Dual Vector Self-Inactivating CRISPR/CAS9 System

RELATED APPLICATIONS

This application claims priority to United States patent application No. 18/583,277 ("US '277"), filed 21 February 2024, and entitled "DUAL VECTOR SELF-INACTIVATING CRISPR/CAS9 SYSTEM". The US '277 application is hereby incorporated herein in its entirety.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in XML format and is hereby incorporated by reference in its entirety. Said XML copy, created on February 07, 2024, is named DTSN-0001_SL.xml and is 13,792 bytes in size.

BACKGROUND

Genome editing or gene-editing is a powerful technique presenting great possibilities in the treatment of numerous gene-associated conditions, including but not limited to both (i) numerous genetic forms of gene-associated conditions and (ii) numerous sporadic forms of gene-associated conditions. In particular, the CRISPR/Cas gene-editing system provides great hope for development of treatments for such diseases/disorders/conditions.

In general, the CRISPR/Cas system utilizes two main components: a guide RNA (gRNA) and an associated Cas nuclease (CRISPR associated nuclease), e.g., Cas9 nuclease. The gRNA has two parts, crRNA, which is nucleotide sequence complementary to the target DNA, and tracrRNA, which is a nucleotide sequence that functions as a binding scaffold for the Cas nuclease. A single guide RNA (sgRNA) is a single RNA molecule in which a crRNA sequence and a tracrRNA sequence are fused together.

During gene-editing, the gRNA/Cas complex recognizes a protospacer adjacent motif (PAM) adjacent the target sequence and the gRNA binds to the target sequence. A double strand break (DSB) at the target sequence is then formed by the Cas9 nuclease. The DSB triggers endogenous DNA repair mechanisms, namely homology directed repair (HDR), and non-homologous end joining (NHEJ). Using this cellular repair

machinery, the CRISPR/Cas system can make insertions, replacements, and/or deletions in the DNA sequence. CRISPR/Cas technology and its use as a gene-editing tool in treating genetic disorders is described in detail in, for example, Zhang et al. (WO 2014/093701) and Zhang et al. (WO 2016/020399), hereby incorporated by reference.

In 1993, the Huntingtin gene (HTT) was isolated. Located on chromosome 4, the Huntingtin locus spans 180 kb and consists of 67 exons. The HTT gene codes for production of a protein called "huntingtin", which is believed to play an important role in nerve cell survival and function. Huntington's Disease (HD) is caused by an abnormal version of HTT which exhibits an unstable trinucleotide repeat in the huntingtin gene, i.e., the CAG trinucleotide repeat. The CAG repeat translates as a polyglutamine repeat in the protein product. In a normal HTT gene, the range of CAG trinucleotide repeats can be fairly broad, for example, 10-26. In the mutant (mHTT) or abnormal HTT gene, the CAG repeats can be >36. HD is autosomal dominant. Thus, an individual need receive only one copy of the abnormal HTT responsible for the excessive CAG repeats from either parent to have Huntington's disease.

As discussed above, CRISPR/Cas technology offers the possibility for the development of treatments for genetic diseases/disorders like HD and other gene-associated conditions, including both genetic forms and sporadic forms of diseases and/or disorders that are associated with genes. However, a difficulty associated with using CRISPR/Cas technology in gene-editing is long term expression of the CRISPR/Cas, specifically expression Cas nuclease protein. This can lead to undesirable immune responses and off-target cleavages inducing mutagenesis of DNA sequences. Consequently, various techniques have been developed to render Cas expression transient and thereby minimize off-target effects.

Chen, et al., Mol. Ther 24, 1508–1510 (2016), disclose an *in vitro* study on a self-restricted CRISPR system using a single lentiviral vector format. The study used a modified lentiCRISPR v2 plasmid that contained a guide RNA targeting the P53 gene, and a second guide RNA to target the Cas9 gene. A human hepatoma cell line, Huh7, was transfected with the lentiviral vector. They observed that cells expressing the self-restricted vector exhibited efficient knockout of the P53 gene while reducing the expression levels of SpCas9 protein. Cas9 protein expression was reduced 2 days post-infection. Compared to cells without SpCas9 sgRNA, this system significantly decreased off-target site occurrences.

Petris, et al., Nat. Commun. 8, 15334. (2017), describe the results of a study on a self-limiting Cas9 delivery system. The study involved a single lentiviral vector containing *Streptocovvus pyogenes* Cas9 (SpCas9), a self-limiting sgRNA targeting Cas9, and a second sgRNA targeting a chosen genomic locus, i.e., enhanced green fluorescent protein (EGFP). In 293T cells, they observed that the self-inactivating system was effective in knocking out the endogenous gene and reduced the off-target events. See also, Ceresto et al. (US 2020/0080090), "Self-Limiting Cas9 circuitry for Enhanced Safety (SLiCES) Plasmid and Lentiviral System Thereof."

Li, et al., Molecular therapy: Methods & clinical development, 12, 111–122 (2018), describe a self-deleting AAV-CRISPR system. A dual-vector system is used wherein one vector expresses SaCas9 driven by a liver-specific promoter and sgRNA for the target gene, and the other vector expresses sgRNA for SaCas9. The AAV-packaged vectors were delivered to liver cells through intraperitoneal injection. The results confirmed that the self-inactivating system effectively knocked out harmful genes associated with the liver and reduced SaCas9 expression to a minimum.

Shen, et al., Nucleic Acids Res 47, e13 (2019), describe a synthetic switch for minimizing CRISPR off-target effects resulting from Cas9 expression by self-restricting both Cas9 transcription, and Cas9 translation. Cas9 sgRNA was used to control transcription of SpCas9 and K-turn/L7Ae was used to regulate translational expression of Cas9. In the design, one vector carried SpCas9 and K-turn/L7Ae elements and another vector carried target gene sgRNA and SpCas9 sgRNA. Despite such strict control of Cas9 expression in the system, they still achieved efficient knockout of the endogenous gene and reduced off-target rates in HEK293T cells.

Kelkar, et al., Molecular therapy 28, 29–41. (2020), describe a single guide RNA system that uses doxycycline (Dox) to deactivate *Streptocovvus pyogenes* Cas9 (SpCas9) nuclease in a doxycycline-dependent manner. The lentiviral system involved two vectors: (a) a first vector carrying Cas9, a reporter, and an sgRNA targeting the gene of interest, and (b) a second vector carrying a Tet-repressor, Cerulean reporter, and tetracycline/Dox-inducible sgRNA targeting Cas9. The lentiviral system was applied in editing endogenous genes in HEK293T cells.

Merienne, et al., Cell Rep 20, 2980–2991 (2017), describe a self-inactivating Cas9 system (KamiCas9 system) for editing CNS disease genes such as Huntington's disease. A dual-vector system was used, with one vector expressing SpCas9 and the other expressing sgRNA for HTT and sgRNA for Cas9. These vectors were

transfected into HEK293T cells carrying 82Q and primary striatal neurons from HD mice KI140Q. Results showed that this system effectively knocked out the HTT gene and significantly reduced expression levels of Cas9.

Li, et al., Human gene therapy, 30(11) (2019), describe self-destructing CRISPR/Cas constructs for targeted gene editing in the retina. The system was a dual AAV vector system with a first vector delivering *Streptocovvus pyogenes* Cas9 (SpCas9) and a second vector delivering sgRNAs against SpCas9 and a target locus (yellow fluorescent protein (YFP)). The system achieved efficient editing in YFP with a decreased Cas9 expression.

SUMMARY OF THE INVENTION

An objective of the present invention is to provide a self-inactivating CRISPR/Cas9 system that optionally minimizes off-target effects and/or provides better control of the gene-editing process.

The present invention relates to a gene-editing method and viral vectors for use therein. As a particular example, the invention relates to a method of treating a patient affected by one or more disease and/or disorder via gene-editing using a self-inactivating CRISPR/Cas9 system.

In a first exemplary embodiment, the present disclosure is directed to a self-inactivating CRISPR/Cas9 delivery system comprising: a first viral vector comprising an expression unit for expression of a Cas9 nuclease and a nucleotide sequence encoding a first sgRNA targeting a specific genomic locus; and a second viral vector comprising a nucleotide sequence encoding an second sgRNA targeting expression of the Cas9 nuclease by the expression unit; wherein the specific genomic locus comprises a nucleotide sequence that (i) is located in a gene and/or (ii) is located in a regulatory region of the gene; wherein the gene (i) causes, (ii) exacerbates, (iii) is otherwise associated with, or (iv) any combination of (i)-(iii) one or more disease and/or disorder.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments, (i) the gene and/or regulatory region is a mutated gene and/or regulatory region; (ii) the gene and/or regulatory region is a wild type gene and/or regulatory region; (iii) the specific genomic locus is located in a regulatory region of the gene; (iv) the specific genomic locus is located in an intron of

the gene; (v) the specific genomic locus is located in an exon of the gene; (vi) one or more disruption and/or cleavage of the specific genomic locus causes and/or results in reduction and/or elimination of expression of the gene; an/or (vii) one or more disruption and/or cleavage of the specific genomic locus causes and/or results in truncation of the protein expressed by the gene.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments, the sgRNA targeting expression of the Cas9 nuclease by the expression unit is encoded by a nucleotide sequence comprising (i) a nucleotide sequence selected from SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, and SEQ ID NO: 12, (ii) a nucleotide sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, or at least 99% identity with one or more of the nucleotide sequence of SEQ ID NO: 3 to SEQ ID NO. 12 and having the same function as one or more of the nucleotide sequences of SEQ ID NO: 3 to SEQ ID NO: 12, (iii) a nucleotide sequence having at most three base pairs different from, at most two base pairs different from, or at most one base pair different from one or more of the nucleotide sequence of SEQ ID NO: 3 to SEQ ID NO. 12 and having the same function as one or more of the nucleotide sequences of SEQ ID NO: 3 to SEQ ID NO: 12, or (iv) a nucleotide sequence obtained from the nucleotide sequence of one of SEQ ID NO: 3 to SEQ ID NO: 12 by deletion, substitution, or addition of 1, 2, 3, 4, 5 or 6 bases and having the same function as one or more of the nucleotide sequences of SEQ ID NO: 3 to SEQ ID NO: 12.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments, the sgRNA targeting expression of the Cas9 nuclease by the expression unit is encoded by a a nucleotide sequence comprising a nucleotide sequence selected from the nucleotide sequences according to SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, and SEQ ID NO: 12.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments, the first and second viral vectors are selected from AAV, lentiviral vectors, adenovirus vectors, herpesvirus vectors, poxvirus vectors, baculovirus vectors, and papillomavirus vectors.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments, the first and second viral vectors are selected from AAV vectors.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments, the first and second viral vectors are selected from AAV1, AA2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV PHP.B and AAV PHP.eB.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments, the first and second viral vectors are not AAV8 viral vectors.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments, the first and second viral vectors are AAV9, AAV PHP.B, or AAV PHP.eB viral vectors.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments, the first and second viral vectors are AAV9 viral vectors.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments, (i) the first viral vector further comprises a CMV promoter to drive expression of the Cas9 nuclease; (ii) the first viral vector further comprises a U6 promoter to drive transcription of the first sgRNA, (iii) the second viral vector further comprises a U6 promoter to drive transcription of the second sgRNA, or (iv) any combination thereof.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments, the second viral vector does not include a nucleotide sequence encoding a reporter gene.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments, the second viral vector does include a nucleotide sequence encoding a reporter gene.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments, the Cas9 nuclease is one or more of SaCas9, SpCas9 nuclease, StCas9 nuclease, CjCas9 nuclease, or NmCas9 nuclease.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments, the Cas9 nuclease is SaCas9 nuclease.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments, the disease or disorder is selected from cystic fibrosis, myotonic dystrophy, spinal muscular dystrophy, alpha- thalassemia, beta-thalassemia, sickle cell anemia, chronic granulomatous disease Canavan disease, Fanconi anemia, Marfan syndrome, retinitis pigmentosa, fragile X syndrome, Gaucher disease, Huntington's disease, hemochromatosis, congenital deafness, Duchenne muscular dystrophy, familial hypercholesterolemia, Farber disease, Neurofibromatosis type 1, Neurofibromatosis t Becker muscular dystrophy type II, hemophilia A, hemophilia B, Tay-Sachs disease, Wiskott-Aldrich syndrome, Hurler syndrome, Hunter syndrome, Purine nucleoside phosphorylase deficiency, Fabry disease, Pompe disease, Gyrate atrophy, Krabbe disease, Wolman disease, Amyotrophic lateral sclerosis, Spinocerebellar Ataxia, Sanfilippo disease, a birth defect, breast cancer, prostate cancer, skin cancer, high blood pressure, high cholesterol, diabetes, Alzheimer disease, schizophrenia, Parkinson’s disease, Frontotemporal dementia, or bipolar disorder.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments, (i) the disease or disorder is a neurodegenerative disease, (ii) the gene is expressed in the CNS, or (iii) both (i) and (ii).

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments, (i) the disease or disorder is selected from Huntington’s disease, Alzheimer’s disease, Parkinson’s disease, Amyotrophic Lateral Sclerosis (ALS), Frontotemporal dementia (FTD) (aka Pick’s disease), spinocerebellar ataxi (SCA), particularly types 1 to 17, Retinitis Pigmentosa, and Age-related macular degeneration (AMD), (ii) the gene is expressed in the CNS, or (iii) both (i) and (ii).

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments, wherein (i) the disease or disorder is

Huntington's disease, (ii) the first sgRNA targeting a specific genomic locus targets Huntingtin (HTT) gene, or (iii) both (i) and (ii).

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments, the nucleotide sequence encoding the first sgRNA targeting a specific genomic locus comprises (i) a nucleotide sequence according to SEQ ID NO. 1 or SEQ ID NO. 2, (ii) a nucleotide sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, or at least 99% identity with the nucleotide sequence of SEQ ID NO. 1 and/or SEQ ID NO. 2 and having the same function as the nucleotide sequence of SEQ ID NO: 1 and/or SEQ ID NO: 2, (iii) a nucleotide sequence having at most three base pairs different from, at most two base pairs different from, or at most one base pair different from of either or both of the nucleotide sequence of SEQ ID NO. 1 or SEQ ID NO. 2 and having the same function as the nucleotide sequence of SEQ ID NO: 1 and/or SEQ ID NO: 2, or (iv) a nucleotide sequence obtained from the nucleotide sequence of SEQ ID NO: 1 or SEQ ID NO: 2 by deletion, substitution or addition of 1, 2, 3, 4, 5 or 6 base pairs and having the same function as the nucleotide sequence of SEQ ID NO: 1 and/or SEQ ID NO: 2.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments, the nucleotide sequence encoding the first sgRNA targeting a specific genomic locus comprises a nucleotide sequence according to SEQ ID NO. 1 or SEQ ID NO. 2.

In another exemplary embodiment, the present disclosure is directed to a pharmaceutical composition comprising (i) one or more self-inactivating CRISPR/Cas9 delivery system according to any of the described exemplary embodiments and/or further exemplary embodiments and (ii) one or more pharmaceutical acceptable carrier and/or excipient.

In another exemplary embodiment, the present disclosure is directed to a method for treating a disease and/or disorder, the method comprising: administering to a patient having the disease and/or disorder (i) one or more effective amount of one or more self-inactivating CRISPR/Cas9 delivery system according to any of the described exemplary embodiments and/or further exemplary embodiments (ii) one or more effective amount of a pharmaceutical composition comprising one or more self-inactivating CRISPR/Cas9 delivery system according to any of the described

exemplary embodiments and/or further exemplary embodiments, or (iii) both (i) and (ii).

