



(12) **DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION**

(13) **A1**

(86) Date de dépôt PCT/PCT Filing Date: 2019/04/29
(87) Date publication PCT/PCT Publication Date: 2019/10/31
(85) Entrée phase nationale/National Entry: 2020/10/05
(86) N° demande PCT/PCT Application No.: US 2019/029744
(87) N° publication PCT/PCT Publication No.: 2019/210325
(30) Priorité/Priority: 2018/04/27 (US62/664,006)

(51) Cl.Int./Int.Cl. *C12N 15/86* (2006.01),
C12N 15/63 (2006.01), *C12N 15/85* (2006.01),
C12N 9/22 (2006.01)
(71) Demandeur/Applicant:
ROCKET PHARMACEUTICALS, LTD., US
(72) Inventeurs/Inventors:
KERAVALA, ANNAHITA, US;
BATTIPROLU, PAVEN, US;
PRABHAKAR, RAJ, US;
WONG, RODERICK, US;
PRATUMSUWAN, PIRATIP, US;
YALAMANCHI, NAVEEN, US
(74) Agent: GOWLING WLG (CANADA) LLP

(54) Titre : THERAPIE GENIQUE CONTRE LA DEGENERESCENCE DU SYSTEME NERVEUX CENTRAL (SNC)
(54) Title: GENE THERAPY FOR CNS DEGENERATION



FIG. 1

(57) **Abrégé/Abstract:**

The present disclosure relates generally to compositions and methods for treating, preventing, inhibiting, or delaying central nervous system degeneration. The disclosure relates to a recombinant gene therapy vector comprising a PARK2, PINK1, DJ-1, LRRK2, SCNA, c-Rel, ATG7, VMAT2, or GBA gene, or functional fragment or variant thereof. The disclosure also relates to CRISPR/Cas-based gene editing systems for treating, preventing, inhibiting, or delaying central nervous system degeneration.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property

Organization

International Bureau

(43) International Publication Date

31 October 2019 (31.10.2019)



(10) International Publication Number

WO 2019/210325 A1

(51) International Patent Classification:

C12N 15/86 (2006.01) C12N 15/85 (2006.01)

C12N 9/22 (2006.01) C12N 15/63 (2006.01)

(21) International Application Number:

PCT/US2019/029744

(22) International Filing Date:

29 April 2019 (29.04.2019)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/664,006 27 April 2018 (27.04.2018) US

(71) Applicant: **ROCKET PHARMACEUTICALS, LTD.**

[US/US]; The Empire State Building, 350 Fifth Avenue, Suite 7530, New York, New York 10118 (US).

(72) **Inventors: KERAVALA, Annahita;** c/o Rocket Pharmaceuticals, Ltd., The Empire State Building, 350 Fifth Avenue, Suite 7530, New York, New York 10118 (US). **BAT-TIPROLU, Paven;** c/o Rocket Pharmaceuticals, Ltd., The Empire State Building, 350 Fifth Avenue, Suite 7530, New York, New York 10118 (US). **PRABHAKAR, Raj;** c/o Rocket Pharmaceuticals, Ltd., The Empire State Building, 350 Fifth Avenue, Suite 7530, New York, New York 10118 (US). **WONG, Roderick;** c/o Rocket Pharmaceuticals, Ltd., The Empire State Building, 350 Fifth Avenue, Suite 7530, New York, New York 10118 (US). **PRATUMSUWAN, Piratip;** c/o Rocket Pharmaceuticals, Ltd., The Empire State Building, 350 Fifth Avenue, Suite 7530, New York, New York 10118 (US). **YALAMANCHI, Naveen;** c/o Rocket Pharmaceuticals, Ltd., The Empire State Building, 350 Fifth Avenue, Suite 7530, New York, New York 10118 (US).

(74) **Agent: CHRISTIANSEN, William** et al.; Cooley LLP, 1299 Pennsylvania Avenue, NW, Suite 700, Washington, District of Columbia 20004 (US).

(81) **Designated States** (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))

(54) **Title:** GENE THERAPY FOR CNS DEGENERATION**FIG. 1**

(57) **Abstract:** The present disclosure relates generally to compositions and methods for treating, preventing, inhibiting, or delaying central nervous system degeneration. The disclosure relates to a recombinant gene therapy vector comprising a PARK2, PINK1, DJ-1, LRRK2, SCNA, c-Rel, ATG7, VMAT2, or GBA gene, or functional fragment or variant thereof. The disclosure also relates to CRISPR/Cas-based gene editing systems for treating, preventing, inhibiting, or delaying central nervous system degeneration.

[Continued on next page]



WO 2019/210325 A1

WO 2019/210325 A1

Published:

- *with international search report (Art. 21(3))*
- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
- *with sequence listing part of description (Rule 5.2(a))*

GENE THERAPY FOR CNS DEGENERATION

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims benefit of priority to U.S. Provisional Patent Application
5 No. 62/664,006, filed April 27, 2018, the disclosure of which is incorporated herein by
reference in its entirety for all purposes.

INCORPORATION OF SEQUENCE LISTING

The Sequence Listing associated with this application is provided in text format
10 in lieu of a paper copy, and is hereby incorporated by reference into the specification.
The name of the text file containing the Sequence Listing is
ROPA_009_01WO_ST25.txt. The text file is 254 KB, was created on April 29, 2019,
and is being submitted electronically via EFS-Web.

FIELD OF THE DISCLOSURE

The present disclosure relates generally to gene therapy and/or gene editing
for treatment of disorders associated with central nervous system degeneration,
such as Parkinson's Disease. In particular, the disclosure provides compositions and
methods for gene therapy or gene repair in neurons both *ex vivo* and *in vivo*.

BACKGROUND

Various genes have been implicated in disorders of central nervous system
degeneration, such as Parkinson's Disease (PD). Those genes include PARK2,
PINK1 (PARK6), DJ-1 (PARK7), LRRK2, α -synuclein, and DJ-1. Creed et al. (2018)
25 *Mov Disord.* 33:717-729; Blesa et al. (2014) *Front. Neuroanat.* 8:1-12; Alcalay et al.
(2010) *Arch Neurol.* 67:1116-1122). PARK2, also known as PRKN, has been
implicated in a form of PD, autosomal recessive juvenile PD. Despite widespread
attempts, there have been few reports of successful gene therapy for central nervous
system degeneration.

30 There is a great need in the art for new compositions and methods of treating
and preventing central nervous system degeneration.

SUMMARY OF THE DISCLOSURE

The present disclosure provides, in part, compositions and methods for treating, preventing, inhibiting, or delaying central nervous system degeneration. In particular, the inventors disclose various embodiments of, and methods related to, a recombinant gene therapy vector comprising a Parkinson protein 2, E3 ubiquitin protein ligase (PARK2) gene, a PTEN-induced putative kinase 1 (PINK1) gene, a protein deglycase DJ-1 (DJ-1) gene, a Leucine Rich Repeat Kinase 2 (LRRK2) gene, an alpha-synuclein (SCNA) gene, a Proto-oncogene c-Rel (c-Rel) gene, a Ubiquitin-like modifier-activating enzyme (ATG7) gene, Synaptic vesicular amine transporter (VMAT2) gene, or glucocerebrosidase (GBA) gene, or functional fragment or variant thereof.

The further inventors disclose various embodiments, and methods related to, of a gene editing system comprising a Cas protein, guide RNA, a repair template comprising a a Parkinson protein 2, E3 ubiquitin protein ligase (PARK2) gene, a PTEN-induced putative kinase 1 (PINK1) gene, a protein deglycase DJ-1 (DJ-1) gene, a Leucine Rich Repeat Kinase 2 (LRRK2) gene, an alpha-synuclein (SCNA) gene, a Proto-oncogene c-Rel (c-Rel) gene, a Ubiquitin-like modifier-activating enzyme (ATG7) gene, Synaptic vesicular amine transporter (VMAT2) gene, or glucocerebrosidase (GBA) gene, or functional fragment or variant thereof.

In a first aspect, the disclosure provides a method of inhibiting degeneration or death of a dopaminergic neuron comprising a mutation in a gene associated with a Parkinson's Disease (PD). The mutated gene can be a Parkinson protein 2, E3 ubiquitin protein ligase (PARK2) gene, a PTEN-induced putative kinase 1 (PINK1) gene, a protein deglycase DJ-1 (DJ-1) gene, a Leucine Rich Repeat Kinase 2 (LRRK2) gene, alpha-synuclein (SCNA) gene, a Proto-oncogene c-Rel (c-Rel) gene, a Ubiquitin-like modifier-activating enzyme (ATG7) gene, Synaptic vesicular amine transporter (VMAT2) gene, or glucocerebrosidase (GBA) gene. In methods of this aspect, the method comprises contacting the neuron with a recombinant gene therapy vector comprising a polynucleotide encoding a wild-type protein expressed by a wild-type version of the mutated gene, or a functional variant or fragment thereof. Following contact with the recombinant gene therapy vector, the neuron expresses the wild-type protein, or functional variant or fragment thereof.

In some embodiments, the PARK2, PINK1, LRRK2, SCNA, c-Rel, ATG7, VMAT2, or GBA protein comprises the amino acid sequence set forth in SEQ ID NOs: 1-9, respectively. In some embodiments, the gene is the PARK2 gene, and the wild-type PARK2 protein comprises the amino acid sequence set forth in any of SEQ ID NOs: 10-17. In some embodiments, the polynucleotide comprises a sequence having at least 70%, 75%, 80%, 85%, 95%, or 99% identity to a PARK2, PINK1, LRRK2, SCNA, c-Rel, ATG7, VMAT2, or GBA polynucleotide sequence set forth in SEQ ID NOs: 18-26, respectively. In some embodiments, the gene is the PARK2 gene, and the polynucleotide comprises a sequence having at least 70%, 75%, 80%, 85%, 95%, or 99% identity to a PARK2 isoform polynucleotide sequence set forth in any of SEQ ID NOs: 27-34. In some embodiments, the polynucleotide is codon-optimized. In some embodiments, the polynucleotide comprises less than 40, less than 30, less than 20, or 10 or fewer CpG islands. In some embodiments, the polynucleotide comprises at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, or at least 10 CpG islands. In some embodiments, it comprises between 5 and 20 CpG islands.

Various viral or non-viral vectors can be used. In some embodiments, the recombinant gene therapy vector is a recombinant adeno-associated virus (AAV). Any of the known serotypes can be used. In some embodiments, the AAV has serotype AAV1, AAV2, AAV5, AAV8, AAV9, AAVrh10, or AAVrh74. In some embodiments, the recombinant gene therapy vector comprises a self-complementary AAV. In some embodiments, the recombinant gene therapy vector comprises a single-stranded AAV. In some embodiments, the AAV is a wild-type AAV or a modified AAV. In some embodiments, the AAV comprises a capsid protein having at least 95% identity to a wild-type VP1, VP2, or VP3 capsid protein.

The recombinant gene therapy vector may include gene regulatory elements. In some embodiments, the recombinant gene therapy vector comprises a polynucleotide comprising, in the following 5' to 3' order, a eukaryotically active promoter sequence and the sequence encoding the wild-type protein, or functional fragment or variant thereof. The sequence encoding the wild-type protein, or functional fragment or variant thereof, is operably linked to the eukaryotically active promoter sequence.

Without being limited by examples of the disclosure, the recombinant gene therapy vector in some embodiments further comprises one or more of a neuron-specific promoter, optionally selected from the group consisting of hSYN1 (human synapsin), INA (alpha-internexin), NES (nestin), TH (tyrosine hydroxylase), FOXA2 (Forkhead box A2), CaMKII (calmodulin-dependent protein kinase II), and NSE (neuron-specific enolase) promoters; a ubiquitous promoter selected from the group consisting of CMV, CAG, UBC, PGK, EF1-alpha, GAPDH, SV40, HBV, and chicken beta-actin promoters; an enhancer; an intron; a poly-A signal; a WPRE (Woodchuck hepatitis virus posttranscriptional regulatory element); and a HPRE (Hepatitis posttranscriptional regulatory element). The WPRE may be a WPRE(r) or a WPRE(x).

In various embodiments of any of the vectors and methods disclosed herein, the vector comprises an expression cassette comprising in 5' to 3' order:

HuBA promoter, the transgene, WPRE(x), and pAGlobin-Oc;

CMV promoter, TPL-eMLP 5' enhancer, the transgene, WPRE(r), and pAGlobin-Oc;

Syn promoter, the transgene, WPRE(r), 3'UTR(globin), and pAGH-Bt;

CBA promoter, the transgene, and pAGH-Bt;

EF1a promoter, the transgene, and pAGlobin-Oc;

HuBA promoter, the transgene, R2V17, and pAGH-Bt;

Syn promoter, the transgene, WPRE(x), 3'UTR(globin), and pAGH-Hs;

CaMKIIa promoter, the transgene, WPRE(r), and pAGH-Hs;

CMV and TPL promoter, the transgene, WPRE(r), and pAGH-Hs;

HuBA promoter, the transgene, and pAGH-Hs;

CMV and TPL promoter, eMPL, the transgene, R2V17, 3'UTR(globin), and pAGH-Bt;

EF1 α promoter, the transgene, WPRE(r), and pAGH-Bt;

Syn promoter, the transgene, R2V17, and pAGlobin-Oc;

CaMKIIa promoter, the transgene, R2V17, and pAGlobin-Oc;

CBA promoter, the transgene, WPRE(x), 3'UTR(globin), and pAGH-Hs.

CBA promoter, the transgene, 3'UTR(globin), and pAGlobin-Oc;

CaMKIIa promoter, the transgene, R2V17, and pAGH-Bt;

EF1a promoter, the transgene, R2V17, 3'aglobin, and pAGH-Hs;.

CMV promoter, the transgene, R2V17, 3'UTR(globin), and pAGH-Hs; or
CMV promoter, the transgene, and pAGH-Hs,
optionally wherein the transgene encodes PARK2.

The methods of the disclosure may have various effects. In some

5 embodiments, the neuron expresses a reduced amount of alpha-synuclein and/or
comprises a reduced amount of Lewy bodies following contact with the recombinant
gene therapy vector. In some embodiments, the neuron expresses a reduced
amount of monoamine oxidases following contact with the recombinant gene therapy
vector. In some embodiments, the neuron produces and/or releases an increased
10 amount of dopamine following contact with the recombinant gene therapy vector. In
some embodiments, the neuron undergoes increased mitophagy following contact
with the recombinant gene therapy vector.

In some embodiments, the neuron expresses a lower amount of monoamine
oxidases as compared to an amount of monoamine oxidases expressed in a neuron
15 not contacted with said recombinant gene therapy vector, optionally wherein said
lower amount is at least 5%, at least 10%, at least 20%, at least 30%, at least 40%,
at least 50%, at least 60%, at least 70%, or at least 80% lower than the amount
expressed in the neuron not contacted with said recombinant gene therapy vector. In
some embodiments, the neuron produces and/or releases an increased amount of
20 dopamine as compared to an amount of dopamine produced and/or released by a
neuron not contacted with said recombinant gene therapy vector, optionally wherein
said increase amount is at least 5%, at least 10%, at least 20%, at least 30%, at
least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at
least two-fold, at least three-fold, at least four-fold, at least five-fold, or at least 10-
25 fold greater than the amount produced and/or released by the neuron not contacted
with said recombinant gene therapy vector. In some embodiments, the neuron
undergoes an increased amount of autophagy as compared to an amount of
autophagy undergone by a neuron not contacted with said recombinant gene
therapy vector, optionally wherein the increased amount is at least 5%, at least 10%,
30 at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at
least 80%, at least 90%, at least two-fold, at least three-fold, at least four-fold, at
least five-fold, or at least 10-fold greater than the amount undergone by the neuron
not contacted with said recombinant gene therapy vector.

The neuron used in the methods of the disclosure may have various characteristics. In some embodiments, the neuron is a primary tyrosine hydroxylase positive neuron. In some embodiments, the neuron was produced from an induced pluripotent stem cell prepared from cells obtained from a subject diagnosed with

5 Parkinson's disease.

In another aspect, the disclosure provides a recombinant gene therapy vector comprising a polynucleotide encoding a wild-type Parkinson protein 2, E3 ubiquitin protein ligase (PARK2) gene, a PTEN-induced putative kinase 1 (PINK1) gene, a protein deglycase DJ-1 (DJ-1) gene, a Leucine Rich Repeat Kinase 2 (LRRK2) gene, an alpha-synuclein (SCNA) gene, a Proto-oncogene c-Rel (c-Rel) gene, a Ubiquitin-like modifier-activating enzyme (ATG7) gene, Synaptic vesicular amine transporter (VMAT2) gene, or glucocerebrosidase (GBA) gene, or a functional variant or fragment thereof; wherein the polynucleotide is operatively linked to a eukaryotically active promoter; and wherein a neuron transduced with said recombinant gene therapy vector expresses the wild-type protein, or functional variant or fragment thereof.

In some embodiments, the functional PARK2, PINK1, LRRK2, SCNA, c-Rel, ATG7, VMAT2, or GBA protein comprises the amino acid sequence set forth in SEQ ID NOs: 1-9, respectively. In some embodiments, the gene is the PARK2 gene, and the wild-type PARK2 protein comprises the amino acid sequence set forth in any of SEQ ID NOs: 10-17. In some embodiments, the polynucleotide comprises a sequence having at least 70%, 75%, 80%, 85%, 95%, or 99% identity to a PARK2, PINK1, LRRK2, SCNA, c-Rel, ATG7, VMAT2, or GBA polynucleotide sequence set forth in SEQ ID NOs: 18-26, respectively. In some embodiments, the gene is the PARK2 gene, and the polynucleotide comprises a sequence having at least 70%, 75%, 80%, 85%, 95%, or 99% identity to a PARK2 isoform polynucleotide sequence set forth in any of SEQ ID NOs: 27-34. In some embodiments, the vector comprises an expression cassette comprising in 5' to 3' order:

HuBA promoter, the transgene, WPRE(x), and pAGlobin-Oc;

CMV promoter, TPL-eMLP 5' enhancer, the transgene, WPRE(r), and pAGlobin-Oc;

Syn promoter, the transgene, WPRE(r), 3'UTR(globin), and pAGH-Bt;

CBA promoter, the transgene, and pAGH-Bt;

EF1a promoter, the transgene, and pAGlobin-Oc;

HuBA promoter, the transgene, R2V17, and pAGH-Bt;

Syn promoter, the transgene, WPRE(x), 3'UTR(globin), and pAGH-Hs;

CaMKIIa promoter, the transgene, WPRE(r), and pAGH-Hs;

5 CMV and TPL promoter, the transgene, WPRE(r), and pAGH-Hs;

HuBA promoter, the transgene, and pAGH-Hs;

CMV and TPL promoter, eMPL, the transgene, R2V17, 3'UTR(globin), and pAGH-Bt;

EF1a promoter, the transgene, WPRE(r), and pAGH-Bt;

10 Syn promoter, the transgene, R2V17, and pAGlobin-Oc;

CaMKIIa promoter, the transgene, R2V17, and pAGlobin-Oc;

CBA promoter, the transgene, WPRE(x), 3'UTR(globin), and pAGH-Hs.

CBA promoter, the transgene, 3'UTR(globin), and pAGlobin-Oc;

CaMKIIa promoter, the transgene, R2V17, and pAGH-Bt;

15 EF1a promoter, the transgene, R2V17, 3'UTR(globin), and pAGH-Hs;.

CMV promoter, the transgene, R2V17, 3'UTR(globin), and pAGH-Hs; or

CMV promoter, the transgene, and pAGH-Hs,

optionally wherein the transgene encodes PARK2.

In some embodiments, the polynucleotide is codon-optimized. In some
20 embodiments, the polynucleotide comprises any of SEQ ID NOs: 35-38. In some
embodiments, the recombinant gene therapy vector is a recombinant adeno-
associated virus (rAAV). In some embodiments, the rAAV has serotype AAV1,
AAV2, AAV5, AAV8, AAV9, AAVrh10, or AAVrh74. In some embodiments, the
recombinant gene therapy vector comprises a self-complementary or a single-
25 stranded AAV genome. In some embodiments, the AAV is a wild-type AAV or a
modified AAV. In some embodiments, the AAV comprises a capsid protein having at
least 95% identity to wild-type VP1, VP2, or VP3 capsid protein.

In another aspect, the disclosure provides a method of treating or inhibiting
onset of a Parkinson's Disease (PD) in a subject suffering from or at risk of the PD,
30 comprising administering a recombinant gene therapy vector comprising a
polynucleotide encoding a wild-type a wild-type Parkinson protein 2, E3 ubiquitin
protein ligase (PARK2) gene, a PTEN-induced putative kinase 1 (PINK1) gene, a
protein deglycase DJ-1 (DJ-1) gene, a Leucine Rich Repeat Kinase 2 (LRRK2) gene,

an alpha-synuclein (SCNA) gene, a Proto-oncogene c-Rel (c-Rel) gene, a Ubiquitin-like modifier-activating enzyme (ATG7) gene, Synaptic vesicular amine transporter (VMAT2) gene, or glucocerebrosidase (GBA) gene, or a functional variant or fragment thereof, to the subject; wherein administration of the recombinant gene therapy vector treats or inhibits onset of the Parkinson's Disease in the subject.

In some embodiments, the PD is an early-onset PD, optionally an early-onset autosomal recessive PD. In some embodiments, the subject comprises a mutation in a PARK2 gene, PINK1 gene, LRRK2 gene, SCNA gene, c-Rel gene, ATG7 gene, VMAT2, or GBA gene. In some embodiments, the PARK2, PINK1, LRRK2, SCNA, c-Rel, ATG7, VMAT2, or GBA protein comprises the amino acid sequence set forth in SEQ ID NOs: 1-9, respectively. In some embodiments, the gene is the PARK2 gene, and the wild-type PARK2 protein comprises the amino acid sequence set forth in any of SEQ ID NOs: 10-17. In some embodiments, the polynucleotide comprises a sequence having at least 70%, 75%, 80%, 85%, 95%, or 99% identity to a PARK2, PINK1, LRRK2, SCNA, c-Rel, ATG7, VMAT2, or GBA polynucleotide sequence set forth in SEQ ID NOs: 18-26, respectively. In some embodiments, the gene is the PARK2 gene, and the polynucleotide comprises a sequence having at least 70%, 75%, 80%, 85%, 95%, or 99% identity to a PARK2 isoform polynucleotide sequence set forth in any of SEQ ID NOs: 27-34. In some embodiments, the polynucleotide is codon-optimized. In some embodiments, the polynucleotide comprises a sequence having at least 70%, 75%, 80%, 85%, 95%, or 99% identity to a sequence set forth in any of SEQ ID NOs: 35-38.

In some embodiments, the recombinant gene therapy vector is a recombinant adeno-associated virus (AAV). In some embodiments, the AAV has serotype AAV1, AAV2, AAV5, AAV8, AAV9, AAVrh10, or AAVrh74. In some embodiments, the recombinant gene therapy vector comprises a self-complementary AAV genome. In some embodiments, the recombinant gene therapy vector comprises a single-stranded AAV. In some embodiments, the AAV is a wild-type AAV or a modified AAV. In some embodiments, the AAV comprises a capsid protein having at least 95% identity to wild-type VP1, VP2, or VP3 capsid protein.

In some embodiments, the recombinant gene therapy vector comprises a polynucleotide comprising in the following 5' to 3' order, a eukaryotically active promoter sequence; and the sequence encoding the wild-type protein, or functional

fragment or variant thereof; wherein the sequence encoding the wild-type protein, or functional fragment or variant thereof, is operably linked to the eukaryotically active promoter sequence.

In some embodiments, the recombinant gene therapy vector further
 5 comprises one or more of a neuron-specific promoter, optionally selected from the group consisting of hSYN1 (human synapsin), INA (alpha-interneuron), NES (nestin), TH (tyrosine hydroxylase), FOXA2 (Forkhead box A2), CaMKII (calmodulin-dependent protein kinase II), and NSE (neuron-specific enolase) promoters; a ubiquitous promoter selected from the group consisting of CMV, CAG, UBC, PGK,
 10 EF1-alpha, GAPDH, SV40, HBV, human beta-actin, and chicken beta-actin promoters; an enhancer; an intron; a poly-A signal; a WPRE (Woodchuck hepatitis virus posttranscriptional regulatory element); and a HPRE (Hepatitis posttranscriptional regulatory element).

The recombinant gene therapy vector or gene editing system can be
 15 administered in various ways. In some embodiments, the administering step comprises systemic, parenteral, intravenous, cerebral, cerebrospinal, intrathecal, intracisternal, intraputaminar, intrahippocampal, intra-striatal, or intra-cerebroventricular administration. In some embodiments, the administering step comprises intravenous, cerebral, cerebrospinal, intrathecal, intracisternal,
 20 intraputaminar, intrahippocampal, intra-striatal, or intra-cerebroventricular injection. In some embodiments, the administering step comprises intrathecal injection with Threndelenburg tilting. In some embodiments, the administering step comprises direct injection into the pars compacta of the substantia nigra of the brain. In some embodiments, the administering step comprises introducing the recombinant gene
 25 therapy vector into the subject's brain or cerebrospinal fluid (CSF).

In some embodiments, 1×10^9 - 1×10^{14} vector genomes per kilogram body mass of the subject (vg/kg) of the gene therapy vector are administered to the subject. In some embodiments, 1×10^9 - 1×10^{14} vector genomes per kilogram body mass of the subject (vg/kg) of the gene therapy vector are administered to the
 30 subject's brain. In some embodiments, 1×10^9 - 1×10^{14} vector genomes per kilogram body mass of the subject (vg/kg) of the gene therapy vector are administered to the subject's CSF. In some embodiments, 1×10^7 - 1×10^9 vector genomes per kilogram

body mass of the subject (vg/kg) of the gene therapy vector are administered to the subject.

The methods of the disclosure relate to both adult and juvenile forms of disease. In some embodiments, the subject is an adult. In some embodiments, the
5 subject is a child.

The methods of the disclosure may have various effects on the subject. In some embodiments, the number of dopaminergic neurons in the subject after the administering step is greater than the number of dopaminergic neurons in the subject before the administering step. In some embodiments, the level of dopamine in the
10 subject after the administering step is greater than the level of dopamine in the subject before the administering step. In some embodiments, the number of dopaminergic neurons in a subject treated by the method is increased compared to the number of dopaminergic neurons in a subject not so treated. In some
15 embodiments, the level of dopamine of a subject treated by the method is increased compared to the level of dopamine in a subject not so treated. In some embodiments, the level of dopamine in the substantia nigra of a subject treated by method is increased compared to the level of dopamine in the substantia nigra of a
20 subject not so treated. In some embodiments, the level of PRKN in the subject's CSF after the administering step is greater than the level of PRKN in the subject's CSF before the administering step. In some embodiments, the Unified Parkinson's Disease Rating Scale (UPDRS) score of the subject before the administering step is improved compared to the UPDRS score of the subject before the administering
25 step. In some embodiments, the level of PRKN in the CSF of a subject treated by the method is increased compared to the level of PRKN in the CSF of a subject not so treated. In some embodiments, the level of PRKN in the subject's substantia nigra after the administering step is greater than the level of PRKN in the subject's
30 substantia nigra before the administering step. In some embodiments, the UPDRS score of a subject treated by the method is improved compared to the UPDRS score of a subject not so treated. In some embodiments, the subject's neurons express a reduced amount of alpha-synuclein and/or comprises a reduced amount of Lewy bodies following contact with the recombinant gene therapy vector.

In another aspect, the disclosure provides a method of inhibiting degeneration or death of a dopaminergic neuron having a mutated Parkin (PRKN) gene,

comprising contacting the neuron with a gene editing system comprising: Cas protein or a polynucleotide encoding a Cas protein; a guide-RNA (gRNA); and a repair template comprising a functional Parkinson protein 2, E3 ubiquitin protein ligase (PARK2) gene, a PTEN-induced putative kinase 1 (PINK1) gene, a protein
 5 deglycase DJ-1 (DJ-1) gene, a Leucine Rich Repeat Kinase 2 (LRRK2) gene, an alpha-synuclein (SCNA) gene, a Proto-oncogene c-Rel (c-Rel) gene, a Ubiquitin-like modifier-activating enzyme (ATG7) gene, Synaptic vesicular amine transporter (VMAT2) gene, or glucocerebrosidase (GBA) gene, or a functional variant or
 10 fragment thereof; wherein the gene editing system is capable of repairing an endogenous gene in the neuron or inserting a functional gene into the genome of the neuron.

In some embodiments, at least one component of the gene editing system is delivered by recombinant AAV. In some embodiments, the gene editing system is delivered by recombinant AAV.

15 In another aspect, the disclosure provides a gene editing system for a cell comprising: Cas protein or a polynucleotide encoding a Cas protein; a guide-RNA (gRNA); and a repair template comprising a functional Parkinson protein 2, E3 ubiquitin protein ligase (PARK2) gene, a PTEN-induced putative kinase 1 (PINK1)
 20 gene, a protein deglycase DJ-1 (DJ-1) gene, a Leucine Rich Repeat Kinase 2 (LRRK2) gene, an alpha-synuclein (SCNA) gene, a Proto-oncogene c-Rel (c-Rel) gene, a Ubiquitin-like modifier-activating enzyme (ATG7) gene, Synaptic vesicular amine transporter (VMAT2) gene, or glucocerebrosidase (GBA) gene, or a functional
 25 variant or fragment thereof; wherein the gene editing system is capable of repairing an endogenous gene in the cell or inserting a functional gene into the genome of the cell.

In some embodiments, at least one component of the gene editing system is delivered by recombinant AAV. In some embodiments, the gene editing system is delivered by recombinant AAV. In some embodiments, the cell is an *ex vivo* neuron. In some embodiments, the cell is a cell of a subject.

30 In another aspect, the disclosure provides recombinant gene therapy vector, comprising a transgene polynucleotide encoding E3 ubiquitin protein ligase (PARK2) gene, wherein the transgene polynucleotide is operably linked to a eukaryotically active promoter sequence.

In some embodiments, the transgene polynucleotide shares at least 95% identity to one of SEQ ID NOs: 35-38.

In some embodiments, the promoter sequence is selected from Table 5.

In some embodiments, the vector further comprises a CMV enhancer.

5 In some embodiments, the vector further comprises a 5' untranslated region (UTR) selected from Table 6.

In some embodiments, the vector further comprises a 3' untranslated region selected from Table 7.

10 In some embodiments, the vector further comprises a polyadenylation sequence (polyA) selected from Table 8.

In some embodiments, the polynucleotide is codon-optimized.

In some embodiments, the expression cassette shares at least 95% sequence identity to any one of SEQ ID NOs: 39-58.

In some embodiments, the vector is an adeno-associated virus (AAV) vector.

15 In some embodiments, the vector comprises two AAV inverted terminal repeats (ITRs) flanking the expression cassette.

In some embodiments, the AAV has serotype AAV1, AAV2, AAV5, AAV8, AAV9, AAVrh10, or AAVrh74.

20 In some embodiments, the recombinant gene therapy vector comprises a self-complementary AAV.

In some embodiments, the recombinant gene therapy vector comprises a single-stranded AAV.

In some embodiments, the AAV is a wild-type AAV or a modified AAV.

25 In some embodiments, the AAV comprises a capsid protein having at least 95% identity to wild-type VP1, VP2, or VP3 capsid protein.

In another aspect, the disclosure provides host cell comprising any of the foregoing recombinant gene therapy vectors.

30 In another aspect, the disclosure provides method of inhibiting degeneration or death of a dopaminergic neuron comprising a mutation in a gene associated with a Parkinson's Disease (PD), wherein the mutated gene is a Parkinson protein 2, E3 ubiquitin protein ligase (PARK2) gene, comprising contacting the neuron with the recombinant gene therapy vector of the disclosure, wherein following contact with the recombinant gene therapy vector, the neuron expresses the wild-type protein.

In some embodiments, the neuron expresses a reduced amount of alpha-synuclein and/or comprises a reduced amount of Lewy bodies following contact with the recombinant gene therapy vector.

5 In some embodiments, the neuron expresses a reduced amount of monoamine oxidases following contact with the recombinant gene therapy vector.

In some embodiments, the neuron produces and/or releases an increased amount of dopamine following contact with the recombinant gene therapy vector.

In some embodiments, the neuron undergoes increased mitophagy following contact with the recombinant gene therapy vector.

10 In some embodiments, the neuron expresses a lower amount of monoamine oxidases as compared to an amount of monoamine oxidases expressed in a neuron not contacted with said recombinant gene therapy vector, optionally wherein said lower amount is at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, or at least 80% lower than the amount
15 expressed in the neuron not contacted with said recombinant gene therapy vector.

In some embodiments, the neuron produces and/or releases an increased amount of dopamine as compared to an amount of dopamine produced and/or released by a neuron not contacted with said recombinant gene therapy vector, optionally wherein said increase amount is at least 5%, at least 10%, at least 20%, at
20 least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least two-fold, at least three-fold, at least four-fold, at least five-fold, or at least 10-fold greater than the amount produced and/or released by the neuron not contacted with said recombinant gene therapy vector.

In some embodiments, the neuron undergoes an increased amount of autophagy as compared to an amount of autophagy undergone by a neuron not contacted with said recombinant gene therapy vector, optionally wherein the increased amount is at least 5%, at least 10%, at least 20%, at least 30%, at least
25 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least two-fold, at least three-fold, at least four-fold, at least five-fold, or at least 10-fold
30 greater than the amount undergone by the neuron not contacted with said recombinant gene therapy vector .

In some embodiments, the neuron is a primary tyrosine hydroxylase positive neuron.

In some embodiments, the neuron was produced from an induced pluripotent stem cell prepared from cells obtained from a subject diagnosed with Parkinson's disease.

5 In another aspect, the disclosure provides, a method of treating or inhibiting onset of a Parkinson's Disease (PD) in a subject suffering from or at risk of the PD, comprising administering a gene therapy vector of the disclosure, wherein administration of the recombinant gene therapy vector treats or inhibits onset of the Parkinson's Disease in the subject.

10 In some embodiments, the PD is an early-onset PD, optionally an early-onset autosomal recessive PD.

In some embodiments, the subject comprises a mutation in a PARK2 gene.

In some embodiments, the PARK2 comprises the amino acid sequence set forth in SEQ ID NO: 1.

15 In some embodiments, administering step comprises systemic, parenteral, intravenous, cerebral, cerebrospinal, intrathecal, intracisternal, intraputaminial, intrahippocampal, intra-striatal, or intra-cerebroventricular administration.

In some embodiments, the administering step comprises intravenous, cerebral, cerebrospinal, intrathecal, intracisternal, intraputaminial, intrahippocampal, intra-striatal, or intra-cerebroventricular injection.

20 In some embodiments, the administering step comprises intrathecal injection with Threndelenburg tilting.

In some embodiments, the administering step comprises direct injection into the pars compacta of the substantia nigra of the brain.

25 In some embodiments, the administering step comprises introducing the recombinant gene therapy vector into the subject's brain or cerebrospinal fluid (CSF).

In some embodiments, 1×10^9 - 1×10^{14} vector genomes per kilogram body mass of the subject (vg/kg) of the gene therapy vector are administered to the subject.

30 In some embodiments, 1×10^9 - 1×10^{14} vector genomes per kilogram body mass of the subject (vg/kg) of the gene therapy vector are administered to the subject's brain.

In some embodiments, 1×10^9 - 1×10^{14} vector genomes per kilogram body mass of the subject (vg/kg) of the gene therapy vector are administered to the subject's CSF.

5 In some embodiments, 1×10^7 - 1×10^9 vector genomes per kilogram body mass of the subject (vg/kg) of the gene therapy vector are administered to the subject.

In some embodiments, 1×10^7 - 1×10^{11} total vector genomes are administered to the subject, e.g., via direct injection into the putamen or substantia nigra.

In some embodiments, the subject is an adult.

10 In some embodiments, the subject is a child.

In some embodiments, the number of dopaminergic neurons in the subject after the administering step is greater than the number of dopaminergic neurons in the subject before the administering step.

15 In some embodiments, the level of dopamine in the subject after the administering step is greater than the level of dopamine in the subject before the administering step.

In some embodiments, the number of dopaminergic neurons in a subject treated by the method is increased compared to the number of dopaminergic neurons in a subject not so treated.

20 In some embodiments, the level of dopamine of a subject treated by the method is increased compared to the level of dopamine in a subject not so treated.

In some embodiments, the level of dopamine in the substantia nigra of a subject treated by method is increased compared to the level of dopamine in the substantia nigra of a subject not so treated.

25 In some embodiments, the level of PRKN in the subject's CSF after the administering step is greater than the level of PRKN in the subject's CSF before the administering step.

In some embodiments, the Unified Parkinson's Disease Rating Scale (UPDRS) score of the subject before the administering step is improved compared to the UPDRS score of the subject before the administering step.

30 In some embodiments, the level of PRKN in the CSF of a subject treated by the method is increased compared to the level of PRKN in the CSF of a subject not so treated.

In some embodiments, the UPDRS score of a subject treated by the method is improved compared to the UPDRS score of a subject not so treated.

In some embodiments, the subject's neurons express a reduced amount of alpha-synuclein and/or comprises a reduced amount of Lewy bodies following

5 contact with the recombinant gene therapy vector.

Other features and advantages of the invention will be apparent from and encompassed by the following detailed description and claims.

BRIEF DESCRIPTION OF DRAWINGS

10 The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

FIG. 1 shows an embodiment of the expression cassettes of the disclosure.

15 **FIGS. 2A-2J** show representative micrographs of gene expression in SH-SY5Y cells for untransfected negative control (**FIG. 2A**) and a positive control (**FIG. 2B**). Expression cassettes were tested in duplicate: WT (**FIG. 2C** and **FIG. 2D**), CO1 (**FIG. 2E** and **FIG. 2F**), CO2 (**FIG. 2G** and **FIG. 2H**), and CO3 (**FIG. 2I** and **FIG. 2J**).

FIG. 2K shows the percentage of GFP+ cells.

FIG. 2L shows the fluorescence intensity of GFP+ cells.

20 **FIG. 3** shows an enzyme-linked immunosorbent assay (ELISA) for Parkin performed on cell lysates from transfected cells.

FIGS. 4A-4D show representative micrographs of gene expression in SH-SY5Y cells for untransfected negative control (**FIG. 4A**), WT (**FIG. 4B**), CO1 (**FIG. 4C**), or CO4 (**FIG. 4D**).

25 **FIG. 4E** shows the percentage of GFP+ cells.

FIG. 4F shows and the fluorescence intensity of GFP+ cells.

FIG. 5 shows an enzyme-linked immunosorbent assay (ELISA) for Parkin performed on cell lysates from transfected cells.

30 **FIGS. 6A-6D** show representative micrographs of gene expression in induced pluripotent stem cell (iPSC)-derived, Parkin knockout dopaminergic precursors cells for untransfected negative control (**FIG. 6A**), WT (**FIG. 6B**), CO1 (**FIG. 6C**), or CO4 (**FIG. 6D**).

FIG. 6E shows the percentage of GFP+ cells and the fluorescence intensity of GFP+ cells. For each of WT, CO1, and CO\$, the left bar indicates % change GFP intensity relative to WT, and the right bar indicates % GFP+ cells.

FIG. 7 shows an embodiment of the AAV vector expression cassettes of the disclosure.

FIG. 8 shows a diagram of transgene cassettes and various elements thereof.

FIG. 9 shows Parkin transgene expression in SH-SY5Y cells for each of the constructs listed in **Table 10**.

FIG. 10 shows Parkin transgene expression in iPSC-derived, Parkin knockout dopaminergic precursor cells for each of the constructs listed in **Table 11**.

FIGS. 11A-11D show representative fluorescent micrographs for iPSC-derived, Parkin knockout dopaminergic precursor cells transfected with each of the constructs in **Table 11** imaged in brightfield (**FIG. 11A**), or by immunofluorescence for Parkin (**FIG. 11B**), neuronal marker NeuN (**FIG. 11C**), or astrocyte marker GFAP (**FIG. 11D**)

FIG. 12A shows an enlarged image of **FIG. 11A**.

FIG. 12B shows an enlarged image of **FIG. 11B**.

FIG. 12C shows an enlarged image of **FIG. 11C**.

FIG. 12D shows an enlarged image of **FIG. 11D**.

FIG. 13 provides diagrams of illustrative construct designs.

DETAILED DESCRIPTION

The present disclosure provides, in part, compositions and methods for treating, preventing, inhibiting, or delaying central nervous system degeneration, e.g., in the treatment of a Parkinson's Disease. In particular, the inventors disclose various embodiments of a recombinant gene therapy vector comprising a Parkinson protein 2, E3 ubiquitin protein ligase (PARK2) gene, a PTEN-induced putative kinase 1 (PINK1) gene, a protein deglycase DJ-1 (DJ-1) gene, a Leucine Rich Repeat Kinase 2 (LRRK2) gene, an alpha-synuclein (SCNA) gene, a Proto-oncogene c-Rel (c-Rel) gene, a Ubiquitin-like modifier-activating enzyme (ATG7) gene, a Synaptic vesicular amine transporter (VMAT2) gene, or a glucocerebrosidase (GBA) gene, or functional fragment or variant thereof. As used herein, the term "gene" or "transgene"

are used interchangeably and refer to a polynucleotide sequence encoding a polypeptide or protein, such as any of the proteins disclosed in **Table 1**.

The disclosure further includes various embodiments of a gene editing system comprising a Cas protein, guide RNA, a repair template comprising a Parkinson protein 2, E3 ubiquitin protein ligase (PARK2) gene, a PTEN-induced putative kinase 1 (PINK1) gene, a protein deglycase DJ-1 (DJ-1) gene, a Leucine Rich Repeat Kinase 2 (LRRK2) gene, an alpha-synuclein (SCNA) gene, a Proto-oncogene c-Rel (c-Rel) gene, a Ubiquitin-like modifier-activating enzyme (ATG7) gene, a Synaptic vesicular amine transporter (VMAT2) gene, or a glucocerebrosidase (GBA) gene, or functional fragment or variant thereof.

The disclosure further includes methods of inhibiting degeneration or death of a dopaminergic neuron having a mutated PARK2 gene, and methods of treating or inhibiting onset or progression of Parkinson's Disease in a subject suffering from or at risk of Parkinson's disease. In particular embodiments, the Parkinson's disease is an early-onset or juvenile Parkinson's Disease. In certain embodiments, it is associated with or caused by an autosomal recessive mutation, e.g., in a subject's PARK2, PINK1, DJ-1, LRRK2, SCNA, c-Rel, ATG7, VMAT2, or GBA gene. In some embodiments, a viral vector, e.g., an adeno-associated virus (AAV), is used to deliver a recombinant gene therapy construct to the body or more particularly the brain of a subject. Also provided are recombinant gene therapy vectors or gene editing systems for PARK2, PINK1, DJ-1, LRRK2, SCNA, c-Rel, ATG7, VMAT2, or GBA. **Table 1** provides protein sequences. Those genes are expressed as various isoforms. For example, and without limiting the disclosure to PARK2, the isoforms of PARK2 are provided in **Table 2**. **Table 3** provides polynucleotide sequences. **Table 4** provides polynucleotide sequences for the isoforms of PARK2. The disclosure provides compositions and methods comprising or encoding any of the isoforms as proteins or polynucleotides, including codon-optimized polynucleotides and spliced or un-spliced variants.

