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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
26 September 2024 (26.09.2024)

WIPO | PCT

(10) International Publication Number
WO 2024/196955 A2

(51) International Patent Classification:
A61K 31/711 (2006.01) *A61P 25/28* (2006.01)

(21) International Application Number:
PCT/US2024/020591

(22) International Filing Date:
19 March 2024 (19.03.2024)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

63/453,186	20 March 2023 (20.03.2023)	US
63/524,024	29 June 2023 (29.06.2023)	US

(71) Applicant: **DUKE UNIVERSITY** [US/US]; 2812 Erwin Road, Suite 406, Durham, North Carolina 27705 (US).

(72) Inventors: **CHIBA-FALEK, Ornit**; 2812 Erwin Road, Suite 406, Durham, North Carolina 27705 (US). **KANTOR, Boris**; 2812 Erwin Road, Suite 406, Durham, North Carolina 27705 (US).

(74) Agent: **MCMULLEN, Michelle L.** et al.; Polsinelli PC, 150 N. Riverside Plaza, Suite 3000, Chicago, Illinois 60606 (US).

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

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

Published:
— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*
— *with sequence listing part of description (Rule 5.2(a))*

(54) Title: APOE-TARGETED MICROGLIA-SPECIFIC GENE THERAPY FOR NEUROLOGICAL DISEASES

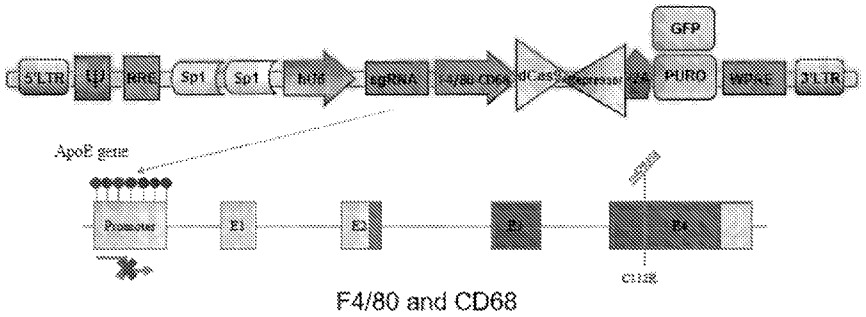


FIG. 4A

(57) Abstract: Disclosed herein are methods of administering precision gene therapy, treating and/or preventing Alzheimer's disease progression, and reducing expression of APOE and APOE e4. Disclosed herein are isolated nucleic acid molecules, viral vectors, AAV vectors, pharmaceutical formulations, host cells, guide RNAs, and plasmids for use in the disclosed methods.

WO 2024/196955 A2

APOE-TARGETED MICROGLIA-SPECIFIC GENE THERAPY FOR NEUROLOGICAL DISEASES

I. CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority to U.S. Provisional Patent Application No. 63/524,024 filed June 29, 2023, and U.S. Provisional Patent Application No. 63/453,186 filed March 20, 2023, both of which are incorporated by reference herein in their entirety.

II. REFERENCE TO THE SEQUENCE LISTING

[0002] This application contains a Sequence Listing that has been submitted electronically as a computer readable XML format via Patent Center and is hereby incorporated by reference in its entirety pursuant to 37 C.F.R. § 1.52(e)(5). The XML file, created on March 15, 2024, is named "23-2069-WO_SL.xml" and is 546,400 bytes in size.

III. BACKGROUND

[0003] Alzheimer's disease (AD) is sixth leading cause of death in the US and the most common cause of dementia in aging. With a rapidly growing aging population the number of AD cases is growing fast and projected to rise drastically over the next three decades. Today, more than 5 million people are living with AD in the United States alone, and by 2050, this number is projected to reach 14 million. Therefore, AD poses a huge economic burden on society placing overwhelming strain on the healthcare system. In 2020, the cost of AD to the US was $301 billion, including $206 billion in Medicare and Medicaid payments, while the caregivers provided $244 billion worth of care (Alzheimer's Association, Alzheimer's Impact Movement: Factsheet 2020). These trends will only worsen with time because there are no therapies to halt or prevent AD (*i.e.*, projected to cost more than $1.1 trillion annually by 2050). Despite all the research effort, money and commitment, clinical trials to identify disease modifying therapies (DMT) for AD have repeatedly failed. To date, there is no cure and no DMT for AD and there are no methods to delay the onset and/or progression of the disease. Most available treatments are palliative and aimed at relieving symptom (Sharma K. (2019) Mol Med Rep. 20:1479-1487; Olivares D, et al. (2012) Curr Alzheimer Res. 9:746-758).

[0004] Late onset AD (LOAD) is a heterogenous disease with various genetic etiologies (Lo MT, et al. (2019). Neurobiol Aging. 84:243 e1-243.e9; Nacmias B, et al. (2018) J Alzheimers Dis. 62:903-911). A major reason for the failure to identify an effective treatment is likely the inaccurate consideration of LOAD as a homogeneous disease. In this respect, increasing evidence

demonstrate the heterogeneity in the underlying pathophysiologic processes of LOAD and show variability in the genetic risk and molecular profiles amongst AD patients (Reitz C. (2016) Ann Transl Med. 4:107; Chiba-Falek O, et al. (2017) Expert Rev Precis Med Drug Dev. 2:47-55). Thus, AD remains an unmet medical need underscoring the urgent need for a paradigm shift in AD clinical research.

[0005] Accordingly, any advancement in LOAD therapy will require the development and validation of new therapeutic targets including those tailored to sub-groups of patients with specific risk factors. Thus, to date many investigators and funding bodies recognize the need to shift the focus to new potential culprits including candidate gene-targets such as LOAD risk genes and rare mutations.

[0006] ApoE is a protein involved in metabolism of fat and is a major lipid and cholesterol carrier in the CNS. There are three major human polymorphisms, apoE2, apoE3, and apoE4, and the genetic expression of APOE4 is considered to be a risk factor for the development of late-onset Alzheimer's disease (AD). Apart from AD, mutations and abnormal expression of APOE has also been implicated in other neurological diseases like Parkinson's disease and other neurodegenerative diseases including Age-Related Macular Degeneration AMD, and glaucoma.

[0007] Accordingly, there is a need for a disease modifying therapy for AD, particularly LOAD, that target specific genes like APOE in an allele specific manner.

IV. BRIEF DESCRIPTION OF THE FIGURES

[0008] **FIG. 1A** provides a UMAP plot showing separation of cell type-specific clusters in single nucleus (sn)RNA-seq data from 12 AD and 12 control temporal cortex. Microglial cluster 1 is circled.

[0009] **FIG. 1B** provides UMAP plot showing differential expression analysis of APOE in AD vs control mice, with identified APOE as differentially expressed in microglia cluster 1.

[0010] **FIG. 1C** is a violin plot representing APOE gene expression in LOAD compared to control for each cell cluster. Differential expression (DE) analysis identified APOE as significant Differential Expressed Gene (DEG) only in Micro1 cluster with $Log_2(FC)=0.45$ and adjusted $p=0.0086$.

[0011] **FIG. 1D** shows single nucleus (sn)ATAC-seq data from the same 12 AD and 12 control temporal cortex for *APOE* gene. The *APOE* gene is located at chromosome 19q13.2. Differential chromatin accessibility analysis, AD vs control, identified differential accessible peak in microglia cluster 1 DAP (red) and open chromatin peaks (orange).

[0012] **FIG. 1E** further shows LOAD Micro1 APOE DAP and co-accessible peaks within the range of gene range +/- 1k bp.

[0013] **FIG. 1G** depicts snRNA-seq of 12 LOAD and 12 aged and sex matched normal controls. RIGHT: dotplot for the clusters that underwent APOE tested in differential expression (DE) analysis. Size of dot = % nuclei expressing APOE, bluer = higher expression value. Micro1 is the only cluster with an obvious difference. LEFT: dotplots representing APOE expression in all brain cell clusters LOAD and control.

[0014] **FIG. 1H** depicts snATAC-seq of 12 LOAD and 12 aged and sex matched normal controls. Chromatin accessibility analysis across APOE genomic locus. UPPER PANELS: coverage plots for all 6 cell-types and micro1 cluster. Micro 1 differential accessible peak (DAP) in the promoter region of APOE is labeled in a red box. MIDDLE PANEL: APOE genomic region and gene structure. LOWER PANEL: Open chromatin peaks are denoted. APOE promoter peak: chr19 44905270-44906059 (790bp), APOE exon 2 peak, Micro1 specific : chr19 44906364-44906667 (304bp).

[0015] **FIG. 1I** is a bar graph showing differential expression results for all clusters in which APOE was tested. Illustrated a bar plot of the logFCs (coef), where Micro1 is clearly the most robust.

[0016] **FIG. 1J** provides a scatter plot showing the adjusted p-value (-log10(fdr)) vs. the logFC (coef) for snRNA-seq of 12 LOAD and 12 aged and sex matched normal controls. APOE as DEG was tested in 5 clusters in which sufficient number of nuclei expressed APOE. Broken line define the thresholds at p=0.05 and logFC=0.25. Micro1 is the only datapoint that passes both thresholds.

[0017] **FIG. 1K** provides a schematic of chromosome 19 with individual gRNAs (SEQ ID NO: 30-33) sequences in the ApoE promoter and intron 1 listed.

[0018] **FIG. 1L** shows clustering for snRNA-seq of 24 brain samples: 12 LOAD and 12 aged and sex matched normal controls. Analysis of genes expressed specifically and exclusively in Micro1 identified FCGR2A as a marker for Micro 1 cluster. UPPER PANEL: UMAP plot showing separation of cell type-specific clusters. Micro1 cluster circled in red. LOWER PANEL: FCGR2A expression is detected specifically in Micro1 cluster.

[0019] **FIG. 1M** shows snATAC-seq of 24 brain samples: 12 LOAD and 12 aged and sex matched normal controls. Chromatin accessibility analysis across the genomic region of FCGR2A - the Micro 1 marker . UPPER PANELS: coverage plots for all cell-types and micro1 cluster. MIDDLE PANEL: FCGR2A genomic region and gene structure. LOWER PANEL: Open chromatin peaks are denoted. The Micro 1 specific open chromatin/regulatory element overlapping FCGR2A promoter region is marked in Red box. 1904bp, coordinates chr1: 161505031-161506934 (hg38). The ATAC-seq peak includes the promoter-like signature (ENCODE: cis-Regulatory Elements (cCREs)) for FCGR2A Position: chr1:161505211-161505558, Genomic Size: 348.

[0020] **FIG. 2** shows measurement of repression capacity of developed AAV Cas repressor constructs. Left panel depicts a schematic of the screening cascade, middle panel depicts the in vitro luciferase assay to characterize repression effects on gene expression and right panel shows that Cas-repressor 6 showed the most robust reduction in in luciferase expression. Normalized luciferase was measured on day 6 after AAV transduction. This data represents the % of luciferase for each treatment group normalized by the control group (CRISPR/dCas with no sgRNA and no effector). Unpaired two-tail t-test with equal variance (α=0.05) was conducted to find significant gene repression (*p<0.01, **p<0.001, ***p<0.0001).

[0021] **FIG. 3A** shows qRT-PCR data for expression of five microglial specific markers. Human induced pluripotent stem cells harboring APOE3/3 (E3) and APOE4/4 (E4) were differentiated to microglia. Upon microglia maturation, RNA was isolated, and qRT-PCR was performed to evaluate the expression of the five microglia markers: IBA1, CD68, CD11B, P2RY12 and TMEM119. The results are displayed in the order of gene expression, with IBA1 having the highest expression in our cells. The results were normalized to GAPDH and PPIA1 internal controls.

[0022] **FIG. 3B** is the UCSC genome browser view of the AIF1 (IBA1) gene locus showing DAPS region as a red bar.

[0023] **FIG. 3C** shows representative immunofluorescence images testing IBA1 promoter activity. The numbers correspond to three iterations of IBA promoter as indicated below. Immortalized microglia HMC3 in 0%FBS EMEM or 0.5% FBS EMEM on matrigel plates were transduced with different GFP reporter AAV vectors (serotype 6) driven by synthetic human IBA1 promoters of 3 lengths.

[0024] **FIG. 3D** is a bar graph showing GFP expression for each of the IBA promoters tested. The medium length promoter (1986: SEQ ID NO: 6) showed the strongest GFP expression.

[0025] **FIG. 4A** is a schematic representation of lentiviral vector system carrying a repressor domain to target promoter and exon 4 regions of APOE gene. Micro1 specific regulatory element replace the constitutive promoter. Promoter sequence of F4/80 or CD68 may be used for microglial specific expression OR the promoter region of FCGR2A: (1904bp) coordinates chr1: 161505031-161506934 (hg38). Repressors: DNMT3A or KRAB-MeCP2 can be used.

[0026] **FIG. 4B** shows representative immunofluorescence images showing expression of GFP in organiods transformed with lentiviral or AAV viral vectors. Human induced pluripotent stem cell derived cerebral organoids were transduced with either Lentivirus or AAV serotype (phpeb) expressing GFP. Transduced organoids were spun down for 45 minutes at 300 g, and the media

was changed the next day. GFP fluorescence was monitored daily. Representative images show a higher efficiency of transduction with the AAV serotype (phpeb).

[0027] **FIG. 4C** are representative immunofluorescence images showing infection efficiency of different AAV serotypes. Human induced pluripotent stem cell derived cerebral organoids were transduced with various AAV serotypes expressing GFP. Transduced organoids were spun down for 45 minutes at 300 g, and the media was changed the next day. GFP fluorescence was monitored daily. Images show infection efficiency at different time points.

[0028] **FIG. 4D** are representative immunofluorescence showing microglial infection efficiency of different AAV serotypes. Immortalized microglia HMC3 in 0% FBS EMEM or 0.5% FBS EMEM on matrigel plates were transduced with 2uL of different serotypes. Media was changed the next day. Images were taken at day 3. Serotype 6 showed the strongest GFP expression.

[0029] **FIG. 4E** are representative immunofluorescence showing microglial infection efficiency of different AAV serotypes. Immortalized microglia HMC3 in 0% FBS EMEM or 0.5% FBS EMEM on matrigel plates were transduced with 2uL of different serotypes. Media was changed the next day. Images were taken at day 6. Serotype 6 showed the strongest GFP expression.

[0030] **FIG. 4F** is a schematic showing in vivo proof-of-concept of the platform efficacy in the hippocampus. Epigenome editing viral vector (AAV) with GFP as reporter were stereotaxically injected bilaterally into the left hemisphere of the hippocampus and a control vector with no repressor into the right hippocampus to provide within animal control.

[0031] **FIG. 4G** are representative immunofluorescence showing a robust and precise reduction in the expression of the reporter gene in the left side.

[0032] **FIG. 4H** is a box plot with statistical analysis of 11 mice, demonstrating stable and significant suppression over a period of 4 and 8 months.

[0033] **FIG. 4I** shows that was no significant change is weight in the injected animals over the duration they were monitored.

[0034] **FIG. 5A** is a schematic showing the *in vivo* validation experiment for the all-in-one AAV/dSaCas9-KRAB-MECP2(TRD) repressor platform targeting mouse endogenous APOE in the hippocampus. 4-month old C57B/L6 mice were used to perform a bilateral stereotaxic injection directly into the mice's dorsal hippocampus (DH), the right DH hemisphere was injected with the AAV-gRNA/dCas9-repressor vectors with gRNA 1 or gRNA 2 or the left DH hemisphere with the control vector with no repressor (AAV-dCas-no repressor).

[0035] **FIG. 5B** shows representative images (2X magnification) of brain slices 6 weeks post-injection, showing expression of mouse APOE in the dorsal hippocampus. A 20X magnification of the image is provide below.

[0036] **FIG. 5C** is a box plot showing that APOE was significantly repressed in the left dorsal hippocampus relative to the right dorsal hippocampus by AAV-gRNA1(ApoE)/dCas-repressor. The box plot displays the ratios of the right DH for mice injected with the repressor vector harboring gRNA 1 (n=8) and gRNA2 (n=8). Each open circle represents the quantified signal right/left for a mouse. (*p<0.05, **p<0.001, ***p<0.0001; Mann Whitney U Test).

[0037] **FIG. 5D** shows that there was no significant change is weight in the injected animals over the duration they were monitored. Daily weight post-injection (day 0) through when the mice were sacrificed were monitored (day 42).

[0038] **FIG. 5E** is a schematic showing the *in vivo* validation experiment for the all-in-one AAV/dSaCas9-KRAB-MECP2(TRD) repressor platform targeting human APOE in the hippocampus of APOE humanized mice model. 4-month old mice were used to perform a bilateral stereotaxic injection directly into the mice's dorsal hippocampus (DH), the right DH hemisphere was injected with the AAV-gRNA/dCas9-repressor vectors with gRNA 1 or gRNA 2 or the left DH hemisphere with the control vector with no repressor (AAV-dCas-no repressor).

[0039] **FIG. 5F** shows representative images (2X magnification) of brain slices 6 weeks post-injection, showing expression of human APOE in the dorsal hippocampus. A 20X magnification of the image is provide below.

[0040] **FIG. 5G** is a box plot showing that APOE was significantly repressed in the left dorsal hippocampus relative to the right dorsal hippocampus. Repression was significantly greater in males than females. APOE gRNA1 mice N =16 (8 female, 8 male), APOE gRNA2 mice N =12 (6 female, 6 male). Values represent mean ± SEM. * $p < 0.05$. Unpaired t-test.

[0041] **FIG. 6A** is a bar graph quantifying the results of APOE mRNA expression in 4 month old organoids. Human induced pluripotent stem cells harboring APOE3/3 (E3) and APOE4/4 (E4) were differentiated to cerebral organoids. Next, 4 months old organoids were transduced with gRNA/dCas-repressor (gRNA) virus harboring gRNA to target promoter region of APOE or no-gRNA (Control) virus. RNA was then isolated, and qRT-PCR was performed to evaluate the expression of APOE. Results show a significant downregulation of APOE expression in both E3 and E4 organoids. The results were normalized to GAPDH, PPIA1 and the controls.

[0042] **FIG. 6B** is a bar graph showing beneficial effect of reduced APOE leading to a decrease in pTAU expression as evidenced by the western blot analysis. The graph represents pTAU band intensity, normalized to total TAU. quantifying the results of APOE mRNA expression in 4 month-old organoids.

[0043] **FIG. 7A** shows *in vitro* validation studies of the microglia-specific APOEe4-targeted dCas9-repressor platform using hiPSC-derived microglia. Immunofluorescence image of hiPSC-derived microglia were stained with a microglia specific marker IBA1.

[0044] **FIG. 7B** is a bar graph showing a significant decrease in APOE-mRNA expression levels. APOEe4 microglia were transduced with gRNA/dCas-repressor or control (no-gRNA) viruses using a vector driven by a synthetic human CD68 regulatory sequence harboring gRNA to target the APOE locus.

[0045] **FIG. 7C** is a bar graph showing that repressing APOEe4 expression in the microglia led to an increase in Aβ uptake, comparable to that of APOEe3 microglia, ** $p < 0.01$, **** $p < 0.0001$

[0046] **FIG. 7D** are bar graphs showing reduction of the APOEe4 expression in the microglia led to an increase in TMEM119 level and decrease CD74, demonstrating rescue of neuroinflammation.

V. BRIEF SUMMARY

[0047] Disclosed herein is an isolated nucleic acid molecule, comprising: (a) a nucleic acid sequence encoding: (i) a deactivated Clustered Regularly Interspaced Short Palindromic Repeats associated (dCas) endonuclease, (ii) at least one polypeptide having an enzymatic activity, and (iii) at least one guide RNA targeting an APOE gene sequence or a regulatory element thereof; and (b) one or more microglia specific regulatory elements operably linked to (a)(i), (a)(ii), (a)(iii), or any combination thereof.

[0048] In an aspect, the one or more microglia specific regulatory element may comprise a promoter, wherein the promoter comprises a wild-type promoter, a synthetic promoter, or a minimal promoter. In an aspect, the one or more microglia specific regulatory elements are operably linked to the nucleic acid sequence encoding the dCas endonuclease. In an aspect, the one or more microglia specific regulatory elements are operably linked to the nucleic acid encoding the at least one polypeptide having enzymatic activity. In an aspect, the one or more microglia specific regulatory elements are operably linked to the nucleic acid encoding the dCas endonuclease and the nucleic acid encoding the at least one polypeptide having enzymatic activity. In an aspect, the one or more microglia specific regulatory elements is operably linked to the at least one guide RNA. Non-limiting examples of micro-glia specific promoters include F4/80, CD68, FCGR2A, HexA, FCGR2A or IBA1 promoter, or a derivative thereof, or any combination thereof. In an aspect, the promoter drives the expression of the at least one gRNA, the dCas endonuclease, the at least one polypeptide, or any combination thereof. In an aspect, the promoter is a CD68 promoter, or a derivative thereof. In an aspect, the promoter is a IBA1 promoter, or a

derivative thereof. In an aspect, the promoter is a F4/80 promoter, or a derivative thereof. In an aspect, the promoter comprises a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequence set forth in SEQ ID NO: 1-7, or a fragment or a derivative thereof.

[0049] The disclosed isolated nucleic acid may comprise a nucleic acid sequence encoding at least one gRNA which targets the APOE gene, or promoter region thereof, or an untranslated region (UTR) thereof, or any combination thereof. In an aspect, the at least one gRNA targets the promoter region, or intron 1, or a flanking sequence thereof, of APOE. In an aspect, the at least one gRNA may target the nucleic acid sequence set forth in SEQ ID NO: 15-33, or a sequence at least about 90% identical thereto.

[0050] The disclosed isolated nucleic acid may comprise a nucleic acid sequence encoding a Cas endonuclease comprising a deactivated Cas9 (dCas9) endonuclease. In an aspect, the dCas endonuclease comprises a deactivated Staphylococcus aureus Cas9 (dSaCas9), a deactivated Streptococcus pyogenes Cas9 (dSpCas9), a deactivated Campylobacter jejuni Cas9 (dCjCas9), or a variant dCas9 endonuclease. In an aspect, the dCas endonuclease may comprise a variant dCas9 endonuclease selected from a VRER, EQR or VQR variant of dCas9. In an aspect, the dCas9 endonuclease comprises an amino acid sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequence set forth in SEQ ID NO: 44-47, or a fragment thereof.

[0051] The disclosed isolated nucleic acid may comprise a nucleic acid sequence encoding at least one polypeptide with an enzymatic activity, for example a transcription activation activity, transcription repression activity, transcription release factor activity, histone modification activity, nucleic acid association activity, methyltransferase activity, demethylase activity, acetyltransferase activity, deacetylase activity, or any combination thereof. Non-limiting examples of polypeptide having enzymatic activity comprises HP1a, HP1b, MBD1, MBD2, Krüppel-Associated Box (KRAB), NIPP1, Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof. In an aspect, the at least one polypeptide having an enzymatic activity comprises Krüppel-associated box (KRAB), the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A or any fusion polypeptide thereof. In an aspect, the fusion polypeptide comprises KRAB-MeCP2 (KRAB-MeCP2). In an aspect, at least one polypeptide having enzymatic activity comprises an amino acid sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least

90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequence set forth in SEQ ID NO: 36-39, or a derivative thereof. In an aspect, the dCas endonuclease comprises dCas9 VRER and the polypeptide having enzymatic activity comprises Krüppel-associated box (KRAB), the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), or a fusion of KRAB-MeCP2 (KRAB-MeCP2). In an aspect, the nucleic acid sequence encodes a fusion protein comprising the dCas endonuclease and the at least one polypeptide having an enzymatic activity. In an aspect, the isolated nucleic acid molecule may comprise one of more of a P2A, T2A, E2A, F2A, and IRES sequence. In an aspect, the fusion protein comprises an amino acid sequence set forth in SEQ ID NO: 36-39, or a sequence at least 80% identical thereto. In an aspect, the isolated nucleic acid molecule may further comprising one or more additional regulatory elements, non-limiting examples of which include primer binding sites (PBS), one or more splice donor (SD) sites, one or more splice acceptor (SA) sites, one or more central polypurine tracts (cPPT), one or more polypurine tracts (PPT), one or more Rev Response elements (RRE), one or more Woodchuck Hepatitis Virus Posttranscriptional Regulatory Elements (WPRE), Sp1 responsive element, a p2A signal, a woodchuck hepatitis virus post-transcriptional regulatory element, a Phi signal-packaging signal, a rev responsive element, a 5'-LTR, and a 3'-LTR one or more retroviral vector packaging elements, or any combination thereof.

[0052] Also disclosed is a viral vector comprising the isolated nucleic acid molecule as provided herein. In an aspect, the vector is a recombinant adenovirus-associated viral vector (rAAV). In an aspect, the viral vector comprises a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequence set forth in the viral vector may a sequence as set forth in SEQ ID NO: 112-128.

[0053] In an aspect, the current disclosure also encompasses a pharmaceutical formulation comprising the isolated nucleic acid molecule as disclosed herein or the rAAV vector as disclosed herein and a pharmaceutically acceptable carrier. In an aspect, the pharmaceutical formulation is for the treatment of a neurological disease associated with APOE overexpression. In an aspect, the neurodegenerative disease is Alzheimer's disease (AD) or Parkinson's disease (PD).

[0054] In an aspect, the current disclosure also encompasses a method of effecting an epigenetic modulation in a cell, comprising contacting the cell with the isolated nucleic acid molecule or the viral vector disclosed herein. In an aspect, the cell is a microglial cell. In an aspect, the epigenetic modulation comprises modification of methylation of at least one nucleotide in the promoter region of APOE.

[0055] In an aspect, the current disclosure also encompasses a method of effecting a precision gene therapy, comprising administering to a subject in need thereof a therapeutically effective amount of the disclosed pharmaceutical composition. In an aspect, the administering of the pharmaceutical composition enhances DNA-methylation of the APOE promoter region in at least one microglial cells.

[0056] Disclosed herein is a method of treating and/or preventing a neurological disease in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the pharmaceutical composition. In an aspect, the subject is a human. In an aspect, the neurological disease is Alzheimer's disease, Parkinson's disease, or a neuroinflammatory disease. In an aspect, the subject is suspected of having or has been diagnosed with having Alzheimer's disease. In an aspect, the method comprises reducing the pathological phenotype associated with Alzheimer's disease. In an aspect, administering the pharmaceutical composition comprises intravenous administration, intracerebral administration, intra-CSF administration, intracerebroventricular (ICV) administration, intraventricular administration, intra-cisterna magna (ICM) administration, intraparenchymal administration, intrathecal (lumbar, cisternal, or both) administration, or any combination thereof. In an aspect, the method further comprises administering to the subject a therapeutically effective amount of a therapeutic agent. In an aspect, the administering to the subject a therapeutically effective amount of an immune modulator.

[0057] Disclosed herein is a method of treating and/or preventing Alzheimer's disease progression in a subject, the method comprising administering to the subject a therapeutically effective amount of the disclosed pharmaceutical composition, wherein the administering of the pharmaceutical composition reduces the expression of APOE at least one microglial cell of the subject, thereby reducing the pathological phenotype associated with Alzheimer's disease. In an aspect, the subject is a human. In an aspect, administering the vector comprises intravenous administration, intracerebral administration, intra-CSF administration, intracerebroventricular (ICV) administration, intraventricular administration, intra-cisterna magna (ICM) administration, intraparenchymal administration, intrathecal (lumbar, cisternal, or both) administration, or any combination thereof. In an aspect, the method further comprising administering to the subject at least one additional therapeutic effective agent. In an aspect, the therapeutically effective agent is an immune modulator.

[0058] In an aspect, the current disclosure also encompasses a method of controlling expression of APOE in a target cell comprising, contacting the target cell with the disclosed isolated nucleic acid molecule, or the viral vector, or the pharmaceutical composition, wherein the APOE expression in the target cell is reduced after contacting the cell. In an aspect, the target cell

comprises a microglial cell. In an aspect, the target cell is located in vivo, ex vivo, or in vitro. In an aspect, the target cell is located in a brain tissue. In an aspect, the target cell is located in a human brain.

VI. DETAILED DESCRIPTION

[0059] The present disclosure describes formulations, compounded compositions, kits, capsules, containers, and/or methods thereof. It is to be understood that the inventive aspects of which are not limited to specific synthetic methods unless otherwise specified, or to particular reagents unless otherwise specified, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular aspects only and is not intended to be limiting. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, example methods and materials are now described.

[0060] All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited. The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention.

A. Alzheimer's Disease

[0061] Alzheimer's disease (AD) is the sixth leading cause of death in the United States and the most common cause of dementia in aging. With a rapidly growing aging population, the number of AD cases is growing fast and is projected to rise drastically over the next three decades. In the U.S. alone today, more than 5 million people are living with AD. By 2050, this number is projected to reach 14 million cases. Therefore, AD poses a huge economic burden on society placing overwhelming strain on the healthcare system. Currently, the cost of AD to the US is $301 billion, including $206 billion in Medicare and Medicaid payments. It is estimated that caregivers provide 244 billion dollars' worth of care (Costs of Alzheimer's to Medicare and Medicaid. (2020); Alzheimer's Association, Alzheimer's Impact Movement: Fact Sheet 2020). In the absence of a treatment that halts or prevents AD, the cost of AD to society is projected to be more than $1.1 trillion annually by 2050. Mainstream research for LOAD therapies continues to focus on the Amyloid Cascade Hypothesis, and as such Aβ has been for decades the leading molecular target (Mawuenyega KG, et al. (2013). Anal Biochem 440:56-62). Despite all the research effort, money, and commitment, numerous clinical trials aimed at identifying disease modifying therapies (DMT) for AD have failed. Current treatments are mostly palliative and aimed at relieving symptoms management (Sharma K. (2019). Mol Med Rep 20:1479-1487; Olivares D, et al. (2012). Curr

Alzheimer Res 9:746-758). Thus, AD remains an unmet medical need underscoring the urgent need for a paradigm shift in AD drug discovery research.

B. Late Onset Alzheimer's Disease (LOAD)

[0062] Late onset AD (LOAD) is a heterogenous disease with various genetic etiologies. A major reason for the failure to identify an effective treatment is likely the inaccurate consideration of LOAD as a homogeneous disease. In this respect, mounting evidence demonstrates the heterogeneity in the underlying pathophysiologic processes of LOAD and shows variability in the genetic risk and molecular profiles amongst AD patients. Advancement in LOAD therapy requires the development and validation of new therapeutic targets including those tailored to a specific sub-group of patients with a specific set of risk factors. *APOE* has emerged as potential promising targets for LOAD treatment.

C. Apolipoprotein E (ApoE)

[0063] Apolipoprotein E is encoded by the APOE gene positioned on chromosome 19q13.32 (GRCh 38: chr19:44,905,795-44,909,392). Two common coding SNPs in exon 4 give rise to three allelic variants (APOE e2, APOE e3, and APOE e4), encoding three corresponding protein isoforms that differ at two amino acid positions 112 and 158. The e4 allele of the apolipoprotein E gene (APOE e4) is the first, strongest, and most firmly established genetic risk factor for LOAD.

[0064] The initial discovery was made nearly 30 years ago by linkage analysis of pedigrees. Over the ensuing years, APOE e4 has become the most highly replicated genetic risk factor *for LOAD*. Subsequent LOAD genome-wide association studies (GWAS) have confirmed strong associations with the *APOE* genomic region, and no other LOAD-association remotely approached the same level of significance.

[0065] Carrying the APOE e4 variant significantly increases the lifetime risk for LOAD, whereas the number of e4 copies affects the level of risk and is associated with *lower* age of clinical disease onset. APOE e3 is natural and APOE e2 confers a protective effect.

[0066] About 40-65% of LOAD patients carry the e4 allele as compared to 10-15% in the general population. Data indicates that ApoE e4 acquired hyperfunction (gain of toxic effects). Data implicates several cellular pathways through which ApoE e4 may exert toxicity associated with LOAD pathologic phenotypes. Thus, decreasing the levels of ApoE e4 has a therapeutic implication. However, ApoE e4 as a target for LOAD remains significantly understudied.

[0067] Disclosed herein are compositions and methods for decreasing the levels of ApoE. In an aspect, the disclosed compositions and methods are effective in decreasing the expression of APOE e4. The allelic discrimination approach is innovative as it allows a precise and fine-tuned downregulation of APOEe4 allele expression.

[0068] Also disclosed herein are compositions and methods for epigenetic editing at specifically targeted APOE loci representing appealing approach for gene therapy mediated by altering the expression of a specific gene. Allele specific modulation combined with epigenetic gene editing described herein provides a precise and targeted approach to treat diseases like Alzheimer's and Parkinson's. This technology offers an unprecedented opportunity to modify epigenetic markers to control the expression of disease-associated genes and variants. The allelic discrimination approach is innovative as it allows a precise and fine-tuned downregulation of APOEe4 allele expression.

[0069] Also disclosed herein is a method of cell-type specific targeting of APOE in the microglia. This method has several benefits, for example minimal therapeutic side effects, often a concern with CRISPR based technologies.

[0070] Finally, also provided herein is an all-in-one vector technology which circumvents several challenges related to gene therapy: (i) optimized AAV backbone for high packaging and delivery efficiencies of the CRISPR/dCas9 components; (ii) low cytotoxicity, immunogenicity; and (iii) inheritable, sustainable expression of the vector to ensure the durability of the epigenetic changes.

D. Relevant Definitions

[0071] Before the present compounds, compositions, articles, systems, devices, and/or methods are disclosed and described, it is to be understood that they are not limited to specific synthetic methods unless otherwise specified, or to particular reagents unless otherwise specified, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular aspects only and is not intended to be limiting. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, example methods and materials are now described.

[0072] This disclosure describes inventive concepts with reference to specific examples. However, the intent is to cover all modifications, equivalents, and alternatives of the inventive concepts that are consistent with this disclosure.

[0073] As used in the specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise.

[0074] The phrase "consisting essentially of" limits the scope of a claim to the recited components in a composition or the recited steps in a method as well as those that do not materially affect the basic and novel characteristic or characteristics of the claimed composition or claimed method. The phrase "consisting of" excludes any component, step, or element that is not recited in the claim. The phrase "comprising" is synonymous with "including," "containing," or "characterized

by," and is inclusive or open-ended. "Comprising" does not exclude additional, unrecited components or steps.

[0075] The phraseology and terminology employed herein are for the purpose of description and should not be regarded as limiting. For example, the use of a singular term, such as, "a" is not intended as limiting of the number of items. Also, the use of relational terms such as, but not limited to, "top," "bottom," "left," "right," "upper," "lower," "down," "up," and "side," are used in the description for clarity in specific reference to the figures and are not intended to limit the scope of the present disclosure or the appended claims.

[0076] Any term of degree such as, but not limited to, "substantially" as used in the description and the appended claims, should be understood to include an exact, or a similar, but not exact configuration. For example, "a substantially planar surface" means having an exact planar surface or a similar, but not exact planar surface. Similarly, the terms "about" or "approximately," as used in the description and the appended claims, should be understood to include the recited values or a value that is three times greater or one third of the recited values. For example, about 3 mm includes all values from 1 mm to 9 mm, and approximately 50 degrees includes all values from 16.6 degrees to 150 degrees. For example, they can refer to less than or equal to ± 5%, such as less than or equal to ± 2%, such as less than or equal to ± 1%, such as less than or equal to ± 0.5%, such as less than or equal to ± 0.2%, such as less than or equal to ± 0.1%, such as less than or equal to ± 0.05%.

[0077] Ranges can be expressed herein as from "about" one particular value, and/or to "about" another particular value. When such a range is expressed, a further aspect includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by use of the antecedent "about," it will be understood that the particular value forms a further aspect. It will be further understood that the endpoints of each of the ranges are significant both in relation to the other endpoint and independently of the other endpoint. It is also understood that there are a number of values disclosed herein, and that each value is also herein disclosed as "about" that particular value in addition to the value itself. For example, if the value "10" is disclosed, then "about 10" is also disclosed. It is also understood that each unit between two particular units are also disclosed. For example, if 10 and 15 are disclosed, then 11, 12, 13, and 14 are also disclosed.

[0078] References in the specification and concluding claims to parts by weight of a particular element or component in a composition denotes the weight relationship between the element or component and any other elements or components in the composition or article for which a part by weight is expressed. Thus, in a compound containing 2 parts by weight component X and 5

parts by weight component Y, X and Y are present at a weight ratio of 2:5, and are present in such ratio regardless of whether additional components are contained in the compound.

[0079] The terms "or" and "and/or," as used herein, are to be interpreted as inclusive or meaning any one or any combination. Therefore, "A, B, or C" or "A, B, and/or C" mean any of the following: "A," "B," or "C"; "A and B"; "A and C"; "B and C"; "A, B, and C." An exception to this definition will occur only when a combination of elements, functions, steps or acts are in some way inherently mutually exclusive.

[0080] Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art to which this disclosure belongs. The following references provide one of skill with a general definition of many of the terms used in this disclosure: Singleton et al., Dictionary of Microbiology and Molecular Biology (2nd ed. 1994); The Cambridge Dictionary of Science and Technology (Walker ed., 1988); The Glossary of Genetics, 5th Ed., R. Rieger et al. (eds.), Springer Verlag (1991); and Hale & Marham, The Harper Collins Dictionary of Biology (1991), all of which are incorporated by reference herein. As used herein, the following terms have the meanings ascribed to them below, unless specified otherwise.

[0081] The phraseology and terminology employed herein are for the purpose of description and should not be regarded as limiting. When introducing elements of the present disclosure or the preferred aspects(s) thereof, the articles "a," "an," "the" and "said" are intended to mean that there are one or more of the elements. The terms "comprising," "including" and "having" are intended to be inclusive and mean that there may be additional elements other than the listed elements. Wherever the terms "comprising" or "including" are used, it should be understood the disclosure also expressly contemplates and encompasses additional aspects "consisting of" the disclosed elements, in which additional elements other than the listed elements are not included.

[0082] As used herein, the terms "optional" or "optionally" means that the subsequently described event or circumstance can or cannot occur, and that the description includes instances where said event or circumstance occurs and instances where it does not. In an aspect, a disclosed method can optionally comprise one or more additional steps, such as, for example, repeating an administering step or altering an administering step.

[0083] Further, as the present disclosure is susceptible to aspects of many different forms, it is intended that the present disclosure be considered as an example of the principles of the present disclosure and not intended to limit the present disclosure to the specific aspects shown and described. Any one of the features of the present disclosure may be used separately or in combination with any other feature. References to the terms "aspect," "aspects," and/or the like

in the description mean that the feature and/or features being referred to are included in, at least, one aspect of the description. Separate references to the terms "aspect," "aspects," and/or the like in the description do not necessarily refer to the same aspect and are also not mutually exclusive unless so stated and/or except as will be readily apparent to those skilled in the art from the description. For example, a feature, structure, process, step, action, or the like described in one aspect may also be included in other aspects but is not necessarily included. Thus, the present disclosure may include a variety of combinations and/or integrations of the aspects described herein. Additionally, all aspects of the present disclosure, as described herein, are not essential for its practice. Likewise, other systems, methods, features, and advantages of the present disclosure will be, or become, apparent to one with skill in the art upon examination of the figures and the description. It is intended that all such additional systems, methods, features, and advantages be included within this description, be within the scope of the present disclosure, and be encompassed by the claims.

[0084] The term "nucleic acid" or "polynucleotide" refers to deoxyribonucleic acids (DNA) or ribonucleic acids (RNA) and polymers thereof in either single- or double-stranded form. Unless specifically limited, the term encompasses nucleic acids containing known analogues of natural nucleotides that have similar binding properties as the reference nucleic acid and are metabolized in a manner similar to naturally occurring nucleotides. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (*e.g.*, degenerate codon substitutions), alleles, orthologs, SNPs, and complementary sequences as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues See, *e.g.*, Batzer et al., Nucleic Acid Res. 19:5081 (1991), the disclosure of which is incorporated in its entirety herein.

[0085] The terms "peptide," "polypeptide," and "protein" are used interchangeably, and refer to a compound comprised of amino acid residues covalently linked by peptide bonds. A protein or peptide must contain at least two amino acids, and no limitation is placed on the maximum number of amino acids that can comprise a protein's or peptide's sequence. Polypeptides include any peptide or protein comprising two or more amino acids joined to each other by peptide bonds. As used herein, the term refers to both short chains, which also commonly are referred to in the art as peptides, oligopeptides and oligomers, for example, and to longer chains, which generally are referred to in the art as proteins, of which there are many types. "Polypeptides" include, for example, biologically active fragments, substantially homologous polypeptides, oligopeptides, homodimers, heterodimers, variants of polypeptides, modified polypeptides, derivatives, analogs,

fusion proteins, among others. A polypeptide includes a natural peptide, a recombinant peptide, or a combination thereof.

[0086] Within the context of the application a protein is represented by an amino acid sequence and correspondingly a nucleic acid molecule or a polynucleotide represented by a nucleic acid sequence. Identity and similarity between sequences: throughout this application, each time one refers to a specific amino acid sequence SEQ ID NO (take SEQ ID NO: Y as example), one may replace it by: a polypeptide represented by an amino acid sequence comprising a sequence that has at least 60% sequence identity or similarity with amino acid sequence SEQ ID NO: Y. Another preferred level of sequence identity or similarity is 65%. Another preferred level of sequence identity or similarity is 70%. Another preferred level of sequence identity or similarity is 75%. Another preferred level of sequence identity or similarity is 80%. Another preferred level of sequence identity or similarity is 85%. Another preferred level of sequence identity or similarity is 90%. Another preferred level of sequence identity or similarity is 95%. Another preferred level of sequence identity or similarity is 98%. Another preferred level of sequence identity or similarity is 99%.

[0087] Each amino acid sequence described herein by virtue of its identity or similarity percentage with a given amino acid sequence respectively has in a further preferred aspect an identity or a similarity of at least 60%, at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% with the given nucleotide or amino acid sequence, respectively. The terms “homology,” “sequence identity” and the like are used interchangeably herein. Sequence identity is described herein as a relationship between two or more amino acid (polypeptide or protein) sequences or two or more nucleic acid (polynucleotide) sequences, as determined by comparing the sequences. In a preferred aspect, sequence identity is calculated based on the full length of two given SEQ ID NO’s or on a part thereof. Part thereof preferably means at least 50%, 60%, 70%, 80%, 90%, or 100% of both SEQ ID NO’s. In the art, “identity” also refers to the degree of sequence relatedness between amino acid or nucleic acid sequences, as the case may be, as determined by the match between strings of such sequences. The degree of sequence identity between two sequences can be determined, for example, by comparing the two sequences using computer programs commonly employed for this purpose, such as global or local alignment algorithms. Non-limiting examples

include BLASTp, BLASTn, Clustal W, MAFFT, Clustal Omega, AlignMe, Praline, GAP, BESTFIT, or another suitable method or algorithm. A Needleman and Wunsch global alignment algorithm can be used to align two sequences over their entire length or part thereof (part thereof may mean at least 50%, 60%, 70%, 80%, 90% of the length of the sequence), maximizing the number of matches and minimizes the number of gaps. Default settings can be used and preferred program is Needle for pairwise alignment (in an aspect, EMBOSS Needle 6.6.0.0, gap open penalty 10, gap extent penalty: 0.5, end gap penalty: false, end gap open penalty: 10 , end gap extent penalty: 0.5 is used) and MAFFT for multiple sequence alignment (in an aspect, MAFFT v7Default value is: BLOSUM62 [bl62], Gap Open: 1.53, Gap extension: 0.123, Order: aligned, Tree rebuilding number: 2, Guide tree output: ON [true], Max iterate: 2, Perform FFTS: none is used).

[0088] "Similarity" between two amino acid sequences is determined by comparing the amino acid sequence and its conserved amino acid substitutes of one polypeptide to the sequence of a second polypeptide. Similar algorithms used for determination of sequence identity may be used for determination of sequence similarity. Optionally, in determining the degree of amino acid similarity, the skilled person may also take into account so-called conservative amino acid substitutions. As used herein, "conservative" amino acid substitutions refer to the interchangeability of residues having similar side chains.

[0089] For example, a group of amino acids having aliphatic side chains is glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having amide-containing side chains is asparagine and glutamine; a group of amino acids having aromatic side chains is phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains is lysine, arginine, and histidine; and a group of amino acids having sulfur-containing side chains is cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, and asparagine-glutamine. Substitutional variants of the amino acid sequence disclosed herein are those in which at least one residue in the disclosed sequences has been removed and a different residue inserted in its place. Preferably, the amino acid change is conservative. Preferred conservative substitutions for each of the naturally occurring amino acids are as follows: Ala to Ser; Arg to Lys; Asn to Gln or His; Asp to Glu; Cys to Ser or Ala; Gln to Asn; Glu to Asp; Gly to Pro; His to Asn or Gln; Ile to Leu or Val; Leu to Ile or Val; Lys to Arg; Gln or Glu; Met to Leu or Ile; Phe to Met, Leu or Tyr; Ser to Thr; Thr to Ser; Trp to Tyr; Tyr to Trp or Phe; and Val to Ile or Leu.