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments, an effective amount comprises a dosage of 10^9 to 10^{14} viral genomes or infectious units of viral vectors per dose.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments, wherein an effective amount comprises a dosage of 10^{10} to 10^{12} viral genomes or infectious units of viral vector per dose.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments, a molar ratio of the first vector to the second vector is selected (i) to maximize one or more beneficial and/or desired physiological effect, (ii) to minimize one or more detrimental and/or undesired physiological effect, or (iii) both (i) and (ii), optionally wherein

(a) the selection comprises: (i)(A) administering to one or more first mouse the first vector and the second vector at a first molar ratio of the first vector to the second vector, the first mouse being a model of a disease, a disorder, or both and/or (A') administering to one or more first population of cells the first vector and the second vector at a first molar ratio of the first vector to the second vector; (ii) and measuring one or more first physiological effect of administering the first molar ratio; (iii)(A) administering to one or more second mouse the first vector and the second vector at a second molar ratio of the first vector to the second vector, the second mouse being a model of the disease, the disorder, or both and/or (A') administering to one or more second population of cells the first vector and the second vector at a second molar ratio of the first vector to the second vector; (iv) measuring one or more second physiological effect of administering the second molar ratio; (v) optionally repeating (a)(i)-(a)(ii) one or more additional times at one or more additional molar ratio, wherein each of the one or more additional molar ratio is independently different from the first molar ratio and the second molar ratio; and (v) selecting a molar ratio from the first molar ratio, the second molar ratio, and optionally one or more additional molar ratio, to produce a selected molar ratio, wherein the selected molar ratio is a molar ratio the administration of which (i) is effective to produce one or more beneficial and/or desired physiological effect and (ii) produces less detrimental and/or fewer detrimental and/or undesired physiological effects than one or more molar ratio that is not selected;

(b) maximizing one or more beneficial and/or desired physiological effect is accomplished by maximizing editing of one or more on-target genomic locus;

(c) minimizing one or more detrimental and/or undesired physiological effect is accomplished by eliminating or reducing editing of one or more off-target genomic locus; or

(d) any combination of (a)-(c).

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments, (a) (i) the disease or disorder is a neurodegenerative disease, (ii) the gene is expressed in the CNS, or (iii) both (i) and (ii) and (b) administration comprises stereotaxic injection (i) into the striatum of the brain of the patient, (ii) into the cortex of the brain of the patient, or (iii) into both the striatum and the cortex of the brain of the patient.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments, the patient is human.

In another exemplary embodiment, the present disclosure is directed to one or more self-inactivating CRISPR/Cas9 delivery system according to any of the described exemplary embodiments and/or further exemplary embodiments formulated for use in treatment of the one or more disease, disorder, or both.

In another exemplary embodiment, the present disclosure is directed to one or more self-inactivating CRISPR/Cas9 delivery system according to any of the described exemplary embodiments and/or further exemplary embodiments formulated for use in treatment of the one or more disease, disorder, or both, wherein (a) the formulation comprises a dosage of 10^9 to 10^{14}, or 10^{10} to 10^{12} viral genomes or infectious units of viral vectors per dose; (b) the formulation comprises a molar ratio of the first vector to the second vector, wherein the molar ratio is selected (i) to maximize one or more beneficial and/or desired physiological effect, (ii) to minimize one or more detrimental and/or undesired physiological effect, or (iii) both (i) and (ii); (c) (A) (i) the disease or disorder is a neurodegenerative disease, (ii) the gene is expressed in the CNS, or (iii) both (i) and (ii) and (B) the formulation is suitable for stereotaxic injection (i) into the striatum of the brain of the patient, (ii) into the cortex of the brain of the patient, or (iii) into both the striatum and the cortex of the brain of the patient; (d) the formulation is suitable for administration to a human; or (e) any combination of (a)-(d).

In another exemplary embodiment, the present disclosure is directed to a medicament comprising one or more self-inactivating CRISPR/Cas9 delivery system according to any of the described exemplary embodiments and/or further exemplary embodiments.

In another exemplary embodiment, the present disclosure is directed to a medicament comprising one or more self-inactivating CRISPR/Cas9 delivery system according to any of the described exemplary embodiments and/or further exemplary embodiments, the medicament being for use in treatment of the one or more disease, disorder, or both, wherein (a) the medicament comprises a dosage of 10^9 to 10^{14}, or 10^{10} to 10^{12} viral genomes or infectious units of viral vectors per dose; (b) the medicament comprises a molar ratio of the first vector to the second vector, wherein the molar ratio is selected (i) to maximize one or more beneficial and/or desired physiological effect, (ii) to minimize one or more detrimental and/or undesired physiological effect, or (iii) both (i) and (ii); (c)(A) (i) the disease or disorder is a neurodegenerative disease, (ii) the gene is expressed in the CNS, or (iii) both (i) and (ii) and (B) the formulation is suitable for stereotaxic injection (i) into the striatum of the brain of the patient, (ii) into the cortex of the brain of the patient, or (iii) into both the striatum and the cortex of the brain of the patient; (d) the medicament is suitable for administration to a human; or (e) any combination of (a)-(d).

In another exemplary embodiment, the present disclosure is directed to a self-inactivating CRISPR/Cas9 delivery system comprising: a first viral vector comprising an expression unit for expression of a Cas9 nuclease and a nucleotide sequence encoding a first sgRNA targeting a specific genomic locus; and a second viral vector comprising a nucleotide sequence encoding a second sgRNA targeting expression of the Cas9 nuclease by the expression unit, wherein the specific genomic locus comprises a nucleotide sequence that (i) is located in a gene and/or (ii) is located in a regulatory region of the gene; wherein the gene (i) causes, (ii) exacerbates, (iii) is otherwise associated with, or (iv) any combination of (i)-(iii), one or more disease and/or disorder; and wherein (i) the disease and/or disorder is Huntington's disease, (ii) the gene is the Huntingtin gene (HTT), or (iii) both (i) and (ii).

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments and/or further exemplary embodiments, the nucleotide sequence encoding the first sgRNA targeting a specific genomic locus comprises (i) a nucleotide sequence according to SEQ ID NO. 1 or SEQ ID NO. 2, (ii) a nucleotide sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, or at least 99% identity with the nucleotide sequence of

SEQ ID NO. 1 and/or SEQ ID NO. 2 and having the same function as the nucleotide sequence of SEQ ID NO: 1 and/or SEQ ID NO: 2, (iii) a nucleotide sequence having at most three base pairs different from, at most two base pairs different from, or at most one base pair different from of either or both of the nucleotide sequence of SEQ ID NO. 1 or SEQ ID NO. 2 and having the same function as the nucleotide sequence of SEQ ID NO: 1 and/or SEQ ID NO: 2, or (iv) a nucleotide sequence obtained from the nucleotide sequence of SEQ ID NO: 1 or SEQ ID NO: 2 by deletion, substitution or addition of 1, 2, 3, 4, 5 or 6 base pairs and having the same function as the nucleotide sequence of SEQ ID NO: 1 and/or SEQ ID NO: 2.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments and/or further exemplary embodiments, the nucleotide sequence encoding the first sgRNA targeting a specific genomic locus comprises a nucleotide sequence according to SEQ ID NO. 1 or SEQ ID NO. 2.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments and/or further exemplary embodiments, (i) the gene and/or regulatory region is a mutated gene and/or regulatory region; (ii) the gene and/or regulatory region is a wild type gene and/or regulatory region; (iii) the specific genomic locus is located in a regulatory region of the gene; (iv) the specific genomic locus is located in an intron of the gene; (v) the specific genomic locus is located in an exon of the gene; (vi) one or more disruption and/or cleavage of the specific genomic locus causes reduction or elimination of expression of the gene; and/or (vii) one or more disruption and/or cleavage of the specific genomic locus causes truncation of the protein expressed by the gene.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments and/or further exemplary embodiments, the sgRNA targeting expression of the Cas9 nuclease by the expression unit is encoded by a nucleotide sequence comprising (i) a nucleotide sequence selected from SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, and SEQ ID NO: 12, (ii) a nucleotide sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, or at least 99% identity with one or more of the nucleotide sequence of SEQ ID NO: 3 to SEQ ID NO. 12 and having the same function as one or more of the nucleotide sequences of SEQ ID NO: 3 to SEQ ID NO: 12, (iii) a nucleotide sequence having at most three base pairs different from, at most two base pairs different from, or at most one base pair different from one or more of

the nucleotide sequence of SEQ ID NO: 3 to SEQ ID NO. 12 and having the same function as one or more of the nucleotide sequences of SEQ ID NO: 3 to SEQ ID NO: 12, or (iv) a nucleotide sequence obtained from the nucleotide sequence of one of SEQ ID NO: 3 to SEQ ID NO: 12 by deletion, substitution, or addition of 1, 2, 3, 4, 5 or 6 bases and having the same function as one or more of the nucleotide sequences of SEQ ID NO: 3 to SEQ ID NO: 12.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments and/or further exemplary embodiments, the sgRNA targeting expression of the Cas9 nuclease by the expression unit is encoded by a nucleotide sequence comprising a nucleotide sequence selected from the nucleotide sequences according to SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, and SEQ ID NO: 12.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments and/or further exemplary embodiments, the first and second viral vectors are selected from AAV, lentiviral vectors, adenovirus vectors, herpesvirus vectors, poxvirus vectors, baculovirus vectors, and papillomavirus vectors.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments and/or further exemplary embodiments, the first and second viral vectors are selected from AAV vectors.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments and/or further exemplary embodiments, the first and second viral vectors are selected from AAV1, AA2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV PHP.B and AAV PHP.eB.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments and/or further exemplary embodiments, the first and second viral vectors are not AAV8 viral vectors.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments and/or further exemplary embodiments, the first and second viral vectors are AAV9, AAV PHP.B, or AAV PHP.eB viral vectors.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments and/or further exemplary embodiments, the first and second viral vectors are AAV9 viral vectors.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments and/or further exemplary embodiments, (i) the first viral vector further comprises a CMV promoter to drive expression of the Cas9 nuclease; (ii) the first viral vector further comprises a U6 promoter to drive transcription of the first sgRNA, (iii) the second viral vector further comprises a U6 promoter to drive transcription of the second sgRNA, or (iv) any combination thereof.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments and/or further exemplary embodiments, the second viral vector does not include a nucleotide sequence encoding a reporter gene.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments and/or further exemplary embodiments, the second viral vector does include a nucleotide sequence encoding a reporter gene.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments and/or further exemplary embodiments, the Cas9 nuclease is one or more of SaCas9 nuclease, SpCas9 nuclease, StCas9 nuclease, CjCas9 nuclease, or NmCas9 nuclease.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments and/or further exemplary embodiments, the Cas9 nuclease is SaCas9 nuclease.

In another exemplary embodiment, the present disclosure is directed to a pharmaceutical composition comprising (i) one or more self-inactivating CRISPR/Cas9 delivery system according to any of the described exemplary embodiments and/or further exemplary embodiments and (ii) one or more pharmaceutical acceptable carrier and/or excipient.

In another exemplary embodiment, the present disclosure is directed to a method for treating a disease and/or disorder, the method comprising: administering to a patient

having the disease and/or disorder (i) one or more effective amount of one or more self-inactivating CRISPR/Cas9 delivery system according to any of the described exemplary embodiments and/or further exemplary embodiments, (ii) one or more effective amount of a pharmaceutical composition according one or more self-inactivating CRISPR/Cas9 delivery system according to any of the described exemplary embodiments and/or further exemplary embodiments, or (iii) both (i) and (ii).

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments and/or further exemplary embodiments, an effective amount comprises a dosage of 10^9 to 10^{14} viral genomes or infectious units of viral vectors per dose.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments and/or further exemplary embodiments, an effective amount comprises a dosage of 10^{10} to 10^{12} viral genomes or infectious units of viral vector per dose.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments and/or further exemplary embodiments, wherein administration comprises stereotaxic injection (i) into the striatum of the brain of the patient, (ii) into the cortex of the brain of the patient, or (iii) into both the striatum and the cortex of the brain of the patient.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments, a molar ratio of the first vector to the second vector is selected (i) to maximize one or more beneficial and/or desired physiological effect, (ii) to minimize one or more detrimental and/or undesired physiological effect, or (iii) both (i) and (ii), optionally wherein

(a) the selection comprises: (i)(A) administering to one or more first mouse the first vector and the second vector at a first molar ratio of the first vector to the second vector, the first mouse being a model of a disease, a disorder, or both and/or (A') administering to one or more first population of cells the first vector and the second vector at a first molar ratio of the first vector to the second vector; (ii) and measuring one or more first physiological effect of administering the first molar ratio; (iii)(A) administering to one or more second mouse the first vector and the second vector at a second molar ratio of the first vector to the second vector, the second mouse being a model of the disease, the disorder, or both and/or (A') administering to

one or more second population of cells the first vector and the second vector at a second molar ratio of the first vector to the second vector; (iv) measuring one or more second physiological effect of administering the second molar ratio; (v) optionally repeating (a)(i)-(a)(ii) one or more additional times at one or more additional molar ratio, wherein each of the one or more additional molar ratio is independently different from the first molar ratio and the second molar ratio; and (v) selecting a molar ratio from the first molar ratio, the second molar ratio, and optionally one or more additional molar ratio, to produce a selected molar ratio, wherein the selected molar ratio is a molar ratio the administration of which (i) is effective to produce one or more beneficial and/or desired physiological effect and (ii) produces less detrimental and/or fewer detrimental and/or undesired physiological effects than one or more molar ratio that is not selected;

(b) maximizing one or more beneficial and/or desired physiological effect is accomplished by maximizing editing of one or more on-target genomic locus;

(c) minimizing one or more detrimental and/or undesired physiological effect is accomplished by eliminating or reducing editing of one or more off-target genomic locus; or

(d) any combination of (a)-(c).

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments and/or further exemplary embodiments, the patient is human.

In another exemplary embodiment, the present disclosure is directed to one or more self-inactivating CRISPR/Cas9 delivery system according to any of the described exemplary embodiments and/or further exemplary embodiments formulated for use in treatment of Huntington's disease.

In another exemplary embodiment, the present disclosure is directed to one or more self-inactivating CRISPR/Cas9 delivery system according to any of the described exemplary embodiments and/or further exemplary embodiments formulated for use in treatment of the Huntington's disease, wherein (a) the formulation comprises a dosage of 10^9 to 10^{14}, or 10^{10} to 10^{12} viral genomes or infectious units of viral vectors per dose; (b) the formulation comprises a molar ratio of the first vector to the second vector, wherein the molar ratio is selected (i) to maximize one or more beneficial and/or desired physiological effect, (ii) to minimize one or more detrimental and/or undesired physiological effect, or (iii) both (i) and (ii); (c) the formulation is suitable for stereotaxic injection (i) into the striatum of the brain of the patient, (ii) into the

cortex of the brain of the patient, or (iii) into both the striatum and the cortex of the brain of the patient; (d) the formulation is suitable for administration to a human; or (e) any combination of (a)-(d).

In another exemplary embodiment, the present disclosure is directed to a medicament comprising one or more self-inactivating CRISPR/Cas9 delivery system according to any of the described exemplary embodiments and/or further exemplary embodiments.