Table 1: Non-Limiting Examples of Genes Associated with CNS Degradation

Gene (Synonym)	Protein Sequence	SEQ ID NO:

PARK2 (PRK2; PRKN; Parkin)	>sp O60260 PRKN_HUMAN E3 ubiquitin- protein ligase parkin OS=Homo sapiens OX=9606 GN=PRKN PE=1 SV=2 MIVFVRFNSSHGFPVEVDSDTSI FQLKEVVAKRQGV PADQ LRVI FAGKELRNDWTVQNC DLDQQSIVHIVQRPWRKGQEM NATGGDDPRNAAGGCERE PQSLTRVDLSSSVLP GDSVGLA VILHTDSRKDSPPAGSPAGRSIYNSFYVYCKGPCQ RVQPG KLRVQCSTCRQATLT LTQGPSCWDDVLI PNRMSGECQSPH CPGTSAEFFFFKCGAHPTSDKETSVALH LIATNSRNITCIT CTDVRS PVLVFQCN SRHVICLD CFHLYCVTRLNDRQ FVHD PQLGYSLPCVAGCPNS LIKELHHFRILGEEQYNRYQQYGA EECVLQMGGVLCPRPGCGAGLLPEPDQRKVTCEGGNGLGC GFAFCRECKEAYHEGECSAVFEASGTTTQAYRVDERAAEQ ARWEAASKETIKKTTKPCPRCHVPVEKNGGCMHMKCPQPQ CRLEWCWNCGCEWNRVCMGDHWF DV	1
PARK6 (PRK6; PINK1)	>sp Q9BXM7 PINK1_HUMAN Serine/threonine- protein kinase PINK1, mitochondrial OS=Homo sapiens OX=9606 GN=PINK1 PE=1 SV=1 MAVRQALGRGLQLGRALLRFTGKPGRAYGLGRPGPAAGC VRGERPGWAAGPGAEP RRVLGLPNRLRFFRQSVAGLAAR LQRQFVVRAWGCAGPCGRAVFLAFGLGLGLIEEKQAESRR AVSACQEIQAIFTQKSKPGPDPLDTRRLQGFRLEEYLIQ SIGKGCSAAVYEATMPTLPQNLEVTKSTGLLPGRGPGTSA PGEQGERAPGAPAFPLAIKMMWNISAGSSSEAILNTMSQE LVPASRVALAGEYGAVTYRKS KRGPQLAPHPNIIRVLRA FTSSVPLLPGALVDYDPDVLPSRLHPEGLGHGRTLFLVMKN YPCTLRQYLCVNTSPRLAAMMLLQLLEGVDHLVQQGIAH RDLKSDNILVELDPDGCPWLVIADFGCCLADESIGLQLPF SSWYVDRGGNGCLMAPEVSTARPGPRAVIDYSKADAWAVG AIAYEIFGLVNPFGYQGKAHLESRSYQEAQLPALPESVPP DVRQLVRALLQREASKRPSARVAANVLHLSLWGEHILALK	2

	NLKLDKMVGWLLQQSAATLLANRLTEKCCVETKMMLFLA NLECETLCQAALLLCSWRAAL	
PARK7 (PRK7; DJ-1)	>sp Q99497 PARK7_HUMAN Protein/nucleic acid deglycase DJ-1 OS=Homo sapiens OX=9606 GN=PARK7 PE=1 SV=2 MASKRALVILAKGAEEMETVIPVDVMRRAGIKVTVAGLAG KDPVQCSRDVVICPDASLEDKKEGPDVVLPGGNLGAQ NLSESAAVKEILKEQENRKGLIAAICAGPTALLAHEIGFG SKVTTHPLAKDKMMNGGHYTYSENVRVEKDGLILTSRGPQT SFEFALAIVEALNGKEVAAQVKAPLVVKD	3
LRRK2 (PARK8; PRK8)	>sp Q5S007 LRRK2_HUMAN Leucine-rich repeat serine/threonine-protein kinase 2 OS=Homo sapiens OX=9606 GN=LRRK2 PE=1 SV=2 MASGSCQGCEEDEETLKKLIVRLNNVQEGKQIETLVQILE DLLVFTYSERASKLFQGKNIHVPLLIVLDSYMRVASVQQV GWSLLCKLIEVCPGTMQSLMGPQDVGNDEVLGVHQLILK MLTVHNASVNLVIGLKTLDLLLTSGKITLLILDEESDIF MLIFDAMHSFPANDEVQKLGCKALHVLFEVSEEQLTEFV ENKDYMILLSALTNFKDEEEIVLHVLHCLHSLAIPCNNVE VLMSGNVRCYNIVVEAMKAFPMSERIQEVSCCLLHRLTLG NFFNILVLNEVHEFVVKAVQQYPENAALQISALSCLALLT ETIFLNQDLEEKNNENQENDDEGEEDKLFWLEACYKALTWH RKNKHVQEAACWALNNLLMYQNSLHEKIGDEDGHFPAHRE VMLSMLMHSSSKEVFQASANALSTLLEQNVNFRKILLSKG IHLNVLELMQKHIHSPEVAESGCKMLNHLFEGSNTSLDIM AAVVPKILTVMKRHETSLPVQLEALRAILHFIVPGMPES REDTEFHHKLNLMVKKQCFKNDIHKLVLAALNRFIGNPGIQ KCGLKVISSIVHFPDALEMLSLEGAMDSVLHTLQMPDDQ EIQCLGLSLIGYLITKKNVFIGTGHLAKILVSSLYRFKD VAEIQTGKFQTI LAILKLSASFSLLVHHSFDLVI FHQMS SNIMEQKDQQFLNLCKCFKAVAMDDYLKNVMLERACDQN	4

NSIMVECLLLLGDANQAKEGSSLICQVCEKESSPKLVEL LLNSGSREQDVRKALTISIGKGDSQIISLLLRRLALDVAN NSICLGGFCIGKVEPSWLGPLFPDKTSNLRKQTNIASTLA RMVIRYQMKSAVEEGTASGSDGNFSEDVLSKFDEWTFIPD SSMSDVFAQSDDL DSEGSEGSFLVKKKSNSISVGEFYRDA VLQRCSPNLQRHSNSLGPIFDHEDLLKRKRKILSSDDSLR SSKLQSHMRHSDSISSLASEREYITSLDLSANELRDIDAL SQKCCISVHLEHLEKLELHQNALTSFPQQLCETLKS LTHL DLHSNKFTSFPSYLLKMSCIANLDVSRNDIGPSVVLDPTV KCPTLKQFNLSYNQLSFVPENLTDVVEKLEQLILEGNKIS GICSPLRLKELKILNLSKNHISSLSENFLACPKVESFSA RMNFLAAMPFLPPSMTILKLSQNKFSCEAILNLPHLRS LDMSSNDIQYLPGPAHWKSLNLRELLFSHNQISILDSEK AYLWSRVEKLHLSHNKLKEIPPEIGCLENLTSLDVSYNLE LRSFPNEMGKLSKIWDLPLDELHLNFDKFKHIGCKAKDIIR FLQQRLLKAVPYNRMKLMIVGNTGSGKTTLLQQLMKT KKS DLGMQSATVGIDVKDWPIQIRDKRKRDLVLNVWDFAGREE FYSTHPHFMTQRALYLAVYDLSKGQAEVDAMKPWLFNIKA RASSSPVILVGTHLDVSDEKQORKACMSKITKELLNKR GFP AIRDYHFVNATEESDALAKLRKTIINESLNFKIRDQLVVG QLIPDCYVELEKIIILSERKNVPIEFVIDRKRLQLVREN QLQLDENELPHAVHFLNESGVLLHFQDPALQLSDLYFVEP KWLCKIMAQILTVKVEGCPKHPKGIISRRDVEKFLSKKRK FPKNYMSQYFKLLEKFQIALPIGEEYLLVPSSLS DHRPVI ELPHCENSEIIIRLYEMPYFPMGFWSRLINRLLEISPYML SGRERALRPNRM YWRQGIYLNWSPEAYCLVGSEVLDNHPE SFLKITVPSCRKGCILLGQVVDHIDSLMEEWFPGLLEIDI CGEGETLLKKWALYSFNDGEEHQKILLDDLMKKAEEGDLL VNPDPRLTIPISQIAPDLILADLPRNIMLNND ELEFEQA PEFLLGDGSFGSVYRAAYEGEEVAVKIFNKHTSLRLLRQE LVVLCHLHHPSLISLLAAGIRPRMLVMELASKGSLDRLLQ QDKASLTRTLQHRIALHVADGLRYLHSAMIIYRDLKPHNV LLFTLYPNAAIIAKIADYGIAQYCCRMGIKTSEGT PGFRA	
--	--

	PEVARGNVIYNQQADVYSFGLLLYDILTTGGRIVEGLKFP NEFDELEIQGKLDPDPVKEYGCAPWPMVEKLIKQCLKENPQ ERPTSAQVFDILNSAELVCLTRRILLPKNVIVECMVATHH NSRNASIWLGCGHTDRGQLSFLDLNTEGYTSEEVADSRIL CLALVHLPVEKESWIVSGTQSGTLLVINTEDGKKRHTLEK MTDSVTCLYCNSFSKQSKQKNFLLVGTADGKLAI FEDKTV KLKGAAPLKILNIGNVSTPLMCLSESTNSTERNVMWGGCG TKIFSFSNDFTIQKLIETRISQLFSYAAFSDSNIIITVVVD TALYIAKQNSPVVEVWDKKTEKLCGLIDCVHFLREVMVKE NKESKHKMSYSGRVKTLCLQKNTALWIGTGGGHILLDLDS TRRLIRVIYNFCNSVRVMMTAQLGSLKNVMLVLGYNRKNT EGTQKQKEIQSCLTVWDINLPHEVQNLEKHIEVRKELAEK MRRTSVE	
alpha-synuclein (PARK1; PRK1)	>sp P37840 SYUA_HUMAN Alpha-synuclein OS=Homo sapiens OX=9606 GN=SNCA PE=1 SV=1 MDVFMKGLSKAKEGVVAAAEKTKQGVAAEAGKTKEGVLYV GSKTKEGVVHGVATVAEKTKEQVTNVGGAVVTGVTAVAQK TVEGAGSIAAATGFVKKDQLGKNEEGAPQEGILEDMPVDP DNEAYEMPSEEGYQDYEPEA	5
c-Rel-NFKB	>sp Q04864 REL_HUMAN Proto-oncogene c- Rel OS=Homo sapiens OX=9606 GN=REL PE=1 SV=1 MASGAYNPYIEIIEQPRQRGMRFRYKCEGRSAGSIPGEHS TDNNRTYPSIQIMNYYGKGKVRITLVTKNDPYKPHPHDLV GKDCRDGYEAEFGQERRPLFFQNLGIRCVKKKEVKEAII TRIKAGINPFNVPEKQLNDIEDCDLNVRLCFQVFLPDEH GNLTALPPVVSNIYDNRAPNTAELRICRVNKNCGSVRG GDEIFLLCDKVQKDDIEVRFVLNDWEAKGIFSQADVHRQV AIVFKTPPYCKAITEPVTVKMLRRPSDQEVSESMDFRYL PDEKDTYGNKAKKQKTTLLFQKLCQDHVETGFRHVDQDGL ELLTSGDPPTLASQSAGITVNFPERPRPGLLSIGEGRYF	6

	KKEPNLFSHDAVVREMTGVSSQAESYYPSPGPISGLSH HASMAPLPSSSWSSVAHPTPRSGNTNPLSSFSTRTLPSNS QGIPPFRLRIPVGNDLNASNACIYNNADDIVGMEASSMPSA DLYGISDPNMLSNCSVNMMTTSSDSMGETDNPRLLSMNLE NPSCNSVLDPRDLRQLHQMSSSSMSAGANSNTTVFVSQSD AFEGSDFSCADNSMINESGPSNSTNPNSHGFVQDSQYSGI GSMQNEQLSDSFPYEFFQV	
ATG7	>sp Q9D906 ATG7_MOUSE Ubiquitin-like modifier-activating enzyme ATG7 OS=Mus musculus OX=10090 GN=Atg7 PE=1 SV=1 MGDPGLAKLQFAPFNSALDVGFWHELTQKKLNEYRLDEAP KDIKGYYYNGDSAGLPTRLTLEFSAFDMSASTPAHCCPAM GTLHNTNTLEAFKTADKLLLEQSANEIWEAIKSGAALEN PMLLNKFLLLTFADLKKYHFYYWFCCPALCLPESIPLIRG PVSLDQRLSPKQIQALEHAYDDLCRAEGVTALPYFLFKYD DDTVLVSLLKHYSDFQGGQRTKITVGVYDPCNLAQYPGWP LRNFLVLAAHRWSGSFQSVEVLCFRDRMQGARDVTHSII FEVKLPMAFSPDCPKAVGWKNQKGGMGRMVNLSGCMD PKRLAESSVDLNLKLMCWRLVPTLDLDKVVSVKCLLLGAG TLGCNVARTLMGWGVRHVTFVDNAKISYSNPVRQPLYEFE DCLGGGKPKALAAAERLQKIFPGVNARGFNMSIPMPGHPV NFSDVTMEQARRDVEQLEQLIDNHDVIFLLMDTRESRWLP TVIAASKRKLVINAAALGFDTFVVMRHGLKKPKQQGAGDLC PSHLVAPADLGSSLFANIPGYKLGCFNDVVAPGDSTRD RTLDQQCTVSRPGLAVIAGALAVELMVSVLQHPEGGYAIA SSSDDRMNEPPTSLGLVPHQIRGFLSRFDNVLPVSLAFDK CTACSPKVLDQYEREGFTFLAKVFNSSHSFLEDLTGLTLL HQETQAAEIWDMSDEETV	7
VMAT2	>sp Q05940 VMAT2_HUMAN Synaptic vesicular amine transporter OS=Homo sapiens OX=9606 GN=SLC18A2 PE=1 SV=2	8

	MALSELALVRWLQESRRSRKLILFIVFLALLLDNMLLTVV VPIIPSYLYSIKHEKNATEIQTARPVHTASISDSFQSIFS YYDNSTMVTGNATRDLT LHQTATQHMVTNASAVPSDCPSE DKDLLNENVQVGLLFASKATVQLITNPFIGLLTNRIGYPI PIFAGFCIMFVSTIMFAFSSSYAFLLIARSLQGIGSSCSS VAGMGMLASVYTDDEERGNVMGIALGGLAMGVLVGPPFGS VLYEFVGKTAPFLVLAALVLLDGAIQLFVLQPSRVQPESQ KGTPLTTLLKDPYILIAAGSICFANMGIAMLEPALPIWMM ETMCSRKWQLGVAFLPASISYLGITNIFGILAHKMGRWLC ALLGMIIVGVSILCIPFAKNIYGLIAPNFGVGFAIGMVDS SMMPIMGYLVDLRHVSVYGSVYAIADVAFCMGYAIGPSAG GAIAKAIGFPWLMTIIGIIDILFAPLCFFLRSPPAKEEKM AILMDHNCPIKTKMYTQNNIQSYPIGEDEESES	
GBA	>sp P04062 GLCM_HUMAN Glucosylceramidase OS=Homo sapiens OX=9606 GN=GBA PE=1 SV=3 MEFSSPSREECPKPLSRVSIMAGSLTGLLLLQAVSWASGA RPCIPKSFGYSSVVCNATYCDSDPPTFPALGTFSTRYE STRSGRMELSMGP IQANHTGTGLLLLTLOPEQKFQKVKG GGAMTDAAALNILALSPPAQNLLLKSYFSEEGIGYNIIRV PMASCDFSIRTYTYADTPDDFQLHNFSLPEEDTKLKIPLI HRALQLAQRPVSLASPWTSPWLKTNGAVNGKGS LKGQP GDIYHQTWARYFVKFLDAYAEHKLQFWAVTAENEPSAGLL SGYPFQCLGFTPEHQ RDFIARDLGPTLANSTHHNVRLML DDQRLLLLPHWAKVVLTDPEAAKYVHGIAVHWYLD FLAPAK ATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGM QYSHSIIITNLLYHVVGWTDWNLALNPEGGPNWVRNFVDSP IIVDITKDTFYKQPMFYHLGHFSKFIPEGSQ RVGLVASQK NDLDAVALMHPDGS AVVVVLNRSSKDVPLTIKDPAVGFLE TISPGYSIHTYLWRRQ	9

Table 2: Isoforms of PARK2

Gene (Isoform)	Protein Sequence	SEQ ID NO:
PARK2 (1)	>sp O60260 PRKN_HUMAN E3 ubiquitin-protein ligase parkin OS=Homo sapiens OX=9606 GN=PRKN PE=1 SV=2 MIVFVRFNSSHGFPVEVDSDTISIFQLKEVVAKRQGV PADQ LRVIFAGKELRNDWTVQNC DLDQQSIVHIVQRPWRKGQEM NATGGDDPRNAAGGCERE PQSLTRVDLSSSVLP GDSVGLA VILHTDSRKDSPPAGSPAGRSIYNSFYVYCKGPCQ RVQPG KLRVQCSTCRQATLTLTQGPSCWDDVLI PNMSGECQSPH CPGTSAEFFFKCGAHPTSDKETSVALH LIATNSRNITCIT CTDVRSPVLV FQCNSRHVICLD CFHLYCVTRLNDRQ FVHD PQLGYSLPCVAGCPNSLIKELHHFRILGEEQYNRYQQYGA EECVLQMG GVLCPRPGCGAGLLPEPDQRKVTCEGGNGLGC GFAFCRECKEAYHEGECSAVFEASGTTTQAYRVDERAAEQ ARWEAASKETIKKTTKPCPRCHVPVEKNGGCMHMKCPQPQ CRLEWCWNCGCEWNRVCMGDHWF DV	10
PARK2 (2)	>sp O60260-2 PRKN_HUMAN Isoform 2 of E3 ubiquitin-protein ligase parkin OS=Homo sapiens OX=9606 GN=PRKN MIVFVRFNSSHGFPVEVDSDTISIFQLKEVVAKRQGV PADQ LRVIFAGKELRNDWTVQNC DLDQQSIVHIVQRPWRKGQEM NATGGDDPRNAAGGCERE PQSLTRVDLSSSVLP GDSVGLA VILHTDSRKDSPPAGSPAGRSIYNSFYVYCKGPCQ RVQPG KLRVQCSTCRQATLTLTQ EFFFFKCGAHPTSDKETSVALHL IATNSRNITCITCTDVRSPVLV FQCNSRHVICLD CFHLYC VTRLNDRQ FVHDPQLGYSLPCVAGCPNSLIKELHHFRILG EEQYNRYQQYGAEECVLQMG GVLCPRPGCGAGLLPEPDQR KVTCEGGNGLGCGFAFCRECKEAYHEGECSAVFEASGTTT QAYRVDERAAEQARWEAASKETIKKTTKPCPRCHVPVEKN GGCMHMKCPQPQCRLEWCWNCGCEWNRVCMGDHWF DV	11

PARK2 (3)	>sp O60260-3 PRKN_HUMAN Isoform 3 of E3 ubiquitin-protein ligase parkin OS=Homo sapiens OX=9606 GN=PRKN MNATGGDDPRNAAGGCEREPQSLTRVDLSSSVLPGDSVGL AVILHTDSRKDSPPAGSPAGRSIYNSFYVYCKGPCQRVQP GKLRVQCSTCRQATLTLTQGPSCWDDVLI PNRMSEGECQSP HCPGTSAEFFFKCGAHPTSDKETSVALHLIATNSRNITCI TCTDVRSPVLVVFQCNSRHVICLD CFHLYCVTRLNDRQFVH DPQLGYSLPCVVCLLP GM	12
PARK2 (4)	>sp O60260-4 PRKN_HUMAN Isoform 4 of E3 ubiquitin-protein ligase parkin OS=Homo sapiens OX=9606 GN=PRKN MSGECQSPHCPGTSAEFFFKCGAHPTSDKETSVALHLIAT NSRNITCITCTDVRSPVLVVFQCNSRHVICLD CFHLYCVTR LNDRQFVHDPQLGYSLPCVAGCPNSLIKELHHFRILGEEQ YNRYQQYGAEECVLQMGGVLCPRPGCGAGLLPEPDQRKVT CEGGNGLGCGFAFCRECKEAYHEGEC SAVFEASGTTTQAY RVDERAAEQARWEAASKETIKKTKPCPRCHVPVEKNGGC MHMKCPQPQCRLEWCWNCGCEWNRVCMGDHWF DV	13
PARK2 (5)	>sp O60260-5 PRKN_HUMAN Isoform 5 of E3 ubiquitin-protein ligase parkin OS=Homo sapiens OX=9606 GN=PRKN MIVFVRFNSSHGFPVEVDS DT SIFQLKEVVAKRQGV PADQ LRVIFAGKELRNDWTVQNC DLDQQSIVHIVQRPWRKGQEM NATGGDDPRNAAGGCEREPQSLTRVDLSSSVLPGDSVGLA VILHTDSRKDSPPAGSPAGRSIYNSFYVYCKGPCQRVQPG KLRVQCSTCRQATLTLTQGPSCWDDVLI PNRMSEGECQSPH CPGTSAEFFFKCGAHPTSDKETSVALHLIATNSRNITCIT CTDVRSPVLVVFQCNSRHVICLD CFHLYCVTRLNDRQFVHD PQLGYSLPCVGTGDTVVLRGALGGFRRGVAGCPNSLIKEL HHFRILGEEQYNRYQQYGAEECVLQMGGVLCPRPGCGAGL LPEPDQRKVTCEGGNGLGCGY GQRRTK	14

PARK2 (6)	>sp O60260-6 PRKN_HUMAN Isoform 6 of E3 ubiquitin-protein ligase parkin OS=Homo sapiens OX=9606 GN=PRKN MIVFVRFNSSHGFPVEVDS DTSIFQLKEVVAKRQGV PADQ LRVIFAGKELRNDWTVQE FFFKCGAHPTSDKETSVALHLI ATNSRNITCITCTDVRSPVLV FQCNSRHVICLDCFHLYCV TRLNDRQFVHDPQLGYSLPCVAGCPNSLIKELHHFRILGE EQYNRYQQYGAEECVLQMG GVLCPRPGCGAGLLPEPDQRK VTCEGGNGLGCGFAFCRECKEAYHEGECSAVFEASGTTTQ AYRVDERAAEQARWEAASKETIKKTTKPCPRCHVPVEKNG GCMHMKCPQPQCRLEWCWNCGCEWNRVCMGDHWFVDV	15
PARK2 (7)	>sp O60260-7 PRKN_HUMAN Isoform 7 of E3 ubiquitin-protein ligase parkin OS=Homo sapiens OX=9606 GN=PRKN MIVFVRFNSSHGFPVEVDS DTSIFQLKEVVAKRQGV PADQ LRVIFAGKELRNDWTVQNC DLDQQSIVHIVQRPWRKGQEM NATGGDDPRNAAGGCERE PQSLTRVDLSSSVLPGDSVGLA VILHTDSRKDSPPAGSPAGRSIYNSFYVYCKGPCQRVQPG KLRVQCSTCRQATLTLTQ E FFFKCGAHPTSDKETSVALHL IATNSRNITCITCTDVRSPVLV FQCNSRHVICLDCFHLYC VTRLNDRQFVHDPQLGYSLPCVAGCPNSLIKELHHFRILG EEQFAFCRECKEAYHEGECSAVFEASGTTTQAYRVDERAA EQARWEAASKETIKKTTKPCPRCHVPVEKNGGCMHMKCPQ PQCRLEWCWNCGCEWNRVCMGDHWFVDV	16
PARK2 (8)	>sp O60260-8 PRKN_HUMAN Isoform 8 of E3 ubiquitin-protein ligase parkin OS=Homo sapiens OX=9606 GN=PRKN MIVFVRFNSSHGFPVEVDS DTSIFQLKEVVAKRQGV PADQ LRVIFAGKELRNDWTVQNC DLDQQSIVHIVQRPWRKGQEM NATGGDDPRNAAGGCERE PQSLTRVDLSSSVLPGDSVGLA VILHTDSRKDSPPAGSPAGRSIYNSFYVYCKGPCQRVQPG KLRVQCSTCRQATLTLTQ GPSCWDDVLI PNRMGECQSPH CPGTSAE FFFKCGAHPTSDKETSVALHLIATNSRNITCIT	17

	CTDVRSPVLVVFQCNSRHVICLD CFHLYCVTRLNDRQFVHD PQLGYSLPCVAGCPNSLIKELHHFRILGEEQFAFCRECKE AYHEGECSAVFEASGTTTQAYRVDERAAEQARWEAASKET IKKTTKPCPRCHVPVEKNGGCMHMKCPQPQCRLEWCWNCG CEWNRVCMGDHWFVDV	
--	--	--

Table 3: Polynucleotides of Non-Limiting Example Genes Associated with CNS Degradation

Gene (Synonym)	Nucleic Acid Sequence	SEQ ID NO:
PARK2 (PRK2; PRKN; Parkin)	>NM_004562.2:135-1532 Homo sapiens parkin RBR E3 ubiquitin protein ligase (PRKN), transcript variant 1, mRNA ATGATAGTGTTTGTCTCAGGTTCAACTCCAGCCATGGTTTCC CAGTGGAGGTCGATTCTGACACCAGCATCTTCCAGCTCAA GGAGGTGGTTGCTAAGCGACAGGGGGTTCCGGCTGACCAG TTGCGTGTGATTTTCGCAGGGAAGGAGCTGAGGAATGACT GGACTGTGCAGAATTGTGACCTGGATCAGCAGAGCATTTGT TCACATTGTGCAGAGACCGTGGAGAAAAGGTCAAGAAATG AATGCAACTGGAGGCGACGACCCCAGAAACGCGGCGGGAG GCTGTGAGCGGGAGCCCCAGAGCTTGACTCGGGTGGACCT CAGCAGCTCAGTCCTCCCAGGAGACTCTGTGGGGCTGGCT GTCATTCTGCACACTGACAGCAGGAAGGACTCACCACCAG CTGGAAGTCCAGCAGGTAGATCAATCTACAACAGCTTTTA TGTGTATTGCAAAGGCCCTGTCAAAGAGTGCAGCCGGGA AAACTCAGGGTACAGTGCAGCACCTGCAGGCAGGCAACGC TCACCTTGACCCAGGGTCCATCTTGCTGGGATGATGTTTT AATTCCAAACCGGATGAGTGGTGAATGCCAATCCCCACAC TGCCCTGGGACTAGTGCAGAATTTTTCTTTAAATGTGGAG CACACCCACCTCTGACAAGGAAACATCAGTAGCTTTGCA CCTGATCGCAACAAATAGTCGGAACATCACTTGCATTACG TGCACAGACGTCAGGAGCCCCGTCCTGGTTTTCCAGTGCA ACTCCCGCCACGTGATTTGCTTAGACTGTTTCCACTTATA	18

	CTGTGTGACAAGACTCAATGATCGGCAGTTTGTTCACGAC CCTCAACTTGGCTACTCCCTGCCTTGTGTGGCTGGCTGTC CCAACCTCCTTGATTAAAGAGCTCCATCACTTCAGGATTCT GGGAGAAGAGCAGTACAACCGGTACCAGCAGTATGGTGCA GAGGAGTGTGTCCTGCAGATGGGGGGCGTGTTATGCCCCC GCCCTGGCTGTGGAGCGGGGCTGCTGCCGGAGCCTGACCA GAGGAAAGTCACCTGCGAAGGGGGCAATGGCCTGGGCTGT GGGTTTGCCTTCTGCCGGGAATGTAAAGAAGCGTACCATG AAGGGGAGTGCAGTGCCGTATTTGAAGCCTCAGGAACAAC TACTCAGGCCTACAGAGTCGATGAAAGAGCCGCCGAGCAG GCTCGTTGGGAAGCAGCCTCCAAAGAAACCATCAAGAAAA CCACCAAGCCCTGTCCCCGCTGCCATGTACCAGTGGAAAA AAATGGAGGCTGCATGCACATGAAGTGTCCGCAGCCCCAG TGCAGGCTCGAGTGGTGCTGGAAGTGTGGCTGCGAGTGGA ACCGCGTCTGCATGGGGGACCACTGGTTCGACGTGTAG	
PARK6 (PRK6; PINK1)	>NM_032409.2:95-1840 Homo sapiens PTEN induced putative kinase 1 (PINK1), mRNA ATGGCGGTGCGACAGGCGCTGGGCCGCGGCCTGCAGCTGG GTCGAGCGCTGCTGCTGCGCTTCACGGGCAAGCCCGGCCG GGCCTACGGCTTGGGGCGGCCGGGCCCGGCGGCGGGCTGT GTCCGCGGGGAGCGTCCAGGCTGGGCCGCGAGACCGGGCG CGGAGCCTCGCAGGGTCGGGCTCGGGCTCCCTAACCGTCT CCGCTTCTTCCGCCAGTCGGTGGCCGGGCTGGCGGCGCGG TTGCAGCGGCAGTTCGTGGTGCGGGCCTGGGGCTGCGCGG GCCCTTGCGGCCGGGCAGTCTTTCTGGCCTTCGGGCTAGG GCTGGGCCTCATCGAGGAAAAACAGGCGGAGAGCCGGCGG GCGGTCTCGGCCTGTCAGGAGATCCAGGCAATTTTTACCC AGAAAAGCAAGCCGGGGCCTGACCCGTGGACACGAGACG CTTGCAGGGCTTTTCGGCTGGAGGAGTATCTGATAGGGCAG TCCATTGGTAAGGGCTGCAGTGCTGCTGTGTATGAAGCCA CCATGCCTACATTGCCCCAGAACCTGGAGGTGACAAAGAG CACCGGGTTGCTTCCAGGGAGAGGCCAGGTACCAGTGCA	19

	CCAGGAGAAGGGCAGGAGCGAGCTCCGGGGGGCCCCCTGCCT TCCCCCTTGCCCATCAAGATGATGTGGAACATCTCGGCAGG TTCCTCCAGCGAAGCCATCTTGAACACAATGAGCCAGGAG CTGGTCCCAGCGAGCCGAGTGGCCTTGGCTGGGGAGTATG GAGCAGTCACTTACAGAAAATCCAAGAGAGGTCCCAAGCA ACTAGCCCCCTCACCCCAACATCATCCGGGTTCTCCGCGCC TTCACCTCTTCCGTGCCGCTGCTGCCAGGGGGCCCTGGTCG ACTACCCTGATGTGCTGCCCTCACGCCTCCACCCTGAAGG CCTGGGCCATGGCCGGACGCTGTTCCCTCGTTATGAAGAAC TATCCCTGTACCCTGCGCCAGTACCTTTGTGTGAACACAC CCAGCCCCCGCCTCGCCGCCATGATGCTGCTGCAGCTGCT GGAAGGCGTGGACCATCTGGTTCAACAGGGCATCGCGCAC AGAGACCTGAAATCCGACAACATCCTTGTGGAGCTGGACC CAGACGGCTGCCCCCTGGCTGGTGATCGCAGATTTTGGCTG CTGCCTGGCTGATGAGAGCATCGGCCTGCAGTTGCCCTTC AGCAGCTGGTACGTGGATCGGGGCGGAAACGGCTGTCTGA TGGCCCCAGAGGTGTCCACGGCCCGTCCTGGCCCCAGGGC AGTGATTGACTACAGCAAGGCTGATGCCTGGGCAGTGGGA GCCATCGCCTATGAAATCTTCGGGCTTGTCAATCCCTTCT ACGGCCAGGGCAAGGCCACCTTGAAAGCCGCAGCTACCA AGAGGCTCAGCTACCTGCACTGCCCCGAGTCAGTGCCTCCA GACGTGAGACAGTTGGTGAGGGCACTGCTCCAGCGAGAGG CCAGCAAGAGACCATCTGCCCCGAGTAGCCGCAAATGTGCT TCATCTAAGCCTCTGGGGTGAACATATTCTAGCCCTGAAG AATCTGAAGTTAGACAAGATGGTTGGCTGGCTCCTCCAAC AATCGGCCGCCACTTTGTTGGCCAACAGGCTCACAGAGAA GTGTTGTGTGGAAACAAAAATGAAGATGCTCTTTCTGGCT AACCTGGAGTGTGAAACGCTCTGCCAGGCAGCCCTCCTCC TCTGCTCATGGAGGGCAGCCCTGTGA	
PARK7 (PRK7; DJ-1)	>NM_007262.4:164-733 Homo sapiens Parkinsonism associated deglycase (PARK7), transcript variant 1, mRNA	20

	ATGGCTTCCAAAAGAGCTCTGGTCATCCTGGCTAAAGGAG CAGAGGAAATGGAGACGGTCATCCCTGTAGATGTCATGAG GCGAGCTGGGATTAAGGTCACCGTTGCAGGCCTGGCTGGA AAAGACCCAGTACAGTGTAGCCGTGATGTGGTCATTTGTC CTGATGCCAGCCTTGAAGATGCAAAAAAAGAGGGACCATA TGATGTGGTGGTTCTACCAGGAGGTAATCTGGGCGCACAG AATTTATCTGAGTCTGCTGCTGTGAAGGAGATACTGAAGG AGCAGGAAAACCGGAAGGGCCTGATAGCCGCCATCTGTGC AGGTCCTACTGCTCTGTTGGCTCATGAAATAGGTTTTGGA AGTAAAGTTACAACACACCCTCTTGCTAAAGACAAAATGA TGAATGGAGGTCATTACACCTACTCTGAGAATCGTGTGGA AAAAGACGGCCTGATTCTTACAAGCCGGGGGCCTGGGACC AGCTTCGAGTTTTCGCTTGCAATTGTTGAAGCCCTGAATG GCAAGGAGGTGGCGGCTCAAGTGAAGGCTCCACTTGTCTCT TAAAGACTAG	
LRRK2 (PARK8; PRK8)	>NM_198578.3:122-7705 Homo sapiens leucine rich repeat kinase 2 (LRRK2), mRNA ATGGCTAGTGGCAGCTGTCAGGGGTGCGAAGAGGACGAGG AAACTCTGAAGAAGTTGATAGTCAGGCTGAACAATGTCCA GGAAGGAAAACAGATAGAAACGCTGGTCCAAATCCTGGAG GATCTGCTGGTGTTCACGTA CTCCGAGCACGCCTCCAAGT TATTTCAAGGCAAAAATATCCATGTGCCTCTGTTGATCGT CTTGGA CTCTATATGAGAGTCGCGAGTGTGCAGCAGGTG GGTTGGTCACTTCTGTGCAAATTAATAGAAGTCTGTCCAG GTACAATGCAAAGCTTAATGGGACCCCAGGATGTTGGAAA TGATTGGGAAGTCCTTGGTGTTCACCAATTGATTCTTAAA ATGCTAACAGTTCATAATGCCAGTGTAACTTGT CAGTGA TTGGACTGAAGACCTTAGATCTCCTCCTAACTTCAGGTAA AATCACCTTGCTGATATTGGATGAAGAAAGTGATATTTTC ATGTTAATTTTTGATGCCATGCACTCATTTCCAGCCAATG ATGAAGTCCAGAACTTGGATGCAAAGCTTTACATGTGCT	21

GTTTGAGAGAGTCTCAGAGGAGCAACTGACTGAATTTGTT GAGAACAAAGATTATATGATATTGTTAAGTGCCTTAACAA ATTTTAAAGATGAAGAGGAAATTGTGCTTCATGTGCTGCA TTGTTTACATTCCCTAGCGATTCCCTTGCAATAATGTGGAA GTCCTCATGAGTGGCAATGTCAGGTGTTATAATATTGTGG TGGAAGCTATGAAAGCATTCCCTATGAGTGAAAGAATTCA AGAAGTGAGTTGCTGTTTGCTCCATAGGCTTACATTAGGT AATTTTTTCAATATCCTGGTATTAAACGAAGTCCATGAGT TTGTGGTGAAAGCTGTGCAGCAGTACCCAGAGAATGCAGC ATTGCAGATCTCAGCGCTCAGCTGTTTGGCCCTCCTCACT GAGACTATTTTCTTAAATCAAGATTTAGAGGAAAAGAATG AGAATCAAGAGAATGATGATGAGGGGGAAGAAGATAAATT GTTTTGGCTGGAAGCCTGTTACAAAGCATTAACTGGCAT AGAAAGAACAAGCACGTGCAGGAGGCCGCATGCTGGGCAC TAAATAATCTCCTTATGTACCAAACAGTTTACATGAGAA GATTGGAGATGAAGATGGCCATTTCCCAGCTCATAGGGAA GTGATGCTCTCCATGCTGATGCATTCTTCATCAAAGGAAG TTTTCCAGGCATCTGCGAATGCATTGTCAACTCTCTTAGA ACAAAATGTTAATTTAGAAAAATACTGTTATCAAAGGA ATACACCTGAATGTTTTGGAGTTAATGCAGAAGCATATAC ATTCTCCTGAAGTGGCTGAAAGTGGCTGTAAAATGCTAAA TCATCTTTTTGAAGGAAGCAACACTTCCCTGGATATAATG GCAGCAGTGGTCCCCAAAATACTAACAGTTATGAAACGTC ATGAGACATCATTACCAGTGCAGCTGGAGGCGCTTCGAGC TATTTTACATTTTATAGTGCCTGGCATGCCAGAAGAATCC AGGGAGGATACAGAATTTATCATAAGCTAAATATGGTTA AAAAACAGTGTTTCAAGAATGATATTCACAACTGGTCCT AGCAGCTTTGAACAGGTTTCATTGGAAATCCTGGGATTGAG AAATGTGGATTAAAAGTAATTTCTTCTATTGTACATTTTC CTGATGCATTAGAGATGTTATCCCTGGAAGGTGCTATGGA TTCAGTGCTTCACACACTGCAGATGTATCCAGATGACCAA GAAATTCAGTGTCTGGGTTTAAAGTCTTATAGGATACTTGA TTACAAAGAAGAATGTGTTTCATAGGAACTGGACATCTGCT	
---	--

GGCAAAAATTCTGGTTTCCAGCTTATACCGATTTAAGGAT GTTGCTGAAATACAGACTAAAGGATTTAGACAATCTTAG CAATCCTCAAATTGTCAGCATCTTTTTCTAAGCTGCTGGT GCATCATTCATTTGACTTAGTAATATTCCATCAAATGTCT TCCAATATCATGGAACAAAAGGATCAACAGTTTCTAAACC TCTGTTGCAAGTGTTTTGCAAAAGTAGCTATGGATGATTA CTTAAAAAATGTGATGCTAGAGAGAGCGTGTGATCAGAAT AACAGCATCATGGTTGAATGCTTGCTTCTATTGGGAGCAG ATGCCAATCAAGCAAAGGAGGGATCTTCTTTAATTTGTCA GGTATGTGAGAAAGAGAGCAGTCCCAAATTGGTGGAACCTC TTACTGAATAGTGGATCTCGTGAACAAGATGTACGAAAAG CGTTGACGATAAGCATTGGGAAAGGTGACAGCCAGATCAT CAGCTTGCTCTTAAGGAGGCTGGCCCTGGATGTGGCCAAC AATAGCATTTGCCTTGAGGATTTTGTATAGGAAAAGTTG AACCTTCTTGGCTTGGTCCTTTATTTCCAGATAAGACTTC TAATTTAAGGAAACAAACAAATATAGCATCTACACTAGCA AGAATGGTGATCAGATATCAGATGAAAAGTGCTGTGGAAG AAGGAACAGCCTCAGGCAGCGATGGAAATTTTCTGAAGA TGTGCTGTCTAAATTTGATGAATGGACCTTTATTCCTGAC TCTTCTATGGACAGTGTGTTTGCTCAAAGTGATGACCTGG ATAGTGAAGGAAGTGAAGGCTCATTTCTTGTAAGAAAGAA ATCTAATTCAATTAGTGTAGGAGAATTTTACCGAGATGCC GTATTACAGCGTTGCTCACCAAATTTGCAAAGACATTCCA ATTCTTGGGGCCCATTTTTGATCATGAAGATTTACTGAA GCGAAAAAGAAAAATATTATCTTCAGATGATTCACCTCAGG TCATCAAACTTCAATCCCATATGAGGCATTCAGACAGCA TTTCTTCTCTGGCTTCTGAGAGAGAATATATTACATCACT AGACCTTTCAGCAAATGAACTAAGAGATATTGATGCCCTA AGCCAGAAATGCTGTATAAGTGTTTCAATTTGGAGCATCTTG AAAAGCTGGAGCTTCACCAGAATGCACTCACGAGCTTTCC ACAACAGCTATGTGAACTCTGAAGAGTTTGACACATTTG GACTTGACAGTAATAAATTTACATCATTTCTTCTTATT TGTTGAAAATGAGTTGTATTGCTAATCTTGATGTCTCTCG	
---	--

AAATGACATTGGACCCTCAGTGGTTTTAGATCCTACAGTG AAATGTCCAACCTCTGAAACAGTTTAAACCTGTCATATAACC AGCTGTCTTTTGTACCTGAGAACCTCACTGATGTGGTAGA GAAACTGGAGCAGCTCATTTTAGAAGGAAATAAAATATCA GGGATATGCTCCCCCTTGAGACTGAAGGAACTGAAGATTT TAAACCTTAGTAAGAACCACATTTTCATCCCTATCAGAGAA CTTTCTTGAGGCTTGTCTAAAGTGGAGAGTTTCAGTGCC AGAATGAATTTTCTTGCTGCTATGCCTTTCTTGCCCTCCTT CTATGACAATCCTAAAATTATCTCAGAACAAATTTTCCTG TATTCCAGAAGCAATTTTAAATCTTCCACACTTGCGGTCT TTAGATATGAGCAGCAATGATATTCAGTACCTACCAGGTC CCGCACACTGGAAATCTTTGAACTTAAGGGAACCTTTATT TAGCCATAATCAGATCAGCATCTTGGACTTGAGTGAAAAA GCATATTTATGGTCTAGAGTAGAGAACTGCATCTTCTC ACAATAAACTGAAAGAGATTCCCTCCTGAGATTGGCTGTCT TGAAAATCTGACATCTCTGGATGTCAGTTACAACCTTGAA CTAAGATCCTTTCCCAATGAAATGGGGAAATTAAGCAAAA TATGGGATCTTCCTTTGGATGAACTGCATCTTAACCTTGA TTTTAAACATATAGGATGTAAAGCCAAAGACATCATAAGG TTTCTTCAACAGCGATTAAAAAAGGCTGTGCCTTATAACC GAATGAAACTTATGATTGTGGGAAATACTGGGAGTGGTAA AACCACCTTATTGCAGCAATTAATGAAAACCAAGAAATCA GATCTTGGAATGCAAAGTGCCACAGTTGGCATAGATGTGA AAGACTGGCCTATCCAAATAAGAGACAAAAGAAAGAGAGA TCTCGTCCTAAATGTGTGGGATTTTGCAGGTCGTGAGGAA TTCTATAGTACTCATCCCCATTTTATGACGCAGCGAGCAT TGTACCTTGCTGTCTATGACCTCAGCAAGGGACAGGCTGA AGTTGATGCCATGAAGCCTTGGCTCTTCAATATAAAGGCT CGCGCTTCTTCTTCCCCTGTGATTCTCGTTGGCACACATT TGGATGTTTCTGATGAGAAGCAACGCAAAGCCTGCATGAG TAAAATCACCAAGGAACTCCTGAATAAGCGAGGGTTCCCT GCCATACGAGATTACCACTTTGTGAATGCCACCGAGGAAT CTGATGCTTTGGCAAACTTCGGAAAACCATCATAAACGA	
---	--