[0090] As used herein, "operably linked" means that expression of a gene is under the control of a regulatory element with which it is spatially connected. In some instance the regulatory element is a promoter and when used in this context, the expression of a gene is under control of the promoter with which it is spatially connected. A regulatory element (*e.g.*, a promoter) can be positioned 5' (upstream) or 3' (downstream) of a gene under its control. The distance between the regulatory element (*e.g.*, promoter) and a gene can be approximately the same as the distance between that regulatory element (promoter) and the gene it controls in the gene from which the regulatory element (*e.g.*, promoter) is derived. As is known in the art, variation in this distance can be accommodated without loss of function (*i.e.*, without loss of promoter function).

[0091] As used herein, "regulatory elements" refer to any sequence elements that regulate, positively or negatively, the expression of an operably linked sequence. "Regulatory elements" include, without being limiting, a promoter, an enhancer, a leader, a transcription start site (TSS), a linker, 5′ and 3′ untranslated regions (UTRs), an intron, a polyadenylation signal, and a termination region or sequence, etc., that are suitable, necessary, or preferred for regulating or allowing expression of the gene or transcribable DNA sequence in a cell. Such additional regulatory element(s) can be optional and used to enhance or optimize expression of the gene or transcribable DNA sequence. A regulatory sequence can, for example, be inducible, non-inducible, constitutive, cell-cycle regulated, metabolically regulated, and the like. A regulatory sequence may be a promoter. As used herein, the term "promoter" refers to a DNA sequence that comprises an RNA polymerase binding site, a transcription start site, and/or a TATA box and assists or promotes the transcription and expression of an associated transcribable polynucleotide sequence and/or gene (or transgene). A promoter can be synthetically produced, varied, or derived from a known or naturally occurring promoter sequence or other promoter sequence. A promoter can also include a chimeric promoter comprising a combination of two or more heterologous sequences. A promoter of the present application can thus include variants of promoter sequences that are similar in composition, but not identical to, other promoter sequence(s) known or provided herein. In some exemplary aspects the promoter sequence is adapted to enable expression of a nucleic acid in the microglial cells. In an aspect, the promoter is a microglia specific promoter or a derivative thereof, non-limiting examples of which include F4/80, CD68, FCGR2A, HexB or IBA1.

[0092] As used herein, the term "microglia specific regulatory element," encompasses a sequence which regulates the expression of a gene-of-interest to which it is operably linked, such that the gene product exhibits enhanced and/or preferential expression in the microglia when compared to other cell-types. In as aspect, the enhanced expression may result in at least about 1.1-fold to about

100-fold or more, expression of the gene-of-interest. A microglia specific regulatory sequence may comprise a microglia specific wild-type promoter, a microglia specific enhancer, a synthetic promoter, a synthetic enhancer, a transcriptional or translational regulatory sequence or any combination thereof. In an aspect, the microglia specific regulatory sequence is a promoter, an enhancer or a combination thereof. As used herein, the term "microglia specific promoter" includes wild-type promoter, or derivatives thereof, synthetic promoter, minimal promoter, and any combination of promoter and enhancers sequence, that drives enhanced expression of a gene-of-interest to which it is operably linked in the microglial cells.

[0093] The terms "derivative," "variant," and "fragment," when used herein with reference to a nucleic acid sequence, refers to a nucleic acid sequence related to a wild type sequence, for example either by the nucleic acid sequence or encoded amino acid sequence, structure (*e.g.*, secondary and/or tertiary), activity (*e.g.*, enzymatic activity) and/or function. Derivatives, variants and fragments of a nucleic acid sequence can comprise one or more nucleotide variations (*e.g.*, mutations, insertions, and deletions), truncations, modifications, or combinations thereof compared to a wild type nucleic acid sequence. The terms "derivative," "variant," and "fragment," when used herein with reference to a polypeptide, refers to a polypeptide related to a wild type polypeptide, for example either by amino acid sequence, structure (*e.g.*, secondary and/or tertiary), activity (*e.g.*, enzymatic activity) and/or function. Derivatives, variants and fragments of a polypeptide can comprise one or more amino acid variations (*e.g.*, mutations, insertions, and deletions), truncations, modifications, or combinations thereof compared to a wild type polypeptide.

[0094] A "fragment" or "portion" of a nucleotide sequence can be understood to mean a nucleotide sequence of reduced length relative (*e.g.*, reduced by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more nucleotides) to a reference nucleic acid or nucleotide sequence and comprising, consisting essentially of, or consisting of a nucleotide sequence of contiguous nucleotides identical or almost identical (*e.g.*, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical) to the reference nucleic acid or nucleotide sequence. Such a nucleic acid fragment or portion according to the disclosure can be, where appropriate, included in a larger polynucleotide of which it is a constituent. In an aspect, a fragment or portion of a nucleotide sequence or nucleic acid sequence can comprise the sequence encoding an exon having one or more mutations. In an aspect, a fragment or portion of a nucleotide sequence or nucleic acid sequence can comprise a target of interest.

[0095] A "fragment" or "portion" of an amino acid sequence can be understood to mean an amino acid sequence of reduced length relative (*e.g.*, reduced by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, or more amino acids) to a reference amino acid sequence and comprising, consisting essentially of, or consisting of an amino acid sequence of contiguous amino acids identical or almost identical (*e.g.*, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical) to the reference amino acid sequence. Such an amino acid fragment or portion according to the disclosure can be, where appropriate, included in a larger amino acid sequence of which it is a constituent.

[0096] As used herein, an "isolated" refers to a biological component (such as a nucleic acid molecule, nucleic acid sequence, protein, or virus) that has been substantially separated or purified away from other biological components (*e.g.*, other chromosomal and extra-chromosomal DNA and RNA, proteins and/or organelles). Nucleic acids, proteins, and/or viruses that have been "isolated" include nucleic acids, proteins, and viruses purified by standard purification methods. The term also embraces nucleic acids, proteins, and viruses prepared by recombinant expression in a host cell, as well as chemically synthesized nucleic acids or proteins. The term "isolated" (or purified) does not require absolute purity; rather, it is intended as a relative term. Thus, for example, an isolated or purified nucleic acid, protein, virus, or other active compound is one that is isolated in whole or in part from associated nucleic acids, proteins, and other contaminants. In an aspect, the term "substantially purified" refers to a nucleic acid, protein, virus or other active compound that has been isolated from a cell, cell culture medium, or other crude preparation and subjected to fractionation to remove various components of the initial preparation, such as proteins, cellular debris, and other components. In an aspect, isolated proteins or nucleic acids, or cells containing such, in some examples are at least 50% pure, such as at least 75%, at least 80%, at least 90%, at least 95%, at least 98%, or at least 100% pure. Isolated nucleic acid molecules may comprise one or more naturally occurring sequences, recombinant sequences, or combinations thereof. An isolated nucleic acid molecule may comprise any one or more of a gene, a transcription regulatory sequence, a translation regulatory sequence, coding sequence, non-coding sequence, plasmids, vector, or viral vector. The isolated nucleic acid molecule may also comprise one or more modified nucleotides.

[0097] A "heterologous" or a "recombinant" nucleotide or amino acid sequence as used interchangeably herein can refer to a nucleotide or an amino acid sequence not naturally associated with a host cell into which it is introduced, including non-naturally occurring multiple copies of a naturally occurring nucleotide or amino acid sequence.

[0098] Different nucleic acids or proteins having homology can be referred to as "homologues." The term homologue includes homologous sequences from the same and other species and orthologous sequences from the same and other species. "Homology" refers to the level of similarity between two or more nucleic acid and/or amino acid sequences in terms of percent of positional identity (*i.e.*, sequence similarity or identity). Homology also refers to the concept of similar functional properties among different nucleic acids or proteins. Thus, the disclosed compositions and disclosed methods can comprise homologues to the disclosed nucleotide sequences and/or disclosed polypeptide sequences.

[0099] "Orthologous," as used herein, can refer to homologous nucleotide sequences and/or amino acid sequences in different species that arose from a common ancestral gene during speciation. A homologue of a disclosed nucleotide sequence or a disclosed polypeptide can have substantial sequence identity (*e.g.*, at least about 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, and/or 100%) to a disclosed nucleotide sequence or a disclosed polypeptide.

[0100] "Complement" or "complementary" as used herein means a nucleic acid can mean Watson-Crick (*e.g.*, A-T/U and C-G) or Hoogsteen base pairing between nucleotides or nucleotide analogs of nucleic acid molecules. "Complementarity" refers to a property shared between two nucleic acid sequences, such that when they are aligned antiparallel to each other, the nucleotide bases at each position will be complementary.

[0101] As used herein, the term "APOE associated diseases or disorders" refers to any disease of disorder that results from an abnormal APOE gene or abnormal APOE gene expression/activity, or both. Non-limiting examples of APOE associated diseases and disorders include Alzheimer's disease, Parkinson's disease, and other neurodegenerative diseases including Age-Related Macular Degeneration (AMD), and glaucoma.

[0102] As used herein, the term "subject" refers to the target of administration, *e.g.*, a human being. The term "subject" also includes domesticated animals (*e.g.*, cats, dogs, etc.), livestock (*e.g.*, cattle, horses, pigs, sheep, goats, etc.), and laboratory animals (*e.g.*, mouse, rabbit, rat, guinea pig, fruit fly, etc.). Thus, the subject of the herein disclosed methods can be a vertebrate, such as a mammal, a fish, a bird, a reptile, or an amphibian. Alternatively, the subject of the herein disclosed methods can be a human, non-human primate, horse, pig, rabbit, dog, sheep, goat, cow, cat, guinea pig, or rodent. The term does not denote a particular age or sex, and thus, adult and child subjects, as well as fetuses, whether male or female, are intended to be covered. In an aspect, a subject can be a human patient. In an aspect, a subject can have or is suspected of having

Alzheimer's disease (*e.g.*, LOAD), be suspected of having Alzheimer's disease, or be at risk of developing and/or acquiring Alzheimer's disease. In an aspect, a subject can have or is suspected of having Parkinson's disease (PD), or other neurodegenerative diseases associated with abnormal APOE gene, expression and/or activity, non-limiting examples of which include Age-Related Macular Degeneration (AMD), and glaucoma.

[0103] As used herein, the term "diagnosed" means having been subjected to an examination by a person of skill, for example, a physician, and found to have a condition that can be diagnosed or treated by one or more of the disclosed agents, disclosed therapeutic agents, disclosed pharmaceutical formulations, or a combination thereof, or by one or more of the disclosed methods. For example, "diagnosed with Alzheimer's disease or LOAD" means having been subjected to an examination by a person of skill, for example, a physician, and found to have a condition that can be treated by one or more of the disclosed isolated nucleic acid molecules, disclosed viral vectors, disclosed lentiviral vectors, disclosed pharmaceutical formulations, disclosed host cells, disclosed gRNAs, disclosed plasmids, or any combination thereof, or by one or more of the disclosed methods. For example, "suspected of having Alzheimer's disease" can mean having been subjected to an examination by a person of skill, for example, a physician, and found to have a condition that can likely be treated by one or more of the disclosed isolated nucleic acid molecules, disclosed viral vectors, disclosed lentiviral vectors, disclosed pharmaceutical formulations, disclosed host cells, disclosed gRNAs, disclosed plasmids, or any combination thereof, or by one or more of the disclosed methods. In an aspect, an examination can be physical, can involve various tests (*e.g.*, blood tests, genotyping, biopsies, etc.) and assays (*e.g.*, enzymatic assay), or a combination thereof.

[0104] A "patient" can refer to a subject that has been diagnosed with or is suspected of having an APOE associated disease or condition. In an aspect, a patient can refer to a subject that has been diagnosed with or is suspected of having an APOE associated disease or condition, and is seeking treatment or receiving treatment for APOE associated disease or condition. A "patient" can refer to a subject that has been diagnosed with or is suspected of having Alzheimer's disease (AD) or late-onset Alzheimer's disease (LOAD). In an aspect, a patient can refer to a subject that has been diagnosed with or is suspected of having AD such as for example, LOAD, and is seeking treatment or receiving treatment for AD or LOAD.

[0105] As used herein, the phrase "identified to be in need of treatment for a disorder," or the like, refers to selection of a subject based upon need for treatment of the disorder. For example, a subject can be identified as having a need for treatment of a disorder (*e.g.*, such as APOE associated disease or disorder) based upon an earlier diagnosis by a person of skill and thereafter

subjected to treatment for the disorder (*e.g.*, AD, LOAD, PD, AMD).For example, a subject can be identified as having a need for treatment of a disorder (*e.g.*, such as AD) based upon an earlier diagnosis by a person of skill and thereafter subjected to treatment for the disorder (*e.g.*, AD or LOAD). In an aspect, the identification can be performed by a person different from the person making the diagnosis. In an aspect, the administration can be performed by one who performed the diagnosis.

[0106] As used herein, "inhibit," "inhibiting," and "inhibition" mean to diminish or decrease an activity, level, response, condition, severity, disease, or other biological parameter. This can include, but is not limited to, the complete ablation of the activity, level, response, condition, severity, disease, or other biological parameter. This can also include, for example, a 10% inhibition or reduction in the activity, level, response, condition, severity, disease, or other biological parameter as compared to the native or control level (*e.g.*, a subject not having Alzheimer's disease). Thus, in an aspect, the inhibition or reduction can be a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or any amount of reduction in between as compared to native or control levels. In an aspect, the inhibition or reduction can be 10-20%, 20-30%, 30-40%, 40-50%, 50-60%, 60-70%, 70-80%, 80-90%, or 90-100% as compared to native or control levels. In an aspect, the inhibition or reduction can be 0-25%, 25-50%, 50-75%, or 75-100% as compared to native or control levels. In an aspect, a native or control level can be a pre-disease or pre-disorder level.

[0107] The words "treat" or "treating" or "treatment" include palliative treatment, that is, treatment designed for the relief of symptoms rather than the curing of the disease, pathological condition, or disorder; preventative treatment, that is, treatment directed to minimizing or partially or completely inhibiting the development of the associated disease, pathological condition, or disorder; and supportive treatment, that is, treatment employed to supplement another specific therapy directed toward the improvement of the associated disease, pathological condition, or disorder (such as Alzheimer's disease or APOE associated diseases). In an aspect, the terms cover any treatment of a subject, including a mammal (*e.g.*, a human), and includes: (i) preventing the undesired physiological change, disease, pathological condition, or disorder from occurring in a subject that can be predisposed to the disease but has not yet been diagnosed as having it; (ii) inhibiting the physiological change, disease, pathological condition, or disorder, *i.e.*, arresting its development; or (iii) relieving the physiological change, disease, pathological condition, or disorder, *i.e.*, causing regression of the disease. For example, in an aspect, treating AD or LOAD can reduce the severity of an established disease in a subject by 1%-100% as compared to a control (such as, for example, an individual not having AD or LOAD). In an aspect, treating can refer to

a 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% reduction in the severity of AD or LOAD. For example, treating Alzheimer's disease can reduce one or more symptoms of AD or LOAD in a subject by 1%-100% as compared to a control (such as, for example, an individual not having AD or LOAD). In an aspect, treating can refer to 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% reduction of one or more symptoms of an established AD (such as LOAD). It is understood that treatment does not necessarily refer to a cure or complete ablation or eradication of AD. However, in an aspect, treatment can refer to a cure or complete ablation or eradication of AD or LOAD.

[0108] As used herein, the term "prevent" or "preventing" or "prevention" refers to precluding, averting, obviating, forestalling, stopping, or hindering something from happening, especially by advance action. It is understood that where reduce, inhibit, or prevent are used herein, unless specifically indicated otherwise, the use of the other two words is also expressly disclosed. In an aspect, preventing AD or LOAD and/or AD or LOAD progression is intended. The words "prevent" and "preventing" and "prevention" also refer to prophylactic or preventative measures for protecting or precluding a subject (*e.g.*, an individual) not having AD or LOAD or an AD or LOAD complication from progressing to that complication. In an aspect, preventing or reducing APOE expression and/or activity is intended.

[0109] As used herein, the terms "administering" and "administration" refer to any method of providing one or more of the disclosed isolated nucleic acid molecules, disclosed pharmaceutical formulations, disclosed vectors, or any combination thereof to a subject. Such methods are well known to those skilled in the art and include, but are not limited to, the following routes: oral administration, transdermal administration, administration by inhalation, nasal administration, topical administration, in utero administration, intrahepatic administration, intravaginal administration, ophthalmic administration, intraaural administration, otic administration, intracerebral administration, rectal administration, sublingual administration, buccal administration, and parenteral administration, including injectable such as intravenous administration, intra-CSF administration, intra-arterial administration, intramuscular administration, and subcutaneous administration. Administration can also include hepatic intra-arterial administration or administration through the hepatic portal vein (HPV). Administration of a disclosed therapeutic agent, a disclosed pharmaceutical composition, or a combination thereof can comprise administration directly into the CNS (*e.g.*, intraparenchymal, intracerebroventriular, inthrathecal cisternal, intrathecal (lumbar), deep gray matter delivery, convection-enhanced delivery to deep gray matter) or the PNS. Administration can be continuous or intermittent.

[0110] In an aspect, a "therapeutic agent" can be a "biologically active agent" or "biologic active agent" or "bioactive agent," which refers to an agent that is capable of providing a local or systemic biological, physiological, or therapeutic effect in the biological system to which it is applied. For example, the bioactive agent can act to control infection or inflammation, enhance cell growth and tissue regeneration, control tumor growth, act as an analgesic, promote anti-cell attachment, and enhance bone growth, among other functions. Other suitable bioactive agents can include anti-viral agents, vaccines, hormones, antibodies (including active antibody fragments sFv, Fv, and Fab fragments), aptamers, peptide mimetics, functional nucleic acids, therapeutic proteins, peptides, or nucleic acids. Other bioactive agents include prodrugs, which are agents that are not biologically active when administered but, upon administration to a subject are converted to bioactive agents through metabolism or some other mechanism. Additionally, any of the compositions of the invention can contain combinations of two or more bioactive agents. It is understood that a biologically active agent can be used in connection with administration to various subjects, for example, to humans (*i.e.*, medical administration) or to animals (*i.e.*, veterinary administration). As used herein, the recitation of a biologically active agent inherently encompasses the pharmaceutically acceptable salts thereof.

[0111] In an aspect, a "therapeutic agent" can be any agent that effects a desired clinical outcome in a subject having AD or LOAD, suspected of having AD or LOAD, and/or likely to develop or acquire AD or LOAD. In an aspect, a disclosed therapeutic agent can be an oligonucleotide therapeutic agent. A disclosed oligonucleotide therapeutic agent can comprise a single-stranded or double-stranded DNA, gRNA, iRNA, shRNA, siRNA, mRNA, non-coding RNA (ncRNA), an antisense molecule, miRNA, a morpholino, a peptide-nucleic acid (PNA), or an analog or conjugate thereof. In an aspect, a disclosed oligonucleotide therapeutic agent can be an ASO or an RNAi. In an aspect, a disclosed oligonucleotide therapeutic agent can comprise one or more modifications at any position applicable.

[0112] In an aspect, a therapeutic agent can be a "drug" or a "vaccine" and means a molecule, group of molecules, complex or substance administered to an organism for diagnostic, therapeutic, preventative medical, or veterinary purposes. This term includes externally and internally administered topical, localized and systemic human and animal pharmaceuticals, treatments, remedies, nutraceuticals, cosmeceuticals, biologicals, devices, diagnostics and contraceptives, including preparations useful in clinical and veterinary screening, prevention, prophylaxis, healing, wellness, detection, imaging, diagnosis, therapy, surgery, monitoring, cosmetics, prosthetics, forensics and the like. This term may also be used in reference to agricultural, workplace, military, industrial and environmental therapeutics or remedies comprising selected

molecules or selected nucleic acid sequences capable of recognizing cellular receptors, membrane receptors, hormone receptors, therapeutic receptors, microbes, viruses or selected targets comprising or capable of contacting plants, animals and/or humans. Examples include but are not limited to a radiosensitizer, the combination of a radiosensitizer and a chemotherapeutic, a steroid, a xanthine, a beta-2-agonist bronchodilator, an anti-inflammatory agent, an analgesic agent, a calcium antagonist, an angiotensin-converting enzyme inhibitors, a beta-blocker, a centrally active alpha-agonist, an alpha-1-antagonist, carbonic anhydrase inhibitors, prostaglandin analogs, a combination of an alpha agonist and a beta blocker, a combination of a carbonic anhydrase inhibitor and a beta blocker, an anticholinergic/antispasmodic agent, a vasopressin analogue, an antiarrhythmic agent, an antiparkinsonian agent, an antiangina/antihypertensive agent, an anticoagulant agent, an antiplatelet agent, a sedative, an ansiolytic agent, a peptidic agent, a biopolymeric agent, an antineoplastic agent, a laxative, an antidiarrheal agent, an antimicrobial agent, an antifungal agent, or a vaccine. In a further aspect, the pharmaceutically active agent can be coumarin, albumin, bromolidine, steroids such as betamethasone, dexamethasone, methylprednisolone, prednisolone, prednisone, triamcinolone, budesonide, hydrocortisone, and pharmaceutically acceptable hydrocortisone derivatives; xanthines such as theophylline and doxophylline; beta-2-agonist bronchodilators such as salbutamol, fenterol, clenbuterol, bambuterol, salmeterol, fenoterol; antiinflammatory agents, including antiasthmatic anti-inflammatory agents, antiarthritis antiinflammatory agents, and non-steroidal antiinflammatory agents, examples of which include but are not limited to sulfides, mesalamine, budesonide, salazopyrin, diclofenac, pharmaceutically acceptable diclofenac salts, nimesulide, naproxene, acetominophen, ibuprofen, ketoprofen and piroxicam; analgesic agents such as salicylates; calcium channel blockers such as nifedipine, amlodipine, and nicardipine; angiotensin-converting enzyme inhibitors such as captopril, benazepril hydrochloride, fosinopril sodium, trandolapril, ramipril, lisinopril, enalapril, quinapril hydrochloride, and moexipril hydrochloride; beta-blockers (*i.e.*, beta adrenergic blocking agents) such as sotalol hydrochloride, timolol maleate, timol hemihydrate, levobunolol hydrochloride, esmolol hydrochloride, carteolol, propanolol hydrochloride, betaxolol hydrochloride, penbutolol sulfate, metoprolol tartrate, metoprolol succinate, acebutolol hydrochloride, atenolol, pindolol, and bisoprolol fumarate; centrally active alpha-2-agonists (*i.e.*, alpha adrenergic receptor agonist) such as clonidine, brimonidine tartrate, and apraclonidine hydrochloride; alpha-1-antagonists such as doxazosin and prazosin; anticholinergic/antispasmodic agents such as dicyclomine hydrochloride, scopolamine hydrobromide, glycopyrrolate, clidinium bromide, flavoxate, and oxybutynin; vasopressin analogues such as vasopressin and desmopressin; prostaglandin analogs such as latanoprost,

travoprost, and bimatoprost; cholinergics (*i.e.*, acetylcholine receptor agonists) such as pilocarpine hydrochloride and carbachol; glutamate receptor agonists such as the N-methyl D-aspartate receptor agonist memantine; anti-Vascular endothelial growth factor (VEGF) aptamers such as pegaptanib; anti-VEGF antibodies (including but not limited to anti-VEGF-A antibodies) such as ranibizumab and bevacizumab; carbonic anhydrase inhibitors such as methazolamide, brinzolamide, dorzolamide hydrochloride, and acetazolamide; antiarrhythmic agents such as quinidine, lidocaine, tocainide hydrochloride, mexiletine hydrochloride, digoxin, verapamil hydrochloride, propafenone hydrochloride, flecaimide acetate, procainamide hydrochloride, moricizine hydrochloride, and diisopyramide phosphate; antiparkinsonian agents, such as dopamine, L-Dopa/Carbidopa, selegiline, dihydroergocryptine, pergolide, lisuride, apomorphine, and bromocryptine; antiangina agents and antihypertensive agents such as isosorbide mononitrate, isosorbide dinitrate, propranolol, atenolol and verapamil; anticoagulant and antiplatelet agents such as coumadin, warfarin, acetylsalicylic acid, and ticlopidine; sedatives such as benzodiazapines and barbiturates; ansiolytic agents such as lorazepam, bromazepam, and diazepam; peptidic and biopolymeric agents such as calcitonin, leuprolide and other LHRH agonists, hirudin, cyclosporin, insulin, somatostatin, protirelin, interferon, desmopressin, somatotropin, thymopentin, pidotimod, erythropoietin, interleukins, melatonin, granulocyte/macrophage-CSF, and heparin; antineoplastic agents such as etoposide, etoposide phosphate, cyclophosphamide, methotrexate, 5-fluorouracil, vincristine, doxorubicin, cisplatin, hydroxyurea, leucovorin calcium, tamoxifen, flutamide, asparaginase, altretamine, mitotane, and procarbazine hydrochloride; laxatives such as senna concentrate, casanthranol, bisacodyl, and sodium picosulphate; antidiarrheal agents such as difenoxine hydrochloride, loperamide hydrochloride, furazolidone, diphenoxylate hydrochloride, and microorganisms; vaccines such as bacterial and viral vaccines; antimicrobial agents such as penicillins, cephalosporins, and macrolides, antifungal agents such as imidazolic and triazolic derivatives; and nucleic acids such as DNA sequences encoding for biological proteins, and antisense oligonucleotides. It is understood that a pharmaceutically active agent can be used in connection with administration to various subjects, for example, to humans (*i.e.*, medical administration) or to animals (*i.e.*, veterinary administration). As used herein, the recitation of a pharmaceutically active agent inherently encompasses the pharmaceutically acceptable salts thereof.

[0113] "AAV" is an abbreviation for adeno-associated virus, and may be used to refer to the virus itself or derivatives thereof. The term covers all subtypes and both naturally occurring and recombinant forms, except where required otherwise. The abbreviation "rAAV" refers to recombinant adeno-associated virus, also referred to as a recombinant AAV vector (or "rAAV

vector"). AAV vector can include naturally isolated serotypes including, but not limited to, AAV1, AAV2, AAV3 (including 3a and 3b), AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAV9, AAV10, AAVrh10, AAV11, AAV12, AAV13, AAVrh39, AAVrh43, AAVcy.7 as well as bovine AAV, caprine AAV, canine AAV, equine AAV, ovine AAV, avian AAV, primate AAV, non-primate AAV, and any other virus classified by the International Committee on Taxonomy of Viruses (ICTV) as an AAV. In an aspect, an AAV capsid can be a chimera either created by capsid evolution or by rational capsid engineering from a naturally isolated AAV variants to capture desirable serotype features such as enhanced or specific tissue tropism and/or a host immune response escape. Naturally isolated AAV variants include, but not limited to, AAV-DJ, AAV-HAE1, AAV-HAE2, AAVM41, AAV-1829, AAV2 Y/F, AAV2 T/V, AAV2i8, AAV2.5, AAV9.45, AAV9.61, AAV-B1, AAV-AS, AAV9.45A-String (*e.g.*, AAV9.45-AS), AAV9.45Angiopep, AAV9.47-Angiopep, and AAV9.47-AS, AAV-PHP.B, AAV-PHP.eB, AAV-PHP.S, AAV-F, AAVcc.47, and AAVcc.81. In an aspect, a disclosed AAV vector can be AAV-Rh74 or a related variant (*e.g.*, capsid variants like RHM4-1).

[0114] An "rAAV vector" as used herein refers to an AAV vector comprising a polynucleotide sequence not of AAV origin (*i.e.* a polynucleotide heterologous to AAV), typically a sequence of interest for introducing into a target cell. In general, the heterologous polynucleotide is flanked by at least one, and generally by two AAV inverted terminal repeat sequences (ITRs). The term rAAV vector encompasses both rAAV vector particles and rAAV vector plasmids.

[0115] An "AAV virus" or "AAV viral particle" refers to a viral particle composed of at least one AAV capsid protein (typically by all of the capsid proteins of a wild-type AAV) and an encapsidated nucleic acid molecule AAV vector. If the particle comprises a heterologous nucleic acid molecule (*i.e.* a nucleic acid molecule other than a wild-type AAV genome, such as a transgene to be delivered to a mammalian cell), it is typically referred to as an "rAAV vector particle" or simply an "rAAV vector."." Thus, production of rAAV particle necessarily includes production of rAAV vector, as such a vector is contained within an rAAV particle.

[0116] "Packaging" refers to a series of intracellular events that result in the assembly and encapsidation of an AAV particle.

[0117] AAV "rep" and "cap" genes refer to polynucleotide sequences encoding replication and encapsidation proteins of adeno-associated virus. AAV rep and cap are referred to herein as AAV "packaging genes."

[0118] A "helper virus" for AAV refers to a virus that allows AAV (*e.g.*, wild-type AAV) to be replicated and packaged by a mammalian cell. A variety of such helper viruses for AAV are known in the art, including adenoviruses, herpesviruses and poxviruses such as vaccinia. The

adenoviruses encompass a number of different subgroups, although Adenovirus type 5 of subgroup C is most commonly used. Numerous adenoviruses of human, non-human mammalian and avian origin are known and available from depositories such as the ATCC. Viruses of the herpes family include, for example, herpes simplex viruses (HSV) and Epstein-Barr viruses (EBV), as well as cytomegaloviruses (CMV) and pseudorabies viruses (PRV); which are also available from depositories such as ATCC.

[0119] "Helper virus function(s)" refers to function(s) encoded in a helper virus genome which allow AAV replication and packaging (in conjunction with other requirements for replication and packaging described herein). As described herein, "helper virus function" may be provided in a number of ways, including by providing helper virus or providing, for example, polynucleotide sequences encoding the requisite function(s) to a producer cell in trans.

[0120] An "infectious" virus or viral particle is one that comprises a nucleic acid molecule component which it is capable of delivering into a cell for which the viral species is tropic. The term does not necessarily imply any replication capacity of the virus. As used herein, an "infectious" virus or viral particle is one that can access a target cell, can infect a target cell, and can express a heterologous nucleic acid in a target cell. Thus, "infectivity" refers to the ability of a viral particle to access a target cell, infect a target cell, and express a heterologous nucleic acid in a target cell. Infectivity can refer to in vitro infectivity or in vivo infectivity. Assays for counting infectious viral particles are described elsewhere in this disclosure and in the art. Viral infectivity can be expressed as the ratio of infectious viral particles to total viral particles. Total viral particles can be expressed as the number of viral genome (vg) copies. The ability of a viral particle to express a heterologous nucleic acid in a cell can be referred to as "transduction." The ability of a viral particle to express a heterologous nucleic acid in a cell can be assayed using a number of techniques, including assessment of a marker gene, such as a green fluorescent protein (GFP) assay (*e.g.* where the virus comprises a nucleotide sequence encoding GFP), where GFP is produced in a cell infected with the viral particle and is detected and/or measured; or the measurement of a produced protein, for example by an enzyme-linked immunosorbent assay (ELISA). Viral infectivity can be expressed as the ratio of infectious viral particles to total viral particles. Methods of determining the ratio of infectious viral particle to total viral particle are known in the art.

[0121] A "replication-competent" virus (*e.g.* a replication-competent AAV) refers to a phenotypically wild-type virus that is infectious, and is also capable of being replicated in an infected cell (*i.e.*, in the presence of a helper virus or helper virus functions). In the case of AAV, replication competence generally requires the presence of functional AAV packaging genes. In

general, rAAV vectors as described herein are replication-incompetent in mammalian cells (especially in human cells) by virtue of the lack of one or more AAV packaging genes. Typically, such rAAV vectors lack any AAV packaging gene sequences in order to minimize the possibility that replication competent AAV are generated by recombination between AAV packaging genes and an incoming rAAV vector. In general, rAAV vector preparations as described herein are those which contain few if any replication competent AAV (rcAAV, also referred to as RCA).

[0122] As used herein, "tropism" refers to the specificity of an AAV capsid protein present in an AAV viral particle, for infecting a particular type of cell or tissue. The tropism of an AAV capsid for a particular type of cell or tissue may be determined by measuring the ability of AAV vector particles comprising the hybrid AAV capsid protein to infect or to transduce a particular type of cell or tissue, using standard assays that are well-known in the art such as those disclosed in the examples of the present application. As used herein, the term "liver tropism" or "hepatic tropism" refers to the tropism for liver or hepatic tissue and cells, including hepatocytes.

[0123] As used herein, "codon optimization" can refer to a process of modifying a nucleic acid sequence for enhanced expression in the host cells of interest by replacing one or more codons or more of the native sequence with codons that are more frequently or most frequently used in the genes of that host cell while maintaining the native amino acid sequence. Various species exhibit particular bias for certain codons of a particular amino acid. As contemplated herein, genes can be tailored for optimal gene expression in a given organism based on codon optimization. Codon usage tables are readily available, for example, at the "Codon Usage Database." Many methods and software tools for codon optimization have been reported previously. (See, for example, genomes.urv.es/OPTIMIZER/).

[0124] As used herein, "expression cassette" or "transgene cassette" can refer to a distinct component of vector DNA comprising a transgene and one or more regulatory sequences to be expressed by a transfected cell. Generally, an expression cassette or transgene cassette can comprise a promoter sequence, an open reading frame (*i.e.*, the transgene), and a 3' untranslated region (*e.g.*, in eukaryotes a polyadenylation site).

[0125] In an aspect, the skilled person can determine an efficacious dose, an efficacious schedule, and an efficacious route of administration for one or more of the disclosed isolated nucleic acid molecules, disclosed pharmaceutical formulations, disclosed vectors, or any combination thereof so as to treat or prevent AD or LOAD. In an aspect, the skilled person can also alter, change, or modify an aspect of an administering step to improve efficacy of one or more of the disclosed isolated nucleic acid molecules, disclosed pharmaceutical formulations, disclosed vectors, or any combination thereof. In an aspect, the skilled person can determine an efficacious dose, an

efficacious schedule, and an efficacious route of administration for any disclosed isolated nucleic acid molecule, disclosed pharmaceutical formulation, disclosed vector, disclosed therapeutic agent, or any combination thereof.

[0126] As used herein, "modifying the method" can comprise modifying or changing one or more features or aspects of one or more steps of a disclosed method. For example, in an aspect, a method can be altered by changing the amount of one or more of the disclosed isolated nucleic acid molecules, disclosed viral vectors, disclosed lentiviral vectors, disclosed pharmaceutical formulations, disclosed host cells, disclosed gRNAs, disclosed plasmids, or any combination thereof, or administered to a subject, or by changing the frequency of administration of one or more of the disclosed isolated nucleic acid molecules, disclosed viral vectors, disclosed lentiviral vectors, disclosed pharmaceutical formulations, disclosed host cells, disclosed gRNAs, disclosed plasmids, or any combination thereof, or by changing the duration of time that the one or more of the disclosed isolated nucleic acid molecules, disclosed viral vectors, disclosed lentiviral vectors, disclosed pharmaceutical formulations, disclosed host cells, disclosed gRNAs, disclosed plasmids, or any combination thereof, or are administered to a subject.

[0127] As used herein, "concurrently" means (1) simultaneously in time, or (2) at different times during the course of a common treatment schedule.

[0128] The term "contacting" as used herein refers to bringing one or more of disclosed isolated nucleic acid molecules, disclosed pharmaceutical formulations, disclosed vectors, or any combination thereof together with a target area or intended target area in such a manner that the one or more of the disclosed isolated nucleic acid molecules, disclosed pharmaceutical formulations, disclosed vectors, or any combination thereof exert an effect on the intended target or targeted area either directly or indirectly. A target area or intended target area can be one or more of a subject's organs (*e.g.*, lungs, heart, liver, kidney, brain, etc.). In an aspect, a target area or intended target area can be any cell or any organ infected by AD or LOAD (such as cholinergic neurons). In an aspect, a target area or intended target area can be the brain or various neuronal populations.

[0129] As used herein, "determining" can refer to measuring or ascertaining the presence and severity of AD such as, for example, LOAD. Methods and techniques used to determine the presence and/or severity of AD are typically known to the medical arts. For example, the art is familiar with the ways to identify and/or diagnose the presence, severity, or both of AD (such as, for example, a LOAD. In an aspect, "determining" can also refer to measuring or ascertaining the level of one or more proteins or peptides in a biosample or measuring or ascertaining the level or

one or more RNAs or miRNAs in a biosample. Methods and techniques for determining the level of proteins/peptides and RNAs/miRNAs are known to the art and are disclosed herein.

[0130] As used herein, "effective amount" and "amount effective" can refer to an amount that is sufficient to achieve the desired result such as, for example, the treatment and/or prevention of AD or LOAD. As used herein, the terms "effective amount" and "amount effective" can refer to an amount that is sufficient to achieve the desired an effect on an undesired condition (*e.g.*, an AD or LOAD). For example, a "therapeutically effective amount" refers to an amount that is sufficient to achieve the desired therapeutic result or to have an effect on undesired symptoms, but is generally insufficient to cause adverse side effects. In an aspect, "therapeutically effective amount" means an amount of a disclosed isolated nucleic acid molecule, a disclosed pharmaceutical formulation, a disclosed vector, or any combination thereof that (i) treats the particular disease, condition, or disorder (*e.g.*, AD or LOAD), (ii) attenuates, ameliorates, or eliminates one or more symptoms of the particular disease, condition, or disorder (*e.g.*, AD or LOAD), or (iii) delays the onset of one or more symptoms of the particular disease, condition, or disorder described herein (*e.g.*, AD or LOAD). The specific therapeutically effective dose level for any particular patient will depend upon a variety of factors including the disorder being treated and the severity of the disorder; the isolated nucleic acid molecules, disclosed pharmaceutical formulations, disclosed vectors, or any combination thereof employed; the disclosed methods employed; the age, body weight, general health, sex and diet of the patient; the time of administration; the route of administration; the rate of excretion of the disclosed isolated nucleic acid molecules, disclosed pharmaceutical formulations, disclosed vectors, or any combination thereof employed; the duration of the treatment; drugs used in combination or coincidental with the disclosed isolated nucleic acid molecules, disclosed pharmaceutical formulations, disclosed vectors, or any combination thereof employed, and other like factors well known in the medical arts. For example, it is well within the skill of the art to start doses of the disclosed isolated nucleic acid molecules, disclosed pharmaceutical formulations, disclosed vectors, or any combination thereof at levels lower than those required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved. If desired, then the effective daily dose can be divided into multiple doses for purposes of administration. Consequently, a single dose of the disclosed isolated nucleic acid molecules, disclosed pharmaceutical formulations, disclosed vectors, or any combination thereof can contain such amounts or submultiples thereof to make up the daily dose. The dosage can be adjusted by the individual physician in the event of any contraindications. Dosage can vary, and can be administered in one or more dose administrations daily, for one or several days. Guidance can be found in the literature for appropriate dosages for

given classes of pharmaceutical products. In further various aspects, a preparation can be administered in a "prophylactically effective amount"; that is, an amount effective for prevention of a disease or condition, such as, for example, AD or LOAD.

[0131] As used herein, the term "pharmaceutically acceptable carrier" refers to sterile aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, as well as sterile powders for reconstitution into sterile injectable solutions or dispersions just prior to use. Examples of suitable aqueous and nonaqueous carriers, diluents, solvents, or vehicles include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol and the like), carboxymethylcellulose and suitable mixtures thereof, vegetable oils (such as olive oil) and injectable organic esters such as ethyl oleate. In an aspect, a pharmaceutical carrier employed can be a solid, liquid, or gas. In an aspect, examples of solid carriers can include lactose, terra alba, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate, and stearic acid. In an aspect, examples of liquid carriers can include sugar syrup, peanut oil, olive oil, and water. In an aspect, examples of gaseous carriers can include carbon dioxide and nitrogen. In preparing a disclosed composition for oral dosage form, any convenient pharmaceutical media can be employed. For example, water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents and the like can be used to form oral liquid preparations such as suspensions, elixirs and solutions; while carriers such as starches, sugars, microcrystalline cellulose, diluents, granulating agents, lubricants, binders, disintegrating agents, and the like can be used to form oral solid preparations such as powders, capsules and tablets. Because of their ease of administration, tablets and capsules are the preferred oral dosage units whereby solid pharmaceutical carriers are employed. Optionally, tablets can be coated by standard aqueous or nonaqueous techniques. Proper fluidity can be maintained, for example, by the use of coating materials such as lecithin, by the maintenance of the required particle size in the case of dispersions and by the use of surfactants. These compositions can also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms can be ensured by the inclusion of various antibacterial and antifungal agents such as paraben, chlorobutanol, phenol, sorbic acid and the like. It can also be desirable to include isotonic agents such as sugars, sodium chloride and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the inclusion of agents, such as aluminum monostearate and gelatin, which delay absorption. Injectable depot forms are made by forming microencapsule matrices of the drug in biodegradable polymers such as polylactide-polyglycolide, poly(orthoesters) and poly(anhydrides). Depending upon the ratio of drug to polymer and the nature of the particular polymer employed, the rate of drug release can be controlled. Depot injectable formulations are also prepared by entrapping the drug in liposomes

or microemulsions that are compatible with body tissues. The injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable media just prior to use. Suitable inert carriers can include sugars such as lactose. Desirably, at least 95% by weight of the particles of the active ingredient have an effective particle size in the range of 0.01 to 10 micrometers.

[0132] As used herein, the term "excipient" refers to an inert substance which is commonly used as a diluent, vehicle, preservative, binder, or stabilizing agent, and includes, but is not limited to, proteins (*e.g.*, serum albumin, etc.), amino acids (*e.g.*, aspartic acid, glutamic acid, lysine, arginine, glycine, histidine, etc.), fatty acids and phospholipids (*e.g.*, alkyl sulfonates, caprylate, etc.), surfactants (*e.g.*, SDS, polysorbate, nonionic surfactant, etc.), saccharides (*e.g.*, sucrose, maltose, trehalose, etc.) and polyols (*e.g.*, mannitol, sorbitol, etc.). See, also, for reference, Remington's Pharmaceutical Sciences, (1990) Mack Publishing Co., Easton, Pa., which is hereby incorporated by reference in its entirety.

[0133] As used herein, the term "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, contraindications and/or warnings concerning the use of such therapeutic products.

[0134] As used herein, the term "in combination" in the context of the administration of one or more of the disclosed agents, disclosed therapeutic agents, disclosed pharmaceutical formulations or a combination thereof includes the use of more than one therapy (*e.g.*, additional therapeutic agents). Administration "in combination with" one or more additional therapeutic agents includes simultaneous (*e.g.*, concurrent) and consecutive administration in any order. The use of the term "in combination" does not restrict the order in which therapies are administered to a subject. By way of non-limiting example, a first therapy (*e.g.*, one or more of the disclosed isolated nucleic acid molecules, disclosed pharmaceutical formulations, disclosed vectors, or any combination thereof) may be administered prior to (*e.g.*, 1 minute, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, or 12 weeks), concurrently, or after (*e.g.*, 1 minute, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, or 12 weeks or longer) the administration of a second therapy (*e.g.*, one or more of the disclosed isolated nucleic acid molecules, disclosed

pharmaceutical formulations, disclosed vectors, or any combination thereof or one or more additional therapeutic agents) to a subject having or diagnosed with AD or LOAD.

[0135] As used herein, "CRISPR or clustered regularly interspaced short palindromic repeat" is an ideal tool for correction of genetic abnormalities associated with diseases such as Alzheimer's disease or LOAD. The system can be designed to target genomic DNA directly. A CRISPR system involves two main components: a Cas9 enzyme and a guide (gRNA). The gRNA contains a targeting sequence for DNA binding (at, for example, the APOE promoter region) and a scaffold sequence for Cas9 binding. Cas9 nuclease is often used to "knockout" target genes such as for example, the APOE e4 allele. Also, multiple gRNAs can be employed to suppress or activate multiple genes simultaneously, hence increasing the treatment efficacy and reducing resistance potentially caused by new mutations in the target genes.

[0136] As used herein, "CRISPR-based endonucleases" include RNA-guided endonucleases that comprise at least one nuclease domain and at least one domain that interacts with a guide RNA. As known to the art, a guide RNA directs the CRISPR-based endonucleases to a targeted site in a nucleic acid at which site the CRISPR-based endonucleases cleaves at least one strand of the targeted nucleic acid sequence. As the guide RNA provides the specificity for the targeted cleavage, the CRISPR-based endonuclease is universal and can be used with different guide RNAs to cleave different target nucleic acid sequences. CRISPR-based endonucleases are RNA-guided endonucleases derived from CRISPR/Cas systems.

[0137] In an aspect, a disclosed CRISPR-based endonuclease can be derived from a CRISPR/Cas type I, type II, or type III system. Non-limiting examples of suitable CRISPR/Cas proteins include Cas3, Cas4, Cas5, Cas5e (or CasD), Cas6, Cas6e, Cas6f, Cas7, Cas8a1, Cas8a2, Cas8b, Cas8c, Cas9, Cas10, Cas10d, CasF, CasG, CasH, Csy1, Csy2, Csy3, Cse1 (or CasA), Cse2 (or CasB), Cse3 (or CasE), Cse4 (or CasC), Csc1, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csz1, Csx15, Csf1, Csf2, Csf3, Csf4, and Cu1966.