In another exemplary embodiment, the present disclosure is directed to a medicament comprising one or more self-inactivating CRISPR/Cas9 delivery system according to any of the described exemplary embodiments and/or further exemplary embodiments for use in treatment of Huntington's disease, wherein (a) the medicament comprises a dosage of 10^9 to 10^{14}, or 10^{10} to 10^{12} viral genomes or infectious units of viral vectors per dose; (b) the medicament comprises a molar ratio of the first vector to the second vector, wherein the molar ratio is selected (i) to maximize one or more beneficial and/or desired physiological effect, (ii) to minimize one or more detrimental and/or undesired physiological effect, or (iii) both (i) and (ii); (c) the medicament is suitable for stereotaxic injection (i) into the striatum of the brain of the patient, (ii) into the cortex of the brain of the patient, or (iii) into both the striatum and the cortex of the brain of the patient; (d) the medicament is suitable for administration to a human; or (e) any combination of (a)-(d).

In another exemplary embodiment, the present disclosure is directed to a self-inactivating CRISPR/Cas9 delivery system comprising: a first viral vector comprising an expression unit for expression of a saCas9 nuclease and a nucleotide sequence encoding a first sgRNA targeting a specific genomic locus; and a second viral vector comprising a nucleotide sequence encoding a second sgRNA targeting expression of the saCas9 nuclease by the expression unit, wherein the specific genomic locus comprises a nucleotide sequence that (i) is located in a gene and/or (ii) is located in a regulatory region of the gene, wherein the gene (i) causes, (ii) exacerbates, (iii) is otherwise associated with, or (iv) any combination of (i)-(iii), one or more disease and/or disorder; and wherein (i) the disease/and or disorder is Huntington's disease, (ii) the gene is the Huntingtin gene (HTT), or (iii) both (i) and (ii).

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments and/or further exemplary embodiments, the nucleotide sequence encoding the first sgRNA targeting a specific genomic locus comprises (i) a nucleotide sequence according to SEQ ID NO. 1 or SEQ ID NO. 2, (ii)

a nucleotide sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, or at least 99% identity with the nucleotide sequence of SEQ ID NO. 1 and/or SEQ ID NO. 2 and having the same function as the nucleotide sequence of SEQ ID NO: 1 and/or SEQ ID NO: 2, (iii) a nucleotide sequence having at most three base pairs different from, at most two base pairs different from, or at most one base pair different from of either or both of the nucleotide sequence of SEQ ID NO. 1 or SEQ ID NO. 2 and having the same function as the nucleotide sequence of SEQ ID NO: 1 and/or SEQ ID NO: 2, or (iv) a nucleotide sequence obtained from the nucleotide sequence of SEQ ID NO: 1 or SEQ ID NO: 2 by deletion, substitution or addition of 1, 2, 3, 4, 5 or 6 base pairs and having the same function as the nucleotide sequence of SEQ ID NO: 1 and/or SEQ ID NO: 2.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments and/or further exemplary embodiments, the nucleotide sequence encoding the first sgRNA targeting a specific genomic locus comprises a nucleotide sequence according to SEQ ID NO. 1 or SEQ ID NO. 2.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments and/or further exemplary embodiments, (i) the gene and/or regulatory region is a mutated gene and/or regulatory region; (ii) the gene and/or regulatory region is a wild type gene and/or regulatory region, (iii) the specific genomic locus is located in a regulatory region of the gene, (iv) the specific genomic locus is located in an intron of the gene; (v) the specific genomic locus is located in an exon of the gene; (vi) one or more disruption and/or cleavage of the specific genomic locus causes reduction or elimination of expression of the gene; and/or (vii) one or more disruption and/or cleavage of the specific genomic locus causes truncation of the protein expressed by the gene.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments and/or further exemplary embodiments, the sgRNA targeting expression of the saCas9 nuclease by the expression unit is encoded by a nucleotide sequence comprising (i) a nucleotide sequence selected from SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, and SEQ ID NO: 12, (ii) a nucleotide sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, or at least 99% identity with one or more of the nucleotide sequence of SEQ ID NO: 3 to SEQ ID NO. 12 and having the same function as one or more of the nucleotide sequences of SEQ ID NO: 3 to SEQ ID NO:

12, (iii) a nucleotide sequence having at most three base pairs different from, at most two base pairs different from, or at most one base pair different from one or more of the nucleotide sequence of SEQ ID NO: 3 to SEQ ID NO. 12 and having the same function as one or more of the nucleotide sequences of SEQ ID NO: 3 to SEQ ID NO: 12, or (iv) a nucleotide sequence obtained from the nucleotide sequence of one of SEQ ID NO: 3 to SEQ ID NO: 12 by deletion, substitution, or addition of 1, 2, 3, 4, 5 or 6 bases and having the same function as one or more of the nucleotide sequences of SEQ ID NO: 3 to SEQ ID NO: 12.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments and/or further exemplary embodiments, the sgRNA targeting expression of the saCas9 nuclease by the expression unit is encoded by a nucleotide sequence comprising a nucleotide sequence selected from the nucleotide sequences according to SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, and SEQ ID NO: 12.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments and/or further exemplary embodiments, the first and second viral vectors are selected from AAV, lentiviral vectors, adenovirus vectors, herpesvirus vectors, poxvirus vectors, baculovirus vectors, and papillomavirus vectors.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments and/or further exemplary embodiments, the first and second viral vectors are selected from AAV vectors.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments and/or further exemplary embodiments, the first and second viral vectors are selected from AAV1, AA2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV PHP.B and AAV PHP.eB.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments and/or further exemplary embodiments, (i) the first and second viral vectors are not AAV8 viral vectors, (ii) the first and second viral vectors are AAV9, AAV PHP.B, or AAV PHP.eB viral vectors, or (iii) both (i) and (ii).

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments and/or further exemplary embodiments, the first and second viral vectors are AAV9 viral vectors.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments and/or further exemplary embodiments, (i) the first viral vector further comprises a CMV promoter to drive expression of the Cas9 nuclease; (ii) the first viral vector further comprises a U6 promoter to drive transcription of the first sgRNA, (iii) the second viral vector further comprises a U6 promoter to drive transcription of the second sgRNA, or (iv) any combination thereof.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments and/or further exemplary embodiments, the second viral vector does not include a nucleotide sequence encoding a reporter gene.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments and/or further exemplary embodiments, the second viral vector does include a nucleotide sequence encoding a reporter gene.

In another exemplary embodiment, the present disclosure is directed to a pharmaceutical composition comprising one or more self-inactivating CRISPR/Cas9 delivery system according to any of the described exemplary embodiments and/or further exemplary embodiments and one or more pharmaceutical acceptable carrier and/or excipient.

In another exemplary embodiment, the present disclosure is directed to a method for treating a Huntington's Disease, the method comprising: administering to a patient having the disease and/or disorder one or more effective amount of one or more self-inactivating CRISPR/Cas9 delivery system according to any of the described exemplary embodiments and/or further exemplary embodiments.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments and/or further exemplary embodiments, an effective amount comprises a dosage of 10^9 to 10^{14} viral genomes or infectious units of viral vectors per dose.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments and/or further exemplary embodiments, an effective amount comprises a dosage of 10^{10} to 10^{12} viral genomes or infectious units of viral vector per dose.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments and/or further exemplary embodiments, administration comprises stereotaxic injection (i) into the striatum of the brain of the patient, (ii) into the cortex of the brain of the patient, or (iii) into both the striatum and the cortex of the brain of the patient.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments and/or further exemplary embodiments, the patient is human.

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments, a method for treating a Huntington's Disease is provided, the method comprising: administering to a patient having the disease and/or disorder one or more effective amount of one or more self-inactivating CRISPR/Cas9 delivery system according to any one of claims 73 to 75; wherein administration of one or more the one or more effective amount comprises administration of the first vector and the second vector at a selected molar ratio of the first vector to the second vector; wherein the selected molar ratio (i) maximizes one or more beneficial and/or desired physiological effect, as compared to one or more ratio that is not selected, (ii) minimizes one or more detrimental and/or undesired physiological effect, as compared to one or more ratio that is not selected, or (iii) both (i) and (ii).

In further embodiments of the present disclosure, including further embodiments of the above or below exemplary embodiments,

(a) selection of a selected molar ratio comprises: (i)(A) administering to one or more first mouse the first vector and the second vector at a first molar ratio of the first vector to the second vector, the first mouse being a model of a disease, a disorder, or both and/or (A') administering to one or more first population of cells the first vector and the second vector at a first molar ratio of the first vector to the second vector; (ii) and measuring one or more first physiological effect of administering the first molar ratio; (iii)(A) administering to one or more second mouse the first vector and the second vector at a second molar ratio of the first vector to the second vector,

the second mouse being a model of the disease, the disorder, or both and/or (A') administering to one or more second population of cells the first vector and the second vector at a second molar ratio of the first vector to the second vector; (iv) measuring one or more second physiological effect of administering the second molar ratio; (v) optionally repeating (a)(i)-(a)(ii) one or more additional times at one or more additional molar ratio, wherein each of the one or more additional molar ratio is independently different from the first molar ratio and the second molar ratio; and (v) selecting a molar ratio from the first molar ratio, the second molar ratio, and optionally one or more additional molar ratio, to produce a selected molar ratio, wherein the selected molar ratio is a molar ratio the administration of which (i) is effective to produce one or more beneficial and/or desired physiological effect and (ii) produces less detrimental and/or fewer detrimental and/or undesired physiological effects than one or more molar ratio that is not selected;

(b) maximizing one or more beneficial and/or desired physiological effect is accomplished by maximizing editing of one or more on-target genomic locus;

(c) minimizing one or more detrimental and/or undesired physiological effect is accomplished by eliminating or reducing editing of one or more off-target genomic locus; or

(d) any combination of (a)-(c).

Combination of the above embodiments in any number, order, or combination is contemplated.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows schematic representations of vector 1 and vector 2 exemplary embodiments according to the invention. The exemplary embodiment of vector 1 includes CMV (cytomegalovirus)-driven nucleotide sequence encoding SaCas9 and U6-driven nucleotide sequence encoding sgRNA targeting HTT gene, between two ITRs (inverted terminal repeats). In vector 1 "NLS" refers to a nuclear localization signal sequence and "HA" refers to the glycol protein human influenza hemagglutinin. The first exemplary embodiment of vector 2 includes CMV (cytomegalovirus)-driven nucleotide sequence encoding EGFP (reporter gene) and U6-driven nucleotide sequence encoding sgRNA targeting Cas9, between two ITRs (inverted terminal repeats). The exemplary second embodiment of vector 2 includes CMV(cytomegalovirus)-driven nucleotide sequence encoding mCherry (reporter gene) and U6-driven nucleotide sequence encoding sgRNA targeting Cas9, between two ITRs. The third exemplary embodiment of vector 2 includes U6-driven

nucleotide sequence encoding sgRNA targeting Cas9, between two ITRs, without any reporter gene.

Figure 2 shows results of Western blot tests for SaCas9 and HTT using SaCas9-gRNAs (sgRNA1 to sgRNA10) according to the invention in HEK293T cells.

Figures 3A and 3B illustrate results of HD mice (mouse model BAC226Q) injected with AAV9-SaCas9-HTTg1/gCas9 or AAV9-SaCas9-HTTg1 (control group: AAV9-SaCas9). Fig. 3A: fluorescent micrograph of brain samples (scale bar: 50 μm). Fig. 3B: graphical representation of quantitative analysis of fluorescence intensity of Fig. 3A and mHTT aggregation signals.

Figure 4A and 4B presents graphical representations of sequential timepoints Motor phenotype analysis of 1st-month-old virus injected BAC226Q mice.

DETAILED DESCRIPTION OF THE INVENTION

In general, this disclosure provides a self-inactivating CRISPR/Cas9 system in the form of a dual vector system that includes (a) a first vector that comprises (i) one or more nucleic acid sequence that provides for expression of a single guide RNA (sgRNA) that targets a specific genomic locus and (ii) one or more nucleic acid sequence that provides Cas9 endonuclease expression and (b) a second vector that comprises one or more nucleic acid sequence that provides for expression of a single guide RNA that targets Cas9 endonuclease. The Cas9 endonuclease may be SaCas9 (a SaCas9 from *Staphylococcus aureus*).

The specific genomic locus may be located in a gene (or its regulatory region) that causes, exacerbates, and/or is otherwise associated with one or more gene-associated disease and/or gene-associated disorder. Methods of using the system to treat (e.g., cure, ameliorate, lessen one or more symptoms of, delay the onset of, delay the morbidity of, and/or delay the mortality of) one or more gene-associated disease and/or gene-associated disorder may be provided. Formulations, medicaments, and methods of making and using them may be provided. Editing of one or more genomic locus intended to be edited may be referred to as “on-target” editing. Editing of one or more genomic locus not intended to be edited may be referred to as “off-target” editing.

As used herein, a “gene-associated disease” and/or “gene-associated disorder” (which may be referred to collectively as “gene-associated diseases/disorders”) is (i) a disease and/or disorder that is caused by, exacerbated by, and/or otherwise associated with the expression of one or more genes and/or (ii) a disease and/or disorder wherein alteration of the expression of one or more genes can eliminate or alleviate one or more symptoms of the disease and/or disorder. “Alteration of expression” can include, for example, (i) one or more alternation to the expression of one or more expression product (e.g., protein, non-coding RNA) expressed by the one or more gene and/or (ii) one or more alternation to one or more characteristics of one or more expression product (e.g., protein, non-coding RNA) expressed by the one or more gene and/or. As non-limiting examples, “alteration of expression” can include, for example, one or more of: increase in expression of one or more expression product, decrease in expression of one or more expression product, elimination of expression of one or more expression product, alteration of one or more characteristic of one or more expression product (e.g., one or more of truncation one or more expression product, alteration to one or more post-translational modification of one or more expression product, alteration of the trafficking of one or more expression product, alteration of the locations in which one or more expression product is expressed (alteration(s) in expression pattern), and/or inappropriate proteolysis of one or more expression product, leading to its over- or underabundance).

As used herein, a “genetic disease” and/or or “genetic disorder” is a gene-associated disease and or a gene-associated disorder resulting from one or more DNA-level abnormality (*e.g.,* one or more DNA-level mutation) in a genome. The abnormality can be inherited or acquired, and when present usually exhibits clear inheritance. Genetic diseases/disorders, as non-limiting examples, (a) can be caused by mutations in a single gene and/or regulatory region (monogenic disorders) through, e.g., one or more point mutations, deletions, truncations, and/or duplication of a gene and/or one or more of its regulatory regions (b) can be caused by a combination of factors such as, as non-limiting examples, (i) one or more mutations in each of multiple (e.g., two or more) genes and/or one mutations in each of multiple (e.g., two or more) regulatory regions or (ii) a combination of one or more genetic mutations and environmental factors (complex or multifactorial disorders), or (c) can be caused by damage to chromosomes. A non-limiting example of a genetic disease is Huntington’s disease, in which a mutation in the Huntingtin gene (HTT) causes expression of abnormal protein, which causes disease.

Gene-associated diseases/disorders caused by, exacerbated by, and/or otherwise associated with one or more gene and/or regulatory region, in which the gene or regulatory region does comprise one or more DNA-level mutation (which usually exhibit clear inheritance) may be referred to as "genetic gene-associated diseases", "genetic gene-associated disorders", "genetic diseases", "genetic disorders", a "genetic form of a disease", a "genetic form of a disorder", and/or the like.

In some cases, certain genes without one or more DNA-level mutation to the gene and or its regulatory region(s) can still be the causes of diseases/disorders via other means. For example, a certain gene "X" which does not comprise a DNA-level mutation in itself or its regulatory region(s) can still cause, exacerbate, or otherwise be associated with a disease and/or disorder. Reasons for this could include, as non-limiting examples, (a) one or more aberration (e.g., DNA-level mutation) in one or more different gene "A" and/or gene A's regulatory regions(s), wherein one or more expression product of gene A affects gene X, gene X's regulatory region(s), or some characteristic of gene X's expression product(s) and/or (b) interaction of one or more more of gene X, gene X's regulatory region(s), or some characteristic of gene X's expression product(s) with one or more environmental factors, where environmental factors may include, for example, factors external to the affected individual and/ or factors internal to the affected individual, such as, as non-limiting examples, the affected individual's microbiome, a disease microenvironment (e.g., tumor microenvironment) present in the affected individual, or the like.