GAGCCTTAATTTCAAGATCCGAGATCAGCTTGTTGTTGGA CAGCTGATTCCAGACTGCTATGTAGAACTTGAAAAATCA TTTTATCGGAGCGTAAAAATGTGCCAATTGAATTTCCCGT AATTGACCGGAAACGATTATTACAAC TAGTGAGAGAAAAT CAGCTGCAGTTAGATGAAAATGAGCTTCCTCACGCAGTTC ACTTTCTAAATGAATCAGGAGTCCTTCTTCATTTTCAAGA CCCAGCACTGCAGTTAAGTGACTTGTACTTTGTGGAACCC AAGTGGCTTTGTAAAATCATGGCACAGATTTTGACAGTGA AAGTGGAAGGTTGTCCAAAACACCCTAAGGGCATTATTTTC GCGTAGAGATGTGGAAAAATTTCTTTCAAAAAAAGGAAA TTTCCAAAGAACTACATGTCACAGTATTTTAAGCTCCTAG AAAAATTCCAGATTGCTTTGCCAATAGGAGAAGAATATTT GCTGGTTCCAAGCAGTTTGTCTGACCACAGGCCTGTGATA GAGCTTCCCCATTGTGAGAACTCTGAAATTATCATCCGAC TATATGAAATGCCTTATTTTCCAATGGGATTTTGGTCAAG ATTAATCAATCGATTACTTGAGATTTTACCTTACATGCTT TCAGGGAGAGAACGAGCACTTCGCCCAAACAGAATGTATT GGCGACAAGGCATTTACTTAAATTGGTCTCCTGAAGCTTA TTGTCTGGTAGGATCTGAAGTCTTAGACAATCATCCAGAG AGTTTCTTAAAATTACAGTTCCTTCTTGTAGAAAAGGCT GTATTCTTTTGGGCCAAGTTGTGGACCACATTGATTCTCT CATGGAAGAATGGTTTCCTGGGTTGCTGGAGATTGATATT TGTGGTGAAGGAGAACTCTGTTGAAGAAATGGGCATTAT ATAGTTTTAATGATGGTGAAGAACATCAAAAAATCTTACT TGATGACTTGATGAAGAAAGCAGAGGAAGGAGATCTCTTA GTAAATCCAGATCAACCAAGGCTCACCATTCCAATATCTC AGATTGCCCCTGACTTGATTTTGGCTGACCTGCCTAGAAA TATTATGTTGAATAATGATGAGTTGGAATTTGAACAAGCT CCAGAGTTTCTCCTAGGTGATGGCAGTTTGGATCAGTTT ACCGAGCAGCCTATGAAGGAGAAGAAGTGGCTGTGAAGAT TTTTAATAAACATACATCACTCAGGCTGTTAAGACAAGAG CTTGTGGTGCTTTGCCACCTCCACCACCCAGTTTGATAT CTTTGCTGGCAGCTGGGATTCGTCCCCGGATGTTGGTGAT	
---	--

GGAGTTAGCCTCCAAGGGTTCCTTGGATCGCCTGCTTCAG CAGGACAAAGCCAGCCTCACTAGAACCCCTACAGCACAGGA TTGCACTCCACGTAGCTGATGGTTTGAGATACCTCCACTC AGCCATGATTATATACCGAGACCTGAAACCCCACAATGTG CTGCTTTTCACACTGTATCCCAATGCTGCCATCATTGCAA AGATTGCTGACTACGGCATTGCTCAGTACTGCTGTAGAAT GGGGATAAAAACATCAGAGGGCACACCAGGGTTTCGTGCA CCTGAAGTTGCCAGAGGAAATGTCATTTATAACCAACAGG CTGATGTTTATTCATTTGGTTTACTACTCTATGACATTTT GACAACTGGAGGTAGAATAGTAGAGGGTTTGAAGTTTCCA AATGAGTTTGATGAATTAGAAATACAAGGAAAATTACCTG ATCCAGTTAAAGAATATGGTTGTGCCCCATGGCCTATGGT TGAGAAATTAATTAAACAGTGTTTGAAAGAAAATCCTCAA GAAAGGCCTACTTCTGCCCAGGTCTTTGACATTTTGAATT CAGCTGAATTAGTCTGTCTGACGAGACGCATTTTATTACC TAAAAACGTAATTGTTGAATGCATGGTTGCTACACATCAC AACAGCAGGAATGCAAGCATTGCGCTGGGCTGTGGGCACA CCGACAGAGGACAGCTCTCATTTCTTGACTTAAATACTGA AGGATACACTTCTGAGGAAGTTGCTGATAGTAGAATATTG TGCTTAGCCTTGGTGCATCTTCCTGTTGAAAAGGAAAGCT GGATTGTGTCTGGGACACAGTCTGGTACTCTCCTGGTCAT CAATACCGAAGATGGGAAAAAGAGACATACCCTAGAAAAG ATGACTGATTCTGTCACTTGTTTGTATTGCAATTCCTTTT CCAAGCAAAGCAAACAAAAAATTTTCTTTTGGTTGGAAC CGCTGATGGCAAGTTAGCAATTTTGAAGATAAGACTGTT AAGCTTAAAGGAGCTGCTCCTTTGAAGATACTAAATATAG GAAATGTCAGTACTCCATTGATGTGTTTGAGTGAATCCAC AAATTC AACGGAAAGAAATGTAATGTGGGGAGGATGTGGC ACAAAGATTTTCTCCTTTTCTAATGATTTACCATTCAGA AACTCATTGAGACAAGAACAAGCCAACGTGTTTTCTTATGC AGCTTTCAGTGATTCCAACATCATAACAGTGGTGGTAGAC ACTGCTCTCTATATTGCTAAGCAAAATAGCCCTGTTGTGG AAGTGTGGGATAAGAAAAC TGAAAACTCTGTGGACTAAT	
---	--

	AGACTGCGTGCACTTTTTAAGGGAGGTAATGGTAAAAGAA AACAAAGGAATCAAAACACAAAATGTCTTATTCTGGGAGAG TGAAAACCCCTCTGCCTTCAGAAGAACACTGCTCTTTGGAT AGGAACTGGAGGAGGCCATATTTTACTCCTGGATCTTTCA ACTCGTCGACTTATACGTGTAATTTACAACCTTTGTAAAT CGGTCAGAGTCATGATGACAGCACAGCTAGGAAGCCTTAA AAATGTCATGCTGGTATTGGGCTACAACCGGAAAAATACT GAAGGTACACAAAAGCAGAAAGAGATACAATCTTGCTTGA CCGTTTGGGACATCAATCTTCCACATGAAGTGCAAAATTT AGAAAAACACATTGAAGTGAGAAAAGAATTAGCTGAAAAA ATGAGACGAACATCTGTTGAGTAA	
alpha- synuclein (PARK1; PRK1)	>NM_000345.3:264-686 Homo sapiens synuclein alpha (SNCA), transcript variant 1, mRNA ATGGATGTATTCATGAAAGGACTTTCAAAGGCCAAGGAGG GAGTTGTGGCTGCTGCTGAGAAAACCAAACAGGGTGTGGC AGAAGCAGCAGGAAAGACAAAAGAGGGTGTCTCTATGTA GGCTCCAAAACCAAGGAGGGAGTGGTGCATGGTGTGGCAA CAGTGGCTGAGAAGACCAAAGAGCAAGTGACAAATGTTGG AGGAGCAGTGGTGACGGGTGTGACAGCAGTAGCCCAGAAG ACAGTGGAGGGAGCAGGGAGCATTGCAGCAGCCACTGGCT TTGTCAAAAAGGACCAGTTGGGCAAGAATGAAGAAGGAGC CCCACAGGAAGGAATTCTGGAAGATATGCCTGTGGATCCT GACAATGAGGCTTATGAAATGCCTTCTGAGGAAGGGTATC AAGACTACGAACCTGAAGCCTAA	22
c-Rel-NFkB	>NM_002908.3:347-2206 Homo sapiens REL proto-oncogene, NF-kB subunit (REL), transcript variant 1, mRNA ATGGCCTCCGGTGCGTATAACCCGTATATAGAGATAATTG AACAAACCCAGGCAGAGGGGAATGCGTTTTAGATACAAATG TGAAGGGCGATCAGCAGGCAGCATTCCAGGGGAGCACAGC	23

ACAGACAACAACCGAACATACCCTTCTATCCAGATTATGA ACTATTATGGAAAAGGAAAAGTGAGAATTACATTAGTAAC AAAGAATGACCCATATAAACCTCATCCTCATGATTTAGTT GGAAAAGACTGCAGAGACGGCTACTATGAAGCAGAATTTG GACAAGAACGCAGACCTTTGTTTTTTCCAAAATTTGGGTAT TCGATGTGTGAAGAAAAAAGAAGTAAAAGAAGCTATTATT ACAAGAATAAAGGCAGGAATCAATCCATTCAATGTCCCTG AAAAACAGCTGAATGATATTGAAGATTGTGACCTCAATGT GGTGAGACTGTGTTTTCAAGTTTTTCTCCCTGATGAACAT GGTAATTTGACGACTGCTCTTCCTCCTGTTGTCTCGAACC CAATTTATGACAACCGTGCTCCAAATACTGCAGAATTAAG GATTTGTCGTGTAAACAAGAATTGTGGAAGTGTGAGAGGA GGAGATGAAATATTTCTACTTTGTGACAAAGTTCAGAAAG ATGACATAGAAGTTCGTTTTGTGTTGAACGATTGGGAAGC AAAAGGCATCTTTTCACAAGCTGATGTACACCGTCAAGTA GCCATTGTTTTCAAACCTCCACCATATTGCAAAGCTATCA CAGAACCCGTAAACAGTAAAAATGCAGTTGCGGAGACCTTC TGACCAGGAAGTTAGTGAATCTATGGATTTTAGATATCTG CCAGATGAAAAAGATACTTACGGCAATAAAGCAAAGAAAC AAAAGACAACCTCTGCTTTTCCAGAACTGTGCCAGGATCA CGTAGAAACAGGGTTTTCGCCATGTTGACCAGGATGGTCTT GAACTCCTGACATCAGGTGATCCACCCACCTTGGCCTCCC AAAGTGCTGGGATTACAGTTAATTTTCCTGAGAGACCAAG ACCTGGTCTCCTCGGTTCAATTGGAGAAGGAAGATACTTC AAAAAAGAACCAACTTGTTTTCTCATGATGCAGTTGTGA GAGAAATGCCTACAGGGGTTTCAAGTCAAGCAGAATCCTA CTATCCCTCACCTGGGCCCATCTCAAGTGGATTGTCACAT CATGCCTCAATGGCACCTCTGCCTTCTTCAAGCTGGTCAT CAGTGGCCCACCCACCCACGCTCAGGCAATACAAACCC ACTGAGTAGTTTTTCAACAAGGACACTTCCTTCTAATTCTG CAAGGTATCCCACCATTCCTGAGAATACCTGTTGGGAATG ATTTAAATGCTTCTAATGCTTGCATTTACAACAATGCCGA TGACATAGTCGGAATGGAAGCGTCATCCATGCCATCAGCA	
---	--

	GATTTATATGGTATTTCTGATCCCAACATGCTGTCTAATT GTTCTGTGAATATGATGACAACCAGCAGTGACAGCATGGG AGAGACTGATAATCCAAGACTTCTGAGCATGAATCTTGAA AACCCCTCATGTAATTCAGTGTTAGACCCAAGAGACTTGA GACAGCTCCATCAGATGTCCTCTTCCAGTATGTCAGCAGG CGCCAATTCCAATACTACTGTTTTTGTTCACAATCAGAT GCATTTGAGGGATCTGACTTCAGTTGTGCAGATAACAGCA TGATAAATGAGTCGGGACCATCAAACAGTACTAATCCAAA CAGTCATGGTTTTTGTTCAGATAGTCAGTATTCAGGTATT GGCAGTATGCAAAATGAGCAATTGAGTGAATCCTTTCCAT ATGAATTTTTTCAAGTATAA	
ATG7	>NM_028835.4:74-2170 Mus musculus autophagy related 7 (Atg7), transcript variant 3, mRNA ATGGGGGACCCTGGACTGGCCAAGTTGCAGTTCGCCCCCT TTAATAGTGCCCTGGACGTTGGCTTCTGGCACGAACTGAC CCAGAAGAAGTTGAACGAGTACCGCCTGGACGAGGCACCC AAAGACATCAAGGGCTATTACTACAATGGTGACTCTGCTG GTCTGCCCACCCGCTTGACGTTGGAGTTCAGTGCTTTTGA CATGAGTGCCTCCACGCCTGCCCACTGCTGCCCGGCCATG GGAACCCTGCACAACACCAACACACTTGAGGCTTTTAAGA CAGCAGACAAGAAGCTCCTTCTGGAGCAGTCAGCAAATGA GATCTGGGAAGCCATAAAGTCAGGTGCTGCTCTCGAAAAC CCCATGCTCCTCAACAAGTTTCTGCTCCTGACCTTCGCGG ACCTAAAGAAGTACCACTTCTACTACTGGTTTTGCTGCCC CGCCCTCTGTCTTCCTGAGAGCATCCCTCTAATCCGGGGA CCTGTGAGCTTGATCAAAGGCTTTCACCAAAACAGATCC AGGCCCTGGAGCATGCCTATGATGATCTGTGTCGAGCCGA AGGCGTCACGGCCCTGCCCTACTTCTTATTCAAGTACGAT GACGACACTGTTCTGGTCTCCTTGCTCAAACACTACAGTG ATTTCTTCCAAGGTCAAAGGACAAAGATAACAGTTGGTGT GTACGATCCCTGTAACCTAGCCCAGTACCCTGGATGGCCT	24

TTGAGGAATTTTTTGGTCCTGGCAGCCACAGATGGAGCG GCAGTTTCCAGTCCGTTGAAGTCCTCTGCTTTCGGGACCG CACCATGCAGGGAGCTAGAGACGTGACACATAGCATCATC TTTGAAGTGAACTTCCAGAAATGGCATTTAGCCCAGATT GTCCTAAAGCTGTTGGCTGGGAGAAGAACCAGAAAGGAGG CATGGGTCCGAGGATGGTGAACCTCAGTGGATGTATGGAC CCCCAAAGGCTGGCTGAGTCATCTGTGGATCTGAATCTCA AGCTGATGTGCTGGCGATTGGTCCCCACCTTGGACTTGGA CAAGGTCGTGTCTGTCAAGTGCCTGCTGCTGGGAGCTGGT ACCTTGGGGTGTAATGTGGCTAGGACACTGATGGGCTGGG GCGTCAGACATGTCACCTTTGTGGATAACGCCAAGATCTC CTACTCCAATCCCGTGAGGCAGCCTCTGTATGAATTTGAA GATTGTCTAGGGGGTGGCAAGCCCAAGGCCCTGGCTGCAG CAGAGCGGCTACAGAAAATATTTCCCGGAGTGAATGCCAG AGGGTTCAACATGAGCATCCCCATGCCAGGACACCCTGTG AACTTCTCTGACGTCACGATGGAGCAGGCCCGCAGAGATG TGGAGCAGCTGGAGCAGCTCATTGATAACCATGATGTCAT CTTCCTGCTAATGGACACCAGGGAGAGCCGGTGGCTTCCT ACTGTTATTGCAGCCAGCAAGCGAAAGCTGGTCATCAACG CTGCCTTGGGGTTTGATACCTTTGTTGTCATGAGACATGG CCTGAAGAAACCCAAGCAGCAGGGAGCCGGAGACCTCTGC CCAAGCCATCTTGTAGCACCTGCTGACCTGGGCTCCTCAC TTTTTGCCAACATCCCTGGATACAAGCTTGGCTGCTACTT CTGCAATGATGTGGTGGCTCCAGGAGATTCAACCAGAGAC CGGACTCTGGACCAGCAGTGCACAGTGAGCCGCCAGGCC TGGCCGTGATTGCAGGTGCCCTGGCTGTGGAGCTGATGGT CTCTGTCCTGCAGCATCCTGAGGGGGGCTACGCCATCGCC AGCAGCAGTGATGACCGCATGAATGAGCCTCCCACCTCGC TGGGACTTGTGCCTCACCAGATCCGGGGTTTTCTGTCACG GTTTCGATAATGTTCTTCCTGTCAGCCTGGCATTGATAAA TGTACAGCCTGTTACCCAAAGTTCTTGATCAGTACGAGC GAGAAGGATTCACCTTCCTAGCGAAGGTTTTTAACTCCTC ACATTCTTCTTAGAAGACTTGACCGGTCTTACCCTGCTC	
--	--

	CATCAAGAGACCCAAGCTGCTGAGATCTGGGACATGAGTG ACGAGGAGACTGTCTGA	
VMAT2	>NM_003054.4:164-1708 Homo sapiens solute carrier family 18 member A2 (SLC18A2), mRNA ATGGCCCTGAGCGAGCTGGCGCTGGTCCGCTGGCTGCAGG AGAGCCGCCGCTCGCGGAAGCTCATCCTGTTTCATCGTGTT CCTGGCGCTGCTGCTGGACAACATGCTGCTCACTGTCGTG GTCCCCATCATCCCAAGTTATCTGTACAGCATTAAAGCATG AGAAGAATGCTACAGAAATCCAGACGGCCAGGCCAGTGCA CACTGCCTCCATCTCAGACAGCTTCCAGAGCATCTTCTCC TATTATGATAACTCGACTATGGTCACCGGGAATGCTACCA GAGACCTGACACTTCATCAGACCGCCACACAGCACATGGT GACCAACGCGTCCGCTGTTCCCTCCGACTGTCCCAGTGAA GACAAAGACCTCCTGAATGAAAACGTGCAAGTTGGTCTGT TGTTTGCCTCGAAAGCCACCGTCCAGCTCATCACCAACCC TTTCATAGGACTACTGACCAACAGAATTGGCTATCCAATT CCCATATTTGCGGGATTCTGCATCATGTTTGTCTCAACAA TTATGTTTGCCTTCTCCAGCAGCTATGCCTTCCTGCTGAT TGCCAGGTCGCTGCAGGGCATCGGCTCGTCCTGCTCCTCT GTGGCTGGGATGGGCATGCTTGCCAGTGTCTACACAGATG ATGAAGAGAGAGGCAACGTCATGGGAATCGCCTTGGGAGG CCTGGCCATGGGGGTCTTAGTGGGCCCCCCTTCGGGAGT GTGCTCTATGAGTTTGTGGGGAAGACGGCTCCGTTCTGG TGCTGGCCGCCCTGGTACTCTTGATGGAGCTATTACAGCT CTTTGTGCTCCAGCCGTCCCGGGTGCAGCCAGAGAGTCAG AAGGGGACACCCCTAACCACGCTGCTGAAGGACCCGTACA TCCTCATTGCTGCAGGCTCCATCTGCTTTGCAAACATGGG CATCGCCATGCTGGAGCCAGCCCTGCCCATCTGGATGATG GAGACCATGTGTTCCCGAAAGTGGCAGCTGGGCGTTGCCT TCTTGCCAGCTAGTATCTTATCTCATTGGAACCAATAT TTTTGGGATACTTGCACACAAAATGGGGAGGTGGCTTTGT	25

	GCTCTTCTGGGAATGATAATTGTTGGAGTCAGCATTTTAT GTATTCCATTTGCAAAAACATTTATGGACTCATAGCTCC GAACTTTGGAGTTGGTTTTGCAATTGGAATGGTGGATTCTG TCAATGATGCCTATCATGGGCTACCTCGTAGACCTGCGGC ACGTGTCCGTCTATGGGAGTGTGTACGCCATTGCGGATGT GGCATTTTGTATGGGGTATGCTATAGGTCCTTCTGCTGGT GGTGCTATTGCAAAGGCAATTGGATTTCCATGGCTCATGA CAATTATTGGGATAATTGATATTCTTTTTGCCCTCTCTG CTTTTTTCTTCGAAGTCCACCTGCCAAAGAAGAAAAAATG GCTATTCTCATGGATCACAACCTGCCCTATTAAAACAAAAA TGTACACTCAGAATAATATCCAGTCATATCCGATAGGTGA AGATGAAGAATCTGAAAGTGAAGTGA	
GBA	>NM_000157.3 Homo sapiens glucosylceramidase beta (GBA), transcript variant 1, mRNA ATCACATGACCCATCCACATCGGGAAGCCGGAATTACTTG CAGGGCTAACCTAGTGCCTATAGCTAAGGCAGGTACCTGC ATCCTTGTTTTTGTGTTAGTGGATCCTCTATCCTTCAGAGA CTCTGGAACCCCTGTGGTCTTCTCTTCATCTAATGACCCT GAGGGGATGGAGTTTTCAAGTCCTTCCAGAGAGGAATGTC CCAAGCCTTTGAGTAGGGTAAGCATCATGGCTGGCAGCCT CACAGGATTGCTTCTACTTCAGGCAGTGTCTGTTGGCATCA GGTGCCCGCCCTGCATCCCTAAAAGCTTCGGCTACAGCT CGGTGGTGTGTGTCTGCAATGCCACATACTGTGACTCCTT TGACCCCCCGACCTTTCCTGCCCTTGGTACCTTCAGCCGC TATGAGAGTACACGCAGTGGGCGACGGATGGAGCTGAGTA TGGGGCCCATCCAGGCTAATCACACGGGCACAGGCCTGCT ACTGACCCTGCAGCCAGAACAGAAGTTCCAGAAAGTGAAG GGATTTGGAGGGGCCATGACAGATGCTGCTGCTCTCAACA TCCTTGCCCTGTCACCCCTGCCCAAATTTGCTACTTAA ATCGTACTTCTCTGAAGAAGGAATCGGATATAACATCATC CGGGTACCCATGGCCAGCTGTGACTTCTCCATCCGCACCT	26

ACACCTATGCAGACACCCCTGATGATTTCAGTTGCACAA CTTCAGCCTCCCAGAGGAAGATACCAAGCTCAAGATACCC CTGATTACCGAGCCCTGCAGTTGGCCCAGCGTCCCGTTT CACTCCTTGCCAGCCCCCTGGACATCACCCACTTGGCTCAA GACCAATGGAGCGGTGAATGGGAAGGGGTCACTCAAGGGA CAGCCCGGAGACATCTACCACCAGACCTGGGCCAGATACT TTGTGAAGTTCCTGGATGCCTATGCTGAGCACAAGTTACA GTTCTGGGCAGTGACAGCTGAAAATGAGCCTTCTGCTGGG CTGTTGAGTGGATACCCCTTCCAGTGCCTGGGCTTCACCC CTGAACATCAGCGAGACTTCATTGCCCCGTGACCTAGGTCC TACCCTCGCCAACAGTACTCACCACAATGTCCGCCTACTC ATGCTGGATGACCAACGCTTGCTGCTGCCCCACTGGGCAA AGGTGGTACTGACAGACCCAGAAGCAGCTAAATATGTTCA TGGCATTGCTGTACATTGGTACCTGGACTTTCTGGCTCCA GCCAAAGCCACCCTAGGGGAGACACACCGCCTGTTCCCCA ACACCATGCTCTTTGCCTCAGAGGCCTGTGTGGGCTCCAA GTTCTGGGAGCAGAGTGTGCGGCTAGGCTCCTGGGATCGA GGGATGCAGTACAGCCACAGCATCATCACGAACCTCCTGT ACCATGTGGTTCGGCTGGACCGACTGGAACCTTGCCCTGAA CCCCGAAGGAGGACCCAATTGGGTGCGTAACTTTGTGAC AGTCCCATCATTGTAGACATCACCAAGGACACGTTTTACA AACAGCCCATGTTCTACCACCTTGGCCACTTCAGCAAGTT CATTCCTGAGGGCTCCCAGAGAGTGGGGCTGGTTGCCAGT CAGAAGAACGACCTGGACGCAGTGGCACTGATGCATCCCG ATGGCTCTGCTGTTGTGGTCGTGCTAAACCGCTCCTCTAA GGATGTGCCTCTTACCATCAAGGATCCTGCTGTGGGCTTC CTGGAGACAATCTCACCTGGCTACTCCATTACACCTACC TGTGGCGTCGCCAGTGATGGAGCAGATACTCAAGGAGGCA CTGGGCTCAGCCTGGGCATTAAAGGGACAGAGTCAGCTCA CACGCTGTCTGTGACTAAAGAGGGCACAGCAGGGCCAGTG TGAGCTTACAGCGACGTAAGCCCAGGGGCAATGGTTTGGG TGACTCACTTTCCCCTCTAGGTGGTGCCAGGGGCTGGAGG CCCCTAGAAAAAGATCAGTAAGCCCCAGTGTCCCCCAGC	
--	--

	CCCCATGCTTATGTGAACATGCGCTGTGTGCTGCTTGCTT TGGAAACTGGGCCTGGGTCCAGGCCTAGGGTGAGCTCACT GTCCGTACAAACACAAGATCAGGGCTGAGGGTAAGGAAAA GAAGAGACTAGGAAAGCTGGGCCCAAACTGGAGACTGTT TGTCTTTCCTGGAGATGCAGAACTGGGCCCGTGGAGCAGC AGTGTCTAGCATCAGGGCGGAAGCCTTAAAGCAGCAGCGGG TGTGCCCAGGCACCCAGATGATTCCTATGGCACCAGCCAG GAAAAATGGCAGCTCTTAAAGGAGAAAATGTTTGAGCCCA GTCA	
--	--	--

Table 4: Polynucleotides for Isoforms of PARK2

Gene (Isoform)	Protein Sequence	SEQ ID NO:
PARK2 (1)	>NM_004562.2:135-1532 Homo sapiens parkin RBR E3 ubiquitin protein ligase (PRKN), transcript variant 1, mRNA ATGATAGTGTTCAGGTTCAACTCCAGCCATGGTTTCC CAGTGGAGGTGATTCTGACACCAGCATCTTCCAGCTCAA GGAGGTGGTTGCTAAGCGACAGGGGGTTCCGGCTGACCAG TTGCGTGTGATTTTCGCAGGGAAGGAGCTGAGGAATGACT GGACTGTGCAGAATTGTGACCTGGATCAGCAGAGCATTGT TCACATTGTGCAGAGACCGTGGAGAAAAGGTCAAGAAATG AATGCAACTGGAGGCGACGACCCAGAAACGCGGCGGGAG GCTGTGAGCGGGAGCCCCAGAGCTTGACTCGGGTGGACCT CAGCAGCTCAGTCCTCCAGGAGACTCTGTGGGGCTGGCT GTCATTCTGCACACTGACAGCAGGAAGGACTCACCACCAG CTGGAAGTCCAGCAGGTAGATCAATCTACAACAGCTTTTA TGTGTATTGCAAAGGCCCTGTCAAAGAGTGCAGCCGGGA AAACTCAGGGTACAGTGCAGCACCTGCAGGCAGGCAACGC TCACCTTGACCCAGGGTCCATCTTGCTGGGATGATGTTTT AATTCCAAACCGGATGAGTGGTGAATGCCAATCCCCACAC TGCCCTGGGACTAGTGCAGAAATTTTCTTTAAATGTGGAG CACACCCACCTCTGACAAGGAAACATCAGTAGCTTTGCA	27

	CCTGATCGCAACAAATAGTCGGAACATCACTTGCATTACG TGCACAGACGTCAGGAGCCCCGTCCTGGTTTTCCAGTGCA ACTCCCGCCACGTGATTTGCTTAGACTGTTTCCACTTATA CTGTGTGACAAGACTCAATGATCGGCAGTTTGTTCACGAC CCTCAACTTGGCTACTCCCTGCCTTGTGTGGCTGGCTGTC CCAACTCCTTGATTAAAGAGCTCCATCACTTCAGGATTCT GGGAGAAGAGCAGTACAACCGGTACCAGCAGTATGGTGCA GAGGAGTGTGTCCTGCAGATGGGGGGCGTGTTATGCCCCC GCCCTGGCTGTGGAGCGGGGCTGCTGCCGGAGCCTGACCA GAGGAAAGTCACCTGCGAAGGGGGCAATGGCCTGGGCTGT GGGTTTGCTTCTGCCGGGAATGTAAAGAAGCGTACCATG AAGGGGAGTGCAGTGCCGTATTTGAAGCCTCAGGAACAAC TACTCAGGCCTACAGAGTCGATGAAAGAGCCGCCGAGCAG GCTCGTTGGGAAGCAGCCTCCAAAGAAACCATCAAGAAAA CCACCAAGCCCTGTCCCCGCTGCCATGTACCAGTGGAAAA AAATGGAGGCTGCATGCACATGAAGTGTCCGCAGCCCCAG TGCAGGCTCGAGTGGTGCTGGAAGTGTGGCTGCGAGTGGA ACCGCGTCTGCATGGGGGACCACTGGTTCGACGTGTAG	
PARK2 (2)	>NM_013987.2:135-1448 Homo sapiens parkin RBR E3 ubiquitin protein ligase (PRKN), transcript variant 2, mRNA ATGATAGTGTGTTGTCAGGTTCAACTCCAGCCATGGTTTCC CAGTGGAGGTGCGATTCTGACACCAGCATCTTCCAGCTCAA GGAGGTGGTTGCTAAGCGACAGGGGGTTCCGGCTGACCAG TTGCGTGTGATTTTCGCAGGGAAGGAGCTGAGGAATGACT GGACTGTGCAGAATTGTGACCTGGATCAGCAGAGCATTGT TCACATTGTGCAGAGACCGTGGAGAAAAGGTCAAGAAATG AATGCAACTGGAGGCGACGACCCCAGAAACGCGGCGGGAG GCTGTGAGCGGGAGCCCCAGAGCTTGACTCGGGTGGACCT CAGCAGCTCAGTCCTCCCAGGAGACTCTGTGGGGCTGGCT GTCATTCTGCACACTGACAGCAGGAAGGACTCACCACCAG CTGGAAGTCCAGCAGGTAGATCAATCTACAACAGCTTTTA	28

	TGTGTATTGCAAAGGCCCTGTCAAAGAGTGCAGCCGGGA AAACCTCAGGGTACAGTGCAGCACCTGCAGGCAGGCAACGC TCACCTTGACCCAGGAATTTTTCTTTAAATGTGGAGCACA CCCCACCTCTGACAAGGAAACATCAGTAGCTTTGCACCTG ATCGCAACAAATAGTCGGAACATCACTTGCATTACGTGCA CAGACGTCAGGAGCCCCGTCCTGGTTTTCCAGTGCAACTC CCGCCACGTGATTTGCTTAGACTGTTTCCACTTATACTGT GTGACAAGACTCAATGATCGGCAGTTTGTTCACGACCCTC AACTTGGCTACTCCCTGCCTTGTGTGGCTGGCTGTCCCAA CTCCTTGATTAAAGAGCTCCATCACTTCAGGATTCTGGGA GAAGAGCAGTACAACCGGTACCAGCAGTATGGTGCAGAGG AGTGTGTCCTGCAGATGGGGGGCGTGTTATGCCCCGCCC TGGCTGTGGAGCGGGGCTGCTGCCGGAGCCTGACCAGAGG AAAGTCACCTGCGAAGGGGGCAATGGCCTGGGCTGTGGGT TTGCCTTCTGCCGGGAATGTAAAGAAGCGTACCATGAAGG GGAGTGCAGTGCCGTATTTGAAGCCTCAGGAACAACACTACT CAGGCCTACAGAGTCGATGAAAGAGCCGCCGAGCAGGCTC GTTGGGAAGCAGCCTCCAAAGAAACCATCAAGAAAACCAC CAAGCCCTGTCCCCGCTGCCATGTACCAGTGGAaaaaaAT GGAGGCTGCATGCACATGAAGTGTCCGCAGCCCCAGTGCA GGCTCGAGTGGTGTGGAAGTGTGGCTGCGAGTGGAACCG CGTCTGCATGGGGGACCACTGGTTCGACGTGTAG	
PARK2 (3)	>Ensembl ENST00000479615.5 ATGAATGCAACTGGAGGCGACGACCCCAGAAACGCGGCGG GAGGCTGTGAGCGGGAGCCCCAGAGCTTGACTCGGGTGGA CCTCAGCAGCTCAGTCCTCCCAGGAGACTCTGTGGGGCTG GCTGTCATTCTGCACACTGACAGCAGGAAGGACTCACCAC CAGCTGGAAGTCCAGCAGGTAGATCAATCTACAACAGCTT TTATGTGTATTGCAAAGGCCCTGTCAAAGAGTGCAGCCG GGAAAACTCAGGGTACAGTGCAGCACCTGCAGGCAGGCAA CGCTCACCTTGACCCAGGGTCCATCTTGCTGGGATGATGT	29

	TTTAATTCCAAACCGGATGAGTGGTGAATGCCAATCCCCA CACTGCCCTGGGACTAGTGCAGAATTTTTCTTTAAATGTG GAGCACACCCACCTCTGACAAGGAAACATCAGTAGCTTT GCACCTGATCGCAACAAATAGTCGGAACATCACTTGCATT ACGTGCACAGACGTCAGGAGCCCCGTCCTGGTTTTCCAGT GCAACTCCCGCCACGTGATTTGCTTAGACTGTTTCCACTT ATACTGTGTGACAAGACTCAATGATCGGCAGTTTGTTCAC GACCCTCAACTTGGCTACTCCCTGCCTTGTGTGGTTTGCC TTCTGCCGGGAATGTAA	
PARK2 (4)	>Ensembl ENST00000338468.7 ATGAGTGGTGAATGCCAATCCCCACACTGCCCTGGGACTA GTGCAGAATTTTTCTTTAAATGTGGAGCACACCCACCTC TGACAAGGAAACATCAGTAGCTTTGCACCTGATCGCAACA AATAGTCGGAACATCACTTGCATTACGTGCACAGACGTCA GGAGCCCCGTCCTGGTTTTCCAGTGCAACTCCCGCCACGT GATTTGCTTAGACTGTTTCCACTTATACTGTGTGACAAGA CTCAATGATCGGCAGTTTGTTCACGACCCTCAACTTGGCT ACTCCCTGCCTTGTGTGGCTGGCTGTCCCAACTCCTTGAT TAAAGAGCTCCATCACTTCAGGATTCTGGGAGAAGAGCAG TACAACCGGTACCAGCAGTATGGTGCAGAGGAGTGTGTCC TGCAGATGGGGGGCGTGTTATGCCCCCGCCCTGGCTGTGG AGCGGGGCTGCTGCCGGAGCCTGACCAGAGGAAAGTCACC TGCGAAGGGGGCAATGGCCTGGGCTGTGGGTTTGCCTTCT GCCGGGAATGTAAAGAAGCGTACCATGAAGGGGAGTGCAG TGCCGTATTTGAAGCCTCAGGAACAACACTCAGGCCTAC AGAGTCGATGAAAGAGCCGCCGAGCAGGCTCGTTGGGAAG CAGCCTCCAAAGAAACCATCAAGAAAACCACCAAGCCCTG TCCCCGCTGCCATGTACCAGTGGAAAAAATGGAGGCTGC ATGCACATGAAGTGTCCGCAGCCCCAGTGCAGGCTCGAGT GGTGCTGGAAGTGTGGCTGCGAGTGGAACCGCGTCTGCAT GGCGGACCACTGGTTCCGACGTGTAG	30

PARK2 (5)	>ENA ALQ33698 ALQ33698.1 Homo sapiens (human) partial parkinson protein 2 E3 ubiquitin protein ligase isoform 1 ATGATAGTGTGTTGTCAGGTTCAACTCCAGCCATGGTTTCC CAGTGGAGGTCGATTCTGACACCAGCATCTTCCAGCTCAA GGAGGTGGTTGCTAAGCGACAGGGGGTTCCGGCTGACCAG TTGCGTGTGATTTTCGCAGGGAAGGAGCTGAGGAATGACT GGACTGTGCAGAATTGTGACCTGGATCAGCAGAGCATTTGT TCACATTGTGCAGAGACCGTGGAGAAAAGGTCAAGAAATG AATGCAACTGGAGGCGACGACCCCAGAAACGCGGCGGGAG GCTGTGAGCGGGAGCCCCAGAGCTTGACTCGGGTGGACCT CAGCAGCTCAGTCCTCCCAGGAGACTCTGTGGGGCTGGCT GTCATTCTGCACACTGACAGCAGGAAGGACTCACCACCAG CTGGAAGTCCAGCAGGTAGATCAATCTACAACAGCTTTTA TGTGTATTGCAAAGGCCCTGTCAAAGAGTGCAGCCGGGA AAACTCAGGGTACAGTGCAGCACCTGCAGGCAGGCAACGC TCACCTTGACCCAGGGTCCATCTTGCTGGGATGATGTTTT AATTCCAAACCGGATGAGTGGTGAATGCCAATCCCCACAC TGCCCTGGGACTAGTGCAGAATTTTTCTTTAAATGTGGAG CACACCCACCTCTGACAAGGAAACATCAGTAGCTTTGCA CCTGATCGCAACAAATAGTCGGAACATCACTTGCATTACG TGCACAGACGTCAGGAGCCCCGTCCTGGTTTTCCAGTGCA ACTCCCGCCACGTGATTTGCTTAGACTGTTTCCACTTATA CTGTGTGACAAGACTCAATGATCGGCAGTTTGTTACGAC CCTCAACTTGGCTACTCCCTGCCTTGTGTGGGAACTGGAG ACACAGTGGTGCTTAGAGGAGCTCTGGGGGGATTTCAGGAG AGGAGTCGCTGGCTGTCCCAACTCCTTGATTAAAGAGCTC CATCACTTCAGGATTCTGGGAGAAGAGCAGTACAACCGGT ACCAGCAGTATGGTGCAGAGGAGTGTGTCCTGCAGATGGG GGGCGTGTTATGCCCCCGCCCTGGCTGTGGAGCGGGGCTG CTGCCGGAGCCTGACCAGAGGAAAGTCACCTGCGAAGGGG GCAATGGCCTGGGCTGTGGGTATGGACAACGAAGAACAAA A	31
-----------	---	----

PARK2 (6)	>NM_013988.2:135-1085 Homo sapiens parkin RBR E3 ubiquitin protein ligase (PRKN), transcript variant 3, mRNA ATGATAGTGTGTTGTCAGGTTCAACTCCAGCCATGGTTTCC CAGTGGAGGTCGATTCTGACACCAGCATCTTCCAGCTCAA GGAGGTGGTTGCTAAGCGACAGGGGGTTCCGGCTGACCAG TTGCGTGTGATTTTCGCAGGGAAGGAGCTGAGGAATGACT GGACTGTGCAGGAATTTTCTTTAAATGTGGAGCACACCC CACCTCTGACAAGGAAACATCAGTAGCTTTGCACCTGATC GCAACAAATAGTCGGAACATCACTTGCATTACGTGCACAG ACGTCAGGAGCCCCGTCCTGGTTTTCCAGTGCAACTCCCG CCACGTGATTTGCTTAGACTGTTTCCACTTATACTGTGTG ACAAGACTCAATGATCGGCAGTTTGTTCACGACCCTCAAC TTGGCTACTCCCTGCCTTGTGTGGCTGGCTGTCCCAACTC CTTGATTAAAGAGCTCCATCACTTCAGGATTCTGGGAGAA GAGCAGTACAACCGGTACCAGCAGTATGGTGCAGAGGAGT GTGTCCTGCAGATGGGGGGCGTGTTATGCCCCCGCCCTGG CTGTGGAGCGGGGCTGCTGCCGGAGCCTGACCAGAGGAAA GTCACCTGCGAAGGGGGCAATGGCCTGGGCTGTGGGTTTG CCTTCTGCCGGAATGTAAAGAAGCGTACCATGAAGGGGA GTGCAGTGCCGTATTTGAAGCCTCAGGAACAACACTACTCAG GCCTACAGAGTCGATGAAAGAGCCGCCGAGCAGGCTCGTT GGGAAGCAGCCTCCAAAGAAACCATCAAGAAAACCAACAA GCCCTGTCCCCGCTGCCATGTACCAGTGGAATAAATGGA GGCTGCATGCACATGAAGTGTCCGCAGCCCCAGTGCAGGC TCGAGTGGTGCTGGAAGTGTGGCTGCGAGTGGAACCGCGT CTGCATGGGGGACCACTGGTTTCGACGTGTAG	32
PARK2 (7)	>ENA ADB91979 ADB91979.1 Homo sapiens (human) parkin variant SV5,9DEL ATGATAGTGTGTTGTCAGGTTCAACTCCAGCCATGGTTTCC CAGTGGAGGTCGATTCTGACACCAGCATCTTCCAGCTCAA	33

	GGAGGTGGTTGCTAAGCGACAGGGGGTTCCGGCTGACCAG TTGCGTGTGATTTTCGCAGGGAAGGAGCTGAGGAATGACT GGACTGTGCAGAATTGTGACCTGGATCAGCAGAGCATTGT TCACATTGTGCAGAGACCGTGGAGAAAAGGTCAAGAAATG AATGCAACTGGAGGCGACGACCCCAGAAACGCGGCGGGAG GCTGTGAGCGGGAGCCCCAGAGCTTGACTCGGGTGGACCT CAGCAGCTCAGTCCTCCCAGGAGACTCTGTGGGGCTGGCT GTCATTCTGCACACTGACAGCAGGAAGGACTCACCACCAG CTGGAAGTCCAGCAGGTAGATCAATCTACAACAGCTTTTA TGTGTATTGCAAAGGCCCTGTCAAAGAGTGCAGCCGGGA AAACTCAGGGTACAGTGCAGCACCTGCAGGCAGGCAACGC TCACCTTGACCCAGGAATTTTCTTTAAATGTGGAGCACA CCCCACCTCTGACAAGGAAACATCAGTAGCTTTGCACCTG ATCGCAACAAATAGTCGGAACATCACTTGCATTACGTGCA CAGACGTCAGGAGCCCCGTCCTGGTTTTCCAGTGCAACTC CCGCCACGTGATTTGCTTAGACTGTTTCCACTTATACTGT GTGACAAGACTCAATGATCGGCAGTTTGTTCACGACCCTC AACTTGGCTACTCCCTGCCTTGTGTGGCTGGCTGTCCCAA CTCCTTGATTAAAGAGCTCCATCACTTCAGGATTCTGGGA GAAGAGCAGTTTGCCTTCTGCCGGGAATGTAAAGAAGCGT ACCATGAAGGGGAGTGCAGTGCCGTATTTGAAGCCTCAGG AACAACTACTCAGGCCTACAGAGTCGATGAAAGAGCCGCC GAGCAGGCTCGTTGGGAAGCAGCCTCCAAAGAAACCATCA AGAAAACCAAGCCCTGTCCCCGCTGCCATGTACCAGT GGAAAAAATGGAGGCTGCATGCACATGAAGTGTCCGCAG CCCCAGTGCAGGCTCGAGTGGTGCTGGAAGTGTGGCTGCG AGTGGAACCGCGTCTGCATGGGGGACCACTGGTTCGACGT GTAG	
PARK2 (8)	>ENA ADB90271 ADB90271.1 Homo sapiens (human) parkin variant SV9DEL ATGATAGTGTGTTGTCAGGTTCAACTCCAGCCATGGTTTCC CAGTGGAGGTCGATTCTGACACCAGCATCTTCCAGCTCAA	34

	GGAGGTGGTTGCTAAGCGACAGGGGGTTCCGGCTGACCAG TTGCGTGTGATTTTCGCAGGGAAGGAGCTGAGGAATGACT GGACTGTGCAGAATTGTGACCTGGATCAGCAGAGCATTGT TCACATTGTGCAGAGACCGTGGAGAAAAGGTCAAGAAATG AATGCAACTGGAGGCGACGACCCCAGAAACGCGGCGGGAG GCTGTGAGCGGGAGCCCCAGAGCTTGACTCGGGTGGACCT CAGCAGCTCAGTCCTCCCAGGAGACTCTGTGGGGCTGGCT GTCATTCTGCACACTGACAGCAGGAAGGACTCACCACCAG CTGGAAGTCCAGCAGGTAGATCAATCTACAACAGCTTTTA TGTGTATTGCAAAGGCCCTGTCAAAGAGTGCAGCCGGGA AAACTCAGGGTACAGTGCAGCACCTGCAGGCAGGCAACGC TCACCTTGACCCAGGGTCCATCTTGCTGGGATGATGTTTT AATTCCAAACCGGATGAGTGGTGAATGCCAATCCCCACAC TGCCCTGGGACTAGTGCAGAATTTTTCTTTAAATGTGGAG CACACCCACCTCTGACAAGGAAACATCAGTAGCTTTGCA CCTGATCGCAACAAATAGTCGGAACATCACTTGCATTACG TGCACAGACGTCAGGAGCCCCGTCCTGGTTTTCCAGTGCA ACTCCCGCCACGTGATTTGCTTAGACTGTTTCCACTTATA CTGTGTGACAAGACTCAATGATCGGCAGTTTGTTCACGAC CCTCAACTTGGCTACTCCCTGCCTTGTGTGGCTGGCTGTC CCAACCTCCTTGATTAAAGAGCTCCATCACTTCAGGATTCT GGGAGAAGAGCAGTTTGCCTTCTGCCGGGAATGTAAAGAA GCGTACCATGAAGGGGAGTGCAGTGCCGTATTTGAAGCCT CAGGAACAACACTCAGGCCTACAGAGTCGATGAAAGAGC CGCCGAGCAGGCTCGTTGGGAAGCAGCCTCCAAAGAAACC ATCAAGAAAACCAAGCCCTGTCCCCGCTGCCATGTAC CAGTGGAAAAAATGGAGGCTGCATGCACATGAAGTGTCC GCAGCCCCAGTGCAGGCTCGAGTGGTGCTGGAACGTGTGGC TGCGAGTGGAACCGCGTCTGCATGGGGGACCACTGGTTTCG ACGTGTAG	
--	---	--

In particular embodiments, the compositions and methods disclosed herein contemplate the use of functional variants and functional fragments of any of these

protein or polynucleotide sequences. Functional fragments and variants retain the biological properties or activities of the corresponding wild-type protein, although the properties or activities may in certain instances be reduced, e.g., to about 50%, about 60%, about 70%, or about 80% as compared to the wild-type protein, or increased, e.g., 1.1-fold, 1.2-fold, 1.3-fold, 1.4-fold, 1.5-fold, 2-fold, 3-fold, 4-fold, or 5-fold as compared to the wild-type protein. In certain embodiments, a functional fragment or variant of a protein has at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least 99% sequence identity to the corresponding wild-type protein. In certain embodiments, a functional fragment of a protein comprises at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least 99% of the corresponding wild-type protein.

