 335

Asp Pro Glu Ala Ala Lys Tyr Val His Gly Ile Ala Val His Trp Tyr
 340 345 350

Leu Asp Phe Leu Ala Pro Ala Lys Ala Thr Leu Gly Glu Thr His Arg

355

360

365

Leu Phe Pro Asn Thr Met Leu Phe Ala Ser Glu Ala Cys Val Gly Ser
 370 375 380

Lys Phe Trp Glu Gln Ser Val Arg Leu Gly Ser Trp Asp Arg Gly Met
 385 390 395 400

Gln Tyr Ser His Ser Ile Ile Thr Asn Leu Leu Tyr His Val Val Gly
 405 410 415

Trp Thr Asp Trp Asn Leu Ala Leu Asn Pro Glu Gly Gly Pro Asn Trp
 420 425 430

Val Arg Asn Phe Val Asp Ser Pro Ile Ile Val Asp Ile Thr Lys Asp
 435 440 445

Thr Phe Tyr Lys Gln Pro Met Phe Tyr His Leu Gly His Phe Ser Lys
 450 455 460

Phe Ile Pro Glu Gly Ser Gln Arg Val Gly Leu Val Ala Ser Gln Lys
 465 470 475 480

Asn Asp Leu Asp Ala Val Ala Leu Met His Pro Asp Gly Ser Ala Val
 485 490 495

Val Val Val Leu Asn Arg Ser Ser Lys Asp Val Pro Leu Thr Ile Lys
 500 505 510

Asp Pro Ala Val Gly Phe Leu Glu Thr Ile Ser Pro Gly Tyr Ser Ile
 515 520 525

His Thr Tyr Leu Trp Arg Arg Gln
 530 535

<210> 15

<211> 1608

<212> DNA

<213> Homo sapiens

<400> 15

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tactgcgaca gtttcgacct tcctaccttt cctgctctgg gcaccttcag cagatacgag	240
agcaccagat ccggcagacg gatggaactg agcatgggac ccatccaggc caatcacaca	300
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cccatggcca gctgcgactt cagcatcagg acctacacct acgccgacac acccgacgat	540
ttccagctgc acaacttcag cctgcctgaa gaggacacca agctgaagat ccctctgata	600
cacagagccc tgcagctggc acaaagacct gtgtcactgc tggcctctcc atggacatct	660
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cagtacagcc acagcatcat caccaacctg ctgtaccacg tcgtcggctg gaccgactgg	1260
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aacgatctgg acgccgtggc tctgatgcac cctgatggat ctgctgtggt ggtggtcctg    1500
aaccgcagca gcaaagatgt gcccctgacc atcaaggatc cgcctgtggg attcctggaa    1560
acaatcagcc ctggctactc catccacacc tacctgtggc gtagacag                    1608

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<210> 16
 <211> 524
 <212> PRT
 <213> Homo sapiens

<400> 16

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Met Tyr Ala Leu Phe Leu Leu Ala Ser Leu Leu Gly Ala Ala Leu Ala
1           5           10          15

```

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Gly Pro Val Leu Gly Leu Lys Glu Cys Thr Arg Gly Ser Ala Val Trp
          20          25          30

```

```

Cys Gln Asn Val Lys Thr Ala Ser Asp Cys Gly Ala Val Lys His Cys
      35           40           45

```

```

Leu Gln Thr Val Trp Asn Lys Pro Thr Val Lys Ser Leu Pro Cys Asp
      50           55           60

```

```

Ile Cys Lys Asp Val Val Thr Ala Ala Gly Asp Met Leu Lys Asp Asn
65           70           75          80

```

```

Ala Thr Glu Glu Glu Ile Leu Val Tyr Leu Glu Lys Thr Cys Asp Trp
          85           90          95

```

```

Leu Pro Lys Pro Asn Met Ser Ala Ser Cys Lys Glu Ile Val Asp Ser
      100          105          110