Gene-associated diseases/disorders caused by, exacerbated by, and/or otherwise associated with one or more gene and/or regulatory region, in which the gene or regulatory region does not comprise a DNA-level mutation (which usually do not exhibit clear inheritance) may be referred to as "sporadic gene-associated diseases", "sporadic gene-associated disorders", "sporadic diseases", "sporadic disorders", a "sporadic form of a disease", a "sporadic form of a disorder", and/or the like.

Some gene-associated diseases and/or gene-associated disorders can be both genetic and sporadic. Parkinson's disease and Alzheimer's disease are non-limiting examples of diseases that can be genetic and/or sporadic. Parkinson's disease can be genetic and/or sporadic, and the alpha-synuclein gene is causative for both types of Parkinson's disease. Alzheimer's disease can also be genetic and/or sporadic, and the APP gene is causative for both types of Alzheimer's disease. In these examples, overexpression and accumulation of wild type alpha-synuclein causes Parkinson's disease pathogenesis; while abnormal proteolysis and accumulation of wild type APP

causes Alzheimer's disease pathogenesis. These are just two non-limiting examples of mechanisms by which wild type genes can still be responsible for gene-associated diseases/disorders.

Systems, processes, methods, and/or products disclosed herein may, as non-limiting examples, be targeted towards one or more genetic forms of a gene-associated disease/disorder and/or towards one or more sporadic forms of a gene-associated disease/disorder. Systems, processes, methods, and/or products disclosed herein may, as non-limiting examples, be used in treatment of one or more genetic form of a gene-associated disease/disorder and/or be used in treatment of one or more sporadic form of a gene-associated disease/disorder. Processes disclosed herein may, as non-limiting examples, (i) be directed to producing one or more system and/or product disclosed herein and/or (ii) be targeted towards treating one or more genetic form of a gene-associated disease/disorder and/or towards treating one or more sporadic form of a gene-associated disease/disorder.

Examples of monogenic genetic disorders include cystic fibrosis, myotonic dystrophy, spinal muscular dystrophy, alpha- thalassemia, beta-thalassemia, sickle cell anemia, chronic granulomatous disease Canavan disease, Fanconi anemia, Marfan syndrome, retinitis pigmentosa, fragile X syndrome, Gaucher disease, Huntington's disease, hemochromatosis, congenital deafness, Duchenne muscular dystrophy, familial hypercholesterolemia, Farber disease, Neurofibromatosis type 1, Neurofibromatosis t Becker muscular dystrophy type II, hemophilia A, hemophilia B, Tay-Sachs disease, Wiskott-Aldrich syndrome, Hurler syndrome, Hunter syndrome, Purine nucleoside phosphorylase deficiency, Fabry disease, Pompe disease, Gyrate atrophy, Krabbe disease, Wolman disease, Amyotrophic lateral sclerosis, Spinocerebellar Ataxia, and Sanfilippo disease.

Examples of complex or multifactorial disorders include birth defects, breast cancer, prostate cancer, skin cancer, high blood pressure, high cholesterol, diabetes, Alzheimer disease, schizophrenia, Parkinson's disease, Frontotemporal dementia, and bipolar disorder.

Numerous genetic diseases/disorders and disease-associated genes including signaling biochemical pathway-associated genes are listed in Tables A, B, and C of US 11,708,588, hereby incorporated by reference.

One genetic disease for which CRISPR/Cas technology could lead to beneficial and effective treatment is Huntington's disease (HD). See, e.g., WO 2016/020399. HD is

an inherited neurodegenerative disorder, characterized by loss of striatal neurons, which causes the progressive breakdown of nerve cells in the brain resulting in movement disorders. These movement disorders include both involuntary and voluntary movements, such as jerking movements, rigidity, muscle contracture, abnormal eye movements, impaired walking, posture and/or balance, and difficulty with speech or swallowing. Huntington's disease can further cause cognitive disorders such as difficulty organizing/focusing on tasks, perseveration, and lack of impulse control.

Embodiments of the invention may include a self-inactivating CRISPR/Cas9 system that utilizes a dual vector system (e.g., a dual AAV vector system). The system includes (i) a first vector that comprises DNA that may be transcribed to (encoding) a first single guide RNA (sgRNA) targeting a specific genomic locus (for example, the HTT gene (HTTg1)) and that delivers Cas9 (e.g., SaCas9) expression, e.g., by comprising a nucleotide sequence encoding the Cas9, and (ii) a second vector that comprises DNA that may be transcribed to (encoding) a second sgRNA targeting the gene encoding the Cas9 (e.g., SaCas9), i.e., guide Cas9 or gCas9. The system components are packaged and implemented through dual vectors. A first vector delivers Cas9 and sgRNA against the target gene and the second vector delivers sgRNA against Cas9.

Upon deployment of the dual AAV vector system, the sgRNA targeting the specific genomic locus (e.g., HTTg1) is transcribed and binds to the target gene and Cas9 begins to be expressed, leading to destruction of the target gene. Excessive Cas9 expression is then disrupted simultaneously by the sgRNA targeting Cas9 (e.g., SaCas9), i.e., gCas9. The gCas9 targets the nucleotide sequence encoding Cas9 in the region encoding the N terminus of Cas9 RuvC-I domain, which plays crucial role in nuclease activity, thereby disrupting expression of Cas9 and rendering the expression of Cas9 transient. As a result, this self-inactivating CRISPR/Cas9 system attenuates Cas9 expression time, resulting in reduction or complete elimination of off-target events.

In embodiments, the self-inactivating CRISPR/Cas9 system according to the invention may offer advantages in comparison to other attempts to provide for self-inactivation. In comparison, for example, (i) to a system wherein two sgRNAs are delivered by one vector and Cas9 alone is delivered by another vector and/or (ii) to a system wherein two sgRNAs and Cas9 are delivered by single vector, the self-inactivating CRISPR/Cas9 system according to the invention may provide more control over one

or both (i) efficient editing of the genomic target gene and (i) expression control of Cas9 nuclease by providing the ability to adjust the molar ratio between the two vectors, where each vector delivers a different sgRNA.Further, in systems where the gRNA for the target gene and the gRNA for Cas9 are packaged together with Cas9 in a single vector, there is a disadvantage Cas9 itself can face the risk of being knocked out during plasmid passaging and viral packaging. As a result, other molecules may be used to suppress Cas9 expression, such as using TetR to suppress expression of Cas9 driven by CMV-TetO or anti-CRISPR molecules to block self-targeting. However, such approaches may introduce unnecessary molecules or cause unnecessary complications.

According to a first aspect, the invention is directed to a self-inactivating CRISPR/Cas9 delivery system comprising:

a first viral vector having an expression unit for expression of a Cas9 nuclease and a nucleotide sequence encoding an sgRNA targeting a specific genomic locus; and

a second viral vector having a nucleotide sequence encoding an sgRNA targeting expression of the Cas9 nuclease by the expression unit.

According to a further aspect, the invention is directed to a self-inactivating CRISPR/Cas9 delivery system comprising:

a first viral vector having an expression unit for expression of a Cas9 nuclease and a nucleotide sequence encoding an sgRNA targeting a specific genomic locus; and

a second viral vector having a nucleotide sequence encoding an sgRNA targeting expression of the Cas9 nuclease by the expression unit,

wherein said first viral vector further comprises a CMV promoter to drive expression the Cas9 nuclease.

According to a further aspect, the invention is directed to a self-inactivating CRISPR/Cas9 delivery system comprising:

a first viral vector having an expression unit for expression of a Cas9 nuclease and a nucleotide sequence encoding an sgRNA targeting a specific genomic locus; and

a second viral vector having a nucleotide sequence encoding an sgRNA targeting expression of the Cas9 nuclease by the expression unit,

wherein said second viral vector does not include a nucleotide sequence encoding a reporter gene.

According to a further aspect, the invention is directed to a self-inactivating CRISPR/Cas9 delivery system comprising:

a first viral vector having an expression unit for expression of a Cas9 nuclease and a nucleotide sequence encoding an sgRNA targeting a specific genomic locus; and

a second viral vector having a nucleotide sequence encoding an sgRNA targeting expression of the Cas9 nuclease by the expression unit,

wherein said first and second viral vectors are not AAV8 viral vectors, and preferably are AAV9, AAV PHP.B, or AAV PHP.eB viral vectors, particularly AAV9 viral vectors.

According to a further aspect, the invention is directed to a self-inactivating CRISPR/Cas9 delivery system comprising:

a first viral vector having an expression unit for expression of a Cas9 nuclease and a nucleotide sequence encoding an sgRNA targeting a specific genomic locus; and

a second viral vector having a nucleotide sequence encoding an sgRNA targeting expression of the Cas9 nuclease by the expression unit,

wherein the sgRNA targeting expression of the Cas9 nuclease by the expression unit is encoded by a nucleotide sequence comprising (i) a nucleotide sequence selected from the nucleotide sequences according to SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, and SEQ ID NO: 12, (ii) a nucleotide sequence having at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology with one or more of the nucleotide sequence of SEQ ID NO: 3 to SEQ ID NO. 12 and having the same function as one or more of the nucleotide sequences of SEQ ID NO: 3 to SEQ ID NO: 12, (iii) a nucleotide sequence having at most three base pairs different from, at most two base pairs different from, or at most one base pair different from one or more of the nucleotide sequence of SEQ ID NO: 3 to SEQ ID NO. 12 and having the same function as one or more of the nucleotide sequences of SEQ ID NO: 3 to SEQ ID NO: 12, or (iv) a nucleotide sequence obtained from the nucleotide sequence of one of SEQ ID NO: 3 to SEQ ID NO: 12 by deletion, substitution, or addition of 1, 2, 3, 4, 5 or 6 bases and having the same function as one or more of the nucleotide sequences of SEQ ID NO: 3 to SEQ ID NO: 12, and

the specific genomic locus is selected from a gene causing, exacerbating, or otherwise related to a neurodegenerative disease, wherein preferably the gene is expressed at least in the CNS, and wherein preferably the neurodegenerative disease is selected from Huntington's disease, Alzheimer's disease, Parkinson's disease, Amyotrophic Lateral Sclerosis (ALS), Frontotemporal dementia (FTD) (aka Pick's disease), spinocerebellar ataxi (SCA), e.g., types 1 to 17, Retinitis Pigmentosa, or Age-related macular degeneration (AMD), particularly Huntington's disease.

In accordance with this disclosure, the term "% homology" is equivalent to the term "% identity".

According to a further aspect, the invention is directed to any of the self-inactivating CRISPR/Cas9 delivery system described above, wherein the Cas9 nuclease is SaCas9.

According to a further aspect, the invention is directed to any of the self-inactivating CRISPR/Cas9 delivery systems described above, wherein the two vectors are both AAV vectors, e.g. AAV9 vectors.

According to a further aspect, the invention is directed to any of the self-inactivating CRISPR/Cas9 delivery systems described above, wherein the specific genomic locus is selected from neurodegenerative disease related to genes in the central nervous system (CNS).

According to a further aspect, the invention is directed to any of the self-inactivating CRISPR/Cas9 delivery systems described above wherein the sgRNA targeting a specific genomic locus targets a gene associated with Huntington's disease, Alzheimer's disease, Parkinson's disease, Amyotrophic Lateral Sclerosis (ALS), Frontotemporal dementia (FTD) (aka Pick's disease), spinocerebellar ataxi (SCA), e.g., types 1 to 17, Retinitis Pigmentosa, or Age-related macular degeneration (AMD).

According to a further aspect, the invention is directed to any of the self-inactivating CRISPR/Cas9 delivery systems as described above, wherein the sgRNA targeting a specific genomic locus targets Huntingtin (HTT) gene.

According to a further aspect, the invention is directed to any of the self-inactivating CRISPR/Cas9 delivery systems as described above, wherein the nucleotide sequence encoding the sgRNA targeting a specific genomic locus comprises (i) a nucleotide sequence according to SEQ ID NO. 1 or SEQ ID NO. 2, (ii) a nucleotide sequence having at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology with the nucleotide sequence of SEQ ID NO. 1 and/or SEQ ID NO. 2 and having the same function as the nucleotide sequence of SEQ ID NO: 1 and/or SEQ ID NO: 2, (iii) a nucleotide sequence having at most three base pairs different from, at most two base pairs different from, or at most one base pair different from of either or both of the nucleotide sequence of SEQ ID NO. 1 or SEQ ID NO. 2 and having the same function as the nucleotide sequence of SEQ ID NO: 1 and/or SEQ ID NO: 2, or (iv) a nucleotide sequence obtained from

the nucleotide sequence of SEQ ID NO: 1 or SEQ ID NO: 2 by deletion, substitution or addition of 1, 2, 3, 4, 5 or 6 base pairs and having the same function as the nucleotide sequence of SEQ ID NO: 1 and/or SEQ ID NO: 2.

According to another aspect, the invention is directed to a pharmaceutical composition comprising a self-inactivating CRISPR/Cas9 delivery system according to any of the self-inactivating CRISPR/Cas9 delivery systems as described above and at least one pharmaceutical acceptable carrier and/or excipient.

According to another aspect, the invention is directed to a method for treating a genetic gene-associated disease/disorder and/or a sporadic gene-associated disease/disorder comprising administering to a patient in need thereof any of the self-inactivating CRISPR/Cas9 delivery systems as described above.

According to another aspect, the invention is directed to a method for treating a Huntington's disease comprising administering to a patient in need thereof any of the self-inactivating CRISPR/Cas9 delivery systems as described above.

In accordance with the invention, the Cas9 nuclease can be, for example, Cas9 from *Staphylococcus aureus* (SaCas9), from *Streptococcus pyogenes* (SpCas9), Cas9 from *Streptococcus thermophilus* (StCas9), Cas9 from *Campylobacter jejuni* (CjCas9), and/or Cas9 from *Neisseria meningitidis* (NmCas9). It is also possible that other Cas nucleases can be used, such as Cas12a, Cas12b, Cas12e, Cas12j, Cas12f1, Cas13a, and Cas14a. In each case, the sgRNA targeting the Cas nuclease must be able to effectively target and deactivate the particular Cas nuclease involved.

Species that produce naturally occurring Cas9 are described in Chylinski et al., RNA Biology 2013, 10:5, 727-737.

In embodiments in accordance with the invention, the vector carrying the nucleotide sequence encoding the single guide RNA (sgRNA) targeting HTT gene may comprise a nucleotide sequence selected from the sequences presented in the following Table.

Name	Sequence	SEQ ID NO:
HTTg1	5'-TGGAAAAGCTGATGAAGGCCT-3'	SEQ ID NO: 1
HTTg2	5'-GAAGGCCTTCATCAGCTTTTC-3'	SEQ ID NO: 2

In other embodiments in accordance with the invention, the nucleotide sequence encoding the sgRNA targeting HTT gene may comprise (i) a nucleotide sequence having at least 95% homology, at least 96% homology, at least 97% homology, at

least 98% homology, or at least 99% homology with the nucleotide sequence of SEQ ID NO. 1 and/or SEQ ID NO. 2 and having the same function as the nucleotide sequence of SEQ ID NO: 1 and/or SEQ ID NO: 2, (ii) a nucleotide sequence having at most three base pairs different from, at most two base pairs different from, or at most one base pair different from of either or both of the nucleotide sequence of SEQ ID NO. 1 or SEQ ID NO. 2 and having the same function as the nucleotide sequence of SEQ ID NO: 1 and/or SEQ ID NO: 2, or (iii) a nucleotide sequence obtained from the nucleotide sequence of SEQ ID NO: 1 or SEQ ID NO: 2 by deletion, substitution or addition of 1, 2, 3, 4, 5 or 6 base pairs and having the same function as the nucleotide sequence of SEQ ID NO: 1 and/or SEQ ID NO: 2.