In another aspect, the disclosure provides recombinant gene therapy vector, comprising a transgene polynucleotide encoding E3 ubiquitin protein ligase (PARK2), wherein the transgene polynucleotide is operably linked to a eukaryotically active promoter sequence. In some embodiments, the transgene polynucleotide shares at least 95% identity to one of SEQ ID NOs: 35-38.

In some embodiments, the disclosure provides a codon-optimized polynucleotide encoding PARK2. In some cases, the entire transgene sequence is codon optimized for expression in a mammalian cell. Codon optimization refers to the discovery that the frequency of occurrence of synonymous codons (*i.e.*, codons that code for the same amino acid) in coding DNA is biased in different species. Such codon degeneracy allows an identical polypeptide to be encoded by a variety of nucleotide sequences. A variety of codon optimization methods is known in the art, and include, e.g., methods disclosed in at least U.S. Pat. Nos. 5,786,464 and 6,114,148.

In some embodiments, the codon-optimized polynucleotide encoding PARK2 comprises fewer CpG islands than the native human polynucleotide sequence encoding human PARK2. For example, in some embodiments, the native human sequence comprises 95 CpG islands, whereas the codon-optimized polynucleotides comprise less than 95, less than 90, less than 85, less than 80, less than 75, less than 70, less than 65, less than 60, less than 55, less than 50, less than 45, less than 40, less than 35, less than 30, less than 25, less than 20, less than 15, less

than 10, less than 5, or no CpG islands. In some embodiments, the codon-optimized polynucleotide sequence comprises 2-20, 5-20, about 5, or about 10 CpG islands. In some embodiments, the codon-optimized polynucleotide sequence comprises one or more CpG island. In some embodiments, the expression cassette shares at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to any one of SEQ ID NOs: 39-58. In some embodiments, the expression cassette comprises, consists essentially of, or consists of a sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to any one of SEQ ID NOs: 39-58.

In some embodiments, the vector is an adeno-associated virus (AAV) vector. In some embodiments, the vector comprises two AAV inverted terminal repeats (ITRs) flanking the expression cassette. In some embodiments, the AAV has serotype AAV1, AAV2, AAV5, AAV8, AAV9, AAVrh10, or AAVrh74. In some embodiments, the recombinant gene therapy vector comprises a self-complementary AAV. In some embodiments, the recombinant gene therapy vector comprises a single-stranded AAV. In some embodiments, the AAV is a wild-type AAV or a modified AAV. In some embodiments, the AAV comprises a capsid protein having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to wild-type VP1, VP2, or VP3 capsid protein.

In another aspect, the disclosure provides host cell comprising any of the foregoing recombinant gene therapy vectors. Exemplary host cells include HEK293, 293T, HeLa, Vero, and Sf9 cells.

In another aspect, the disclosure provides a method of inhibiting, reducing, or delaying degeneration or death of a dopaminergic neuron comprising a mutation in a gene associated with a Parkinson's Disease (PD), wherein the mutated gene is a Parkinson protein 2, E3 ubiquitin protein ligase (PARK2) gene, comprising contacting the neuron with the recombinant gene therapy vector of the disclosure, wherein following contact with the recombinant gene therapy vector, the neuron expresses the wild-type protein. The method may be practiced in vitro, or in vivo, e.g., in a subject in need thereof.

In some embodiments, the neuron expresses a reduced amount of alpha-synuclein and/or comprises a reduced amount of Lewy bodies following contact with the recombinant gene therapy vector. In some embodiments, alpha-synuclein and/or comprises a reduced amount of Lewy bodies are reduced by at least 5%, at least 5 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40% or more.

In some embodiments, the neuron expresses a reduced amount of monoamine oxidases following contact with the recombinant gene therapy vector. In some embodiments, the amount of monoamine oxidases is reduced by at least 5%, 10 at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40% or more.

In some embodiments, the neuron produces and/or releases an increased amount of dopamine following contact with the recombinant gene therapy vector. In some embodiments, the amount of dopamine produced and/or released is increased 15 at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40% or more.

In some embodiments, the neuron undergoes increased mitophagy following contact with the recombinant gene therapy vector. In some embodiments, mitophagy is increased at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at 20 least 30%, at least 35%, at least 40% or more.

In some embodiments, the neuron expresses a lower amount of monoamine oxidases as compared to an amount of monoamine oxidases expressed in a neuron not contacted with said recombinant gene therapy vector, optionally wherein said lower amount is at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, 25 at least 50%, at least 60%, at least 70%, or at least 80% lower than the amount expressed in the neuron not contacted with said recombinant gene therapy vector.

In some embodiments, the neuron produces and/or releases an increased amount of dopamine as compared to an amount of dopamine produced and/or released by a neuron not contacted with said recombinant gene therapy vector, 30 optionally wherein said increase amount is at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least two-fold, at least three-fold, at least four-fold, at least five-fold, or

at least 10-fold greater than the amount produced and/or released by the neuron not contacted with said recombinant gene therapy vector.

In some embodiments, the neuron undergoes an increased amount of autophagy as compared to an amount of autophagy undergone by a neuron not contacted with said recombinant gene therapy vector, optionally wherein the increased amount is at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least two-fold, at least three-fold, at least four-fold, at least five-fold, or at least 10-fold greater than the amount undergone by the neuron not contacted with said recombinant gene therapy vector .

In some embodiments, the neuron is a primary tyrosine hydroxylase positive neuron. In some embodiments, the neuron was produced from an induced pluripotent stem cell prepared from cells obtained from a subject diagnosed with Parkinson's disease.

In another aspect, the disclosure provides a method of treating or inhibiting or delaying onset or progression of a Parkinson's Disease (PD) in a subject suffering from or at risk of the PD, comprising administering a gene therapy vector of the disclosure to the subject, wherein administration of the recombinant gene therapy vector treats or inhibits or delays onset or progression of the Parkinson's Disease in the subject.

In some embodiments, the PD is an early-onset PD. In some embodiments, the PD is an early-onset autosomal recessive PD. In some embodiments, the subject comprises a mutation in a PARK2 gene.

In some embodiments, the gene therapy vector comprises an expression cassette comprising a transgene that encodes for PARK2 or a functional fragment or variant thereof. In some embodiments, the PARK2 comprises the amino acid sequence set forth in SEQ ID NO: 1 or an amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% identical thereto. In some embodiments, the transgene polynucleotide shares at least 95% identity to one of SEQ ID NOs: 35-38.

In some embodiments, administering step comprises systemic, parenteral, intravenous, cerebral, cerebrospinal, intrathecal, intracisternal, intraputaminar, intrahippocampal, intra-striatal, or intra-cerebroventricular administration.

In some embodiments, the administering step comprises intravenous, cerebral, cerebrospinal, intrathecal, intracisternal, intraputaminial, intrahippocampal, intra-striatal, or intra-cerebroventricular injection.

5 In some embodiments, the administering step comprises intrathecal injection with Threndelenburg tilting.

In some embodiments, the administering step comprises direct injection into the pars compacta of the substantia nigra of the brain.

10 In some embodiments, the administering step comprises introducing the recombinant gene therapy vector into the subject's brain or cerebrospinal fluid (CSF).

In some embodiments, 1×10^9 - 1×10^{14} vector genomes per kilogram body mass of the subject (vg/kg) of the gene therapy vector are administered to the subject.

15 In some embodiments, 1×10^9 - 1×10^{14} vector genomes per kilogram body mass of the subject (vg/kg) of the gene therapy vector are administered to the subject's brain.

In some embodiments, 1×10^9 - 1×10^{14} vector genomes per kilogram body mass of the subject (vg/kg) of the gene therapy vector are administered to the subject's CSF.

20 In some embodiments, 1×10^7 - 1×10^9 vector genomes per kilogram body mass of the subject (vg/kg) of the gene therapy vector are administered to the subject.

In some embodiments, the subject is an adult or a child.

25 In some embodiments, the number of dopaminergic neurons in the subject after the administering step is greater than the number of dopaminergic neurons in the subject before the administering step.

In some embodiments, the level of dopamine in the subject after the administering step is greater than the level of dopamine in the subject before the administering step.

30 In some embodiments, the number of dopaminergic neurons in a subject treated by the method is increased compared to the number of dopaminergic neurons in a subject not so treated.

In some embodiments, the level of dopamine of a subject treated by the method is increased compared to the level of dopamine in a subject not so treated.

In some embodiments, the level of dopamine in the substantia nigra of a subject treated by method is increased compared to the level of dopamine in the substantia nigra of a subject not so treated.

In some embodiments, the level of PRKN in the subject's CSF after the administering step is greater than the level of PRKN in the subject's CSF before the administering step.

In some embodiments, the Unified Parkinson's Disease Rating Scale (UPDRS) score of the subject before the administering step is improved compared to the UPDRS score of the subject before the administering step.

In some embodiments, the level of PRKN in the CSF of a subject treated by the method is increased compared to the level of PRKN in the CSF of a subject not so treated.

In some embodiments, the UPDRS score of a subject treated by the method is improved compared to the UPDRS score of a subject not so treated.

In some embodiments, the subject's neurons express a reduced amount of alpha-synuclein and/or comprises a reduced amount of Lewy bodies following contact with the recombinant gene therapy vector.

Definitions

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are expressly incorporated by reference in their entirety. In cases of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples described herein are illustrative only and are not intended to be limiting.

All publications and patents mentioned herein are hereby incorporated by reference in their entirety as if each individual publication or patent was specifically and individually indicated to be incorporated by reference. In case of conflict, the

present application, including any definitions herein, will control. However, mention of any reference, article, publication, patent, patent publication, and patent application cited herein is not, and should not be taken as an acknowledgment, or any form of suggestion, that they constitute valid prior art or form part of the common general knowledge in any country in the world.

In the present description, any concentration range, percentage range, ratio range, or integer range is to be understood to include the value of any integer within the recited range and, when appropriate, fractions thereof (such as one tenth and one hundredth of an integer), unless otherwise indicated. The term "about", when immediately preceding a number or numeral, means that the number or numeral ranges plus or minus 10%. It should be understood that the terms "a" and "an" as used herein refer to "one or more" of the enumerated components unless otherwise indicated. The use of the alternative (e.g., "or") should be understood to mean either one, both, or any combination thereof of the alternatives. The term "and/or" should be understood to mean either one, or both of the alternatives. As used herein, the terms "include" and "comprise" are used synonymously.

The abbreviations PRKN and PARK2 are used interchangeably herein.

The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

Adeno-Associated Virus (AAV)

As used herein, the term "AAV" is a standard abbreviation for adeno-associated virus or a recombinant vector thereof. Adeno-associated virus is a single-stranded DNA parvovirus that grows only in cells in which certain functions are provided by a co-infecting helper virus. General information and reviews of AAV can be found in, for example, Carter, 1989, Handbook of Parvoviruses, Vol. 1, pp. 169-228, and Berns, 1990, Virology, pp. 1743-1764, Raven Press, (New York). It is fully expected that the same principles described in these reviews will be applicable to additional AAV serotypes characterized after the publication dates of the reviews because it is well known that the various serotypes are quite closely related, both structurally and functionally, even at the genetic level. (See, for example, Blacklowe, 1988, pp. 165-174 of Parvoviruses and Human Disease, J. R. Pattison, ed.; and Rose, Comprehensive Virology 3: 1-61 (1974)). For example, all AAV serotypes apparently exhibit very similar replication properties mediated by homologous rep

genes; and all bear three related capsid proteins such as those expressed in AAV2. The degree of relatedness is further suggested by heteroduplex analysis which reveals extensive cross-hybridization between serotypes along the length of the genome; and the presence of analogous self-annealing segments at the termini that correspond to "inverted terminal repeat sequences" (ITRs). The similar infectivity patterns also suggest that the replication functions in each serotype are under similar regulatory control.

As used herein, an "AAV vector" or "rAAV vector" refers to a recombinant vector comprising one or more polynucleotides of interest (or transgenes) that are flanked by AAV terminal repeat sequences (ITRs). Such AAV vectors can be replicated and packaged into infectious viral particles when present in a host cell that has been transfected with a plasmid encoding and expressing *rep* and *cap* gene products. Alternatively, AAV vectors can be packaged into infectious particles using a host cell that has been stably engineered to express *rep* and *cap* genes.

As used herein, an "AAV virion" or "AAV viral particle" or "AAV vector particle" refers to a viral particle composed of at least one AAV capsid protein and an encapsidated polynucleotide AAV vector. As used herein, if the particle comprises a heterologous polynucleotide (i.e. a polynucleotide other than a wild-type AAV genome such as a transgene to be delivered to a mammalian cell), it is typically referred to as an "AAV vector particle" or simply an "AAV vector." Thus, production of AAV vector particle necessarily includes production of AAV vector, as such a vector is contained within an AAV vector particle.

Adeno-associated virus (AAV) is a replication-deficient parvovirus, the single-stranded DNA genome of which is about 4.7 kb in length including two 145-nucleotide inverted terminal repeat (ITRs). There are multiple known variants of AAV, also sometimes called serotypes when classified by antigenic epitopes. The nucleotide sequences of the genomes of the AAV serotypes are known. For example, the complete genome of AAV-1 is provided in GenBank Accession No. NC_002077; the complete genome of AAV-2 is provided in GenBank Accession No. NC_001401 and Srivastava et al., J. Virol., 45: 555-564 { 1983}; the complete genome of AAV-3 is provided in GenBank Accession No. NC_1829; the complete genome of AAV-4 is provided in GenBank Accession No. NC_001829; the AAV-5 genome is provided in GenBank Accession No. AF085716; the complete genome of

AAV-6 is provided in GenBank Accession No. NC_001862; at least portions of AAV-7 and AAV-8 genomes are provided in GenBank Accession Nos. AX753246 and AX753249, respectively; the AAV-9 genome is provided in Gao et al., J. Virol., 78: 6381-6388 (2004); the AAV-10 genome is provided in Mol. Ther., 13(1): 67-76 (2006); and the AAV-11 genome is provided in Virology, 330(2): 375-383 (2004). The sequence of the AAVrh.74 genome is provided in U.S. Patent 9,434,928, incorporated herein by reference. Cis-acting sequences directing viral DNA replication (rep), encapsidation/packaging and host cell chromosome integration are contained within the AAV ITRs. Three AAV promoters (named p5, p19, and p40 for their relative map locations) drive the expression of the two AAV internal open reading frames encoding rep and cap genes. The two rep promoters (p5 and p19), coupled with the differential splicing of the single AAV intron (at nucleotides 2107 and 2227), result in the production of four rep proteins (rep78, rep68, rep52, and rep40) from the rep gene. Rep proteins possess multiple enzymatic properties that are ultimately responsible for replicating the viral genome. The cap gene is expressed from the p40 promoter and it encodes the three capsid proteins VP1, VP2, and VP3. Alternative splicing and non-consensus translational start sites are responsible for the production of the three related capsid proteins. A single consensus polyadenylation site is located at map position 95 of the AAV genome. The life cycle and genetics of AAV are reviewed in Muzyczka, Current Topics in Microbiology and Immunology, 158: 97-129 (1992).

AAV possesses unique features that make it attractive as a vector for delivering foreign DNA to cells, for example, in gene therapy. AAV infection of cells in culture is noncytopathic, and natural infection of humans and other animals is silent and asymptomatic. Moreover, AAV infects many mammalian cells allowing the possibility of targeting many different tissues in vivo. Moreover, AAV transduces slowly dividing and non-dividing cells, and can persist essentially for the lifetime of those cells as a transcriptionally active nuclear episome (extrachromosomal element). The AAV proviral genome is inserted as cloned DNA in plasmids, which makes construction of recombinant genomes feasible. Furthermore, because the signals directing AAV replication and genome encapsidation are contained within the ITRs of the AAV genome, some or all of the internal approximately 4.3 kb of the genome (encoding replication and structural capsid proteins, rep-cap) may be

replaced with foreign DNA. To generate AAV vectors, the rep and cap proteins may be provided in trans. Another significant feature of AAV is that it is an extremely stable and hearty virus. It easily withstands the conditions used to inactivate adenovirus (56° to 65°C for several hours), making cold preservation of AAV less critical. AAV may even be lyophilized. Finally, AAV-infected cells are not resistant to superinfection.

AAV DNA in the rAAV genomes may be from any AAV variant or serotype for which a recombinant virus can be derived including, but not limited to, AAV variants or serotypes AAV-1, AAV-2, AAV-3, AAV-4, AAV-5, AAV-6, AAV-7, AAV-8, AAV-9, AAV- 10, AAV-11, AAV- 12, AAV-13 and AAVrh10. Production of pseudotyped rAAV is disclosed in, for example, WO 01/83692. Other types of rAAV variants, for example rAAV with capsid mutations, are also contemplated. *See, for example*, Marsic et al., Molecular Therapy, 22(11): 1900-1909 (2014). The nucleotide sequences of the genomes of various AAV serotypes are known in the art. To promote eye-specific expression, AAV6, AAV8 or AAV9 may be used.

In some cases, the rAAV comprises a self-complementary genome. As defined herein, an rAAV comprising a “self-complementary” or “double stranded” genome refers to an rAAV which has been engineered such that the coding region of the rAAV is configured to form an intra-molecular double-stranded DNA template, as described in McCarty et al. Self-complementary recombinant adeno-associated virus (scAAV) vectors promote efficient transduction independently of DNA synthesis. Gene Therapy. 8 (16): 1248–54 (2001). The present disclosure contemplates the use, in some cases, of an rAAV comprising a self-complementary genome because upon infection (such transduction), rather than waiting for cell mediated synthesis of the second strand of the rAAV genome, the two complementary halves of scAAV will associate to form one double stranded DNA (dsDNA) unit that is ready for immediate replication and transcription. It will be understood that instead of the full coding capacity found in rAAV (4.7-6kb), rAAV comprising a self-complementary genome can only hold about half of that amount (≈2.4kb).

In other cases, the rAAV vector comprises a single stranded genome. As defined herein, a “single standard” genome refers to a genome that is not self-complementary. In most cases, non-recombinant AAVs are have singled stranded DNA genomes. There have been some indications that rAAVs should be scAAVs to

achieve efficient transduction of cells, such as ocular cells. The present disclosure contemplates, however, rAAV vectors that maybe have singled stranded genomes, rather than self-complementary genomes, with the understanding that other genetic modifications of the rAAV vector may be beneficial to obtain optimal gene

5 transcription in target cells. In some cases, the present disclosure relates to single-stranded rAAV vectors capable of achieving efficient gene transfer to anterior segment in the mouse eye. See Wang et al. Single stranded adeno-associated virus achieves efficient gene transfer to anterior segment in the mouse eye. PLoS ONE 12(8): e0182473 (2017).

10 In some cases, the rAAV vector is of the serotype AAV1, AAV2, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV13, or AAVrh10.

Production of pseudotyped rAAV is disclosed in, for example, WO 01/83692. Other types of rAAV variants, for example rAAV with capsid mutations, are also contemplated. See, *for example*, Marsic et al., Molecular Therapy, 22(11): 1900-

15 1909 (2014). In some cases, the rAAV vector is of the serotype AAV9. In some embodiments, said rAAV vector is of serotype AAV9 and comprises a single stranded genome. In some embodiments, said rAAV vector is of serotype AAV9 and comprises a self-complementary genome. In some embodiments, a rAAV vector comprises the inverted terminal repeat (ITR) sequences of AAV2. In some
20 embodiments, the rAAV vector comprises an AAV2 genome, such that the rAAV vector is an AAV-2/9 vector, an AAV-2/6 vector, or an AAV-2/8 vector.

Full-length sequences and sequences for capsid genes for most known AAVs are provided in US Patent No. 8,524,446, which is incorporated herein in its entirety.

AAV vectors may comprise wild-type AAV sequence or they may comprise
25 one or more modifications to a wild-type AAV sequence. In certain embodiments, an AAV vector comprises one or more amino acid modifications, e.g., substitutions, deletions, or insertions, within a capsid protein, e.g., VP1, VP2 and/or VP3. In particular embodiments, the modification provides for reduced immunogenicity when the AAV vector is provided to a subject.

30 Promoters

In some embodiments, the polynucleotide sequence encoding wild-type PARK2, PINK1, LRRK2, SCNA, c-Rel, ATG7, VMAT2, or GBA protein, or functional variant or fragment thereof is operably linked to a CMV promoter. The present

disclosure further contemplates the use of other promoter sequences. Promoters useful in embodiments of the present disclosure include, without limitation, a cytomegalovirus (CMV) promoter, phosphoglycerate kinase (PGK) promoter, or a promoter sequence comprised of the CMV enhancer and portions of the chicken beta-actin promoter and the rabbit beta-globin gene (CAG). In some cases, the promoter may be a synthetic promoter. Exemplary synthetic promoters are provided by Schlabach et al. Synthetic design of strong promoters. Proc Natl Acad Sci U S A. 2010 Feb 9; 107(6): 2538–2543.

In some cases, a polynucleotide sequence encoding a therapeutic protein or a wild-type PARK2, PINK1, LRRK2, SCNA, c-Rel, ATG7, VMAT2, or GBA protein, or functional variant or fragment thereof, is operatively linked to an inducible promoter. A polynucleotide sequence operatively linked to an inducible promoter may be configured to cause the polynucleotide sequence to be transcriptionally expressed or not transcriptionally expressed in response to addition or accumulation of an agent or in response to removal, degradation, or dilution of an agent. The agent may be a drug. The agent may be tetracycline or one of its derivatives, including, without limitation, doxycycline. In some cases, the inducible promoter is a tet-on promoter, a tet-off promoter, a chemically-regulated promoter, a physically-regulated promoter (i.e. a promoter that responds to presence or absence of light or to low or high temperature). This list of inducible promoters is non-limiting.

As used herein, “eukaryotically active promoter” or “promoter” are used interchangeably and refer to promoter capable of promoting initiation of RNA transcription from a polynucleotide in a eukaryotic cell. In some cases, the promoter is a tissue-specific promoter, such as a promoter capable of driving expression in a neuron to a greater extent than in a non-neuronal cell. In some embodiments, tissue-specific promoter is selected from a list of neuron-specific promoters consisting of: hSYN1 (human synapsin), INA (alpha-internexin), NES (nestin), TH (tyrosine hydroxylase), FOXA2 (Forkhead box A2), CaMKII (calmodulin-dependent protein kinase II), and NSE (neuron-specific enolase). In some cases, the promoter is a ubiquitous promoter. A “ubiquitous promoter” refers to a promoter that is not tissue-specific under experimental or clinical conditions. In some cases, the ubiquitous promoter is selected from the group consisting of: CMV, CAG, UBC, PGK, EF1-alpha, GAPDH, SV40, HBV, chicken beta-actin, and human beta-actin.

In some embodiments, the promoter sequence is selected from Table 5, and sequences having at least 95%, at least 98%, or least 99% identity thereto.

Table 5

PROMOTER	SEQUENCE	SEQ ID NO:
Human beta-actin (HuBa)	GCCCAGCACCCCAAGGCGGCCAACGCCAAACT CTCCCTCCTCCTCTTCCTCAATCTCGCTCTCGC TCTTTTTTTTTTTTCGCAAAGGAGGGGAGAGGG GGTAATAAATGCTGCACTGTGCGGCGAAGCCG GTGAGTGAGCGGCGCGGGGCCAATCAGCGTGCG CCGTTCCGAAAGTTGCCTTTTATGGCTCGAGCG GCCGCGGCGGCGCCCTATAAAACCCAGCGGCGC GACGCGCCACCACCGCCGAGTC	59
Chicken beta-actin (CBA)	GGTCGAGGTGAGCCCCACGTTCTGCTTCACTCT CCCCATCTCCCCCCCCCTCCCCACCCCAATTTT GTATTTATTTATTTTTTAATTATTTGTGCAGC GATGGGGGCGGGGGGGGGGGGGGCGCGGCCAG GCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGG CGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGA GCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGA GGCGGCGGCGGCGGCGGCCCTATAAAAAGCGAA GCGCGCGGCGGGCGGGA	60
Cytomegalovirus (CMV)	TGGTGATGCGGTTTTTGGCAGTACACCAATGGGC GTGGATAGCGGTTTGACTCACGGGGATTTCCAA GTCTCCACCCATTGACGTCAATGGGAGTTTGT TTTGGCACCAAAATCAACGGGACTTTCCAAAAT GTCGTAATAACCCCGCCCCGTTGACGCAAATGG GCGGTAGGCGTGACGGTGGGAGGTCTATATAA GCAGAGCTCGTTTAGTGAACCG	61
Human EF1-alpha (EF1- α)	CAACCTTTGGAGCTAAGCCAGCAATGGTAGAGG GAAGATTCTGCACGTCCCTTCCAGGCGGCCTCC CCGTCACCACCCCCCAACCCGCCCCGACCGG AGCTGAGAGTAATTCATACAAAAGGACTCGCCC	62

	CTGCCTTGGGGAATCCCAGGGACCGTCGTTAAA CTCCCCTAACGTAGAACCCAGAGATCGCTGCG TTCCCGCCCCCTCACCCGCCCCGCTCTCGTCATC ACTGAGGTGGAGAATAGCATGCGTGAGGCTCCG GTGCCCCGTCAGTGGGCAGAGCGCACATCGCCCA CAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCA ATTGAACGGGTGCCTAGAGAAGGTGGCGCGGGG TAAACTGGGAAAGTGATGTCGTGTACTGGCTCC GCCTTTTTTCCCGAGGGTGGGGGAGAACCGTATA TAAGTGCAGTAGTCGCCGTGAACGTT	
Human Synapsin1 (Syn)	CTGCAGAGGGCCCTGCGTATGAGTGCAAGTGGG TTTTAGGACCAGGATGAGGCGGGGTGGGGGTGC CTACCTGACGACCGACCCCGACCCACTGGACAA GCACCCAACCCCCATTCCCCAAATTGCGCATCC CCTATCAGAGAGGGGGAGGGGAAACAGGATGCG GCGAGGCGCGTGCGCACTGCCAGCTTCAGCACC GCGGACAGTGCCTTCGCCCCCGCCTGGCGGCGC GCGCCACCGCCGCCTCAGCACTGAAGGCGCGCT GACGTCACCTCGCCGGTCCCCCGCAAACCTCCCCT TCCCGGCCACCTTGGTCGCGTCCGCGCCGCCGC CGGCCCAGCCGGACCGCACCCACGCGAGGCGCGA GATAGGGGGGCACGGGCGCGACCATCTGCGCTG CGGCGCCGGCGACTCAGCGCTGCCTC	63
Human CamKIIa (CaMKIIa)	ACTTGTGGACAAAGTTTGCTCTATTCCACCTCC TCCAGGCCCTCCTTGGGTCCATCACCCAGGGG TGCTGGGTCCATCCCACCCCCAGGCCACACAG GCTTGCAGTATTGTGTGCGGTATGGTCAGGGCG TCCGAGAGCAGGTTTCGCAGTGGAAGGCAGGCA GGTGTGGGGAGGCAGTTACCGGGGCAACGGGA ACAGGGCGTTTTGGAGGTGGTTGCCATGGGGAC CTGGATGCTGACGAAGGCTCGCGAGGCTGTGAG CAGCCACAGTGCCCTGC	64

Further illustrative examples of promoters are the SV40 late promoter from simian virus 40, the Baculovirus polyhedron enhancer/promoter element, Herpes Simplex Virus thymidine kinase (HSV tk), the immediate early promoter from cytomegalovirus (CMV) and various retroviral promoters including LTR elements.

Inducible promoters include heavy metal ion inducible promoters (such as the mouse mammary tumor virus (mMTV) promoter or various growth hormone promoters), and the promoters from T7 phage which are active in the presence of T7 RNA polymerase. By way of illustration, examples of tissue-specific promoters include various surfactin promoters (for expression in the lung), myosin promoters (for expression in muscle), and albumin promoters (for expression in the liver). A large variety of other promoters are known and generally available in the art, and the sequences of many such promoters are available in sequence databases such as the GenBank database.

In some embodiments, the vector further comprises a CMV enhancer.

Other Regulatory Elements

In some cases, vectors of the present disclosure further comprise one or more regulatory elements selected from the group consisting of an enhancer, an intron, a poly-A signal, a 2A peptide encoding sequence, a WPRE (Woodchuck hepatitis virus posttranscriptional regulatory element), and a HPRE (Hepatitis B posttranscriptional regulatory element).

In certain embodiments, the vectors comprise one or more enhancers. In particular embodiments, the enhancer is a CMV enhancer sequence, a GAPDH enhancer sequence, a β -actin enhancer sequence, or an EF1- α enhancer sequence. Sequences of the foregoing are known in the art. For example, the sequence of the CMV immediate early (IE) enhancer is:

```
CGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGC
CCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCAT
TGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTG
TATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGG
CATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTA
TTAGTCATCGCTATTACCA
```

(SEQ ID NO: 79)

In certain embodiments, the vectors comprise one or more introns. In particular embodiments, the intron is a rabbit globin intron sequence, a chicken β -actin intron sequence, a synthetic intron sequence, or an EF1- α intron sequence.

In certain embodiments, the vectors comprise a polyA sequence. In particular
 5 embodiments, the polyA sequence is a rabbit globin polyA sequence, a human growth hormone polyA sequence, a bovine growth hormone polyA sequence, a PGK polyA sequence, an SV40 polyA sequence, or a TK polyA sequence. In some embodiments, the poly-A signal may be a bovine growth hormone polyadenylation signal (bGHpA).

10 In certain embodiments, the vectors comprise one or more transcript stabilizing element. In particular embodiments, the transcript stabilizing element is a WPRE sequence, a HPRE sequence, a scaffold-attachment region, a 3' UTR, or a 5' UTR. In particular embodiments, the vectors comprise both a 5' UTR and a 3' UTR.

In some embodiments, the vector comprises a 5' untranslated region (UTR)
 15 selected from Table 6.

Table 6

5' UNTRANSLATED REGION	SEQUENCE	SEQ ID NO:
Human beta-actin exon/intron	CGCGTCCGCCCGCGAGCACAGAGCCTCGCCTTTGC CGATCCGCCGCCCGTCCACACCCGCCGCCAGGTAA GCCCCGCCAGCCGACCGGGGCATGCGGCCGCGGCC CTTCGCCCCGTGCAGAGCCGCCGTCTGGGCCGCAGC GGGGGGCGCATGGGGCGGAACCGGACCGCCGTGGG GGGCGCGGGAGAAGCCCCCTGGGCCTCCGGAGATGG GGGACACCCCACGCCAGTTCGCAGGCGCGAGGCCG CGCTCGGGCGGGCGCGCTCCGGGGGTGCCGTCTC GGGGCGGGGGCAACCGGCGGGGTCTTTGTCTGAGC CGGGCTCTTGCCAATGGGGATCGCACGGTGGGCGC GGCGTAGCCCCCGTCAGGCCCGGTGGGGGCTGGGG CGCCATGCGCGTGCGCGCTGGTCCTTTGGGCGCTA ACTGCGTGCGCGCTGGGAATTGGCGCTAATTGCGC GTGCGCGCTGGGACTCAATGGCGCTAATCGCGCGT	65

	GCGTTCTGGGGCCCCGGGCGCTTGCGCCACTTCCTG CCCGAGCCGCTGGCGCCCCGAGGGTGTGGCCGCTGC GTGCGCGCGCGCGACCCGGTCGCTGTTTGAACCGG GCGGAGGCGGGGCTGGCGCCCCGGTTGGGAGGGGGT TGGGGCCTGGCTTCCTGCCGCGCGCCGCGGGGACG CCTCCGACCAGTGTTTGCCTTTTATGGTAATAACG CGGCCGGCCCCGGCTTCCTTTGTCCCCAATCTGGGC GCGCGCCGGCGCCCCCTGGCGGCCTAAGGACTCGG CGCGCCGGAAGTGGCCAGGGCGGCAGCGGCTGCTC TTGGCGGCCCCGAGGTGACTATAGCCTTCTTTTGT GTCTTGATAGTTCGCCAGCCTCTGCTAACCATGTT CATGCCTTCTTCTTTTTCTACAGCTCCTGGGCAA CGTGCTGGTTATTGTGCTGTCTCATTTTTGGCA AAGAATTC	
Chicken beta-actin exon/intron + rabbit globin intron	GTCGCTGCGCGCTGCCTTCGCCCCGTGCCCCGCTC CGCCGCCGCTCGCGCCGCCCGCCCCGGCTCTGAC TGACCGCGTTACTCCCACAGGTGAGCGGGCGGGAC GGCCCTTCTCCTCCGGGCTGTAATTAGCGCTTGGT TTAATGACGGCTTGTTTCTTTTCTGTGGCTGCGTG AAAGCCTTGAGGGGCTCCGGGAGGGCCCTTTGTGC GGGGGGAGCGGCTCGGGGGGTGCGTGCGTGTGTGT GTGCGTGGGGAGCGCCGCGTGCGGCTCCGCGCTGC CCGGCGGCTGTGAGCGCTGCGGGCGCGGCGCGGGG CTTTGTGCGCTCCGCAGTGTCGCGAGGGGAGCGC GGCCGGGGGCGGTGCCCCGCGGTGCGGGGGGGGCT GCGAGGGGAACAAAGGCTGCGTGCGGGGTGTGTGC GTGGGGGGGTGAGCAGGGGGTGTGGGCGCGTCGGT CGGGCTGCAACCCCCCTGCACCCCCCTCCCCGAG TTGCTGAGCACGGCCCGGCTTCGGGTGCGGGGCTC CGTACGGGGCGTGCGCGGGGCTCGCCGTGCCGGG CGGGGGGTGGCGGCAGGTGGGGGTGCCGGGCGGGG CGGGGCCGCTCGGGCCGGGGAGGGCTCGGGGGAG GGGCGCGGCGGCCCCCGGAGCGCCGGCGGCTGTCTG	66

	AGGCGCGGCGAGCCGCAGCCATTGCCTTTTATGGT AATCGTGCGAGAGGGCGCAGGGACTTCCTTTGTCC CAAATCTGTGCGGAGCCGAAATCTGGGAGGCGCCG CCGCACCCCCCTCTAGCGGGCGCGGGGCGAAGCGGT GCGGCGCCGGCAGGAAGGAAATGGGCGGGGAGGGC CTTCGTGCGTCGCCGCGCCGCGTCCCCCTTCTCCC TCTCCAGCCTCGGGGCTGTCCGCGGGGGGACGGCT GCCTTCGGGGGGGACGGGGCAGGGCGGGGTTCGGC TTCTGGCGTGTGACCGGCGGCTCTAGAGCCTCTGC TAACCATGTTTCATGCCTTCTTCTTTTCTACAGC TCCTGGGCAACGTGCTGGTTATTGTGCTGTCTCAT CATTTTGGCAAAGAATTC	
Synapsin1 exon	AGTCTGCGGTGGGCAGCGGAGGAGTCGTGTCGTGC CTGAGAGCGCAGCTGTGCTCCTGGGCACCGCGCAG TCCGCCCCCGCGGCTCCTGGCCAGACCACCCCTAG GACCCCCTGCCCCAAGTCGCAG	67
CMV IE exon	TCAGATCGCCTGGAGAGGCCATCCACGCTGTTTTG ACCTCCATAGTGGACACCGGGACCGATCCAGCCTC CGCGGCCGGGAACGGTGCATTGGAACGCGGATTCC CCGTGCCAAGAGTGAC	68
TPL-eMLP (<i>adenovirus derived enhancer element</i>)	CTCACTCTCTTCCGCATCGCTGTCTGCGAGGGCCA GCTGTTGGGCTCGCGGTTGAGGACAACTCTTCGC GGTCTTTCCAGTACTCTTGGATCGGAAACCCGTCG GCCTCCGAACGGTACTCCGCCACCGAGGGACCTGA GCGAGTCCGCATCGACCGGATCGGAAAACCTCTCG AGAAAGGCGTCTAACCAGTCACAGTCGCAAGGTAG GCTGAGCACCGTGGCGGGCGGCAGCGGGTGGCGGT CGGGGTGTTTCTGGCGGAGGTGCTGCTGATGATG TAATTAAAGTAGGCGGTCTTGAGACGGCGGATGGT CGAGGTGAGGTGTGGCAGGCTTGAGATCCAGCTGT TGGGGTGAGTACTCCCTCTCAAAGCGGGCATTAC TTCTGCGCTAAGATTGTCAGTTTCCAAAACGAGG AGGATTTGATATTACCTGGCCCGATCTGGCCATA	69

	CACTTGAGTGACAATGACATCCACTTTGCCTTTCT CTCCACAGGTGTCCACTCCCAG	
Human EF1a intron/exon	CTTTTTCGCAACGGGTTTGCCGCCAGAACACAGGT AAGTGCCGTGTGTGGTTCCCGCGGGCCTGGCCTCT TTACGGGTTATGGCCCTTGCGTGCCTTGAATTACT TCCACCTGGCTCCAGTACGTGATTCTTGATCCCGA GCTGGAGCCAGGGGCGGGCCTTGCGCTTTAGGAGC CCCTTCGCCTCGTGCTTGAGTTGAGGCCTGGCCTG GGCGCTGGGGCCGCCGCGTGCGAATCTGGTGGCAC CTTCGCGCCTGTCTCGCTGCTTTCGATAAGTCTCT AGCCATTTAAAATTTTGGATGACGTGCTGCGACGC TTTTTTTCTGGCAAGATAGTCTTGTAATGCGGGC CAGGATCTGCACACTGGTATTTTCGGTTTTTGGGCC CGCGGCCGCGACGGGGCCCGTGCGTCCCAGCGCA CATGTTTCGGCGAGGCGGGGCCTGCGAGCGCGGCCA CCGAGAATCGGACGGGGGTAGTCTCAAGCTGGCCG GCCTGCTCTGGTGCCTGGCCTCGCGCCGCCGTGTA TCGCCCCGCCCTGGGCGGCAAGGCTGGCCCGGTCTG GCACCAGTTGCGTGAGCGGAAAGATGGCCGCTTCC CGGCCCTGCTCCAGGGGGCTCAAAATGGAGGACGC GGCGCTCGGGAGAGCGGGCGGGTGAGTCACCCACA CAAAGGAAAAGGGCCTTTCCGTCCTCAGCCGTCTGC TTCATGTGACTCCACGGAGTACCGGGCGCCGTCCA GGCACCTCGATTAGTTCTGGAGCTTTTGGAGTACG TCGTCTTTAGGTTGGGGGGAGGGGTTTTATGCGAT GGAGTTTCCCCACACTGAGTGGGTGGAGACTGAAG TTAGGCCAGCTTGGCACTTGATGTAATTCTCCTTG GAATTTGGCCTTTTTGAGTTTGGATCTTGTTTCAT TCTCAAGCCTCAGACAGTGGTTCAAAGTTTTTTTC TTCCATTTTCAG	70
5' UTR human CamKIIa	TCAGAAGCCCCGGGCTCGTCAGTCAAACCGGTTCT CTGTTTGCCTCGGCAGCACGGGCAGGCAAGTGGT CCCTAGGTTTCGGG	71

In some embodiments, the vector comprises a 3' untranslated region selected from Table 7.