```

```

Tyr Leu Pro Val Ile Leu Asp Ile Ile Lys Gly Glu Met Ser Arg Pro

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115

120

125

Gly Glu Val Cys Ser Ala Leu Asn Leu Cys Glu Ser Leu Gln Lys His
 130 135 140

Leu Ala Glu Leu Asn His Gln Lys Gln Leu Glu Ser Asn Lys Ile Pro
 145 150 155 160

Glu Leu Asp Met Thr Glu Val Val Ala Pro Phe Met Ala Asn Ile Pro
 165 170 175

Leu Leu Leu Tyr Pro Gln Asp Gly Pro Arg Ser Lys Pro Gln Pro Lys
 180 185 190

Asp Asn Gly Asp Val Cys Gln Asp Cys Ile Gln Met Val Thr Asp Ile
 195 200 205

Gln Thr Ala Val Arg Thr Asn Ser Thr Phe Val Gln Ala Leu Val Glu
 210 215 220

His Val Lys Glu Glu Cys Asp Arg Leu Gly Pro Gly Met Ala Asp Ile
 225 230 235 240

Cys Lys Asn Tyr Ile Ser Gln Tyr Ser Glu Ile Ala Ile Gln Met Met
 245 250 255

Met His Met Gln Pro Lys Glu Ile Cys Ala Leu Val Gly Phe Cys Asp
 260 265 270

Glu Val Lys Glu Met Pro Met Gln Thr Leu Val Pro Ala Lys Val Ala
 275 280 285

Ser Lys Asn Val Ile Pro Ala Leu Glu Leu Val Glu Pro Ile Lys Lys
 290 295 300

His Glu Val Pro Ala Lys Ser Asp Val Tyr Cys Glu Val Cys Glu Phe

305 310 315 320
 Leu Val Lys Glu Val Thr Lys Leu Ile Asp Asn Asn Lys Thr Glu Lys
 325 330 335
 Glu Ile Leu Asp Ala Phe Asp Lys Met Cys Ser Lys Leu Pro Lys Ser
 340 345 350
 Leu Ser Glu Glu Cys Gln Glu Val Val Asp Thr Tyr Gly Ser Ser Ile
 355 360 365
 Leu Ser Ile Leu Leu Glu Glu Val Ser Pro Glu Leu Val Cys Ser Met
 370 375 380
 Leu His Leu Cys Ser Gly Thr Arg Leu Pro Ala Leu Thr Val His Val
 385 390 395 400
 Thr Gln Pro Lys Asp Gly Gly Phe Cys Glu Val Cys Lys Lys Leu Val
 405 410 415
 Gly Tyr Leu Asp Arg Asn Leu Glu Lys Asn Ser Thr Lys Gln Glu Ile
 420 425 430
 Leu Ala Ala Leu Glu Lys Gly Cys Ser Phe Leu Pro Asp Pro Tyr Gln
 435 440 445
 Lys Gln Cys Asp Gln Phe Val Ala Glu Tyr Glu Pro Val Leu Ile Glu
 450 455 460
 Ile Leu Val Glu Val Met Asp Pro Ser Phe Val Cys Leu Lys Ile Gly
 465 470 475 480
 Ala Cys Pro Ser Ala His Lys Pro Leu Leu Gly Thr Glu Lys Cys Ile
 485 490 495
 Trp Gly Pro Ser Tyr Trp Cys Gln Asn Thr Glu Thr Ala Ala Gln Cys

500

505

510

Asn Ala Val Glu His Cys Lys Arg His Val Trp Asn
 515 520

<210> 17
 <211> 1572
 <212> DNA
 <213> Homo sapiens

<400> 17
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 gactgcggcg ccgtgaagca ctgcctgcag accgtgtgga acaagcccac cgtgaagagc 180
 ctgccctgcg acatctgcaa ggacgtggtg accgccgccg gcgacatgct gaaggacaac 240
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 aacatgagcg ccagctgcaa ggagatcgtg gacagctacc tgcccgtgat cctggacatc 360
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 tgcattccaga tggtagaccg catccagacc gccgtgcgca ccaacagcac cttcgtgcag 660
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 tgcaagaact acatcagcca gtacagcgag atcgccatcc agatgatgat gcacatgcag 780
 cccaaggaga tctgcgccct ggtgggcttc tgcgacgagg tgaaggagat gcccatgcag 840
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cacgtgtgga ac                                                              1572

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<210> 18
 <211> 478
 <212> PRT
 <213> Homo sapiens

<400> 18

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Met Gly Arg Cys Cys Phe Tyr Thr Ala Gly Thr Leu Ser Leu Leu Leu
1           5           10          15

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Leu Val Thr Ser Val Thr Leu Leu Val Ala Arg Val Phe Gln Lys Ala
          20          25          30