In embodiments in accordance with the invention, the vector carrying the nucleotide sequence encoding the single guide RNA (sgRNA) targeting Cas9, particularly SaCas9, may comprise a nucleotide sequence selected from the sequences presented in the following Table.

Name	Sequence	SEQ ID NO:
gRNA1	5'-GGTGATGCCGATGTCCAGGCC	SEQ ID NO: 3
gRNA2	5'-GTGAGCCAGAAGCTGAGCGAGG	SEQ ID NO: 4
gRNA3	5'-GAGGTCCCTCATAGTAGGTCCG	SEQ ID NO: 5
gRNA4	5'-GCTCAGGATGAAGTCGTCCAC	SEQ ID NO: 6
gRNA5	5'-GTTGTTCAGCAGATCTTCCAG	SEQ ID NO: 7
gRNA6	5'-GACTTCATCAACCGGAACCTG	SEQ ID NO: 8
gRNA7	5'-GTGCCCGAGATCGAAACCGAGC	SEQ ID NO: 9
gRNA8	5'-GATGGAACAGTACGGCGACGAG	SEQ ID NO: 10
gRNA9	5'-GACGCCATTGTCCAGGTACACG	SEQ ID NO: 11
gRNA10	5'-GAACATGATCGACATCACCTACC	SEQ ID NO: 12

These sequences are described Li, et al., Molecular therapy: Methods & clinical development, 12, 111–122 (2018), describe a self-deleting AAV-CRISPR system.

In other embodiments in accordance with the invention, the vector carrying the nucleotide sequence encoding the single guide RNA (sgRNA) targeting Cas9, particularly SaCas9, comprises a nucleotide sequence comprising (i) a nucleotide sequence having at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology with one or more of the nucleotide sequences of SEQ ID NO: 3 to SEQ ID NO. 12 and having the same function as one or more of the nucleotide sequences of SEQ ID NO: 3 to SEQ ID NO: 12, (ii) a nucleotide sequence having at most three base pairs different from, at most two base pairs different from, or at most one base pair different from one or more of

the nucleotide sequence of SEQ ID NO: 3 to SEQ ID NO. 12 and having the same function as one or more of the nucleotide sequences of SEQ ID NO: 3 to SEQ ID NO: 12, or (iii) a nucleotide sequence obtained from the nucleotide sequence of one of SEQ ID NO: 3 to SEQ ID NO: 12 by deletion, substitution, or addition of 1, 2, 3, 4, 5 or 6 bases and having the same function as one or more of the nucleotide sequences of SEQ ID NO: 3 to SEQ ID NO: 12,

The viral vectors for use with the invention can be selected from AAV, lentiviral vectors, adenovirus vectors, herpesvirus vectors, poxvirus vectors, baculovirus vectors, and papillomavirus vectors.

The viral vectors for use in the invention are preferably adeno-associated (AAV) vectors, particularly AAV vectors of human origin. In particular, the AAV vector is selected from the group comprising serotypes AAV1, AA2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV PHP.B, and AAV PHP.eB. In a preferred situation, the AAV vector is not AAV8. In a further preferred embodiment, the AAV vector is AAV9, AAV PHP.B, or AAV PHP.eB, especially AAV9.

The invention further includes pharmaceutical compositions. In embodiments, a pharmaceutical composition according to the invention may comprise an exemplary self-inactivating CRISPR/Cas9 delivery system in accordance with the invention in the form of dual viral vectors, one or more pharmaceutically acceptable carrier, one or more optionally pharmaceutically acceptable diluents, one or more excipients known to those skilled in the art, and/or or combinations thereof.

Examples of pharmaceutically acceptable carriers include aqueous carries (e.g., water or salt solutions such as phosphate-buffered saline), alcohols, and polyethylene glycols. Pharmaceutically acceptable diluents/excipients can include, for example, wetting agents, emulsifying, and suspending agents, salt for influencing osmotic pressure, and/or buffers. For example, the diluent/excipient can be selected from one or more of polyethylene glycol, propylene glycol, vegetable oil, and mineral oil. In a specific example, the preservative is selected from one or more of sorbic acid, methyl sorbate, methyl paraben, ethyl paraben, propyl paraben, butyl paraben, benzyl paraben, sodium methylparaben, benzoic acid, and benzyl alcohol. In another one of the specific examples, the buffering agent may be selected from one or more of sodium hydrogen phosphate, sodium dihydrogen phosphate, sodium citrate, sodium tartrate, and sodium acetate. In another one of the specific examples, the disintegrant may be selected from one or more of cross-linked sodium carboxymethyl cellulose,

sodium carboxymethyl starch, cross-linked polyvinylpyrrolidone, or low-substituted hydroxypropyl cellulose. In another one of the specific examples, the antioxidant may be selected from one or more of ethylenediaminetetraacetic acid, ethylenediaminetetraacetic acid disodium salt, dibutylhydroxytoluene, glycine, inositol, ascorbic acid, sodium ascorbate, lecithin, malic acid, hydroquinone, citric acid, succinic acid, and sodium metabisulfite. In another one of the specific examples, the co-suspension agent may be selected from one or more of beeswax, ethyl hydroxyethyl cellulose, chitin, chitosan, methyl cellulose, carboxymethyl cellulose, agar, hydroxypropyl methyl cellulose, and xanthan gum. In another one of the specific examples, the colorant may be selected from one or more of carbon black, iron black, iron brown, iron red, and titanium dioxide. In another one of the specific examples, the excipient may be selected from one or more of mannitol, glucose, lactose, dextran, dextrose, and sodium chloride.

The invention further includes methods of treatment. Thus, the invention includes a method for treating a genetic gene-associated disease/disorder and/or a sporadic gene-associated disease/disorder comprising administering to a patient in need thereof an effective amount of any of the self-inactivating CRISPR/Cas9 delivery systems as described above.

According to one method embodiment, the invention includes a method for treating a genetic gene-associated neurodegenerative disease/disorder and/or a sporadic gene-associated neurodegenerative disease/disorder related to one or more genes in the CNS, comprising administering to a patient in need thereof an effective amount any of the self-inactivating CRISPR/Cas9 delivery systems as described above. According to another method embodiment, the invention includes a method for treating a neurodegenerative disease selected from Huntington's disease, Alzheimer's disease, Parkinson's disease, Amyotrophic Lateral Sclerosis (ALS), Frontotemporal dementia (FTD) (aka Pick's disease), spinocerebellar ataxi (SCA), particularly types 1 to 17, Retinitis Pigmentosa, and Age-related macular degeneration (AMD) comprising administering to a patient in need thereof an effective amount any of the self-inactivating CRISPR/Cas9 delivery systems as described above.

In a particular embodiment, the invention includes a method for treating a Huntington's disease comprising administering to a patient in need thereof an effective amount any of the self-inactivating CRISPR/Cas9 delivery systems as described above.

The term "effective amount" refers to an amount sufficient and/or necessary to obtain and/or produce one or more beneficial and/or desired biological and/or physiological effect. A beneficial and/or desired biological and/or physiological effect may, as a non-limiting example, be measured molecularly, such as, for example, a reduction and/or cessation in the production and/or an increase in the elimination of one or more molecule that causes, contributes to, or exacerbates a disease or disorder, such as a reduction and/or cessation in the production and/or an increase in the elimination of HTT, of, e.g., the entire protein, of the full-length protein, or the mutant variant of the protein. A beneficial and/or desired biological and/or physiological effect may, as a non-limiting example, be measured cellularly, such as, for example, a reduction and/or cessation in the production and/or an increase in the elimination of one or more cells that cause, contribute to, or exacerbate a disease or disorder. A beneficial and/or desired biological and/or physiological effect may, as a non-limiting example, be measured by changes in fine or gross physiology, such as, for example a reduction or cessation of one or more symptoms associated with the disease. A beneficial and/or desired biological and/or physiological effect may be, for example, life-long (e.g., permanent), intermittent, or temporary. The physiological effect may be achieved by one application dose or by repeated applications (e.g., daily over several days, weekly, monthly). The administered dosage will, of course, vary depending upon various factors based on the particular composition administered, the mode of administration, the disease/disorder to be treated, and the patient (e.g., human) to be treated, and can be adjusted by one skilled in the art. Such factors may include, for example, the composition's physiological characteristics; age, health, sex, and weight of the subject; nature/ extent of the symptoms; any concurrent treatment; frequency of administration; and desired effect.

Editing of one or more off-target (e.g., one that is not intended to be edited) genomic locus may produce one or more detrimental and/or undesired biological and/or physiological effect. A detrimental and/or undesired biological and/or physiological effect may, as a non-limiting example, be measured molecularly, such as, for example, a reduction and/or cessation in the production and/or an increase in the elimination of one or more beneficial and/or desired molecule, for example a molecule that is beneficial, desired, and/or necessary to sustaining life and/or to avoiding a disease or disorder. A detrimental and/or undesired biological and/or physiological effect may, as a non-limiting example, be measured cellularly, such as, for example, a reduction and/or cessation in the production and/or an increase in the elimination of one or more cells that is beneficial, desired, and/or necessary to sustaining life and/or to avoiding a disease or disorder. A detrimental and/or undesired

biological and/or physiological effect may, as a non-limiting example, be measured by changes in fine or gross physiology, such as, for example a appearance or increase in detrimental, unpleasant, and/or undesirable symptoms and/or decrease in health and/or length of life. A detrimental and/or undesired physiological effect may be, for example, life-long (e.g., permanent), intermittent, or temporary.

In many cases, off-target editing may not result in any detrimental and/or undesired physiological effect. In many cases, off-target editing may not result in any detrimental and/or undesired physiological effect that causes observable symptoms and/or may not result in any measurable or noticeable detrimental and/or undesired physiological effect. Further, a detrimental and/or undesired physiological effect may vary in severity, and the production of one or more detrimental and/or undesired physiological effect by treatment with a product or process described herein does not necessarily, and usually will not, preclude its use. Accordingly, off-target editing product or process described herein does not necessarily, and usually will not, preclude its use.

In comparison, for example, (i) to a system wherein two sgRNAs are delivered by one vector and Cas9 alone is delivered by another vector and/or (ii) to a system wherein two sgRNAs and Cas9 are delivered by single vector, the self-inactivating CRISPR/Cas9 system according to the invention may provide more control over one or both (i) efficient editing of the genomic target gene and (i) expression control of Cas9 nuclease by providing the ability to adjust the molar ratio between the two vectors, where each vector delivers a different sgRNA.

For example, the molar ratio of the first vector to the second vector may be selected (i) to maximize one or more beneficial and/or desired physiological effect, (ii) to minimize one or more detrimental and/or undesired physiological effect, or (iii) both (i) and (ii).

Maximizing one or more beneficial and/or desired physiological effect may be accomplished, for example, by maximizing editing of one or more on-target genomic locus. Editing of one or more on-target genomic locus, or the degree of such editing, may be evaluated by, for example, observing and/or measuring one or more beneficial and/or desired biological and/or physiological effect. Minimizing one or more detrimental and/or undesired physiological effect may be accomplished, for example, by eliminating or reducing editing of one or more off-target genomic locus. Editing of one or more off-target genomic locus, or the degree of such editing, may be evaluated

by, for example, observing and/or measuring one or more detrimental and/or undesired biological and/or physiological effect.

Selection of a desirable molar ratio may be via suitable methods, such as, for example, experiments performed *in vivo* and/or *in vitro*. Experiments performed *in vivo* may, for example, be performed on non-human animals, such as mice or other rodents. The non-human animal may be a model of a gene-associated disease and/or a gene-associated disorder. As an example, the non-human animal may naturally possess the characteristics of a disease/disorder model (e.g., a wild type of the non-human animal is a model of the gene-associated disease and/or a gene-associated disorder). As another example, the non-human animal may be genetically modified to possess the characteristics of a disease/disorder model. As another example, the non-human animal may be engrafted to possess tissue having the characteristics of a disease/disorder model. Combinations thereof are also envisioned. Experiments performed *in vitro* may, for example, be performed on cells, such as populations of cells. As examples, the cells may naturally comprise the target gene (the gene to be cleaved by the dual vector system) in their genome, may naturally express the target gene, may be genetically modified to comprise the target gene in their genome and/or extra-chromosomally, and/or may be genetically modified to express the target gene. As other example, cells may naturally be and/or be genetically modified to be more informative models of a disease/disorder. For example, cells may naturally express or be modified to express a certain transcription factor, a certain proteolytic enzyme, or the like. Combinations thereof are also envisioned.

Selection of a desirable molar ratio may, as a non-limiting example, comprise the following:

(a) administering to one or more first non-human animal the first vector and the second vector at a first molar ratio of the first vector to the second vector and/or administering to one or more first population of cells the first vector and the second vector at a first molar ratio of the first vector to the second vector;

(b) and measuring one or more first physiological effect of administering the first molar ratio;

(c) administering to one or more second non-human animal the first vector and the second vector at a second molar ratio of the first vector to the second vector and/or administering to one or more second population of cells the first vector and the second vector at a second molar ratio of the first vector to the second vector;

(d) measuring one or more second physiological effect of administering the second molar ratio;

(e) optionally repeating (a)-(b) one or more additional times at one or more additional molar ratio, wherein each of the one or more additional molar ratio may independently be different from the first molar ratio and the second molar ratio; and
(f) selecting a molar ratio from the first molar ratio, the second molar ratio, and optionally one or more additional molar ratio. The selected molar ratio may be a molar ratio the administration of which (i) is effective to produce one or more beneficial and/or desired physiological effect and (ii) produces less detrimental and/or fewer detrimental and/or undesired physiological effects than one or more molar ratio that is not selected.

Suitable dose ranges for are generally about 10^9 to 10^{14} (e.g., 10^9, 10^{10}, 10^{11}, 10^{12}, $10^{13,}$ or 10^{14}) viral genomes or infectious units of viral vector per dose, preferably 10^9 to 10^{13} viral genomes or infectious units of viral vector per dose, more preferably 10^{10} to 10^{12} viral genomes or infectious units of viral vector per dose. It should be understood that the aforementioned dosage ranges are merely exemplary dosage. Those skilled in the art will understand that this dosage may be varied in light of the factors discussed above.

The mode of administration used in the method of treatment may include all known administration modes used for viral vector systems. In the case of a method for treatment of Huntington's disease, the self-inactivating CRISPR/Cas9 delivery systems can, for example, by administered by local administration to deliver the vector system to the striatum and/or cortical regions of the brain, e.g., injection in the human striatum. Brain stereotaxic injection to striatum and cortex is preferred and the delivery method can be, for example, without limitation, intraperitoneal injection, subarachnoid injection, lateral ventricular injection, cerebellar medullary pool injection intravenous injection, or another acceptable delivery method.

The above description will be more fully understand when considered in conjunction with the following Examples and accompanying figures. The following Examples are exemplary in nature with regards to methods for practicing the invention are not intended to be limiting.

Examples

The following examples are intended to exemplify the invention and are not to be as construed as limiting the invention.