Table 7

3' UNTRANSLATED REGION	SEQUENCE	SEQ ID NO:
WPRES(x) (mutated woodchuck hepatitis regulatory element)	TTCCTGTAAATCAACCTCTGGATTACAAAATTTGT GAAAGATTGACTGGTATTCTTAACTATGTTGCTCC TTTTACGCTATGTGGATACGCTGCTTTAATGCCTT TGTATCATGCTATTGCTTCCCGTATGGCTTTCATT TTCTCCTCCTTGTATAAATCCTGGTTGCTGTCTCT TTATGAGGAGTTGTGGCCCGTTGTCAGGCAACGTG GCGTGGTGTGCACTGTGTTTGCTGACGCAACCCCC ACTGGTTGGGGCATTGCCACCACCTGTCAGCTCCT TTCCGGGACTTTCGCTTTCCCCCTCCCTATTGCCA CGGCGGAACATCATCGCCGCTGCCTTGCCCGCTGC TGGACAGGGGCTCGGCTGTTGGGCACTGACAATTC CGTGGTGTGTCGGGGAAGCTGACGTCCCTTCCGC GGCTGCTCGCCTGTGTTGCCACCTGGATTCTGCGC GGGACGTCCCTTCTGCTACGTCCCTTCGGCCCTCAA TCCAGCGGACCTTCCTTCCCGCGGCCTGCTGCCGG CTCTGCGGCCTCTTCCGCCTCTTCGCCTTCGCCCT CAGACGAGTCGGATCTCCCTTTGGGCGCCTCCCC GCCCATGTATCTTTTTCACCTGTGCCTTGTTTTTG CCTGTGTTCCGCGTCCTACTTTTCAAGCCTCCAAG CTGTGCCTTGGGCGGCTTTGGGGCATGGACATAGA TCCCTATAAAGAATTTGGTTCATCTTATCAGTTGT TGAATTTTCTTCCTTTGGAC	72
R2V17 (<i>HepB</i> derived enhancer element)	TTCCTGTAAACAGGCCTATTGATTGGAAAGTTTGT CAACGAATTGTGGGTCTTTTGGGGTTTGCTGCCCC TTTTACGCAATGTGGATATCCTGCTTTAATGCCTT TATATGCATGTATACAAGCAAAACAGGCTTTTACT TTCTCGCCAACTTACAAGGCCTTTCTCAGTAAACA	73

	GTATATGACCCTTTACCCCGTTGCTCGGCAACGGC CTGGTCTGTGCCAAGTGTTTGCTGACGCAACCCCC ACTGGTTGGGGCTTGGCCATAGGCCATCAGCGCAT GCGTGGAACCTTTGTGTCTCCTCTGCCGATCCATA CTGCGGAACCTCCTAGCCGCTTGTTTTGCTCGCAGC TGGACTGGAGCAAACCTCATCGGGACCGACAATTC TGTCGTA CTCTCCCGCAAGCACTCACCGTTTCCGC GGCTGCTCGCCTGTGTTGCCACCTGGATTCTGCGC GGGACGTCCTTCTGCTACGTCCCTTCGGCCCTCAA TCCAGCGGACCTTCCTTCCCGCGGCCTGCTGCCGG CTCTGCGGCCTCTTCCGCCTCTTCGCCTTCGCCCT CAGACGAGTCGGATCTCCCTTTGGGCGCCTCCCC GCCCATGTATCTTTTTACCTGTGCCTTGTTTTTG CCTGTGTTCCGCGTCCTACTTTTCAAGCCTCCAAG CTGTGCCTTGGGCGGCTTTGGGGCATGGACATAGA TCCCTATAAAGAATTGGTTCATCTTATCAGTTGT TGAATTTTCTTCCTTTGGAC	
3'UTR(globin)	GCTGGAGCCTCGGTAGCCGTTCCCTCCTGCCCGCTG GGCCCTCCCAACGGGCCCCTCCTCCCCTCCTTGCACC GGCCCTTCCTGGTCTTTGAATAAA	74
WPRE(r)	ATTCGAGCATCTTACCGCCATTTATTCCCATATTT GTTCTGTTTTTCTTGATTTGGGTATACATTTAAAT GTTAATAAAACAAAATGGTGGGGCAATCATTTACA TTTT TAGGGATATGTAATTACTAGTTCAGGTGTAT TGCCACAAGACAAACATGTTAAGAAACTTTCCCGT TATTTACGCTCTGTTCCCTGTTAATCAACCTCTGGA TTACAAAATTTGTGAAAGATTGACTGATATTCTTA ACTATGTTGCTCCTTTTACGCTGTGTGGATATGCT GCTTTAATGCCTCTGTATCATGCTATTGCTTCCCG TACGGCTTTCGTTTTCTCCTCCTTGATAAAATCCT GGTTGCTGTCTCTTTATGAGGAGTTGTGGCCCGTT GTCCGTCAACGTGGCGTGGTGTGCTCTGTGTTTGC TGACGCAACCCCCACTGGCTGGGGCATTGCCACCA	75

	CCTGTCAACTCCTTTCTGGGACTTTCGCTTTCCCC CTCCCGATCGCCACGGCAGAACTCATCGCCGCCTG CCTTGCCCGCTGCTGGACAGGGGCTAGGTTGCTGG GCACTGATAATTCCGTGGTGTTCGCGGGAAGGGC C	
--	--	--

In some embodiments, the vector comprises a polyadenylation sequence (polyA) selected from Table 8.

Table 8

POLYADENYLATION	SEQUENCE	SEQ ID NO:
Rabbit globin (pAGlobin-Oc)	TGGCTAATAAAGGAAATTTATTTTCATTGCAATA GTGTGTTGGAATTTTTTGTGTCTCTCACTCGGAA GAACATATGGGAGGGCAAATCATTTAAAACATCA GAATGAGTATTTGGTTTAGAGTTTGGCAACATAT GCCCATATGCTGGCTGCCATGAACAAAGGTTGGC TATAAAGAGGTCATCAGTATATGAAACAGCCCCC TGCTGTCCATTCTTATTCCATAGAAAAGCCTTG ACTTGAGGTTAGATTTTTTTTATATTTTGTTTTG TGTTATTTTTTTCTTTAACATCCCTAAAATTTTC CTTACATGTTTTACTAGCCAGATTTTTCCTCCTC TCCTGACTACTCCCAGTCATAGCTGTCCCTCTTC TCTTATGGAGATC	76
Bovine growth hormone (pAGH-Bt)	TTGCCAGCCATCTGTTGTTTGCCCCCTCCCCGTG CCTTCCTTGACCCTGGAAGGTGCCACTCCCCTG TCCTTTCCTAATAAAAATGAGGAAATTGCATCGCA TTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGT GGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGG AATACAATAGCAGGCATGCTGGGGATGCGGTGGG CTCTATGGGTACCCAGGTGCTGAAGAATTGACCC GGTTCCTCCTGGG	77

Human growth hormone (pAGH-Hs)	CTGCCCCGGGTGGCATCCCTGTGACCCCTCCCCAG TGCTCTCCTGGCCCTGGAAGTTGCCACTCCAGT GCCACCAGCCTTGTCTAATAAAATTAAGTTGC ATCATTTTGTCTGACTAGGTGTCCTTCTATAATA TTATGGGGTGGAGGGGGTGGTATGGAGCAAGGG GCCCAAGTTGGGAAGAAACCTGTAGGGCCTGC	78
-----------------------------------	---	----

Illustrative expression cassettes are depicted in FIG. 13 and provided as SEQ ID NOs: 39-58, listed in Table 10. In some embodiments, the expression cassette comprises, consists essentially of, or consists of a polynucleotide sequence that shares at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity with any one of SEQ ID NOs: 39-58. In some embodiments, the expression cassette comprises, consists essentially of, or consists of a polynucleotide sequence that shares at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity with any one of SEQ ID NOs: 39-58 excluding the sequence encoding the therapeutic gene product. In some embodiments, the sequence encoding the therapeutic gene product is replaced by a sequence encoding a different therapeutic gene product.

In an embodiment, the expression cassette comprises, in 5' to 3' order, HuBA promoter, the transgene, WPRE(x), and pAGlobin-Oc. In certain embodiments, the transgene encodes PARK2, PINK1 (PARK6), DJ-1 (PARK7), LRRK2, α -synuclein, and DJ-1. In particular embodiments, it encodes PARK2, and in certain embodiments, it comprises a sequence set forth in any of SEQ ID NOs: 27 or 35-38.

In an embodiment, the expression cassette comprises, in 5' to 3' order, CMV promoter, TPL-eMLP enhancer, the transgene, WPRE(r), and pAGlobin-Oc. In certain embodiments, the transgene encodes PARK2, PINK1 (PARK6), DJ-1 (PARK7), LRRK2, α -synuclein, and DJ-1. In particular embodiments, it encodes PARK2, and in certain embodiments, it comprises a sequence set forth in any of SEQ ID NOs: 27 or 35-38.

In an embodiment, the expression cassette comprises, in 5' to 3' order, Syn promoter, the transgene, WPRE(r), 3'UTR (globin), and pAGH-Bt. In certain embodiments, the transgene encodes PARK2, PINK1 (PARK6), DJ-1 (PARK7), LRRK2, α -synuclein, and DJ-1. In particular embodiments, it encodes PARK2, and in

certain embodiments, in comprises a sequence set forth in any of SEQ ID NOs: 27 or 35-38.

In an embodiment, the expression cassette comprises, in 5' to 3' order, CBA promoter, the transgene, and pAGH-Bt. In certain embodiments, the transgene
5 encodes PARK2, PINK1 (PARK6), DJ-1 (PARK7), LRRK2, α -synuclein, and DJ-1. In particular embodiments, it encodes PARK2, and in certain embodiments, in comprises a sequence set forth in any of SEQ ID NOs: 27 or 35-38.

In an embodiment, the expression cassette comprises, in 5' to 3' order, EF1 α promoter, the transgene, and pAGlobin-Oc. In certain embodiments, the transgene
10 encodes PARK2, PINK1 (PARK6), DJ-1 (PARK7), LRRK2, α -synuclein, and DJ-1. In particular embodiments, it encodes PARK2, and in certain embodiments, in comprises a sequence set forth in any of SEQ ID NOs: 27 or 35-38.

In an embodiment, the expression cassette comprises, in 5' to 3' order, HuBA promoter, the transgene, R2V17, and pAGH-Bt. In certain embodiments, the
15 transgene encodes PARK2, PINK1 (PARK6), DJ-1 (PARK7), LRRK2, α -synuclein, and DJ-1. In particular embodiments, it encodes PARK2, and in certain embodiments, in comprises a sequence set forth in any of SEQ ID NOs: 27 or 35-38.

In an embodiment, the expression cassette comprises, in 5' to 3' order, Syn promoter, the transgene, WPRE(x), 3'UTR (globin), and pAGH-Hs. In certain
20 embodiments, the transgene encodes PARK2, PINK1 (PARK6), DJ-1 (PARK7), LRRK2, α -synuclein, and DJ-1. In particular embodiments, it encodes PARK2, and in certain embodiments, in comprises a sequence set forth in any of SEQ ID NOs: 27 or 35-38.

In an embodiment, the expression cassette comprises, in 5' to 3' order,
25 CaMKIIa promoter, the transgene, WPRE(r), and pAGH-Hs. In certain embodiments, the transgene encodes PARK2, PINK1 (PARK6), DJ-1 (PARK7), LRRK2, α -synuclein, and DJ-1. In particular embodiments, it encodes PARK2, and in certain embodiments, in comprises a sequence set forth in any of SEQ ID NOs: 27 or 35-38.

In an embodiment, the expression cassette comprises, in 5' to 3' order, CMV
30 promoter, TPL-eMLP enhancer, the transgene, WPRE(r), and pAGH-Hs. In certain embodiments, the transgene encodes PARK2, PINK1 (PARK6), DJ-1 (PARK7), LRRK2, α -synuclein, and DJ-1. In particular embodiments, it encodes PARK2, and in

certain embodiments, in comprises a sequence set forth in any of SEQ ID NOs: 27 or 35-38.

In an embodiment, the expression cassette comprises, in 5' to 3' order, HuBA promoter, the transgene, and pAGH-Hs. In certain embodiments, the transgene
5 encodes PARK2, PINK1 (PARK6), DJ-1 (PARK7), LRRK2, α -synuclein, and DJ-1. In particular embodiments, it encodes PARK2, and in certain embodiments, in comprises a sequence set forth in any of SEQ ID NOs: 27 or 35-38.

In an embodiment, the expression cassette comprises, in 5' to 3' order, CMV promoter, TPL/eMLP enhancer, the transgene, R2V17, 3'UTR (globin), and pAGH-
10 Bt. In certain embodiments, the transgene encodes PARK2, PINK1 (PARK6), DJ-1 (PARK7), LRRK2, α -synuclein, and DJ-1. In particular embodiments, it encodes PARK2, and in certain embodiments, in comprises a sequence set forth in any of SEQ ID NOs: 27 or 35-38.

In an embodiment, the expression cassette comprises, in 5' to 3' order, EF1 α promoter, the transgene, WPRE(r), and pAGH-Bt. In certain embodiments, the
15 transgene encodes PARK2, PINK1 (PARK6), DJ-1 (PARK7), LRRK2, α -synuclein, and DJ-1. In particular embodiments, it encodes PARK2, and in certain embodiments, in comprises a sequence set forth in any of SEQ ID NOs: 27 or 35-38.

In an embodiment, the expression cassette comprises, in 5' to 3' order, Syn promoter, the transgene, R2V17, and pAGlobin-Oc. In certain embodiments, the
20 transgene encodes PARK2, PINK1 (PARK6), DJ-1 (PARK7), LRRK2, α -synuclein, and DJ-1. In particular embodiments, it encodes PARK2, and in certain embodiments, in comprises a sequence set forth in any of SEQ ID NOs: 27 or 35-38.

In an embodiment, the expression cassette comprises, in 5' to 3' order, CaMKIIa promoter, the transgene, R2V17, and pAGlobin-Oc. In certain
25 embodiments, the transgene encodes PARK2, PINK1 (PARK6), DJ-1 (PARK7), LRRK2, α -synuclein, and DJ-1. In particular embodiments, it encodes PARK2, and in certain embodiments, in comprises a sequence set forth in any of SEQ ID NOs: 27 or 35-38.

In an embodiment, the expression cassette comprises, in 5' to 3' order, CBA promoter, the transgene, WPRE(x), 3'UTR (globin), and pAGH-Hs. In certain
30 embodiments, the transgene encodes PARK2, PINK1 (PARK6), DJ-1 (PARK7), LRRK2, α -synuclein, and DJ-1. In particular embodiments, it encodes PARK2, and in

certain embodiments, in comprises a sequence set forth in any of SEQ ID NOs: 27 or 35-38.

In an embodiment, the expression cassette comprises, in 5' to 3' order, CBA promoter, the transgene, 3'UTR (globin), and pAGlobin-Oc. In certain embodiments, the transgene encodes PARK2, PINK1 (PARK6), DJ-1 (PARK7), LRRK2, α -synuclein, and DJ-1. In particular embodiments, it encodes PARK2, and in certain
5 embodiments, in comprises a sequence set forth in any of SEQ ID NOs: 27 or 35-38.

In an embodiment, the expression cassette comprises, in 5' to 3' order, CaMKIIa promoter, the transgene, R2V17, and pAGH-Bt. In certain embodiments, the transgene encodes PARK2, PINK1 (PARK6), DJ-1 (PARK7), LRRK2, α -synuclein, and DJ-1. In particular embodiments, it encodes PARK2, and in certain
10 embodiments, in comprises a sequence set forth in any of SEQ ID NOs: 27 or 35-38.

In an embodiment, the expression cassette comprises, in 5' to 3' order, EF1 α promoter, the transgene, R2V17, 3'UTR (globin), and pAGH-Hs. In certain
15 embodiments, the transgene encodes PARK2, PINK1 (PARK6), DJ-1 (PARK7), LRRK2, α -synuclein, and DJ-1. In particular embodiments, it encodes PARK2, and in certain embodiments, in comprises a sequence set forth in any of SEQ ID NOs: 27 or 35-38.

In an embodiment, the expression cassette comprises, in 5' to 3' order, CMV promoter, the transgene, R2V17, 3'UTR (globin), and pAGH-Hs. In certain
20 embodiments, the transgene encodes PARK2, PINK1 (PARK6), DJ-1 (PARK7), LRRK2, α -synuclein, and DJ-1. In particular embodiments, it encodes PARK2, and in certain embodiments, in comprises a sequence set forth in any of SEQ ID NOs: 27 or 35-38.

In an embodiment, the expression cassette comprises, in 5' to 3' order, CMV promoter, the transgene, and pAGH-Hs. In certain embodiments, the transgene
25 encodes PARK2, PINK1 (PARK6), DJ-1 (PARK7), LRRK2, α -synuclein, and DJ-1. In particular embodiments, it encodes PARK2, and in certain embodiments, in comprises a sequence set forth in any of SEQ ID NOs: 27 or 35-38.

In embodiments of the foregoing, the order of the elements 5' to the transgene are reversed so that the promoter precedes the enhancer elements or the enhancer element precedes the promoter element.
30

Therapeutic Compositions and Methods

As used herein, the term “patient in need” or “subject in need” refers to a patient or subject at risk of, or suffering from, a disease, disorder or condition that is amenable to treatment or amelioration with a recombinant gene therapy vector or gene editing system disclosed herein. A patient or subject in need may, for instance, be a patient or subject diagnosed with a disorder associated with central nervous system degradation. A subject may have a mutation or a malfunction in a PARK2, PARK6, PARK7, LRRK2, or α -synuclein, gene or protein. “Subject” and “patient” are used interchangeably herein. The subject treated by the methods described herein may be an adult or a child. Subjects may range in age. The subject may be a person identified as at risk for a Parkinson’s Disease, e.g., an early-onset Parkinson’s Disease.

Combination therapies are also contemplated by the invention. Combination as used herein includes simultaneous treatment or sequential treatment.

Combinations of methods of the invention with standard medical treatments (e.g., corticosteroids or topical pressure reducing medications) are specifically contemplated, as are combinations with novel therapies. In some cases, a subject may be treated with a steroid to prevent or to reduce an immune response to administration of a rAAV described herein. In certain cases, a subject may receive topical pressure reducing medications before, during, or after administering of a rAAV described herein.

A therapeutically effective amount of the rAAV vector is a dose of rAAV ranging from about $1e7$ vg/kg to about $5e15$ vg/kg, or about $1e7$ vg/kg to about $1e14$ vg/kg, or about $1e8$ vg/kg to about $1e14$ vg/kg, or about $1e9$ vg/kg to about $1e13$ vg/kg, or about $1e9$ vg/kg to about $1e12$ vg/kg, or about $1e7$ vg/kg to about $5e7$ vg/kg, or about $1e8$ vg/kg to about $5e8$ vg/kg, or about $1e9$ vg/kg to about $5e9$ vg/kg, or about $1e10$ vg/kg to about $5e10$ vg/kg, or about $1e11$ vg/kg to about $5e11$ vg/kg, or about $1e12$ vg/kg to about $5e12$ vg/kg, or about $1e13$ vg/kg to about $5e13$ vg/kg, or about $1e14$ vg/kg to about $5e14$ vg/kg, or about $1e15$ vg/kg to about $5e15$ vg/kg.

The invention also comprises compositions comprising these ranges of rAAV vector.

For example, in particular embodiments, a therapeutically effective amount of rAAV vector is a dose of about $1e10$ vg/kg, about $2e10$ vg/kg, about $3e10$ vg/kg, about $4e10$ vg/kg, about $5e10$ vg/kg, about $6e10$ vg/kg, about $7e10$ vg/kg, about

8e10 vg/kg, about 9e10 vg/kg, about 1e12 vg/kg, about 2e12 vg/kg, about 3e12 vg/kg, about 4e12 vg/kg and 5e12 vg/kg. The invention also comprises compositions comprising these doses of rAAV vector.

5 In some embodiments, for example where direct injection into substantia nigra is performed, a therapeutically effective amount of rAAV vector is a dose in the range of 1e7 vg to 1e11 vg, or about 1e7 vg, about 1e8 vg, about 1e9 vg, about 1e10 vg, or about 1e11 vg.

10 In some embodiments, for example where direct injection into intraputaminial is performed, a therapeutically effective amount of rAAV vector is a dose in the range of 1e7 vg to 1e11 vg, or about 1e7 vg, about 1e8 vg, about 1e9 vg, about 1e10 vg, or about 1e11 vg.

15 In some cases, the therapeutic composition comprises more than about 1e9, 1e10, or 1e11 genomes of the rAAV vector per volume of therapeutic composition injected. In some cases, the therapeutic composition comprises more than about 1e9, 1e10, or 1e11 genomes of the rAAV vector per volume of therapeutic composition injected. In some cases, the therapeutic composition comprises more than approximately 1e10, 1e11, 1e12, or 1e13 genomes of the rAAV vector per mL. In certain embodiments, the therapeutic composition comprises less than about 1e14, 1e13 or 1e12 genomes of the rAAV vector per mL.

20 **Administration of Compositions**

Administration of an effective dose of the compositions may be by routes standard in the art including, but not limited to, systemic, local, direct injection, parenteral, intravenous, cerebral, cerebrospinal, intrathecal, intracisternal, intraputaminial, intrahippocampal, intra-striatal, or intra-cerebroventricular administration. In some cases, administration comprises intravenous, cerebral, cerebrospinal, intrathecal, intracisternal, intraputaminial, intrahippocampal, intra-striatal, or intra-cerebroventricular injection. Administration may be performed by intrathecal injection with Threndelenberg tilting. Route(s) of administration and serotype(s) of AAV components of the rAAV (in particular, the AAV ITRs and capsid protein) of the invention may be chosen and/or matched by those skilled in the art taking into account the disorder being treated and the target cells/tissue(s) that are to express the repaired and/or exogenously provided gene.

25
30

In certain embodiment, the disclosure provides for local administration and systemic administration of an effective dose of rAAV and compositions of the invention. For example, systemic administration may be administration into the circulatory system so that the entire body is affected. Systemic administration
5 includes enteral administration such as absorption through the gastrointestinal tract and parental administration through injection, infusion or implantation.

In particular, actual administration of rAAV of the present invention may be accomplished by using any physical method that will transport the rAAV recombinant vector into the target tissue of an animal. Administration according to the invention
10 includes, but is not limited to, injection into the central nervous system (CNS) or cerebrospinal fluid (CSF) and/or directly into the brain.

Capsid proteins of a rAAV may be modified so that the rAAV is targeted to a particular target tissue of interest such as neurons or more particularly a dopaminergic neuron. *See, for example*, Albert et al. AAV Vector-Mediated Gene
15 Delivery to Substantia Nigra Dopamine Neurons: Implications for Gene Therapy and Disease Models. Genes. 2017 Feb 8; *see also* US Patent No. 6,180,613 and U.S. Patent Pub. No. US20120082650A1, the disclosures of both of which are incorporated by reference herein. In some embodiments, the rAAV is directly injected into the substantia nigra of the subject.

For purposes of administration, e.g., by injection, various solutions can be employed, such as sterile aqueous solutions. Such aqueous solutions can be buffered, if desired, and the liquid diluent first rendered isotonic with saline or glucose. Solutions of rAAV as a free acid (DNA contains acidic phosphate groups) or a pharmacologically acceptable salt can be prepared in water suitably mixed with a
25 surfactant such as hydroxypropylcellulose. A dispersion of rAAV can also be prepared in glycerol, liquid polyethylene glycols and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms. In this connection, the sterile aqueous media employed are all readily obtainable by standard techniques well-known to those
30 skilled in the art.

The pharmaceutical forms suitable for injectable use include but are not limited to sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all

cases the form is sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating actions of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of a dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal and the like. In many cases it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by use of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions may be prepared by incorporating rAAV in the required amount in the appropriate solvent with various other ingredients enumerated above, as required, followed by filter sterilization. Generally, dispersions are prepared by incorporating the sterilized active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying technique that yield a powder of the active ingredient plus any additional desired ingredient from the previously sterile-filtered solution thereof.

Transduction with rAAV may also be carried out *in vitro*. In one embodiment, desired target cells are removed from the subject, transduced with rAAV and reintroduced into the subject.

Suitable methods for the transduction and reintroduction of transduced cells into a subject are known in the art. In one embodiment, cells can be transduced *in vitro* by combining rAAV with cells, e.g., in appropriate media, and screening for those cells harboring the DNA of interest using conventional techniques such as Southern blots and/or PCR, or by using selectable markers. Transduced cells can then be formulated into pharmaceutical compositions, and the composition introduced into the subject by various techniques, such as by systemic, local, direct

injection, parenteral, intravenous, cerebral, cerebrospinal, intrathecal, intracisternal, intraputamin, intrahippocampal, intra-striatal, or intra-cerebroventricular administration. In some cases, administration comprises intravenous, cerebral, cerebrospinal, intrathecal, intracisternal, intraputamin, intrahippocampal, intra-striatal, or intra-cerebroventricular injection. Administration may be performed by intrathecal injection with Trendelenburg tilting.

Transduction of cells with rAAV of the invention results in sustained expression of a gene of interest, such as PARK2, PARK6, PARK7, LRRK2, or α -synuclein. The present invention thus provides methods of administering or delivering recombinant gene therapy vectors (e.g. rAAV vectors) which express a gene related to a CNS degeneration to a mammalian subject, preferably a human being. These methods include transducing tissues (including, but not limited to, the tissues of the brain) with one or more rAAV of the present invention. Transduction may be carried out with gene cassettes comprising tissue specific control elements. For example, one embodiment of the invention provides methods of transducing neuronal cells and brain tissues directed by neuron-specific control elements, including, but not limited to, those derived from neuron-enriched promoters, and other control elements.

Gene-Editing System

As used herein, a gene-editing system is a system comprising one or more proteins or polynucleotides capable of editing an endogenous target gene or locus in a sequence specific manner. In some embodiments, the gene-editing system is a protein-based gene regulating system comprising a protein comprising one or more zinc-finger binding domains and an enzymatic domain. In some embodiments, the protein-based gene regulating system comprises a protein comprising a Transcription activator-like effector nuclease (TALEN) domain and an enzymatic domain. Such embodiments are referred to herein as "TALENs".

1. Zinc finger-based systems

Zinc finger-based systems comprise a fusion protein comprising two protein domains: a zinc finger DNA binding domain and an enzymatic domain. A "zinc finger DNA binding domain", "zinc finger protein", or "ZFP" is a protein, or a domain within a larger protein, that binds DNA in a sequence-specific manner through one or more zinc fingers, which are regions of amino acid sequence within the binding domain

whose structure is stabilized through coordination of a zinc ion. The zinc finger domain, by binding to a target DNA sequence, directs the activity of the enzymatic domain to the vicinity of the sequence and, hence, induces modification of the endogenous target gene in the vicinity of the target sequence. A zinc finger domain can be engineered to bind to virtually any desired sequence. Accordingly, after identifying a target genetic locus containing a target DNA sequence at which cleavage or recombination is desired (e.g., a target locus in a target gene referenced in Table 1), one or more zinc finger binding domains can be engineered to bind to one or more target DNA sequences in the target genetic locus. Expression of a fusion protein comprising a zinc finger binding domain and an enzymatic domain in a cell, effects modification in the target genetic locus.

In some embodiments, a zinc finger binding domain comprises one or more zinc fingers. Miller *et al.* (1985) EMBO J. 4:16010-1714; Rhodes (1993) Scientific American February:56-65; U.S. Pat. No. 6,453,242. Typically, a single zinc finger domain is about 30 amino acids in length. An individual zinc finger binds to a three-nucleotide (*i.e.*, triplet) sequence (or a four-nucleotide sequence which can overlap, by one nucleotide, with the four-nucleotide binding site of an adjacent zinc finger). Therefore, the length of a sequence to which a zinc finger binding domain is engineered to bind (e.g., a target sequence) will determine the number of zinc fingers in an engineered zinc finger binding domain. For example, for ZFPs in which the finger motifs do not bind to overlapping subsites, a six-nucleotide target sequence is bound by a two-finger binding domain; a nine-nucleotide target sequence is bound by a three-finger binding domain, etc. Binding sites for individual zinc fingers (*i.e.*, subsites) in a target site need not be contiguous, but can be separated by one or several nucleotides, depending on the length and nature of the amino acid sequences between the zinc fingers (*i.e.*, the inter-finger linkers) in a multi-finger binding domain. In some embodiments, the DNA-binding domains of individual ZFNs comprise between three and six individual zinc finger repeats and can each recognize between 9 and 18 basepairs.

Zinc finger binding domains can be engineered to bind to a sequence of choice. See, for example, Beerli *et al.* (2002) Nature Biotechnol. 20:135-141; Pabo *et al.* (2001) Ann. Rev. Biochem. 70:313-340; Isalan *et al.* (2001) Nature Biotechnol. 19:656-660; Segal *et al.* (2001) Curr. Opin. Biotechnol. 12:632-637; Choo *et al.*

(2000) Curr. Opin. Struct. Biol. 10:411-416. An engineered zinc finger binding domain can have a novel binding specificity, compared to a naturally-occurring zinc finger protein. Engineering methods include, but are not limited to, rational design and various types of selection.

5 Selection of a target DNA sequence for binding by a zinc finger domain can be accomplished, for example, according to the methods disclosed in U.S. Pat. No. 6,453,242. It will be clear to those skilled in the art that simple visual inspection of a nucleotide sequence can also be used for selection of a target DNA sequence. Accordingly, any means for target DNA sequence selection can be used in the
10 methods described herein. A target site generally has a length of at least 9 nucleotides and, accordingly, is bound by a zinc finger binding domain comprising at least three zinc fingers. However binding of, for example, a 4-finger binding domain to a 12-nucleotide target site, a 5-finger binding domain to a 15-nucleotide target site or a 6-finger binding domain to an 18-nucleotide target site, is also possible. As will
15 be apparent, binding of larger binding domains (*e.g.*, 7-, 8-, 9-finger and more) to longer target sites is also possible.

 In some embodiments, the zinc finger binding domains bind to a target DNA sequence that is at least 90% identical to a target DNA sequence within a target locus of a target gene selected those listed in Table 1. In some embodiments, the
20 zinc finger binding domains bind to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a target DNA sequence within a target locus of a target gene selected those listed in Table 1. In some embodiments, the zinc finger binding domains bind to a target DNA sequence that is 100% identical to a target DNA sequence within a target locus of a target gene selected those listed in Table 1.

25 The enzymatic domain portion of the zinc finger fusion proteins can be obtained from any endo- or exonuclease. Exemplary endonucleases from which an enzymatic domain can be derived include, but are not limited to, restriction endonucleases and homing endonucleases. See, for example, 2002-2003 Catalogue, New England Biolabs, Beverly, Mass.; and Belfort *et al.* (1997) Nucleic
30 Acids Res. 25:3379-3388. Additional enzymes which cleave DNA are known (*e.g.*, 51 Nuclease; mung bean nuclease; pancreatic DNase I; micrococcal nuclease; yeast HO endonuclease; see also Linn *et al.* (eds.) Nucleases, Cold Spring Harbor

Laboratory Press, 1993). One or more of these enzymes (or functional fragments thereof) can be used as a source of cleavage domains.

Exemplary restriction endonucleases (restriction enzymes) suitable for use as an enzymatic domain of the ZFPs described herein are present in many species and are capable of sequence-specific binding to DNA (at a recognition site), and cleaving DNA at or near the site of binding. Certain restriction enzymes (e.g., Type IIS) cleave DNA at sites removed from the recognition site and have separable binding and cleavage domains. For example, the Type IIS enzyme Fok I catalyzes double-stranded cleavage of DNA, at 9 nucleotides from its recognition site on one strand and 13 nucleotides from its recognition site on the other. See, for example, U.S. Pat. Nos. 5,356,802; 5,436,150 and 5,487,994; as well as Li *et al.* (1992) Proc. Natl. Acad. Sci. USA 89:4275-4279; Li *et al.* (1993) Proc. Natl. Acad. Sci. USA 90:2764-2768; Kim *et al.* (1994a) Proc. Natl. Acad. Sci. USA 91:883-887; Kim *et al.* (1994b) J. Biol. Chem. 269:31,978-31,982. Thus, in one embodiment, fusion proteins comprise the enzymatic domain from at least one Type IIS restriction enzyme and one or more zinc finger binding domains.

An exemplary Type IIS restriction enzyme, whose cleavage domain is separable from the binding domain, is Fok I. This particular enzyme is active as a dimer. Bitinaite *et al.* (1998) Proc. Natl. Acad. Sci. USA 95: 10,570-10,575. Thus, for targeted double-stranded DNA cleavage using zinc finger-Fok I fusions, two fusion proteins, each comprising a FokI enzymatic domain, can be used to reconstitute a catalytically active cleavage domain. Alternatively, a single polypeptide molecule containing a zinc finger binding domain and two FokI enzymatic domains can also be used. Exemplary ZFPs comprising FokI enzymatic domains are described in US Patent No. 9,782,437.

2. TALEN-based systems

TALEN-based systems comprise a protein comprising a TAL effector DNA binding domain and an enzymatic domain. They are made by fusing a TAL effector DNA-binding domain to a DNA cleavage domain (a nuclease which cuts DNA strands). The FokI restriction enzyme described above is an exemplary enzymatic domain suitable for use in TALEN-based gene regulating systems.

TAL effectors are proteins that are secreted by *Xanthomonas* bacteria via their type III secretion system when they infect plants. The DNA binding domain

contains a repeated, highly conserved, 33–34 amino acid sequence with divergent 12th and 13th amino acids. These two positions, referred to as the Repeat Variable Diresidue (RVD), are highly variable and strongly correlated with specific nucleotide recognition. Therefore, the TAL effector domains can be engineered to bind specific target DNA sequences by selecting a combination of repeat segments containing the appropriate RVDs. The nucleic acid specificity for RVD combinations is as follows: HD targets cytosine, NI targets adenine, NG targets thymine, and NN targets guanine (though, in some embodiments, NN can also bind adenine with lower specificity).

In some embodiments, the TAL effector domains bind to a target DNA sequence that is at least 90% identical to a target DNA sequence within a target locus of a target gene selected those listed in Table 1. In some embodiments, the TAL effector domains bind to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a target DNA sequence within a target locus of a target gene selected those listed in Table 1. In some embodiments, the TAL effector domains bind to a target DNA sequence that is 100% identical to a target DNA sequence within a target locus of a target gene selected those listed in Table 1.

Methods and compositions for assembling the TAL-effector repeats are known in the art. See *e.g.*, Cermak *et al*, Nucleic Acids Research, 39:12, 2011, e82. Plasmids for constructions of the TAL-effector repeats are commercially available from Addgene.

In some embodiments, the gene-editing system is a combination gene-regulating system comprising a site-directed modifying polypeptide and a nucleic acid guide molecule. Herein, a "site-directed modifying polypeptide" refers to a polypeptide that binds to a nucleic acid guide molecule, is targeted to a target nucleic acid sequence, such as, for example, a DNA sequence, by the nucleic acid guide molecule to which it is bound, and modifies the target DNA sequence (*e.g.*, cleavage, mutation, or methylation of target DNA). A site-directed modifying polypeptide comprises two portions, a portion that binds the nucleic acid guide and an activity portion. In some embodiments, a site-directed modifying polypeptide comprises an activity portion that exhibits site-directed enzymatic activity (*e.g.*, DNA methylation, DNA cleavage, histone acetylation, histone methylation, etc.), wherein the site of enzymatic activity is determined by the guide nucleic acid.

The nucleic acid guide comprises two portions: a first portion that is complementary to, and capable of binding with, an endogenous target DNA sequence (referred to herein as a “DNA-binding segment”), and a second portion that is capable of interacting with the site-directed modifying polypeptide (referred to herein as a “protein-binding segment”). In some embodiments, the DNA-binding segment and protein-binding segment of a nucleic acid guide are comprised within a single polynucleotide molecule. In some embodiments, the DNA-binding segment and protein-binding segment of a nucleic acid guide are each comprised within separate polynucleotide molecules, such that the nucleic acid guide comprises two polynucleotide molecules that associate with each other to form the functional guide.

The nucleic acid guide mediates the target specificity of the combined protein/nucleic gene regulating systems by specifically hybridizing with a target DNA sequence comprised within the DNA sequence of a target gene. Reference herein to a target gene encompasses the full-length DNA sequence for that particular gene and a full-length DNA sequence for a particular target gene will comprise a plurality of target genetic loci, which refer to portions of a particular target gene sequence (e.g., an exon or an intron). Within each target genetic loci are shorter stretches of DNA sequences referred to herein as “target DNA sequences” or “target sequences” that can be modified by the gene-regulating systems described herein. Further, each target genetic loci comprises a “target modification site,” which refers to the precise location of the modification induced by the gene-regulating system (e.g., the location of an insertion, a deletion, or mutation, the location of a DNA break, or the location of an epigenetic modification). The gene-regulating systems described herein may comprise a single nucleic acid guide, or may comprise a plurality of nucleic acid guides (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more nucleic acid guides).

The CRISPR/Cas systems described below are exemplary embodiments of a combination protein/nucleic acid system.

3. CRISPR/Cas Gene Regulating systems

In some embodiments, the gene editing systems described herein are CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)/Cas (CRISPR Associated) nuclease systems. In such embodiments, the site-directed modifying polypeptide is a CRISPR-associated endonuclease (a “Cas endonuclease”) and the nucleic acid guide molecule is a guide RNA (gRNA).

A Cas polypeptide refers to a polypeptide that can interact with a gRNA molecule and, in concert with the gRNA molecule, homes or localizes to a target DNA sequence and includes naturally occurring Cas proteins and engineered, altered, or otherwise modified Cas proteins that differ by one or more amino acid residues from a naturally-occurring Cas sequence.

In some embodiments, the Cas protein is a Cas9 protein. Cas9 is a multi-domain enzyme that uses an HNH nuclease domain to cleave the target strand of DNA and a RuvC-like domain to cleave the non-target strand. In some embodiments, mutants of Cas9 can be generated by selective domain inactivation enabling the conversion of WT Cas9 into an enzymatically inactive mutant (e.g., dCas9), which is unable to cleave DNA, or a nickase mutant, which is able to produce single-stranded DNA breaks by cleaving one or the other of the target or non-target strand.

A guide RNA (gRNA) comprises two segments, a DNA-binding segment and a protein-binding segment. In some embodiments, the protein-binding segment of a gRNA is comprised in one RNA molecule and the DNA-binding segment is comprised in another separate RNA molecule. Such embodiments are referred to herein as "double-molecule gRNAs" or "two-molecule gRNA" or "dual gRNAs." In some embodiments, the gRNA is a single RNA molecule and is referred to herein as a "single-guide RNA" or an "sgRNA." The term "guide RNA" or "gRNA" is inclusive, referring both to two-molecule guide RNAs and sgRNAs.

The protein-binding segment of a gRNA comprises, in part, two complementary stretches of nucleotides that hybridize to one another to form a double stranded RNA duplex (dsRNA duplex), which facilitates binding to the Cas protein.

The DNA-binding segment (or "DNA-binding sequence") of a gRNA comprises a nucleotide sequence that is complementary to and capable of binding to a specific sequence target DNA sequence. The protein-binding segment of the gRNA interacts with a Cas polypeptide and the interaction of the gRNA molecule and site-directed modifying polypeptide results in Cas binding to the endogenous DNA and produces one or more modifications within or around the target DNA sequence. The precise location of the target modification site is determined by both (i) base-pairing complementarity between the gRNA and the target DNA sequence; and (ii) the location of a short motif, referred to as the protospacer adjacent motif (PAM), in

the target DNA sequence. The PAM sequence is required for Cas binding to the target DNA sequence. A variety of PAM sequences are known in the art and are suitable for use with a particular Cas endonuclease (e.g., a Cas9 endonuclease) are known in the art (See e.g., Nat Methods. 2013 Nov; 10(11): 1116–1121 and Sci Rep. 2014; 4: 5405). In some embodiments, the PAM sequence is located within 50 base pairs of the target modification site. In some embodiments, the PAM sequence is located within 10 base pairs of the target modification site. The DNA sequences that can be targeted by this method are limited only by the relative distance of the PAM sequence to the target modification site and the presence of a unique 20 base pair sequence to mediate sequence-specific, gRNA-mediated Cas binding. In some embodiments, the target modification site is located at the 5' terminus of the target locus. In some embodiments, the target modification site is located at the 3' end of the target locus. In some embodiments, the target modification site is located within an intron or an exon of the target locus.

In some embodiments, the present disclosure provides a polynucleotide encoding a gRNA. In some embodiments, a gRNA-encoding nucleic acid is comprised in an expression vector, e.g., a recombinant expression vector. In some embodiments, the present disclosure provides a polynucleotide encoding a site-directed modifying polypeptide. In some embodiments, the polynucleotide encoding a site-directed modifying polypeptide is comprised in an expression vector, e.g., a recombinant expression vector.

a. Cas proteins

In some embodiments, the site-directed modifying polypeptide is a Cas protein. Cas molecules of a variety of species can be used in the methods and compositions described herein, including Cas molecules derived from *S. pyogenes*, *S. aureus*, *N. meningitidis*, *S. thermophiles*, *Acidovorax avenae*, *Actinobacillus pleuropneumoniae*, *Actinobacillus succinogenes*, *Actinobacillus suis*, *Actinomyces* sp., *Cytophila denitrificans*, *Aminomonas paucivorans*, *Bacillus cereus*, *Bacillus smithii*, *Bacillus thuringiensis*, *Bacteroides* sp., *Blastopirellula marina*, *Bradyrhizobium* sp., *Brevibacillus laterosporus*, *Campylobacter coli*, *Campylobacter jejuni*, *Campylobacter lari*, *Candidatus puniceispirillum*, *Clostridium cellulolyticum*, *Clostridium perfringens*, *Corynebacterium accolens*, *Corynebacterium diphtheria*, *Corynebacterium matruchotii*, *Dinoroseobacter shibae*, *Eubacterium dolichum*,

Gammaproteobacterium, *Gluconacetobacter diazotrophicus*, *Haemophilus parainfluenzae*, *Haemophilus sputomm*, *Helicobacter canadensis*, *Helicobacter cinaedi*, *Helicobacter mustelae*, *Ilyobacter polytropus*, *Kingella kingae*, *Lactobacillus crispatus*, *Listeria ivanovii*, *Listeria monocytogenes*, *Listeriaceae bacterium*,
 5 *Methylocystis* sp., *Methylosinus trichosporium*, *Mobiluncus mulieris*, *Neisseria bacilliformis*, *Neisseria cinerea*, *Neisseria flavescens*, *Neisseria lactamica*, *Neisseria meningitidis*, *Neisseria* sp., *Neisseria wadsworthii*, *Nitrosomonas* sp., *Parvibaculum lavamentivorans*, *Pasteurella multocida*, *Phascolarctobacterium succinatutens*,
Ralstonia syzygii, *Rhodopseudomonas palustris*, *Rhodovulum* sp., *Simonsiella*
 10 *muelleri*, *Sphingomonas* sp., *Sporolactobacillus vineae*, *Staphylococcus aureus*, *Staphylococcus lugdunensis*, *Streptococcus* sp., *Subdoligranulum* sp., *Tistrella mobilis*, *Treponema* sp., or *Verminephrobacter eiseniae*.

In some embodiments, the Cas protein is a Cas9 protein or a Cas9 ortholog and is selected from the group consisting of SpCas9, SpCas9-HF1, SpCas9-HF2,
 15 SpCas9-HF3, SpCas9-HF4, SaCas9, FnCpf, FnCas9, eSpCas9, and NmeCas9. In some embodiments, the endonuclease is selected from the group consisting of C2C1, C2C3, Cpf1 (also referred to as Cas12a), CasI, CasIB, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas9 (also known as CsnI and Csx12), Cas10, CsyI, Csy2, Csy3, CseI, Cse2, CseI, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6,
 20 CmrI, Cmr3, Cmr4, Cmr5, Cmr6, CsbI, Csb2, Csb3, CsxI7, CsxI4, Csx10, Csx16, CsaX, Csx3, CsxI, CsxI5, CsfI, Csf2, Csf3, and Csf4. Additional Cas9 orthologs are described in International PCT Publication No. WO 2015/071474.

In some embodiments, the Cas9 protein is a naturally-occurring Cas9 protein. Exemplary naturally occurring Cas9 molecules are described in Chylinski *et al.*, RNA
 25 Biology 2013 10:5, 727-737. Such Cas9 molecules include Cas9 molecules of a cluster 1 bacterial family, cluster 2 bacterial family, cluster 3 bacterial family, cluster 4 bacterial family, cluster 5 bacterial family, cluster 6 bacterial family, a cluster 7 bacterial family, a cluster 8 bacterial family, a cluster 9 bacterial family, a cluster 10 bacterial family, a cluster 11 bacterial family, a cluster 12 bacterial family, a cluster
 30 13 bacterial family, a cluster 14 bacterial family, a cluster 15 bacterial family, a cluster 16 bacterial family, a cluster 17 bacterial family, a cluster 18 bacterial family, a cluster 19 bacterial family, a cluster 20 bacterial family, a cluster 21 bacterial family, a cluster 22 bacterial family, a cluster 23 bacterial family, a cluster 24

bacterial family, a cluster 25 bacterial family, a cluster 26 bacterial family, a cluster 27 bacterial family, a cluster 28 bacterial family, a cluster 29 bacterial family, a cluster 30 bacterial family, a cluster 31 bacterial family, a cluster 32 bacterial family, a cluster 33 bacterial family, a cluster 34 bacterial family, a cluster 35 bacterial family, a cluster 36 bacterial family, a cluster 37 bacterial family, a cluster 38 bacterial family, a cluster 39 bacterial family, a cluster 40 bacterial family, a cluster 41 bacterial family, a cluster 42 bacterial family, a cluster 43 bacterial family, a cluster 44 bacterial family, a cluster 45 bacterial family, a cluster 46 bacterial family, a cluster 47 bacterial family, a cluster 48 bacterial family, a cluster 49 bacterial family, a cluster 50 bacterial family, a cluster 51 bacterial family, a cluster 52 bacterial family, a cluster 53 bacterial family, a cluster 54 bacterial family, a cluster 55 bacterial family, a cluster 56 bacterial family, a cluster 57 bacterial family, a cluster 58 bacterial family, a cluster 59 bacterial family, a cluster 60 bacterial family, a cluster 61 bacterial family, a cluster 62 bacterial family, a cluster 63 bacterial family, a cluster 64 bacterial family, a cluster 65 bacterial family, a cluster 66 bacterial family, a cluster 67 bacterial family, a cluster 68 bacterial family, a cluster 69 bacterial family, a cluster 70 bacterial family, a cluster 71 bacterial family, a cluster 72 bacterial family, a cluster 73 bacterial family, a cluster 74 bacterial family, a cluster 75 bacterial family, a cluster 76 bacterial family, a cluster 77 bacterial family, or a cluster 78 bacterial family.