[0138] In an aspect, a disclosed CRISPR-based endonuclease can be derived from a type II CRISPR/Cas system. For example, in an aspect, a CRISPR-based endonuclease can be derived from a Cas9 protein. The Cas9 protein can be from *Streptococcus pyogenes*, *Streptococcus thermophilus*, *Streptococcus sp*, *Nocardiopsis dassonvillei*, *Streptomyces pristinaespiralis*, *Streptomyces viridochromogenes*, *Streptomyces viridochromogenes*, *Streptosporangium roseum*, *Streptosporangium roseum*, *Alicyclobacillus acidocaldarius*, *Bacillus pseudomycoides*, *Bacillus selenitireducens*, *Exiguobacterium sibiricum*, *Lactobacillus delbrueckii*, *Lactobacillus salivarius*, *Microscilla marina*, *Burkholderiales bacterium*, *Polaromonas naphthalenivorans*, *Polaromonas*

sp., *Crocosphaera watsonii*, *Cyanothece sp.*, *Microcystis aeruginosa*, *Synechococcus sp.*, *Acetohalobium arabaticum*, *Ammonifex degensii*, *Caldicelulosiruptor becscii*, *Candidatus Desulforudis*, *Clostridium botulinum*, *Clostridium difficile*, *Finegoldia magna*, *Natranaerobius thermophilus*, *Pelotomaculum thermopropionicum*, *Acidithiobacillus caldus*, *Acidithiobacillus ferrooxidans*, *Allochromatium vinosum*, *Marinobacter sp.*, *Nitrosococcus halophilus*, *Nitrosococcus watsoni*, *Pseudoalteromonas haloplanktis*, *Ktedonobacter racemifer*, *Methanohalobium evestigatum*, *Anabaena variabilis*, *Nodularia spumigena*, *Nostoc sp.*, *Arthrospira maxima*, *Arthrospira platensis*, *Arthrospira sp.*, *Lyngbya sp.*, *Microcoleus chthonoplastes*, *Oscillatoria sp.*, *Petrotoga mobilis*, *Thermosipho africanus*, *or Acaryochloris* marina. In an aspect, the CRISPR-based nuclease can be derived from a Cas9 protein from Streptococcus pyogenes. In an aspect, the CRISPR-based nuclease can comprise the sequence set forth in any one of SEQ ID NO: 36-40, or a derivative thereof, or a sequence at least about 80% identical thereto.

[0139] As used herein, "CRISPRa" refers to CRISPR Activation, which is using a dCas9 or dCas9-activator with a gRNA to increase transcription of a target gene.

[0140] As used herein, "CRISPRi" refers to CRISPR Interference, which is using a dCas9 or dCas9-repressor with a gRNA to repress/decrease transcription of a target gene.

[0141] As used herein, "dCas9" refers to enzymatically inactive form of Cas9, which can bind, but cannot cleave, DNA.

[0142] As used herein, "Protospacer Adjacent Motif" or "PAM" refers to a sequence adjacent to the target sequence that is necessary for Cas enzymes to bind target DNA.

[0143] Disclosed are the components to be used to prepare the disclosed isolated nucleic acid molecules, disclosed pharmaceutical formulations, disclosed vectors, or any combination thereof as well the disclosed isolated nucleic acid molecules, disclosed pharmaceutical formulations, disclosed vectors, or any combination thereof used within the methods disclosed herein. These and other materials are disclosed herein, and it is understood that when combinations, subsets, interactions, groups, etc. of these materials are disclosed that while specific reference of each various individual and collective combinations and permutation of these compounds cannot be explicitly disclosed, each is specifically contemplated and described herein. For example, if a particular compound is disclosed and discussed and a number of modifications that can be made to a number of molecules including the compounds are discussed, specifically contemplated is each and every combination and permutation of the compound and the modifications that are possible unless specifically indicated to the contrary. Thus, if a class of molecules A, B, and C are disclosed as well as a class of molecules D, E, and F and an example of a combination molecule,

A-D is disclosed, then even if each is not individually recited each is individually and collectively contemplated meaning combinations, A-E, A-F, B-D, B-E, B-F, C-D, C-E, and C-F are considered disclosed. Likewise, any subset or combination of these is also disclosed. Thus, for example, the sub-group of A-E, B-F, and C-E would be considered disclosed. This concept applies to all aspects of this application including, but not limited to, steps in methods of making and using the compositions of the invention. Thus, if there are a variety of additional steps that can be performed it is understood that each of these additional steps can be performed with any specific aspects or combination of aspects of the disclosed methods.

E. Compositions

1. Isolated Nucleic Acid Molecules

[0144] Disclosed herein is an isolated nucleic acid molecule comprising (a) a nucleic acid sequence encoding (i) a deactivated Clustered Regularly Interspaced Short Palindromic Repeats associated (dCas) endonuclease, (ii) at least one polypeptide having an enzymatic activity, and (iii) at least one guide RNA targeting an *APOE* gene sequence or a regulatory element thereof; and (b) one or more microglia specific regulatory elements operably linked to (a)(i), (a)(ii), (a)(iii), or any combination thereof.

Microglia specific regulatory elements

[0145] In an aspect, the microglia specific regulatory sequence may comprise a wild-type promoter, a microglia specific enhancer, a synthetic promoter, a synthetic enhancer, a transcriptional or translational regulatory sequence or any combination thereof. In an aspect, the microglia specific regulatory sequence is a microglia specific promoter. As used herein, the term "microglia specific promoter" includes wild-type promoter, or derivatives thereof, synthetic promoter, minimal promoter, and any combination of promoter and enhancers sequence, that drives enhanced expression of a gene-of-interest to which it is operably linked in the microglial cells. In an exemplary aspect, the microglia specific promoter may drive enhanced expression of the gene-of-interest to which it is operably linked, in the microglia compared to other brain cells, for example neurons. In an aspect, the microglia specific promoter may be functional in enhancing expression in the microglia, of gene-of-interest to which it is operably linked, by about 1.1 fold to about 100-fold or more. In an aspect, the microglia specific promoter may be functional in enhancing expression in the microglia, of gene-of-interest to which it is operably linked, by about 1.1-fold to about 2-fold, about 2-fold to about 10-fold, about 10-fold to about 20-fold, about 20-fold to about 30-fold, about 30-fold to about 40-fold, about 40-fold to about 50-fold, about 50-fold to about 60-fold, about 60-fold to about 70-fold, about 70-fold to about 80-fold, about 80-fold to about 90-fold, about 90-fold to about 100-fold or more compared to other brain cells.

[0146] In an aspect, the microglia specific promoter can be any promoter known in the art, that exhibits enhanced activity in the microglia. Non-limiting examples of microglia specific promoters include F4/80, CD68, FCGR2A, HexB or IBA1 promoter, or a derivative thereof.

[0147] In an aspect, the promoter is a CD68 promoter, or a derivative thereof. In an aspect, the promoter may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one or more of the sequences set forth in SEQ ID NO: 1-3, or a fragment, or a derivative thereof. In an aspect, the promoter comprises a sequence as set forth in any one of SEQ ID NO: 1-3.

[0148] In an aspect, the promoter is a F4/80 promoter, or a derivative thereof. In an aspect, the promoter may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequence set forth in SEQ ID NO: 4, or a fragment, or a derivative thereof. In an aspect, the promoter comprises a sequence as set forth in SEQ ID NO: 4.

[0149] In an aspect, the promoter is a IBA1 promoter, or a derivative thereof. In an aspect, the promoter may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequence set forth in SEQ ID NO: 5-7, or a fragment, or a derivative thereof. In an aspect, the promoter comprises a sequence as set forth in any one of SEQ ID NO: 5-7.

[0150] In an aspect, the promoter is a FCGR2A promoter, or a derivative thereof. In an aspect, the promoter may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequence set forth in SEQ ID NO: 8, or a fragment, or a derivative thereof. In an aspect, the promoter comprises a sequence as set forth in SEQ ID NO: 8.

[0151] In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) can be operably linked to the dCas endonuclease and the fusion protein having one or more enzymatic activities. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the dCas endonuclease and the fusion protein having one or more enzymatic activities can comprise a F4/80 promoter, or a derivative thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the dCas endonuclease and the fusion protein having one or more enzymatic

activities can comprise a CD68 promoter, or a derivative thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the dCas endonuclease and the fusion protein having one or more enzymatic activities can comprise a IBA1 promoter, or a derivative thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the dCas endonuclease and the fusion protein having one or more enzymatic activities can comprise a FCGR2A promoter, or a derivative thereof.

Encoded dCas endonucleases

[0152] In an aspect, the isolated nucleic acid molecule disclosed herein may comprising a nucleic acid sequence encoding a deactivated Cas (dCas) endonuclease. In an aspect, the nucleic acid sequence encoding the dCas may be operably linked to the microglia specific regulatory element disclosed herein above. In an aspect, the nucleic acid sequence encoding the dCas may be operably linked to the microglia specific promoter disclosed herein above. In an aspect, the microglia specific promoter may comprise the nucleic acid sequence as set forth in any one of SEQ ID NOS: 1-8, or a sequence at least about 60% identical thereto.

[0153] In an aspect, a disclosed dCas endonuclease can comprise a dCas9 endonuclease. In an aspect, a disclosed dCas9 endonuclease can comprise a deactivated Staphylococcus aureus Cas9 (dSaCas9), a deactivated Streptococcus pyogenes Cas9 (dSpCas9), a deactivated Campylobacter jejuni Cas9 (dCjCas9), or a variant dCas9 endonuclease. In an aspect, a disclosed variant dCas9 can comprise a variant dSaCas9, a variant dSpCas9, or a variant dCjCa9. In an aspect, a disclosed variant dSpCas9 can comprise dVQR, dEQR, or dVRER.

[0154] In an aspect, the nucleic acid sequence encoding the disclosed dSpCas9 may comprise a sequence as set forth in any one of SEQ ID NO: 40 or SEQ ID NO: 41, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed dSpCas9 endonuclease may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 40 or SEQ ID NO: 41, or a fragment thereof. In an aspect, a disclosed dSpCas9 endonuclease can comprise the amino acid sequence as set forth in SEQ ID NO: 36 or SEQ ID NO: 37, or a fragment, or a derivative thereof. In an aspect, a disclosed dSpCas9 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 36 or SEQ ID NO: 37, or a fragment thereof.

[0155] In an aspect, the nucleic acid sequence encoding the disclosed dSaCas9 may comprise a sequence as set forth in SEQ ID NO: 42, or a fragment, or a derivative thereof. In an aspect, the

sequence encoding the disclosed dSaCas9 endonuclease may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequences set forth in SEQ ID NO: 42, or a fragment thereof. In an aspect, a disclosed dSaCas9 endonuclease can comprise the amino acid sequence as set forth in SEQ ID NO: 38 or a fragment, or a derivative thereof. In an aspect, a disclosed dSaCas9 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 38, or a fragment thereof.

[0156] In an aspect, the nucleic acid sequence encoding the disclosed dCjCas9 may comprise a sequence as set forth in SEQ ID NO: 43, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed dCjCas9 endonuclease may comprise a nucleic acid sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 43, or a fragment thereof. In an aspect, a disclosed dCjCas9 endonuclease can comprise the amino acid sequence as set forth in SEQ ID NO: 39, or a fragment, or a derivative thereof. In an aspect, a disclosed dCjCas9 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 39 or a fragment thereof.

[0157] In an aspect, a disclosed variant of dCas9 dVQR can comprise D1135V, R1335Q, and T1337R with reference to SEQ ID NO: 36. In an aspect, a disclosed variant of dCas9 dEQR can comprise D1135E, R1335Q, and T1337R with reference to SEQ ID NO: 36. In an aspect, a disclosed variant of dCas9 dVRER can comprise D1135V, G1218R, R1335E, and T1337R with reference to SEQ ID NO: 36. In an aspect, a disclosed dVRER can comprise the sequence set forth in SEQ ID NO: 37 or a fragment thereof.

[0158] In an aspect, the nucleic acid sequence encoding the disclosed dCas9 endonuclease may comprise a sequence as set forth in any one of SEQ ID NO: 40-43. In an aspect, the sequence encoding the disclosed dCas9 endonuclease may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 40-43, or a fragment, or a derivative thereof.

Encoded Polypeptides having Enzymatic Activity

[0159] In an aspect, the isolated nucleic acid molecule may comprise a nucleic acid sequence encoding at least one polypeptide having an enzymatic activity. In an aspect, a disclosed encoded

polypeptide can comprise transcription activation activity, transcription repression activity, transcription release factor activity, histone modification activity, nucleic acid association activity, methyltransferase activity, demethylase activity, acetyltransferase activity, deacetylase activity, or any combination thereof.

[0160] In an aspect, a disclosed encoded polypeptide can comprise HP1a, HP1b, MBD1, MBD2, Krüppel-Associated Box (KRAB), NIPP1, the Transcription Repression Domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof. In an aspect, a disclosed fusion can comprise HP1a-HP1a, HP1a-HP1b, HP1a-MBD1, HP1a-MBD2, HP1a-KRAB, HP1a-NIPP1, HP1a-MeCP2, HP1a-DNMT3A, HP1b-HP1b, HP1b-HP1a, HP1b-MBD1, HP1b-MBD2, HP1b-KRAB, HP1b-NIPP1, HP1b-MeCP2, HP1b-DNMT3A, MBD1-MBD1, MBD1-HP1a, MBD1-HP1b, MBD1-MBD2, MBD1-KRAB, MBD1-NIPP1, MBD1-MeCP2, MBD1-DNMT3A, MBD2-MBD2, MBD2-HP1a, MBD2-HP1b, MBD2-MBD1, MBD2-KRAB, MBD2-NIPP1, MBD2-MeCP2, MBD2-DNMT3A, KRAB-KRAB, KRAB-HP1a, KRAB-HP1b, KRAB-MBD1, KRAB-MBD2, KRAB-NIPP1, KRAB-MeCP2, KRAB-DNMT3A, NIPP1-NIPP1, NIPP1-HP1a, NIPP1-HP1b, NIPP1-MBD1, NIPP1-MBD2, NIPP1-KRAB, NIPP1-MeCP2, NIPP1-DNMT3A, MeCP2-MeCP2, MeCP2-HP1a, MeCP2-HP1b, MeCP2-MBD1, MeCP2-MBD2, MeCP2-KRAB, MeCP2-NIPP1, MeCP2-DNMT3A, DNMT3A-DNMT3A, DNMT3A-HP1a, DNMT3A-HP1b, DNMT3A-MBD1, DNMT3A-MBD2, DNMT3A-KRAB, DNMT3A-NIPP1, or DNMT3A-MeCP2. Examples of suitable polypeptides sequences for use with the current disclosure are provided in PCT/US2022/078260, the contents of which are incorporated herein by reference, in their entirety.

[0161] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide HP1a may comprise a sequence as set forth in any one of SEQ ID NO: 59 or SEQ ID NO: 60, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide HP1a may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 59 or SEQ ID NO: 60, or a fragment thereof. In an aspect, a disclosed polypeptide HP1a can comprise the amino acid sequence as set forth in SEQ ID NO: 44 or SEQ ID NO: 45, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide HP1a can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 44 or SEQ ID NO: 45, or a fragment thereof.

[0162] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide HP1b may comprise a sequence as set forth in any one of SEQ ID NO: 61 or SEQ ID NO: 62, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide HP1b may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 61 or SEQ ID NO: 62, or a fragment thereof. In an aspect, a disclosed polypeptide HP1b can comprise the amino acid sequence as set forth in SEQ ID NO: 46 or SEQ ID NO: 47, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide HP1b can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 46 or SEQ ID NO: 47, or a fragment thereof.

[0163] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide MBD1 may comprise a sequence as set forth in any one of SEQ ID NO: 63 or SEQ ID NO: 64, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide MBD1 may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 63 or SEQ ID NO: 64, or a fragment thereof. In an aspect, a disclosed polypeptide MBD1 can comprise the amino acid sequence as set forth in any one of SEQ ID NO: 48-50, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide MDB1 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in any one of SEQ ID NO: 48-50, or a fragment thereof.

[0164] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide MBD2 may comprise a sequence as set forth in any one of SEQ ID NO: 65 or SEQ ID NO: 66, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide MBD2 may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 65 or SEQ ID NO: 66, or a fragment thereof. In an aspect, a disclosed polypeptide MBD2 can comprise the amino acid sequence as set forth in SEQ ID NO: 51 or SEQ ID NO: 52, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide MBD2 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or

at least 95% identity to the sequence set forth in SEQ ID NO: 51 or SEQ ID NO: 52, or a fragment thereof.

[0165] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide NIPP1 may comprise a sequence as set forth in any one of SEQ ID NO: 67 or SEQ ID NO: 68, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide NIPP1 may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 67 or SEQ ID NO: 68, or a fragment thereof. In an aspect, a disclosed polypeptide NIPP1 can comprise the amino acid sequence as set forth in SEQ ID NO: 53 or SEQ ID NO: 54, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide NIPP1 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 53 or SEQ ID NO: 54, or a fragment thereof.

[0166] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide DNMT3A may comprise a sequence as set forth in SEQ ID NO: 69, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide DNMT3A may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 69, or a fragment thereof. In an aspect, a disclosed polypeptide DNMT3A can comprise the amino acid sequence as set forth in SEQ ID NO: 55, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide DNMT3A can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 55, or a fragment thereof.

[0167] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide KRAB may comprise a sequence as set forth in any one of SEQ ID NO: 70 or SEQ ID NO: 71, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide KRAB may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 70 or SEQ ID NO: 71, or a fragment thereof. In an aspect, a disclosed polypeptide KRAB can comprise the amino acid sequence as set forth in SEQ ID NO: 56, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide KRAB can comprise a sequence having at least 60%, at least

65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 56, or a fragment thereof.

[0168] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide KRAB may comprise a sequence as set forth in any one of SEQ ID NO: 70 or SEQ ID NO: 71, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide KRAB may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 70 or SEQ ID NO: 71, or a fragment thereof. In an aspect, a disclosed polypeptide KRAB can comprise the amino acid sequence as set forth in SEQ ID NO: 56, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide KRAB can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 56, or a fragment thereof.

[0169] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide MeCP2 may comprise a sequence as set forth in any one of SEQ ID NO: 72, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide MeCP2 may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequences set forth in SEQ ID NO: 72, or a fragment thereof. In an aspect, a disclosed polypeptide MeCP2 can comprise the amino acid sequence as set forth in SEQ ID NO: 57, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide MeCP2 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 57, or a fragment thereof.

[0170] In an aspect, the nucleic acid sequence encoding the disclosed fusion polypeptide KRAB-MeCP2 may comprise a sequence as set forth in any one of SEQ ID NO: 73 or SEQ ID NO: 74, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide KRAB-MeCP2 may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequences set forth in SEQ ID NO: 73 or SEQ ID NO: 74, or a fragment thereof. In an aspect, a disclosed polypeptide KRAB-MeCP2 can comprise the amino acid sequence as set forth in SEQ ID NO: 58, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide KRAB-MeCP2 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%,

at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 58, or a fragment thereof.

[0171] In an aspect, a disclosed encoded polypeptide can comprise transcription activation activity. In an aspect, a disclosed encoded polypeptide comprising transcription activation activity can comprise a transactivation domain. In an aspect, a disclosed transactivation domain can comprise a VP16 protein, a series of linked VP16 proteins, a p65 domain of NFκB, or any combination thereof.

[0172] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide VP16 may comprise a sequence as set forth in any one of SEQ ID NO: 132 or SEQ ID NO: 133, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide VP16 may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequences set forth in SEQ ID NO: 132 or SEQ ID NO: 133, or a fragment thereof. In an aspect, a disclosed polypeptide VP16 can comprise the amino acid sequence as set forth in SEQ ID NO: 131, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide VP16 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 131, or a fragment thereof.

[0173] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide p65 domain of NFκB or RELA may comprise a sequence as set forth in any one of SEQ ID NO: 135 or SEQ ID NO: 136, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide p65 domain of NFκB or RELA may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequences set forth in SEQ ID NO: 135 or SEQ ID NO: 136, or a fragment thereof. In an aspect, a disclosed polypeptide p65 domain of NFκB or RELA can comprise the amino acid sequence as set forth in SEQ ID NO: 134, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide p65 domain of NFκB or RELA can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 134, or a fragment thereof.

[0174] In an aspect, a disclosed encoded polypeptide can comprise transcription repression activity. In an aspect, a disclosed encoded polypeptide comprising transcription repression activity can comprise a Krüppel associated box domain, an ERF repressor domain, a MXI1 repressor domain, a SID4x repressor domain, a fused KRAB-MeCP2 domain, a MeCP2 TRD domain, a

MAS-SID repressor domain, a TATA box binding protein activity, or any combination thereof. In an aspect, a disclosed encoded polypeptide comprising transcription repression activity can comprise HP1 repressor activity, MeCP2 repressor activity, MBD1 repressor activity, MBD2 repressor activity, MBD3 repressor activity, MBD4 repressor activity, KRAB repressor activity, SUV39H1 repressor activity, SUV39H2 repressor activity, CTCF insulator-repressor activity, LSD-1 histone-demethylase repressor activity, or any combination thereof.

[0175] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide comprising Eukaryotic Release Factor 1 (ERF1) activity or Eukaryotic Release Factor 3 (ERF3) activity may comprise a sequence as set forth in any one of SEQ ID NO: 138, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide comprising ERF1 activity or ERF3 activity may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequences set forth in SEQ ID NO: 138, or a fragment thereof. In an aspect, a disclosed polypeptide comprising ERF1 activity or ERF3 activity can comprise the amino acid sequence as set forth in SEQ ID NO: 137, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide comprising ERF1 activity or ERF3 activity can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 137, or a fragment thereof.

[0176] In an aspect, a disclosed encoded polypeptide can comprise histone modification activity. In an aspect, a disclosed encoded polypeptide comprising histone modification activity can comprise histone acetyltransferase, histone deacetylase, histone demethylase, histone methyltransferase activity, or any combination thereof.

[0177] In an aspect, a disclosed encoded polypeptide can comprise nucleic acid association activity. In an aspect, a disclosed encoded polypeptide comprising nucleic acid association activity can comprise a helix-turn-helix region, a leucine zipper region, a winged helix region, a winged helix-turn-helix region, a helix-loop-helix region, an immunoglobulin fold, a B3 domain, a zinc finger, a HMG-box, a Wor3 domain, a TAL effector DNA-binding domain, or any combination thereof.

[0178] In an aspect, a disclosed encoded polypeptide can comprise methyltransferase activity. In an aspect, a disclosed encoded polypeptide comprising methyltransferase activity can comprise DNA (cytosine-5)-methyltransferase 3a (DNMT3A). In an aspect, a disclosed encoded polypeptide can comprise demethylase activity. In an aspect, a disclosed encoded polypeptide comprising demethylase activity can comprise ten-eleven translocation methylcytosine

dioxygenase 1 (TET1) or lysine-specific histone demethylase 1 (LSD1). In an aspect, a disclosed encoded polypeptide can comprise acetyltransferase activity. In an aspect, a disclosed encoded polypeptide comprising acetyltransferase activity can comprise histone acetyltransferase. In an aspect, a disclosed encoded polypeptide can comprise deacetylase activity. In an aspect, a disclosed encoded polypeptide comprising deacetylase activity can comprise histone deacetylase.

Exemplary Combinations of dCas Endonucleases and Polypeptides Having Enzymatic Activity

[0179] In an aspect, a disclosed dCas endonuclease can comprise dSpCas9 and a disclosed encoded polypeptide can comprise HP1a, HP1b, MBD1, MBD2, Krüppel-associated box (KRAB), NIPP1, the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof. In an aspect, a disclosed dCas endonuclease can comprise dSaCas9 and a disclosed encoded polypeptide can comprise HP1a, HP1b, MBD1, MBD2, Krüppel-associated box (KRAB), NIPP1, the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof. In an aspect, a disclosed dCas endonuclease can comprise dCjCas9 and a disclosed polypeptide can comprise HP1a, HP1b, MBD1, MBD2, Krüppel-associated box (KRAB), NIPP1, the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof. In an aspect, a disclosed dCas endonuclease can comprise dVQR, dEQR, or dVRER and a disclosed encoded polypeptide can comprise HP1a, HP1b, MBD1, MBD2, Krüppel-associated box (KRAB), NIPP1, the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof.

[0180] In an aspect, a disclosed dCas endonuclease can comprise dSpCas9, dSaCas9, dCjCas9, or a variant dCas9 (*e.g.*, dVQR, dEQR or dVRER and a disclosed fusion can comprise HP1a-HP1a, HP1a-HP1b, HP1a-MBD1, HP1a-MBD2, HP1a-KRAB, HP1a-NIPP1, HP1a-MeCP2, HP1a-DNMT3A, HP1b-HP1b, HP1b-HP1a, HP1b-MBD1, HP1b-MBD2, HP1b-KRAB, HP1b-NIPP1, HP1b-MeCP2, HP1b-DNMT3A, MBD1-MBD1, MBD1-HP1a, MBD1-HP1b, MBD1-MBD2, MBD1-KRAB, MBD1-NIPP1, MBD1-MeCP2, MBD1-DNMT3A, MBD2-MBD2, MBD2-HP1a, MBD2-HP1b, MBD2-MBD1, MBD2-KRAB, MBD2-NIPP1, MBD2-MeCP2, MBD2-DNMT3A, KRAB-KRAB, KRAB-HP1a, KRAB-HP1b, KRAB-MBD1, KRAB-MBD2, KRAB-NIPP1, KRAB-MeCP2, KRAB-DNMT3A, NIPP1-NIPP1, NIPP1-HP1a, NIPP1-HP1b, NIPP1-MBD1, NIPP1-MBD2, NIPP1-KRAB, NIPP1-MeCP2, NIPP1-DNMT3A, MeCP2-MeCP2, MeCP2-HP1a, MeCP2-HP1b, MeCP2-MBD1, MeCP2-MBD2, MeCP2-KRAB, MeCP2-NIPP1, MeCP2-DNMT3A, DNMT3A-DNMT3A, DNMT3A-HP1a, DNMT3A-HP1b, DNMT3A-MBD1, DNMT3A-MBD2, DNMT3A-KRAB, DNMT3A-NIPP1, or DNMT3A-

MeCP2. In an aspect, a disclosed dCas endonuclease can comprise dSpCas9, dSaCas9, or dCjCas9 and a disclosed encoded polypeptide can comprise KRAB-MeCP2. In an aspect, a disclosed dCas endonuclease can comprise dSaCas9, and a disclosed encoded polypeptide (*i.e.*, a disclosed fusion) can comprise KRAB-MeCP2.

[0181] In an aspect, an isolated nucleic acid can encode for a dCas endonuclease fused to the at least one encoded polypeptide having an enzymatic activity. In these or related aspects, the isolated nucleic acid can encode for a "fusion protein" wherein the fusion protein comprises a first component comprising a dCas endonuclease and a second component comprising at least one polypeptide having an enzymatic activity. In an aspect, the encoded fusion protein can comprise a dSpCas9 and a polypeptide having enzymatic activity comprising HP1a, HP1b, MBD1, MBD2, Krüppel-associated box (KRAB), NIPP1, the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof. In an aspect, the encoded fusion protein can comprise a dSaCas9 and a polypeptide having enzymatic activity comprising HP1a, HP1b, MBD1, MBD2, Krüppel-associated box (KRAB), NIPP1, the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof. In an aspect, the encoded fusion protein can comprise a dCjCas9 and a polypeptide having enzymatic activity comprising HP1a, HP1b, MBD1, MBD2, Krüppel-associated box (KRAB), NIPP1, the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof. In an aspect, the encoded fusion protein can comprise dVQR, dEQR, or dVRER and a polypeptide having enzymatic activity comprising HP1a, HP1b, MBD1, MBD2, Krüppel-associated box (KRAB), NIPP1, the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof.

[0182] In an aspect, the encoded fusion protein can comprise dSpCas9, dSaCas9, dCjCas9, or a variant dCas9 (*e.g.*, dVQR, dEQR or dVRER) and one or more of HP1a-HP1a, HP1a-HP1b, HP1a-MBD1, HP1a-MBD2, HP1a-KRAB, HP1a-NIPP1, HP1a-MeCP2, HP1a-DNMT3A, HP1b-HP1b, HP1b-HP1a, HP1b-MBD1, HP1b-MBD2, HP1b-KRAB, HP1b-NIPP1, HP1b-MeCP2, HP1b-DNMT3A, MBD1-MBD1, MBD1-HP1a, MBD1-HP1b, MBD1-MBD2, MBD1-KRAB, MBD1-NIPP1, MBD1-MeCP2, MBD1-DNMT3A, MBD2-MBD2, MBD2-HP1a, MBD2-HP1b, MBD2-MBD1, MBD2-KRAB, MBD2-NIPP1, MBD2-MeCP2, MBD2-DNMT3A, KRAB-KRAB, KRAB-HP1a, KRAB-HP1b, KRAB-MBD1, KRAB-MBD2, KRAB-NIPP1, KRAB-MeCP2, KRAB-DNMT3A, NIPP1-NIPP1, NIPP1-HP1a, NIPP1-HP1b, NIPP1-MBD1, NIPP1-MBD2, NIPP1-KRAB, NIPP1-MeCP2, NIPP1-DNMT3A, MeCP2-MeCP2, MeCP2-HP1a, MeCP2-HP1b, MeCP2-MBD1, MeCP2-MBD2, MeCP2-KRAB, MeCP2-NIPP1, MeCP2-

DNMT3A, DNMT3A-DNMT3A, DNMT3A-HP1a, DNMT3A-HP1b, DNMT3A-MBD1, DNMT3A-MBD2, DNMT3A-KRAB, DNMT3A-NIPP1, or DNMT3A-MeCP2. In an aspect, the encoded fusion protein can comprise a dCas9 selected from dSpCas9, dSaCas9, or dCjCas9 and a fusion comprising KRAB-MeCP2. In an aspect, the encoded fusion protein can comprise a dSaCas9 and KRAB-MeCP2.

[0183] In an aspect, the nucleic acid sequence encoding the fusion protein comprising dSpCas9 and DNMT3A or dVRER and DNMT3A may comprise a sequence as set forth in SEQ ID NO: 75 or SEQ ID NO: 76 respectively, or a derivative thereof. In an aspect, the nucleic acid sequence encoding the fusion protein comprising dSpCas9 and DNMT3A or dVRER and DNMT3A may comprise a sequence as set forth in SEQ ID NO: 77 or SEQ ID NO: 78 respectively, or a derivative thereof. In an aspect, the nucleic acid sequence encoding the fusion protein comprising dSpCas9 and DNMT3A may comprise a sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any of the sequences set forth in SEQ ID NO: 77 or SEQ ID NO: 78, or a fragment thereof. In an aspect, a disclosed polypeptide dSpCas9 can comprise the amino acid sequence as set forth in SEQ ID NO: 75 or SEQ ID NO: 76, or a fragment, or a derivative thereof.

gRNAs and Genes of Interest

[0184] In an aspect, the isolated nucleic acid molecule, comprises a nucleic acid sequence encoding at least one guide RNA targeting a gene of interest. In an aspect, a gene of interest can be any gene which has a disease linked mutation and/or exhibits abnormal expression or activity in the microglia, linked to a disease or disorder. Examples of such genes are known in the art. In an aspect, a disclosed gene of interest or portion thereof can demonstrate a reduced expression and/or activity level when compared to wild-type or control expression level. In an aspect, a disclosed gRNA can target a gene of interest or portion thereof that demonstrates a reduced expression and/or activity level when compared to wild-type or control expression level. In an aspect, a disclosed gene of interest or portion thereof can demonstrate an increased expression and/or activity level when compared to wild-type or control expression level. In an aspect, the gene of interest can be any gene which exhibits unusual expression in microglia in a diseased condition. In an aspect, the gene-of-interest is the *APOE* gene.

[0185] In an aspect, a disclosed isolated nucleic acid can comprise at least one gRNA that can target a gene of interest or portion thereof (*e.g.*, an *APOE* gene). In an aspect, a disclosed gRNA targeting a gene of interest or portion thereof can comprise at least two gRNAs. In an aspect, disclosed gRNAs can target the same gene of interest or portion thereof. In an aspect, disclosed

gRNAs can target different genes of interest or portions thereof. In an aspect, a disclosed gRNA can target a gene or portion thereof that demonstrates a loss of function. In an aspect, a disclosed gRNA can target a gene or portion thereof that demonstrates a gain of function.

[0186] As is understood in the art, a suitable gRNA that "targets" a nucleic acid (*i.e.*, a "target nucleic acid") is designed to hybridize to a sequence that is complementary to the "target nucleic acid."." This, in effect, results in a gRNA that comprises an RNA sequence having a certain percent identity to the target nucleic acid (DNA), with the understanding that uracils in the RNA sequence are equivalent to thymidine sin the DNA target sequence. Accordingly, in an aspect, the gRNA can comprise an RNA sequence corresponding to the "target nucleic acid."." In an aspect, a disclosed gRNA scaffold

[0187] In an aspect, a disclosed gRNA can target the *APOE* gene sequence or a regulatory element thereof. In an aspect, the at least one gRNA can target the *APOE* gene, or promoter region thereof, or an untranslated region (UTR) thereof, or any combination thereof. In an aspect, the at least one gRNA may target the promoter region, or intron 1, or a flanking sequence thereof, of *APOE* gene. In an aspect, a disclosed gRNA can target exon 4 of the *APOE* gene. In an aspect, a disclosed gRNA can target a protospacer-adjacent motif (PAM) created by a SNP rs429358 in exon 4 of the *APOE* gene.

[0188] In an aspect, the at least one gRNA may target a region in the *APOE* gene comprising a nucleic acid sequence as set forth in any one of SEQ ID NO: 11-14, or a complement thereof. In an aspect, the at least one gRNA targets a nucleic acid sequence comprising at least 10 consecutive nucleotides as set forth in SEQ ID NO: 11-14, or a complement thereof. In an aspect, the disclosed gRNA may comprise a nucleic acid sequence as set forth in SEQ ID NO: 15-33. In an aspect, the disclosed gRNA may comprise a nucleic acid sequence as set forth in SEQ ID NO: 15-33, or a complement thereof. In an aspect, the disclosed gRNA may comprise a nucleic acid sequence with at least about 90%, or about 91%, or about 92%, or about 93%, or about 94%, or about 95%, or about 96%, or about 97%, or about 98%, or about 99%, or more sequence identity to any one of the sequences set forth in SEQ ID NO: 15-33, or a complement thereof, or a fragment thereof.

[0189] In an aspect of a disclosed nucleic acid molecule, the nucleic acid molecule can further comprise a gRNA scaffold. In an aspect, a disclosed gRNA scaffold can comprise the sequence set forth in SEQ ID NO: 129, SEQ ID NO: 130, or a fragment thereof.

Additional Elements in Isolated Nucleic Acid Molecules

[0190] In an aspect, a disclosed isolated nucleic acid molecule can further comprise one or more regulatory elements (*i.e.*, promoters) in addition to the microglia specific regulatory element (*e.g.*, microglia specific promoter). In an aspect, a disclosed additional promoter can comprise a U6

promoter, a chicken β-actin promoter, an EF-1α, a CMV promoter, a CMV promoter/enhancer, a fragment thereof, a derivative thereof, or any combination thereof. In an aspect, a disclosed U6 promoter can comprise the sequence set forth in SEQ ID NO: 81, or a fragment thereof. In an aspect, a disclosed U6 promoter can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% identity to the sequence set forth in SEQ ID NO: 81 or a fragment thereof. In an aspect, a disclosed EF-1α promoter can comprise the sequence set forth in SEQ ID NO: 79, SEQ ID NO: 80, or a fragment thereof. In an aspect, a disclosed EF-1α promoter can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% identity to the sequence set forth in SEQ ID NO: 79, SEQ ID NO:80, or a fragment thereof. In an aspect, a promoter can be a short EF1alpha (EFS-NF) promoter.

[0191] In an aspect, a disclosed promoter operably can be linked to the at least one guide RNA targeting a gene of interest or portion thereof. In an aspect, a disclosed promoter operably linked to the at least one guide RNA can comprise a U6 promoter. In an aspect, a disclosed promoter operably linked to the at least one guide RNA targeting an *APOE* gene can comprise a U6 promoter.

[0192] In an aspect, a disclosed isolated nucleic acid molecule can further comprise two or more promoters, wherein a first promoter can be operably linked to the dCas endonuclease, a polypeptide having enzymatic activity, a fusion of two or more polypeptides having enzymatic activities or any combination thereof, and wherein a second promoter can be operably linked to the at least one guide RNA targeting a gene of interest or portion thereof. In an aspect, a disclosed isolated nucleic acid molecule can further comprise one or more promoters, wherein a first promoter comprises a microglia specific promoter that is operably linked to the dCas endonuclease, a polypeptide having enzymatic activity, a fusion of two or more polypeptides having enzymatic activities or any combination thereof, and wherein a second promoter can be operably linked to the at least one guide RNA targeting a gene of interest thereof. In an aspect, the second promoter operably linked to the gRNA may not be a microglia specific promoter. In an aspect, the isolated nucleic acid can comprise a first promoter selected from a F4/80 promoter, a CD68 promoter, a IBA1 promoter, or a FCGR2A promoter or derivatives thereof, and a second promoter comprising a U6 promoter, or a derivative thereof.

[0193] In an aspect, a disclosed isolated nucleic acid molecule can further comprise a nucleic acid sequencing encoding one or more regulatory elements. In an aspect, a disclosed regulatory element can comprise a promoter, an enhancer, a promoter/enhancer, an internal ribosomal entry

site, a transcription terminal signal, a polyadenylation signal, a Sp1 and/or NF-kB transcriptional factor binding site, a p2A signal, a woodchuck hepatitis virus post-transcriptional regulatory element, a Phi signal-packaging signal, a rev responsive element, a 5'-ITR, a 3'-ITR, an inverted terminal repeat, a nuclear localization signal (NLS), or any combination thereof.

[0194] In an aspect, a disclosed 5'-ITR may comprise the sequence as set forth in any one of SEQ ID NO: 82 or SEQ ID NO: 83. In an aspect, a disclosed 3'-ITR may comprise the sequence as set forth in any one of SEQ ID NO: 84 or SEQ ID NO: 85.

[0195] In an aspect, a disclosed NLS can comprise the sequence set forth in any one of SEQ ID NO: 88 – SEQ ID NO: 94, or a fragment thereof. In an aspect, a disclosed PolyA sequence can comprise the sequence set forth in SEQ ID NO: 86, SEQ ID NO: 87, or a fragment thereof.

[0196] In an aspect, a disclosed nucleic acid sequence can be CpG depleted and codon-optimized for expression in a human cell. In an aspect, "CpG-free" can mean completely free of CpGs or partially free of CpGs. In an aspect, "CpG-free" can mean "CpG-depleted."." In an aspect, "CpG-depleted" can mean "CpG-free." In an aspect, "CpG-depleted" can mean completely depleted of CpGs or partially depleted of CpGs. In an aspect, "CpG-free" can mean "CpG-optimized" for a desired and/or ideal expression level. CpG depletion and/or optimization is known to the skilled person in the art. In an aspect, any disclosed Cas9 endonuclease, a disclosed polypeptide having enzymatic activities, a disclosed fusion product or a disclosed fusion protein, or any combination thereof can be codon-optimized.

[0197] In an aspect, a disclosed isolated nucleic acid molecule can comprise a sequence that is about 4.5 kilobases or less than about 4.5 kilobases.

[0198] Disclosed herein is an isolated nucleic acid molecule, comprising: a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, wherein the dCas is a dSpCas9, dSaCas9, or dCjCas9, (ii) a fusion protein having one or more enzymatic activities, wherein the fusion product comprises any combination of HP1a, HP1b, MBD1, MBD2, KRAB, NIPP1, MeCP2, or DNMT3A, and (iii) at least one guide RNA targeting a gene of interest or portion thereof.

[0199] Disclosed herein is an isolated nucleic acid molecule, comprising: a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, wherein the dCas is a dSpCas9, dSaCas9, or dCjCas9, (ii) a fusion protein having one or more enzymatic activities, wherein the fusion product comprises any combination of HP1a, HP1b, MBD1, MBD2, KRAB, NIPP1, MeCP2, or DNMT3A, (iii) at least one guide RNA targeting a gene of interest or portion thereof, (iv) a promoter operably linked to the dCas endonuclease and the fusion protein, and (v) a promoter operably linked to the at least one guide RNA.

[0200] Disclosed herein is an isolated nucleic acid molecule, comprising: a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, wherein the dCas is a dSpCas9, dSaCas9, or dCjCas9, (ii) a fusion protein having one or more enzymatic activities, wherein the fusion protein comprises any combination of HP1a, HP1b, MBD1, MBD2, KRAB, NIPP1, MeCP2, or DNMT3A, and (iii) at least one guide RNA targeting *APOE* or portion thereof.

[0201] Disclosed herein is an isolated nucleic acid molecule, comprising: a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, wherein the dCas is a dSpCas9, dSaCas9, or dCjCas9, (ii) a fusion protein having one or more enzymatic activities, wherein the fusion protein comprises any combination of HP1a, HP1b, MBD1, MBD2, KRAB, NIPP1, MeCP2, or DNMT3A, (iii) at least one guide RNA targeting *APOE* or portion thereof, (iv) a microglia specific promoter (*e.g.* F4/80, CD68, IBA1, FCGR2A or derivatives thereof) operably linked to the dCas endonuclease and the at least one polypeptide, and (v) a promoter operably linked to the at least one guide RNA.

[0202] In an aspect, a disclosed isolated nucleic acid molecule can treat and/or prevent Alzheimer's disease progression. In an aspect, a disclosed isolated nucleic acid molecule can treat and/or prevent Parkinson's disease progression.

2. Vectors

Viral Vectors

[0203] Disclosed herein is a viral vector comprising a disclosed isolated nucleic acid molecule. Disclosed herein is a viral vector comprising a disclosed nucleic acid molecule. Disclosed herein is a viral vector comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, (ii) at least one polypeptide having an enzymatic activity, and (iii) at least one guide RNA targeting a gene of interest or portion thereof; and (b) one or more microglia specific regulatory element operably linked to (a)(i), (a)(ii), (a)(iii) or any combination thereof. In an aspect, In an aspect, the gene of interest is *APOE,* or an allele thereof.

[0204] In an aspect, a disclosed viral vector can be an adenovirus vector, an AAV vector, a herpes simplex virus vector, a retrovirus vector, a lentivirus vector, and alphavirus vector, a flavivirus vector, a rhabdovirus vector, a measles virus vector, a Newcastle disease viral vector, a poxvirus vector, or a picornavirus vector. In an aspect, a disclosed viral vector can be a lentiviral vector.

[0205] Disclosed herein is a recombinant AAV vector comprising a disclosed isolated nucleic acid molecule. Disclosed herein is a recombinant AAV vector comprising a disclosed isolated nucleic acid molecule comprising (a) a nucleic acid sequence encoding (i) a deactivated Clustered Regularly Interspaced Short Palindromic Repeats associated (dCas) endonuclease, (ii) at least one polypeptide having an enzymatic activity, and (iii) at least one guide RNA targeting an *APOE*

gene sequence or a regulatory element thereof; and (b) one or more microglia specific regulatory elements operably linked to (a)(i), (a)(ii), (a)(iii), or any combination thereof.

[0206] Disclosed herein is a recombinant AAV vector comprising a disclosed isolated nucleic acid molecule comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, (ii) at least one polypeptide having an enzymatic activity, and (iii) at least one guide RNA targeting a gene of interest or portion thereof; and (b) one or more microglia specific promoter operably linked to (a)(i), (a)(ii), (a)(iii) or any combination thereof. In an aspect, the gene of interest is *APOE,* or an allele thereof.

[0207] Disclosed herein is a recombinant AAV vector comprising a disclosed isolated nucleic acid molecule comprising (a) (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, (ii) a fusion product having one or more enzymatic activities, and (iii) at least one guide RNA targeting a gene of interest or portion thereof, and (b) one or more microglia specific regulatory element operably linked to (a)(i), (a)(ii), (a)(iii) or any combination thereof. In an aspect, the gene of interest is *APOE,* or an allele thereof.

[0208] Disclosed herein is a recombinant AAV vector comprising a disclosed isolated nucleic acid molecule comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, (ii) a fusion product having one or more enzymatic activities, and (iii) at least one guide RNA targeting a gene of interest or portion thereof, and (b) one or more microglia specific promoter operably linked to (a)(i), (a)(ii), (a)(iii) or any combination thereof. In an aspect, the gene of interest is *APOE,* or an allele thereof.