Vector cloning (See Fig. 1)

A single vector AAV-SaCas9 system containing nucleotide sequence encoding Cas9 from *Staphylococcus aureus* (SaCas9) and its sgRNA scaffold was obtained from Addgene (plasmid #61591). sgRNAs targeting human HTT and SaCas9 were designed based on PAM sequence (5'-NNGRRT-3') and by CRISPR RGEN Tools (http://www.rgenome.net/). AAV-EGFP-sgCas9 or AAV-mCherry-sgCas9 which contain EGFP or mCherry were constructed by replacing SaCas9 on vector AAV-SaCas9. Top and bottom strands of oligos for each sgRNA were phosphorylated and annealed into duplex. sgRNA for HTT was inserted into AAV-SaCas9 vector through BsaI enzyme digestion. Cas9 was replaced by EGFP or mCherry through AgeI and EcoRI enzyme digestion to obtain vector AAV-EGFP and AAV-mCherry, respectively. sgCas9 was also inserted into AAV-GFP and AAV-mCherry vector through BsaI enzyme digestion. Transformation of ligation product into Stbl3™ competent cells and isolated the plasmid DNA from cultures by using a QIAprep spin miniprep kit (QIAGEN, 27104) according to the manufacturer's instructions. Sequences of sgRNAs were verified by sequencing from the LKO.1 5' primer: 5'-GAC TAT CAT ATG CTT ACC GT-3' (SEQ ID NO: 13). Sequences of EGFP were verified using EGFP-forward primer: 5'-CGA AGG CTA CGT CCA GGA GC-3' (SEQ ID NO: 14). Sequences of mCherry were verified using mCherry-forward primer: 5'-ACA ACC GGT ATG GTG AGC AAG-3' (SEQ ID NO: 15).

Figure 1 shows schematic representations of vector 1 and vector 2 exemplary embodiments according to the invention. The exemplary embodiment of vector 1 includes CMV (cytomegalovirus)-driven nucleotide sequence encoding SaCas9 and U6-driven nucleotide sequence encoding sgRNA targeting HTT gene, between two ITRs (inverted terminal repeats). The first embodiment of vector 2 includes CMV (cytomegalovirus)-driven nucleotide sequence encoding EGFP (reporter gene) and U6-driven nucleotide sequence encoding sgRNA targeting Cas9, between two ITRs (inverted terminal repeat). The exemplary second embodiment of vector 2 includes CMV(cytomegalovirus)-driven nucleotide sequence encoding mCherry (reporter gene) and U6-driven nucleotide sequence encoding sgRNA targeting Cas9 between two ITRs. The exemplary third embodiment of vector 2 includes U6-driven nucleotide sequence encoding sgRNA targeting Cas9, between two ITRs, without any reporter gene.

While these vectors used the CMV and U6 promotors, one skilled in the art is well aware other suitable promoters for both the first vector and the second vector. For example, CaMKII, Synapsin, EF-1α, NSE, or PGK promoter could be used to drive

Cas9 and reporter protein expression, and H1 or 7SK promoter could be used to drive sgRNA expression.

In terms of promoter for the first viral vector that includes the expression unit for expression of a Cas9 nuclease and the nucleotide sequence encoding an sgRNA targeting a specific genomic locus, preferably a CMV promoter is utilized.

AAV vector production and purification.

AAV plasmids were extracted by EndoFree plasmid maxi kit (QIAGEN, 12362) for following virus production and purification. HEK293T cells were plated on 15-cm dishes and transfected at 80% confluence with linear polyethylenimine (PEI, Sigma-Aldrich, 765090). The transfection mixture was prepared with 70 μg AAV9 vector (AAV-SaCas9-HTTg1, AAV-SaCas9, AAV-GFP-sgCas9 or AAV-GFP), 200 μg Ad-Helper plasmid, 70 μg AAV-Rep/Cap plasmid and PEI (1 μg/μL), the PEI to DNA ratio was 5:1 (v/g) in this experiment. The mixture was filled to 50 mL with DMEM and allowed to sit room temperature for 15min. Cells were harvested 60 hours after transfection and re-suspended in 5 mL cell lysis buffer (150 mM NaCl, 20 mM Tris, pH 8.0). The cell lysate was freeze-thawed completely for 3 times between liquid nitrogen and 37°C water bath. Cell lysate was added with $MgCl_2$ and Benzonase (Sigma, E8263-25k) to a final concentration of 1 mM and 250 U/mL respectively, followed by incubation at 37°C for 15 min.

The supernatant was collected after centrifugation of the cell lysate at 4000 rpm for 30 min at 4°C. The viral solution was transferred on the top layer of the discontinuous iodixanol gradient solution, which is listed in the order of 6 mL of 17% (5 mL 10×PBS, 0.05 mL 1 M $MgCl_2$, 0.125 mL 1 M KCl, 10 mL 5 M NaCl, 12.5 mL Optiprep (Sigma, D1556) and H2O up to 50 mL), 6 mL 25% (5 mL 10×PBS, 0.05 mL 1 M $MgCl_2$, 0.125 mL 1 M KCl, 20 mL Optiprep and H2O up to 50 mL), 5 mL of 40% (5 mL 10×PBS, 0.05 mL 1 M $MgCl_2$, 0.125 mL 1 M KCl, 33.3 mL Optiprep and H2O up to 50 mL) and 4 mL of 60% (0.05 mL 1 M $MgCl_2$, 0.125 mL 1 M KCl, 50 mL Optiprep) from the bottom. The gradient was centrifuged at 53000 rpm for 160 min at 14°C and the viral fraction was harvested in the 40% layer with a syringe.

The viral fraction was transferred into the activated concentrated column Amacon 100K filter (Millipore Sigma, UFC910008) and filled with PBS solution containing F188 (1:10000, Polomaxer, Sigma). Then, it was centrifuged at 3500 rpm for 20 min at 4°C. The filtrate was discarded, and the viral fraction was again filled with F188-containing PBS solution to be repeatedly spun and added PBS for 3 times. The filtrate

was discarded again, and the viral fraction was mixed with PBS to be concentrated by centrifugation at 3500 rmp for 20 min at 4°C until the viral volume was about 500 μL. Virus titer was measured by quantitative PCR. Virus quality was determined by SDS-PAGE followed by Coomassie blue staining which would show virus capsid protein.

A single vector AAV-SaCas9-gHTT system containing nucleotide sequence encoding Cas9 from Staphylococcus aureus (SaCas9) and its sgRNA scaffold was obtained from Addgene (plasmid #61591). Single guide RNAs targeting human HTT and SaCas9 were designed based on PAM sequence (5'-NNGRRT-3') and by CRISPR RGEN Tools (http://www.rgenome.net/). Top and bottom strands of oligos for each sgRNA were phosphorylated and annealed into duplex. Single guide RNAs (HTTg1/HTTg2) were inserted into AAV-SaCas9 vector through BsaI enzyme digestion. Sequences of gRNA were verified by sequencing from the LKO.1 5' primer: 5'-GACTATCATATGCTTACCGT-3' (SEQ ID NO: 13). AAV vector production and purification was carried out as set forth above.

Culture and transfection of HEK293T cell.

HEK293T cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Thermo Fisher) supplemented with 10% (v/v) fetal bovine serum (FBS, Thermo Fisher), 1% (v/v) penicillin-streptomycin (Thermo Fisher) and 1% (v/v) L-glutamine (Thermo Fisher) in a humidified 5% CO_2 atmosphere at 37°C. Cells were co-transfected with AAV-SaCas9-HTTg1/ AAV-GFP-gCas9 and pEGFPc3-120 CAG with 0.8 μg of each vector at 6-well plate at 70% confluency with lipofectamine 2000 (Invitrogen, 11668030).

Western blot (See Fig. 2).

Lysates from 293T cells were prepared in Triton X-100 buffer (150 mM NaCl, 50 mM Tris-HCl, pH 7.4, 1 mM EDTA, 1% Triton X-100). The lysates were incubated at 4°C for 30 min, followed by centrifugation at 4°C for 20 min at 16,100 g to remove insoluble components. Protein concentration was measured by BCA protein assay. Protein samples were prepared for loading by heating in 4×SDS sample buffer and heated for 10 min at 95°C. A total amount of 30 μg of protein was loaded onto SDS-PAGE gels using running buffer, and wet-transferred to an Immobilon-FL PVDF membrane. Blots were incubated with Odyssey blocking buffer (LI-COR, 927-40000) for 60 min. After washing with TBST, blots were incubated with primary antibodies diluted in blocking buffer at 4°C overnight. Blots were washed 3 times (10 min each) and incubated with fluorescently labeled IRDye 680RD goat anti-mouse (1:10000, LI-

COR, 926-68070) or goat anti-rabbit (1:10000, LI-COR, 926-68071) secondary antibodies in TBST for 60 min at room temperature. Proteins were detected in the 700 nm channel using Odyssey CLx imager (LI-COR). The following primary antibodies were used: 1C2 (1:5000, mouse, Millipore, MAB1574), HA (1:2000, mouse, Abcam, ab18181), β-actin (1:2000, rabbit, Cell Signaling, 4970). As shown in Fig. 2, Western blot analysis validated that the tested sequences, sgRNA1 to sgRNA10, were able to knock out SaCas9 without compromising the gene editing efficiency of HTT-gRNA to hHTT-120Q.

Stereotaxic injection.

Stereotaxic administration of AAV vectors were performed on 1-month-old of BAC226Q mice. Mice were anesthetized with 1.5% isoflurane inhalation and stabilized in a stereotaxic instrument (RWD, 68019). Small holes were drilled in the skull at both side of striatum and cortex. A total of 2.5 μL AAV was injected into mouse striatum at a rate of 0.3 μL/min (coordinates: +0.8 mm rostral to Bregma, ±2.1 mm lateral to medial and -3.1 mm ventral from brain surface). And 0.5 μL of AAV was injected into mouse primary motor cortex at 0.1 μL/min (coordinates: +1.5 mm rostral to Bregma, ±1.5 mm lateral to medial and -1.0 mm ventral from brain surface). Two viruses were co-injected at 1:1 molar ratio. Each virus titer was 2×10^{12} viral genomes/mL with the total volume of 3 μL, therefore 6×10^{9} viral genomes of each virus were injected into one mouse brain. Injection was performed through a microinjection pump (RWD, 788130)-connected Hamilton syringe. A 1701 Hamilton microsyringe (Hamilton, 7853-01) with 33-gauge needle (Hamilton, 7803-05) was used to deliver the virus.

Immunofluorescent staining (Figs. 3A and 3B).

Mice were anesthetized, perfused with fresh 4% paraformaldehyde in PBS, and post-fixed overnight in the same fixative. Fixed brains were sliced at 40 μm thickness with a vibrating blade microtome (Leica, VT1200S). The brain slices were incubated in PBST (0.3% Triton X-100) for 30min in room temperature and then blocked with 10% goat serum in PBS supplemented with 0.1% Triton X-100 for 60 min at room temperature. Following incubation of brain slices with primary antibodies at 4°C overnight and washes, fluor-conjugated secondary antibodies (anti-mouse or anti-rabbit Alexa Fluor 594 or 647, 1:1000, Thermo Fisher Scientific) were added to the samples for 60 min at room temperature. Images were taken by Zeiss LSM 710. Following primary antibodies were used: mEM48 (1:1000, mouse, Millipore, MAB5374), HA (1:1000, rabbit, Cell Signaling Technology, 3724). As shown in Fig.

3A and 3B, BAC226Q mice, after injection of AAV9-SaCas9-HTTg1, showed significant decrease of mHTT. And injection of AAV9-SaCas9-HTTg1/gCas9 showed similar reduction of mHTT along with decreased SaCas9 expression.

Rota-rod test (Fig. 4A).

Mice were trained on a rota-rod treadmill (Med Associates, Inc., ENV-574M) for 3 trials per day for 3 consecutive days. Mice were trained at a constant speed of 10 rpm/min for 1 min per trial. During the training trials, mice that fell were gently returned to the rota-rod treadmill. Animals were tested for 3 trials per day for 3 consecutive days every week, allowing at least 15 min of rest between each trial. Mice were tested with the rota-rod accelerating from 5 to 40 rpm with a maximum period of 300 sec. The latency to fall was recorded and the mean of all trials was used for statistical analysis. As shown in Fig. 4A, BAC226Q mice, after injection of AAV9-SaCas9-HTTg1 or AAV9-SaCas9-HTTg1/gCas9, showed significant improvement in duration time on rod.

Open-field test (Fig 4B).

The open field consisted of a clear glass box (28×28 cm, Med Associates, Inc., ENV-510). Mice were tested for 3 trials per day for 3 consecutive days every 2 weeks. Mice were put into the open-field boxes over a 10 min period in every trial according to an established protocol. The total travel distance was recorded and the mean of all trials was used for statistical analysis. As shown in Fig. 4B, both the AAV9-HTTg1/gCas9 and AAV9-HTTg1 injected groups exhibited reduced hyperactivity compared with the HD-SaCas9 control group at relative timepoints.

Although the foregoing subject matter has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be understood by those skilled in the art that certain changes and modifications can be practiced within the scope of the appended claims.

All publications, patent applications, patents, and other references mentioned herein and incorporated by reference to the same extent as if each individual publication, patent application, patent, and other reference was specifically and individually indicated to be incorporated by reference.

Patent Claims

What is claimed is:

1. A self-inactivating CRISPR/Cas9 delivery system comprising:

a first viral vector comprising an expression unit for expression of a Cas9 nuclease and a nucleotide sequence encoding a first sgRNA targeting a specific genomic locus; and

a second viral vector comprising a nucleotide sequence encoding a second sgRNA targeting expression of the Cas9 nuclease by the expression unit,

wherein the specific genomic locus comprises a nucleotide sequence that (i) is located in a gene and/or (ii) is located in a regulatory region of the gene, wherein

the gene (i) causes, (ii) exacerbates, (iii) is otherwise associated with, or (vi) any combination of (i)-(iii), one or more disease and/or disorder.

2. The delivery system according to claim 1, wherein the gene and/or regulatory region is a mutated gene and/or regulatory region.

3. The delivery system according to claim 1, wherein the gene and/or regulatory region is a wild type gene and/or regulatory region.

4. The delivery system according to any one of claims 1 to 3, wherein the specific genomic locus is located in a regulatory region of the gene.

5. The delivery system according to any one of claims 1 to 3, wherein the specific genomic locus is located in an intron of the gene.

6. The delivery system according to any one of claims 1 to 3, wherein the specific genomic locus is located in an exon of the gene.

7. The delivery system according to any one of claims 1 to 6, wherein one or more disruption and/or cleavage of the specific genomic locus causes reduction or elimination of expression of the gene.

8. The delivery system according to any one of claims 1 to 7, wherein one or more disruption and/or cleavage of the specific genomic locus causes truncation of the protein expressed by the gene.

9. The delivery system according to any one of claims 1 to 8, wherein the sgRNA targeting expression of the Cas9 nuclease by the expression unit is encoded by a nucleotide sequence comprising (i) a nucleotide sequence selected from SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, and SEQ ID NO: 12, (ii) a nucleotide sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, or at least 99% identity with one or more of the nucleotide sequence of SEQ ID NO: 3 to SEQ ID NO. 12 and having the same function as one or more of the nucleotide sequences of SEQ ID NO: 3 to SEQ ID NO: 12, (iii) a nucleotide sequence having at most three base pairs different from, at most two base pairs different from, or at most one base pair different from one or more of the nucleotide sequence of SEQ ID NO: 3 to SEQ ID NO. 12 and having the same function as one or more of the nucleotide sequences of SEQ ID NO: 3 to SEQ ID NO: 12, or (iv) a nucleotide sequence obtained from the nucleotide sequence of one of SEQ ID NO: 3 to SEQ ID NO: 12 by deletion, substitution, or addition of 1, 2, 3, 4, 5 or 6 bases and having the same function as one or more of the nucleotide sequences of SEQ ID NO: 3 to SEQ ID NO: 12.