In some embodiments, a Cas9 protein comprises an amino acid sequence having at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a Cas9 amino acid sequence described in Chylinski *et al.*, RNA Biology 2013 10:5, 727-737; Hou *et al.*, PNAS Early Edition 2013, 1-6).

In some embodiments, a Cas polypeptide comprises one or more of the following activities:

- a) a nickase activity, *i.e.*, the ability to cleave a single strand, *e.g.*, the non-complementary strand or the complementary strand, of a nucleic acid molecule;
- b) a double stranded nuclease activity, *i.e.*, the ability to cleave both strands of a double stranded nucleic acid and create a double stranded break, which in an embodiment is the presence of two nickase activities;

- c) an endonuclease activity;
- d) an exonuclease activity; and/or
- e) a helicase activity, *i.e.*, the ability to unwind the helical structure of a double stranded nucleic acid.

5 In some embodiments, the Cas9 is a wildtype (WT) Cas9 protein or ortholog. WT Cas9 comprises two catalytically active domains (HNH and RuvC). Binding of WT Cas9 to DNA based on gRNA specificity results in double-stranded DNA breaks that can be repaired by non-homologous end joining (NHEJ) or homology-directed repair (HDR). In some embodiments, Cas9 is fused to heterologous proteins that

10 recruit DNA-damage signaling proteins, exonucleases, or phosphatases to further increase the likelihood or the rate of repair of the target sequence by one repair mechanism or another. In some embodiments, a WT Cas9 is co-expressed with a nucleic acid repair template to facilitate the incorporation of an exogenous nucleic acid sequence by homology-directed repair.

15 In some embodiments, different Cas9 proteins (*i.e.*, Cas9 proteins from various species) may be advantageous to use in the various provided methods in order to capitalize on various enzymatic characteristics of the different Cas9 proteins (*e.g.*, for different PAM sequence preferences; for increased or decreased enzymatic activity; for an increased or decreased level of cellular toxicity; to change the balance

20 between NHEJ, homology-directed repair, single strand breaks, double strand breaks, etc.).

In some embodiments, the Cas protein is a Cas9 protein derived from *S. pyogenes* and recognizes the PAM sequence motif NGG, NAG, NGA (Mali et al, Science 2013; 339(6121): 823-826). In some embodiments, the Cas protein is a

25 Cas9 protein derived from *S. thermophiles* and recognizes the PAM sequence motif NGGNG and/or NNAGAAW (W = A or T) (See, *e.g.*, Horvath et al, Science, 2010; 327(5962): 167-170, and Deveau et al, J BACTERIOL 2008; 190(4): 1390-1400). In some embodiments, the Cas protein is a Cas9 protein derived from *S. mutans* and recognizes the PAM sequence motif NGG and/or NAAR (R = A or G) (See, *e.g.*,

30 Deveau et al, J BACTERIOL 2008; 190(4): 1390-1400). In some embodiments, the Cas protein is a Cas9 protein derived from *S. aureus* and recognizes the PAM sequence motif NNGRR (R = A or G). In some embodiments, the Cas protein is a Cas9 protein derived from *S. aureus* and recognizes the PAM sequence motif N

GRRT (R = A or G). In some embodiments, the Cas protein is a Cas9 protein derived from *S. aureus* and recognizes the PAM sequence motif N GRRV (R = A or G). In some embodiments, the Cas protein is a Cas9 protein derived from *N. meningitidis* and recognizes the PAM sequence motif N GATT or N GCTT (R = A or G, V = A, G or C) (See, e.g., Hou et al., PNAS 2013, 1-6). In the aforementioned embodiments, N can be any nucleotide residue, e.g., any of A, G, C or T.

In some embodiments, a polynucleotide encoding a Cas protein is provided. In some embodiments, the polynucleotide encodes a Cas protein that is at least 90% identical to a Cas protein described in International PCT Publication No. WO 2015/071474 or Chylinski *et al.*, RNA Biology 2013 10:5, 727-737. In some embodiments, the polynucleotide encodes a Cas protein that is at least 95%, 96%, 97%, 98%, or 99% identical to a Cas protein described in International PCT Publication No. WO 2015/071474 or Chylinski *et al.*, RNA Biology 2013 10:5, 727-737. In some embodiments, the polynucleotide encodes a Cas protein that is 100% identical to a Cas protein described in International PCT Publication No. WO 2015/071474 or Chylinski *et al.*, RNA Biology 2013 10:5, 727-737.

i. Cas Mutants

In some embodiments, the Cas polypeptides are engineered to alter one or more properties of the Cas polypeptide. For example, in some embodiments, the Cas polypeptide comprises altered enzymatic properties, e.g., altered nuclease activity, (as compared with a naturally occurring or other reference Cas molecule) or altered helicase activity. In some embodiments, an engineered Cas polypeptide can have an alteration that alters its size, e.g., a deletion of amino acid sequence that reduces its size without significant effect on another property of the Cas polypeptide. In some embodiments, an engineered Cas polypeptide comprises an alteration that affects PAM recognition. For example, an engineered Cas polypeptide can be altered to recognize a PAM sequence other than the PAM sequence recognized by the corresponding wild-type Cas protein.

Cas polypeptides with desired properties can be made in a number of ways, including alteration of a naturally occurring Cas polypeptide or parental Cas polypeptide, to provide a mutant or altered Cas polypeptide having a desired property. For example, one or more mutations can be introduced into the sequence of a parental Cas polypeptide (e.g., a naturally occurring or engineered Cas

polypeptide). Such mutations and differences may comprise substitutions (*e.g.*, conservative substitutions or substitutions of non-essential amino acids); insertions; or deletions. In some embodiments, a mutant Cas polypeptide comprises one or more mutations (*e.g.*, at least 1, 2, 3, 4, 5, 10, 15, 20, 30, 40 or 50 mutations) relative to a parental Cas polypeptide.

In an embodiment, a mutant Cas polypeptide comprises a cleavage property that differs from a naturally occurring Cas polypeptide. In some embodiments, the Cas is a Cas nickase mutant. Cas nickase mutants comprise only one catalytically active domain (either the HNH domain or the RuvC domain). The Cas nickase mutants retain DNA binding based on gRNA specificity, but are capable of cutting only one strand of DNA resulting in a single-strand break (*e.g.* a “nick”). In some embodiments, two complementary Cas nickase mutants (*e.g.*, one Cas nickase mutant with an inactivated RuvC domain, and one Cas nickase mutant with an inactivated HNH domain) are expressed in the same cell with two gRNAs corresponding to two respective target sequences; one target sequence on the sense DNA strand, and one on the antisense DNA strand. This dual-nickase system results in staggered double stranded breaks and can increase target specificity, as it is unlikely that two off-target nicks will be generated close enough to generate a double stranded break. In some embodiments, a Cas nickase mutant is co-expressed with a nucleic acid repair template to facilitate the incorporation of an exogenous nucleic acid sequence by homology-directed repair.

In some embodiments, the Cas polypeptides described herein can be engineered to alter the PAM specificity of the Cas polypeptide. In some embodiments, a mutant Cas polypeptide has a PAM specificity that is different from the PAM specificity of the parental Cas polypeptide. For example, a naturally occurring Cas protein can be modified to alter the PAM sequence that the mutant Cas polypeptide recognizes to decrease off target sites, improve specificity, or eliminate a PAM recognition requirement. In some embodiments, a Cas protein can be modified to increase the length of the PAM recognition sequence. In some embodiments, the length of the PAM recognition sequence is at least 4, 5, 6, 7, 8, 9, 10 or 15 amino acids in length. Cas polypeptides that recognize different PAM sequences and/or have reduced off-target activity can be generated using directed evolution. Exemplary methods and systems that can be used for directed evolution

of Cas polypeptides are described, e.g., in Esvelt *et al.* Nature 2011, 472(7344): 499-503.

Exemplary Cas mutants are described in International PCT Publication No. WO 2015/161276, which is incorporated herein by reference in its entirety.

5 2. gRNAs

The present disclosure provides guide RNAs (gRNAs) that direct a site-directed modifying polypeptide to a specific target DNA sequence. A gRNA comprises a DNA-targeting segment and protein-binding segment. The DNA-targeting segment of a gRNA comprises a nucleotide sequence that is
10 complementary to a sequence in the target DNA sequence. As such, the DNA-targeting segment of a gRNA interacts with a target DNA in a sequence-specific manner via hybridization (*i.e.*, base pairing), and the nucleotide sequence of the DNA-targeting segment determines the location within the target DNA that the gRNA will bind. The DNA-targeting segment of a gRNA can be modified (e.g., by genetic
15 engineering) to hybridize to any desired sequence within a target DNA sequence.

The protein-binding segment of a guide RNA interacts with a site-directed modifying polypeptide (e.g. a Cas9 protein) to form a complex. The guide RNA guides the bound polypeptide to a specific nucleotide sequence within target DNA via the above-described DNA-targeting segment. The protein-binding segment of a
20 guide RNA comprises two stretches of nucleotides that are complementary to one another and which form a double stranded RNA duplex.

In some embodiments, a gRNA comprises two separate RNA molecules. In such embodiments, each of the two RNA molecules comprises a stretch of nucleotides that are complementary to one another such that the complementary
25 nucleotides of the two RNA molecules hybridize to form the double-stranded RNA duplex of the protein-binding segment. In some embodiments, a gRNA comprises a single RNA molecule (sgRNA).

The specificity of a gRNA for a target loci is mediated by the sequence of the DNA-binding segment, which comprises about 20 nucleotides that are
30 complementary to a target DNA sequence within the target locus. In some embodiments, the corresponding target DNA sequence is approximately 20 nucleotides in length. In some embodiments, the DNA-binding segments of the gRNA sequences of the present invention are at least 90% complementary to a

target DNA sequence within a target locus. In some embodiments, the DNA-binding segments of the gRNA sequences of the present invention are at least 95%, 96%, 97%, 98%, or 99% complementary to a target DNA sequence within a target locus. In some embodiments, the DNA-binding segments of the gRNA sequences of the present invention are 100% complementary to a target DNA sequence within a target locus.

In some embodiments, the DNA-binding segments of the gRNA sequences bind to a target DNA sequence that is at least 90% identical to a target DNA sequence within a target locus of a target gene selected those listed in Table 1. In some embodiments, the DNA-binding segments of the gRNA sequences bind to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a target DNA sequence within a target locus of a target gene selected those listed in Table 1. In some embodiments, the DNA-binding segments of the gRNA sequences bind to a target DNA sequence that is 100% identical to a target DNA sequence within a target locus of a target gene selected those listed in Table 1.

In some embodiments, the DNA-binding segments of the gRNA sequences described herein are designed to minimize off-target binding using algorithms known in the art (e.g., Cas-OFF finder) to identify target sequences that are unique to a particular target locus or target gene.

In some embodiments, the gRNAs described herein can comprise one or more modified nucleosides or nucleotides which introduce stability toward nucleases. In such embodiments, these modified gRNAs may elicit a reduced innate immune as compared to a non-modified gRNA. The term "innate immune response" includes a cellular response to exogenous nucleic acids, including single stranded nucleic acids, generally of viral or bacterial origin, which involves the induction of cytokine expression and release, particularly the interferons, and cell death.

In some embodiments, the gRNAs described herein are modified at or near the 5' end (e.g., within 1-10, 1-5, or 1-2 nucleotides of their 5' end). In some embodiments, the 5' end of a gRNA is modified by the inclusion of a eukaryotic mRNA cap structure or cap analog (e.g., a G(5')ppp(5')G cap analog, a m7G(5')ppp(5')G cap analog, or a 3'-O-Me-m7G(5')ppp(5')G anti reverse cap analog (ARCA)). In some embodiments, an *in vitro* transcribed gRNA is modified by treatment with a phosphatase (e.g., calf intestinal alkaline phosphatase) to remove

the 5' triphosphate group. In some embodiments, a gRNA comprises a modification at or near its 3' end (e.g., within 1-10, 1-5, or 1-2 nucleotides of its 3' end). For example, in some embodiments, the 3' end of a gRNA is modified by the addition of one or more (e.g., 25-200) adenine (A) residues.

5 In some embodiments, modified nucleosides and modified nucleotides can be present in a gRNA, but also may be present in other gene-regulating systems, e.g., mRNA, RNAi, or siRNA- based systems. In some embodiments, modified nucleosides and nucleotides can include one or more of:

- 10 a) alteration, e.g., replacement, of one or both of the non-linking phosphate oxygens and/or of one or more of the linking phosphate oxygens in the phosphodiester backbone linkage;
- b) alteration, e.g., replacement, of a constituent of the ribose sugar, e.g., of the 2' hydroxyl on the ribose sugar;
- 15 c) wholesale replacement of the phosphate moiety with "dephospho" linkers;
- d) modification or replacement of a naturally occurring nucleobase;
- e) replacement or modification of the ribose-phosphate backbone;
- f) modification of the 3' end or 5' end of the oligonucleotide, e.g., removal, modification or replacement of a terminal phosphate group or
- 20 conjugation of a moiety; and
- g) modification of the sugar.

In some embodiments, the modifications listed above can be combined to provide modified nucleosides and nucleotides that can have two, three, four, or more modifications. For example, in some embodiments, a modified nucleoside or

25 nucleotide can have a modified sugar and a modified nucleobase. In some embodiments, every base of a gRNA is modified. In some embodiments, each of the phosphate groups of a gRNA molecule are replaced with phosphorothioate groups.

In some embodiments, a software tool can be used to optimize the choice of gRNA within a user's target sequence, e.g., to minimize total off-target activity across

30 the genome. Off target activity may be other than cleavage. For example, for each possible gRNA choice using *S. pyogenes* Cas9, software tools can identify all potential off-target sequences (preceding either NAG or NGG PAMs) across the genome that contain up to a certain number (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) of

mismatched base-pairs. The cleavage efficiency at each off-target sequence can be predicted, *e.g.*, using an experimentally-derived weighting scheme. Each possible gRNA can then be ranked according to its total predicted off-target cleavage; the top-ranked gRNAs represent those that are likely to have the greatest on-target and the least off-target cleavage. Other functions, *e.g.*, automated reagent design for gRNA vector construction, primer design for the on-target Surveyor assay, and primer design for high-throughput detection and quantification of off-target cleavage via next-generation sequencing, can also be included in the tool.

The invention is further described in the following Examples, which do not limit the scope of the invention described in the claims.

Examples

Example 1

Evaluation of Parkin Transgene Variants

A series of plasmid vectors were generated to evaluate expression of Parkin transgene variants. The expression cassette (**FIG. 1**) contained, in 5' to 3' order, a CMV immediate early (IE) enhancer/promoter and 5' UTR, a Parkin transgene 2A-linked to enhanced green fluorescence protein (eGFP), a 3' UTR, and rabbit globin polyadenylation sequence (polyA). For the Parkin transgene, either the wild-type sequence of human *PRKN* (WT) or one of four codon optimized variants (CO1 to CO4) was tested. The sequences of the wild-type and codon-optimized Parkin transgenes are provided in SEQ ID NOs: 27, 35, 36, 37 and 38.

One consideration in design of codon-optimized CO1 to CO4 was the number of CpG sites. CpG islands are regions of DNA where a cytosine nucleotide is followed by a guanine nucleotide in the linear sequence of bases along its 5' → 3' direction. CpG islands (which are typically defined as a polynucleotide sequence of at least 200 bp, a GC percentage greater than 50%, and an observed-to-expected CpG ratio greater than 60%) are known to be associated with vector immunogenicity. Therefore, it was expected that decreasing the number of CpG sites would improve vector performance by decreasing immunogenicity. The number of CpG sites (5'-C-G-3') in each Parkin codon variant is given in **Table 9**.

Table 9: CpG Sites in Exemplary Parkin Codon Variants

Construct	Number of CpG Islands	SEQ ID NO:
Wild type (WT)	95	27
Construct 1 (CO1)	0	35
Construct 2 (CO2)	0	36
Construct 3 (CO3)	0	37
Construct 4 (CO4)	10	38

SH-SY5Y cells. A human neuroblastoma cell line, SH-SY5Y, was used to evaluate gene expression in neuronal lineage cells. SH-SY5Y cells were cultured on 96-well plates at a seeding density of 10,000 cells per well. After 24 hours, the cells were transfected with 0.10 µg of WT, CO1, CO2, or CO3 plasmid complexed with 4 µL Eugene HD per µg plasmid. Cells were cultured for an additional 48 hours and then assayed for eGFP expression by fluorescence microscopy.

Representative micrographs are shown in **FIGS. 2A-2J**. Controls were untransfected cells as negative control (**FIG. 2A**) and a known plasmid as a positive control (**FIG. 2B**). Expression cassettes were tested in duplicate: WT (**FIGS. 2C-2D**), CO1 (**FIGS. 2E-2F**), CO2 (**FIGS. 2G-2H**), and CO3 (**FIGS. 2I-2J**). The percentage of GFP+ cell and the fluorescence intensity of GFP+ cells are plotted in **FIG. 2H** and **FIG. 2K**, respectively. Cell lysate was collected seven days after transfection and assayed by enzyme-linked immunosorbent assay (ELISA) for Parkin, as shown in **FIG. 3**. These experiments demonstrated that CO1 to CO3 did not increase Parkin expression and indeed decreased the percentage of cells that expressed Parkin and the overall level of Parkin expression.

Codon variant CO4 was then tested against WT and CO1. Fluorescence micrographs of SH-SY5Y cells for untransfected negative control (**FIG. 4A**), WT (**FIG. 4B**), CO1 (**FIG. 4C**), or CO4 (**FIG. 4D**) are shown, and the results were quantitated for percent GFP+ (**FIG. 4E**) and intensity (**FIG. 4F**). Cell lysates were assayed for Parkin expression by ELISA (**FIG 5**). In SH-SY5Y cells, CO4 expression was similar to WT and CO1.

iPSC-derived, Parkin knockout dopaminergic precursor cells. Human induced pluripotent stem cell (iPSC)-derived, Parkin knockout dopaminergic

precursor cells were cultured in 96-well plates at a seeding density of 10,000 cells per well. After 7 days, the cells were transfected with 0.15 µg WT, CO1, and CO4 plasmid complexed with 4 µL ViaFect per µg plasmid). Transgene expression was evaluated 7 days after transfection in untransfected wells (**FIG. 6A**), WT (**FIG. 6B**), CO1 (**FIG. 6C**), or CO4 (**FIG. 6D**) wells. As shown in **FIG. 6E**, the three transgenes (WT, CO1, and CO4) resulted in similar percentages of GFP+ cells, but, surprisingly, CO4 caused a 20% increase in GFP intensity compared to WT.

Example 2

Selection of Cassette for Expression of CO4 Parkin Transgene

To evaluate other regulatory elements, a variety of AAV expression cassettes were constructed in the form of transfer plasmids for use in a helper-free AAV packaging system. The AAV expression cassettes (**FIG. 7**) contained, in 5' to 3' order, the 5' ITR of AAV2, the CMV enhancer (Enh) or no enhancer, a promoter selected from **Table 5**, a 5' untranslated region (UTR) selected from **Table 6**, the Parkin transgene variant CO4 (SEQ ID NO: 38), a 3' untranslated region selected from **Table 7**, a polyadenylation sequence (polyA) selected from **Table 8**, and the 3' ITR of AAV2. A diagram of the cassette and various elements is provided in **FIG. 8**. For detection of transgene expression in *in vitro* testing, a polynucleotide sequence (SEQ ID NO: 80) encoding an N-terminal FLAG/HA-tag (SEQ ID NO: 81) was inserted after the start codon on the Parkin transgene in each of the sequences listed in **Table 10**.

SH-SY5Y cells. SH-SY5Y cells were cultured on 24-well plates at a seeding density of 50,000 cells per well. After 24 hours, the cells were transfected with 0.75 µg of plasmid complexed with 4 µL Fugene6 per µg plasmid. Cells were cultured for an additional 48 hours, and Parkin expression was assayed by performing an ELISA on cell lysates with an anti-Parkin primary antibody. Results for each construct are shown in **Table 10** and graphed in **FIG. 9**. Notably, protein expression was lower with the neuro-specific promoters (Syn and CaMKIIa).

Table 10. Expression of Parkin in SH-SY5Y Cells (Average of Replicates)

No.	Promoter/5'UTR/3'UTR/polyA	Expression cassette SEQ ID NO:	pg Parkin per ug total protein

1	HuBA/WPRE(x)/pAGlobin-Oc	39	931
2	CMV/TPL-eMLP/WPRE(r)/pAGlobin-Oc	40	207
3	Syn/WPRE(r)/3'UTR(globin)/pAGH-Bt	41	74
4	CBA/pAGH-Bt	42	200
5	EF1 α /pAGlobin-Oc	43	221
6	HuBA/R2V17/pAGH-Bt	44	234
7	Syn/WPRE(x)/3'UTR(globin)/pAGH-Hs	45	58
8	CaMKIIa/WPRE(r)/pAGH-Hs	46	68
9	CMV/TPL-eMLP/WPRE(r)/pAGH-Hs	47	290
10	HuBA/pAGH-Hs	48	336
11	CMV/TPL-eMPL/R2V17/3'UTR(globin)/pAGH-Bt	49	365
12	EF1 α /WPRE(r)/pAGH-Bt	50	251
13	Syn/R2V17/pAGlobin-Oc	51	40
14	CaMKIIa/R2V17/pAGlobin-Oc	52	38
15	CBA/WPRE(x)/3'UTR(globin)/pAGH-Hs	53	722
16	CBA/ 3'UTR(globin)/pAGlobin-Oc	54	533
17	CaMKIIa/R2V17/pAGH-Bt	55	46
18	EF1a/R2V17/3'UTR(globin)/pAGH-Hs	56	667
19	CMV/R2V17/3'UTR(globin)/pAGH-Hs	57	228
20	CMV/pAGH-Hs	58	191

iPSC-derived, Parkin knockout dopaminergic precursor cells. iPSC-

derived, Parkin knockout dopaminergic precursors cells were cultured in 96-well plates at a seeding density of 10,000 cells per well. After 15 days, the cells were transfected with 0.15 μ g plasmid complexed with 4 μ L ViaFect per μ g plasmid).

Parkin expression was assayed 2 days after by performing an ELISA on cell lysates with an anti-Parkin primary antibody. Results for each construct are shown in **Table 11** and graphed in **FIG. 10** (three replicates shown for each construct).

Table 11. Expression of Parkin in iPSC-derived Cells (Average of Replicates)

No.	Promoter/5'UTR/3'UTR/polyA	Expression cassette	Average pg Parkin per total ug protein
-----	----------------------------	---------------------	--

		SEQ ID NO:	
1	HuBA/WPRE(x)/pAGlobin-Oc	39	58
7	Syn/WPRE(x)/3'UTR(globin)/pAGH-Hs	45	3.2
8	CaMKIIa/WPRE(x)/pAGH-Hs	46	2.1
9	CMV/TPL-eMLP/WPRE(r)/pAGH-Hs	47	34.7
11	CMV/TPL- eMLP/R2V17/3'UTR(globin)/pAGH-Bt	49	35.5
13	Syn/ R2V17/pAGlobin-Oc	51	2.9
14	CaMKIIa/ R2V17/pAGlobin-Oc	52	1.1
15	CBA/WPRE(x)-3'UTR(globin)/pAGH- Hs	53	70.9
18	EF1 α /R2V17/3'UTR(globin) /pAGH-Hs	56	24.6

For fluorescence microscopy, transfected cells were fixed 8 days after transfection, stained, and imaged in brightfield (**FIG. 11A** and **FIG. 12A**), or by immunofluorescence for Parkin (**FIG. 11B** and **FIG. 12B**), neuronal marker NeuN (**FIG. 11C** and **FIG. 12C**), or astrocyte marker GFAP (**FIG. 11D** and **FIG. 12D**). **FIGS. 12A-12D** show enlarged images of **FIGS. 11A-11D**.

Example 3

In Vivo and Clinical Testing of Parkin AAV Gene Therapy

AAV vectors are generated by packaging of AAV vector genomes from constructs 1 to 20 in Example 2, in particular constructs 1, 7, 11, and 15 from Table 11,. Testing is performed in rats and one or more constructs are selected for testing in non-human primates (NHPs). Dose finding studies are performed and a starting dose for clinical trials is determined by measurement of protein expression and toxicity observed.

Clinical trials are performed in subjects identified as having recessive mutations in the *PRKN* gene (also known as *PRK2*). Protein expression and observed toxicity is used to determine an optimal dose. Efficacy is evaluated using improvement on the Unified Parkinson's Disease Rating Scale (UPDRS).

Example 4

In Vitro Testing

Recombinant cassettes are tested in a human neuroblastoma cell line (SH-SY5Y). (See Jiang et al. Extracellular dopamine induces the oxidative toxicity of SH-SY5Y cells. Synapse. 2008 Nov;62(11):797-803. doi: 10.1002/syn.20554.) The genes of interest tested are a Parkinson protein 2, E3 ubiquitin protein ligase (PARK2) gene, a PTEN-induced putative kinase 1 (PINK1) gene, a protein deglycase DJ-1 (DJ-1) gene, a Leucine Rich Repeat Kinase 2 (LRRK2) gene, an alpha-synuclein (SCNA) gene, a Proto-oncogene c-Rel (c-Rel) gene, a Ubiquitin-like modifier-activating enzyme (ATG7) gene, Synaptic vesicular amine transporter (VMAT2) gene, or glucocerebrosidase (GBA) gene.

Each cell line is treated with a recombinant gene therapy vector for each gene of interest. Expression of the gene of interest is increased. Function of the gene of interest is improved. In some cases, improved mitophagy, reduced cellular toxicity, and/or reduced oxidative stress is observed.

Example 5

In Vivo Testing

A rodent or non-human primate having a combination of one or more known mutations in a gene of interest is treated with a recombinant gene therapy vector for that gene of interest. The genes of interest tested are a Parkinson protein 2, E3 ubiquitin protein ligase (PARK2) gene, a PTEN-induced putative kinase 1 (PINK1) gene, a protein deglycase DJ-1 (DJ-1) gene, a Leucine Rich Repeat Kinase 2 (LRRK2) gene, an alpha-synuclein (SCNA) gene, a Proto-oncogene c-Rel (c-Rel) gene, an Ubiquitin-like modifier-activating enzyme (ATG7) gene, Synaptic vesicular amine transporter (VMAT2) gene, or glucocerebrosidase (GBA) gene

The test subject has one or more of the following features: Loss of dopamine (DA), $\geq 20\%$ nigral cell loss, dyskinesia, Lewy bodies, indications of mitochondrial dysfunction, ROS, inflammation, other locomotor behavioral deficits, and neurodegenerative symptoms.

Animals in which the methods of the present disclosure are tested include non-diseased and diseased animals and mutation-carrier and non-carrier animals.

Animal models and illustrative motor behavior readouts in which the methods of the present disclosure are tested are provided in **Table 12**.

Table 12

	Animal model	Motor behavior
Toxin-based	MPTP Mice	Reduced locomotion, bradykinesia
	MPTP Monkeys	Reduced locomotion, altered behavior, tremor and rigidity
	6-OHDA rat	Reduced locomotion, altered behavior
	Rotenone	Reduced locomotion
	Paraquat/maneb	Reduced locomotion
	MET/MDMA	Reduced locomotion
Genetic mutations	α -Synuclein	Altered behavior, reduced or increased motor activity
	LRKK2	Mild behavioral alteration
	PINK1	No obvious alterations or reduced locomotion
	PARKIN	No obvious alterations or reduced locomotion
	PRKN Exon3 mutated mice	No obvious alterations or reduced locomotion
	PRKN knockout rats	No obvious alterations or reduced locomotion
	DJ-1	Decreased locomotor activity
	ATP13A2	Late onset sensorimotor deficits
	SHH	Reduced locomotion
	Nurr1	Reduced locomotion
Others	Engrailed 1	Reduced locomotion
	Pitx3	Reduced locomotion
	C-Rel-NFKB	Gait, bradykinesia, rigidity
	MitoPark	Reduced locomotion, tremor, and rigidity
	Atg7	Late onset locomotor deficits

	VMAT2	Reduced locomotion and altered behavior
--	-------	---

The test subject exhibits improvement in any of the motor behavior readouts or one or more of the following features: Gain of DA in the striatum, nigral cell gain, reduced lewy bodies, improved behavioral or other locomotor deficits, improved mitochondrial function, reduced inflammatory markers, improved life span.

Example 6

Clinical Testing

The diagnosis of parkin type of early-onset Parkinson disease is considered primarily in individuals with early-onset parkinsonism (age <40 years), particularly if autosomal recessive inheritance is suspected. PRKN (formerly termed PARK2), the gene encoding the protein parkin, is the primary gene in which pathogenic variants are known to cause parkin type of early-onset Parkinson disease. The diagnosis of parkin type of early-onset Parkinson disease can only be confirmed when pathogenic variants are identified on both alleles of PRKN (i.e., the individual is homozygous for the same pathogenic allele or compound heterozygous for two different pathogenic alleles). The variant detection frequency varies by family history and age of onset.

Other mutants related to Parkinson's Disease are provided in **Table 13**.

Table 13

Gene	Human Mutations	Mode of inheritance	Rat model mutations
α -Synuclein (PRK 1)	A53T, A53E, A30P, H50Q, E46K, G51D	Dominant	Human E46K expressed via BAC transgenic
LRRK2 (PRK8)	N1437H, R1441C/G/H, Y1699C, G2019S, I2020T	Dominant	Human G2019S expressed via BAC transgenic; human R1441C expressed via BAC transgenic

Parkin (PRK2)	Deletions, Insertions, frameshifts, missense and nonsense mutations in every exon; deletions and duplications of 1 or more exons are most common mutations	Recessive	5-bp deletion in exon 4
PINK1 (PRK6)	Missense and nonsense mutations Q21X, G440E, Q456X, Q1290fxX157, W437X, A271D, G309D, exon deletions or duplications	Recessive	26-bp deletion in exon 4
DJ-1 (PRK7)	L166P, D149A, R98Q, exon deletions or splice-site alterations	Recessive	9-bp deletion and 1-bp insertion in exon 5

Human subjects having one or more mutations associated with Parkinson's Disease are treated with recombinant gene therapy vectors encoding wild-type or functional variants of the mutated gene. The subjects exhibit improvement in any of the motor behavior readouts listed in Table 12, or any of the following features:

5 increased dopamine in the brain, especially the substantia nigra, increased numbers of dopaminergic neurons, increased expression of genes of interesting, including PRKN, or improvement on the Unified Parkinson's Disease Rating Scale (UPDRS).

10 All of the above U.S. patents, U.S. patent application publications, U.S. patent applications, foreign patents, foreign patent applications and non-patent publications referred to in this specification and/or listed in the Application Data Sheet, are incorporated herein by reference, in their entirety.

15 The present invention may be embodied in other specific forms without departing from its structures, methods, or other essential characteristics as broadly described herein and claimed hereinafter. The described embodiments are to be considered in all respects only as illustrative, and not restrictive. The scope of the

invention is, therefore, indicated by the appended claims, rather than by the foregoing description. All changes that come within the meaning and range of equivalency of the claims are to be embraced within their scope.

CLAIMS

1. A recombinant gene therapy vector comprising an expression cassette comprising a transgene encoding an E3 ubiquitin protein ligase (PARK2), a PTEN-induced putative kinase 1 (PINK1), a protein deglycase DJ-1 (DJ-1), a Leucine Rich Repeat Kinase 2 (LRRK2), an alpha-synuclein (SCNA), a Proto-oncogene c-Rel (c-Rel), a Ubiquitin-like modifier-activating enzyme (ATG7), a Synaptic vesicular amine transporter (VMAT2), or a glucocerebrosidase (GBA), wherein the transgene polynucleotide is operably linked to a eukaryotically active promoter sequence.
2. The vector of claim 1, wherein the transgene encodes the PARK2, and the transgene polynucleotide sequence shares at least 95% identity to one of SEQ ID NOs: 35-38.
3. The vector of claim 1 or claim 2, wherein the promoter sequence is selected from Table 5.
4. The vector of any one of claim 1 to 3, wherein the expression cassette further comprises a CMV enhancer.
5. The vector of any one of claims 1 to 4, wherein the expression cassette further comprises a 5' untranslated region (UTR) selected from Table 6.
6. The vector of any one of claims 1 to 6, wherein the expression cassette further comprises a 3' untranslated region selected from Table 7.
7. The vector of any one of claims 1 to 6, wherein the expression cassette further comprises a polyadenylation sequence (polyA) selected from Table 8.
8. The vector of any one of claims 1 to 7, wherein the transgene is codon-optimized.

9. The vector of any one of claim 1 to 8, wherein the expression cassette shares at least 95% sequence identity to any one of SEQ ID NOs: 39-58.
10. The vector of any one of claims 1-9, wherein the expression cassette comprises in 5' to 3' order:
 - HuBA promoter, the transgene, WPRE(x), and pAGlobin-Oc;
 - CMV promoter, TPL-eMLP enhancer, the transgene, WPRE(r), and pAGlobin-Oc;
 - Syn promoter, the transgene, WPRE(r), 3'UTR(globin), and pAGH-Bt;
 - CBA promoter, the transgene, and pAGH-Bt;
 - EF1 α promoter, the transgene, and pAGlobin-Oc;
 - HuBA promoter, the transgene, R2V17, and pAGH-Bt;
 - Syn promoter, the transgene, WPRE(x), 3'UTR(globin), and pAGH-Hs;
 - CaMKIIa promoter, the transgene, WPRE(r), and pAGH-Hs;
 - CMV promoter, TPL-eMLP 5'enhancer, the transgene, WPRE(r), and pAGH-Hs;
 - HuBA promoter, the transgene, and pAGH-Hs;
 - CMV and TPL promoter, eMPL, the transgene, R2V17, 3'UTR(globin), and pAGH-Bt;
 - EF1 α promoter, the transgene, WPRE(r), and pAGH-Bt;
 - Syn promoter, the transgene, R2V17, and pAGlobin-Oc;
 - CaMKIIa promoter, the transgene, R2V17, and pAGlobin-Oc;
 - CBA promoter, the transgene, WPRE(x), 3'UTR(globin), and pAGH-Hs.
 - CBA promoter, the transgene, 3'UTR(globin), and pAGlobin-Oc;
 - CaMKIIa promoter, the transgene, R2V17, and pAGH-Bt;
 - EF1 α promoter, the transgene, R2V17, 3'UTR(globin), and pAGH-Hs;.
 - CMV promoter, the transgene, R2V17, 3'UTR(globin), and pAGH-Hs; or
 - CMV promoter, the transgene, and pAGH-Hs,
 - optionally wherein the transgene encodes PARK2.
11. The vector of any one of claims 1 to 10, wherein the vector is an adeno-associated virus (AAV) vector.

12. The vector of claim 11, wherein the vector comprises two AAV inverted terminal repeats (ITRs) flanking the expression cassette.
13. The vector of claim 11 or 12, wherein the AAV has serotype AAV1, AAV2, AAV5, AAV8, AAV9, AAVrh10, or AAVrh74.
14. The vector of any one of claims 11 to 13, wherein the recombinant gene therapy vector comprises a self-complementary AAV or a single stranded AAV.
15. The vector of any one of claims 11 to 14, wherein the AAV is a wild-type AAV or a modified AAV.
16. The vector of any one of claims 11 to 15, wherein the AAV comprises a capsid protein having at least 95% identity to wild-type VP1, VP2, or VP3 capsid protein.
17. A host cell, comprising the vector of any one of claims 1 to 16.
18. A method of inhibiting degeneration or death of a dopaminergic neuron comprising a mutation in a gene associated with a Parkinson's Disease (PD), comprising:
 - contacting the neuron with the recombinant gene therapy vector of any one of claim 1 to 16;
 - wherein following contact with the recombinant gene therapy vector, the neuron expresses the PARK2, PINK1, DJ-1, LRRK2, SCNA, c-Rel, ATG7, VMAT2, or GBA,
 - optionally wherein the neuron comprises a mutation in a PARK2 gene, and wherein the expression cassette encodes PARK2.
19. The method of claim 18, wherein the neuron expresses a reduced amount of alpha-synuclein and/or comprises a reduced amount of Lewy bodies following contact with the recombinant gene therapy vector.

20. The method of claim 18 or 19, wherein the neuron expresses a reduced amount of monoamine oxidases following contact with the recombinant gene therapy vector.
21. The method of any of claims 18 to 20, wherein the neuron produces and/or releases an increased amount of dopamine following contact with the recombinant gene therapy vector.
22. The method of any of claims 18 to 21, wherein the neuron undergoes increased mitophagy following contact with the recombinant gene therapy vector.
23. The method of any of claims 18 to 22, wherein the neuron expresses a lower amount of monoamine oxidases as compared to an amount of monoamine oxidases expressed in a neuron not contacted with said recombinant gene therapy vector, optionally wherein said lower amount is at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, or at least 80% lower than the amount expressed in the neuron not contacted with said recombinant gene therapy vector.
24. The method of any of claims 18 to 23, wherein the neuron produces and/or releases an increased amount of dopamine as compared to an amount of dopamine produced and/or released by a neuron not contacted with said recombinant gene therapy vector, optionally wherein said increase amount is at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least two-fold, at least three-fold, at least four-fold, at least five-fold, or at least 10-fold greater than the amount produced and/or released by the neuron not contacted with said recombinant gene therapy vector.
25. The method of any of claims 18 to 24, wherein the neuron undergoes an increased amount of autophagy as compared to an amount of autophagy undergone by a neuron not contacted with said recombinant gene therapy vector, optionally wherein the increased amount is at least 5%, at least 10%, at least

20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least two-fold, at least three-fold, at least four-fold, at least five-fold, or at least 10-fold greater than the amount undergone by the neuron not contacted with said recombinant gene therapy vector .

26. The method of any of claims 18 to 25, wherein the neuron is a primary tyrosine hydroxylase positive neuron.
27. The method of any of claims 18 to 26, wherein the neuron was produced from an induced pluripotent stem cell prepared from cells obtained from a subject diagnosed with Parkinson's disease.
28. A method of treating or inhibiting onset of a Parkinson's Disease (PD) in a subject suffering from or at risk of the PD, comprising:
 - administering the vector of any one of claims 1 to 16 to the subject;
 - wherein administration of the recombinant gene therapy vector treats or inhibits onset of the Parkinson's Disease in the subject.
29. The method of claim 28, wherein the PD is an early-onset PD, optionally an early-onset autosomal recessive PD.
30. The method of claim 28 or claim 29, wherein the subject comprises a mutation in a PARK2 gene.
31. The method of any of claims 28 to 30, wherein the PARK2 comprises the amino acid sequence set forth in SEQ ID NO: 1.
32. The method of any of claims 28 to 31, wherein administering step comprises systemic, parenteral, intravenous, cerebral, cerebrospinal, intrathecal, intracisternal, intraputamina, intrahippocampal, intra-striatal, or intra-cerebroventricular administration.

- 113

42. The method of any of claims 28 to 40, wherein the subject is a child.
43. The method of any of claims 28 to 42, wherein the number of dopaminergic neurons in the subject after the administering step is greater than the number of dopaminergic neurons in the subject before the administering step.
44. The method of any of claims 28 to 43, wherein the level of dopamine in the subject after the administering step is greater than the level of dopamine in the subject before the administering step.
45. The method of any of claims 28 to 44, wherein the number of dopaminergic neurons in a subject treated by the method is increased compared to the number of dopaminergic neurons in a subject not so treated.
46. The method of any of claims 28 to 45, wherein the level of dopamine of a subject treated by the method is increased compared to the level of dopamine in a subject not so treated.
47. The method of any one of claims 28 to 46, wherein the level of dopamine in the substantia nigra of a subject treated by method is increased compared to the level of dopamine in the substantia nigra of a subject not so treated.
48. The method of any of claims 28 to 47, wherein the level of PRKN in the subject's CSF after the administering step is greater than the level of PRKN in the subject's CSF before the administering step.
49. The method of any of claims 28 to 48, wherein the Unified Parkinson's Disease Rating Scale (UPDRS) score of the subject before the administering step is improved compared to the UPDRS score of the subject before the administering step.

50. The method of any of claims 28 to 49, wherein the level of PRKN in the CSF of a subject treated by the method is increased compared to the level of PRKN in the CSF of a subject not so treated.
51. The method of any of claims 28 to 59, wherein the UPDRS score of a subject treated by the method is improved compared to the UPDRS score of a subject not so treated.
52. The method of any of claims 28 to 51, wherein the subject's neurons express a reduced amount of alpha-synuclein and/or comprises a reduced amount of Lewy bodies following contact with the recombinant gene therapy vector.
53. A method of inhibiting degeneration or death of a dopaminergic neuron comprising a mutation in a gene associated with a Parkinson's Disease (PD), wherein the mutated gene is: a Parkinson protein 2, E3 ubiquitin protein ligase (PARK2) gene, a PTEN-induced putative kinase 1 (PINK1) gene, a protein deglycase DJ-1 (DJ-1) gene, a Leucine Rich Repeat Kinase 2 (LRRK2) gene, an alpha-synuclein (SCNA) gene, a Proto-oncogene c-Rel (c-Rel) gene, a Ubiquitin-like modifier-activating enzyme (ATG7) gene, Synaptic vesicular amine transporter (VMAT2) gene, or glucocerebrosidase (GBA) gene, comprising:
- contacting the neuron with a recombinant gene therapy vector comprising a polynucleotide encoding a wild-type protein expressed by a wild-type version of the mutated gene, or a functional variant or fragment thereof;
 - wherein following contact with the recombinant gene therapy vector, the neuron expresses the wild-type protein, or functional variant or fragment thereof.
54. The method of claim 53, wherein the wild-type PARK2, PINK1, LRRK2, SCNA, c-Rel, ATG7, VMAT2, or GBA protein comprises the amino acid sequence set forth in SEQ ID NOs: 1-9, respectively.

55. The method of claim 53, wherein the gene is the PARK2 gene, and the wild-type PARK2 protein comprises the amino acid sequence set forth in any of SEQ ID NOs: 10-17.
56. The method of claim 53, wherein the polynucleotide comprises a sequence having at least 70%, 75%, 80%, 85%, 95%, or 99% identity to a PARK2, PINK1, LRRK2, SCNA, c-Rel, ATG7, VMAT2, or GBA polynucleotide sequence set forth in SEQ ID NOs: 18-26, respectively.
57. The method of claim 53, wherein the gene is the PARK2 gene, and the polynucleotide comprises a sequence having at least 70%, 75%, 80%, 85%, 95%, or 99% identity to a PARK2 isoform polynucleotide sequence set forth in any of SEQ ID NOs: 27-34.
58. The method of any of claims 53 to 57, wherein the polynucleotide is codon-optimized.
59. The method of any of claims 53 to 58, wherein the recombinant gene therapy vector is a recombinant adeno-associated virus (AAV).
60. The method of claim 59, wherein the AAV has serotype AAV1, AAV2, AAV5, AAV8, AAV9, AAVrh10, or AAVrh74.
61. The method of claim 59 or claim 60, wherein the recombinant gene therapy vector comprises a self-complementary AAV.
62. The method of any of claims 59 to 61, wherein the recombinant gene therapy vector comprises a single-stranded AAV.
63. The method of any of claims 59 to 62, wherein the AAV is a wild-type AAV or a modified AAV.