[0209] In an aspect of a disclosed viral vector or disclosed recombinant AAV vector, the microglia specific regulatory sequence may comprise a wild-type promoter, a microglia specific enhancer, a synthetic promoter, a synthetic enhancer, a transcriptional or translational regulatory sequence or any combination thereof. In an aspect, the microglia specific regulatory sequence is a microglia specific promoter. In an aspect, the microglia specific promoter can be any promoter known in the art, that exhibits enhanced activity in the microglia. Non-limiting examples of microglia specific promoters include F4/80, CD68, FCGR2A, HexB or IBA1 promoter, or a derivative thereof.

[0210] In an aspect, the promoter is a CD68 promoter, or a derivative thereof. In an aspect, the promoter may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one or more of the sequences set forth in SEQ ID NO: 1-3, or a fragment, or a derivative thereof. In an aspect, the promoter comprises a sequence as set forth in any one of SEQ ID NO: 1-3.

[0211] In an aspect, the promoter is a F4/80 promoter, or a derivative thereof. In an aspect, the promoter may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequence set forth in SEQ ID NO: 4, or a fragment, or a derivative thereof. In an aspect, the promoter comprises a sequence as set forth in SEQ ID NO: 4.

[0212] In an aspect, the promoter is a IBA1 promoter, or a derivative thereof. In an aspect, the promoter may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequence set forth in SEQ ID NO: 5-7, or a fragment, or a derivative thereof. In an aspect, the promoter comprises a sequence as set forth in any one of SEQ ID NO: 5-7.

[0213] In an aspect, the promoter is a FCGR2A promoter, or a derivative thereof. In an aspect, the promoter may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequence set forth in SEQ ID NO: 8, or a fragment, or a derivative thereof. In an aspect, the promoter comprises a sequence as set forth in SEQ ID NO: 8.

[0214] In an aspect of a disclosed viral vector or disclosed recombinant AAV vector, the disclosed viral vector or disclosed recombinant AAV vector may comprise at least a microglia specific regulatory element (*i.e.*, a microglia specific promoter) which may be operably linked to the dCas endonuclease and the fusion protein having one or more enzymatic activities. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the dCas endonuclease and the fusion protein having one or more enzymatic activities can comprise a F4/80 promoter, or a derivative thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the dCas endonuclease and the fusion protein having one or more enzymatic activities can comprise a CD68 promoter, or a derivative thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the dCas endonuclease and the fusion protein having one or more enzymatic activities can comprise a IBA1 promoter, or a derivative thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the dCas endonuclease and the fusion protein having one or more enzymatic activities can comprise a FCGR2A promoter, or a derivative thereof.

[0215] In an aspect of a disclosed viral vector or disclosed recombinant AAV vector, the disclosed dCas endonuclease can comprise a dCas9 endonuclease. In an aspect, a disclosed dCas9 endonuclease can comprise a deactivated Staphylococcus aureus Cas9 (dSaCas9), a deactivated Streptococcus pyogenes Cas9 (dSpCas9), a deactivated Campylobacter jejuni Cas9 (dCjCas9), or a variant dCas9 endonuclease. In an aspect, a disclosed variant dCas9 can comprise a variant dSaCas9, a variant dSpCas9, or a variant dCjCa9. In an aspect, a disclosed variant dSpCas9 can comprise dVQR, dEQR, or dVRER.

[0216] In an aspect of a disclosed viral vector or disclosed recombinant AAV vector, the disclosed dSpCas9 may comprise a sequence as set forth in any one of SEQ ID NO: 40 or SEQ ID NO: 41, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed dSpCas9 endonuclease may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 40 or SEQ ID NO: 41, or a fragment thereof. In an aspect, a disclosed dSpCas9 endonuclease can comprise the amino acid sequence as set forth in SEQ ID NO: 36 or SEQ ID NO: 37, or a fragment, or a derivative thereof. In an aspect, a disclosed dSpCas9 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 36 or SEQ ID NO: 37, or a fragment thereof.

[0217] In an aspect of a disclosed viral vector or disclosed recombinant AAV vector, the disclosed dSaCas9 may comprise a sequence as set forth in SEQ ID NO: 42, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed dSaCas9 endonuclease may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequences set forth in SEQ ID NO: 42, or a fragment thereof. In an aspect, a disclosed dSaCas9 endonuclease can comprise the amino acid sequence as set forth in SEQ ID NO: 38 or a fragment, or a derivative thereof. In an aspect, a disclosed dSaCas9 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 38, or a fragment thereof.

[0218] In an aspect of a disclosed viral vector or disclosed recombinant AAV vector, the disclosed dCjCas9 may comprise a sequence as set forth in SEQ ID NO: 43, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed dCjCas9 endonuclease may comprise a nucleic acid sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%,

at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 43, or a fragment thereof. In an aspect, a disclosed dCjCas9 endonuclease can comprise the amino acid sequence as set forth in SEQ ID NO: 39, or a fragment, or a derivative thereof. In an aspect, a disclosed dCjCas9 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 39 or a fragment thereof.

[0219] In an aspect, a disclosed variant of dCas9 dVQR can comprise D1135V, R1335Q, and T1337R with reference to SEQ ID NO: 36. In an aspect, a disclosed variant of dCas9 dEQR can comprise D1135E, R1335Q, and T1337R with reference to SEQ ID NO: 36. In an aspect, a disclosed variant of dCas9 dVRER can comprise D1135V, G1218R, R1335E, and T1337R with reference to SEQ ID NO: 36. In an aspect, a disclosed dVRER can comprise the sequence set forth in SEQ ID NO: 37 or a fragment thereof.

[0220] In an aspect of a disclosed viral vector or disclosed recombinant AAV vector, the disclosed dCas9 endonuclease may comprise a sequence as set forth in any one of SEQ ID NO: 40-43. In an aspect, the sequence encoding the disclosed dCas9 endonuclease may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 40-43, or a fragment, or a derivative thereof.

[0221] In an aspect of a disclosed viral vector or disclosed recombinant AAV vector, the disclosed viral vector or disclosed recombinant AAV vector may comprise a nucleic acid sequence encoding at least one polypeptide having an enzymatic activity. In an aspect, a disclosed encoded polypeptide can comprise transcription activation activity, transcription repression activity, transcription release factor activity, histone modification activity, nucleic acid association activity, methyltransferase activity, demethylase activity, acetyltransferase activity, deacetylase activity, or any combination thereof.

[0222] In an aspect, the disclosed encoded polypeptide can comprise HP1a, HP1b, MBD1, MBD2, Krüppel-Associated Box (KRAB), NIPP1, the Transcription Repression Domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof. In an aspect, a disclosed fusion can comprise HP1a-HP1a, HP1a-HP1b, HP1a-MBD1, HP1a-MBD2, HP1a-KRAB, HP1a-NIPP1, HP1a-MeCP2, HP1a-DNMT3A, HP1b-HP1b, HP1b-HP1a, HP1b-MBD1, HP1b-MBD2, HP1b-KRAB, HP1b-NIPP1, HP1b-MeCP2, HP1b-DNMT3A, MBD1-MBD1, MBD1-HP1a, MBD1-HP1b, MBD1-MBD2, MBD1-KRAB, MBD1-

NIPP1, MBD1-MeCP2, MBD1-DNMT3A, MBD2-MBD2, MBD2-HP1a, MBD2-HP1b, MBD2-MBD1, MBD2-KRAB, MBD2-NIPP1, MBD2-MeCP2, MBD2-DNMT3A, KRAB-KRAB, KRAB-HP1a, KRAB-HP1b, KRAB-MBD1, KRAB-MBD2, KRAB-NIPP1, KRAB-MeCP2, KRAB-DNMT3A, NIPP1-NIPP1, NIPP1-HP1a, NIPP1-HP1b, NIPP1-MBD1, NIPP1-MBD2, NIPP1-KRAB, NIPP1-MeCP2, NIPP1-DNMT3A, MeCP2-MeCP2, MeCP2-HP1a, MeCP2-HP1b, MeCP2-MBD1, MeCP2-MBD2, MeCP2-KRAB, MeCP2-NIPP1, MeCP2-DNMT3A, DNMT3A-DNMT3A, DNMT3A-HP1a, DNMT3A-HP1b, DNMT3A-MBD1, DNMT3A-MBD2, DNMT3A-KRAB, DNMT3A-NIPP1, or DNMT3A-MeCP2. Examples of suitable polypeptides sequences for use with the current disclosure are provided in PCT/US2022/078260, the contents of which are incorporated herein by reference, in their entirety.

[0223] In an aspect of a disclosed viral vector or disclosed recombinant AAV vector, the disclosed viral vector or disclosed recombinant AAV vector encoding the polypeptide HP1a may comprise a sequence as set forth in any one of SEQ ID NO: 59 or SEQ ID NO: 60, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide HP1a may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 59 or SEQ ID NO: 60, or a fragment thereof. In an aspect, a disclosed polypeptide HP1a can comprise the amino acid sequence as set forth in SEQ ID NO: 44 or SEQ ID NO: 45, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide HP1a can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 44 or SEQ ID NO: 45, or a fragment thereof.

[0224] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide HP1b may comprise a sequence as set forth in any one of SEQ ID NO: 61 or SEQ ID NO: 62, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide HP1b may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 61 or SEQ ID NO: 62, or a fragment thereof. In an aspect, a disclosed polypeptide HP1b can comprise the amino acid sequence as set forth in SEQ ID NO: 46 or SEQ ID NO: 47, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide HP1b can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at

least 95% identity to the sequence set forth in SEQ ID NO: 46 or SEQ ID NO: 47, or a fragment thereof.

[0225] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide MBD1 may comprise a sequence as set forth in any one of SEQ ID NO: 63 or SEQ ID NO: 64, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide MBD1 may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 63 or SEQ ID NO: 64, or a fragment thereof. In an aspect, a disclosed polypeptide MBD1 can comprise the amino acid sequence as set forth in any one of SEQ ID NO: 48-50, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide MDB1 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in any one of SEQ ID NO: 48-50, or a fragment thereof.

[0226] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide MBD2 may comprise a sequence as set forth in any one of SEQ ID NO: 65 or SEQ ID NO: 66, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide MBD2 may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 65 or SEQ ID NO: 66, or a fragment thereof. In an aspect, a disclosed polypeptide MBD2 can comprise the amino acid sequence as set forth in SEQ ID NO: 51 or SEQ ID NO: 52, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide MBD2 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 51 or SEQ ID NO: 52, or a fragment thereof.

[0227] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide NIPP1 may comprise a sequence as set forth in any one of SEQ ID NO: 67 or SEQ ID NO: 68, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide NIPP1 may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 67 or SEQ ID NO: 68, or a fragment thereof. In an aspect, a disclosed polypeptide NIPP1 can comprise the amino acid sequence as set forth in SEQ ID NO: 53 or SEQ ID NO: 54, or a fragment, or a

derivative thereof. In an aspect, a disclosed polypeptide NIPP1 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 53 or SEQ ID NO: 54, or a fragment thereof.

[0228] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide DNMT3A may comprise a sequence as set forth in SEQ ID NO: 69, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide DNMT3A may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 69, or a fragment thereof. In an aspect, a disclosed polypeptide DNMT3A can comprise the amino acid sequence as set forth in SEQ ID NO: 55, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide DNMT3A can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 55, or a fragment thereof.

[0229] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide KRAB may comprise a sequence as set forth in any one of SEQ ID NO: 70 or SEQ ID NO: 71, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide KRAB may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 70 or SEQ ID NO: 71, or a fragment thereof. In an aspect, a disclosed polypeptide KRAB can comprise the amino acid sequence as set forth in SEQ ID NO: 56, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide KRAB can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 56, or a fragment thereof.

[0230] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide KRAB may comprise a sequence as set forth in any one of SEQ ID NO: 70 or SEQ ID NO: 71, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide KRAB may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 70 or SEQ ID NO: 71, or a fragment thereof. In an aspect, a disclosed polypeptide KRAB can comprise the amino acid sequence as set forth in SEQ ID NO: 56, or a fragment, or a derivative thereof. In

an aspect, a disclosed polypeptide KRAB can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 56, or a fragment thereof.

[0231] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide MeCP2 may comprise a sequence as set forth in any one of SEQ ID NO: 72, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide MeCP2 may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequences set forth in SEQ ID NO: 72, or a fragment thereof. In an aspect, a disclosed polypeptide MeCP2 can comprise the amino acid sequence as set forth in SEQ ID NO: 57, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide MeCP2 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 57, or a fragment thereof.

[0232] In an aspect, the nucleic acid sequence encoding the disclosed fusion polypeptide KRAB-MeCP2 may comprise a sequence as set forth in any one of SEQ ID NO: 73 or SEQ ID NO: 74, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide KRAB-MeCP2 may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequences set forth in SEQ ID NO: 73 or SEQ ID NO: 74, or a fragment thereof. In an aspect, a disclosed polypeptide KRAB-MeCP2 can comprise the amino acid sequence as set forth in SEQ ID NO: 58, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide KRAB-MeCP2 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 58, or a fragment thereof.

[0233] In an aspect, a disclosed encoded polypeptide can comprise transcription activation activity. In an aspect, a disclosed encoded polypeptide comprising transcription activation activity can comprise a transactivation domain. In an aspect, a disclosed transactivation domain can comprise a VP16 protein, a series of linked VP16 proteins, a p65 domain of NFκB, or any combination thereof.

[0234] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide VP16 may comprise a sequence as set forth in any one of SEQ ID NO: 132 or SEQ ID NO: 133, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide VP16 may

comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequences set forth in SEQ ID NO: 132 or SEQ ID NO: 133, or a fragment thereof. In an aspect, a disclosed polypeptide VP16 can comprise the amino acid sequence as set forth in SEQ ID NO: 131, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide VP16 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 131, or a fragment thereof.

[0235] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide p65 domain of NFκB or RELA may comprise a sequence as set forth in any one of SEQ ID NO: 135 or SEQ ID NO: 136, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide p65 domain of NFκB or RELA may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequences set forth in SEQ ID NO: 135 or SEQ ID NO: 136, or a fragment thereof. In an aspect, a disclosed polypeptide p65 domain of NFκB or RELA can comprise the amino acid sequence as set forth in SEQ ID NO: 134, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide p65 domain of NFκB or RELA can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 134, or a fragment thereof.

[0236] In an aspect, a disclosed encoded polypeptide can comprise transcription repression activity. In an aspect, a disclosed encoded polypeptide comprising transcription repression activity can comprise a Krüppel associated box domain, an ERF repressor domain, a MXI1 repressor domain, a SID4x repressor domain, a fused KRAB-MeCP2 domain, a MeCP2 TRD domain, a MAS-SID repressor domain, a TATA box binding protein activity, or any combination thereof. In an aspect, a disclosed encoded polypeptide comprising transcription repression activity can comprise HP1 repressor activity, MeCP2 repressor activity, MBD1 repressor activity, MBD2 repressor activity, MBD3 repressor activity, MBD4 repressor activity, KRAB repressor activity, SUV39H1 repressor activity, SUV39H2 repressor activity, CTCF insulator-repressor activity, LSD-1 histone-demethylase repressor activity, or any combination thereof.

[0237] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide comprising Eukaryotic Release Factor 1 (ERF1) activity or Eukaryotic Release Factor 3 (ERF3) activity may comprise a sequence as set forth in any one of SEQ ID NO: 138, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide comprising ERF1 activity

or ERF3 activity may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequences set forth in SEQ ID NO: 138, or a fragment thereof. In an aspect, a disclosed polypeptide comprising ERF1 activity or ERF3 activity can comprise the amino acid sequence as set forth in SEQ ID NO: 137, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide comprising ERF1 activity or ERF3 activity can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 137, or a fragment thereof.

[0238] In an aspect, a disclosed encoded polypeptide can comprise histone modification activity. In an aspect, a disclosed encoded polypeptide comprising histone modification activity can comprise histone acetyltransferase, histone deacetylase, histone demethylase, histone methyltransferase activity, or any combination thereof.

[0239] In an aspect, a disclosed encoded polypeptide can comprise nucleic acid association activity. In an aspect, a disclosed encoded polypeptide comprising nucleic acid association activity can comprise a helix-turn-helix region, a leucine zipper region, a winged helix region, a winged helix-turn-helix region, a helix-loop-helix region, an immunoglobulin fold, a B3 domain, a zinc finger, a HMG-box, a Wor3 domain, a TAL effector DNA-binding domain, or any combination thereof.

[0240] In an aspect, a disclosed encoded polypeptide can comprise methyltransferase activity. In an aspect, a disclosed encoded polypeptide comprising methyltransferase activity can comprise DNA (cytosine-5)-methyltransferase 3a (DNMT3A). In an aspect, a disclosed encoded polypeptide can comprise demethylase activity. In an aspect, a disclosed encoded polypeptide comprising demethylase activity can comprise ten-eleven translocation methylcytosine dioxygenase 1 (TET1) or lysine-specific histone demethylase 1 (LSD1). In an aspect, a disclosed encoded polypeptide can comprise acetyltransferase activity. In an aspect, a disclosed encoded polypeptide comprising acetyltransferase activity can comprise histone acetyltransferase. In an aspect, a disclosed encoded polypeptide can comprise deacetylase activity. In an aspect, a disclosed encoded polypeptide comprising deacetylase activity can comprise histone deacetylase.

[0241] In an aspect of a disclosed viral vector or disclosed recombinant AAV vector, the vector may comprise a nucleic acid sequence encoding a disclosed dCas endonuclease, comprising dSpCas9 and a disclosed encoded polypeptide comprising any one or more of HP1a, HP1b, MBD1, MBD2, Krüppel-associated box (KRAB), NIPP1, the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any

fusion thereof. In an aspect, a disclosed dCas endonuclease can comprise dSaCas9 and a disclosed encoded polypeptide can comprise HP1a, HP1b, MBD1, MBD2, Krüppel-associated box (KRAB), NIPP1, the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof. In an aspect, a disclosed dCas endonuclease can comprise dCjCas9 and a disclosed polypeptide can comprise HP1a, HP1b, MBD1, MBD2, Krüppel-associated box (KRAB), NIPP1, the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof. In an aspect, a disclosed dCas endonuclease can comprise dVQR, dEQR, or dVRER and a disclosed encoded polypeptide can comprise HP1a, HP1b, MBD1, MBD2, Krüppel-associated box (KRAB), NIPP1, the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof.

[0242] In an aspect, a disclosed dCas endonuclease can comprise dSpCas9, dSaCas9, dCjCas9, or a variant dCas9 (*e.g.*, dVQR, dEQR or dVRER and a disclosed fusion can comprise HP1a-HP1a, HP1a-HP1b, HP1a-MBD1, HP1a-MBD2, HP1a-KRAB, HP1a-NIPP1, HP1a-MeCP2, HP1a-DNMT3A, HP1b-HP1b, HP1b-HP1a, HP1b-MBD1, HP1b-MBD2, HP1b-KRAB, HP1b-NIPP1, HP1b-MeCP2, HP1b-DNMT3A, MBD1-MBD1, MBD1-HP1a, MBD1-HP1b, MBD1-MBD2, MBD1-KRAB, MBD1-NIPP1, MBD1-MeCP2, MBD1-DNMT3A, MBD2-MBD2, MBD2-HP1a, MBD2-HP1b, MBD2-MBD1, MBD2-KRAB, MBD2-NIPP1, MBD2-MeCP2, MBD2-DNMT3A, KRAB-KRAB, KRAB-HP1a, KRAB-HP1b, KRAB-MBD1, KRAB-MBD2, KRAB-NIPP1, KRAB-MeCP2, KRAB-DNMT3A, NIPP1-NIPP1, NIPP1-HP1a, NIPP1-HP1b, NIPP1-MBD1, NIPP1-MBD2, NIPP1-KRAB, NIPP1-MeCP2, NIPP1-DNMT3A, MeCP2-MeCP2, MeCP2-HP1a, MeCP2-HP1b, MeCP2-MBD1, MeCP2-MBD2, MeCP2-KRAB, MeCP2-NIPP1, MeCP2-DNMT3A, DNMT3A-DNMT3A, DNMT3A-HP1a, DNMT3A-HP1b, DNMT3A-MBD1, DNMT3A-MBD2, DNMT3A-KRAB, DNMT3A-NIPP1, or DNMT3A-MeCP2. In an aspect, a disclosed dCas endonuclease can comprise dSpCas9, dSaCas9, or dCjCas9 and a disclosed encoded polypeptide can comprise KRAB-MeCP2. In an aspect, a disclosed dCas endonuclease can comprise dSaCas9, and a disclosed encoded polypeptide (*i.e.*, a disclosed fusion) can comprise KRAB-MeCP2.

[0243] In an aspect of a disclosed viral vector or disclosed recombinant AAV vector, the disclosed viral vector or disclosed recombinant AAV vector may comprise a nucleic acid sequence encoding a dCas endonuclease fused to the at least one encoded polypeptide having an enzymatic activity. In these or related aspects, the isolated nucleic acid can encode for a "fusion protein" wherein the fusion protein comprises a first component comprising a dCas endonuclease and a second component comprising at least one polypeptide having an enzymatic activity. In an aspect, the

encoded fusion protein can comprise a dSpCas9 and a polypeptide having enzymatic activity comprising HP1a, HP1b, MBD1, MBD2, Krüppel-associated box (KRAB), NIPP1, the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof. In an aspect, the encoded fusion protein can comprise a dSaCas9 and a polypeptide having enzymatic activity comprising HP1a, HP1b, MBD1, MBD2, Krüppel-associated box (KRAB), NIPP1, the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof. In an aspect, the encoded fusion protein can comprise a dCjCas9 and a polypeptide having enzymatic activity comprising HP1a, HP1b, MBD1, MBD2, Krüppel-associated box (KRAB), NIPP1, the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof. In an aspect, the encoded fusion protein can comprise dVQR, dEQR, or dVRER and a polypeptide having enzymatic activity comprising HP1a, HP1b, MBD1, MBD2, Krüppel-associated box (KRAB), NIPP1, the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof.

[0244] In an aspect, the encoded fusion protein can comprise dSpCas9, dSaCas9, dCjCas9, or a variant dCas9 (*e.g.*, dVQR, dEQR or dVRER) and one or more of HP1a-HP1a, HP1a-HP1b, HP1a-MBD1, HP1a-MBD2, HP1a-KRAB, HP1a-NIPP1, HP1a-MeCP2, HP1a-DNMT3A, HP1b-HP1b, HP1b-HP1a, HP1b-MBD1, HP1b-MBD2, HP1b-KRAB, HP1b-NIPP1, HP1b-MeCP2, HP1b-DNMT3A, MBD1-MBD1, MBD1-HP1a, MBD1-HP1b, MBD1-MBD2, MBD1-KRAB, MBD1-NIPP1, MBD1-MeCP2, MBD1-DNMT3A, MBD2-MBD2, MBD2-HP1a, MBD2-HP1b, MBD2-MBD1, MBD2-KRAB, MBD2-NIPP1, MBD2-MeCP2, MBD2-DNMT3A, KRAB-KRAB, KRAB-HP1a, KRAB-HP1b, KRAB-MBD1, KRAB-MBD2, KRAB-NIPP1, KRAB-MeCP2, KRAB-DNMT3A, NIPP1-NIPP1, NIPP1-HP1a, NIPP1-HP1b, NIPP1-MBD1, NIPP1-MBD2, NIPP1-KRAB, NIPP1-MeCP2, NIPP1-DNMT3A, MeCP2-MeCP2, MeCP2-HP1a, MeCP2-HP1b, MeCP2-MBD1, MeCP2-MBD2, MeCP2-KRAB, MeCP2-NIPP1, MeCP2-DNMT3A, DNMT3A-DNMT3A, DNMT3A-HP1a, DNMT3A-HP1b, DNMT3A-MBD1, DNMT3A-MBD2, DNMT3A-KRAB, DNMT3A-NIPP1, or DNMT3A-MeCP2. In an aspect, the encoded fusion protein can comprise a dCas9 selected from dSpCas9, dSaCas9, or dCjCas9 and a fusion comprising KRAB-MeCP2. In an aspect, the encoded fusion protein can comprise a dSaCas9 and KRAB-MeCP2.

[0245] In an aspect, the nucleic acid sequence encoding the fusion protein comprising dSpCas9 and DNMT3A or dVRER and DNMT3A may comprise a sequence as set forth in SEQ ID NO: 77 or SEQ ID NO: 78 respectively, or a derivative thereof. In an aspect, the nucleic acid sequence

encoding the fusion protein comprising dSpCas9 and DNMT3A may comprise a sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any of the sequences set forth in SEQ ID NO: 77 or SEQ ID NO: 78, or a fragment thereof. In an aspect, a disclosed polypeptide dSpCas9 can comprise the amino acid sequence as set forth in SEQ ID NO: 75 or SEQ ID NO: 76, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide KRAB-MeCP2 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 75 or SEQ ID NO: 76, or a fragment thereof.

[0246] In an aspect of a disclosed viral vector or disclosed recombinant AAV vector, the disclosed viral vector or disclosed recombinant AAV vector may further encode at least one guide RNA targeting a gene of interest. In an aspect, a disclosed gene of interest or portion thereof can demonstrate a reduced expression and/or activity level when compared to wild-type or control expression level. In an aspect, a disclosed gRNA can target a gene of interest or portion thereof that demonstrates a reduced expression and/or activity level when compared to wild-type or control expression level. In an aspect, a disclosed gene of interest or portion thereof can demonstrate an increased expression and/or activity level when compared to wild-type or control expression level. In an aspect, the gene of interest can be any gene which exhibits unusual expression in microglia in a diseased condition. In an aspect, the gene-of-interest is the *APOE* gene.

[0247] In an aspect, the at least one gRNA can target a gene of interest or portion thereof (*e.g.*, an *APOE* gene). In an aspect, the viral vector encoding gRNAs targeting a gene of interest or portion thereof can comprise at least two gRNAs. In an aspect, disclosed gRNAs can target the same gene of interest or portion thereof. In an aspect, disclosed gRNAs can target different genes of interest or portions thereof. In an aspect, a disclosed gRNA can target a gene or portion thereof that demonstrates a loss of function. In an aspect, a disclosed gRNA can target a gene or portion thereof that demonstrates a gain of function.

[0248] As is understood in the art, a suitable gRNA that "targets" a nucleic acid (*i.e.*, a "target nucleic acid") is designed to hybridize to a sequence that is complementary to the "target nucleic acid." This, in effect, results in a gRNA that comprises an RNA sequence having a certain percent identity to the target nucleic acid (DNA), with the understanding that uracil in the RNA sequence is equivalent to thymidine in the DNA target sequence. Accordingly, in an aspect, the gRNA can comprise an RNA sequence corresponding to the "target nucleic acid." In an aspect, a disclosed gRNA scaffold

[0249] In an aspect, a disclosed gRNA can target the *APOE* gene sequence or a regulatory element thereof. In an aspect, the at least one gRNA can target the *APOE* gene, or promoter region thereof, or an untranslated region (UTR) thereof, or any combination thereof. In an aspect, the at least one gRNA may target the promoter region, or intron 1, or a flanking sequence thereof, of *APOE* gene. In an aspect, a disclosed gRNA can target exon 4 of the *APOE* gene. In an aspect, a disclosed gRNA can target a protospacer-adjacent motif (PAM) created by a SNP rs429358 in exon 4 of the *APOE* gene.

[0250] In an aspect, the at least one gRNA may target a region in the *APOE* gene comprising a nucleic acid sequence as set forth in any one of SEQ ID NO: 11-14, or a complement thereof. In an aspect, the at least one gRNA targets a nucleic acid sequence comprising at least 10 consecutive nucleotides as set forth in SEQ ID NO: 11-14, or a complement thereof. . In an aspect, the disclosed gRNA may comprise a nucleic acid sequence as set forth in SEQ ID NO: 15-33, or a complement thereof. In an aspect, the disclosed gRNA may comprise a nucleic acid sequence as set forth in SEQ ID NO: 15-33, or a complement thereof. In an aspect, the disclosed gRNA may comprise a nucleic acid sequence with at least about 90%, or about 91%, or about 92%, or about 93%, or about 94%, or about 95%, or about 96%, or about 97%, or about 98%, or about 99%, or more sequence identity to any one of the sequences set forth in SEQ ID NO: 15-33, or a complement thereof, or a fragment thereof.

[0251] In an aspect of a disclosed viral vector or disclosed recombinant AAV vector, the disclosed viral vector or disclosed recombinant AAV vector can further comprise one or more regulatory elements (*i.e.*, promoters) in addition to the microglia specific regulatory element (*e.g.*, microglia specific promoter). In an aspect, a disclosed additional promoter can comprise a U6 promoter, a chicken β-actin promoter, an EF-1α, a CMV promoter, a CMV promoter/enhancer, a fragment thereof, a derivative thereof, or any combination thereof. In an aspect, a disclosed U6 promoter can comprise the sequence set forth in SEQ ID NO: 81, or a fragment thereof. In an aspect, a disclosed U6 promoter can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% identity to the sequence set forth in SEQ ID NO: 81 or a fragment thereof. In an aspect, a disclosed EF-1α promoter can comprise the sequence set forth in SEQ ID NO: 79, SEQ ID NO: 80, or a fragment thereof. In an aspect, a disclosed EF-1α promoter can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% identity to the sequence set forth in SEQ ID NO: 79, SEQ ID NO:80, or a fragment thereof. In an aspect, a promoter can be a short EF1alpha (EFS-NF) promoter.

[0252] In an aspect, a disclosed promoter operably can be linked to the at least one guide RNA targeting a gene of interest or portion thereof. In an aspect, a disclosed promoter operably linked to the at least one guide RNA can comprise a U6 promoter. In an aspect, a disclosed promoter operably linked to the at least one guide RNA targeting an *APOE* gene can comprise a U6 promoter.

[0253] In an aspect of a disclosed viral vector or disclosed recombinant AAV vector, the disclosed viral vector or disclosed recombinant AAV vector can further comprise two or more promoters, wherein a first promoter can be operably linked to the dCas endonuclease, a polypeptide having enzymatic activity, a fusion of two or more polypeptides having enzymatic activities or any combination thereof, and wherein a second promoter can be operably linked to the at least one guide RNA targeting a gene of interest or portion thereof. In an aspect, a disclosed isolated nucleic acid molecule can further comprise one or more promoters, wherein a first promoter comprises a microglia specific promoter that is operably linked to the dCas endonuclease, a polypeptide having enzymatic activity, a fusion of two or more polypeptides having enzymatic activities or any combination thereof, and wherein a second promoter can be operably linked to the at least one guide RNA targeting a gene of interest thereof. In an aspect, the second promoter operably linked to the gRNA may not be a microglia specific promoter. In an aspect, the isolated nucleic acid can comprise a first promoter selected from a F4/80 promoter, a CD68 promoter, a IBA1 promoter, or a FCGR2A promoter or derivatives thereof, and a second promoter comprising a U6 promoter, or a derivative thereof.

[0254] In an aspect of a disclosed viral vector or disclosed recombinant AAV vector, the disclosed viral vector or disclosed recombinant AAV vector can further comprise one or more regulatory elements. In an aspect, a disclosed regulatory element can comprise a promoter, an enhancer, a promoter/enhancer, an internal ribosomal entry site, a transcription terminal signal, a polyadenylation signal, a Sp1 and/or NF-kB transcriptional factor binding site, a p2A signal, a woodchuck hepatitis virus post-transcriptional regulatory element, a Phi signal-packaging signal, a rev responsive element, a 5'-ITR, a 3'-ITR, an inverted terminal repeat, a nuclear localization signal (NLS), or any combination thereof.

[0255] In an aspect, a disclosed 5'-ITR may comprise the sequence as set forth in any one of SEQ ID NO: 82 or SEQ ID NO: 83. In an aspect, a disclosed 3'-ITR may comprise the sequence as set forth in any one of SEQ ID NO: 84 or SEQ ID NO: 85.

[0256] In an aspect, a disclosed NLS can comprise the sequence set forth in any one of SEQ ID NO: 88 – SEQ ID NO: 94, or a fragment thereof. In an aspect, a disclosed PolyA sequence can comprise the sequence set forth in SEQ ID NO: 86, SEQ ID NO: 87, or a fragment thereof.

[0257] In an aspect of a disclosed viral vector or disclosed recombinant AAV vector, the disclosed viral vector or disclosed recombinant AAV vector sequence can be CpG depleted and codon-optimized for expression in a human cell. In an aspect, "CpG-free" can mean completely free of CpGs or partially free of CpGs. In an aspect, "CpG-free" can mean "CpG-depleted." In an aspect, "CpG-depleted" can mean "CpG-free." In an aspect, "CpG-depleted" can mean completely depleted of CpGs or partially depleted of CpGs. In an aspect, "CpG-free" can mean "CpG-optimized" for a desired and/or ideal expression level. CpG depletion and/or optimization is known to the skilled person in the art. In an aspect, any disclosed Cas9 endonuclease, a disclosed polypeptide having enzymatic activities, a disclosed fusion product or a disclosed fusion protein, or any combination thereof can be codon-optimized.

[0258] In an aspect of a disclosed viral vector or disclosed recombinant AAV vector, the disclosed viral vector or disclosed recombinant AAV vector can comprise a sequence that is about 4.5 kilobases or less than about 4.5 kilobases.

[0259] Disclosed herein is a viral vector or a recombinant AAV vector comprising a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, wherein the dCas is a dSpCas9, dSaCas9, or dCjCas9, (ii) a fusion protein having one or more enzymatic activities, wherein the fusion product comprises any combination of HP1a, HP1b, MBD1, MBD2, KRAB, NIPP1, MeCP2, KRAB-MeCP2 or DNMT3A, and (iii) at least one guide RNA targeting *APOE* (*e.g.* *APOE* e4 allele) or portion thereof.

[0260] Disclosed herein is a viral vector or a recombinant AAV vector comprising a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, wherein the dCas is a dSpCas9, dSaCas9, or dCjCas9, (ii) a fusion protein having one or more enzymatic activities, wherein the fusion product comprises any combination of HP1a, HP1b, MBD1, MBD2, KRAB, NIPP1, MeCP2, KRAB-MeCP2 or DNMT3A, (iii) at least one guide RNA targeting *APOE* (*e.g.* *APOE* e4 allele) or portion thereof, (iv) a microglia specific regulatory sequence (e. g. promoter) operably linked to the dCas endonuclease and the fusion protein, and (v) a promoter operably linked to the at least one guide RNA.

[0261] In an aspect, a disclosed recombinant AAV vector can include naturally isolated serotypes including, but not limited to, AAV1, AAV2, AAV3 (including 3a and 3b), AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAV9, AAV10, AAVrh10, AAV11, AAV12, AAV13, AAVrh39, AAVrh43, AAVcy.7 as well as bovine AAV, caprine AAV, canine AAV, equine AAV, ovine AAV, avian AAV, primate AAV, non-primate AAV, and any other virus classified by the International Committee on Taxonomy of Viruses (ICTV) as an AAV. In an aspect, an AAV capsid can be a chimera either created by capsid evolution or by rational capsid engineering from

a naturally isolated AAV variants to capture desirable serotype features such as enhanced or specific tissue tropism and/or a host immune response escape. Naturally isolated AAV variants include, but not limited to, AAV-DJ, AAV-HAE1, AAV-HAE2, AAVM41, AAV-1829, AAV2 Y/F, AAV2 T/V, AAV2i8, AAV2.5, AAV9.45, AAV9.61, AAV-B1, AAV-AS, AAV9.45A-String (*e.g.*, AAV9.45-AS), AAV9.45Angiopep, AAV9.47-Angiopep, and AAV9.47-AS, AAV-PHP.B, AAV-PHP.eB, AAV-PHP.S, AAV-F, AAVcc.47, and AAVcc.81. In an aspect, a disclosed AAV vector can be AAV-Rh74 or a related variant (*e.g.*, capsid variants like RHM4-1). In an aspect, a disclosed AAV vector can be AAV8. In an aspect, a disclosed AAV vector can be AAVhum.8. In an aspect, a disclosed AAV vector can be AAV9.

[0262] In an aspect, a disclosed viral vector or disclosed recombinant AAV vector can comprise the sequence set forth in any of SEQ ID NO: 112 – SEQ ID NO: 128, or a variant thereof, or a fragment thereof, or a portion thereof. In an aspect, a disclosed viral vector or disclosed recombinant AAV vector can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 112 – SEQ ID NO: 128, or a variant thereof, or a fragment thereof, or a portion thereof.

[0263] Disclosed herein is a lentiviral vector comprising the disclosed isolated nucleic acid molecule. Disclosed herein is a lentiviral vector comprising the disclosed isolated nucleic acid molecule comprising (a) a nucleic acid sequence encoding (i) a deactivated Clustered Regularly Interspaced Short Palindromic Repeats associated (dCas) endonuclease, (ii) at least one polypeptide having an enzymatic activity, and (iii) at least one guide RNA targeting a gene of interest sequence or a regulatory element thereof; and (b) one or more microglia specific regulatory elements operably linked to (a)(i), (a)(ii), (a)(iii), or any combination thereof. In an aspect, the gene of interest is *APOE,* or an allele thereof.

[0264] Disclosed herein is a lentiviral vector comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, (ii) at least one polypeptide having an enzymatic activity, and (iii) at least one guide RNA targeting a gene of interest (*e.g. APOE*) or portion thereof. Disclosed herein is a lentiviral vector comprising the nucleic acid sequence encoding (i) a deactivated Clustered Regularly Interspaced Short Palindromic Repeats associated (dCas) endonuclease, (ii) at least one polypeptide having an enzymatic activity, and (iii) at least one guide RNA targeting a gene-of-interest or a regulatory element thereof; and (b) one or more promoters elements operably linked to (a)(i), (a)(ii), (a)(iii), or any combination thereof, wherein at least one of the promoter is a microglia specific promoter. In an aspect, the gene of interest is *APOE,* or an allele thereof.

[0265] Disclosed herein is a lentiviral vector comprising (a) a nucleic acid sequence encoding (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, (ii) a fusion product having one or more enzymatic activities, and (iii) at least one guide RNA targeting a gene of interest or portion thereof, and (b) one or more microglia specific regulatory element operably linked to (a)(i), (a)(ii), (a)(iii) or any combination thereof. In an aspect, the gene of interest is *APOE,* or an allele thereof.

[0266] Disclosed herein is a lentiviral vector comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, (ii) a fusion product having one or more enzymatic activities, and (iii) at least one guide RNA targeting a gene of interest or portion thereof, and (b) one or more microglia specific promoter operably linked to (a)(i), (a)(ii), (a)(iii) or any combination thereof. In an aspect, the gene of interest is *APOE,* or an allele thereof.

[0267] In an aspect of the disclosed lentiviral vector, the lentiviral vector may comprise a sequence encoding any one of the disclosed dCas9, any one or more of the disclosed polypeptides having an enzymatic activity, any one or more of the disclosed gRNA. In an aspect of the disclosed lentiviral vector, the lentiviral vector may further comprise one or more of the disclosed microglia specific regulatory elements (e. g. microglia specific promoter) and optionally additional disclosed promoters. Disclosed herein is a lentiviral vector comprising the disclosed isolated nucleic acid molecule comprising (a) a nucleic acid sequence encoding (i) a deactivated Clustered Regularly Interspaced Short Palindromic Repeats associated (dCas) endonuclease comprising an amino acid sequence as set forth in any one of SEQ ID NO: 36- SEQ ID NO: 38, or a fragment, or derivative thereof, or a sequence at least about 60% identical thereto (ii) at least one polypeptide having an enzymatic activity comprising an amino acid sequence as set forth in any one or more of SEQ ID NO: 44- SEQ ID NO: 58, or a sequence at least about 60% identical thereto and (iii) at least one guide RNA targeting an *APOE* gene sequence or a regulatory element thereof comprising, wherein the targeted sequence comprises a nucleic acid sequence as set forth in SEQ ID NO: 15 – SEQ ID NO: 33, or a complement thereof, or a sequence at least about 60% identical thereto; and (b) one or more microglia specific regulatory elements operably linked to (a)(i), (a)(ii), (a)(iii), or any combination thereof, wherein the microglia specific regulatory element comprises a nucleic acid sequence as set forth in any one of SEQ ID NO: 1- SEQ ID NO: 8, or a fragment, or a derivative thereof, or a sequence at least about 60% identical thereto.

[0268] In an aspect, a disclosed lentiviral vector can comprise the sequence set forth in any of SEQ ID NO: 105 – SEQ ID NO: 111, or a variant thereof, or a fragment thereof, or a portion thereof. In an aspect, a disclosed viral vector or disclosed recombinant AAV vector can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least

85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 105 – SEQ ID NO: 111, or a variant thereof, or a fragment thereof, or a portion thereof.

[0269] In an aspect, a disclosed viral vector or a disclosed recombinant AAV vector of lentiviral vector can treat and/or prevent Alzheimer's disease progression. In an aspect, a disclosed viral vector or a disclosed recombinant AAV vector can treat and/or prevent Parkinson's disease progression.

Plasmid vectors

[0270] Disclosed herein is a plasmid vector comprising a disclosed isolated nucleic acid molecule. Disclosed herein is a plasmid vector comprising a disclosed nucleic acid molecule. Disclosed herein is a plasmid vector comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, (ii) at least one polypeptide having an enzymatic activity, and (iii) at least one guide RNA targeting a gene of interest or portion thereof; and (b) one or more microglia specific regulatory element operably linked to (a)(i), (a)(ii), (a)(iii) or any combination thereof. In an aspect, the gene of interest is *APOE,* or an allele thereof.

[0271] Disclosed herein is a plasmid vector comprising a disclosed isolated nucleic acid molecule. Disclosed herein is a plasmid vector comprising a disclosed isolated nucleic acid molecule comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, (ii) at least one polypeptide having an enzymatic activity, and (iii) at least one guide RNA targeting a gene of interest or portion thereof; and (b) one or more microglia specific promoter operably linked to (a)(i), (a)(ii), (a)(iii) or any combination thereof. In an aspect, the gene of interest is *APOE,* or an allele thereof.

[0272] Disclosed herein is a plasmid vector comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, (ii) a fusion product having one or more enzymatic activities, and (iii) at least one guide RNA targeting a gene of interest or portion thereof, and (b) one or more microglia specific regulatory element operably linked to (a)(i), (a)(ii), (a)(iii) or any combination thereof. In an aspect, the gene of interest is *APOE,* or an allele thereof.

[0273] Disclosed herein is a plasmid vector comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, (ii) a fusion product having one or more enzymatic activities, and (iii) at least one guide RNA targeting a gene of interest or portion thereof, and (b) one or more microglia specific promoter operably linked to (a)(i), (a)(ii), (a)(iii) or any combination thereof. In an aspect, the gene of interest is *APOE,* or an allele thereof.

[0274] In an aspect of a disclosed plasmid vector, the microglia specific regulatory sequence may comprise a wild-type promoter, a microglia specific enhancer, a synthetic promoter, a synthetic enhancer, a transcriptional or translational regulatory sequence or any combination thereof. In an

aspect, the microglia specific regulatory sequence is a microglia specific promoter. In an aspect, the microglia specific promoter can be any promoter known in the art, that exhibits enhanced activity in the microglia. Non-limiting examples of microglia specific promoters include F4/80, CD68, FCGR2A, HexB or IBA1 promoter, or a derivative thereof.

[0275] In an aspect, the promoter is a CD68 promoter, or a derivative thereof. In an aspect, the promoter may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one or more of the sequences set forth in SEQ ID NO: 1-3, or a fragment, or a derivative thereof. In an aspect, the promoter comprises a sequence as set forth in any one of SEQ ID NO: 1-3.

[0276] In an aspect, the promoter is a F4/80 promoter, or a derivative thereof. In an aspect, the promoter may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequence set forth in SEQ ID NO: 4, or a fragment, or a derivative thereof. In an aspect, the promoter comprises a sequence as set forth in SEQ ID NO: 4.

[0277] In an aspect, the promoter is a IBA1 promoter, or a derivative thereof. In an aspect, the promoter may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequence set forth in SEQ ID NO: 5-7, or a fragment, or a derivative thereof. In an aspect, the promoter comprises a sequence as set forth in any one of SEQ ID NO: 5-7.

[0278] In an aspect, the promoter is a FCGR2A promoter, or a derivative thereof. In an aspect, the promoter may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequence set forth in SEQ ID NO: 8, or a fragment, or a derivative thereof. In an aspect, the promoter comprises a sequence as set forth in SEQ ID NO: 8.

[0279] In an aspect of a disclosed plasmid vector, the disclosed plasmid vector may comprise at least a microglia specific regulatory element (*i.e.*, a microglia specific promoter) which may be operably linked to the dCas endonuclease and the fusion protein having one or more enzymatic activities. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the dCas endonuclease and the fusion protein having one or more enzymatic activities can comprise a F4/80 promoter, or a derivative thereof. In an aspect, a

disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the dCas endonuclease and the fusion protein having one or more enzymatic activities can comprise a CD68 promoter, or a derivative thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the dCas endonuclease and the fusion protein having one or more enzymatic activities can comprise a IBA1 promoter, or a derivative thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the dCas endonuclease and the fusion protein having one or more enzymatic activities can comprise a FCGR2A promoter, or a derivative thereof.