10. The delivery system according to claim 9, wherein the sgRNA targeting expression of the Cas9 nuclease by the expression unit is encoded by a nucleotide sequence comprising a nucleotide sequence selected from the nucleotide sequences according to SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, and SEQ ID NO: 12.

11. The delivery system according to any one of claims 1 to 10, wherein the first and second viral vectors are selected from AAV, lentiviral vectors, adenovirus vectors, herpesvirus vectors, poxvirus vectors, baculovirus vectors, and papillomavirus vectors.

12. The delivery system according to claim 10, wherein the first and second viral vectors are selected from AAV vectors.

13. The delivery system according to claim 11, wherein the first and second viral vectors are selected from AAV1, AA2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV PHP.B and AAV PHP.eB.

14. The delivery system according to any one of claims 1 to 12, wherein the first and second viral vectors are not AAV8 viral vectors.

15. The delivery system according to any one of claims 11 to 14, wherein the first and second viral vectors are AAV9, AAV PHP.B, or AAV PHP.eB viral vectors.

16. The delivery system according to any one of claims 11-15, wherein the first and second viral vectors are AAV9 viral vectors.

17. The delivery system according to any one of claims 1 to 15, wherein (i) the first viral vector further comprises a CMV promoter to drive expression of the Cas9 nuclease; (ii) the first viral vector further comprises a U6 promoter to drive transcription of the first sgRNA, (iii) the second viral vector further comprises a U6 promoter to drive transcription of the second sgRNA, or (iv) any combination thereof.

18. The delivery system according to any one of claims 1 to 17, wherein the second viral vector does not include a nucleotide sequence encoding a reporter gene.

19. The delivery system according to any one of claims 1 to 17, wherein the second viral vector does include a nucleotide sequence encoding a reporter gene.

20. The delivery system according to any one of claims 1 to 19, wherein the Cas9 nuclease is one or more of SaCas9 nuclease, SpCas9 nuclease, StCas9 nuclease, CjCas9 nuclease, or NmCas9 nuclease.

21. The delivery system according to claim 20, wherein the Cas9 nuclease is SaCas9 nuclease.

22. The delivery system according to any one of claims 1 to 21, wherein the disease or disorder is selected from cystic fibrosis, myotonic dystrophy, spinal muscular dystrophy, alpha- thalassemia, beta-thalassemia, sickle cell anemia, chronic granulomatous disease Canavan disease, Fanconi anemia, Marfan syndrome, retinitis pigmentosa, fragile X syndrome, Gaucher disease, Huntington's disease, hemochromatosis, congenital deafness, Duchenne muscular dystrophy, familial hypercholesterolemia, Farber disease, Neurofibromatosis type 1, Neurofibromatosis t Becker muscular dystrophy type II, hemophilia A, hemophilia B, Tay-Sachs disease,

Wiskott-Aldrich syndrome, Hurler syndrome, Hunter syndrome, Purine nucleoside phosphorylase deficiency, Fabry disease, Pompe disease, Gyrate atrophy, Krabbe disease, Wolman disease, Amyotrophic lateral sclerosis, Spinocerebellar Ataxia, Sanfilippo disease, a birth defect, breast cancer, prostate cancer, skin cancer, high blood pressure, high cholesterol, diabetes, Alzheimer disease, schizophrenia, Parkinson's disease, Frontotemporal dementia, or bipolar disorder.

23. The delivery system according to any one of claims 1 to 22, wherein (i) the disease or disorder is a neurodegenerative disease, (ii) the gene is expressed in the CNS, or (iii) both (i) and (ii).

24. The delivery system according to any one of claims 1 to 23, wherein (i) the disease or disorder is Huntington's disease, Alzheimer's disease, Parkinson's disease, Amyotrophic Lateral Sclerosis (ALS), Frontotemporal dementia (FTD) (aka Pick's disease), spinocerebellar ataxi (SCA), e.g., types 1 to 17, Retinitis Pigmentosa, or Age-related macular degeneration (AMD), (ii) the gene is expressed in the CNS, or (iii) both (i) and (ii)..

25. The delivery system according to any one of claims 1-24, wherein (i) the neurodegenerative disease is Huntington's disease, (ii) the gene is the Huntingtin gene (HTT), or (iii) both (i) and (ii).

26. The delivery system according to claim 25, wherein the nucleotide sequence encoding the first sgRNA targeting a specific genomic locus comprises (i) a nucleotide sequence according to SEQ ID NO. 1 or SEQ ID NO. 2, (ii) a nucleotide sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, or at least 99% identity with the nucleotide sequence of SEQ ID NO. 1 and/or SEQ ID NO. 2 and having the same function as the nucleotide sequence of SEQ ID NO: 1 and/or SEQ ID NO: 2, (iii) a nucleotide sequence having at most three base pairs different from, at most two base pairs different from, or at most one base pair different from of either or both of the nucleotide sequence of SEQ ID NO. 1 or SEQ ID NO. 2 and having the same function as the nucleotide sequence of SEQ ID NO: 1 and/or SEQ ID NO: 2, or (iv) a nucleotide sequence obtained from the nucleotide sequence of SEQ ID NO: 1 or SEQ ID NO: 2 by deletion, substitution or addition of 1, 2, 3, 4, 5 or 6 base pairs and having the same function as the nucleotide sequence of SEQ ID NO: 1 and/or SEQ ID NO: 2.

27. The delivery system according to claim 26, wherein the nucleotide sequence encoding the first sgRNA targeting a specific genomic locus comprises a nucleotide sequence according to SEQ ID NO. 1 or SEQ ID NO. 2,.

28. A pharmaceutical composition comprising one or more self-inactivating CRISPR/Cas9 delivery system according to any one of claims 1 to 27 and one or more pharmaceutical acceptable carrier and/or excipient.

29. A method for treating a disease and/or disorder, the method comprising:

administering to a patient having the disease and/or disorder (i) one or more effective amount of one or more self-inactivating CRISPR/Cas9 delivery system according to any one of claims 1 to 28, (ii) one or more effective amount of a pharmaceutical composition according to claim 29, or (iii) both (i) and (ii).

30. The method according to claim 29, wherein an effective amount comprises a dosage of 10^9 to 10^{14} viral genomes or infectious units of viral vectors per dose.

31. The method according to claim 29, wherein an effective amount comprises a dosage of 10^{10} to 10^{12} viral genomes or infectious units of viral vector per dose.

32. The method according to any one of claims 27 to 31, wherein a molar ratio of the first vector to the second vector is selected (i) to maximize one or more beneficial and/or desired physiological effect, (ii) to minimize one or more detrimental and/or undesired physiological effect, or (iii) both (i) and (ii), optionally wherein

(a) the selection comprises:

(i)(A) administering to one or more first mouse the first vector and the second vector at a first molar ratio of the first vector to the second vector, the first mouse being a model of a disease, a disorder, or both and/or (A') administering to one or more first population of cells the first vector and the second vector at a first molar ratio of the first vector to the second vector;

(ii) and measuring one or more first physiological effect of administering the first molar ratio;

(iii)(A) administering to one or more second mouse the first vector and the second vector at a second molar ratio of the first vector to the second vector, the

second mouse being a model of the disease, the disorder, or both and/or (A') administering to one or more second population of cells the first vector and the second vector at a second molar ratio of the first vector to the second vector;

(iv) measuring one or more second physiological effect of administering the second molar ratio;

(v) optionally repeating (a)(i)-(a)(ii) one or more additional times at one or more additional molar ratio, wherein each of the one or more additional molar ratio is independently different from the first molar ratio and the second molar ratio; and

(v) selecting a molar ratio from the first molar ratio, the second molar ratio, and optionally one or more additional molar ratio, to produce a selected molar ratio, wherein the selected molar ratio is a molar ratio the administration of which (i) is effective to produce one or more beneficial and/or desired physiological effect and (ii) produces less detrimental and/or fewer detrimental and/or undesired physiological effects than one or more molar ratio that is not selected;

(b) maximizing one or more beneficial and/or desired physiological effect is accomplished by maximizing editing of one or more on-target genomic locus;

(c) minimizing one or more detrimental and/or undesired physiological effect is accomplished by eliminating or reducing editing of one or more off-target genomic locus; or

(d) any combination of (a)-(c).

33. The method of any one of claims 29 to 32, wherein (a) (i) the disease or disorder is a neurodegenerative disease, (ii) the gene is expressed in the CNS, or (iii) both (i) and (ii) and (b) administration comprises stereotaxic injection (i) into the striatum of the brain of the patient, (ii) into the cortex of the brain of the patient, or (iii) into both the striatum and the cortex of the brain of the patient.

34. The method of any one of claims 29 to 33, wherein the patient is human.

35. The self-inactivating CRISPR/Cas9 delivery system according to any one of claims 1 to 27 formulated for use in treatment of the one or more disease, disorder, or both.

36. The self-inactivating CRISPR/Cas9 delivery system according to any one of claims 1 to 27 formulated for use in treatment of the one or more disease, disorder, or both, wherein

(a) the formulation comprises a dosage of 10^9 to 10^{14}, or 10^{10} to 10^{12} viral genomes or infectious units of viral vectors per dose;

(b) the formulation comprises a molar ratio of the first vector to the second vector, wherein the molar ratio is selected (i) to maximize one or more beneficial and/or desired physiological effect, (ii) to minimize one or more detrimental and/or undesired physiological effect, or (iii) both (i) and (ii);

(c) (A) (i) the disease or disorder is a neurodegenerative disease, (ii) the gene is expressed in the CNS, or (iii) both (i) and (ii) and (B) the formulation is suitable for stereotaxic injection (i) into the striatum of the brain of the patient, (ii) into the cortex of the brain of the patient, or (iii) into both the striatum and the cortex of the brain of the patient;

(d) the formulation is suitable for administration to a human;

or (e) any combination of (a)-(d).

37. A medicament comprising the self-inactivating CRISPR/Cas9 delivery system according to any one of claims 1 to 27.

38. A medicament comprising the self-inactivating CRISPR/Cas9 delivery system according to any one of claims 1 to 27 for use in treatment of the one or more disease, disorder, or both, wherein

(a) the medicament comprises a dosage of 10^9 to 10^{14}, or 10^{10} to 10^{12} viral genomes or infectious units of viral vectors per dose;

(b) the medicament comprises a molar ratio of the first vector to the second vector, wherein the molar ratio is selected (i) to maximize one or more beneficial and/or desired physiological effect, (ii) to minimize one or more detrimental and/or undesired physiological effect, or (iii) both (i) and (ii);

(c) (A) (i) the disease or disorder is a neurodegenerative disease, (ii) the gene is expressed in the CNS, or (iii) both (i) and (ii) and (B) the medicament is suitable for stereotaxic injection (i) into the striatum of the brain of the patient, (ii) into the cortex of the brain of the patient, or (iii) into both the striatum and the cortex of the brain of the patient;

(d) the medicament is suitable for administration to a human; or

(e) any combination of (a)-(d).

39. A self-inactivating CRISPR/Cas9 delivery system comprising:

a first viral vector comprising an expression unit for expression of a Cas9 nuclease and a nucleotide sequence encoding a first sgRNA targeting a specific genomic locus; and

a second viral vector comprising a nucleotide sequence encoding a second sgRNA targeting expression of the Cas9 nuclease by the expression unit,

wherein the specific genomic locus comprises a nucleotide sequence that (i) is located in a gene and/or (ii) is located in a regulatory region of the gene; wherein

the gene (i) causes, (ii) exacerbates, (iii) is otherwise associated with, or (iv) any combination of (i)-(iii), one or more disease and/or disorder; and wherein

(i) the disease and/or disorder is Huntington's disease, (ii) the gene is the Huntingtin gene (HTT), or (iii) both (i) and (ii).

40. The delivery system according to claim 39, wherein the nucleotide sequence encoding the first sgRNA targeting a specific genomic locus comprises (i) a nucleotide sequence according to SEQ ID NO. 1 or SEQ ID NO. 2, (ii) a nucleotide sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, or at least 99% identity with the nucleotide sequence of SEQ ID NO. 1 and/or SEQ ID NO. 2 and having the same function as the nucleotide sequence of SEQ ID NO: 1 and/or SEQ ID NO: 2, (iii) a nucleotide sequence having at most three base pairs different from, at most two base pairs different from, or at most one base pair different from of either or both of the nucleotide sequence of SEQ ID NO. 1 or SEQ ID NO. 2 and having the same function as the nucleotide sequence of SEQ ID NO: 1 and/or SEQ ID NO: 2, or (iv) a nucleotide sequence obtained from the nucleotide sequence of SEQ ID NO: 1 or SEQ ID NO: 2 by deletion, substitution or addition of 1, 2, 3, 4, 5 or 6 base pairs and having the same function as the nucleotide sequence of SEQ ID NO: 1 and/or SEQ ID NO: 2.

41. The delivery system according to claim 40, wherein the nucleotide sequence encoding the first sgRNA targeting a specific genomic locus comprises a nucleotide sequence according to SEQ ID NO. 1 or SEQ ID NO. 2,.

42. The delivery system according to any one of claims 39 to 41, wherein the gene and/or regulatory region is a mutated gene and/or regulatory region.

43. The delivery system according to any one of claims 39 to 41, wherein the gene and/or regulatory region is a wild type gene and/or regulatory region.

44. The delivery system according to any one of claims any one of claims 39 to 43, wherein the specific genomic locus is located in a regulatory region of the gene.

45. The delivery system according to any one of claims any one of claims 39 to 44, wherein the specific genomic locus is located in an intron of the gene.

46. The delivery system according to any one of any one of claims 39 to 45, wherein the specific genomic locus is located in an exon of the gene.

47. The delivery system according to any one of claims 39 to 46, wherein one or more disruption and/or cleavage of the specific genomic locus causes reduction or elimination of expression of the gene.

48. The delivery system according to any one of claims 39 to 47, wherein one or more disruption and/or cleavage of the specific genomic locus causes truncation of the protein expressed by the gene.

49. The delivery system according to any one of claims 39 to 48, wherein the sgRNA targeting expression of the Cas9 nuclease by the expression unit is encoded by a nucleotide sequence comprising (i) a nucleotide sequence selected from SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, and SEQ ID NO: 12, (ii) a nucleotide sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, or at least 99% identity with one or more of the nucleotide sequence of SEQ ID NO: 3 to SEQ ID NO. 12 and having the same function as one or more of the nucleotide sequences of SEQ ID NO: 3 to SEQ ID NO: 12, (iii) a nucleotide sequence having at most three base pairs different from, at most two base pairs different from, or at most one base pair different from one or more of the nucleotide sequence of SEQ ID NO: 3 to SEQ ID NO. 12 and having the same function as one or more of the nucleotide sequences of SEQ ID NO: 3 to SEQ ID NO: 12, or (iv) a nucleotide sequence obtained from the nucleotide sequence of one of SEQ ID NO: 3 to SEQ ID NO: 12 by deletion, substitution, or addition of 1, 2, 3, 4, 5 or 6 bases and having the same function as one or more of the nucleotide sequences of SEQ ID NO: 3 to SEQ ID NO: 12.

50. The delivery system according to claim 49, wherein the sgRNA targeting expression of the Cas9 nuclease by the expression unit is encoded by a

nucleotide sequence comprising a nucleotide sequence selected from the nucleotide sequences according to SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, and SEQ ID NO: 12.

51. The delivery system according to any one of claims 39 to 50, wherein the first and second viral vectors are selected from AAV, lentiviral vectors, adenovirus vectors, herpesvirus vectors, poxvirus vectors, baculovirus vectors, and papillomavirus vectors.