64. The method of any of claims 59 to 63, wherein the AAV comprises a capsid protein having at least 95% identity to wild-type VP1, VP2, or VP3 capsid protein.
65. The method of any of claims 53 to 64, wherein the recombinant gene therapy vector comprises a polynucleotide comprising in the following 5' to 3' order:
- a. a eukaryotically active promoter sequence; and
 - b. the sequence encoding the wild-type protein, or functional fragment or variant thereof;
- wherein the sequence encoding the wild-type protein, or functional fragment or variant thereof, is operably linked to the eukaryotically active promoter sequence.
66. The method of any of claim 53 to 65, wherein the recombinant gene therapy vector further comprises one or more of:
- a neuron-specific promoter, optionally selected from the group consisting of hSYN1 (human synapsin), INA (alpha-interneuron), NES (nestin), TH (tyrosine hydroxylase), FOXA2 (Forkhead box A2), CaMKII (calmodulin-dependent protein kinase II), and NSE (neuron-specific enolase) promoters;
 - a ubiquitous promoter selected from the group consisting of CMV, CAG, UBC, PGK, EF1-alpha, GAPDH, SV40, HBV, and chicken beta-actin, human beta-actin promoters;
 - an enhancer;
 - an intron;
 - a poly-A signal;
 - a sequence encoding an 2A peptide; and
 - a WPRE (Woodchuck hepatitis virus posttranscriptional regulatory element).
67. The method of any of claims 53 to 64, wherein the neuron expresses a reduced amount of alpha-synuclein and/or comprises a reduced amount of Lewy bodies following contact with the recombinant gene therapy vector.
68. The method of any of claims 53 to 67, wherein the neuron expresses a reduced amount of monoamine oxidases following contact with the recombinant gene therapy vector.

69. The method of any of claims 53 to 68, wherein the neuron produces and/or releases an increased amount of dopamine following contact with the recombinant gene therapy vector.
70. The method of any of claims 53 to 69, wherein the neuron undergoes increased mitophagy following contact with the recombinant gene therapy vector.
71. The method of any of claims 53 to 70, wherein the neuron expresses a lower amount of monoamine oxidases as compared to an amount of monoamine oxidases expressed in a neuron not contacted with said recombinant gene therapy vector, optionally wherein said lower amount is at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, or at least 80% lower than the amount expressed in the neuron not contacted with said recombinant gene therapy vector.
72. The method of any of claims 53 to 71, wherein the neuron produces and/or releases an increased amount of dopamine as compared to an amount of dopamine produced and/or released by a neuron not contacted with said recombinant gene therapy vector, optionally wherein said increase amount is at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least two-fold, at least three-fold, at least four-fold, at least five-fold, or at least 10-fold greater than the amount produced and/or released by the neuron not contacted with said recombinant gene therapy vector.
73. The method of any of claims 53 to 72, wherein the neuron undergoes an increased amount of autophagy as compared to an amount of autophagy undergone by a neuron not contacted with said recombinant gene therapy vector, optionally wherein the increased amount is at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least two-fold, at least three-fold, at least four-fold, at least

five-fold, or at least 10-fold greater than the amount undergone by the neuron not contacted with said recombinant gene therapy vector .

74. The method of any of claims 53 to 73, wherein the neuron is a primary tyrosine hydroxylase positive neuron.
75. The method of any of claims 53 to 74, wherein the neuron was produced from an induced pluripotent stem cell prepared from cells obtained from a subject diagnosed with Parkinson's disease.
76. A recombinant gene therapy vector comprising a polynucleotide encoding a wild-type Parkinson protein 2, E3 ubiquitin protein ligase (PARK2) gene, a PTEN-induced putative kinase 1 (PINK1) gene, a protein deglycase DJ-1 (DJ-1) gene, a Leucine Rich Repeat Kinase 2 (LRRK2) gene, an alpha-synuclein (SCNA) gene, a Proto-oncogene c-Rel (c-Rel) gene, a Ubiquitin-like modifier-activating enzyme (ATG7) gene, Synaptic vesicular amine transporter (VMAT2) gene, or glucocerebrosidase (GBA) gene, or a functional variant or fragment thereof; wherein the polynucleotide is operatively linked to a eukaryotically active promoter; and wherein a neuron transduced with said recombinant gene therapy vector expresses the wild-type protein, or functional variant or fragment thereof.
77. The recombinant gene therapy vector of claim 76, wherein the wild-type PARK2, PINK1, LRRK2, SCNA, c-Rel, ATG7, VMAT2, or GBA protein comprises the amino acid sequence set forth in SEQ ID NOs: 1-9, respectively.
78. The recombinant gene therapy vector of claim 76, wherein the gene is the PARK2 gene, and the wild-type PARK2 protein comprises the amino acid sequence set forth in any of SEQ ID NOs: 10-17.
79. The recombinant gene therapy vector of claim 76, wherein the polynucleotide comprises a sequence having at least 70%, 75%, 80%, 85%, 95%, or 99% identity to a PARK2, PINK1, LRRK2, SCNA, c-Rel, ATG7, VMAT2, or GBA polynucleotide sequence set forth in SEQ ID NOs: 18-26, respectively.

80. The recombinant gene therapy vector of claim 76, wherein the gene is the PARK2 gene, and the polynucleotide comprises a sequence having at least 70%, 75%, 80%, 85%, 95%, or 99% identity to a PARK2 isoform polynucleotide sequence set forth in any of SEQ ID NOs: 27-34.
81. The recombinant gene therapy vector of claim 76, wherein the polynucleotide is codon-optimized.
82. The recombinant gene therapy vector of any of claims 76 to 81, wherein the recombinant gene therapy vector is a recombinant adeno-associated virus (rAAV).
83. The recombinant gene therapy vector of claim 82, wherein the rAAV has serotype AAV1, AAV2, AAV5, AAV8, AAV9, AAVrh10, or AAVrh74.
84. The recombinant gene therapy vector of any of claims 76 to 83, wherein the recombinant gene therapy vector comprises a self-complementary AAV or a single-stranded AAV.
85. The recombinant gene therapy vector of any of claims 76 to 83, wherein the AAV is a wild-type AAV or a modified AAV.
86. The recombinant gene therapy vector of any of claims 76 to 85, wherein the AAV comprises a capsid protein having at least 95% identity to wild-type VP1, VP2, or VP3 capsid protein.
87. A method of treating or inhibiting onset of a Parkinson's Disease (PD) in a subject suffering from or at risk of the PD, comprising:
administering a recombinant gene therapy vector comprising a polynucleotide sequence encoding a wild-type Parkinson protein 2, E3 ubiquitin protein ligase (PARK2), a PTEN-induced putative kinase 1 (PINK1), a protein deglycase DJ-1 (DJ-1), a Leucine Rich Repeat Kinase 2 (LRRK2),

an alpha-synuclein (SCNA), a Proto-oncogene c-Rel (c-Rel), a Ubiquitin-like modifier-activating enzyme (ATG7), Synaptic vesicular amine transporter (VMAT2), or glucocerebrosidase (GBA) protein, or a functional variant or fragment thereof, to the subject;

wherein administration of the recombinant gene therapy vector treats or inhibits onset of the Parkinson's Disease in the subject.

88. The method of claim 87, wherein the PD is an early-onset PD, optionally an early-onset autosomal recessive PD.
89. The method of claim 87 or claim 88, wherein the subject comprises a mutation in a PARK2 gene, PINK1 gene, LRRK2 gene, SCNA gene, c-Rel gene, ATG7 gene, VMAT2, or GBA gene.
90. The method of any of claims 87 to 89, wherein the PARK2, PINK1, LRRK2, SCNA, c-Rel, ATG7, VMAT2, or GBA protein comprises the amino acid sequence set forth in SEQ ID NOs: 1-9, respectively.
91. The method of any of claims 87 to 89, wherein the sequence encodes the PARK2 gene, and the wild-type PARK2 protein comprises the amino acid sequence set forth in any of SEQ ID NOs: 10-17.
92. The method of any of claims 87 to 89, wherein the polynucleotide comprises a sequence having at least 70%, 75%, 80%, 85%, 95%, or 99% identity to a PARK2, PINK1, LRRK2, SCNA, c-Rel, ATG7, VMAT2, or GBA polynucleotide sequence set forth in SEQ ID NOs: 18-26, respectively.
93. The method of any of claims 87 to 89, wherein the sequence encodes the PARK2 gene, and the polynucleotide comprises a sequence having at least 70%, 75%, 80%, 85%, 95%, or 99% identity to a PARK2 isoform polynucleotide sequence set forth in any of SEQ ID NOs: 27-34.

94. The method of any of claims 87 to 93, wherein the sequence encoding the the PARK2, PINK1, LRRK2, SCNA, c-Rel, ATG7, VMAT2, or GBA protein, or functional variant or fragment thereof is codon-optimized, optionally wherein the sequence comprises any of SEQ ID NOs: 35-38.
95. The method of any of claims 87 to 94, wherein the recombinant gene therapy vector is a recombinant adeno-associated virus (AAV).
96. The method of claim 95, wherein the AAV has serotype AAV1, AAV2, AAV5, AAV8, AAV9, AAVrh10, or AAVrh74.
97. The method of claim 95 or claim 96, wherein the recombinant gene therapy vector comprises a self-complementary AAV or a single-stranded AAV.
98. The method of any of claims 95 to 97, wherein the AAV is a wild-type AAV or a modified AAV.
99. The method of any of claims 95 to 98, wherein the AAV comprises a capsid protein having at least 95% identity to wild-type VP1, VP2, or VP3 capsid protein.
100. The method of any of claims 95 to 99, wherein the recombinant gene therapy vector comprises a polynucleotide comprising in the following 5' to 3' order:
- a. a eukaryotically active promoter sequence; and
 - b. the sequence encoding the wild-type protein, or functional fragment or variant thereof;
- wherein the sequence encoding the wild-type protein, or functional fragment or variant thereof, is operably linked to the eukaryotically active promoter sequence.
101. The method of claim 101, wherein the recombinant gene therapy vector further comprises one or more of:
- a neuron-specific promoter, optionally selected from the group consisting of hSYN1 (human synapsin), INA (alpha-internexin), NES (nestin), TH (tyrosine

hydroxylase), FOXA2 (Forkhead box A2), CaMKII (calmodulin-dependent protein kinase II), and NSE (neuron-specific enolase) promoters;

a ubiquitous promotor selected from the group consisting of CMV, CAG, UBC, PGK, EF1-alpha, GAPDH, SV40, HBV, and chicken beta-actin, human beta-actin promoters;

an enhancer;

an intron;

a poly-A signal;

a sequence encoding an 2A peptide; and

a WPRE (Woodchuck hepatitis virus posttranscriptional regulatory element).

102. The method of claim 101, wherein the recombinant gene therapy vector comprises in 5' to 3' order:

HuBA promoter, the transgene, WPRE(x), and pAGlobin-Oc;

CMV promoter, TPL-eMLP enhancer, the transgene, WPRE(r), and pAGlobin-Oc;

Syn promoter, the transgene, WPRE(r), 3'UTR(globin), and pAGH-Bt;

CBA promoter, the transgene, and pAGH-Bt;

EF1 α promoter, the transgene, and pAGlobin-Oc;

HuBA promoter, the transgene, R2V17, and pAGH-Bt;

Syn promoter, the transgene, WPRE(x), 3'UTR(globin), and pAGH-Hs;

CaMKIIa promoter, the transgene, WPRE(r), and pAGH-Hs;

CMV promoter, TPL-eMLP enhancer, the transgene, WPRE(r), and pAGH-Hs;

HuBA promoter, the transgene, and pAGH-Hs;

CMV and TPL promoter, eMPL, the transgene, R2V17, 3'UTR(globin), and pAGH-Bt;

EF1 α promoter, the transgene, WPRE(r), and pAGH-Bt;

Syn promoter, the transgene, R2V17, and pAGlobin-Oc;

CaMKIIa promoter, the transgene, R2V17, and pAGlobin-Oc;

CBA promoter, the transgene, WPRE(x), 3'UTR(globin), and pAGH-Hs.

CBA promoter, the transgene, 3'UTR(globin), and pAGlobin-Oc;

CaMKIIa promoter, the transgene, R2V17, and pAGH-Bt;

EF1 α promoter, the transgene, R2V17, 3'UTR(globin), and pAGH-Hs;.

CMV promoter, the transgene, R2V17, 3'UTR(globin), and pAGH-Hs; or
CMV promoter, the transgene, and pAGH-Hs,
optionally wherein the transgene encodes PARK2.

103. The method of any of claims 87 to 102, wherein administering step comprises systemic, parenteral, intravenous, cerebral, cerebrospinal, intrathecal, intracisternal, intraputamina, intrahippocampal, intra-striatal, or intra-cerebroventricular administration.
104. The method claim 103, wherein the administering step comprises intravenous, cerebral, cerebrospinal, intrathecal, intracisternal, intraputamina, intrahippocampal, intra-striatal, or intra-cerebroventricular injection.
105. The method of claim 103, wherein the administering step comprises intrathecal injection with Trendelenberg tilting.
106. The method of claim 103, wherein the administering step comprises direct injection into the pars compacta of the substantia nigra of the brain.
107. The method of any of claims 87 to 106, wherein the administering step comprises introducing the recombinant gene therapy vector into the subject's brain or cerebrospinal fluid (CSF).
108. The method of any of claims 87 to 107, wherein 1×10^9 - 1×10^{14} vector genomes per kilogram body mass of the subject (vg/kg) of the gene therapy vector are administered to the subject.
109. The method of any of claims 87 to 108, wherein 1×10^9 - 1×10^{14} vector genomes per kilogram body mass of the subject (vg/kg) of the gene therapy vector are administered to the subject's brain.

110. The method of any of claims 87 to 108, wherein 1×10^9 - 1×10^{14} vector genomes per kilogram body mass of the subject (vg/kg) of the gene therapy vector are administered to the subject's CSF.
111. The method of any of claims 87 to 110, wherein 1×10^7 - 1×10^9 vector genomes per kilogram body mass of the subject (vg/kg) of the gene therapy vector are administered to the subject.
112. The method of any of claims 87 to 111, wherein the subject is an adult.
113. The method of any of claims 87 to 112, wherein the subject is a child.
114. The method of any of claims 87 to 113, wherein the number of dopaminergic neurons in the subject after the administering step is greater than the number of dopaminergic neurons in the subject before the administering step.
115. The method of any of claims 87 to 114, wherein the level of dopamine in the subject after the administering step is greater than the level of dopamine in the subject before the administering step.
116. The method of any of claims 87 to 115, wherein the number of dopaminergic neurons in a subject treated by the method is increased compared to the number of dopaminergic neurons in a subject not so treated.
117. The method of any of claims 87 to 116, wherein the level of dopamine of a subject treated by the method is increased compared to the level of dopamine in a subject not so treated.
118. The method of claim 87 or claim 117, wherein the level of dopamine in the substantia nigra of a subject treated by method is increased compared to the level of dopamine in the substantia nigra of a subject not so treated.

119. The method of any of claims 87 to 118, wherein the level of PRKN in the subject's CSF after the administering step is greater than the level of PRKN in the subject's CSF before the administering step.
120. The method of any of claims 87 to 119, wherein the Unified Parkinson's Disease Rating Scale (UPDRS) score of the subject before the administering step is improved compared to the UPDRS score of the subject before the administering step.
121. The method of any of claims 87 to 120, wherein the level of PRKN in the CSF of a subject treated by the method is increased compared to the level of PRKN in the CSF of a subject not so treated.
122. The method of any of claims 87 to 121, wherein the UPDRS score of a subject treated by the method is improved compared to the UPDRS score of a subject not so treated.
123. The method of any of claims 87 to 112, wherein the subject's neurons express a reduced amount of alpha-synuclein and/or comprises a reduced amount of Lewy bodies following contact with the recombinant gene therapy vector.
124. A method of inhibiting degeneration or death of a dopaminergic neuron having a mutated Parkin (PRKN) gene, comprising contacting the neuron with a gene editing system comprising:
- a Cas protein or a polynucleotide encoding a Cas protein;
 - a guide-RNA (gRNA); and
 - a repair template comprising a functional Parkinson protein 2, E3 ubiquitin protein ligase (PARK2) gene, a PTEN-induced putative kinase 1 (PINK1) gene, a protein deglycase DJ-1 (DJ-1) gene, a Leucine Rich Repeat Kinase 2 (LRRK2) gene, an alpha-synuclein (SCNA) gene, a Proto-oncogene c-Rel (c-Rel) gene, a Ubiquitin-like modifier-activating enzyme (ATG7) gene, Synaptic vesicular amine transporter (VMAT2)

gene, or glucocerebrosidase (GBA) gene, or a functional variant or fragment thereof;

wherein the gene editing system is capable of repairing an endogenous gene in the neuron or inserting a functional gene into the genome of the neuron.

125. The method of claim 124, wherein at least one component of the gene editing system is delivered by recombinant AAV.

126. The method of claim 124, wherein the gene editing system is delivered by recombinant AAV.

127. A gene editing system for a cell comprising:

- a. a Cas protein or a polynucleotide encoding a Cas protein;
- b. a guide-RNA (gRNA); and
- c. a repair template comprising a functional Parkinson protein 2, E3 ubiquitin protein ligase (PARK2) gene, a PTEN-induced putative kinase 1 (PINK1) gene, a protein deglycase DJ-1 (DJ-1) gene, a Leucine Rich Repeat Kinase 2 (LRRK2) gene, an alpha-synuclein (SCNA) gene, a Proto-oncogene c-Rel (c-Rel) gene, a Ubiquitin-like modifier-activating enzyme (ATG7) gene, Synaptic vesicular amine transporter (VMAT2) gene, or glucocerebrosidase (GBA) gene, or a functional variant or fragment thereof;

wherein the gene editing system is capable of repairing an endogenous gene in the cell or inserting a functional gene into the genome of the cell.

128. The gene editing system of claim 127, wherein at least one component of the gene editing system is delivered by recombinant AAV.

129. The gene editing system of claim 127, wherein the gene editing system is delivered by recombinant AAV.

130. The gene editing system of any of claims 127 to 129, wherein the cell is an *ex vivo* neuron.

131. The gene editing system of any of claims 127 to 130, wherein the cell is a cell of a subject.

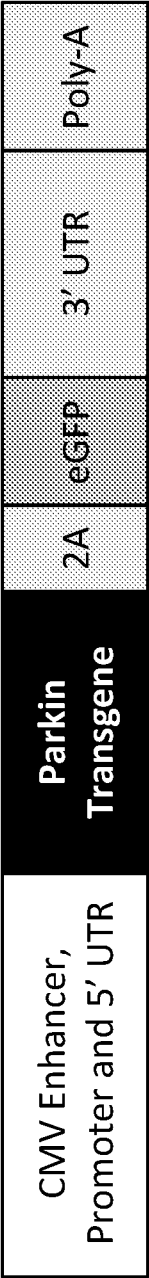


FIG. 1

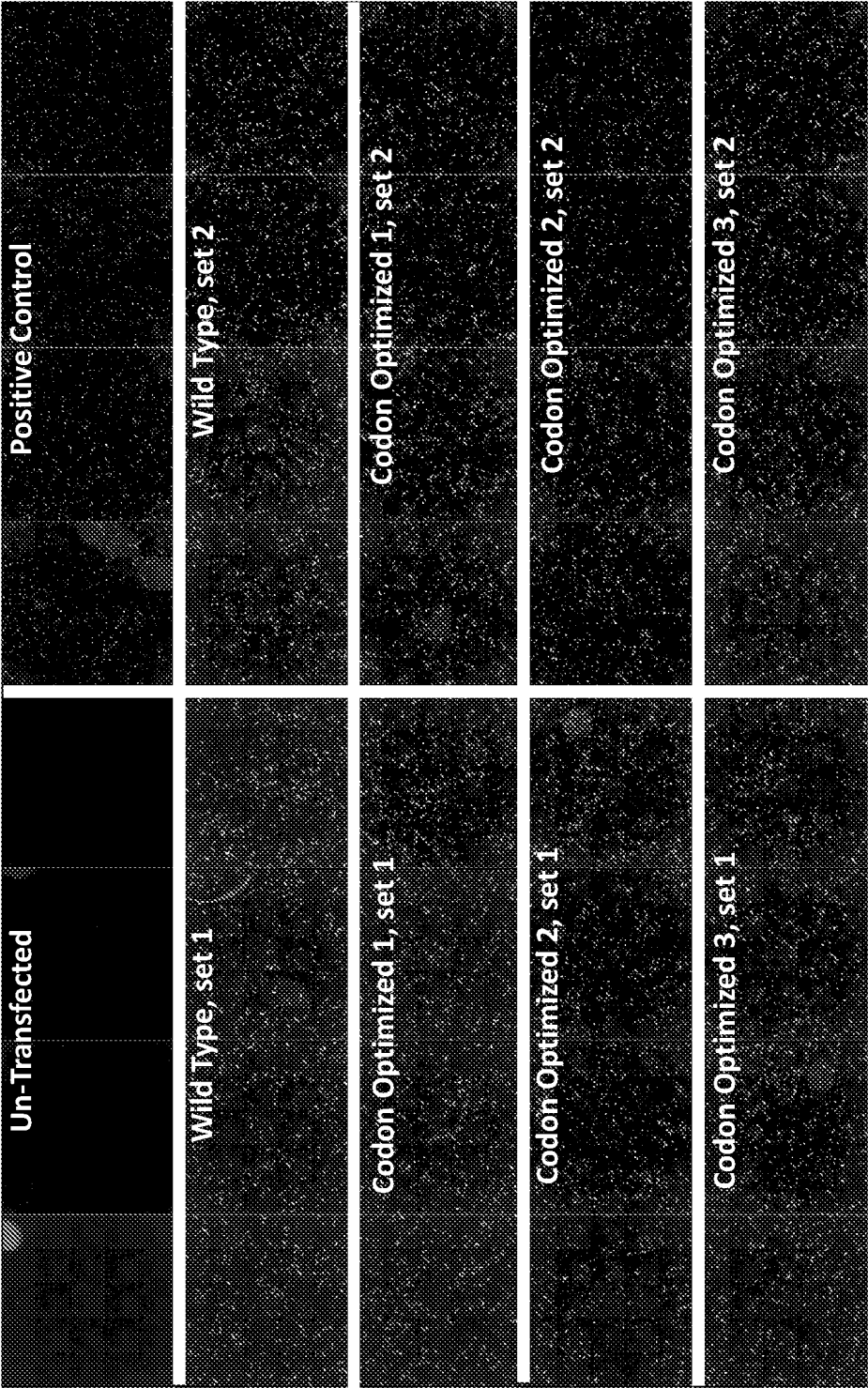


FIG. 2A

FIG. 2B

FIG. 2C

FIG. 2D

FIG. 2E

FIG. 2F

FIG. 2G

FIG. 2H

FIG. 2I

FIG. 2J

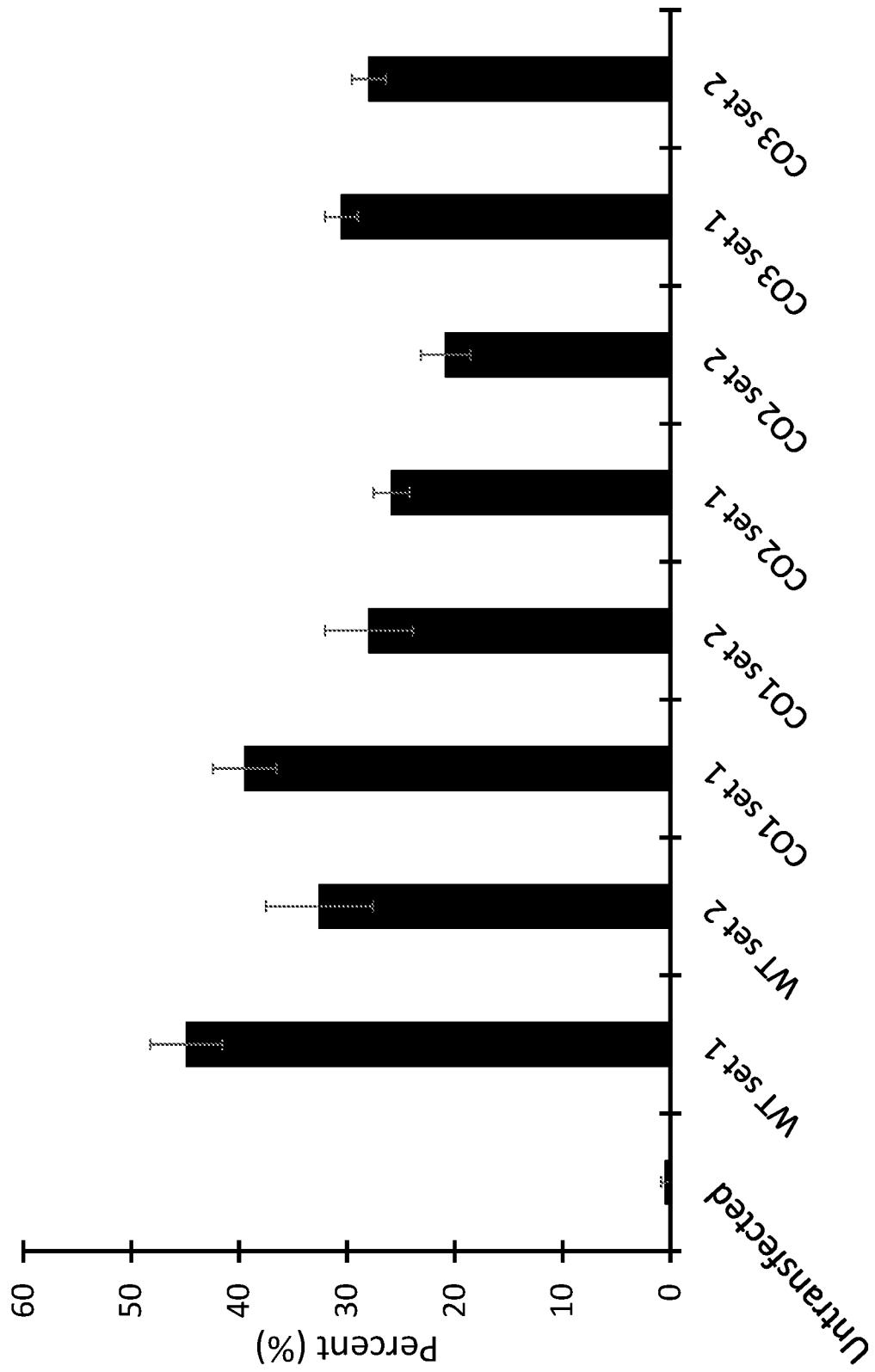
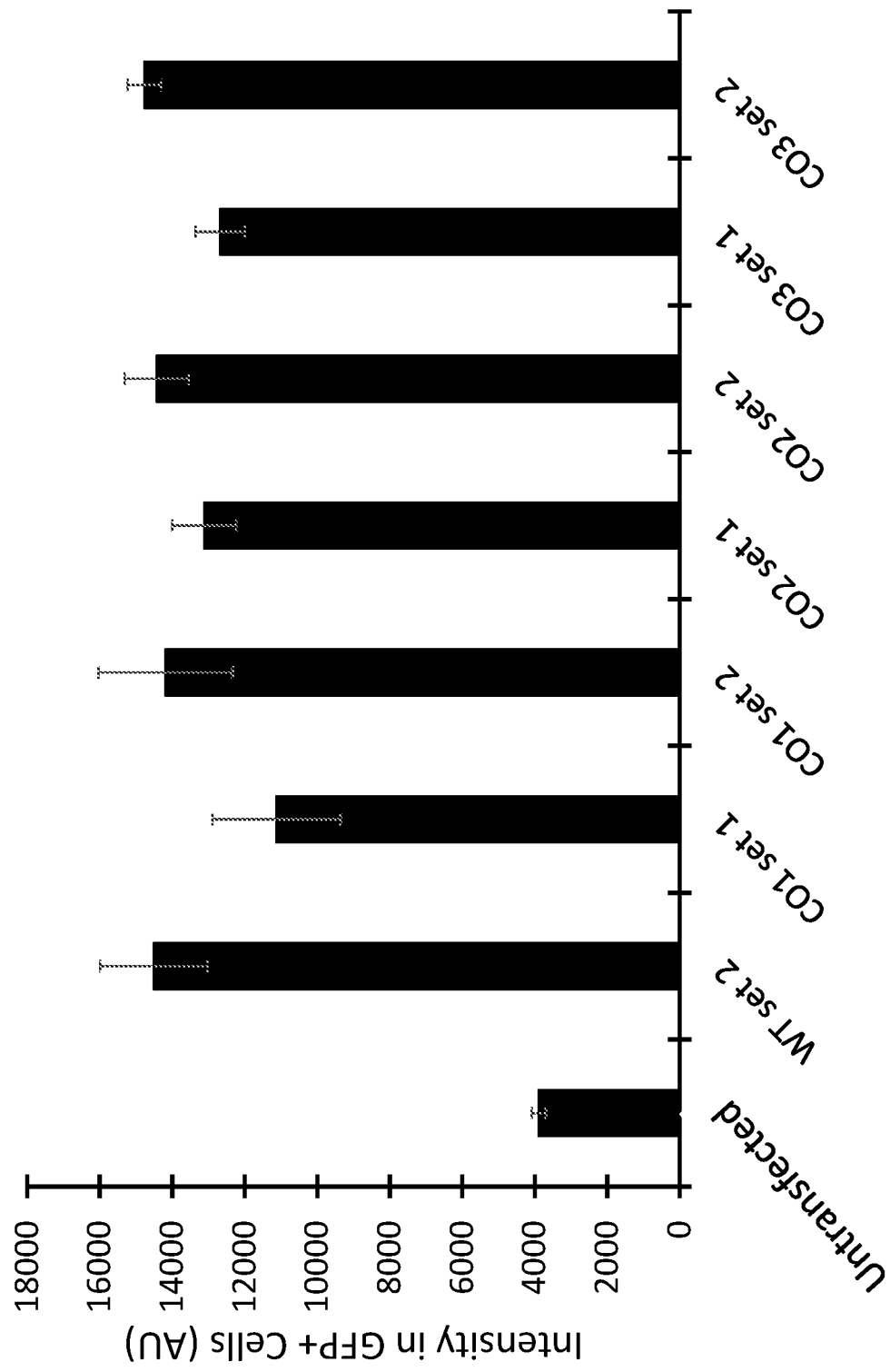


FIG. 2K

**FIG. 2L**

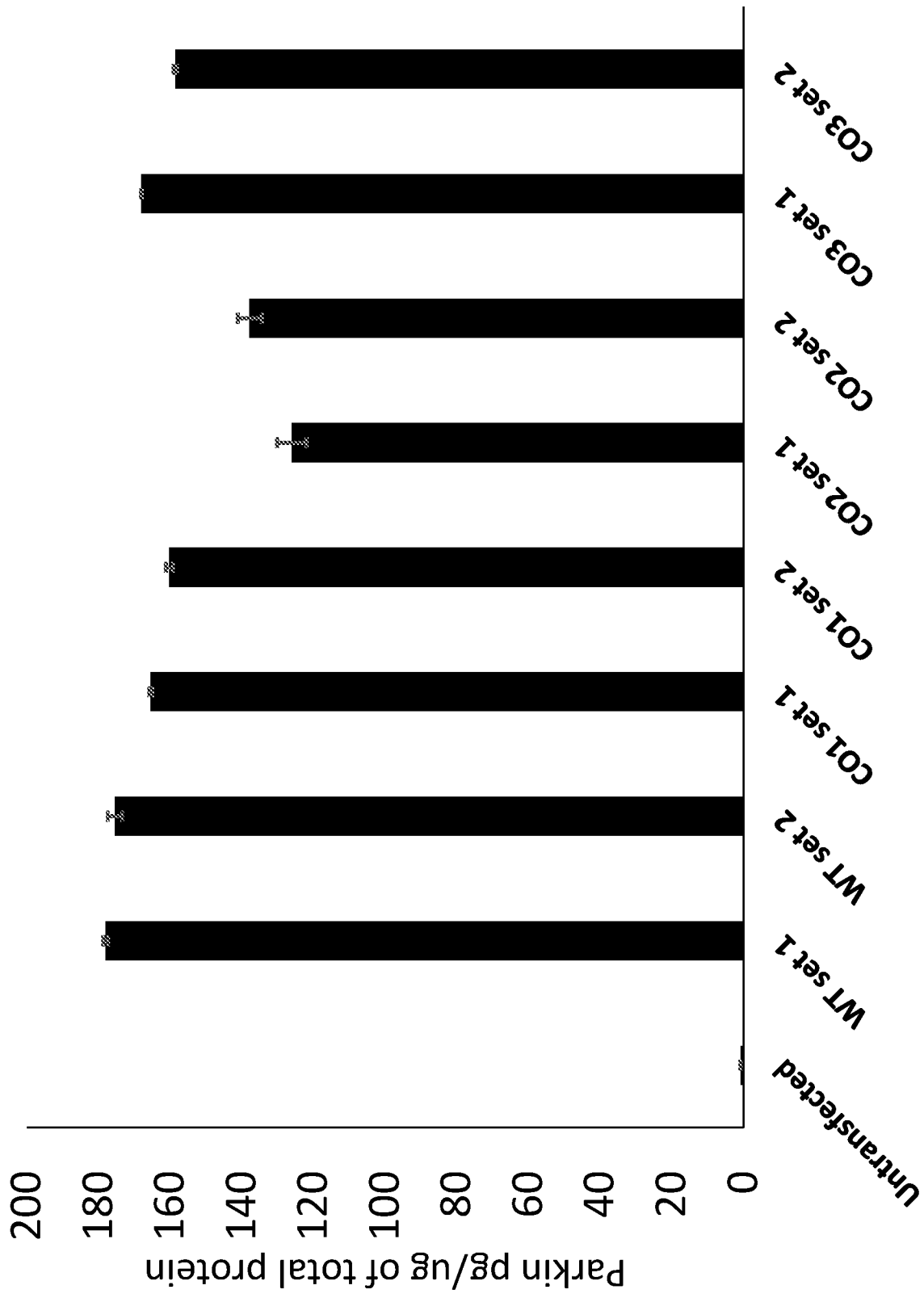
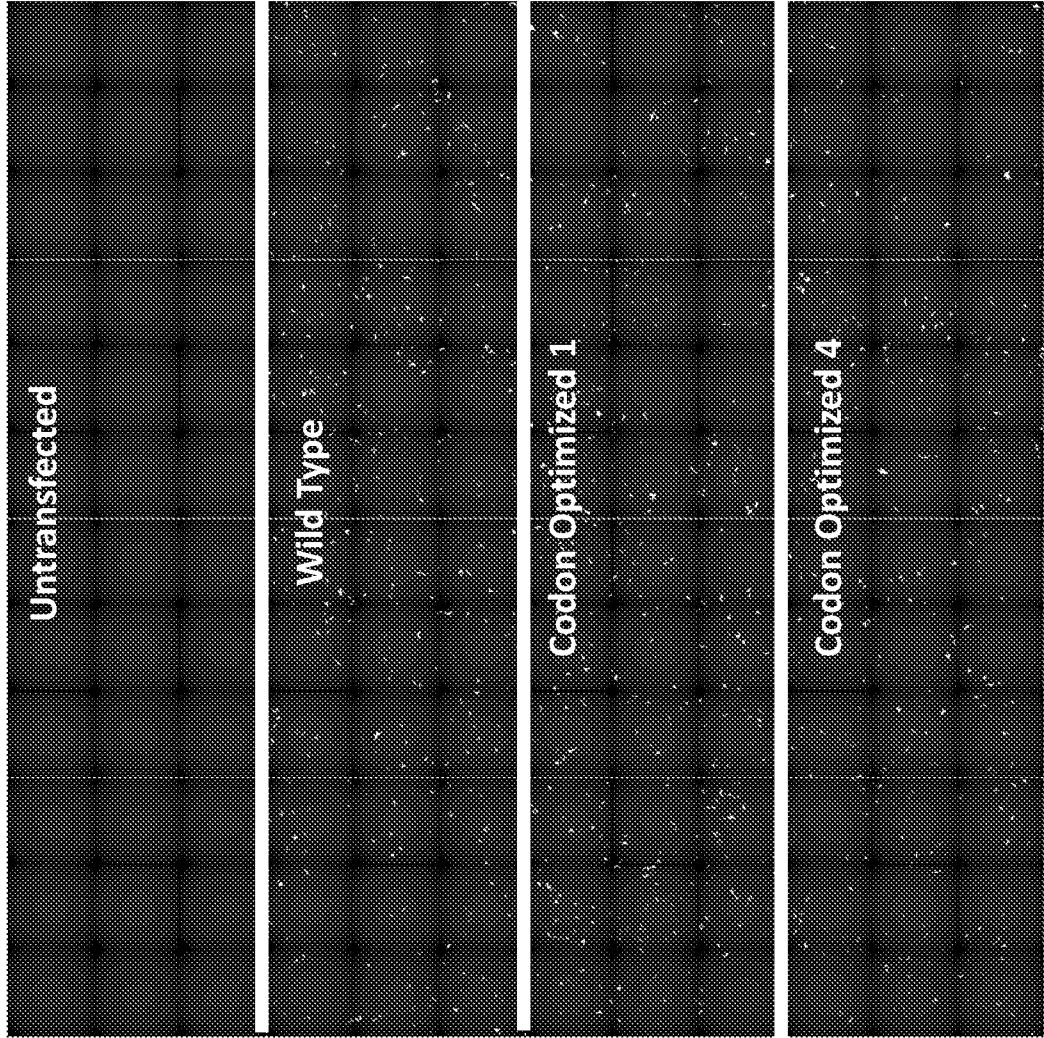
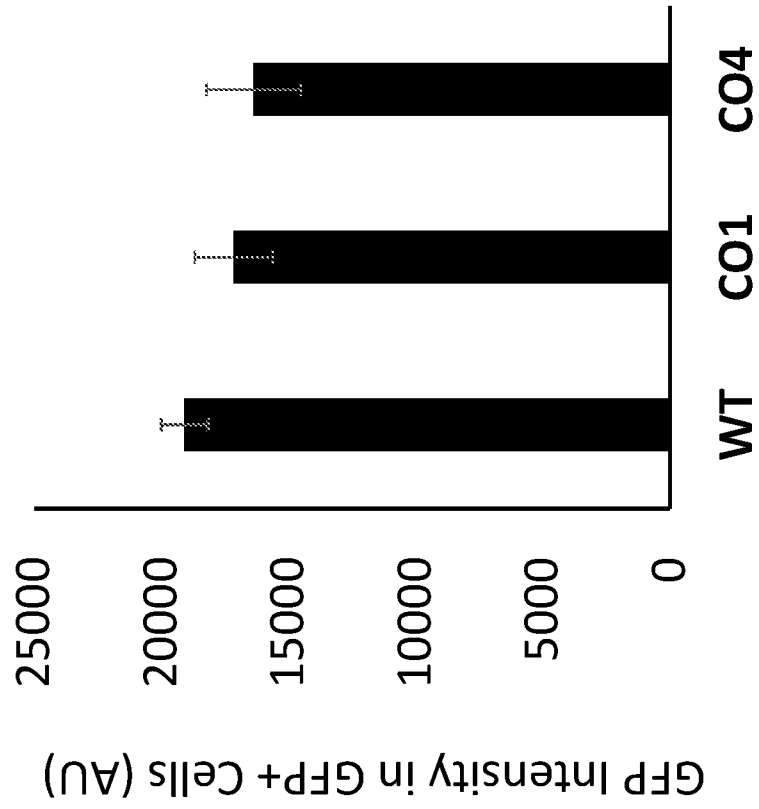
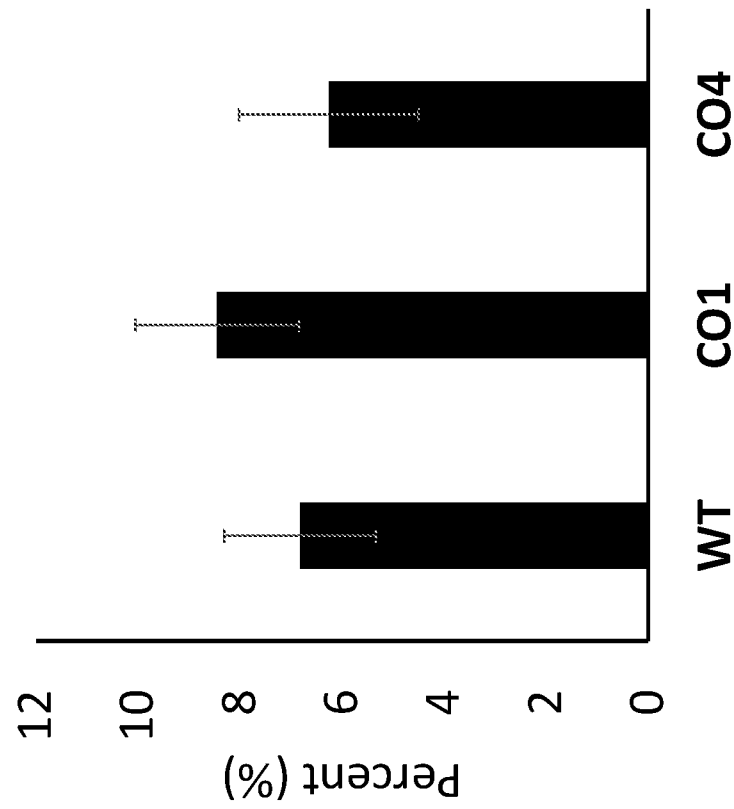