[0280] In an aspect of a disclosed plasmid vector, the disclosed dCas endonuclease can comprise a dCas9 endonuclease. In an aspect, a disclosed dCas9 endonuclease can comprise a deactivated Staphylococcus aureus Cas9 (dSaCas9), a deactivated Streptococcus pyogenes Cas9 (dSpCas9), a deactivated Campylobacter jejuni Cas9 (dCjCas9), or a variant dCas9 endonuclease. In an aspect, a disclosed variant dCas9 can comprise a variant dSaCas9, a variant dSpCas9, or a variant dCjCa9. In an aspect, a disclosed variant dSpCas9 can comprise dVQR, dEQR, or dVRER.

[0281] In an aspect of a disclosed plasmid vector, the disclosed dSpCas9 may comprise a sequence as set forth in any one of SEQ ID NO: 40 or SEQ ID NO: 41, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed dSpCas9 endonuclease may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 40 or SEQ ID NO: 41, or a fragment thereof. In an aspect, a disclosed dSpCas9 endonuclease can comprise the amino acid sequence as set forth in SEQ ID NO: 36 or SEQ ID NO: 37, or a fragment, or a derivative thereof. In an aspect, a disclosed dSpCas9 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 36 or SEQ ID NO: 37, or a fragment thereof.

[0282] In an aspect of a disclosed plasmid vector, the disclosed dSaCas9 may comprise a sequence as set forth in SEQ ID NO: 42, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed dSaCas9 endonuclease may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequences set forth in SEQ ID NO: 42, or a fragment thereof. In an aspect, a disclosed dSaCas9 endonuclease can comprise the amino acid sequence as set forth in SEQ ID NO: 38 or a fragment, or a derivative thereof. In an aspect, a disclosed dSaCas9 can comprise a sequence having at least

60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 38, or a fragment thereof.

[0283] In an aspect of a disclosed plasmid vector, the disclosed dCjCas9 may comprise a sequence as set forth in SEQ ID NO: 43, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed dCjCas9 endonuclease may comprise a nucleic acid sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 43, or a fragment thereof. In an aspect, a disclosed dCjCas9 endonuclease can comprise the amino acid sequence as set forth in SEQ ID NO: 39, or a fragment, or a derivative thereof. In an aspect, a disclosed dCjCas9 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 39 or a fragment thereof.

[0284] In an aspect, a disclosed variant of dCas9 dVQR can comprise D1135V, R1335Q, and T1337R with reference to SEQ ID NO: 36. In an aspect, a disclosed variant of dCas9 dEQR can comprise D1135E, R1335Q, and T1337R with reference to SEQ ID NO: 36. In an aspect, a disclosed variant of dCas9 dVRER can comprise D1135V, G1218R, R1335E, and T1337R with reference to SEQ ID NO: 36. In an aspect, a disclosed dVRER can comprise the sequence set forth in SEQ ID NO: 37 or a fragment thereof.

[0285] In an aspect of a disclosed plasmid vector, the disclosed dCas9 endonuclease may comprise a sequence as set forth in any one of SEQ ID NO: 40-43. In an aspect, the sequence encoding the disclosed dCas9 endonuclease may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 40-43, or a fragment, or a derivative thereof.

[0286] In an aspect of a disclosed plasmid vector, the disclosed plasmid vector may comprise a nucleic acid sequence encoding at least one polypeptide having an enzymatic activity. In an aspect, a disclosed encoded polypeptide can comprise transcription activation activity, transcription repression activity, transcription release factor activity, histone modification activity, nucleic acid association activity, methyltransferase activity, demethylase activity, acetyltransferase activity, deacetylase activity, or any combination thereof.

[0287] In an aspect, the disclosed encoded polypeptide can comprise HP1a, HP1b, MBD1, MBD2, Krüppel-Associated Box (KRAB), NIPP1, the Transcription Repression Domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof. In an aspect, a disclosed fusion can comprise HP1a-HP1a, HP1a-HP1b, HP1a-MBD1,

HP1a-MBD2, HP1a-KRAB, HP1a-NIPP1, HP1a-MeCP2, HP1a-DNMT3A, HP1b-HP1b, HP1b-HP1a, HP1b-MBD1, HP1b-MBD2, HP1b-KRAB, HP1b-NIPP1, HP1b-MeCP2, HP1b-DNMT3A, MBD1-MBD1, MBD1-HP1a, MBD1-HP1b, MBD1-MBD2, MBD1-KRAB, MBD1-NIPP1, MBD1-MeCP2, MBD1-DNMT3A, MBD2-MBD2, MBD2-HP1a, MBD2-HP1b, MBD2-MBD1, MBD2-KRAB, MBD2-NIPP1, MBD2-MeCP2, MBD2-DNMT3A, KRAB-KRAB, KRAB-HP1a, KRAB-HP1b, KRAB-MBD1, KRAB-MBD2, KRAB-NIPP1, KRAB-MeCP2, KRAB-DNMT3A, NIPP1-NIPP1, NIPP1-HP1a, NIPP1-HP1b, NIPP1-MBD1, NIPP1-MBD2, NIPP1-KRAB, NIPP1-MeCP2, NIPP1-DNMT3A, MeCP2-MeCP2, MeCP2-HP1a, MeCP2-HP1b, MeCP2-MBD1, MeCP2-MBD2, MeCP2-KRAB, MeCP2-NIPP1, MeCP2-DNMT3A, DNMT3A-DNMT3A, DNMT3A-HP1a, DNMT3A-HP1b, DNMT3A-MBD1, DNMT3A-MBD2, DNMT3A-KRAB, DNMT3A-NIPP1, or DNMT3A-MeCP2. Examples of suitable polypeptides sequences for use with the current disclosure are provided in PCT/US2022/078260, the contents of which are incorporated herein by reference, in their entirety.

[0288] In an aspect of a disclosed plasmid vector, the polypeptide HP1a may comprise a sequence as set forth in any one of SEQ ID NO: 59 or SEQ ID NO: 60, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide HP1a may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 59 or SEQ ID NO: 60, or a fragment thereof. In an aspect, a disclosed polypeptide HP1a can comprise the amino acid sequence as set forth in SEQ ID NO: 44 or SEQ ID NO: 45, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide HP1a can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 44 or SEQ ID NO: 45, or a fragment thereof.

[0289] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide HP1b may comprise a sequence as set forth in any one of SEQ ID NO: 61 or SEQ ID NO: 62, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide HP1b may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 61 or SEQ ID NO: 62, or a fragment thereof. In an aspect, a disclosed polypeptide HP1b can comprise the amino acid sequence as set forth in SEQ ID NO: 46 or SEQ ID NO: 47, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide HP1b can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at

least 95% identity to the sequence set forth in SEQ ID NO: 46 or SEQ ID NO: 47, or a fragment thereof.

[0290] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide MBD1 may comprise a sequence as set forth in any one of SEQ ID NO: 63 or SEQ ID NO: 64, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide MBD1 may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 63 or SEQ ID NO: 64, or a fragment thereof. In an aspect, a disclosed polypeptide MBD1 can comprise the amino acid sequence as set forth in any one of SEQ ID NO: 48-50, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide MDB1 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in any one of SEQ ID NO: 48-50, or a fragment thereof.

[0291] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide MBD2 may comprise a sequence as set forth in any one of SEQ ID NO: 65 or SEQ ID NO: 66, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide MBD2 may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 65 or SEQ ID NO: 66, or a fragment thereof. In an aspect, a disclosed polypeptide MBD2 can comprise the amino acid sequence as set forth in SEQ ID NO: 51 or SEQ ID NO: 52, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide MBD2 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 51 or SEQ ID NO: 52, or a fragment thereof.

[0292] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide NIPP1 may comprise a sequence as set forth in any one of SEQ ID NO: 67 or SEQ ID NO: 68, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide NIPP1 may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 67 or SEQ ID NO: 68, or a fragment thereof. In an aspect, a disclosed polypeptide NIPP1 can comprise the amino acid sequence as set forth in SEQ ID NO: 53 or SEQ ID NO: 54, or a fragment, or a

derivative thereof. In an aspect, a disclosed polypeptide NIPP1 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 53 or SEQ ID NO: 54, or a fragment thereof.

[0293] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide DNMT3A may comprise a sequence as set forth in SEQ ID NO: 69, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide DNMT3A may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 69, or a fragment thereof. In an aspect, a disclosed polypeptide DNMT3A can comprise the amino acid sequence as set forth in SEQ ID NO: 55, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide DNMT3A can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 55, or a fragment thereof.

[0294] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide KRAB may comprise a sequence as set forth in any one of SEQ ID NO: 70 or SEQ ID NO: 71, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide KRAB may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 70 or SEQ ID NO: 71, or a fragment thereof. In an aspect, a disclosed polypeptide KRAB can comprise the amino acid sequence as set forth in SEQ ID NO: 56, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide KRAB can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 56, or a fragment thereof.

[0295] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide KRAB may comprise a sequence as set forth in any one of SEQ ID NO: 70 or SEQ ID NO: 71, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide KRAB may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 70 or SEQ ID NO: 71, or a fragment thereof. In an aspect, a disclosed polypeptide KRAB can comprise the amino acid sequence as set forth in SEQ ID NO: 56, or a fragment, or a derivative thereof. In

an aspect, a disclosed polypeptide KRAB can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 56, or a fragment thereof.

[0296] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide MeCP2 may comprise a sequence as set forth in any one of SEQ ID NO: 72, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide MeCP2 may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequences set forth in SEQ ID NO: 72, or a fragment thereof. In an aspect, a disclosed polypeptide MeCP2 can comprise the amino acid sequence as set forth in SEQ ID NO: 57, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide MeCP2 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 57, or a fragment thereof.

[0297] In an aspect, the nucleic acid sequence encoding the disclosed fusion polypeptide KRAB-MeCP2 may comprise a sequence as set forth in any one of SEQ ID NO: 73 or SEQ ID NO: 74, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide KRAB-MeCP2 may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequences set forth in SEQ ID NO: 73 or SEQ ID NO: 74, or a fragment thereof. In an aspect, a disclosed polypeptide KRAB-MeCP2 can comprise the amino acid sequence as set forth in SEQ ID NO: 58, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide KRAB-MeCP2 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 58, or a fragment thereof.

[0298] In an aspect, a disclosed encoded polypeptide can comprise transcription activation activity. In an aspect, a disclosed encoded polypeptide comprising transcription activation activity can comprise a transactivation domain. In an aspect, a disclosed transactivation domain can comprise a VP16 protein, a series of linked VP16 proteins, a p65 domain of NFκB, or any combination thereof.

[0299] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide VP16 may comprise a sequence as set forth in any one of SEQ ID NO: 132 or SEQ ID NO: 133, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide VP16 may

comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequences set forth in SEQ ID NO: 132 or SEQ ID NO: 133, or a fragment thereof. In an aspect, a disclosed polypeptide VP16 can comprise the amino acid sequence as set forth in SEQ ID NO: 131, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide VP16 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 131, or a fragment thereof.

[0300] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide p65 domain of NFκB or RELA may comprise a sequence as set forth in any one of SEQ ID NO: 135 or SEQ ID NO: 136, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide p65 domain of NFκB or RELA may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequences set forth in SEQ ID NO: 135 or SEQ ID NO: 136, or a fragment thereof. In an aspect, a disclosed polypeptide p65 domain of NFκB or RELA can comprise the amino acid sequence as set forth in SEQ ID NO: 134, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide p65 domain of NFκB or RELA can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 134, or a fragment thereof.

[0301] In an aspect, a disclosed encoded polypeptide can comprise transcription repression activity. In an aspect, a disclosed encoded polypeptide comprising transcription repression activity can comprise a Krüppel associated box domain, an ERF repressor domain, a MXI1 repressor domain, a SID4x repressor domain, a fused KRAB-MeCP2 domain, a MeCP2 TRD domain, a MAS-SID repressor domain, a TATA box binding protein activity, or any combination thereof. In an aspect, a disclosed encoded polypeptide comprising transcription repression activity can comprise HP1 repressor activity, MeCP2 repressor activity, MBD1 repressor activity, MBD2 repressor activity, MBD3 repressor activity, MBD4 repressor activity, KRAB repressor activity, SUV39H1 repressor activity, SUV39H2 repressor activity, CTCF insulator-repressor activity, LSD-1 histone-demethylase repressor activity, or any combination thereof.

[0302] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide comprising Eukaryotic Release Factor 1 (ERF1) activity or Eukaryotic Release Factor 3 (ERF3) activity may comprise a sequence as set forth in any one of SEQ ID NO: 138, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide comprising ERF1 activity

or ERF3 activity may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequences set forth in SEQ ID NO: 138, or a fragment thereof. In an aspect, a disclosed polypeptide comprising ERF1 activity or ERF3 activity can comprise the amino acid sequence as set forth in SEQ ID NO: 137, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide comprising ERF1 activity or ERF3 activity can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 137, or a fragment thereof.

[0303] In an aspect, a disclosed encoded polypeptide can comprise histone modification activity. In an aspect, a disclosed encoded polypeptide comprising histone modification activity can comprise histone acetyltransferase, histone deacetylase, histone demethylase, histone methyltransferase activity, or any combination thereof.

[0304] In an aspect, a disclosed encoded polypeptide can comprise nucleic acid association activity. In an aspect, a disclosed encoded polypeptide comprising nucleic acid association activity can comprise a helix-turn-helix region, a leucine zipper region, a winged helix region, a winged helix-turn-helix region, a helix-loop-helix region, an immunoglobulin fold, a B3 domain, a zinc finger, a HMG-box, a Wor3 domain, a TAL effector DNA-binding domain, or any combination thereof.

[0305] In an aspect, a disclosed encoded polypeptide can comprise methyltransferase activity. In an aspect, a disclosed encoded polypeptide comprising methyltransferase activity can comprise DNA (cytosine-5)-methyltransferase 3a (DNMT3A). In an aspect, a disclosed encoded polypeptide can comprise demethylase activity. In an aspect, a disclosed encoded polypeptide comprising demethylase activity can comprise ten-eleven translocation methylcytosine dioxygenase 1 (TET1) or lysine-specific histone demethylase 1 (LSD1). In an aspect, a disclosed encoded polypeptide can comprise acetyltransferase activity. In an aspect, a disclosed encoded polypeptide comprising acetyltransferase activity can comprise histone acetyltransferase. In an aspect, a disclosed encoded polypeptide can comprise deacetylase activity. In an aspect, a disclosed encoded polypeptide comprising deacetylase activity can comprise histone deacetylase.

[0306] In an aspect of a disclosed plasmid vector may comprise a nucleic acid sequence encoding a disclosed dCas endonuclease, comprising dSpCas9 and a disclosed encoded polypeptide comprising any one or more of HP1a, HP1b, MBD1, MBD2, Krüppel-associated box (KRAB), NIPP1, the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof. In an aspect, a disclosed dCas

endonuclease can comprise dSaCas9 and a disclosed encoded polypeptide can comprise HP1a, HP1b, MBD1, MBD2, Krüppel-associated box (KRAB), NIPP1, the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof. In an aspect, a disclosed dCas endonuclease can comprise dCjCas9 and a disclosed polypeptide can comprise HP1a, HP1b, MBD1, MBD2, Krüppel-associated box (KRAB), NIPP1, the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof. In an aspect, a disclosed dCas endonuclease can comprise dVQR, dEQR, or dVRER and a disclosed encoded polypeptide can comprise HP1a, HP1b, MBD1, MBD2, Krüppel-associated box (KRAB), NIPP1, the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof.

[0307] In an aspect, a disclosed dCas endonuclease can comprise dSpCas9, dSaCas9, dCjCas9, or a variant dCas9 (*e.g.*, dVQR, dEQR or dVRER and a disclosed fusion can comprise HP1a-HP1a, HP1a-HP1b, HP1a-MBD1, HP1a-MBD2, HP1a-KRAB, HP1a-NIPP1, HP1a-MeCP2, HP1a-DNMT3A, HP1b-HP1b, HP1b-HP1a, HP1b-MBD1, HP1b-MBD2, HP1b-KRAB, HP1b-NIPP1, HP1b-MeCP2, HP1b-DNMT3A, MBD1-MBD1, MBD1-HP1a, MBD1-HP1b, MBD1-MBD2, MBD1-KRAB, MBD1-NIPP1, MBD1-MeCP2, MBD1-DNMT3A, MBD2-MBD2, MBD2-HP1a, MBD2-HP1b, MBD2-MBD1, MBD2-KRAB, MBD2-NIPP1, MBD2-MeCP2, MBD2-DNMT3A, KRAB-KRAB, KRAB-HP1a, KRAB-HP1b, KRAB-MBD1, KRAB-MBD2, KRAB-NIPP1, KRAB-MeCP2, KRAB-DNMT3A, NIPP1-NIPP1, NIPP1-HP1a, NIPP1-HP1b, NIPP1-MBD1, NIPP1-MBD2, NIPP1-KRAB, NIPP1-MeCP2, NIPP1-DNMT3A, MeCP2-MeCP2, MeCP2-HP1a, MeCP2-HP1b, MeCP2-MBD1, MeCP2-MBD2, MeCP2-KRAB, MeCP2-NIPP1, MeCP2-DNMT3A, DNMT3A-DNMT3A, DNMT3A-HP1a, DNMT3A-HP1b, DNMT3A-MBD1, DNMT3A-MBD2, DNMT3A-KRAB, DNMT3A-NIPP1, or DNMT3A-MeCP2. In an aspect, a disclosed dCas endonuclease can comprise dSpCas9, dSaCas9, or dCjCas9 and a disclosed encoded polypeptide can comprise KRAB-MeCP2. In an aspect, a disclosed dCas endonuclease can comprise dSaCas9, and a disclosed encoded polypeptide (*i.e.*, a disclosed fusion) can comprise KRAB-MeCP2.

[0308] In an aspect of a disclosed plasmid vector may comprise a nucleic acid sequence encoding a dCas endonuclease fused to the at least one encoded polypeptide having an enzymatic activity. In these or related aspects, the isolated nucleic acid can encode for a "fusion protein" wherein the fusion protein comprises a first component comprising a dCas endonuclease and a second component comprising at least one polypeptide having an enzymatic activity. In an aspect, the encoded fusion protein can comprise a dSpCas9 and a polypeptide having enzymatic activity

comprising HP1a, HP1b, MBD1, MBD2, Krüppel-associated box (KRAB), NIPP1, the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof. In an aspect, the encoded fusion protein can comprise a dSaCas9 and a polypeptide having enzymatic activity comprising HP1a, HP1b, MBD1, MBD2, Krüppel-associated box (KRAB), NIPP1, the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof. In an aspect, the encoded fusion protein can comprise a dCjCas9 and a polypeptide having enzymatic activity comprising HP1a, HP1b, MBD1, MBD2, Krüppel-associated box (KRAB), NIPP1, the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof. In an aspect, the encoded fusion protein can comprise dVQR, dEQR, or dVRER and a polypeptide having enzymatic activity comprising HP1a, HP1b, MBD1, MBD2, Krüppel-associated box (KRAB), NIPP1, the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof.

[0309] In an aspect, the encoded fusion protein can comprise dSpCas9, dSaCas9, dCjCas9, or a variant dCas9 (*e.g.*, dVQR, dEQR or dVRER) and one or more of HP1a-HP1a, HP1a-HP1b, HP1a-MBD1, HP1a-MBD2, HP1a-KRAB, HP1a-NIPP1, HP1a-MeCP2, HP1a-DNMT3A, HP1b-HP1b, HP1b-HP1a, HP1b-MBD1, HP1b-MBD2, HP1b-KRAB, HP1b-NIPP1, HP1b-MeCP2, HP1b-DNMT3A, MBD1-MBD1, MBD1-HP1a, MBD1-HP1b, MBD1-MBD2, MBD1-KRAB, MBD1-NIPP1, MBD1-MeCP2, MBD1-DNMT3A, MBD2-MBD2, MBD2-HP1a, MBD2-HP1b, MBD2-MBD1, MBD2-KRAB, MBD2-NIPP1, MBD2-MeCP2, MBD2-DNMT3A, KRAB-KRAB, KRAB-HP1a, KRAB-HP1b, KRAB-MBD1, KRAB-MBD2, KRAB-NIPP1, KRAB-MeCP2, KRAB-DNMT3A, NIPP1-NIPP1, NIPP1-HP1a, NIPP1-HP1b, NIPP1-MBD1, NIPP1-MBD2, NIPP1-KRAB, NIPP1-MeCP2, NIPP1-DNMT3A, MeCP2-MeCP2, MeCP2-HP1a, MeCP2-HP1b, MeCP2-MBD1, MeCP2-MBD2, MeCP2-KRAB, MeCP2-NIPP1, MeCP2-DNMT3A, DNMT3A-DNMT3A, DNMT3A-HP1a, DNMT3A-HP1b, DNMT3A-MBD1, DNMT3A-MBD2, DNMT3A-KRAB, DNMT3A-NIPP1, or DNMT3A-MeCP2. In an aspect, the encoded fusion protein can comprise a dCas9 selected from dSpCas9, dSaCas9, or dCjCas9 and a fusion comprising KRAB-MeCP2. In an aspect, the encoded fusion protein can comprise a dSaCas9 and KRAB-MeCP2.

[0310] In an aspect, the nucleic acid sequence encoding the fusion protein comprising dSpCas9 and DNMT3A or dVRER and DNMT3A may comprise a sequence as set forth in SEQ ID NO: 77 or SEQ ID NO: 78 respectively, or a derivative thereof. In an aspect, the nucleic acid sequence encoding the fusion protein comprising dSpCas9 and DNMT3A may comprise a sequence having

at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any of the sequences set forth in SEQ ID NO: 77 or SEQ ID NO: 78, or a fragment thereof. In an aspect, a disclosed polypeptide dSpCas9 can comprise the amino acid sequence as set forth in SEQ ID NO: 75 or SEQ ID NO: 76, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide KRAB-MeCP2 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 75 or SEQ ID NO: 76, or a fragment thereof.

[0311] In an aspect of a disclosed plasmid vector may further encode at least one guide RNA targeting a gene of interest. In an aspect, a disclosed gene of interest or portion thereof can demonstrate a reduced expression and/or activity level when compared to wild-type or control expression level. In an aspect, a disclosed gRNA can target a gene of interest or portion thereof that demonstrates a reduced expression and/or activity level when compared to wild-type or control expression level. In an aspect, a disclosed gene of interest or portion thereof can demonstrate an increased expression and/or activity level when compared to wild-type or control expression level. In an aspect, the gene of interest can be any gene which exhibits unusual expression in microglia in a diseased condition. In an aspect, the gene-of-interest is the *APOE* gene.

[0312] In an aspect, the at least one gRNA can target a gene of interest or portion thereof (*e.g.*, an *APOE* gene). In an aspect, the plasmid vector encoding gRNAs targeting a gene of interest or portion thereof can comprise at least two gRNAs. In an aspect, disclosed gRNAs can target the same gene of interest or portion thereof. In an aspect, disclosed gRNAs can target different genes of interest or portions thereof. In an aspect, a disclosed gRNA can target a gene or portion thereof that demonstrates a loss of function. In an aspect, a disclosed gRNA can target a gene or portion thereof that demonstrates a gain of function.

[0313] As is understood in the art, a suitable gRNA that "targets" a nucleic acid (*i.e.*, a "target nucleic acid") is designed to hybridize to a sequence that is complementary to the "target nucleic acid." This, in effect, results in a gRNA that comprises an RNA sequence having a certain percent identity to the target nucleic acid (DNA), with the understanding that uracil in the RNA sequence are equivalent to thymidine in the DNA target sequence. Accordingly, in an aspect, the gRNA can comprise an RNA sequence corresponding to the "target nucleic acid." In an aspect, a disclosed gRNA scaffold

[0314] In an aspect, a disclosed gRNA can target the *APOE* gene sequence or a regulatory element thereof. In an aspect, the at least one gRNA can target the *APOE* gene, or promoter region thereof,

or an untranslated region (UTR) thereof, or any combination thereof. In an aspect, the at least one gRNA may target the promoter region, or intron 1, or a flanking sequence thereof, of *APOE* gene. In an aspect, a disclosed gRNA can target exon 4 of the *APOE* gene. In an aspect, a disclosed gRNA can target a protospacer-adjacent motif (PAM) created by a SNP rs429358 in exon 4 of the *APOE* gene.

[0315] In an aspect, the at least one gRNA may target a region in the *APOE* gene comprising a nucleic acid sequence as set forth in any one of SEQ ID NO: 11-14, or a complement thereof. In an aspect, the at least one gRNA targets a nucleic acid sequence comprising at least 10 consecutive nucleotides as set forth in SEQ ID NO: 11-14, or a complement thereof. . In an aspect, the disclosed gRNA may comprise a nucleic acid sequence as set forth in SEQ ID NO: 15-33, or a complement thereof. In an aspect, the disclosed gRNA may comprise a nucleic acid sequence as set forth in SEQ ID NO: 15-33, or a complement thereof. In an aspect, the disclosed gRNA may comprise a nucleic acid sequence with at least about 90%, or about 91%, or about 92%, or about 93%, or about 94%, or about 95%, or about 96%, or about 97%, or about 98%, or about 99%, or more sequence identity to any one of the sequences set forth in SEQ ID NO: 15-33, or a complement thereof, or a fragment thereof.

[0316] In an aspect of a disclosed plasmid vector can further comprise one or more regulatory elements (*i.e.*, promoters) in addition to the microglia specific regulatory element (*e.g.*, microglia specific promoter). In an aspect, a disclosed additional promoter can comprise a U6 promoter, a chicken β-actin promoter, an EF-1α, a CMV promoter, a CMV promoter/enhancer, a fragment thereof, a derivative thereof, or any combination thereof. In an aspect, a disclosed U6 promoter can comprise the sequence set forth in SEQ ID NO: 81, or a fragment thereof. In an aspect, a disclosed U6 promoter can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% identity to the sequence set forth in SEQ ID NO: 81 or a fragment thereof. In an aspect, a disclosed EF-1α promoter can comprise the sequence set forth in SEQ ID NO: 79, SEQ ID NO: 80, or a fragment thereof. In an aspect, a disclosed EF-1α promoter can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% identity to the sequence set forth in SEQ ID NO: 79, SEQ ID NO: 80, or a fragment thereof. In an aspect, a promoter can be a short EF1alpha (EFS-NF) promoter.

[0317] In an aspect, a disclosed promoter operably can be linked to the at least one guide RNA targeting a gene of interest or portion thereof. In an aspect, a disclosed promoter operably linked to the at least one guide RNA can comprise a U6 promoter. In an aspect, a disclosed promoter

operably linked to the at least one guide RNA targeting an *APOE* gene can comprise a U6 promoter.

[0318] In an aspect of a disclosed plasmid vector can further comprise two or more promoters, wherein a first promoter can be operably linked to the dCas endonuclease, a polypeptide having enzymatic activity, a fusion of two or more polypeptides having enzymatic activities or any combination thereof, and wherein a second promoter can be operably linked to the at least one guide RNA targeting a gene of interest or portion thereof. In an aspect, a disclosed isolated nucleic acid molecule can further comprise one or more promoters, wherein a first promoter comprises a microglia specific promoter that is operably linked to the dCas endonuclease, a polypeptide having enzymatic activity, a fusion of two or more polypeptides having enzymatic activities or any combination thereof, and wherein a second promoter can be operably linked to the at least one guide RNA targeting a gene of interest thereof. In an aspect, the second promoter operably linked to the gRNA may not be a microglia specific promoter. In an aspect, the isolated nucleic acid can comprise a first promoter selected from a F4/80 promoter, a CD68 promoter, a IBA1 promoter, or a FCGR2A promoter or derivatives thereof, and a second promoter comprising a U6 promoter, or a derivative thereof.

[0319] In an aspect of a disclosed plasmid vector can further comprise one or more regulatory elements. In an aspect, a disclosed regulatory element can comprise a promoter, an enhancer, a promoter/enhancer, an internal ribosomal entry site, a transcription terminal signal, a polyadenylation signal, a Sp1 and/or NF-kB transcriptional factor binding site, a p2A signal, a woodchuck hepatitis virus post-transcriptional regulatory element, a Phi signal-packaging signal, a rev responsive element, a nuclear localization signal (NLS), or any combination thereof.

[0320] In an aspect, a disclosed NLS can comprise the sequence set forth in any one of SEQ ID NO: 88 – SEQ ID NO: 94, or a fragment thereof. In an aspect, a disclosed PolyA sequence can comprise the sequence set forth in SEQ ID NO: 86, SEQ ID NO: 87, or a fragment thereof.

[0321] In an aspect of a disclosed plasmid vector can be CpG depleted and codon-optimized for expression in a human cell. In an aspect, “CpG-free” can mean completely free of CpGs or partially free of CpGs. In an aspect, “CpG-free” can mean “CpG-depleted.” In an aspect, “CpG-depleted” can mean “CpG-free.” In an aspect, “CpG-depleted” can mean completely depleted of CpGs or partially depleted of CpGs. In an aspect, “CpG-free” can mean “CpG-optimized” for a desired and/or ideal expression level. CpG depletion and/or optimization is known to the skilled person in the art. In an aspect, any disclosed Cas9 endonuclease, a disclosed polypeptide having enzymatic activities, a disclosed fusion product or a disclosed fusion protein, or any combination thereof can be codon-optimized.

[0322] Disclosed herein is a plasmid vector comprising a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, wherein the dCas is a dSpCas9, dSaCas9, or dCjCas9, (ii) a fusion protein having one or more enzymatic activities, wherein the fusion product comprises any combination of HP1a, HP1b, MBD1, MBD2, KRAB, NIPP1, MeCP2, KRAB-MeCP2 or DNMT3A, and (iii) at least one guide RNA targeting *APOE* (*e.g. APOE* e4 allele) or portion thereof.

[0323] Disclosed herein is a plasmid vector comprising a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, wherein the dCas is a dSpCas9, dSaCas9, or dCjCas9, (ii) a fusion protein having one or more enzymatic activities, wherein the fusion product comprises any combination of HP1a, HP1b, MBD1, MBD2, KRAB, NIPP1, MeCP2, KRAB-MeCP2 or DNMT3A, (iii) at least one guide RNA targeting *APOE* (*e.g. APOE* e4 allele) or portion thereof, (iv) a microglia specific regulatory sequence (e. g. promoter) operably linked to the dCas endonuclease and the fusion protein, and (v) a promoter operably linked to the at least one guide RNA.

[0324] In an aspect, a disclosed plasmid vector can comprise the sequence set forth in any of SEQ ID NO: 95 – SEQ ID NO: 104, or a variant thereof, or a fragment thereof, or a portion thereof. In an aspect, a disclosed plasmid vector can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 95 – SEQ ID NO: 104, or a variant thereof, or a fragment thereof, or a portion thereof.

3. Host cells

[0325] Disclosed herein is a host cell or a host cell line comprising an isolated nucleic acid molecule comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, (ii) at least one polypeptide having an enzymatic activity, and (iii) at least one guide RNA targeting a gene of interest or portion thereof; and (b) one or more microglia specific regulatory element operably linked to (a)(i), (a)(ii), (a)(iii) or any combination thereof. In an aspect, the gene of interest is *APOE,* or an allele thereof.

[0326] Disclosed herein is a host cell or a host cell line comprising an isolated nucleic acid molecule comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, (ii) at least one polypeptide having an enzymatic activity, and (iii) at least one guide RNA targeting a gene of interest or portion thereof; and (b) one or more microglia specific promoter operably linked to (a)(i), (a)(ii), (a)(iii) or any combination thereof. In an aspect, the gene of interest is *APOE,* or an allele thereof.

[0327] Disclosed herein is a host cell or a host cell line comprising an isolated nucleic acid molecule comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, (ii) a fusion product having one or more enzymatic activities, and (iii) at least one guide RNA targeting a gene of interest or portion thereof, and (b) one or more microglia specific regulatory element operably linked to (a)(i), (a)(ii), (a)(iii) or any combination thereof. In an aspect, the gene of interest is *APOE,* or an allele thereof.

[0328] Disclosed herein is a host cell or a host cell line comprising an isolated nucleic acid molecule comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, (ii) a fusion product having one or more enzymatic activities, and (iii) at least one guide RNA targeting a gene of interest or portion thereof, and (b) one or more microglia specific promoter operably linked to (a)(i), (a)(ii), (a)(iii) or any combination thereof. In an aspect, the gene of interest is *APOE,* or an allele thereof.

[0329] Disclosed herein is a host cell or a host cell line comprising an isolated nucleic acid molecule comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, wherein the dCas is a dSpCas9, dSaCas9, or dCjCas9, (ii) a fusion product having one or more enzymatic activities, wherein the fusion product comprises any combination of HP1a, HP1b, MBD1, MBD2, KRAB, NIPP1, MeCP2, or DNMT3A, and (iii) at least one guide RNA targeting a gene of interest or portion thereof and (b) one or more microglia specific regulatory elements operably linked to (a)(i), (a)(ii), (a)(iii) or any combination thereof. Disclosed herein is a host cell or a host cell line comprising an isolated nucleic acid molecule comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, wherein the dCas is a dSpCas9, dSaCas9, or dCjCas9, (ii) a fusion product having one or more enzymatic activities, wherein the fusion product comprises any combination of HP1a, HP1b, MBD1, MBD2, KRAB, NIPP1, MeCP2, or DNMT3A, and (iii) at least one guide RNA targeting *APOE* or portion thereof and (b) one or more microglia specific promoters operably linked to (a)(i), (a)(ii), (a)(iii) or any combination thereof.

[0330] Disclosed herein is a host cell or a host cell line comprising an isolated nucleic acid molecule comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, wherein the dCas is a dSpCas9, dSaCas9, or dCjCas9, (ii) a fusion product having one or more enzymatic activities, wherein the fusion product comprises any combination of HP1a, HP1b, MBD1, MBD2, KRAB, NIPP1, MeCP2, or DNMT3A, and (iii) at least one guide RNA targeting *APOE* or portion thereof, (b) one or more microglia specific regulatory elements operably linked to (a)(i) and/or (a)(ii), and (c) a promoter operably linked to (a)(iii). Disclosed herein is a host cell or a host cell line comprising an isolated nucleic acid molecule comprising (a)

a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, wherein the dCas is a dSpCas9, dSaCas9, or dCjCas9, (ii) a fusion product having one or more enzymatic activities, wherein the fusion product comprises any combination of HP1a, HP1b, MBD1, MBD2, KRAB, NIPP1, MeCP2, or DNMT3A, and (iii) at least one guide RNA targeting *APOE* or portion thereof, (b) one or more neuron specific promoters operably linked to (a)(i) and/or (a)(ii), and (c) a promoter operably linked to (a)(iii).

[0331] In an aspect of the disclosed host cell or a host cell line, the isolated nucleic acid molecule may comprise a sequence encoding any one of the disclosed dCas9, any one or more of the disclosed polypeptides having an enzymatic activity, any one or more of the disclosed gRNA. In an aspect of the disclosed host cell of a host cell line, the isolated nucleic acid molecule may further comprise one or more of the disclosed microglia specific regulatory elements (e. g. microglia specific promoter) and optionally additional disclosed promoters. Disclosed herein is a host cell or a host cell line comprising the disclosed isolated nucleic acid molecule comprising (a) a nucleic acid sequence encoding (i) a deactivated Clustered Regularly Interspaced Short Palindromic Repeats associated (dCas) endonuclease comprising an amino acid sequence as set forth in any one of SEQ ID NO: 36- SEQ ID NO: 38, or a fragment, or derivative thereof, or a sequence at least about 60% identical thereto (ii) at least one polypeptide having an enzymatic activity comprising an amino acid sequence as set forth in any one or more of SEQ ID NO: 44- SEQ ID NO: 58, or a sequence at least about 60% identical thereto and (iii) at least one guide RNA targeting an *APOE* gene sequence or a regulatory element thereof comprising, wherein the targeted sequence comprises a nucleic acid sequence as set forth in SEQ ID NO: 15 – SEQ ID NO: 33, or a complement thereof, or a sequence at least about 60% identical thereto; and (b) one or more microglia specific regulatory elements operably linked to (a)(i), (a)(ii), (a)(iii), or any combination thereof, wherein the microglia specific regulatory element comprises a nucleic acid sequence as set forth in any one of SEQ ID NO: 1- SEQ ID NO: 8, or a fragment, or a derivative thereof, or a sequence at least about 60% identical thereto.

[0332] Disclosed herein is a host cell or a host cell line transduced by a viral vector comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, (ii) at least one polypeptide having an enzymatic activity, and (iii) at least one guide RNA targeting a gene of interest or portion thereof; and (b) one or more microglia specific regulatory element operably linked to (a)(i), (a)(ii), (a)(iii) or any combination thereof. In an aspect, the gene of interest is *APOE,* or an allele thereof.

[0333] Disclosed herein is a host cell or a host cell line transduced by a viral vector comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, (ii) at least one

polypeptide having an enzymatic activity, and (iii) at least one guide RNA targeting a gene of interest or portion thereof; and (b) one or more microglia specific promoter operably linked to (a)(i), (a)(ii), (a)(iii) or any combination thereof. In an aspect, the gene of interest is *APOE,* or an allele thereof.

[0334] Disclosed herein is a host cell or a host cell line transduced by a viral vector comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, (ii) a fusion product having one or more enzymatic activities, and (iii) at least one guide RNA targeting a gene of interest or portion thereof, and (b) one or more microglia specific regulatory element operably linked to (a)(i), (a)(ii), (a)(iii) or any combination thereof. In an aspect, the gene of interest is *APOE,* or an allele thereof.

[0335] Disclosed herein is a host cell or a host cell line transduced by a viral vector comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, (ii) a fusion product having one or more enzymatic activities, and (iii) at least one guide RNA targeting a gene of interest or portion thereof, and (b) one or more microglia specific promoter operably linked to (a)(i), (a)(ii), (a)(iii) or any combination thereof. In an aspect, the gene of interest is *APOE,* or an allele thereof.

[0336] In an aspect of the disclosed host cell or a host cell line transduced by a viral vector comprises a nucleic acid sequence encoding any one of the disclosed dCas9, any one or more of the disclosed polypeptides having an enzymatic activity, and any one or more of the disclosed gRNA. In an aspect of the disclosed host cell of a host cell line transduced by a viral vector comprises a nucleic acid sequence further comprising one or more of the disclosed microglia specific regulatory elements (e. g. microglia specific promoter) and optionally additional disclosed promoters. Disclosed herein is a host cell or a host cell line transduced by a viral vector comprising comprising (a) a nucleic acid sequence encoding (i) a deactivated Clustered Regularly Interspaced Short Palindromic Repeats associated (dCas) endonuclease comprising an amino acid sequence as set forth in any one of SEQ ID NO: 36- SEQ ID NO: 38, or a fragment, or derivative thereof, or a sequence at least about 60% identical thereto (ii) at least one polypeptide having an enzymatic activity comprising an amino acid sequence as set forth in any one or more of SEQ ID NO: 44-SEQ ID NO: 58, or a sequence at least about 60% identical thereto and (iii) at least one guide RNA targeting an *APOE* gene sequence or a regulatory element thereof comprising, wherein the targeted sequence comprises a nucleic acid sequence as set forth in SEQ ID NO: 15 – SEQ ID NO: 33, or a complement thereof, or a sequence at least about 60% identical thereto; and (b) one or more microglia specific regulatory elements operably linked to (a)(i), (a)(ii), (a)(iii), or any combination thereof, wherein the microglia specific regulatory element comprises a nucleic acid

sequence as set forth in any one of SEQ ID NO: 1- SEQ ID NO: 8, or a fragment, or a derivative thereof, or a sequence at least about 60% identical thereto.

[0337] Disclosed herein is a host cell or a host cell line transduced by a AAV viral vector comprising a sequence as set forth in any one of SEQ ID NO: 112-SEQ ID NO: 128, or a sequence at least about 60% identical thereto. Disclosed herein is a host cell or a host cell line transduced by a lentiviral vector comprising a sequence as set forth in any one of SEQ ID NO: 105-SEQ ID NO: 111, or a sequence at least about 60% identical thereto.

[0338] Disclosed herein is a host cell or a host cell line transfected by a plasmid comprising the sequence set forth in any one of SEQ ID NO: 95 - SEQ ID NO: 104, or a sequence at least about 60% identical thereto.

4. Pharmaceutical Formulations

[0339] Disclosed herein is a pharmaceutical formulation comprising a disclosed isolated nucleic acid molecule and a pharmaceutically acceptable carrier. Disclosed herein is pharmaceutical formulation comprising (1) an isolated nucleic acid molecule comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, (ii) at least one polypeptide having an enzymatic activity, and (iii) at least one guide RNA targeting a gene of interest or portion thereof; and (b) one or more microglia specific regulatory element operably linked to (a)(i), (a)(ii), (a)(iii) or any combination thereof; and (2) a pharmaceutically acceptable carrier. In an aspect, the gene of interest is *APOE,* or an allele thereof.

[0340] Disclosed herein is a pharmaceutical formulation comprising a disclosed isolated nucleic acid molecule and a pharmaceutically acceptable carrier. Disclosed herein is pharmaceutical formulation comprising (1) an isolated nucleic acid molecule comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, (ii) at least one polypeptide having an enzymatic activity, and (iii) at least one guide RNA targeting a gene of interest or portion thereof; and (b) one or more microglia specific promoter operably linked to (a)(i), (a)(ii), (a)(iii) or any combination thereof; and (2) a pharmaceutically acceptable carrier. In an aspect, the gene of interest is *APOE,* or an allele thereof.

[0341] Disclosed herein is a pharmaceutical formulation comprising an isolated nucleic acid molecule and a pharmaceutically acceptable carrier. Disclosed herein is a pharmaceutical formulation comprising (1) an isolated nucleic acid comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, (ii) a fusion product having one or more enzymatic activities, and (iii) at least one guide RNA targeting a gene of interest or portion thereof, and (b) one or more microglia specific regulatory element operably linked to (a)(i), (a)(ii), (a)(iii)

or any combination thereof; and (2) a pharmaceutically acceptable carrier. In an aspect, the gene of interest is *APOE,* or an allele thereof.

[0342] Disclosed herein is a pharmaceutical formulation comprising an isolated nucleic acid molecule and a pharmaceutically acceptable carrier. Disclosed herein is a pharmaceutical formulation comprising (1) an isolated nucleic acid comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, (ii) a fusion product having one or more enzymatic activities, and (iii) at least one guide RNA targeting a gene of interest or portion thereof, and (b) one or more microglia specific promoter operably linked to (a)(i), (a)(ii), (a)(iii) or any combination thereof ; and (2) a pharmaceutically acceptable carrier. In an aspect, the gene of interest is *APOE,* or an allele thereof.

[0343] Disclosed herein is a pharmaceutical formulation comprising an isolated nucleic acid molecule and a pharmaceutically acceptable carrier. Disclosed herein is a pharmaceutical formulation comprising (1) an isolated nucleic acid comprising: (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, wherein the dCas is a dSpCas9, dSaCas9, or dCjCas9, (ii) a fusion product having one or more enzymatic activities, wherein the fusion product comprises any combination of HP1a, HP1b, MBD1, MBD2, KRAB, NIPP1, MeCP2, or DNMT3A, and (iii) at least one guide RNA targeting a gene of interest or portion thereof, and (b) one or more microglia specific regulatory element operably linked to (a)(i), (a)(ii), (a)(iii) or any combination thereof; and (2) a pharmaceutically acceptable carrier.

[0344] Disclosed herein is a pharmaceutical formulation comprising an isolated nucleic acid molecule and a pharmaceutically acceptable carrier. Disclosed herein is a pharmaceutical formulation comprising (1) an isolated nucleic acid comprising: (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, wherein the dCas is a dSpCas9, dSaCas9, or dCjCas9, (ii) a fusion product having one or more enzymatic activities, wherein the fusion product comprises any combination of HP1a, HP1b, MBD1, MBD2, KRAB, NIPP1, MeCP2, or DNMT3A, and (iii) at least one guide RNA targeting a gene of interest or portion thereof, and (b) one or more microglia specific promoter operably linked to (a)(i), (a)(ii), (a)(iii) or any combination thereof; and (2) a pharmaceutically acceptable carrier.

[0345] Disclosed herein is a pharmaceutical formulation comprising an isolated nucleic acid molecule and a pharmaceutically acceptable carrier. Disclosed herein is a pharmaceutical formulation comprising (1) an isolated nucleic acid comprising: (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, wherein the dCas is a dSpCas9, dSaCas9, or dCjCas9, (ii) a fusion product having one or more enzymatic activities, wherein the fusion product comprises any combination of HP1a, HP1b, MBD1, MBD2, KRAB, NIPP1, MeCP2, or

DNMT3A, and (iii) at least one guide RNA targeting a gene of interest or portion thereof, (b) a microglia specific regulatory element operably linked to (a)(i) and/or (a)(ii) and (c) a promoter operably linked to (a)(iii); and (2) a pharmaceutically acceptable carrier.