```

```

Val Asp Gln Ser Ile Glu Lys Lys Ile Val Leu Arg Asn Gly Thr Glu
          35          40          45

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```

Ala Phe Asp Ser Trp Glu Lys Pro Pro Leu Pro Val Tyr Thr Gln Phe
          50          55          60

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Tyr Phe Phe Asn Val Thr Asn Pro Glu Glu Ile Leu Arg Gly Glu Thr
65           70           75           80

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Pro Arg Val Glu Glu Val Gly Pro Tyr Thr Tyr Arg Glu Leu Arg Asn

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85

90

95

Lys Ala Asn Ile Gln Phe Gly Asp Asn Gly Thr Thr Ile Ser Ala Val
 100 105 110

Ser Asn Lys Ala Tyr Val Phe Glu Arg Asp Gln Ser Val Gly Asp Pro
 115 120 125

Lys Ile Asp Leu Ile Arg Thr Leu Asn Ile Pro Val Leu Thr Val Ile
 130 135 140

Glu Trp Ser Gln Val His Phe Leu Arg Glu Ile Ile Glu Ala Met Leu
 145 150 155 160

Lys Ala Tyr Gln Gln Lys Leu Phe Val Thr His Thr Val Asp Glu Leu
 165 170 175

Leu Trp Gly Tyr Lys Asp Glu Ile Leu Ser Leu Ile His Val Phe Arg
 180 185 190

Pro Asp Ile Ser Pro Tyr Phe Gly Leu Phe Tyr Glu Lys Asn Gly Thr
 195 200 205

Asn Asp Gly Asp Tyr Val Phe Leu Thr Gly Glu Asp Ser Tyr Leu Asn
 210 215 220

Phe Thr Lys Ile Val Glu Trp Asn Gly Lys Thr Ser Leu Asp Trp Trp
 225 230 235 240

Ile Thr Asp Lys Cys Asn Met Ile Asn Gly Thr Asp Gly Asp Ser Phe
 245 250 255

His Pro Leu Ile Thr Lys Asp Glu Val Leu Tyr Val Phe Pro Ser Asp
 260 265 270

Phe Cys Arg Ser Val Tyr Ile Thr Phe Ser Asp Tyr Glu Ser Val Gln

275

280

285

Gly Leu Pro Ala Phe Arg Tyr Lys Val Pro Ala Glu Ile Leu Ala Asn
 290 295 300

Thr Ser Asp Asn Ala Gly Phe Cys Ile Pro Glu Gly Asn Cys Leu Gly
 305 310 315 320

Ser Gly Val Leu Asn Val Ser Ile Cys Lys Asn Gly Ala Pro Ile Ile
 325 330 335

Met Ser Phe Pro His Phe Tyr Gln Ala Asp Glu Arg Phe Val Ser Ala
 340 345 350

Ile Glu Gly Met His Pro Asn Gln Glu Asp His Glu Thr Phe Val Asp
 355 360 365

Ile Asn Pro Leu Thr Gly Ile Ile Leu Lys Ala Ala Lys Arg Phe Gln
 370 375 380

Ile Asn Ile Tyr Val Lys Lys Leu Asp Asp Phe Val Glu Thr Gly Asp
 385 390 395 400

Ile Arg Thr Met Val Phe Pro Val Met Tyr Leu Asn Glu Ser Val His
 405 410 415

Ile Asp Lys Glu Thr Ala Ser Arg Leu Lys Ser Met Ile Asn Thr Thr
 420 425 430

Leu Ile Ile Thr Asn Ile Pro Tyr Ile Ile Met Ala Leu Gly Val Phe
 435 440 445

Phe Gly Leu Val Phe Thr Trp Leu Ala Cys Lys Gly Gln Gly Ser Met
 450 455 460

Asp Glu Gly Thr Ala Asp Glu Arg Ala Pro Leu Ile Arg Thr

465

470

475

<210> 19

<211> 1434

<212> DNA

<213> Homo sapiens

<400> 19

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