52. The delivery system according to claim 51, wherein the first and second viral vectors are selected from AAV vectors.

53. The delivery system according to claim 52, wherein the first and second viral vectors are selected from AAV1, AA2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV PHP.B and AAV PHP.eB.

54. The delivery system according to any one of claims 1 to 53, wherein the first and second viral vectors are not AAV8 viral vectors.

55. The delivery system according to any one of claims 52 to 53, wherein the first and second viral vectors are AAV9, AAV PHP.B, or AAV PHP.eB viral vectors.

56. The delivery system according to any one of claims 52-55, wherein the first and second viral vectors are AAV9 viral vectors.

57. The delivery system according to any one of claims 39 to 56, wherein (i) the first viral vector further comprises a CMV promoter to drive expression of the Cas9 nuclease; (ii) the first viral vector further comprises a U6 promoter to drive transcription of the first sgRNA, (iii) the second viral vector further comprises a U6 promoter to drive transcription of the second sgRNA, or (iv) any combination thereof.

58. The delivery system according to any one of claims 39 to 57, wherein the second viral vector does not include a nucleotide sequence encoding a reporter gene.

59. The delivery system according to any one of claims 39 to 58, wherein the second viral vector does include a nucleotide sequence encoding a reporter gene.

60. The delivery system according to any one of claims 39 to 59, wherein the Cas9 nuclease is one or more of SaCas9 nuclease, SpCas9 nuclease, StCas9 nuclease, CjCas9 nuclease, or NmCas9 nuclease.

61. The delivery system according to claim 60, wherein the Cas9 nuclease is SaCas9 nuclease.

62. A pharmaceutical composition comprising one or more self-inactivating CRISPR/Cas9 delivery system according to any one of claims 39 to 61 and one or more pharmaceutical acceptable carrier and/or excipient.

63. A method for treating a disease and/or disorder, the method comprising:

administering to a patient having the disease and/or disorder (i) one or more effective amount of one or more self-inactivating CRISPR/Cas9 delivery system according to any one of claims 39 to 61, (ii) one or more effective amount of a pharmaceutical composition according to claim 62, or (iii) both (i) and (ii).

64. The method according to claim 63, wherein an effective amount comprises a dosage of 10^9 to 10^{14} viral genomes or infectious units of viral vectors per dose.

65. The method according to claim 63, wherein an effective amount comprises a dosage of 10^{10} to 10^{12} viral genomes or infectious units of viral vector per dose.

66. The method of any one of claims 63 to 65, wherein administration comprises stereotaxic injection (i) into the striatum of the brain of the patient, (ii) into the cortex of the brain of the patient, or (iii) into both the striatum and the cortex of the brain of the patient.

67. The method according to any one of claims 63 to 65, wherein a molar ratio of the first vector to the second vector is selected (i) to maximize one or more beneficial and/or desired physiological effect, (ii) to minimize one or more

detrimental and/or undesired physiological effect, or (iii) both (i) and (ii), optionally wherein

(a) the selection comprises:

(i)(A) administering to one or more first mouse the first vector and the second vector at a first molar ratio of the first vector to the second vector, the first mouse being a model of a disease, a disorder, or both and/or (A') administering to one or more first population of cells the first vector and the second vector at a first molar ratio of the first vector to the second vector;

(ii) and measuring one or more first physiological effect of administering the first molar ratio;

(iii)(A) administering to one or more second mouse the first vector and the second vector at a second molar ratio of the first vector to the second vector, the second mouse being a model of the disease, the disorder, or both and/or (A') administering to one or more second population of cells the first vector and the second vector at a second molar ratio of the first vector to the second vector;

(iv) measuring one or more second physiological effect of administering the second molar ratio;

(v) optionally repeating (a)(i)-(a)(ii) one or more additional times at one or more additional molar ratio, wherein each of the one or more additional molar ratio is independently different from the first molar ratio and the second molar ratio; and

(v) selecting a molar ratio from the first molar ratio, the second molar ratio, and optionally one or more additional molar ratio, to produce a selected molar ratio, wherein the selected molar ratio is a molar ratio the administration of which (i) is effective to produce one or more beneficial and/or desired physiological effect and (ii) produces less detrimental and/or fewer detrimental and/or undesired physiological effects than one or more molar ratio that is not selected;

(b) maximizing one or more beneficial and/or desired physiological effect is accomplished by maximizing editing of one or more on-target genomic locus;

(c) minimizing one or more detrimental and/or undesired physiological effect is accomplished by eliminating or reducing editing of one or more off-target genomic locus; or

(d) any combination of (a)-(c).

68. The method of any one of claims 63 to 66, wherein the patient is human.

69. The self-inactivating CRISPR/Cas9 delivery system according to any one of claims 39 to 61 formulated for use in treatment of Huntington's disease.

70. The self-inactivating CRISPR/Cas9 delivery system according to any one of claims 39 to 61 formulated for use in treatment of the Huntington's disease, wherein

(a) the formulation comprises a dosage of 10^9 to 10^{14}, or 10^{10} to 10^{12} viral genomes or infectious units of viral vectors per dose;

(b) the formulation comprises a molar ratio of the first vector to the second vector, wherein the molar ratio is selected (i) to maximize one or more beneficial and/or desired physiological effect, (ii) to minimize one or more detrimental and/or undesired physiological effect, or (iii) both (i) and (ii);

(c) the formulation is suitable for stereotaxic injection (i) into the striatum of the brain of the patient, (ii) into the cortex of the brain of the patient, or (iii) into both the striatum and the cortex of the brain of the patient;

(d) the formulation is suitable for administration to a human;

or (e) any combination of (a)-(d).

71. A medicament comprising the self-inactivating CRISPR/Cas9 delivery system according to any one of claims 39 to 61.

72. A medicament comprising the self-inactivating CRISPR/Cas9 delivery system according to any one of claims 39 to 61 for use in treatment of Huntington's disease, wherein

(a) the medicament comprises a dosage of 10^9 to 10^{14}, or 10^{10} to 10^{12} viral genomes or infectious units of viral vectors per dose;

(b) the medicament comprises a molar ratio of the first vector to the second vector, wherein the molar ratio is selected (i) to maximize one or more beneficial and/or desired physiological effect, (ii) to minimize one or more detrimental and/or undesired physiological effect, or (iii) both (i) and (ii);

(c) the medicament is suitable for stereotaxic injection (i) into the striatum of the brain of the patient, (ii) into the cortex of the brain of the patient, or (iii) into both the striatum and the cortex of the brain of the patient;

(d) the medicament is suitable for administration to a human; or

(e) any combination of (a)-(d).

73. A self-inactivating CRISPR/Cas9 delivery system comprising:

a first viral vector comprising an expression unit for expression of a saCas9 nuclease and a nucleotide sequence encoding a first sgRNA targeting a specific genomic locus; and

a second viral vector comprising a nucleotide sequence encoding a second sgRNA targeting expression of the saCas9 nuclease by the expression unit,

wherein the specific genomic locus comprises a nucleotide sequence that (i) is located in a gene and/or (ii) is located in a regulatory region of the gene, wherein

the gene (i) causes, (ii) exacerbates, (iii) is otherwise associated with, or (iv) any combination of (i)-(iii), one or more disease and/or disorder; and wherein

(i) the disease/and or disorder is Huntington's disease, (ii) the gene is the Huntingtin gene (HTT), or (iii) both (i) and (ii).

74. The delivery system according to claim 73, wherein the nucleotide sequence encoding the first sgRNA targeting a specific genomic locus comprises (i) a nucleotide sequence according to SEQ ID NO. 1 or SEQ ID NO. 2, (ii) a nucleotide sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, or at least 99% identity with the nucleotide sequence of SEQ ID NO. 1 and/or SEQ ID NO. 2 and having the same function as the nucleotide sequence of SEQ ID NO: 1 and/or SEQ ID NO: 2, (iii) a nucleotide sequence having at most three base pairs different from, at most two base pairs different from, or at most one base pair different from of either or both of the nucleotide sequence of SEQ ID NO. 1 or SEQ ID NO. 2 and having the same function as the nucleotide sequence of SEQ ID NO: 1 and/or SEQ ID NO: 2, or (iv) a nucleotide sequence obtained from the nucleotide sequence of SEQ ID NO: 1 or SEQ ID NO: 2 by deletion, substitution or addition of 1, 2, 3, 4, 5 or 6 base pairs and having the same function as the nucleotide sequence of SEQ ID NO: 1 and/or SEQ ID NO: 2.

75. The delivery system according to claim 73, wherein the nucleotide sequence encoding the first sgRNA targeting a specific genomic locus comprises a nucleotide sequence according to SEQ ID NO. 1 or SEQ ID NO. 2.

76. The delivery system according to any one of claims 73 to 75, wherein the gene and/or regulatory region is a mutated gene and/or regulatory region.

77. The delivery system according to any one of claims 73 to 75, wherein the gene and/or regulatory region is a wild type gene and/or regulatory region.

78. The delivery system according to any one of claims any one of claims 73 to 75, wherein the specific genomic locus is located in a regulatory region of the gene.

79. The delivery system according to any one of claims any one of claims 73 to 75, wherein the specific genomic locus is located in an intron of the gene.

80. The delivery system according to any one of any one of claims 73 to 75, wherein the specific genomic locus is located in an exon of the gene.

81. The delivery system according to any one of claims 73 to 75, wherein one or more disruption and/or cleavage of the specific genomic locus causes reduction or elimination of expression of the gene.

82. The delivery system according to any one of claims 73 to 75, wherein one or more disruption and/or cleavage of the specific genomic locus causes truncation of the protein expressed by the gene.

83. The delivery system according to any one of claims 73 to 75, wherein the sgRNA targeting expression of the saCas9 nuclease by the expression unit is encoded by a nucleotide sequence comprising (i) a nucleotide sequence selected from SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, and SEQ ID NO: 12, (ii) a nucleotide sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, or at least 99% identity with one or more of the nucleotide sequence of SEQ ID NO: 3 to SEQ ID NO. 12 and having the same function as one or more of the nucleotide sequences of SEQ ID NO: 3 to SEQ ID NO: 12, (iii) a nucleotide sequence having at most three base pairs different from, at most two base pairs different from, or at most one base pair different from one or more of the nucleotide sequence of SEQ ID NO: 3 to SEQ ID NO. 12 and having the same function as one or more of the nucleotide sequences of SEQ ID NO: 3 to SEQ ID NO: 12, or (iv) a nucleotide sequence obtained from the nucleotide sequence of one of SEQ ID NO: 3 to SEQ ID NO: 12 by deletion, substitution, or addition of 1, 2, 3, 4, 5 or 6 bases and having the same function as one or more of the nucleotide sequences of SEQ ID NO: 3 to SEQ ID NO: 12.

84. The delivery system according to any one of claims 73 to 75, wherein the sgRNA targeting expression of the saCas9 nuclease by the expression unit is

encoded by a nucleotide sequence comprising a nucleotide sequence selected from the nucleotide sequences according to SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, and SEQ ID NO: 12.

85. The delivery system according to any one of claims 73 to 75, wherein the first and second viral vectors are selected from AAV, lentiviral vectors, adenovirus vectors, herpesvirus vectors, poxvirus vectors, baculovirus vectors, and papillomavirus vectors.

86. The delivery system according to claim 85, wherein the first and second viral vectors are selected from AAV vectors.

87. The delivery system according to claim 85, wherein the first and second viral vectors are selected from AAV1, AA2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV PHP.B and AAV PHP.eB.

88. The delivery system according to any one of claims 73 to 75, wherein (i) the first and second viral vectors are not AAV8 viral vectors, (ii) the first and second viral vectors are AAV9, AAV PHP.B, or AAV PHP.eB viral vectors, or (iii) both (i) and (ii).

89. The delivery system according to any one of claims 73 to 75, wherein the first and second viral vectors are AAV9 viral vectors.

90. The delivery system according to any one of claims 73 to 75, wherein (i) the first viral vector further comprises a CMV promoter to drive expression of the Cas9 nuclease; (ii) the first viral vector further comprises a U6 promoter to drive transcription of the first sgRNA, (iii) the second viral vector further comprises a U6 promoter to drive transcription of the second sgRNA, or (iv) any combination thereof.

91. The delivery system according to any one of claims 73 to 75, wherein the second viral vector does not include a nucleotide sequence encoding a reporter gene.

92. The delivery system according to any one of claims 73 to 75, wherein the second viral vector does include a nucleotide sequence encoding a reporter gene.

93. A pharmaceutical composition comprising one ore more self-inactivating CRISPR/Cas9 delivery system according to any one of claims 73 to 75 and one or more pharmaceutical acceptable carrier and/or excipient.

94. A method for treating a Huntington's Disease, the method comprising: administering to a patient having the disease and/or disorder one or more effective amount of one or more self-inactivating CRISPR/Cas9 delivery system according to any one of claims 73 to 75.

95. The method according to claim 94, wherein an effective amount comprises a dosage of 10^9 to 10^{14} viral genomes or infectious units of viral vectors per dose.

96. The method according to claim 94, wherein an effective amount comprises a dosage of 10^{10} to 10^{12} viral genomes or infectious units of viral vector per dose.

97. The method according to claim 94, wherein administration comprises stereotaxic injection (i) into the striatum of the brain of the patient, (ii) into the cortex of the brain of the patient, or (iii) into both the striatum and the cortex of the brain of the patient.

98. The method of according to claim 94, wherein the patient is human.

99. A method for treating a Huntington's Disease, the method comprising: administering to a patient having the disease and/or disorder one or more effective amount of one or more self-inactivating CRISPR/Cas9 delivery system according to any one of claims 73 to 75; wherein

administration of one or more the one or more effective amount comprises administration of the first vector and the second vector at a selected molar ratio of the first vector to the second vector; wherein

the selected molar ratio (i) maximizes one or more beneficial and/or desired physiological effect, as compared to one or more ratio that is not selected, (ii) minimizes one or more detrimental and/or undesired physiological effect, as compared to one or more ratio that is not selected, or (iii) both (i) and (ii).

100. The method according to claim 99, wherein

(a) selection of a selected molar ratio comprises:

(i)(A) administering to one or more first mouse the first vector and the second vector at a first molar ratio of the first vector to the second vector, the first mouse being a model of a disease, a disorder, or both and/or (A') administering to one or more first population of cells the first vector and the second vector at a first molar ratio of the first vector to the second vector;

(ii) and measuring one or more first physiological effect of administering the first molar ratio;

(iii)(A) administering to one or more second mouse the first vector and the second vector at a second molar ratio of the first vector to the second vector, the second mouse being a model of the disease, the disorder, or both and/or (A') administering to one or more second population of cells the first vector and the second vector at a second molar ratio of the first vector to the second vector;

(iv) measuring one or more second physiological effect of administering the second molar ratio;

(v) optionally repeating (a)(i)-(a)(ii) one or more additional times at one or more additional molar ratio, wherein each of the one or more additional molar ratio is independently different from the first molar ratio and the second molar ratio; and

(v) selecting a molar ratio from the first molar ratio, the second molar ratio, and optionally one or more additional molar ratio, to produce a selected molar ratio, wherein the selected molar ratio is a molar ratio the administration of which (i) is effective to produce one or more beneficial and/or desired physiological effect and (ii) produces less detrimental and/or fewer detrimental and/or undesired physiological effects than one or more molar ratio that is not selected;

(b) maximizing one or more beneficial and/or desired physiological effect is accomplished by maximizing editing of one or more on-target genomic locus;

(c) minimizing one or more detrimental and/or undesired physiological effect is accomplished by eliminating or reducing editing of one or more off-target genomic locus; or

(d) any combination of (a)-(c).