[0346] Disclosed herein is a pharmaceutical formulation comprising an isolated nucleic acid molecule and a pharmaceutically acceptable carrier. Disclosed herein is a pharmaceutical formulation comprising (1) an isolated nucleic acid comprising: (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, wherein the dCas is a dSpCas9, dSaCas9, or dCjCas9, (ii) a fusion product having one or more enzymatic activities, wherein the fusion product comprises any combination of HP1a, HP1b, MBD1, MBD2, KRAB, NIPP1, MeCP2, or DNMT3A, and (iii) at least one guide RNA targeting a gene of interest or portion thereof, (b) a microglia specific promoter operably linked to (a)(i) and/or (a)(ii) and (c) a promoter operably linked to (a)(iii); and (2) a pharmaceutically acceptable carrier.

[0347] Disclosed herein is pharmaceutical formulation comprising (1) a viral vector comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, (ii) at least one polypeptide having an enzymatic activity, and (iii) at least one guide RNA targeting a gene of interest or portion thereof and (b) one or more microglia specific regulatory element operably linked to (a)(i), (a)(ii), (a)(iii) or any combination thereof; and (2) a pharmaceutically acceptable carrier.

[0348] Disclosed herein is pharmaceutical formulation comprising (1) a viral vector comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, (ii) at least one polypeptide having an enzymatic activity, and (iii) at least one guide RNA targeting a gene of interest or portion thereof and (b) one or more microglia specific promoter operably linked to (a)(i), (a)(ii), (a)(iii) or any combination thereof; and (2) a pharmaceutically acceptable carrier.

[0349] Disclosed herein is pharmaceutical formulation comprising a (1) viral vector comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, wherein the dCas is a dSpCas9, dSaCas9, or dCjCas9, (ii) a fusion product having one or more enzymatic activities, wherein the fusion product comprises any combination of HP1a, HP1b, MBD1, MBD2, KRAB, NIPP1, MeCP2, or DNMT3A, and (iii) at least one guide RNA targeting a gene of interest or portion thereof, and (b) one or more microglia specific regulatory elements operably linked to (a)(i), (a)(ii), (a)(iii) or any combination thereof, and (2) a pharmaceutically acceptable carrier.

[0350] Disclosed herein is pharmaceutical formulation comprising a (1) viral vector comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, wherein the dCas is a dSpCas9, dSaCas9, or dCjCas9, (ii) a fusion product having one or more enzymatic activities, wherein the fusion product comprises any combination of HP1a, HP1b, MBD1, MBD2, KRAB,

NIPP1, MeCP2, or DNMT3A, and (iii) at least one guide RNA targeting a gene of interest or portion thereof, and (b) one or more microglia specific promoters operably linked to (a)(i), (a)(ii), (a)(iii) or any combination thereof, and (2) a pharmaceutically acceptable carrier.

[0351] Disclosed herein is a pharmaceutical formulation comprising 1) viral vector comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, wherein the dCas is a dSpCas9, dSaCas9, or dCjCas9, (ii) a fusion product having one or more enzymatic activities, wherein the fusion product comprises any combination of HP1a, HP1b, MBD1, MBD2, KRAB, NIPP1, MeCP2, or DNMT3A, and (iii) at least one guide RNA targeting a gene of interest or portion thereof, (b) one or more microglia specific regulatory elements operably linked to (a)(i) and/or (a)(ii), and (c) a promoter operably linked to (a)(iii), and (2) a pharmaceutically acceptable carrier.

[0352] Disclosed herein is a pharmaceutical formulation comprising 1) viral vector comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, wherein the dCas is a dSpCas9, dSaCas9, or dCjCas9, (ii) a fusion product having one or more enzymatic activities, wherein the fusion product comprises any combination of HP1a, HP1b, MBD1, MBD2, KRAB, NIPP1, MeCP2, or DNMT3A, and (iii) at least one guide RNA targeting a gene of interest or portion thereof, (b) one or more microglia specific promoters operably linked to (a)(i) and/or (a)(ii), and (c) a promoter operably linked to (a)(iii), and (2) a pharmaceutically acceptable carrier.

[0353] In an aspect, a disclosed formulation can comprise (i) one or more active agents, (ii) biologically active agents, (iii) one or more pharmaceutically active agents, (iv) one or more immune-based therapeutic agents, (v) one or more clinically approved agents, or (vi) a combination thereof. In an aspect, a disclosed composition can comprise one or more proteasome inhibitors. In an aspect, a disclosed composition can comprise one or more immunosuppressives or immunosuppressive agents. In an aspect, an immunosuppressive agent can be anti-thymocyte globulin (ATG), cyclosporine (CSP), mycophenolate mofetil (MMF), or a combination thereof. In an aspect, a disclosed formulation can comprise a RNA therapeutic. An RNA therapeutic can comprise RNA-mediated interference (RNAi) and/or antisense oligonucleotides (ASO). In an aspect, a disclosed formulation can comprise a disclosed small molecule.

[0354] In an aspect, a disclosed pharmaceutical formulation can treat and/or prevent Alzheimer's disease progression. In an aspect, a disclosed pharmaceutical formulation can treat and/or prevent Parkinson's disease progression.

F. Methods of Effecting Precision Epigenetic Modulation

[0355] Disclosed herein is a method of effecting precision epigenetic modulation of microglial cells, the method comprising contacting one or more microglial cells with a therapeutically

effective amount of a disclosed isolated nucleic acid molecule, wherein the expression and/or activity of one or more genes of interest in the one or more microglial cells is modulated. Disclosed herein is a method of effecting precision epigenetic modulation, the method comprising contacting one or more microglial cells with a therapeutically effective amount of a disclosed isolated nucleic acid molecule, wherein the expression and/or activity of one or more genes of interest in the one or more microglial cells is reduced or decreased. Disclosed herein is a method of effecting precision epigenetic modulation, the method comprising contacting one or more microglial cells with a therapeutically effective amount of a disclosed isolated nucleic acid molecule, wherein the expression and/or activity of one or more genes of interest in the one or more microglial cells is augmented or increased.

[0356] Disclosed herein is a method of effecting precision epigenetic modulation, the method comprising contacting one or more microglial cells with a therapeutically effective amount of a disclosed isolated nucleic acid molecule, wherein the expression and/or activity of APOE in the one or more microglial cells is reduced or decreased.

[0357] Disclosed herein is a method of effecting precision epigenetic modulation in microglial cells, the method comprising contacting one or more microglial cells with a therapeutically effective amount of a disclosed isolated nucleic acid molecule comprising: (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, (ii) at least one polypeptide having an enzymatic activity, and (iii) at least one guide RNA targeting a gene of interest or portion thereof and (b) at least one microglia specific regulatory element operably linked to (a)(i), (a)(ii), (a)(iii) or any combination thereof, wherein the expression and/or activity the gene of interest in the one or more microglial cells is modulated.

[0358] Disclosed herein is a method of effecting precision epigenetic modulation in microglial cells, the method comprising contacting one or more microglial cells with a therapeutically effective amount of a disclosed isolated nucleic acid molecule comprising: (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, (ii) at least one polypeptide having an enzymatic activity and (iii) at least one guide RNA targeting a gene of interest or portion thereof and (b) at least one microglia specific promoter operably linked to (a)(i), (a)(ii), (a)(iii) or any combination thereof, wherein the expression and/or activity the gene of interest in the one or more microglial cells is modulated.

[0359] Disclosed herein is a method of effecting precision epigenetic modulation in microglial cells, the method comprising contacting one or more microglial cells with a therapeutically effective amount of a disclosed isolated nucleic acid molecule, comprising: (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, wherein the dCas is a dSpCas9,

dSaCas9, or dCjCas9, (ii) a fusion product having one or more enzymatic activities, wherein the fusion product comprises any combination of HP1a, HP1b, MBD1, MBD2, KRAB, NIPP1, MeCP2, or DNMT3A, and (iii) at least one guide RNA targeting a gene of interest or portion thereof and (b) at least one microglia specific regulatory element operably linked to (a)(i), (a)(ii), (a)(iii) or any combination thereof, wherein the expression and/or activity the gene of interest in the one or more microglial cells is modulated.

[0360] Non-limiting examples of microglia specific promoters include F4/80 promoter (SEQ ID NO: 4), CD68 promoter (SEQ ID NO: 1-3), FCGR2A promoter (SEQ ID NO: 8), or IBA1 promoter (SEQ ID NO: 5-7), or a derivative thereof, a derivative thereof, a fragment thereof, or any combination thereof. In some aspects, the gene of interest in the *APOE* gene.

[0361] Disclosed herein is a method of effecting precision epigenetic modulation in microglial cells, the method comprising contacting one or more microglial cells with a therapeutically effective amount of a disclosed isolated nucleic acid molecule, comprising: (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, wherein the dCas is a dSpCas9, dSaCas9, or dCjCas9, (ii) a fusion product having one or more enzymatic activities, wherein the fusion product comprises any combination of HP1a, HP1b, MBD1, MBD2, KRAB, NIPP1, MeCP2, or DNMT3A, and (iii) at least one guide RNA targeting a gene of interest or portion thereof and (b) at least one microglia specific promoter operably linked to (a)(i), (a)(ii), (a)(iii) or any combination thereof, wherein the expression and/or activity the gene of interest in the one or more microglial cells is modulated. In some aspects, the microglia specific promoter of b) may comprise F4/80 promoter (SEQ ID NO: 4), CD68 promoter (SEQ ID NO: 1-3), FCGR2A promoter (SEQ ID NO: 8), or IBA1 promoter (SEQ ID NO: 5-7), or a derivative thereof, a derivative thereof, a fragment thereof, or any combination thereof. In some aspects, the gene of interest in the *APOE* gene.

[0362] Disclosed herein is a method of effecting precision epigenetic modulation in microglial cells, the method comprising contacting one or more microglial cells with a therapeutically effective amount of a disclosed isolated nucleic acid molecule, comprising: (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, wherein the dCas is a dSpCas9, dSaCas9, or dCjCas9, (ii) a fusion product having one or more enzymatic activities, wherein the fusion product comprises any combination of HP1a, HP1b, MBD1, MBD2, KRAB, NIPP1, MeCP2, or DNMT3A, and (iii) at least one guide RNA targeting a gene of interest or portion thereof, (b) a microglia specific regulatory element operably linked (a)(i) and/or (a)(ii), and (c) a promoter operably linked to (a)(iii), wherein the expression and/or activity the gene of interest in the one or more microglial cells is modulated. In some aspects, the microglia specific regulatory

element of (b) may comprise F4/80 promoter (SEQ ID NO: 4), CD68 promoter (SEQ ID NO: 1-3), FCGR2A promoter (SEQ ID NO: 8), or IBA1 promoter (SEQ ID NO: 5-7), any derivative thereof, any fragment thereof, or any combination thereof. In some aspects, the promoter of (c) comprises a U6 promoter, a derivative thereof, or a fragment thereof. In some aspects, the gene of interest in the *APOE* gene.

[0363] Disclosed herein is a method of effecting precision epigenetic modulation in microglial cells, the method comprising contacting one or more microglial cells with a therapeutically effective amount of a disclosed isolated nucleic acid molecule, comprising: (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, wherein the dCas is a dSpCas9, dSaCas9, or dCjCas9, (ii) a fusion product having one or more enzymatic activities, wherein the fusion product comprises any combination of HP1a, HP1b, MBD1, MBD2, KRAB, NIPP1, MeCP2, or DNMT3A, (iii) at least one guide RNA targeting a gene of interest or portion thereof, (b) a microglia specific promoter operably linked to (a)(i) and/or (a)(ii), and (c) a promoter operably linked to (a)(iii) wherein the expression and/or activity the gene of interest in the one or more microglial cells is modulated. In some aspects, the microglia specific promoter of (b) may comprise a F4/80 promoter (SEQ ID NO: 4), CD68 promoter (SEQ ID NO: 1-3), FCGR2A promoter (SEQ ID NO: 8), or IBA1 promoter (SEQ ID NO: 5-7), a derivative thereof, a fragment thereof, or any combination thereof. In some aspects, the promoter of (v) comprises a U6 promoter a derivative thereof or a fragment thereof. In some aspects, the gene of interest in the *APOE* gene.

[0364] Disclosed herein is a method of effecting precision epigenetic modulation in microglial cells in a subject, the method comprising contacting one or more microglial cells in a subject with a therapeutically effective amount of a disclosed viral vector, wherein the expression and/or activity of one or more genes of interest in the one or more cells is modulated. Disclosed herein is a method of effecting precision epigenetic modulation in microglial cells, the method comprising contacting one or more cells in a subject with a therapeutically effective amount of a disclosed viral vector, wherein the expression and/or activity of one or more genes of interest in the one or more microglial cells is reduced or decreased. Disclosed herein is a method of effecting precision epigenetic modulation in microglial cells, the method comprising contacting one or more cells in a subject with a therapeutically effective amount of a disclosed viral vector, wherein the expression and/or activity of one or more genes of interest in the one or more microglial cells is augmented or increased. Disclosed herein is a method of effecting precision epigenetic modulation, the method comprising contacting one or more cells in a subject with a therapeutically effective amount of a disclosed viral vector, wherein the expression and/or activity of APOE in the one or more microglial cells is reduced or decreased.

[0365] Disclosed herein is a method of effecting precision epigenetic modulation in microglial cells in a subject, the method comprising contacting one or more microglial cells in a subject with a therapeutically effective amount of a viral vector comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, (ii) at least one polypeptide having an enzymatic activity and (iii) at least one guide RNA targeting a gene of interest or portion thereof, and (b) at least one microglia specific regulatory element operably linked to (a)(i), (a)(ii), (a)(iii) or a combination thereof, wherein the expression and/or activity of one or more genes of interest in the one or more microglial cells is modulated. In an aspect, the at least one guide RNA of (a)(iii) targets the *APOE* gene or a portion thereof. In an aspect, the microglia specific regulatory element of (b) can comprise a F4/80 promoter (SEQ ID NO: 4), CD68 promoter (SEQ ID NO: 1-3), FCGR2A promoter (SEQ ID NO: 8), or IBA1 promoter (SEQ ID NO: 5-7), a derivative thereof, a fragment thereof, or any combination thereof.

[0366] Disclosed herein is a method of effecting precision epigenetic modulation in microglial cells in a subject, the method comprising contacting one or more microglial cells in a subject with a therapeutically effective amount of a viral vector comprising a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, (ii) at least one polypeptide having an enzymatic activity, (iii) at least one guide RNA targeting a gene of interest or portion thereof, and (b) at least one microglia specific promoter operably linked to (a)(i), (a)(ii), (a)(iii) or a combination thereof, wherein the expression and/or activity of one or more genes of interest in the one or more cells is modulated. In an aspect, the at least one guide RNA of (a)(iii) targets the *APOE* gene or a portion thereof. In an aspect, the microglia specific promoter of (b) can comprise a F4/80 promoter (SEQ ID NO: 4), CD68 promoter (SEQ ID NO: 1-3), FCGR2A promoter (SEQ ID NO: 8), or IBA1 promoter (SEQ ID NO: 5-7), a derivative thereof, a fragment thereof, or any combination thereof.

[0367] Disclosed herein is a method of effecting precision epigenetic modulation in microglial cells in a subject, the method comprising administering to a subject a therapeutically effective amount of a viral vector comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, (ii) a fusion product having one or more enzymatic activities, (iii) at least one guide RNA targeting the *APOE* gene or a portion of the *APOE* gene and (b) at least one microglia specific regulatory element operably linked to (a)(i), (a)(ii), (a)(iii), or a combination thereof, wherein the expression and/or activity of one or more genes of interest in the one or more microglial cells is modulated.

[0368] Disclosed herein is a method of effecting precision epigenetic modulation in microglial cells in a subject, the method comprising administering to a subject a therapeutically effective amount of a viral vector comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas

(dCas) endonuclease, (ii) a fusion product having one or more enzymatic activities, (iii) at least one guide RNA targeting the APOE gene or a portion of the APOE gene and (b) at least one microglia specific promoter operably linked to (a)(i), (a)(ii), (a)(iii), or a combination thereof, wherein the expression and/or activity of one or more genes of interest in the one or more microglial cells is modulated.

[0369] Disclosed herein is a method of effecting precision epigenetic modulation in microglial cells in a subject, the method comprising contacting one or more microglial cells in a subject with a therapeutically effective amount of a viral vector comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, wherein the dCas is a dSpCas9, dSaCas9, or dCjCas9, (ii) a fusion product having one or more enzymatic activities, wherein the fusion product comprises any combination of HP1a, HP1b, MBD1, MBD2, KRAB, NIPP1, MeCP2, or DNMT3A, and (iii) at least one guide RNA targeting a gene of interest or portion thereof, and (b) at least one microglia specific regulatory element operably linked to (a)(i), (a)(ii), (a)(iii) or any combination thereof wherein the expression and/or activity of one or more genes of interest in the one or more cells is modulated. In an aspect, the dCas of (a)(i) is dSaCas9. In an aspect, the fusion product of (a)(ii) can comprise KRAB-MeCP2. In an aspect, the at least one guide RNA of (a)(iii) targets the *APOE* gene or a portion thereof. In an aspect, the microglia specific regulatory element of (b) can comprise a F4/80 promoter (SEQ ID NO: 4), CD68 promoter (SEQ ID NO: 1-3), FCGR2A promoter (SEQ ID NO: 8), or IBA1 promoter (SEQ ID NO: 5-7), a derivative thereof, a fragment thereof, or any combination thereof.

[0370] Disclosed herein is a method of effecting precision epigenetic modulation in microglial cells in a subject, the method comprising contacting one or more microglial cells in a subject with a therapeutically effective amount of a viral vector comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, wherein the dCas is a dSpCas9, dSaCas9, or dCjCas9, (ii) a fusion product having one or more enzymatic activities, wherein the fusion product comprises any combination of HP1a, HP1b, MBD1, MBD2, KRAB, NIPP1, MeCP2, or DNMT3A, and (iii) at least one guide RNA targeting a gene of interest or portion thereof, and (b) at least one microglia specific promoter operably linked to (a)(i), (a)(ii), (a)(iii) or a combination thereof wherein the expression and/or activity of one or more genes of interest in the one or more cells is modulated. In an aspect, the dCas of (a)(i) is dSaCas9. In an aspect, the fusion product of (a)(ii) can comprise KRAB-MeCP2. In an aspect, the at least one guide RNA of (a)(iii) targets the *APOE* gene or a portion thereof. In an aspect, the microglia specific promoter of (b) can comprise F4/80 promoter (SEQ ID NO: 4), CD68 promoter (SEQ ID NO: 1-3), FCGR2A promoter (SEQ

ID NO: 8), or IBA1 promoter (SEQ ID NO: 5-7) a derivative thereof, a fragment thereof, or any combination thereof.

[0371] Disclosed herein is a method of effecting precision epigenetic modulation in microglial cells in a subject, the method comprising contacting one or more cells in a subject with a therapeutically effective amount of a viral vector comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, wherein the dCas is a dSpCas9, dSaCas9, or dCjCas9, (ii) a fusion product having one or more enzymatic activities, wherein the fusion product comprises any combination of HP1a, HP1b, MBD1, MBD2, KRAB, NIPP1, MeCP2, or DNMT3A, (iii) at least one guide RNA targeting a gene of interest or portion thereof, (b) a microglia specific regulatory element operably linked to (a)(i) and/or (a)(ii), and (c) a promoter operably linked to (a)(iii), wherein the expression and/or activity of one or more genes of interest in the one or more cells is modulated. In an aspect, the dCas of (a)(i) is dSaCas9. In an aspect, the fusion product of (a)(ii) can comprise KRAB-MeCP2. In an aspect, the at least one guide RNA of (a)(iii) targets the *APOE* gene or a portion thereof. In an aspect, the microglia specific regulatory element of (b) can comprise a F4/80 promoter (SEQ ID NO: 4), CD68 promoter (SEQ ID NO: 1-3), FCGR2A promoter (SEQ ID NO: 8), or IBA1 promoter (SEQ ID NO: 5-7), a derivative thereof, a fragment thereof, or any combination thereof. In an aspect, the promoter of (c) can comprise any promoter known in the art.

[0372] Disclosed herein is a method of effecting precision epigenetic modulation in microglial cells in a subject, the method comprising contacting one or more cells in a subject with a therapeutically effective amount of a viral vector comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, wherein the dCas is a dSpCas9, dSaCas9, or dCjCas9, (ii) a fusion product having one or more enzymatic activities, wherein the fusion product comprises any combination of HP1a, HP1b, MBD1, MBD2, KRAB, NIPP1, MeCP2, or DNMT3A, and (iii) at least one guide RNA targeting a gene of interest or portion thereof, (b) a microglia specific promoter element operably linked to (a)(i) and/or (a)(ii) and (c) a promoter operably linked to (a)(iii), wherein the expression and/or activity of one or more genes of interest in the one or more cells is modulated. In an aspect, the dCas of (a)(i) is dSaCas9. In an aspect, the fusion product of (a)(ii) can comprise KRAB-MeCP2. In an aspect, the at least one guide RNA of (a)(iii) targets the *APOE* gene or a portion thereof. In an aspect, the microglia specific promoter of (b) can comprise a F4/80 promoter (SEQ ID NO: 4), CD68 promoter (SEQ ID NO: 1-3), FCGR2A promoter (SEQ ID NO: 8), or IBA1 promoter (SEQ ID NO: 5-7), a derivative thereof, a fragment thereof, or a combination thereof. In an aspect, the promoter of (c) can comprise a U6 promoter, a derivative thereof, or a fragment thereof.

[0373] In an aspect, a disclosed gene of interest or portion thereof can demonstrate a reduced expression and/or activity level when compared to wild-type or control expression level. In an aspect, a disclosed gene of interest or portion thereof can demonstrate an increased expression and/or activity level when compared to wild-type or control expression level. In an aspect, a disclosed gRNA can target a gene of interest or portion thereof that demonstrates an increased expression level when compared to wild-type or control expression level. In an aspect, a disclosed gRNA can target a gene of interest or portion thereof that demonstrates a reduced expression and/or activity level when compared to wild-type or control expression level.

[0374] In an aspect, a disclosed method of effecting precision epigenetic modulation comprises measuring the level of expression and/or the level of activity of the disclosed gene of interest. In an aspect, a disclosed method can comprise repeating the measuring step one or more times.

[0375] In an aspect, a disclosed gRNA targeting a gene of interest or portion thereof can comprise at least two gRNAs. In an aspect, disclosed gRNAs can target the same gene of interest or portion thereof. In an aspect, disclosed gRNAs can target different genes of interest or portions thereof. In an aspect, a disclosed gRNA can target a gene or portion thereof that demonstrates a loss of function. In an aspect, a disclosed gRNA can target a gene or portion thereof that demonstrates a gain of function. In an aspect, a disclosed gRNA can target the *APOE* gene sequence or a regulatory element thereof. In an aspect, the at least one gRNA can target the *APOE* gene, or promoter region thereof, or an untranslated region (UTR) thereof, or any combination thereof. In an aspect, the at least one gRNA may target the promoter region, or intron 1, or a flanking sequence thereof, of *APOE* gene. In an aspect, a disclosed gRNA can target exon 4 of the *APOE* gene. In an aspect, a disclosed gRNA can target a protospacer-adjacent motif (PAM) created by a SNP rs429358 in exon 4 of the *APOE* gene.

[0376] In an aspect of a disclosed method, a disclosed dCas endonuclease can comprise a dCas9 endonuclease. In an aspect, a disclosed dCas9 endonuclease can comprise a deactivated *Staphylococcus aureus* Cas9 (dSaCas9), a deactivated *Streptococcus pyogenes* Cas9 (dSpCas9), a deactivated *Campylobacter jejuni* Cas9 (dCjCas9), or a variant dCas9 endonuclease. In an aspect, a disclosed variant dCas9 can comprise a variant dSaCas9, a variant dSpCas9, or a variant dCjCa9. In an aspect, a disclosed variant dSpCas9 can comprise dVQR, dEQR, or dVRER.

[0377] In an aspect of a disclosed method, a nucleic acid sequence the disclosed dSpCas9 may comprise a sequence as set forth in any one of SEQ ID NO: 40 or SEQ ID NO: 41, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed dSpCas9 endonuclease may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at

least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 40 or SEQ ID NO: 41, or a fragment thereof. In an aspect, a disclosed dSpCas9 endonuclease can comprise the amino acid sequence as set forth in SEQ ID NO: 36 or SEQ ID NO: 37, or a fragment, or a derivative thereof. In an aspect, a disclosed dSpCas9 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 36 or SEQ ID NO: 37, or a fragment thereof.

[0378] In an aspect, the nucleic acid sequence encoding the disclosed dSaCas9 may comprise a sequence as set forth in SEQ ID NO: 42, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed dSaCas9 endonuclease may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequences set forth in SEQ ID NO: 42, or a fragment thereof. In an aspect, a disclosed dSaCas9 endonuclease can comprise the amino acid sequence as set forth in SEQ ID NO: 38 or a fragment, or a derivative thereof. In an aspect, a disclosed dSaCas9 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 38, or a fragment thereof.

[0379] In an aspect, the nucleic acid sequence encoding the disclosed dCjCas9 may comprise a sequence as set forth in SEQ ID NO: 43, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed dCjCas9 endonuclease may comprise a nucleic acid sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 43, or a fragment thereof. In an aspect, a disclosed dCjCas9 endonuclease can comprise the amino acid sequence as set forth in SEQ ID NO: 39, or a fragment, or a derivative thereof. In an aspect, a disclosed dCjCas9 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 39 or a fragment thereof.

[0380] In an aspect, a disclosed variant of dCas9 dVQR can comprise D1135V, R1335Q, and T1337R with reference to SEQ ID NO: 36. In an aspect, a disclosed variant of dCas9 dEQR can comprise D1135E, R1335Q, and T1337R with reference to SEQ ID NO: 36. In an aspect, a disclosed variant of dCas9 dVRER can comprise D1135V, G1218R, R1335E, and T1337R with reference to SEQ ID NO: 36. In an aspect, a disclosed dVRER can comprise the sequence set forth in SEQ ID NO: 37 or a fragment thereof.

[0381] In an aspect, the nucleic acid sequence encoding the disclosed dCas9 endonuclease may comprise a sequence as set forth in any one of SEQ ID NO: 40-43. In an aspect, the sequence encoding the disclosed dCas9 endonuclease may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 40-43, or a fragment, or a derivative thereof.

[0382] In an aspect of a disclosed method, a disclosed encoded polypeptide can comprise transcription activation activity, transcription repression activity, transcription release factor activity, histone modulation activity, nucleic acid association activity, methyltransferase activity, demethylase activity, acetyltransferase activity, deacetylase activity, or any combination thereof.

[0383] In an aspect of a disclosed method, a disclosed encoded polypeptide can comprise HP1a, HP1b, MBD1, MBD2, Krüppel-Associated Box (KRAB), NIPP1, the Transcription Repression Domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof.

[0384] In an aspect of a disclosed method, a disclosed fusion or disclosed fusion product can comprise HP1a-HP1a, HP1a-HP1b, HP1a-MBD1, HP1a-MBD2, HP1a-KRAB, HP1a-NIPP1, HP1a-MeCP2, HP1a-DNMT3A, HP1b-HP1b, HP1b-HP1a, HP1b-MBD1, HP1b-MBD2, HP1b-KRAB, HP1b-NIPP1, HP1b-MeCP2, HP1b-DNMT3A, MBD1-MBD1, MBD1-HP1a, MBD1-HP1b, MBD1-MBD2, MBD1-KRAB, MBD1-NIPP1, MBD1-MeCP2, MBD1-DNMT3A, MBD2-MBD2, MBD2-HP1a, MBD2-HP1b, MBD2-MBD1, MBD2-KRAB, MBD2-NIPP1, MBD2-MeCP2, MBD2-DNMT3A, KRAB-KRAB, KRAB-HP1a, KRAB-HP1b, KRAB-MBD1, KRAB-MBD2, KRAB-NIPP1, KRAB-MeCP2, KRAB-DNMT3A, NIPP1-NIPP1, NIPP1-HP1a, NIPP1-HP1b, NIPP1-MBD1, NIPP1-MBD2, NIPP1-KRAB, NIPP1-MeCP2, NIPP1-DNMT3A, MeCP2-MeCP2, MeCP2-HP1a, MeCP2-HP1b, MeCP2-MBD1, MeCP2-MBD2, MeCP2-KRAB, MeCP2-NIPP1, MeCP2-DNMT3A, DNMT3A-DNMT3A, DNMT3A-HP1a, DNMT3A-HP1b, DNMT3A-MBD1, DNMT3A-MBD2, DNMT3A-KRAB, DNMT3A-NIPP1, or DNMT3A-MeCP2. In an aspect of the disclosed method, a disclosed fusion or disclosed fusion product can comprise KRAB-MeCP2.

[0385] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide HP1a may comprise a sequence as set forth in any one of SEQ ID NO: 59 or SEQ ID NO: 60, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide HP1a may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 59 or

SEQ ID NO: 60, or a fragment thereof. In an aspect, a disclosed polypeptide HP1a can comprise the amino acid sequence as set forth in SEQ ID NO: 44 or SEQ ID NO: 45, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide HP1a can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 44 or SEQ ID NO: 45, or a fragment thereof.

[0386] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide HP1b may comprise a sequence as set forth in any one of SEQ ID NO: 61 or SEQ ID NO: 62, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide HP1b may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 61 or SEQ ID NO: 62, or a fragment thereof. In an aspect, a disclosed polypeptide HP1b can comprise the amino acid sequence as set forth in SEQ ID NO: 46 or SEQ ID NO: 47, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide HP1b can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 46 or SEQ ID NO: 47, or a fragment thereof.

[0387] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide MBD1 may comprise a sequence as set forth in any one of SEQ ID NO: 63 or SEQ ID NO: 64, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide MBD1 may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 63 or SEQ ID NO: 64, or a fragment thereof. In an aspect, a disclosed polypeptide MBD1 can comprise the amino acid sequence as set forth in any one of SEQ ID NO: 48-50, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide MDB1 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in any one of SEQ ID NO: 48-50, or a fragment thereof.

[0388] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide MBD2 may comprise a sequence as set forth in any one of SEQ ID NO: 65 or SEQ ID NO: 66, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide MBD2 may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at

least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 65 or SEQ ID NO: 66, or a fragment thereof. In an aspect, a disclosed polypeptide MBD2 can comprise the amino acid sequence as set forth in SEQ ID NO: 51 or SEQ ID NO: 52, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide MBD2 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 51 or SEQ ID NO: 52, or a fragment thereof.

[0389] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide NIPP1 may comprise a sequence as set forth in any one of SEQ ID NO: 67 or SEQ ID NO: 68, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide NIPP1 may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 67 or SEQ ID NO: 68, or a fragment thereof. In an aspect, a disclosed polypeptide NIPP1 can comprise the amino acid sequence as set forth in SEQ ID NO: 53 or SEQ ID NO: 54, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide NIPP1 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 53 or SEQ ID NO: 54, or a fragment thereof.

[0390] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide DNMT3A may comprise a sequence as set forth in SEQ ID NO: 69, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide DNMT3A may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 69, or a fragment thereof. In an aspect, a disclosed polypeptide DNMT3A can comprise the amino acid sequence as set forth in SEQ ID NO: 55, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide DNMT3A can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 55, or a fragment thereof.

[0391] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide KRAB may comprise a sequence as set forth in any one of SEQ ID NO: 70 or SEQ ID NO: 71, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide KRAB may

comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 70 or SEQ ID NO: 71, or a fragment thereof. In an aspect, a disclosed polypeptide KRAB can comprise the amino acid sequence as set forth in SEQ ID NO: 56, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide KRAB can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 56, or a fragment thereof.

[0392] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide KRAB may comprise a sequence as set forth in any one of SEQ ID NO: 70 or SEQ ID NO: 71, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide KRAB may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 70 or SEQ ID NO: 71, or a fragment thereof. In an aspect, a disclosed polypeptide KRAB can comprise the amino acid sequence as set forth in SEQ ID NO: 56, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide KRAB can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 56, or a fragment thereof.

[0393] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide MeCP2 may comprise a sequence as set forth in any one of SEQ ID NO: 72, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide MeCP2 may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequences set forth in SEQ ID NO: 72, or a fragment thereof. In an aspect, a disclosed polypeptide MeCP2 can comprise the amino acid sequence as set forth in SEQ ID NO: 57, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide MeCP2 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 57, or a fragment thereof.

[0394] In an aspect, the nucleic acid sequence encoding the disclosed fusion polypeptide KRAB-MeCP2 may comprise a sequence as set forth in any one of SEQ ID NO: 73 or SEQ ID NO: 74, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide KRAB-MeCP2 may comprise a nucleic acid sequence having at least 60% at least

65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequences set forth in SEQ ID NO: 73 or SEQ ID NO: 74, or a fragment thereof. In an aspect, a disclosed polypeptide KRAB-MeCP2 can comprise the amino acid sequence as set forth in SEQ ID NO: 58, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide KRAB-MeCP2 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 58, or a fragment thereof.

[0395] In an aspect, a disclosed encoded polypeptide can comprise transcription activation activity. In an aspect, a disclosed encoded polypeptide comprising transcription activation activity can comprise a transactivation domain. In an aspect, a disclosed transactivation domain can comprise a VP16 protein, a series of linked VP16 proteins, a p65 domain of NFκB, or any combination thereof.

[0396] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide VP16 may comprise a sequence as set forth in any one of SEQ ID NO: 132 or SEQ ID NO: 133, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide VP16 may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequences set forth in SEQ ID NO: 132 or SEQ NO: 133, or a fragment thereof. In an aspect, a disclosed polypeptide VP16 can comprise the amino acid sequence as set forth in SEQ ID NO: 131, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide VP16 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 131, or a fragment thereof.

[0397] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide p65 domain of NFκB or RELA may comprise a sequence as set forth in any one of SEQ ID NO: 135 or SEQ ID NO: 136, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide p65 domain of NFκB or RELA may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequences set forth in SEQ ID NO: 135 or SEQ ID NO: 136, or a fragment thereof. In an aspect, a disclosed polypeptide p65 domain of NFκB or RELA can comprise the amino acid sequence as set forth in SEQ ID NO: 134, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide p65 domain of NFκB or RELA can comprise a sequence having at least 60%, at least 65%, at least

70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 134, or a fragment thereof.

[0398] In an aspect, a disclosed encoded polypeptide can comprise transcription repression activity. In an aspect, a disclosed encoded polypeptide comprising transcription repression activity can comprise a Krüppel associated box domain, an ERF repressor domain, a MXI1 repressor domain, a SID4x repressor domain, a fused KRAB-MeCP2 domain, a MeCP2 TRD domain, a MAS-SID repressor domain, a TATA box binding protein activity, or any combination thereof. In an aspect, a disclosed encoded polypeptide comprising transcription repression activity can comprise HP1 repressor activity, MeCP2 repressor activity, MBD1 repressor activity, MBD2 repressor activity, MBD3 repressor activity, MBD4 repressor activity, KRAB repressor activity, SUV39H1 repressor activity, SUV39H2 repressor activity, CTCF insulator-repressor activity, LSD-1 histone-demethylase repressor activity, or any combination thereof.

[0399] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide comprising Eukaryotic Release Factor 1 (ERF1) activity or Eukaryotic Release Factor 3 (ERF3) activity may comprise a sequence as set forth in any one of SEQ ID NO: 138, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide comprising ERF1 activity or ERF3 activity may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequences set forth in SEQ ID NO: 138, or a fragment thereof. In an aspect, a disclosed polypeptide comprising ERF1 activity or ERF3 activity can comprise the amino acid sequence as set forth in SEQ ID NO: 137, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide comprising ERF1 activity or ERF3 activity can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 137, or a fragment thereof.

[0400] In an aspect of a disclosed method, a disclosed encoded polypeptide can comprise histone modulation activity. A disclosed encoded polypeptide comprising histone modulation activity can comprise histone acetyltransferase, histone deacetylase, histone demethylase, histone methyltransferase activity, or any combination thereof.

[0401] In an aspect of a disclosed method, a disclosed encoded polypeptide can comprise nucleic acid association activity. A disclosed encoded polypeptide comprising nucleic acid association activity can comprise a helix-turn-helix region, a leucine zipper region, a winged helix region, a winged helix-turn-helix region, a helix-loop-helix region, an immunoglobulin fold, a B3 domain, a zinc finger, a HMG-box, a Wor3 domain, a TAL effector DNA-binding domain, or any

combination thereof. In an aspect of a disclosed method, a disclosed encoded polypeptide can comprise methyltransferase activity. A disclosed encoded polypeptide comprising methyltransferase activity can comprise DNA (cytosine-5)-methyltransferase 3a (DNMT3A).

[0402] In an aspect of a disclosed method, a disclosed encoded polypeptide can comprise demethylase activity. A disclosed encoded polypeptide comprising demethylase activity can comprise ten-eleven translocation methylcytosine dioxygenase 1 (TET1) or lysine-specific histone demethylase 1 (LSD1).

[0403] In an aspect of a disclosed method, a disclosed encoded polypeptide can comprise acetyltransferase activity. A disclosed encoded polypeptide comprising acetyltransferase activity can comprise histone acetyltransferase. In In an aspect of a disclosed method, a disclosed encoded polypeptide can comprise deacetylase activity. A disclosed encoded polypeptide comprising deacetylase activity can comprise histone deacetylase.

[0404] In an aspect of a disclosed method, a disclosed dCas endonuclease can comprise dSpCas9 and a disclosed encoded polypeptide can comprise HP1a, HP1b, MBD1, MBD2, Krüppel-associated box (KRAB), NIPP1, the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof. In an aspect, a disclosed dCas endonuclease can comprise dSaCas9 and a disclosed encoded polypeptide can comprise HP1a, HP1b, MBD1, MBD2, Krüppel-associated box (KRAB), NIPP1, the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof. In an aspect, a disclosed dCas endonuclease can comprise dCjCas9 and a disclosed polypeptide can comprise HP1a, HP1b, MBD1, MBD2, Krüppel-associated box (KRAB), NIPP1, the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof.

[0405] In an aspect of a disclosed method, a disclosed dCas endonuclease can comprise dVQR, dEQR, or dVRER and a disclosed encoded polypeptide can comprise HP1a, HP1b, MBD1, MBD2, Krüppel-associated box (KRAB), NIPP1, the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof.

[0406] In an aspect of a disclosed method, a disclosed dCas endonuclease can comprise dSpCas9, dSaCas9, or dCjCas9 and a disclosed encoded polypeptide can comprise KRAB-MeCP2.

[0407] In an aspect of a disclosed method, a disclosed dCas endonuclease can comprise dSpCas9, dSaCas9, or dCjCas9, and a disclosed fusion can comprise HP1a-HP1a, HP1a-HP1b, HP1a-MBD1, HP1a-MBD2, HP1a-KRAB, HP1a-NIPP1, HP1a-MeCP2, HP1a-DNMT3A, HP1b-HP1b, HP1b-HP1a, HP1b-MBD1, HP1b-MBD2, HP1b-KRAB, HP1b-NIPP1, HP1b-MeCP2, HP1b-

DNMT3A, MBD1-MBD1, MBD1-HP1a, MBD1-HP1b, MBD1-MBD2, MBD1-KRAB, MBD1-NIPP1, MBD1-MeCP2, MBD1-DNMT3A, MBD2-MBD2, MBD2-HP1a, MBD2-HP1b, MBD2-MBD1, MBD2-KRAB, MBD2-NIPP1, MBD2-MeCP2, MBD2-DNMT3A, KRAB-KRAB, KRAB-HP1a, KRAB-HP1b, KRAB-MBD1, KRAB-MBD2, KRAB-NIPP1, KRAB-MeCP2, KRAB-DNMT3A, NIPP1-NIPP1, NIPP1-HP1a, NIPP1-HP1b, NIPP1-MBD1, NIPP1-MBD2, NIPP1-KRAB, NIPP1-MeCP2, NIPP1-DNMT3A, MeCP2-MeCP2, MeCP2-HP1a, MeCP2-HP1b, MeCP2-MBD1, MeCP2-MBD2, MeCP2-KRAB, MeCP2-NIPP1, MeCP2-DNMT3A, DNMT3A-DNMT3A, DNMT3A-HP1a, DNMT3A-HP1b, DNMT3A-MBD1, DNMT3A-MBD2, DNMT3A-KRAB, DNMT3A-NIPP1, or DNMT3A-MeCP2.

[0408] In an aspect of a disclosed method, an isolated nucleic acid or viral vector can encode for a dCas endonuclease fused to the at least one encoded polypeptide having an enzymatic activity. In these or related aspects, the isolated nucleic acid can encode for a "fusion protein" wherein the fusion protein comprises a first component comprising a dCas endonuclease and a second component comprising at least one polypeptide having an enzymatic activity. In an aspect, the encoded fusion protein can comprise a dSpCas9 and a polypeptide having enzymatic activity comprising HP1a, HP1b, MBD1, MBD2, Krüppel-associated box (KRAB), NIPP1, the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof. In an aspect, the encoded fusion protein can comprise a dSaCas9 and a polypeptide having enzymatic activity comprising HP1a, HP1b, MBD1, MBD2, Krüppel-associated box (KRAB), NIPP1, the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof. In an aspect, the encoded fusion protein can comprise a dCjCas9 and a polypeptide having enzymatic activity comprising HP1a, HP1b, MBD1, MBD2, Krüppel-associated box (KRAB), NIPP1, the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof. In an aspect, the encoded fusion protein can comprise dVQR, dEQR, or dVRER and a polypeptide having enzymatic activity comprising HP1a, HP1b, MBD1, MBD2, Krüppel-associated box (KRAB), NIPP1, the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof.

[0409] In an aspect, the encoded fusion protein can comprise dSpCas9, dSaCas9, dCjCas9, or a variant dCas9 (*e.g.*, dVQR, dEQR or dVRER) and one or more of HP1a-HP1a, HP1a-HP1b, HP1a-MBD1, HP1a-MBD2, HP1a-KRAB, HP1a-NIPP1, HP1a-MeCP2, HP1a-DNMT3A, HP1b-HP1b, HP1b-HP1a, HP1b-MBD1, HP1b-MBD2, HP1b-KRAB, HP1b-NIPP1, HP1b-MeCP2, HP1b-DNMT3A, MBD1-MBD1, MBD1-HP1a, MBD1-HP1b, MBD1-MBD2, MBD1-KRAB,

MBD1-NIPP1, MBD1-MeCP2, MBD1-DNMT3A, MBD2-MBD2, MBD2-HP1a, MBD2-HP1b, MBD2-MBD1, MBD2-KRAB, MBD2-NIPP1, MBD2-MeCP2, MBD2-DNMT3A, KRAB-KRAB, KRAB-HP1a, KRAB-HP1b, KRAB-MBD1, KRAB-MBD2, KRAB-NIPP1, KRAB-MeCP2, KRAB-DNMT3A, NIPP1-NIPP1, NIPP1-HP1a, NIPP1-HP1b, NIPP1-MBD1, NIPP1-MBD2, NIPP1-KRAB, NIPP1-MeCP2, NIPP1-DNMT3A, MeCP2-MeCP2, MeCP2-HP1a, MeCP2-HP1b, MeCP2-MBD1, MeCP2-MBD2, MeCP2-KRAB, MeCP2-NIPP1, MeCP2-DNMT3A, DNMT3A-DNMT3A, DNMT3A-HP1a, DNMT3A-HP1b, DNMT3A-MBD1, DNMT3A-MBD2, DNMT3A-KRAB, DNMT3A-NIPP1, or DNMT3A-MeCP2. In an aspect, the encoded fusion protein can comprise a variant dCas9 selected from dVQR, dEQR, or dVRER and a fusion comprising KRAB-MeCP2. In an aspect, the encoded fusion protein can comprise a dCas9 selected from dSpCas9, dSaCas9, or dCjCas9 and a fusion comprising KRAB-MeCP2. In an aspect, the encoded fusion protein can comprise a dCas9 selected from dSpCas9, dSaCas9, or dCjCas9 and a fusion comprising KRAB-MeCP2. In an aspect, the encoded fusion protein can comprise a dSaCas9 and KRAB-MeCP2. In an aspect, the encoded fusion protein can comprise a dCas9 selected from dSpCas9, dSaCas9, or dCjCas9 and DNMTA. In an aspect, the encoded fusion protein can comprise a dSaCas9 and DNMTA.

[0410] In an aspect, the nucleic acid sequence encoding the fusion protein comprising dSpCas9 and DNMT3A or dVRER and DNMT3A may comprise a sequence as set forth in SEQ ID NO: 75 or SEQ ID NO: 76 respectively, or a derivative thereof. In an aspect, the nucleic acid sequence encoding the fusion protein comprising dSpCas9 and DNMT3A or dVRER and DNMT3A may comprise a sequence as set forth in SEQ ID NO: 77 or SEQ ID NO: 78 respectively, or a derivative thereof. In an aspect, the nucleic acid sequence encoding the fusion protein comprising dSpCas9 and DNMT3A may comprise a sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any of the sequences set forth in SEQ ID NO: 77 or SEQ ID NO: 78, or a fragment thereof. In an aspect, a disclosed polypeptide dSpCas9 can comprise the amino acid sequence as set forth in SEQ ID NO: 75 or SEQ ID NO: 76, or a fragment, or a derivative thereof.