FIG. 3

**FIG. 4A****FIG. 4B****FIG. 4C****FIG. 4D**

**FIG. 4F****FIG. 4E**

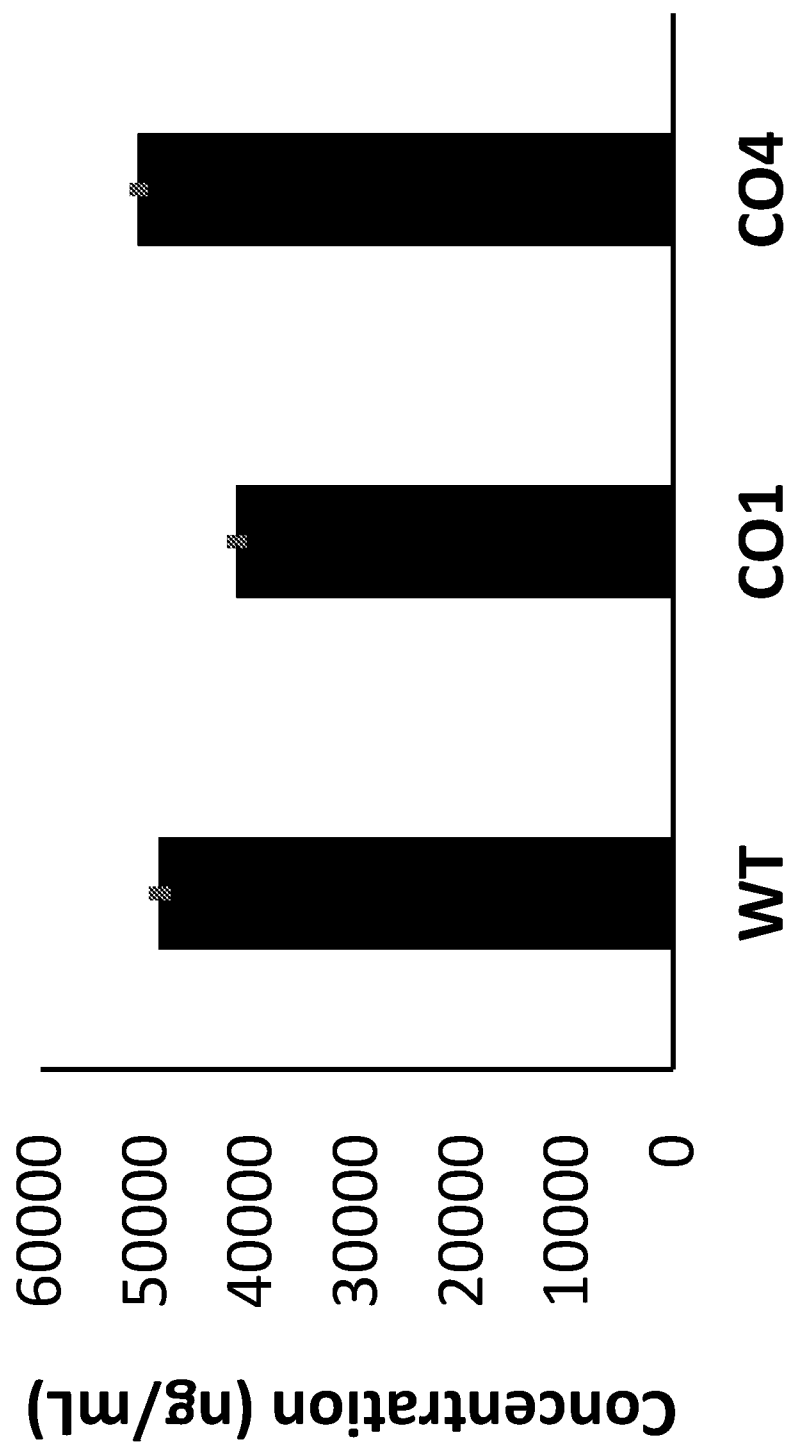
**FIG. 5**

FIG. 6A

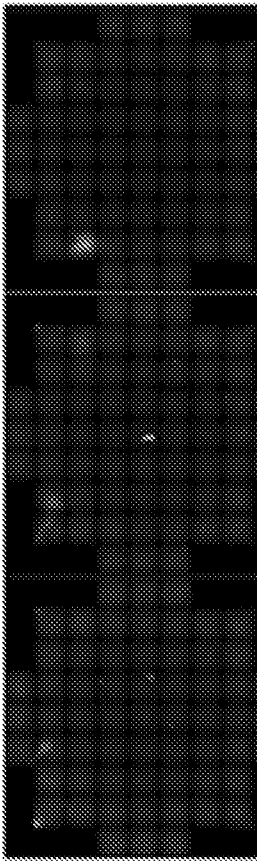


FIG. 6B

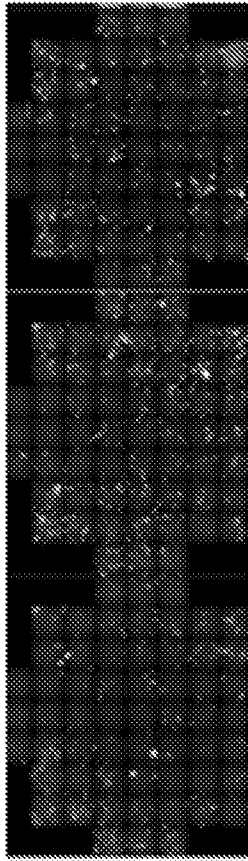


FIG. 6C

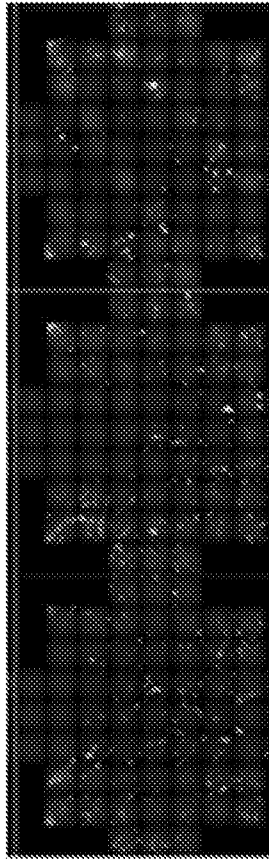
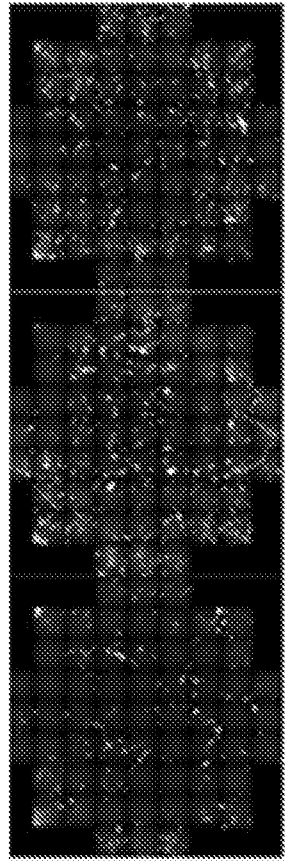


FIG. 6D



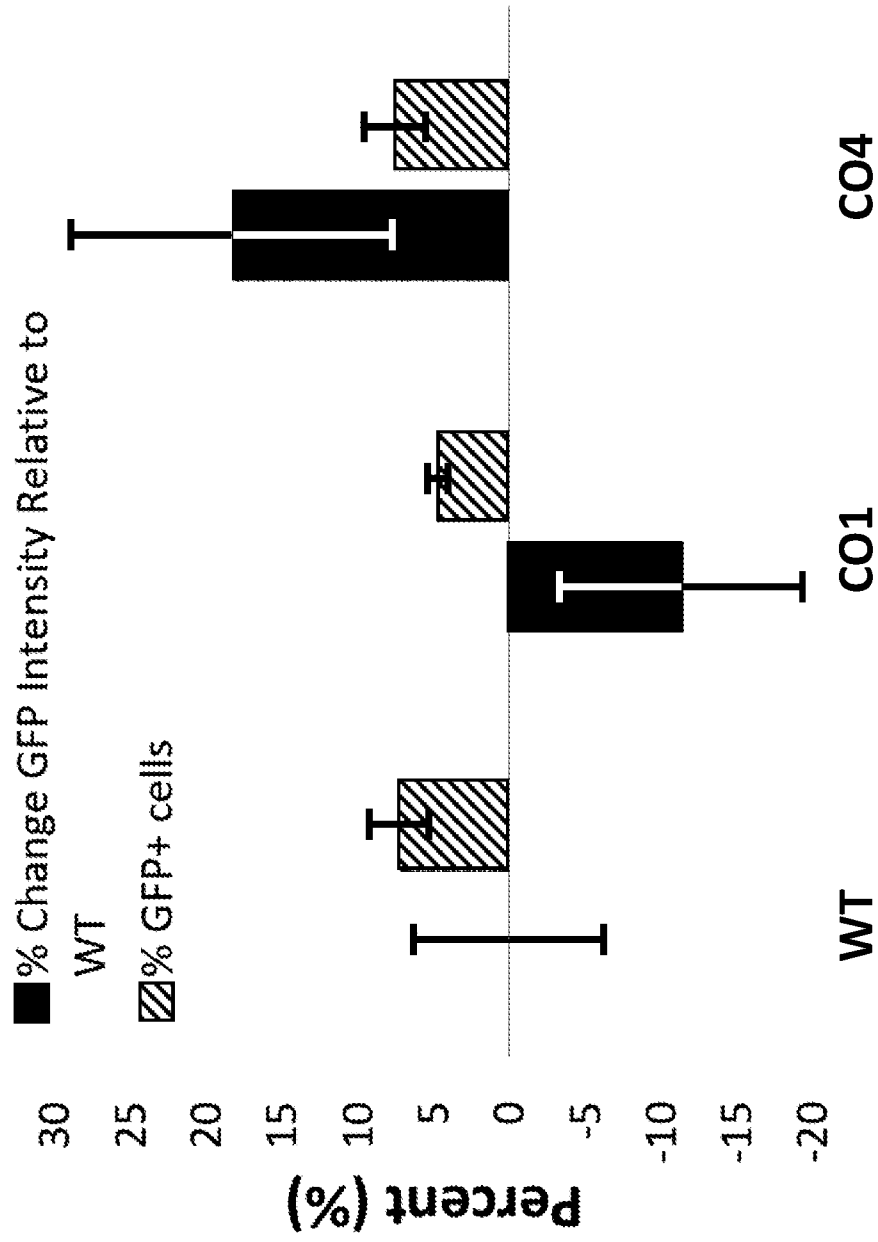


FIG. 6E



FIG. 7

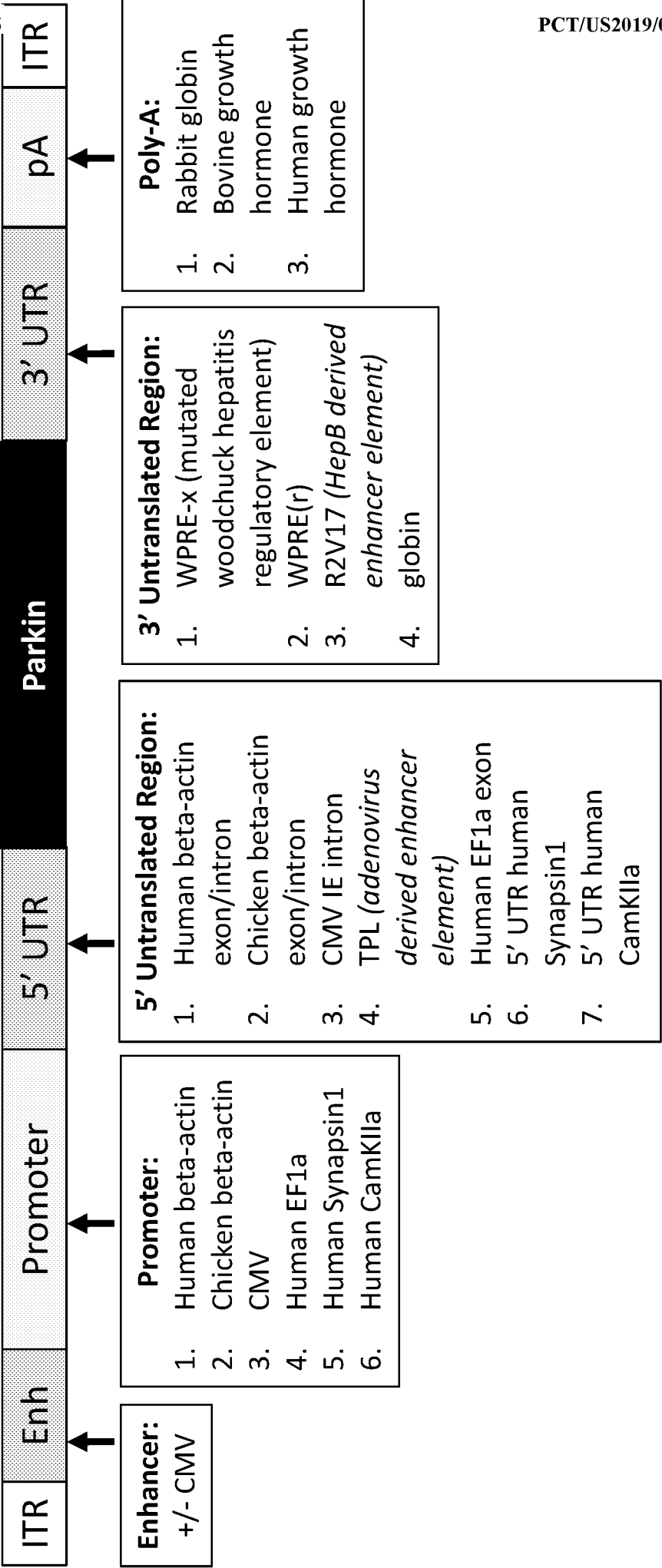


FIG. 8

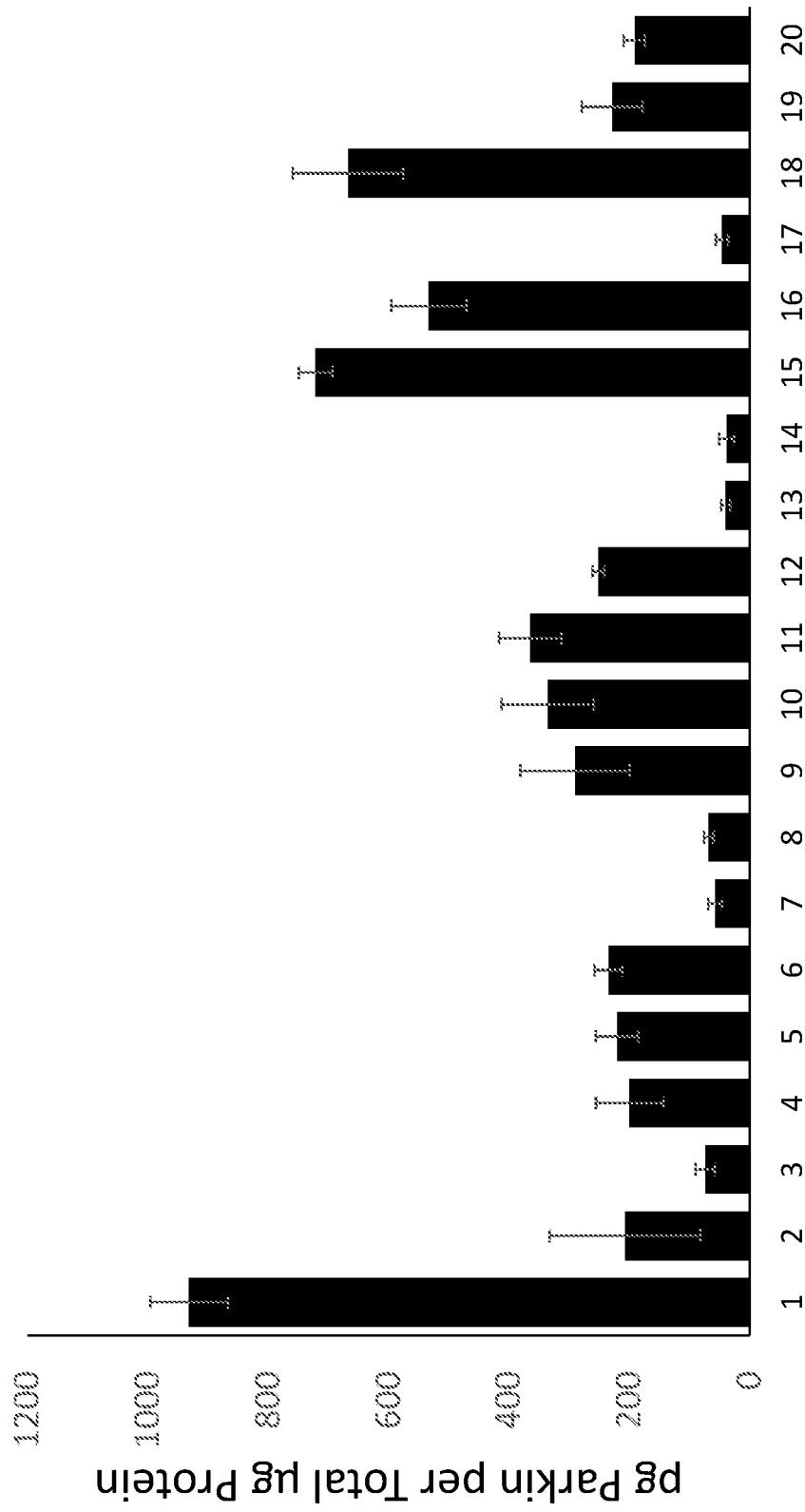
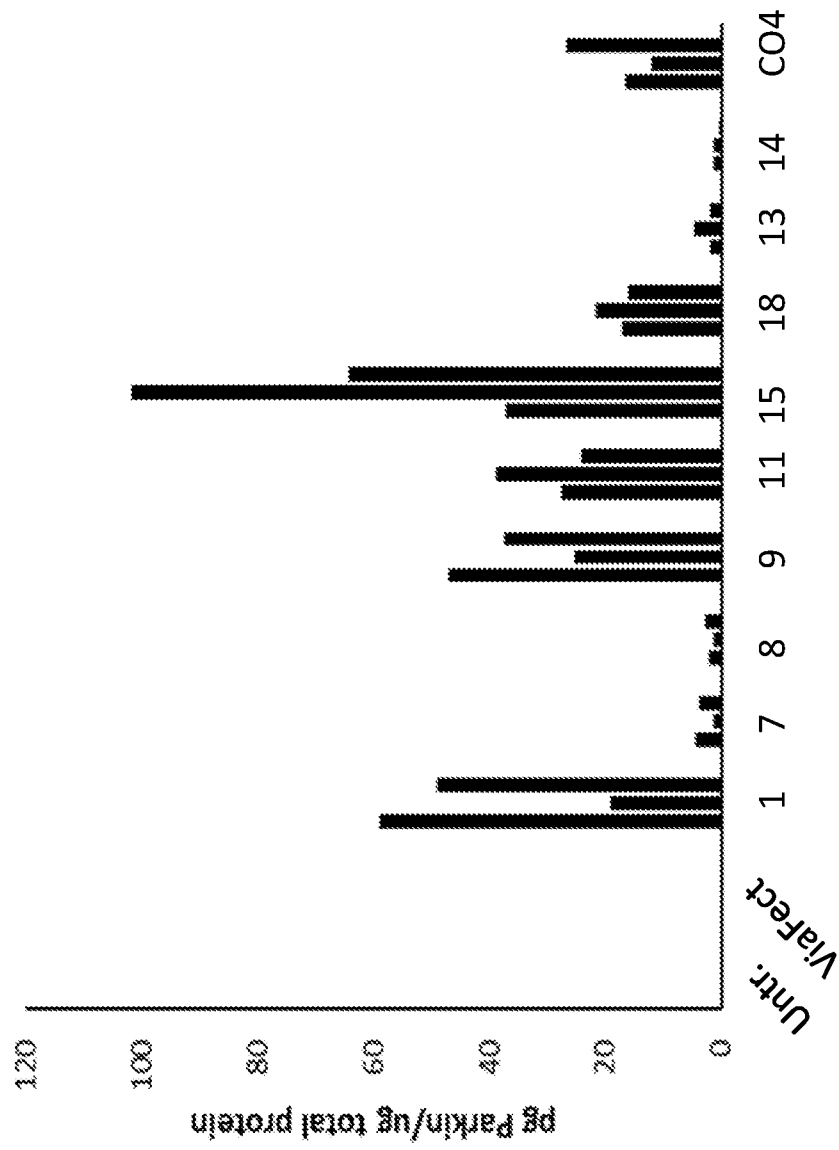
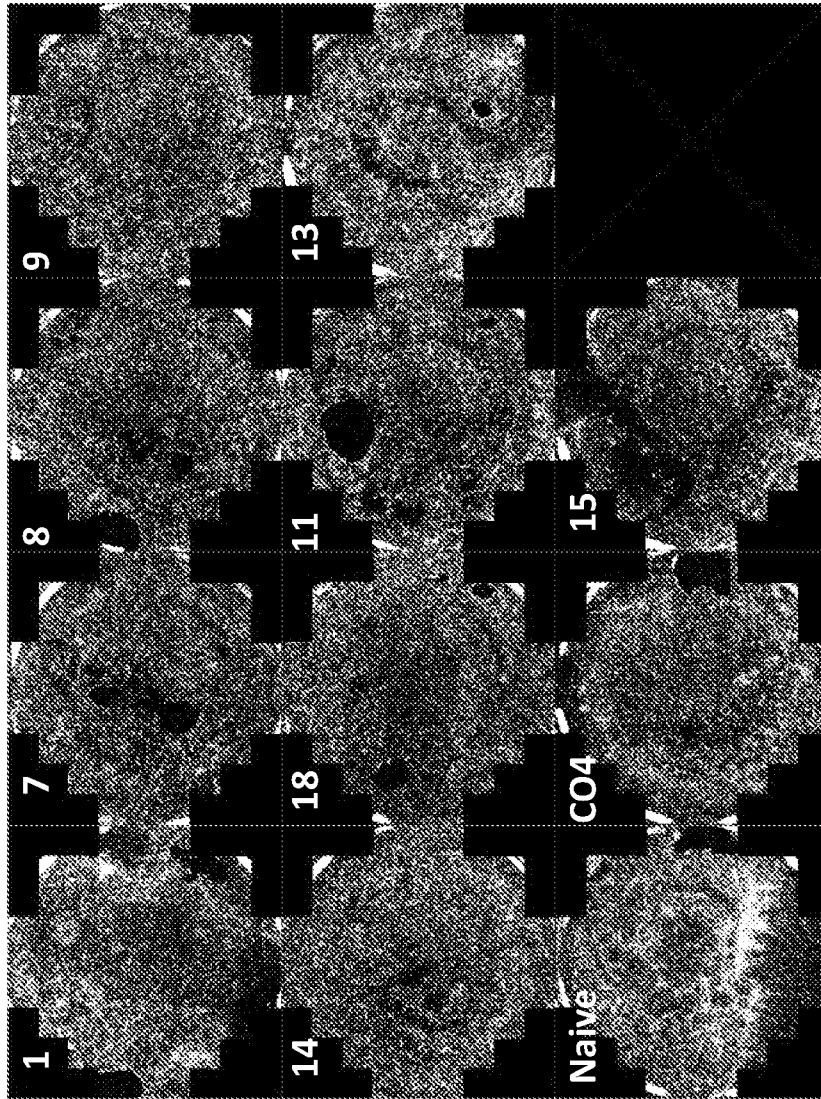
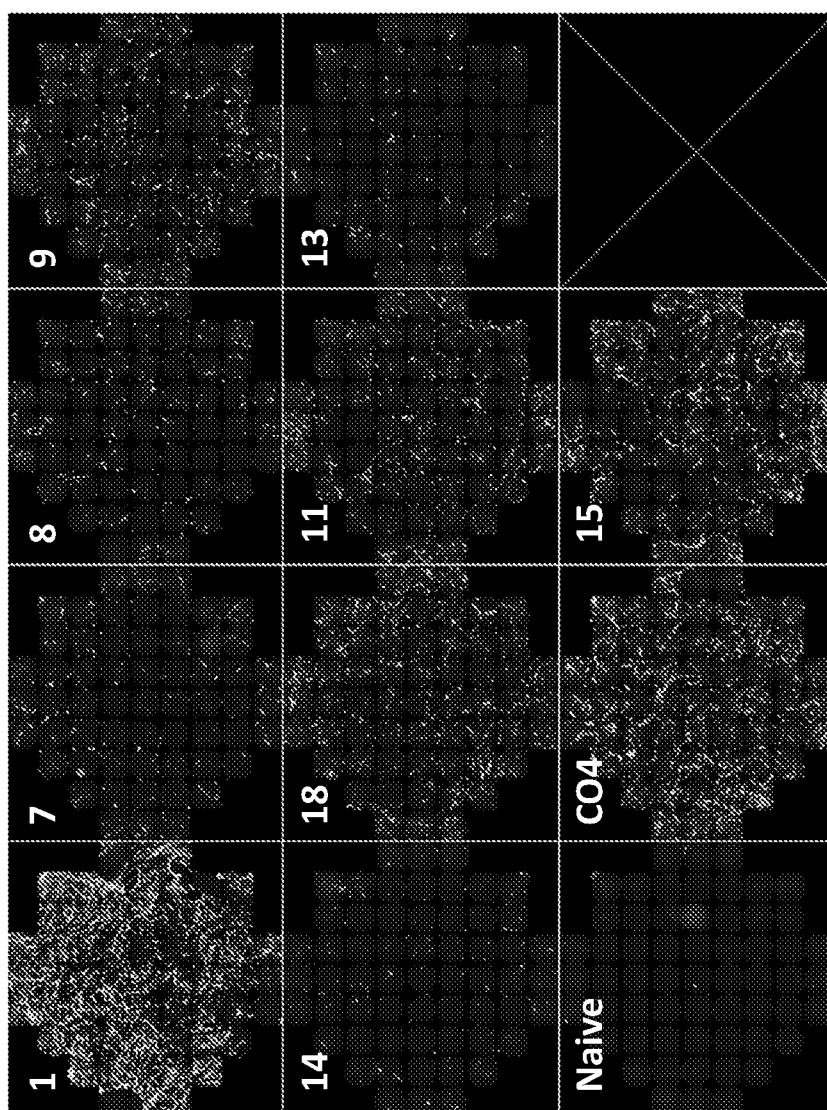


FIG. 9

**FIG. 10**

**FIG. 11A**

**FIG. 11B**

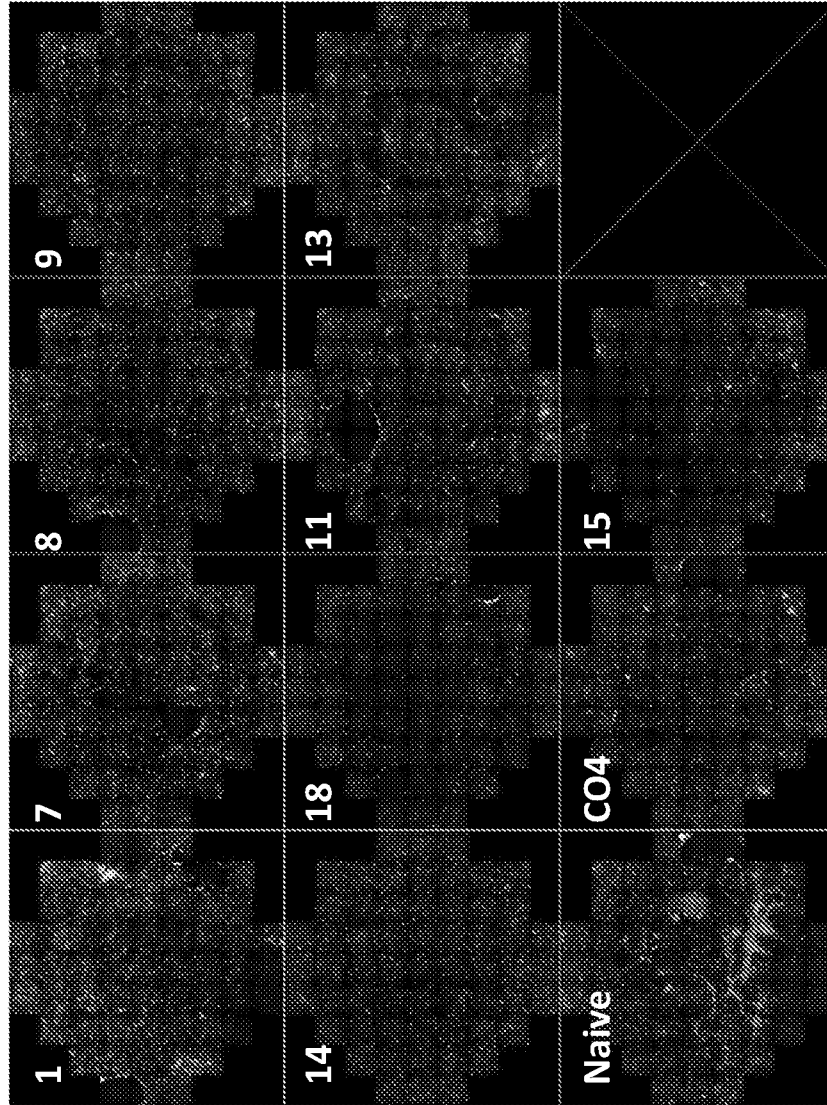
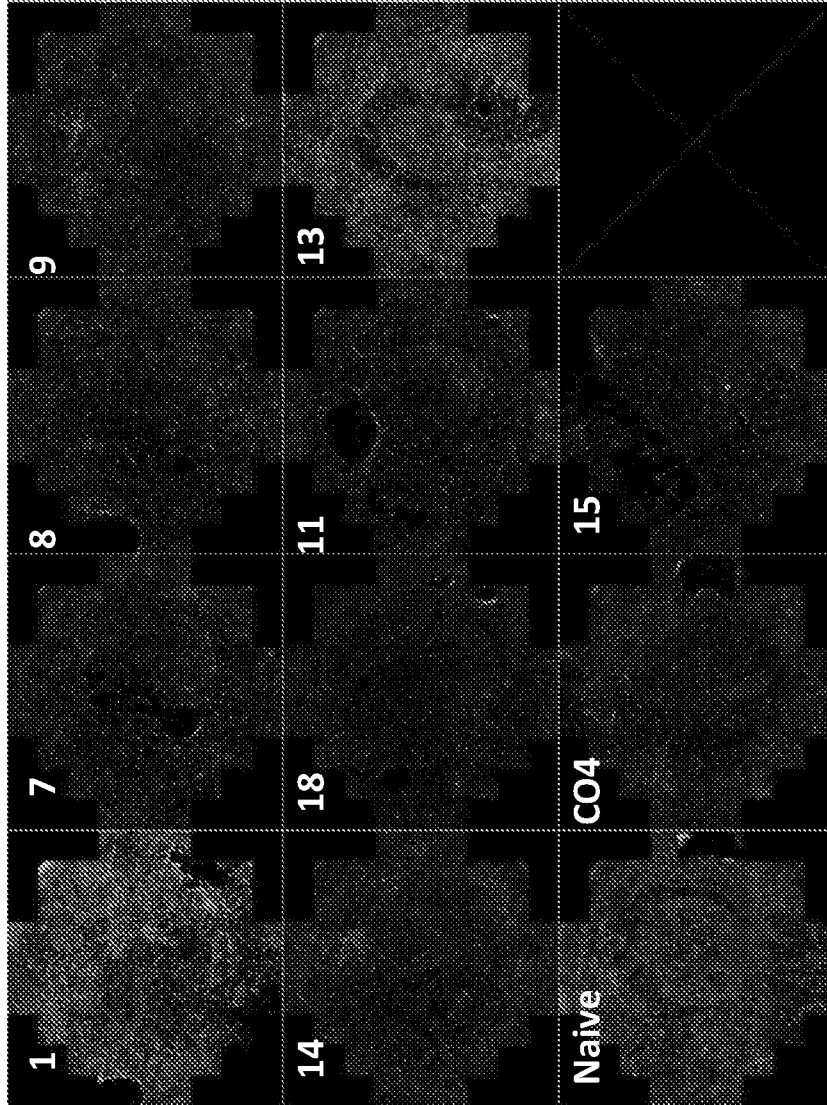


FIG. 11C

**FIG. 11D**

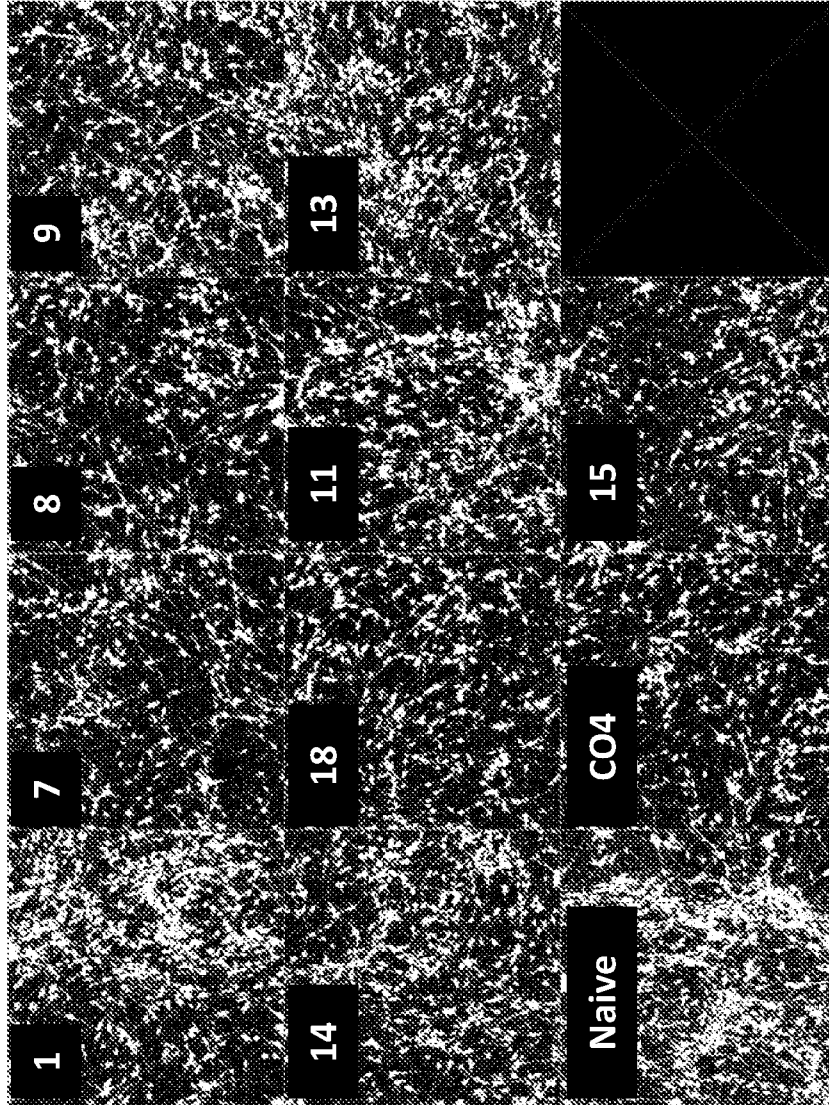
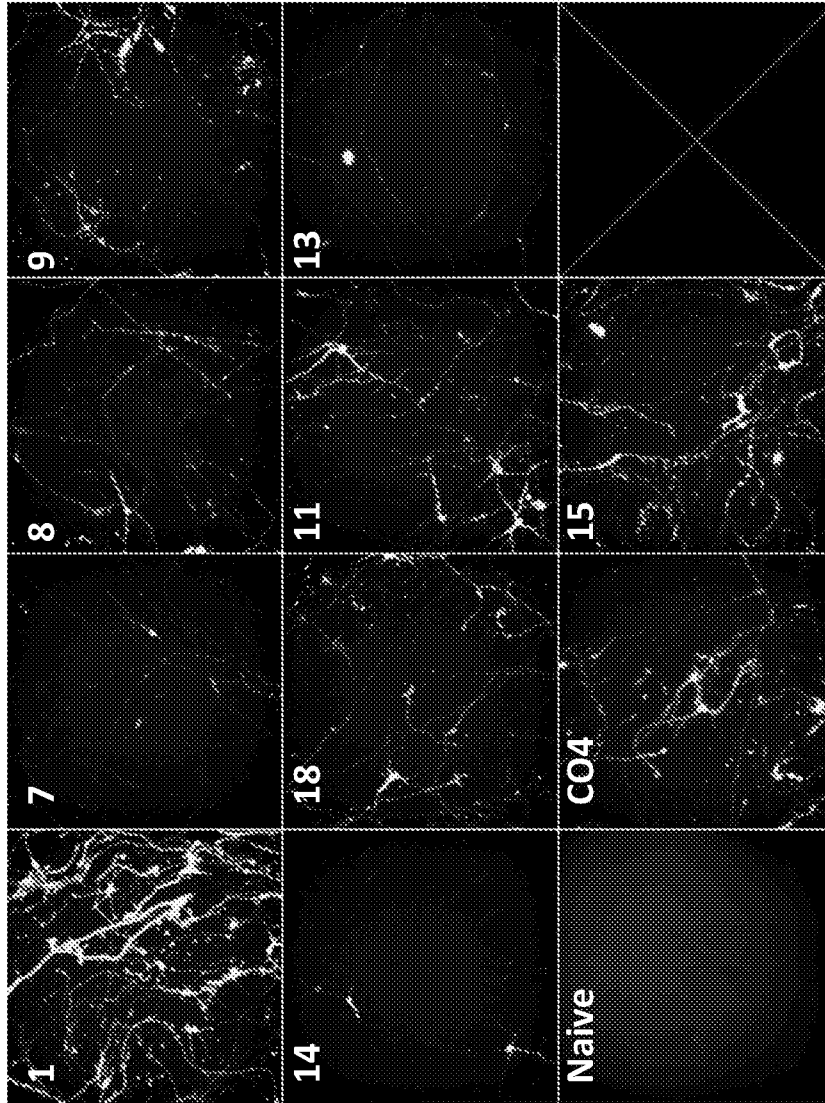


FIG. 12A

**FIG. 12B**

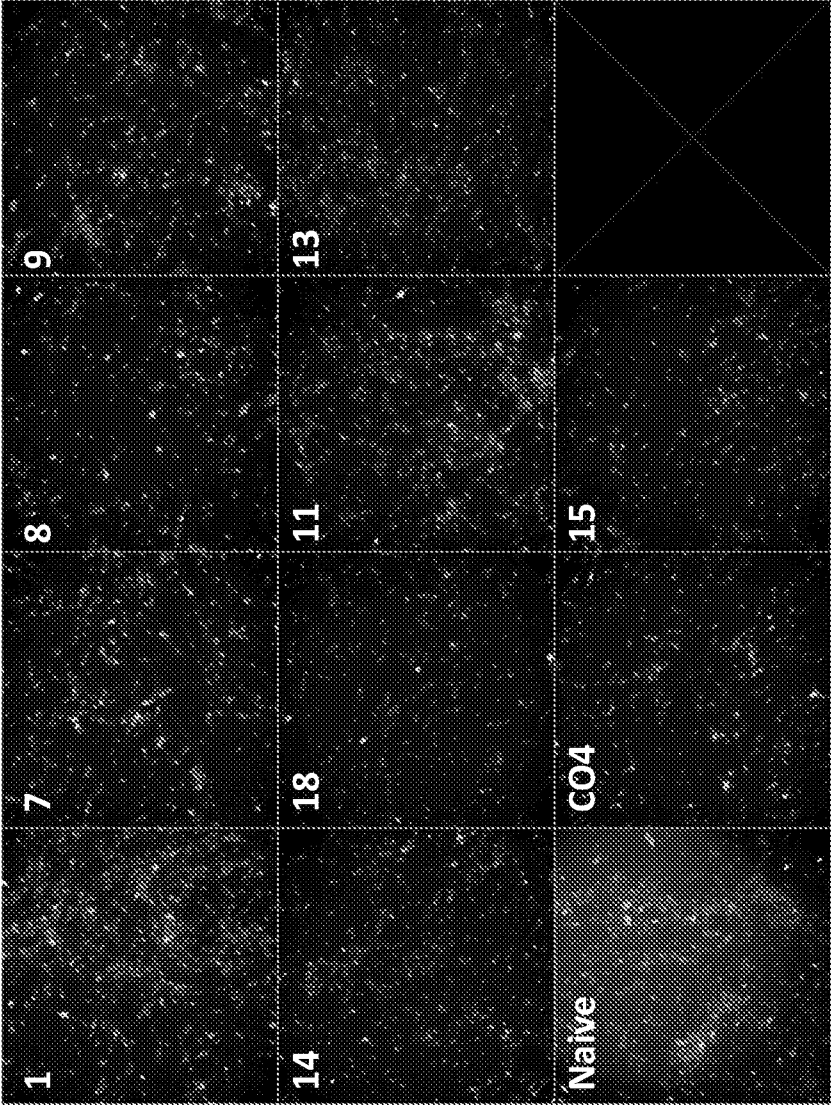
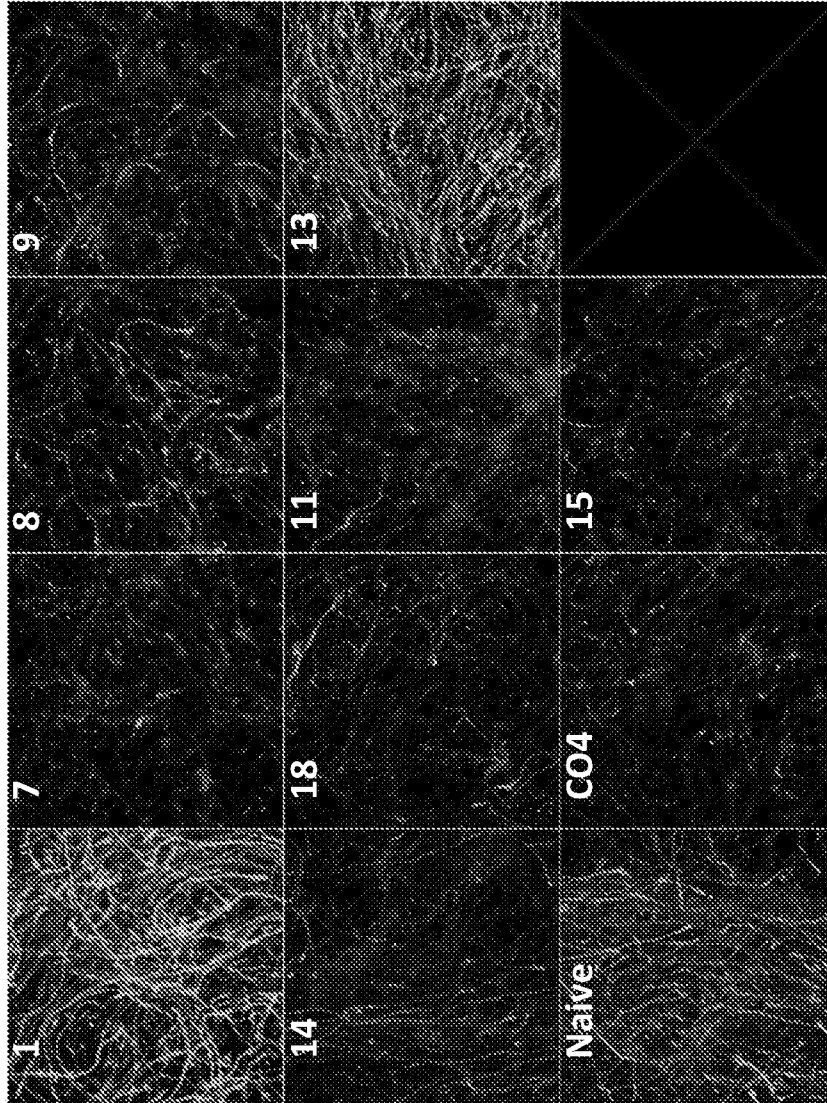


FIG. 12C

**FIG. 12D**

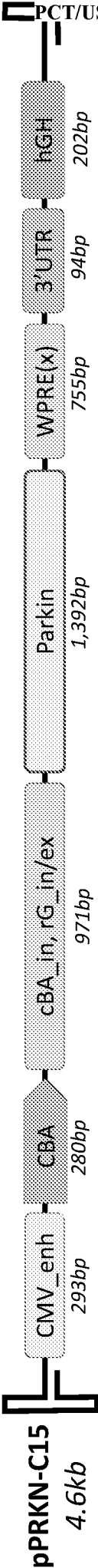
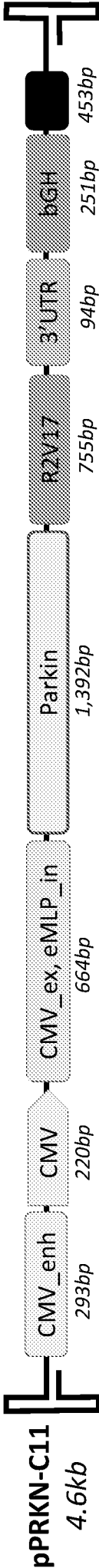
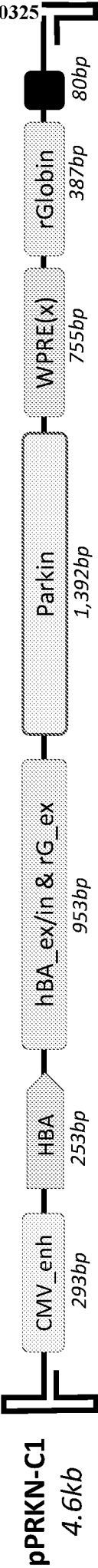


FIG. 13



FIG. 1