[0411] In an aspect of a disclosed method, a disclosed gene of interest or portion thereof can demonstrate a reduced expression level when compared to wild-type or control expression level. In an aspect of a disclosed method, a disclosed gRNA can targes a gene of interest or portion thereof that demonstrates a reduced expression level when compared to wild-type or control expression level. In an aspect of a disclosed method, a disclosed gene of interest or portion thereof can demonstrate an increased expression level when compared to wild-type or control expression

level. In an aspect of a disclosed method, a disclosed gRNA can target a gene of interest or portion thereof that demonstrates an increased expression level when compared to wild-type or control expression level.

[0412] In an aspect of a disclosed method, a disclosed gRNA targeting a gene of interest or portion thereof can comprise at least two gRNAs. In an aspect, disclosed gRNAs can target the same gene of interest or portion thereof. In an aspect, disclosed gRNAs can target different genes of interest or portions thereof. In an aspect of a disclosed method, a disclosed gRNA can target a gene or portion thereof that demonstrates a loss of function. In an aspect, a disclosed gRNA can target a gene or portion thereof that demonstrates a gain of function. Disclosed target genes and disclosed genes of interest are discussed supra.

[0413] In an aspect of a disclosed method, a disclosed gRNA can target the *APOE* gene. In an aspect, the at least one gRNA may target a region in the *APOE* gene comprising a nucleic acid sequence as set forth in any one of SEQ ID NO: 11-14, or a complement thereof. In an aspect, the at least one gRNA targets a nucleic acid sequence comprising at least 10 consecutive nucleotides as set forth in SEQ ID NO: 11-14, or a complement thereof. In an aspect, a disclosed gRNA can target the promoter of the APOE gene. In an aspect, a disclosed gRNA can target exon 1 of the APOE gene. In an aspect, a disclosed gRNA can target exon 1 of the APOE gene. In an aspect, a disclosed gRNA can target intron 1 of the APOE gene. In an aspect, a disclosed gRNA can target intron 4 of the APOE gene. In an aspect, a disclosed gRNA can target a CpG island region of intron 1 of the APOE gene. The CpG island region can include CpG1, CpG2, CpG3, CpG4, CpG5, CpG6, CpG7, CpG8, CpG9, CpG10, CpG11, CpG12, CpG13, CpG14, CpG15, CpG16, CpG17, CpG18, CpG19, CpG20, CpG21, CpG22, CpG23, or a combination thereof. For example, the CpG island region can include CpG1, CpG3, CpG6, CpG7, CpG8, CpG9, CpG18, CpG19, CpG20, CpG21, CpG22, or a combination thereof. In an aspect, the disclosed gRNA may comprise a nucleic acid sequence as set forth in SEQ ID NO: 15-33. In an aspect, the disclosed gRNA may comprise a nucleic acid sequence as set forth in SEQ ID NO: 15-33, or a complement thereof. In an aspect, the disclosed gRNA may comprise a nucleic acid sequence with at least about 90%, or about 91%, or about 92%, or about 93%, or about 94%, or about 95%, or about 96%, or about 97%, or about 98%, or about 99%, or more sequence identity to any one of the sequences set forth in SEQ ID NO: 15-33, or a complement thereof, or a fragment thereof.

[0414] In an aspect of a disclosed method, a disclosed viral vector or disclosed recombinant AAV vector can further comprise a gRNA scaffold. In an aspect, a disclosed gRNA scaffold can comprise the sequence set forth in SEQ ID NO: 129, SEQ ID NO: 130, or a fragment thereof.

[0415] In an aspect of a disclosed method, a disclosed isolated nucleic acid or disclosed viral vector comprises one or more microglia specific regulatory elements. In an aspect, the one or more microglia specific regulatory elements can comprise a microglia specific wild-type promoter, a microglia specific enhancer, a synthetic promoter, a synthetic enhancer, a transcriptional or translational regulatory sequence or any combination thereof. In an aspect, the one or more microglia specific regulatory elements comprise at least one microglia specific promoter (*e.g.*, a microglia specific wild-type promoter or a microglia specific synthetic promoter). In an aspect, the microglia specific regulatory element (*e.g.*, promoter) may be specific for a certain type of microglial cell (*e.g.*, a dopaminergic or a cholinergic microglial cell). In these instances, the microglia specific regulatory element may include a dopaminergic specific regulatory element (*i.e.*, a dopaminergic promoter) and/or a cholinergic microglia specific regulatory element (*i.e.*, a cholinergic promoter). In an aspect, the microglia specific regulatory element can comprise a microglia specific promoter. In an aspect, a disclosed microglia specific promoter can comprise a F4/80 promoter, CD68 promoter, FCGR2A promoter, HexB promoter or IBA1 promoter, a derivative thereof, a fragment thereof, or any combination thereof.

[0416] In an aspect, the promoter is a CD68 promoter, or a derivative thereof. In an aspect, the promoter may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one or more of the sequences set forth in SEQ ID NO: 1-3, or a fragment, or a derivative thereof. In an aspect, the promoter comprises a sequence as set forth in any one of SEQ ID NO: 1-3.

[0417] In an aspect, the promoter is a F4/80 promoter, or a derivative thereof. In an aspect, the promoter may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequence set forth in SEQ ID NO: 4, or a fragment, or a derivative thereof. In an aspect, the promoter comprises a sequence as set forth in SEQ ID NO: 4.

[0418] In an aspect, the promoter is a IBA1 promoter, or a derivative thereof. In an aspect, the promoter may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequence set forth in SEQ ID NO: 5-7, or a fragment, or a derivative thereof. In an aspect, the promoter comprises a sequence as set forth in any one of SEQ ID NO: 5-7.

[0419] In an aspect, the promoter is a FCGR2A promoter, or a derivative thereof. In an aspect, the promoter may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequence set forth in SEQ ID NO: 8, or a fragment, or a derivative thereof. In an aspect, the promoter comprises a sequence as set forth in SEQ ID NO: 8.

[0420] In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) can be operably linked to the dCas endonuclease and the fusion protein having one or more enzymatic activities. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the dCas endonuclease and the fusion protein having one or more enzymatic activities can comprise a F4/80 promoter, or a derivative thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the dCas endonuclease and the fusion protein having one or more enzymatic activities can comprise a CD68 promoter, or a derivative thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the dCas endonuclease and the fusion protein having one or more enzymatic activities can comprise a IBA1 promoter, or a derivative thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the dCas endonuclease and the fusion protein having one or more enzymatic activities can comprise a FCGR2A promoter, or a derivative thereof.

[0421] In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the dCas endonuclease can be operably linked to the at least one polypeptide having enzymatic activity. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the at least one polypeptide having enzymatic activity can comprise a F4/80promoter, a derivative thereof, or a fragment thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the at least one polypeptide having enzymatic activity can comprise a CD68 promoter, a derivative thereof, or a fragment thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the at least one polypeptide having enzymatic activity can comprise a IBA1 promoter, a derivative thereof, or a fragment thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to at least one polypeptide having enzymatic activity can comprise a FCGR2A promoter, a derivative thereof, or a fragment thereof.

[0422] In an aspect of a disclosed method, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) can be operably linked to the dCas endonuclease and the at least one polypeptide having enzymatic activity. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the dCas endonuclease and the at least one polypeptide having enzymatic activity can comprise a FGCR2A promoter, a derivative thereof, or a fragment thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the at dCas endonuclease and the at least one polypeptide having enzymatic activity can comprise a F4/80 promoter, a derivative thereof, or a fragment thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the dCas endonuclease and the at least one polypeptide having enzymatic activity can comprise a CD68 promoter, a derivative thereof, or a fragment thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the dCas endonuclease and the at least one polypeptide having enzymatic activity can comprise a IBA1 promoter, a derivative thereof, or a fragment thereof.

[0423] In an aspect of a disclosed method, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) can be operably linked to the fusion protein having one or more enzymatic activities. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the fusion protein having one or more enzymatic activities can comprise a F4/80 promoter, a derivative thereof, or a fragment thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the fusion protein having one or more enzymatic activities can comprise a CD68 promoter, a derivative thereof, or a fragment thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the fusion protein having one or more enzymatic activities can comprise a IBA1 promoter, a derivative thereof, or a fragment thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the fusion protein having one or more enzymatic activities can comprise a FCGR2A promoter, a derivative thereof, or a fragment thereof.

[0424] In an aspect of a disclosed method, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) can be operably linked to the dCas endonuclease and the fusion protein having one or more enzymatic activities. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the dCas endonuclease and the fusion protein having one or more enzymatic activities can comprise a F4/80 promoter, a derivative thereof, or a fragment thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the dCas endonuclease and the

fusion protein having one or more enzymatic activities can comprise a CD68 promoter, a derivative thereof, or a fragment thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.,* a microglia specific promoter) operably linked to the dCas endonuclease and the fusion protein having one or more enzymatic activities can comprise a IBA1 promoter, a derivative thereof, or a fragment thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.,* a microglia specific promoter) operably linked to the dCas endonuclease and the fusion protein having one or more enzymatic activities can comprise a FCGR2A promoter, a derivative thereof, or a fragment thereof.

[0425] In an aspect of a disclosed method, a disclosed isolated nucleic acid or disclosed viral vector can further comprise one or more regulatory elements (*i.e.*, promoters) in addition to the microglia specific regulatory element (*e.g.*, microglia specific promoter). In an aspect, a disclosed additional promoter can comprise a U6 promoter, a chicken β-actin promoter, an EF-1α, a CMV promoter, a CMV promoter/enhancer, a fragment thereof, a derivative thereof, or any combination thereof. In an aspect, a disclosed U6 promoter can comprise the sequence set forth in SEQ ID NO: 81, or a fragment thereof. In an aspect, a disclosed U6 promoter can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% identity to the sequence set forth in SEQ ID NO: 81 or a fragment thereof. In an aspect, a disclosed EF-1α promoter can comprise the sequence set forth in SEQ ID NO: 79, SEQ ID NO: 80, or a fragment thereof. In an aspect, a disclosed EF-1α promoter can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% identity to the sequence set forth in SEQ ID NO: 79, SEQ ID NO:80, or a fragment thereof. In an aspect, a promoter can be a short EF1alpha (EFS-NF) promoter.

[0426] In an aspect of a disclosed method, a disclosed promoter operably can be linked to the at least one guide RNA targeting a gene of interest or portion thereof. In an aspect, a disclosed promoter operably linked to the at least one guide RNA can comprise a U6 promoter, a derivative thereof, or a fragment thereof. In an aspect, a disclosed promoter operably linked to the at least one guide RNA targeting an *APOE* gene can comprise a U6 promoter, a derivative thereof, or a fragment thereof.

[0427] In an aspect of the disclosed method, a disclosed isolated nucleic acid molecule or viral vector can further two or more promoters, wherein a first promoter can be operably linked to the dCas endonuclease, a polypeptide having enzymatic activity, a fusion of two or more polypeptides having enzymatic activities or any combination thereof, and wherein a second promoter can be operably linked to the at least one guide RNA targeting a gene of interest or portion thereof. In an

aspect, a disclosed isolated nucleic acid molecule can further comprise one or more promoters, wherein a first promoter comprises a microglia specific promoter that is operably linked to the dCas endonuclease, a polypeptide having enzymatic activity, a fusion of two or more polypeptides having enzymatic activities or any combination thereof, and wherein a second promoter can be operably linked to the at least one guide RNA targeting a gene of interest thereof. In an aspect, the second promoter operably linked to the gRNA may not be a microglia specific promoter. In an aspect, the isolated nucleic acid can comprise a first promoter selected from a F4/80 promoter, a CD68 promoter, a IBA1 promoter, or a FCGR2A promoter, a fragment thereof, a derivative thereof, or any combination thereof, and a second promoter comprising a U6 promoter.

[0428] In an aspect of the disclosed method, a disclosed isolated nucleic acid molecule or viral vector can further comprise one or more additional regulatory elements. In an aspect, a disclosed additional regulatory element can comprise a promoter (described supra), an enhancer, a promoter/enhancer, an internal ribosomal entry site, a transcription terminal signal, a polyadenylation signal, a Sp1 and/or NF-kB transcriptional factor binding site, a p2A signal, a woodchuck hepatitis virus post-transcriptional regulatory element, a Phi signal-packaging signal, a rev responsive element, a 5'-ITR, a 3'-ITR, an inverted terminal repeat, a nuclear localization signal (NLS), or any combination thereof.

[0429] In an aspect of the disclosed method, a disclosed 5'-ITR may comprise the sequence as set forth in any one of SEQ ID NO: 82 or SEQ ID NO: 83. In an aspect, a disclosed 3'-ITR may comprise the sequence as set forth in any one of SEQ ID NO: 84 or SEQ ID NO: 85. In an aspect, a disclosed NLS can comprise the sequence set forth in any one of SEQ ID NO: 88 – SEQ ID NO: 94, or a fragment thereof. In an aspect, a disclosed PolyA sequence can comprise the sequence set forth in SEQ ID NO: 86, SEQ ID NO: 87, or a fragment thereof.

[0430] In an aspect of a disclosed method, a disclosed viral vector or isolated nucleic acid can comprise a nucleic acid that is CpG depleted and codon-optimized for expression in a human cell. In an aspect, "CpG-free" can mean completely free of CpGs or partially free of CpGs. In an aspect, "CpG-free" can mean "CpG-depleted." In an aspect, "CpG-depleted" can mean "CpG-free." In an aspect, "CpG-depleted" can mean completely depleted of CpGs or partially depleted of CpGs. In an aspect, "CpG-free" can mean "CpG-optimized" for a desired and/or ideal expression level. CpG depletion and/or optimization is known to the skilled person in the art. In an aspect, any disclosed Cas9 endonuclease, a disclosed polypeptide having enzymatic activities, a disclosed fusion product or a disclosed fusion protein, or any combination thereof can be codon-optimized.

[0431] In an aspect of a disclosed method, a disclosed viral vector or disclosed recombinant AAV vector can comprise a sequence that is about 4.5 kilobases or less than about 4.5 kilobases.

[0432] In an aspect of a disclosed method of effecting precision epigenetic modulation, modulating can comprise increasing expression and/or activity of one or more genes of interest. In an aspect of a disclosed method of effecting precision epigenetic modulation, modulating can comprise decreasing expression and/or activity of one or more genes of interest.

[0433] In an aspect, a disclosed method of effecting precision epigenetic modulation can comprise reducing expression and/or activity of APOE.

[0434] In an aspect, the disclosed cells can be microglial cells. In an aspect, the disclosed cells can be cells affected by an overexpression level and/or activity level of one or more genes of interest or one or more target genes.

[0435] In an aspect, the disclosed cells can be in a subject. In an aspect of a disclosed method, a subject can be a human. In an aspect, a subject can be suspected of having or can be diagnosed with having Alzheimer's disease. In an aspect, a subject can be suspected of having or can be diagnosed with having Parkinson's disease. In an aspect, a disclosed subject can be symptomatic or asymptomatic.

[0436] In an aspect, a disclosed method of effecting precision epigenetic modulation can comprise reducing the pathological phenotype associated with Alzheimer's disease. In an aspect, reducing the pathological phenotype associated with Alzheimer's disease can comprise reducing the number and/or amount of Tau aggregates. In an aspect, a disclosed method can comprise diagnosing the subject with Alzheimer's disease.

[0437] In an aspect, of a disclosed method of effecting precision epigenetic modulation can comprise reducing the pathological phenotype associated with Parkinson's disease. In an aspect, reducing the pathological phenotype associated with Parkinson's disease can comprise reducing the number and/or amount of Tau aggregates. In an aspect, a disclosed method can comprise diagnosing the subject with Parkinson's disease.

[0438] In an aspect of a disclosed method of effecting precision epigenetic modulation, by knowing what disease or disorder is affecting the subject, the skilled person can identify the relevant gene or genes. In an aspect, a subject can be a subject having a disease or disorder. In an aspect, a disease or disorder can be any disease or disorder disclosed herein, for example, characterized by an overexpression and/or over-activity or characterized by a loss of function.

[0439] In an aspect, a subject can be a subject in need of treatment of a disclosed disease or disorder (*e.g.*, a genetic disease or disorder).

[0440] In an aspect, a disclosed method of effecting precision epigenetic modulation can restore the functionality and/or structural integrity of a missing, deficient, and/or mutant protein or enzyme. In an aspect, a disclosed method of effecting precision epigenetic modulation can

comprise restoring one or more aspects of cellular homeostasis and/or cellular functionality and/or metabolic dysregulation.

[0441] In an aspect, restoring one or more aspects of cellular homeostasis and/or cellular functionality and/or metabolic dysregulation can comprise reducing the expression and/or activity level of at least one gene of interest or at least one target gene. In an aspect, a gene of interest can be a gene that demonstrates over expression and/or over activity.

[0442] In an aspect, restoring one or more aspects of cellular homeostasis and/or cellular functionality and/or metabolic dysregulation can comprise increasing the expression and/or activity level of at least one gene of interest or at least one target gene. In an aspect, a gene of interest can be a gene that demonstrates a loss of function or reduced expression and/or activity level.

[0443] In an aspect, restoring one or more aspects of cellular homeostasis and/or cellular functionality can comprise one or more of the following: (i) correcting cell starvation in one or more cell types; (ii) normalizing aspects of the autophagy pathway (such as, for example, correcting, preventing, reducing, and/or ameliorating autophagy); (iii) improving, enhancing, restoring, and/or preserving mitochondrial functionality and/or structural integrity; (iv) improving, enhancing, restoring, and/or preserving organelle functionality and/or structural integrity; (v) correcting enzyme dysregulation; (vi) reversing, inhibiting, preventing, stabilizing, and/or slowing the rate of progression of the multi-systemic manifestations of a genetic disease or disorder; (vii) reversing, inhibiting, preventing, stabilizing, and/or slowing the rate of progression of a genetic disease or disorder, or (viii) any combination thereof.

[0444] In an aspect, restoring one or more aspects of cellular homeostasis can comprise improving, enhancing, restoring, and/or preserving one or more aspects of cellular structural and/or functional integrity.

[0445] In an aspect, restoring the activity and/or functionality of a missing, deficient, and/or mutant protein or enzyme can comprise a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or any amount of restoration when compared to a pre-existing level such as, for example, a pre-treatment level. In an aspect, the amount of restoration can be 10-20%, 20-30%, 30-40%, 40-50%, 50-60%, 60-70%, 70-80%, 80-90%, or 90-100% more than a pre-existing level such as, for example, a pre-treatment level. In an aspect, restoration can be measured against a control level or a reference level (*e.g.*, determined, for example, using one or more subjects not having a missing, deficient, and/or mutant protein or enzyme). In an aspect, restoration can be a partial or incomplete restoration. In an aspect, restoration can be complete or near complete restoration such that the level of expression, activity, and/or functionality is like that of a wild-type or control level.

[0446] In an aspect, restoring the activity and/or functionality of a gene of interest can comprise increasing or enhancing the expression and/or activity level of that gene. In an aspect, increasing or enhancing can comprise an elevation of at least about 5%, 10%, 15%, 20%, 25%, 35%, 50%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, 100%, 200%, 300%, 400%, 500%, or more as compared to a control (such as a pre-treatment level). In an aspect, restoring the activity and/or functionality of a gene of interest can comprise decreasing or reducing the expression and/or activity level of that gene. In an aspect, decreasing or reducing can comprise a decrease of at least about 5%, 10%, 15%, 20%, 25%, 35%, 50%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, 100%, 200%, 300%, 400%, 500%, or more as compared to a control (such as a pre-treatment level).

[0447] In an aspect of a disclosed method of effecting precision epigenetic modulation, techniques to monitor, measure, and/or assess the restoring one or more aspects of cellular homeostasis and/or cellular functionality can comprise qualitative (or subjective) means as well as quantitative (or objective) means. These means are known to the skilled person. For example, representative regulated variables and sensors relating to systemic homeostasis are discussed supra.

[0448] In an aspect of a disclosed method of effecting precision epigenetic modulation, administering can comprise intravenous administration, intracerebral administration, intra-CSF administration, intracerebroventricular (ICV) administration, intraventricular administration, intra-cisterna magna (ICM) administration, intraparenchymal administration, intrathecal (lumbar, cisternal, or both) administration, intrahepatic administration, hepatic intra-arterial administration, hepatic portal vein (HPV) administration, or any combination thereof. In an aspect, a disclosed vector can be administered via LNP administration.

[0449] In an aspect of a disclosed method of effecting precision epigenetic modulation, administering can comprise use of non-viral vectors to deliver a disclosed isolated nucleic acid. These non-viral vectors can include, but are not limited to, nanoparticles or liposomes. Alternatively, the disclosed isolated nucleic acids may be delivered via viral vectors (such as the disclosed viral vectors provided herein). Exemplary viral vectors are described supra and include adenovirus, adeno-associated virus (AAV), and lentivirus (LV). Viral vectors are used more commonly due to their robust delivery, long-lasting expression, low immunogenicity and toxicity, and compatibility with the advanced manufacturing techniques. Most research in gene therapy has utilized AAV or LV due to their lowered immunogenicity compared to adenovirus. Derived from HIV, LV integrates its viral genome into the host gene to create permanent change in gene expression.

[0450] In an aspect, a disclosed method of effecting precision epigenetic modulation can employ multiple routes of administration to the subject. In an aspect, a disclosed method can employ a

first route of administration that can be the same or different as a second and/or subsequent routes of administration. In an aspect, a disclosed nucleic acid molecule, a disclosed vector, and/or a disclosed pharmaceutical formulation can be concurrently and/or serially administered to a subject via multiple routes of administration. For example, in an aspect, administering a disclosed nucleic acid molecule, a disclosed vector, and/or a disclosed pharmaceutical formulation can comprise intravenous administration and intra-cistern magna (ICM) administration. In an aspect, administering a disclosed nucleic acid molecule, a disclosed vector, and/or a disclosed pharmaceutical formulation can comprise IV administration and intrathecal (ITH) administration.

[0451] In an aspect of a disclosed method of effecting precision epigenetic modulation, a therapeutically effective amount of disclosed vector can be delivered via intravenous (IV) administration and can comprise a range of about 1 x 10^{10} vg/kg to about 2 x 10^{14} vg/kg. In an aspect, for example, a disclosed vector can be administered at a dose of about 1 x 10^{11} vg/kg to about 8 x 10^{13} vg/kg or about 1 x 10^{12} vg/kg to about 8 x 10^{13} vg/kg. In an aspect, a disclosed vector can be administered at a dose of about 1 x 10^{13} vg/kg to about 6 x 10^{13} vg/kg. In an aspect, a disclosed vector can be administered at a dose of at least about 1 x 10^{10} vg/kg, at least about 5 x 10^{10} vg/kg, at least about 1 x 10^{11} vg/kg, at least about 5 x 10^{11} vg/kg, at least about 1 x 10^{12} vg/kg, at least about 5 x 10^{12} vg/kg, at least about 1 x 10^{13} vg/kg, at least about 5 x 10^{13} vg/kg, or at least about 1 x 10^{14} vg/kg. In an aspect, a disclosed vector can be administered at a dose of no more than about 1 x 10^{10} vg/kg, no more than about 5 x 10^{10} vg/kg, no more than about 1 x 10^{11} vg/kg, no more than about 5 x 10^{11} vg/kg, no more than about 1 x 10^{12} vg/kg, no more than about 5 x 10^{12} vg/kg, no more than about 1 x 10^{13} vg/kg, no more than about 5 x 10^{13}, or no more than about 1 x 10^{14} vg/kg. In an aspect, a disclosed vector can be administered at a dose of about 1 x 10^{12} vg/kg. In an aspect, a disclosed vector can be administered at a dose of about 1 x 10^{11} vg/kg. In an aspect, a disclosed vector can be administered in a single dose, or in multiple doses (such as 2, 3, 4, 5, 6, 7, 8, 9 or 10 doses) as needed for the desired therapeutic results.

[0452] In an aspect, a disclosed method of effecting precision epigenetic modulation can further comprise monitoring the subject for adverse effects. In an aspect, in the absence of adverse effects, the method can further comprise continuing to treat the subject. In an aspect, in the presence of adverse effects, the method can further comprise modifying the treating step. Methods of monitoring a subject's well-being can include both subjective and objective criteria (and are discussed supra). Such methods are known to the skilled person.

[0453] In an aspect, a disclosed method of effecting precision epigenetic modulation can further comprise administering to the subject a therapeutically effective amount of a therapeutic agent. A therapeutic agent can be any disclosed agent that effects a desired clinical outcome.

[0454] In an aspect, a disclosed method of effecting precision epigenetic modulation can further comprise administering to the subject a therapeutically effective amount of an agent that can correct one or more aspects of a dysregulated metabolic or enzymatic pathway. In an aspect, such an agent can comprise an enzyme for enzyme replacement therapy. In an aspect, a disclosed enzyme can replace any enzyme in a dysregulated or dysfunctional metabolic or enzymatic pathway. In an aspect, a disclosed method can comprise replacing one or more enzymes in a dysregulated or dysfunctional metabolic pathway.

[0455] In an aspect, a disclosed method of effecting precision epigenetic modulation can further comprise administering one or more immune modulators. In an aspect, a disclosed immune modulator can be methotrexate, rituximab, intravenous gamma globulin, or bortezomib, or a combination thereof. In an aspect, a disclosed immune modulator can be bortezomib or SVP-Rapamycin. In an aspect, a disclosed immune modulator can be Tacrolimus. In an aspect, a disclosed immune modulator such as methotrexate can be administered at a transient low to high dose. In an aspect, a disclosed immune modulator can be administered at a dose of about 0.1 mg/kg body weight to about 0.6 mg/kg body weight. In an aspect, a disclosed immune modulator can be administered at a dose of about 0.4 mg/kg body weight. In an aspect, a disclosed immune modulator can be administered at about a daily dose of 0.4 mg/kg body weight for 3 to 5 or greater cycles, with up to three days per cycle. In an aspect, a disclosed immune modulator can be administered at about a daily dose of 0.4 mg/kg body weight for a minimum of 3 cycles, with three days per cycle. In an aspect, a person skilled in the art can determine the appropriate number of cycles. In an aspect, a disclosed immune modulator can be administered as many times as necessary to achieve a desired clinical effect.

[0456] In an aspect, a disclosed immune modulator can be administered orally about one hour before a disclosed therapeutic agent. In an aspect, a disclosed immune modulator can be administered subcutaneously about 15 minutes before a disclosed therapeutic agent. In an aspect, a disclosed immune modulator can be administered concurrently with a disclosed therapeutic agent. In an aspect, a disclosed immune modulator can be administered orally about one hour or a few days before a disclosed isolated nucleic acid molecule, a disclosed vector, a disclosed pharmaceutical formulation, or a combination thereof. In an aspect, a disclosed immune modulator can be administered subcutaneously about 15 minutes before or a few days before a disclosed isolated nucleic acid molecule, a disclosed vector, a disclosed pharmaceutical formulation, or a combination thereof. In an aspect, a disclosed immune modulator can be administered concurrently with a disclosed isolated nucleic acid molecule, a disclosed vector, a disclosed pharmaceutical formulation, or a combination thereof.

[0457] In an aspect, a disclosed method of effecting precision epigenetic modulation can further comprise administering one or more proteasome inhibitors (*e.g.*, bortezomib, carfilzomib, marizomib, ixazomib, and oprozomib). In an aspect, a proteasome inhibitor can be an agent that acts on plasma cells (*e.g.*, daratumumab). In an aspect, an agent that acts on a plasma cell can be melphalan hydrochloride, melphalan, pamidronate disodium, carmustine, carfilzomib, carmustine, cyclophosphamide, daratumumab, doxorubicin hydrochloride liposome, doxorubicin hydrochloride liposome, elotuzumab, melphalan hydrochloride, panobinostat, ixazomib citrate, carfilzomib, lenalidomide, melphalan, melphalan hydrochloride, plerixafor, ixazomib citrate, pamidronate disodium, panobinostat, plerixafor, pomalidomide, pomalidomide, lenalidomide, selinexor, thalidomide, thalidomide, bortezomib, selinexor, zoledronic acid, or zoledronic acid.

[0458] In an aspect, a disclosed method of effecting precision epigenetic modulation can further comprise administering one or more proteasome inhibitors or agents that act on plasma cells prior to administering a disclosed isolated nucleic acid molecule, a disclosed vector, or a disclosed pharmaceutical formulation. In an aspect, a disclosed method can comprise administering one or more proteasome inhibitors or one or more agents that act on plasma cells concurrently with administering a disclosed isolated nucleic acid molecule, a disclosed vector, or a disclosed pharmaceutical formulation. In an aspect, a disclosed method can comprise administering one or more proteasome inhibitors or one or more agents that act on plasma cells subsequent to administering a disclosed isolated nucleic acid molecule, a disclosed vector, or a disclosed pharmaceutical formulation. In an aspect, a disclosed method can further comprise administering one or more proteasome inhibitors more than 1 time. In an aspect, a disclosed method can comprise administering one or more proteasome inhibitors repeatedly over time.

[0459] In an aspect, a disclosed method of effecting precision epigenetic modulation can further comprise administering one or more immunosuppressive agents. In an aspect, an immunosuppressive agent can be, but is not limited to, azathioprine, methotrexate, sirolimus, anti-thymocyte globulin (ATG), cyclosporine (CSP), mycophenolate mofetil (MMF), steroids, or a combination thereof. In an aspect, a disclosed method can comprise administering one or more immunosuppressive agents more than 1 time. In an aspect, a disclosed method can comprise administering one or more one or more immunosuppressive agents repeatedly over time. In an aspect, a disclosed method can comprise administering a compound that targets or alters antigen presentation or humoral or cell mediated or innate immune responses.

[0460] In an aspect, a disclosed method of effecting precision epigenetic modulation can further comprise administering a compound that exerts a therapeutic effect against B cells and/or a compound that targets or alters antigen presentation or humoral or cell mediated immune response.

In an aspect, a disclosed compound can be rituximab, methotrexate, intravenous gamma globulin, anti CD4 antibody, anti CD2, an anti-FcRN antibody, a BTK inhibitor, an anti-IGF1R antibody, a CD19 antibody (*e.g.*, inebilizumab), an anti-IL6 antibody (*e.g.*, tocilizumab), an antibody to CD40, an IL2 mutein, or a combination thereof. Also disclosed herein are Treg infusions that can be administered as a way to help with immune tolerance (*e.g.*, antigen specific Treg cells to AAV).

[0461] In an aspect of a disclosed method, a subject can be a human. In an aspect, a subject can be suspected of having or can be diagnosed with having Alzheimer's disease (such as, for example, PD). In an aspect, a disclosed subject can be symptomatic or asymptomatic.

[0462] In an aspect, a disclosed method can comprise repeating one or more steps of the method and/or modifying one or more steps of the method (such as, for example, an administering step).

[0463] In an aspect, a disclosed method of effecting precision epigenetic modulation can comprise modifying one or more of the disclosed steps. For example, modifying one or more of steps of a disclosed method can comprise modifying or changing one or more features or aspects of one or more steps of a disclosed method. For example, in an aspect, a method can be altered by changing the amount of one or more of the disclosed isolated nucleic acid molecules, disclosed vectors, disclosed pharmaceutical formulations, or a combination thereof administered to a subject, or by changing the frequency of administration of one or more of the disclosed isolated nucleic acid molecules, disclosed vectors, disclosed pharmaceutical formulations, or a combination thereof to a subject, or by changing the duration of time one or more of the disclosed isolated nucleic acid molecules, disclosed vectors, disclosed pharmaceutical formulations, or a combination are administered to a subject.

[0464] In an aspect, a disclosed method of effecting precision epigenetic modulation can be altered by changing the amount of one or more disclosed therapeutic agents, disclosed immune modulators, disclosed proteasome inhibitors, disclosed immunosuppressive agents, disclosed compounds that exert therapeutic effect against B cells and/or disclosed compounds that targets or alters antigen presentation or humoral or cell mediated immune response administered to a subject, or by changing the frequency of administration of one or more of the disclosed therapeutic agents, disclosed immune modulators, disclosed proteasome inhibitors, disclosed immunosuppressive agents, disclosed compounds that exert therapeutic effect against B cells and/or disclosed compounds that targets or alters antigen presentation or humoral or cell mediated immune response administered to a subject.

[0465] In an aspect, a disclosed method of effecting precision epigenetic modulation can further comprise generating and/or validating one or more of the disclosed isolated nucleic acid

molecules, one or more of the disclosed vectors, one or more of the disclosed pharmaceutical formulations, or any combination thereof.

[0466] In an aspect, a disclosed method of effecting precision epigenetic modulation can further comprise contacting the cells with a second disclosed isolated nucleic acid molecule, a second disclosed vector, a second disclosed pharmaceutical formulations, or any combination thereof. In an aspect, a disclosed method of effecting precision epigenetic modulation can further comprise contacting the cells with additional disclosed isolated nucleic acid molecules, additional disclosed vectors, additional disclosed pharmaceutical formulations, or any combination thereof.

G. Methods of Treating and/or Preventing Alzheimer's and other APOE Associated Diseases or Disorders

[0467] Disclosed herein is a method of treating and/or preventing progression of an APOE associated disease or disorder, the method comprising administering to a subject a therapeutically effective amount of a disclosed isolated nucleic acid molecule, thereby reducing the pathological phenotype associated with the APOE associated disease or disorder. Disclosed herein is a method of treating and/or preventing an APOE associated disease or disorder. In an aspect, the method comprises administering to a subject a therapeutically effective amount of a disclosed viral vector, thereby reducing the pathological phenotype associated with the APOE associated disease or disorder.

[0468] Disclosed herein is a method of treating and/or preventing a tauopathy. In an aspect, the method comprises administering to a subject a therapeutically effective amount of a disclosed isolated nucleic acid molecule, thereby reducing the pathological phenotype associated with the tauopathy. Disclosed herein is a method of treating and/or preventing a tauopathy. In an aspect, the method comprises administering to a subject a therapeutically effective amount of a disclosed viral vector, thereby reducing the pathological phenotype associated with the tauopathy.

[0469] Disclosed herein is a method of treating and/or preventing progression of a tauopathy or other APOE associated disease or disorder in a subject, the method comprising administering to a subject a therapeutically effective amount of a disclosed isolated nucleic acid molecule, comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, (ii) at least one polypeptide having an enzymatic activity, and (iii) at least one guide RNA targeting a APOE gene or portion thereof and (b) at least one microglia specific regulatory element operably linked to (a)(i), (a)(ii), (a)(iii) or any combination thereof, thereby reducing the pathological phenotype associated with the Alzheimer's or other APOE associated disease or disorder. Disclosed herein is a method of treating and/or preventing progression of a Alzheimer's or other APOE associated disease or disorder in a subject, the method comprising administering to a

subject a therapeutically effective amount of a disclosed isolated nucleic acid molecule, comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, (ii) at least one polypeptide having an enzymatic activity, and (iii) at least one guide RNA targeting a APOE gene or portion thereof and (b) at least one microglia specific promoter operably linked to (a)(i), (a)(ii), (a)(iii) or any combination thereof, thereby reducing the pathological phenotype associated with the Alzheimer's or other APOE associated disease or disorder.

[0470] Disclosed herein is a method of treating and/or preventing progression of a Alzheimer's or other APOE associated disease or disorder in a subject, the method comprising administering to a subject a therapeutically effective amount of an isolated nucleic acid comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, (ii) a fusion product having one or more enzymatic activities, (iii) at least one guide RNA targeting the *APOE* gene or a portion of the *APOE* gene, and (b) at least one microglia specific promoter operably linked to (a)(i), (a)(ii), (a)(iii) or any combination thereof, thereby reducing the pathological phenotype associated with the Alzheimer's or other APOE associated disease or disorder.

[0471] Disclosed herein is a method of treating and/or preventing progression of a Alzheimer's or other APOE associated disease or disorder in a subject, the method comprising administering to a subject a therapeutically effective amount of an isolated nucleic acid molecule, comprising: (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, wherein the dCas is a dSpCas9, dSaCas9, or dCjCas9, (ii) a fusion product having one or more enzymatic activities, wherein the fusion product comprises any combination of HP1a, HP1b, MBD1, MBD2, KRAB, NIPP1, MeCP2, or DNMT3A, and (iii) at least one guide RNA targeting an *APOE* gene or portion thereof and b) at least one microglia specific regulatory element operably linked to (a)(i), (a)(ii), (a)(iii) or any combination thereof, wherein the expression and/or activity the *APOE* gene in the one or more microglial cells is modulated, thereby reducing the pathological phenotype associated with the Alzheimer's or other APOE associated disease or disorder. In some aspects, the microglia specific regulatory element of (b) can comprise a F4/80 promoter (SEQ ID NO: 4), CD68 promoter (SEQ ID NO: 1-3), FCGR2A promoter (SEQ ID NO: 8), or IBA1 promoter (SEQ ID NO: 5-7), a derivative thereof, a fragment thereof, or a combination thereof, a derivative thereof, a fragment thereof, or any combination thereof.

[0472] Disclosed herein is a method of treating and/or preventing progression of a Alzheimer's or other APOE associated disease or disorder in a subject, the method comprising administering to a subject a therapeutically effective amount of an isolated nucleic acid molecule, comprising: (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, wherein the dCas is a dSpCas9, dSaCas9, or dCjCas9, (ii) a fusion product having one or more enzymatic activities,

wherein the fusion product comprises any combination of HP1a, HP1b, MBD1, MBD2, KRAB, NIPP1, MeCP2, or DNMT3A, and (iii) at least one guide RNA targeting the *APOE* gene or a portion of the *APOE* gene and (b) at least one microglia specific promoter operably linked to (a)(i), (a)(ii), (a)(iii) or any combination thereof, thereby reducing the pathological phenotype associated with the Alzheimer's or other APOE associated disease or disorder. In some aspects, the microglia specific promoter of (b) can comprise a F4/80 promoter (SEQ ID NO: 4), CD68 promoter (SEQ ID NO: 1-3), FCGR2A promoter (SEQ ID NO: 8), or IBA1 promoter (SEQ ID NO: 5-7), a derivative thereof, a fragment thereof, or a combination thereof, a derivative thereof, a fragment thereof, or any combination thereof.

[0473] Disclosed herein is a method of treating and/or preventing progression of a Alzheimer's or other APOE associated disease or disorder in a subject, the method comprising administering to a subject a therapeutically effective amount of an isolated nucleic acid molecule, comprising: (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, wherein the dCas is a dSpCas9, dSaCas9, or dCjCas9, (ii) a fusion product having one or more enzymatic activities, wherein the fusion product comprises any combination of HP1a, HP1b, MBD1, MBD2, KRAB, NIPP1, MeCP2, or DNMT3A, and (iii) at least one guide RNA targeting the *APOE* gene or a portion of the *APOE* gene, (b) a microglia specific regulatory element operably linked to (a)(i) and/or (a)(ii), and (c) a promoter operably linked to (a)(iii), thereby reducing the pathological phenotype associated with the Alzheimer's or other APOE associated disease or disorder. In some aspects, the microglia specific regulatory element of (iv) may comprise a (b) can comprise a F4/80 promoter (SEQ ID NO: 4), CD68 promoter (SEQ ID NO: 1-3), FCGR2A promoter (SEQ ID NO: 8), or IBA1 promoter (SEQ ID NO: 5-7), a derivative thereof, a fragment thereof, or a combination thereof. In some aspects, the promoter of (c) comprises a U6 promoter, a derivative thereof or a fragment thereof.

[0474] Disclosed herein is a method of treating and/or preventing progression of a Alzheimer's or other APOE associated disease or disorder in a subject, the method comprising administering to a subject a therapeutically effective amount of a disclosed viral vector, thereby reducing the pathological phenotype associated with the Alzheimer's or other APOE associated disease or disorder.

[0475] Disclosed herein is a method of treating and/or preventing progression of a Alzheimer's or other APOE associated disease or disorder in a subject, the method comprising administering to a subject a therapeutically effective amount of a viral vector comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, (ii) at least one polypeptide having an enzymatic activity, and (iii) at least one guide RNA targeting the *APOE* gene or a portion of

the *APOE* gene and (b) at least one microglia specific regulatory element operably linked to (a)(i), (a)(ii), (a)(iii) or a combination thereof, thereby reducing the pathological phenotype associated with the Alzheimer's or other APOE associated disease or disorder. Disclosed herein is a method of treating and/or preventing progression of a Alzheimer's or other APOE associated disease or disorder in a subject, the method comprising administering to a subject a therapeutically effective amount of a disclosed viral vector, comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, (ii) at least one polypeptide having an enzymatic activity, and (iii) at least one guide RNA targeting a *APOE* gene or portion thereof and (b) at least one microglia specific promoter operably linked to (a)(i), (a)(ii), (a)(iii) or any combination thereof, thereby reducing the pathological phenotype associated with the Alzheimer's or other APOE associated disease or disorder.

[0476] Disclosed herein is a method of treating and/or preventing progression of a Alzheimer's or other APOE associated disease or disorder in a subject, the method comprising administering to a subject a therapeutically effective amount of a viral vector comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, (ii) a fusion product having one or more enzymatic activities, (iii) at least one guide RNA targeting the *APOE* gene or a portion of the *APOE* gene and (b) at least one microglia specific regulatory element operably linked to (a)(i), (a)(ii), (a)(iii), or a combination thereof, thereby reducing the pathological phenotype associated with the Alzheimer's or other APOE associated disease or disorder.

[0477] Disclosed herein is a method of treating and/or preventing progression of a Alzheimer's or other APOE associated disease or disorder in a subject, the method comprising administering to a subject a therapeutically effective amount of a viral vector comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, (ii) a fusion product having one or more enzymatic activities, (iii) at least one guide RNA targeting the *APOE* gene or a portion of the *APOE* gene and (b) at least one microglia specific promoter operably linked to (a)(i), (a)(ii), (a)(iii), or a combination thereof, thereby reducing the pathological phenotype associated with the Alzheimer's or other APOE associated disease or disorder.

[0478] Disclosed herein is a method of treating and/or preventing progression of a Alzheimer's or other APOE associated disease or disorder in a subject, the method comprising administering to a subject a therapeutically effective amount of a viral vector comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, wherein the dCas is a dSpCas9, dSaCas9, or dCjCas9, (ii) a fusion product having one or more enzymatic activities, wherein the fusion product comprises any combination of HP1a, HP1b, MBD1, MBD2, KRAB, NIPP1, MeCP2, or DNMT3A, (iii) at least one guide RNA targeting the *APOE* gene or a portion of the

APOE gene, and (b) at least one microglia specific regulatory element operably linked to (a)(i), (a)(ii), (a)(iii), or any combination thereof, thereby reducing the pathological phenotype associated with the Alzheimer's or other APOE associated disease or disorder. Disclosed herein is a method of treating and/or preventing progression of a Alzheimer's or other APOE associated disease or disorder in a subject, the method comprising administering to a subject a therapeutically effective amount of a viral vector comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, wherein the dCas is a dSpCas9, dSaCas9, or dCjCas9, (ii) a fusion product having one or more enzymatic activities, wherein the fusion product comprises any combination of HP1a, HP1b, MBD1, MBD2, KRAB, NIPP1, MeCP2, or DNMT3A, (iii) at least one guide RNA targeting the APOE gene or a portion of the *APOE* gene, and (b) at least one microglia specific promoter operably linked to (a)(i), (a)(ii), (a)(iii), or any combination thereof, thereby reducing the pathological phenotype associated with the Alzheimer's or other APOE associated disease or disorder.

[0479] Disclosed herein is a method of treating and/or preventing progression of a Alzheimer's or other APOE associated disease or disorder in a subject, the method comprising administering to a subject a therapeutically effective amount of a viral vector comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, wherein the dCas is a dSpCas9, dSaCas9, or dCjCas9, (ii) a fusion product having one or more enzymatic activities, wherein the fusion product comprises any combination of HP1a, HP1b, MBD1, MBD2, KRAB, NIPP1, MeCP2, or DNMT3A, and (iii) at least one guide RNA targeting the *APOE* gene or a portion of the APOE gene, (b) a microglia specific regulatory element operably linked to the (a)(i) and/or (a)(ii), and (c) a promoter operably linked to (a)(iii), thereby reducing the pathological phenotype associated with the Alzheimer's or other APOE associated disease or disorder. Disclosed herein is a method of treating and/or preventing progression of a Alzheimer's or other APOE associated disease or disorder in a subject, the method comprising administering to a subject a therapeutically effective amount of a viral vector comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, wherein the dCas is a dSpCas9, dSaCas9, or dCjCas9, (ii) a fusion product having one or more enzymatic activities, wherein the fusion product comprises any combination of HP1a, HP1b, MBD1, MBD2, KRAB, NIPP1, MeCP2, or DNMT3A, and (iii) at least one guide RNA targeting the *APOE* gene or a portion of the *APOE* gene, (b) a microglia specific promoter operably linked to the (a)(i) and/or (a)(ii), and (c) a promoter operably linked to (a)(iii), thereby reducing the pathological phenotype associated with the Alzheimer's or other APOE associated disease or disorder. In an aspect, the microglia specific regulatory element or

microglia specific promoter of (b) can comprise Alzheimer's. In an aspect, the fusion product of (a)(ii) can comprise KRAB-MeCP2.

[0480] Disclosed herein is a method of treating and/or preventing progression of a Alzheimer's or other APOE associated disease or disorder in a subject, the method comprising administering to a subject a therapeutically effective amount of a viral vector comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, wherein the dCas is a dSpCas9, dSaCas9, or dCjCas9, (ii) a fusion product having one or more enzymatic activities, wherein the fusion product comprises any combination of HP1a, HP1b, MBD1, MBD2, KRAB, NIPP1, MeCP2, or DNMT3A, and (iii) at least one guide RNA targeting the *APOE* gene or a portion of the *APOE* gene, (b) a microglia specific regulatory element operably linked to (a)(i) and/or (a)(ii), and (c) a promoter operably linked to (a)(iii), thereby reducing the pathological phenotype associated with the Alzheimer's or other APOE associated disease or disorder. Disclosed herein is a method of treating and/or preventing progression of a Alzheimer's or other APOE associated disease or disorder in a subject, the method comprising administering to a subject a therapeutically effective amount of a viral vector comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, wherein the dCas is a dSpCas9, dSaCas9, or dCjCas9, (ii) a fusion product having one or more enzymatic activities, wherein the fusion product comprises any combination of HP1a, HP1b, MBD1, MBD2, KRAB, NIPP1, MeCP2, or DNMT3A, and (iii) at least one guide RNA targeting the *APOE* gene or a portion of the *APOE* gene, (b) a microglia specific promoter operably linked to (a)(i) and/or (a)(ii), and (c) a promoter operably linked to (a)(iii), thereby reducing the pathological phenotype associated with the Alzhiemer's or other APOE associated disease or disorder. In an aspect, the microglia specific regulatory element or microglia specific promoter of (b) can comprise a (b) can comprise a F4/80 promoter (SEQ ID NO: 4), CD68 promoter (SEQ ID NO: 1-3), FCGR2A promoter (SEQ ID NO: 8), or IBA1 promoter (SEQ ID NO: 5-7), a derivative thereof, a fragment thereof, or a combination thereof. In an aspect, the fusion product of (a)(ii) can comprise KRAB-MeCP2. In an aspect, the promoter of (c) comprises a U6 promoter, a derivative thereof, or a fragment thereof.

[0481] In an aspect, a disclosed method of treating and/or preventing progression of Alzheimer's or other APOE associated disease or disorder can reduce and/or decrease the expression and/or activity of APOE. In an aspect of a disclosed method of treating and/or preventing progression of Alzheimer's or other APOE associated disease or disorder, the expression and/or activity of APOE is reduced and/or decreased. In an aspect, a disclosed method can comprise diagnosing the subject with Alzheimer's or other APOE associated disease or disorder.

[0482] In an aspect, a disclosed method of treating and/or preventing Alzheimer's or other APOE associated disease or disorder comprises measuring the level of expression and/or the level of activity of the APOE gene. In an aspect, a disclosed method can comprise repeating the measuring step one or more times.

[0483] In an aspect, prior to the administering step, the expression and/or activity level of APOE can be elevated and/or increased when compared to wild-type or control expression level. In an aspect, following the administering step, the expression and/or activity level of APOE can be reduced and/or decreased when compared to the pre-administering step level. In an aspect, a disclosed gRNA targeting a portion of the APOE gene can comprise at least two gRNAs. In an aspect, disclosed gRNAs can target the same portion of the APOE gene or different portions of the APOE gene. In an aspect, a disclosed gRNA can target the promoter of the APOE gene. In an aspect, a disclosed gRNA can target exon 1 of the APOE gene. In an aspect, a disclosed gRNA can target exon 1 of the APOE gene. In an aspect, a disclosed gRNA can target intron 1 of the APOE gene. In an aspect, a disclosed gRNA can target intron 4 of the APOE gene. In an aspect, a disclosed gRNA can target a CpG island region of intron 1 of the APOE gene. The CpG island region can include CpG1, CpG2, CpG3, CpG4, CpG5, CpG6, CpG7, CpG8, CpG9, CpG10, CpG11, CpG12, CpG13, CpG14, CpG15, CpG16, CpG17, CpG18, CpG19, CpG20, CpG21, CpG22, CpG23, or a combination thereof. For example, the CpG island region can include CpG1, CpG3, CpG6, CpG7, CpG8, CpG9, CpG18, CpG19, CpG20, CpG21, CpG22, or a combination thereof. In an aspect, the disclosed gRNA may comprise a nucleic acid sequence as set forth in SEQ ID NO: 15-33. In an aspect, the disclosed gRNA may comprise a nucleic acid sequence as set forth in SEQ ID NO: 15-33, or a complement thereof. In an aspect, the disclosed gRNA may comprise a nucleic acid sequence with at least about 90%, or about 91%, or about 92%, or about 93%, or about 94%, or about 95%, or about 96%, or about 97%, or about 98%, or about 99%, or more sequence identity to any one of the sequences set forth in SEQ ID NO: 15-33, or a complement thereof, or a fragment thereof.

[0484] In an aspect of a disclosed method, a disclosed viral vector or disclosed recombinant AAV vector can further comprise a gRNA scaffold. In an aspect, a disclosed gRNA scaffold can comprise the sequence set forth in SEQ ID NO: 129, SEQ ID NO: 130, or a fragment thereof.

[0485] In an aspect of a disclosed method, a disclosed dCas endonuclease can comprise a dCas9 endonuclease. In an aspect, a disclosed dCas9 endonuclease can comprise a deactivated *Staphylococcus aureus* Cas9 (dSaCas9), a deactivated *Streptococcus pyogenes* Cas9 (dSpCas9), a deactivated *Campylobacter jejuni* Cas9 (dCjCas9), or a variant dCas9 endonuclease. In an

aspect, a disclosed variant dCas9 can comprise a variant dSaCas9, a variant dSpCas9, or a variant dCjCa9. In an aspect, a disclosed variant dSpCas9 can comprise dVQR, dEQR, or dVRER.

[0486] In an aspect of a disclosed method, a disclosed dCas endonuclease can comprise a dCas9 endonuclease. In an aspect, a disclosed dCas9 endonuclease can comprise a deactivated *Staphylococcus aureus* Cas9 (dSaCas9), a deactivated *Streptococcus pyogenes* Cas9 (dSpCas9), a deactivated *Campylobacter jejuni* Cas9 (dCjCas9), or a variant dCas9 endonuclease. In an aspect, a disclosed variant dCas9 can comprise a variant dSaCas9, a variant dSpCas9, or a variant dCjCa9. In an aspect, a disclosed variant dSpCas9 can comprise dVQR, dEQR, or dVRER.

[0487] In an aspect of a disclosed method, a nucleic acid sequence the disclosed dSpCas9 may comprise a sequence as set forth in any one of SEQ ID NO: 40 or SEQ ID NO: 41, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed dSpCas9 endonuclease may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 40 or SEQ ID NO: 41, or a fragment thereof. In an aspect, a disclosed dSpCas9 endonuclease can comprise the amino acid sequence as set forth in SEQ ID NO: 36 or SEQ ID NO: 37, or a fragment, or a derivative thereof. In an aspect, a disclosed dSpCas9 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 36 or SEQ ID NO: 37, or a fragment thereof.

[0488] In an aspect, the nucleic acid sequence encoding the disclosed dSaCas9 may comprise a sequence as set forth in SEQ ID NO: 42, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed dSaCas9 endonuclease may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequences set forth in SEQ ID NO: 42, or a fragment thereof. In an aspect, a disclosed dSaCas9 endonuclease can comprise the amino acid sequence as set forth in SEQ ID NO: 38 or a fragment, or a derivative thereof. In an aspect, a disclosed dSaCas9 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 38, or a fragment thereof.

[0489] In an aspect, the nucleic acid sequence encoding the disclosed dCjCas9 may comprise a sequence as set forth in SEQ ID NO: 43, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed dCjCas9 endonuclease may comprise a nucleic acid sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity

to any one of the sequences set forth in SEQ ID NO: 43, or a fragment thereof. In an aspect, a disclosed dCjCas9 endonuclease can comprise the amino acid sequence as set forth in SEQ ID NO: 39, or a fragment, or a derivative thereof. In an aspect, a disclosed dCjCas9 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 39 or a fragment thereof.

[0490] In an aspect, a disclosed variant of dCas9 dVQR can comprise D1135V, R1335Q, and T1337R with reference to SEQ ID NO: 36. In an aspect, a disclosed variant of dCas9 dEQR can comprise D1135E, R1335Q, and T1337R with reference to SEQ ID NO: 36. In an aspect, a disclosed variant of dCas9 dVRER can comprise D1135V, G1218R, R1335E, and T1337R with reference to SEQ ID NO: 36. In an aspect, a disclosed dVRER can comprise the sequence set forth in SEQ ID NO: 37 or a fragment thereof.

[0491] In an aspect, the nucleic acid sequence encoding the disclosed dCas9 endonuclease may comprise a sequence as set forth in any one of SEQ ID NO: 40-43. In an aspect, the sequence encoding the disclosed dCas9 endonuclease may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 40-43, or a fragment, or a derivative thereof.

[0492] In an aspect of a disclosed method, a disclosed encoded polypeptide can comprise transcription activation activity, transcription repression activity, transcription release factor activity, histone modulation activity, nucleic acid association activity, methyltransferase activity, demethylase activity, acetyltransferase activity, deacetylase activity, or any combination thereof.

[0493] In an aspect of a disclosed method, a disclosed encoded polypeptide can comprise HP1a, HP1b, MBD1, MBD2, Krüppel-Associated Box (KRAB), NIPP1, the Transcription Repression Domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof.

[0494] In an aspect of a disclosed method, a disclosed fusion or disclosed fusion product can comprise HP1a-HP1a, HP1a-HP1b, HP1a-MBD1, HP1a-MBD2, HP1a-KRAB, HP1a-NIPP1, HP1a-MeCP2, HP1a-DNMT3A, HP1b-HP1b, HP1b-HP1a, HP1b-MBD1, HP1b-MBD2, HP1b-KRAB, HP1b-NIPP1, HP1b-MeCP2, HP1b-DNMT3A, MBD1-MBD1, MBD1-HP1a, MBD1-HP1b, MBD1-MBD2, MBD1-KRAB, MBD1-NIPP1, MBD1-MeCP2, MBD1-DNMT3A, MBD2-MBD2, MBD2-HP1a, MBD2-HP1b, MBD2-MBD1, MBD2-KRAB, MBD2-NIPP1, MBD2-MeCP2, MBD2-DNMT3A, KRAB-KRAB, KRAB-HP1a, KRAB-HP1b, KRAB-MBD1, KRAB-MBD2, KRAB-NIPP1, KRAB-MeCP2, KRAB-DNMT3A, NIPP1-NIPP1, NIPP1-HP1a,

NIPP1-HP1b, NIPP1-MBD1, NIPP1-MBD2, NIPP1-KRAB, NIPP1-MeCP2, NIPP1-DNMT3A, MeCP2-MeCP2, MeCP2-HP1a, MeCP2-HP1b, MeCP2-MBD1, MeCP2-MBD2, MeCP2-KRAB, MeCP2-NIPP1, MeCP2-DNMT3A, DNMT3A-DNMT3A, DNMT3A-HP1a, DNMT3A-HP1b, DNMT3A-MBD1, DNMT3A-MBD2, DNMT3A-KRAB, DNMT3A-NIPP1, or DNMT3A-MeCP2. In an aspect of the disclosed method, a disclosed fusion or disclosed fusion product can comprise KRAB-MeCP2.

[0495] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide HP1a may comprise a sequence as set forth in any one of SEQ ID NO: 59 or SEQ ID NO: 60, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide HP1a may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 59 or SEQ ID NO: 60, or a fragment thereof. In an aspect, a disclosed polypeptide HP1a can comprise the amino acid sequence as set forth in SEQ ID NO: 44 or SEQ ID NO: 45, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide HP1a can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 44 or SEQ ID NO: 45, or a fragment thereof.

[0496] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide HP1b may comprise a sequence as set forth in any one of SEQ ID NO: 61 or SEQ ID NO: 62, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide HP1b may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 61 or SEQ ID NO: 62, or a fragment thereof. In an aspect, a disclosed polypeptide HP1b can comprise the amino acid sequence as set forth in SEQ ID NO: 46 or SEQ ID NO: 47, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide HP1b can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 46 or SEQ ID NO: 47, or a fragment thereof.

[0497] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide MBD1 may comprise a sequence as set forth in any one of SEQ ID NO: 63 or SEQ ID NO: 64, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide MBD1 may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at

least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 63 or SEQ ID NO: 64, or a fragment thereof. In an aspect, a disclosed polypeptide MBD1 can comprise the amino acid sequence as set forth in any one of SEQ ID NO: 48-50, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide MDB1 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in any one of SEQ ID NO: 48-50, or a fragment thereof.

[0498] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide MBD2 may comprise a sequence as set forth in any one of SEQ ID NO: 65 or SEQ ID NO: 66, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide MBD2 may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 65 or SEQ ID NO: 66, or a fragment thereof. In an aspect, a disclosed polypeptide MBD2 can comprise the amino acid sequence as set forth in SEQ ID NO: 51 or SEQ ID NO: 52, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide MBD2 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 51 or SEQ ID NO: 52, or a fragment thereof.

[0499] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide NIPP1 may comprise a sequence as set forth in any one of SEQ ID NO: 67 or SEQ ID NO: 68, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide NIPP1 may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 67 or SEQ ID NO: 68, or a fragment thereof. In an aspect, a disclosed polypeptide NIPP1 can comprise the amino acid sequence as set forth in SEQ ID NO: 53 or SEQ ID NO: 54, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide NIPP1 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 53 or SEQ ID NO: 54, or a fragment thereof.

[0500] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide DNMT3A may comprise a sequence as set forth in SEQ ID NO: 69, or a fragment, or a derivative thereof. In an

aspect, the sequence encoding the disclosed polypeptide DNMT3A may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 69, or a fragment thereof. In an aspect, a disclosed polypeptide DNMT3A can comprise the amino acid sequence as set forth in SEQ ID NO: 55, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide DNMT3A can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 55, or a fragment thereof.

[0501] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide KRAB may comprise a sequence as set forth in any one of SEQ ID NO: 70 or SEQ ID NO: 71, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide KRAB may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 70 or SEQ ID NO: 71, or a fragment thereof. In an aspect, a disclosed polypeptide KRAB can comprise the amino acid sequence as set forth in SEQ ID NO: 56, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide KRAB can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 56, or a fragment thereof.

[0502] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide KRAB may comprise a sequence as set forth in any one of SEQ ID NO: 70 or SEQ ID NO: 71, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide KRAB may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 70 or SEQ ID NO: 71, or a fragment thereof. In an aspect, a disclosed polypeptide KRAB can comprise the amino acid sequence as set forth in SEQ ID NO: 56, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide KRAB can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 56, or a fragment thereof.

[0503] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide MeCP2 may comprise a sequence as set forth in any one of SEQ ID NO: 72, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide MeCP2 may comprise a

nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequences set forth in SEQ ID NO: 72, or a fragment thereof. In an aspect, a disclosed polypeptide MeCP2 can comprise the amino acid sequence as set forth in SEQ ID NO: 57, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide MeCP2 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 57, or a fragment thereof.

[0504] In an aspect, the nucleic acid sequence encoding the disclosed fusion polypeptide KRAB-MeCP2 may comprise a sequence as set forth in any one of SEQ ID NO: 73 or SEQ ID NO: 74, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide KRAB-MeCP2 may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequences set forth in SEQ ID NO: 73 or SEQ ID NO: 74, or a fragment thereof. In an aspect, a disclosed polypeptide KRAB-MeCP2 can comprise the amino acid sequence as set forth in SEQ ID NO: 58, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide KRAB-MeCP2 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 58, or a fragment thereof.

[0505] In an aspect, a disclosed encoded polypeptide can comprise transcription activation activity. In an aspect, a disclosed encoded polypeptide comprising transcription activation activity can comprise a transactivation domain. In an aspect, a disclosed transactivation domain can comprise a VP16 protein, a series of linked VP16 proteins, a p65 domain of NFκB, or any combination thereof.

[0506] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide VP16 may comprise a sequence as set forth in any one of SEQ ID NO: 132 or SEQ ID NO: 133, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide VP16 may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequences set forth in SEQ ID NO: 132 or SEQ ID NO: 133, or a fragment thereof. In an aspect, a disclosed polypeptide VP16 can comprise the amino acid sequence as set forth in SEQ ID NO: 131, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide VP16 can comprise a sequence having at least 60%, at least 65%,

at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 131, or a fragment thereof.

[0507] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide p65 domain of NFκB or RELA may comprise a sequence as set forth in any one of SEQ ID NO: 135 or SEQ ID NO: 136, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide p65 domain of NFκB or RELA may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequences set forth in SEQ ID NO: 135 or SEQ ID NO: 136, or a fragment thereof. In an aspect, a disclosed polypeptide p65 domain of NFκB or RELA can comprise the amino acid sequence as set forth in SEQ ID NO: 134, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide p65 domain of NFκB or RELA can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 134, or a fragment thereof.

[0508] In an aspect, a disclosed encoded polypeptide can comprise transcription repression activity. In an aspect, a disclosed encoded polypeptide comprising transcription repression activity can comprise a Krüppel associated box domain, an ERF repressor domain, a MXI1 repressor domain, a SID4x repressor domain, a fused KRAB-MeCP2 domain, a MeCP2 TRD domain, a MAS-SID repressor domain, a TATA box binding protein activity, or any combination thereof. In an aspect, a disclosed encoded polypeptide comprising transcription repression activity can comprise HP1 repressor activity, MeCP2 repressor activity, MBD1 repressor activity, MBD2 repressor activity, MBD3 repressor activity, MBD4 repressor activity, KRAB repressor activity, SUV39H1 repressor activity, SUV39H2 repressor activity, CTCF insulator-repressor activity, LSD-1 histone-demethylase repressor activity, or any combination thereof.

[0509] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide comprising Eukaryotic Release Factor 1 (ERF1) activity or Eukaryotic Release Factor 3 (ERF3) activity may comprise a sequence as set forth in any one of SEQ ID NO: 138, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide comprising ERF1 activity or ERF3 activity may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequences set forth in SEQ ID NO: 138, or a fragment thereof. In an aspect, a disclosed polypeptide comprising ERF1 activity or ERF3 activity can comprise the amino acid sequence as set forth in SEQ ID NO: 137, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide comprising ERF1 activity

or ERF3 activity can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 137, or a fragment thereof.

[0510] In an aspect of a disclosed method, a disclosed encoded polypeptide can comprise histone modulation activity. A disclosed encoded polypeptide comprising histone modulation activity can comprise histone acetyltransferase, histone deacetylase, histone demethylase, histone methyltransferase activity, or any combination thereof.

[0511] In an aspect of a disclosed method, a disclosed encoded polypeptide can comprise nucleic acid association activity. A disclosed encoded polypeptide comprising nucleic acid association activity can comprise a helix-turn-helix region, a leucine zipper region, a winged helix region, a winged helix-turn-helix region, a helix-loop-helix region, an immunoglobulin fold, a B3 domain, a zinc finger, a HMG-box, a Wor3 domain, a TAL effector DNA-binding domain, or any combination thereof. In an aspect of a disclosed method, a disclosed encoded polypeptide can comprise methyltransferase activity. A disclosed encoded polypeptide comprising methyltransferase activity can comprise DNA (cytosine-5)-methyltransferase 3a (DNMT3A).

[0512] In an aspect of a disclosed method, a disclosed encoded polypeptide can comprise demethylase activity. A disclosed encoded polypeptide comprising demethylase activity can comprise ten-eleven translocation methylcytosine dioxygenase 1 (TET1) or lysine-specific histone demethylase 1 (LSD1).

[0513] In an aspect of a disclosed method, a disclosed encoded polypeptide can comprise acetyltransferase activity. A disclosed encoded polypeptide comprising acetyltransferase activity can comprise histone acetyltransferase. In In an aspect of a disclosed method, a disclosed encoded polypeptide can comprise deacetylase activity. A disclosed encoded polypeptide comprising deacetylase activity can comprise histone deacetylase.

[0514] In an aspect of a disclosed method, a disclosed dCas endonuclease can comprise dSpCas9 and a disclosed encoded polypeptide can comprise HP1a, HP1b, MBD1, MBD2, Krüppel-associated box (KRAB), NIPP1, the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof. In an aspect, a disclosed dCas endonuclease can comprise dSaCas9 and a disclosed encoded polypeptide can comprise HP1a, HP1b, MBD1, MBD2, Krüppel-associated box (KRAB), NIPP1, the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof. In an aspect, a disclosed dCas endonuclease can comprise dCjCas9 and a disclosed polypeptide can comprise HP1a, HP1b, MBD1, MBD2,

Krüppel-associated box (KRAB), NIPP1, the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof.

[0515] In an aspect of a disclosed method, a disclosed dCas endonuclease can comprise dVQR, dEQR, or dVRER and a disclosed encoded polypeptide can comprise HP1a, HP1b, MBD1, MBD2, Krüppel-associated box (KRAB), NIPP1, the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof.

[0516] In an aspect of a disclosed method, a disclosed dCas endonuclease can comprise dSpCas9, dSaCas9, or dCjCas9 and a disclosed encoded polypeptide can comprise KRAB-MeCP2.

[0517] In an aspect of a disclosed method, a disclosed dCas endonuclease can comprise dSpCas9, dSaCas9, or dCjCas9, and a disclosed fusion can comprise HP1a-HP1a, HP1a-HP1b, HP1a-MBD1, HP1a-MBD2, HP1a-KRAB, HP1a-NIPP1, HP1a-MeCP2, HP1a-DNMT3A, HP1b-HP1b, HP1b-HP1a, HP1b-MBD1, HP1b-MBD2, HP1b-KRAB, HP1b-NIPP1, HP1b-MeCP2, HP1b-DNMT3A, MBD1-MBD1, MBD1-HP1a, MBD1-HP1b, MBD1-MBD2, MBD1-KRAB, MBD1-NIPP1, MBD1-MeCP2, MBD1-DNMT3A, MBD2-MBD2, MBD2-HP1a, MBD2-HP1b, MBD2-MBD1, MBD2-KRAB, MBD2-NIPP1, MBD2-MeCP2, MBD2-DNMT3A, KRAB-KRAB, KRAB-HP1a, KRAB-HP1b, KRAB-MBD1, KRAB-MBD2, KRAB-NIPP1, KRAB-MeCP2, KRAB-DNMT3A, NIPP1-NIPP1, NIPP1-HP1a, NIPP1-HP1b, NIPP1-MBD1, NIPP1-MBD2, NIPP1-KRAB, NIPP1-MeCP2, NIPP1-DNMT3A, MeCP2-MeCP2, MeCP2-HP1a, MeCP2-HP1b, MeCP2-MBD1, MeCP2-MBD2, MeCP2-KRAB, MeCP2-NIPP1, MeCP2-DNMT3A, DNMT3A-DNMT3A, DNMT3A-HP1a, DNMT3A-HP1b, DNMT3A-MBD1, DNMT3A-MBD2, DNMT3A-KRAB, DNMT3A-NIPP1, or DNMT3A-MeCP2.

[0518] In an aspect of a disclosed method, an isolated nucleic acid or viral vector can encode for a dCas endonuclease fused to the at least one encoded polypeptide having an enzymatic activity. In these or related aspects, the isolated nucleic acid can encode for a "fusion protein" wherein the fusion protein comprises a first component comprising a dCas endonuclease and a second component comprising at least one polypeptide having an enzymatic activity. In an aspect, the encoded fusion protein can comprise a dSpCas9 and a polypeptide having enzymatic activity comprising HP1a, HP1b, MBD1, MBD2, Krüppel-associated box (KRAB), NIPP1, the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof. In an aspect, the encoded fusion protein can comprise a dSaCas9 and a polypeptide having enzymatic activity comprising HP1a, HP1b, MBD1, MBD2, Krüppel-associated box (KRAB), NIPP1, the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any

fusion thereof. In an aspect, the encoded fusion protein can comprise a dCjCas9 and a polypeptide having enzymatic activity comprising HP1a, HP1b, MBD1, MBD2, Krüppel-associated box (KRAB), NIPP1, the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof. In an aspect, the encoded fusion protein can comprise dVQR, dEQR, or dVRER and a polypeptide having enzymatic activity comprising HP1a, HP1b, MBD1, MBD2, Krüppel-associated box (KRAB), NIPP1, the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof.

[0519] In an aspect, the encoded fusion protein can comprise dSpCas9, dSaCas9, dCjCas9, or a variant dCas9 (*e.g.*, dVQR, dEQR or dVRER) and one or more of HP1a-HP1a, HP1a-HP1b, HP1a-MBD1, HP1a-MBD2, HP1a-KRAB, HP1a-NIPP1, HP1a-MeCP2, HP1a-DNMT3A, HP1b-HP1b, HP1b-HP1a, HP1b-MBD1, HP1b-MBD2, HP1b-KRAB, HP1b-NIPP1, HP1b-MeCP2, HP1b-DNMT3A, MBD1-MBD1, MBD1-HP1a, MBD1-HP1b, MBD1-MBD2, MBD1-KRAB, MBD1-NIPP1, MBD1-MeCP2, MBD1-DNMT3A, MBD2-MBD2, MBD2-HP1a, MBD2-HP1b, MBD2-MBD1, MBD2-KRAB, MBD2-NIPP1, MBD2-MeCP2, MBD2-DNMT3A, KRAB-KRAB, KRAB-HP1a, KRAB-HP1b, KRAB-MBD1, KRAB-MBD2, KRAB-NIPP1, KRAB-MeCP2, KRAB-DNMT3A, NIPP1-NIPP1, NIPP1-HP1a, NIPP1-HP1b, NIPP1-MBD1, NIPP1-MBD2, NIPP1-KRAB, NIPP1-MeCP2, NIPP1-DNMT3A, MeCP2-MeCP2, MeCP2-HP1a, MeCP2-HP1b, MeCP2-MBD1, MeCP2-MBD2, MeCP2-KRAB, MeCP2-NIPP1, MeCP2-DNMT3A, DNMT3A-DNMT3A, DNMT3A-HP1a, DNMT3A-HP1b, DNMT3A-MBD1, DNMT3A-MBD2, DNMT3A-KRAB, DNMT3A-NIPP1, or DNMT3A-MeCP2. In an aspect, the encoded fusion protein can comprise a variant dCas9 selected from dVQR, dEQR, or dVRER and a fusion comprising KRAB-MeCP2. In an aspect, the encoded fusion protein can comprise a dCas9 selected from dSpCas9, dSaCas9, or dCjCas9 and a fusion comprising KRAB-MeCP2. In an aspect, the encoded fusion protein can comprise a dCas9 selected from dSpCas9, dSaCas9, or dCjCas9 and a fusion comprising KRAB-MeCP2. In an aspect, the encoded fusion protein can comprise a dSaCas9 and KRAB-MeCP2. In an aspect, the encoded fusion protein can comprise a dCas9 selected from dSpCas9, dSaCas9, or dCjCas9 and DNMTA. In an aspect, the encoded fusion protein can comprise a dSaCas9 and DNMTA.

[0520] In an aspect, the nucleic acid sequence encoding the fusion protein comprising dSpCas9 and DNMT3A or dVRER and DNMT3A may comprise a sequence as set forth in SEQ ID NO: 75 or SEQ ID NO: 76 respectively, or a derivative thereof. In an aspect, the nucleic acid sequence encoding the fusion protein comprising dSpCas9 and DNMT3A or dVRER and DNMT3A may comprise a sequence as set forth in SEQ ID NO: 77 or SEQ ID NO: 78 respectively, or a derivative

thereof. In an aspect, the nucleic acid sequence encoding the fusion protein comprising dSpCas9 and DNMT3A may comprise a sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any of the sequences set forth in SEQ ID NO: 77 or SEQ ID NO: 78, or a fragment thereof. In an aspect, a disclosed polypeptide dSpCas9 can comprise the amino acid sequence as set forth in SEQ ID NO: 75 or SEQ ID NO: 76, or a fragment, or a derivative thereof.

[0521] In an aspect of a disclosed method, a disclosed gene of interest or portion thereof can demonstrate a reduced expression level when compared to wild-type or control expression level. In an aspect of a disclosed method, a disclosed gRNA can targes a gene of interest or portion thereof that demonstrates a reduced expression level when compared to wild-type or control expression level. In an aspect of a disclosed method, a disclosed gene of interest or portion thereof can demonstrate an increased expression level when compared to wild-type or control expression level. In an aspect of a disclosed method, a disclosed gRNA can target a gene of interest or portion thereof that demonstrates an increased expression level when compared to wild-type or control expression level.

[0522] In an aspect of a disclosed method, a disclosed gRNA can target the *APOE* gene. In an aspect, the at least one gRNA may target a region in the *APOE* gene comprising a nucleic acid sequence as set forth in any one of SEQ ID NO: 11-14, or a complement thereof. In an aspect, the at least one gRNA targets a nucleic acid sequence comprising at least 10 consecutive nucleotides as set forth in SEQ ID NO: 11-14, or a complement thereof. In an aspect, a disclosed gRNA can target the promoter of the APOE gene. In an aspect, a disclosed gRNA can target exon 1 of the APOE gene. In an aspect, a disclosed gRNA can target exon 1 of the APOE gene. In an aspect, a disclosed gRNA can target intron 1 of the APOE gene. In an aspect, a disclosed gRNA can target intron 4 of the APOE gene. In an aspect, a disclosed gRNA can target a CpG island region of intron 1 of the APOE gene. The CpG island region can include CpG1, CpG2, CpG3, CpG4, CpG5, CpG6, CpG7, CpG8, CpG9, CpG10, CpG11, CpG12, CpG13, CpG14, CpG15, CpG16, CpG17, CpG18, CpG19, CpG20, CpG21, CpG22, CpG23, or a combination thereof. For example, the CpG island region can include CpG1, CpG3, CpG6, CpG7, CpG8, CpG9, CpG18, CpG19, CpG20, CpG21, CpG22, or a combination thereof. In an aspect, the disclosed gRNA may comprise a nucleic acid sequence as set forth in SEQ ID NO: 15-33. In an aspect, the disclosed gRNA may comprise a nucleic acid sequence as set forth in SEQ ID NO: 15-33, or a complement thereof. In an aspect, the disclosed gRNA may comprise a nucleic acid sequence with at least about 90%, or about 91%, or about 92%, or about 93%, or about 94%, or about 95%, or about

96%, or about 97%, or about 98%, or about 99%, or more sequence identity to any one of the sequences set forth in SEQ ID NO: 15-33, or a complement thereof, or a fragment thereof.

[0523] In an aspect of a disclosed method, a disclosed viral vector or disclosed recombinant AAV vector can further comprise a gRNA scaffold. In an aspect, a disclosed gRNA scaffold can comprise the sequence set forth in SEQ ID NO: 129, SEQ ID NO: 130, or a fragment thereof.

[0524] In an aspect of a disclosed method, a disclosed isolated nucleic acid or disclosed viral vector comprises one or more microglia specific regulatory elements. In an aspect, the one or more microglia specific regulatory elements can comprise a microglia specific wild-type promoter, a microglia specific enhancer, a synthetic promoter, a synthetic enhancer, a transcriptional or translational regulatory sequence or any combination thereof. In an aspect, the one or more microglia specific regulatory elements comprise at least one microglia specific promoter (*e.g.*, a microglia specific wild-type promoter or a microglia specific synthetic promoter). In an aspect, the microglia specific regulatory element (*e.g.*, promoter) may be specific for a certain type of microglial cell (*e.g.*, a dopaminergic or a cholinergic microglial cell). In these instances, the microglia specific regulatory element may include a dopaminergic specific regulatory element (*i.e.*, a dopaminergic promoter) and/or a cholinergic microglia specific regulatory element (*i.e.*, a cholinergic promoter). In an aspect, the microglia specific regulatory element can comprise a microglia specific promoter. In an aspect, a disclosed microglia specific promoter can comprise a F4/80 promoter, CD68 promoter, FCGR2A promoter, HexB promoter or IBA1 promoter, a derivative thereof, a fragment thereof, or any combination thereof.

[0525] In an aspect, the promoter is a CD68 promoter, or a derivative thereof. In an aspect, the promoter may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one or more of the sequences set forth in SEQ ID NO: 1-3, or a fragment, or a derivative thereof. In an aspect, the promoter comprises a sequence as set forth in any one of SEQ ID NO: 1-3.

[0526] In an aspect, the promoter is a F4/80 promoter, or a derivative thereof. In an aspect, the promoter may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequence set forth in SEQ ID NO: 4, or a fragment, or a derivative thereof. In an aspect, the promoter comprises a sequence as set forth in SEQ ID NO: 4.

[0527] In an aspect, the promoter is a IBA1 promoter, or a derivative thereof. In an aspect, the promoter may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at

least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequence set forth in SEQ ID NO: 5-7, or a fragment, or a derivative thereof. In an aspect, the promoter comprises a sequence as set forth in any one of SEQ ID NO: 5-7.

[0528] In an aspect, the promoter is a FCGR2A promoter, or a derivative thereof. In an aspect, the promoter may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequence set forth in SEQ ID NO: 8, or a fragment, or a derivative thereof. In an aspect, the promoter comprises a sequence as set forth in SEQ ID NO: 8.

[0529] In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) can be operably linked to the dCas endonuclease and the fusion protein having one or more enzymatic activities. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the dCas endonuclease and the fusion protein having one or more enzymatic activities can comprise a F4/80 promoter, or a derivative thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the dCas endonuclease and the fusion protein having one or more enzymatic activities can comprise a CD68 promoter, or a derivative thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the dCas endonuclease and the fusion protein having one or more enzymatic activities can comprise a IBA1 promoter, or a derivative thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the dCas endonuclease and the fusion protein having one or more enzymatic activities can comprise a FCGR2A promoter, or a derivative thereof.

[0530] In an aspect, a disclosed microglia specific regulatory element (*i.e.,* a microglia specific promoter) operably linked to the dCas endonuclease can be operably linked to the at least one polypeptide having enzymatic activity. In an aspect, a disclosed microglia specific regulatory element (*i.e.,* a microglia specific promoter) operably linked to the at least one polypeptide having enzymatic activity can comprise a F4/80promoter, a derivative thereof, or a fragment thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.,* a microglia specific promoter) operably linked to the at least one polypeptide having enzymatic activity can comprise a CD68 promoter, a derivative thereof, or a fragment thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.,* a microglia specific promoter) operably linked to the at least one polypeptide having enzymatic activity can comprise a IBA1 promoter, a derivative thereof, or a

fragment thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to at least one polypeptide having enzymatic activity can comprise a FCGR2A promoter, a derivative thereof, or a fragment thereof.

[0531] In an aspect of a disclosed method, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) can be operably linked to the dCas endonuclease and the at least one polypeptide having enzymatic activity. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the dCas endonuclease and the at least one polypeptide having enzymatic activity can comprise a FGCR2A promoter, a derivative thereof, or a fragment thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the at dCas endonuclease and the at least one polypeptide having enzymatic activity can comprise a F4/80 promoter, a derivative thereof, or a fragment thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the dCas endonuclease and the at least one polypeptide having enzymatic activity can comprise a CD68 promoter, a derivative thereof, or a fragment thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the dCas endonuclease and the at least one polypeptide having enzymatic activity can comprise a IBA1 promoter, a derivative thereof, or a fragment thereof.

[0532] In an aspect of a disclosed method, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) can be operably linked to the fusion protein having one or more enzymatic activities. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the fusion protein having one or more enzymatic activities can comprise a F4/80 promoter, a derivative thereof, or a fragment thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the fusion protein having one or more enzymatic activities can comprise a CD68 promoter, a derivative thereof, or a fragment thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the fusion protein having one or more enzymatic activities can comprise a IBA1 promoter, a derivative thereof, or a fragment thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the fusion protein having one or more enzymatic activities can comprise a FCGR2A promoter, a derivative thereof, or a fragment thereof.

[0533] In an aspect of a disclosed method, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) can be operably linked to the dCas endonuclease and the fusion protein having one or more enzymatic activities. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the dCas endonuclease

and the fusion protein having one or more enzymatic activities can comprise a F4/80 promoter, a derivative thereof, or a fragment thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the dCas endonuclease and the fusion protein having one or more enzymatic activities can comprise a CD68 promoter, a derivative thereof, or a fragment thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the dCas endonuclease and the fusion protein having one or more enzymatic activities can comprise a IBA1 promoter, a derivative thereof, or a fragment thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the dCas endonuclease and the fusion protein having one or more enzymatic activities can comprise a FCGR2A promoter, a derivative thereof, or a fragment thereof.

[0534] In an aspect of a disclosed method, a disclosed isolated nucleic acid or disclosed viral vector can further comprise one or more regulatory elements (*i.e.*, promoters) in addition to the microglia specific regulatory element (*e.g.*, microglia specific promoter). In an aspect, a disclosed additional promoter can comprise a U6 promoter, a chicken β-actin promoter, an EF-1α, a CMV promoter, a CMV promoter/enhancer, a fragment thereof, a derivative thereof, or any combination thereof. In an aspect, a disclosed U6 promoter can comprise the sequence set forth in SEQ ID NO: 81, or a fragment thereof. In an aspect, a disclosed U6 promoter can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% identity to the sequence set forth in SEQ ID NO: 81 or a fragment thereof. In an aspect, a disclosed EF-1α promoter can comprise the sequence set forth in SEQ ID NO: 79, SEQ ID NO: 80, or a fragment thereof. In an aspect, a disclosed EF-1α promoter can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% identity to the sequence set forth in SEQ ID NO: 79, SEQ ID NO: 80, or a fragment thereof. In an aspect, a promoter can be a short EF1alpha (EFS-NF) promoter.

[0535] In an aspect of a disclosed method, a disclosed promoter operably can be linked to the at least one guide RNA targeting a gene of interest or portion thereof. In an aspect, a disclosed promoter operably linked to the at least one guide RNA can comprise a U6 promoter, a derivative thereof, or a fragment thereof. In an aspect, a disclosed promoter operably linked to the at least one guide RNA targeting an *APOE* gene can comprise a U6 promoter, a derivative thereof, or a fragment thereof.

[0536] In an aspect of the disclosed method, a disclosed isolated nucleic acid molecule or viral vector can further two or more promoters, wherein a first promoter can be operably linked to the

dCas endonuclease, a polypeptide having enzymatic activity, a fusion of two or more polypeptides having enzymatic activities or any combination thereof, and wherein a second promoter can be operably linked to the at least one guide RNA targeting a gene of interest or portion thereof. In an aspect, a disclosed isolated nucleic acid molecule can further comprise one or more promoters, wherein a first promoter comprises a microglia specific promoter that is operably linked to the dCas endonuclease, a polypeptide having enzymatic activity, a fusion of two or more polypeptides having enzymatic activities or any combination thereof, and wherein a second promoter can be operably linked to the at least one guide RNA targeting a gene of interest thereof. In an aspect, the second promoter operably linked to the gRNA may not be a microglia specific promoter. In an aspect, the isolated nucleic acid can comprise a first promoter selected from a F4/80 promoter, a CD68 promoter, a IBA1 promoter, or a FCGR2A promoter, a fragment thereof, a derivative thereof, or any combination thereof, and a second promoter comprising a U6 promoter.

[0537] In an aspect of the disclosed method, a disclosed isolated nucleic acid molecule or viral vector can further comprise one or more additional regulatory elements. In an aspect, a disclosed additional regulatory element can comprise a promoter (described supra), an enhancer, a promoter/enhancer, an internal ribosomal entry site, a transcription terminal signal, a polyadenylation signal, a Sp1 and/or NF-kB transcriptional factor binding site, a p2A signal, a woodchuck hepatitis virus post-transcriptional regulatory element, a Phi signal-packaging signal, a rev responsive element, a 5'-ITR, a 3'-ITR, an inverted terminal repeat, a nuclear localization signal (NLS), or any combination thereof.

[0538] In an aspect of the disclosed method, a disclosed 5'-ITR may comprise the sequence as set forth in any one of SEQ ID NO: 82 or SEQ ID NO: 83. In an aspect, a disclosed 3'-ITR may comprise the sequence as set forth in any one of SEQ ID NO: 84 or SEQ ID NO: 85. In an aspect, a disclosed NLS can comprise the sequence set forth in any one of SEQ ID NO: 88 – SEQ ID NO: 94, or a fragment thereof. In an aspect, a disclosed PolyA sequence can comprise the sequence set forth in SEQ ID NO: 86, SEQ ID NO: 87, or a fragment thereof.

[0539] In an aspect of a disclosed method, a disclosed viral vector or isolated nucleic acid can comprise a nucleic acid that is CpG depleted and codon-optimized for expression in a human cell. In an aspect, "CpG-free" can mean completely free of CpGs or partially free of CpGs. In an aspect, "CpG-free" can mean "CpG-depleted." In an aspect, "CpG-depleted" can mean "CpG-free." In an aspect, "CpG-depleted" can mean completely depleted of CpGs or partially depleted of CpGs. In an aspect, "CpG-free" can mean "CpG-optimized" for a desired and/or ideal expression level. CpG depletion and/or optimization is known to the skilled person in the art. In an aspect, any

disclosed Cas9 endonuclease, a disclosed polypeptide having enzymatic activities, a disclosed fusion product or a disclosed fusion protein, or any combination thereof can be codon-optimized.

[0540] In an aspect of a disclosed method, a disclosed viral vector or disclosed recombinant AAV vector can comprise a sequence that is about 4.5 kilobases or less than about 4.5 kilobases.

[0541] In an aspect of a disclosed method of treating and/or preventing Alzheimer's diseases or an APOE related disease can comprise increasing expression and/or activity of one or more genes of interest. In an aspect of a disclosed method of effecting precision epigenetic modulation, modulating can comprise decreasing expression and/or activity of one or more genes of interest.

[0542] In an aspect, a disclosed method of effecting precision epigenetic modulation can comprise reducing expression and/or activity of APOE.

[0543] In an aspect, the disclosed cells can be microglial cells. In an aspect, the disclosed cells can be cells affected by an overexpression level and/or activity level of one or more genes of interest or one or more target genes.

[0544] In an aspect, the disclosed cells can be in a subject. In an aspect of a disclosed method, a subject can be a human. In an aspect, a subject can be suspected of having or can be diagnosed with having Alzheimer's disease. In an aspect, a subject can be suspected of having or can be diagnosed with having Parkinson's disease. In an aspect, a disclosed subject can be symptomatic or asymptomatic.

[0545] In an aspect of a disclosed method of treating and/or preventing progression of a Alzheimer's or other APOE associated disease or disorder, techniques to monitor, measure, and/or assess the restoring one or more aspects of cellular homeostasis and/or cellular functionality can comprise qualitative (or subjective) means as well as quantitative (or objective) means. These means are known to the skilled person. For example, representative regulated variables and sensors relating to systemic homeostasis are discussed supra.

[0546] In an aspect of a disclosed method of treating and/or preventing progression of a Alzheimer's or other APOE associated disease or disorder, administering can comprise intravenous administration, intracerebral administration, intra-CSF administration, intracerebroventricular (ICV) administration, intraventricular administration, intra-cisterna magna (ICM) administration, intraparenchymal administration, intrathecal (lumbar, cisternal, or both) administration, intrahepatic administration, hepatic intra-arterial administration, hepatic portal vein (HPV) administration, or any combination thereof. In an aspect, a disclosed vector can be administered via LNP administration.

[0547] In an aspect of a disclosed method of treating and/or preventing progression of a Alzheimer's or other APOE associated disease or disorder, administering can comprise use of

non-viral vectors to deliver a disclosed isolated nucleic acid. These non-viral vectors can include, but are not limited to, nanoparticles or liposomes. Alternatively, the disclosed isolated nucleic acids may be delivered via viral vectors (such as the disclosed viral vectors provided herein). Exemplary viral vectors are described supra and include adenovirus, adeno-associated virus (AAV), and lentivirus (LV). Viral vectors are used more commonly due to their robust delivery, long-lasting expression, low immunogenicity and toxicity, and compatibility with the advanced manufacturing techniques. Most research in gene therapy has utilized AAV or LV due to their lowered immunogenicity compared to adenovirus. Derived from HIV, LV integrates its viral genome into the host gene to create permanent change in gene expression.

[0548] In an aspect, a disclosed method of treating and/or preventing progression of a Alzheimer's or other APOE associated disease or disorder can employ multiple routes of administration to the subject. In an aspect, a disclosed method can employ a first route of administration that can be the same or different as a second and/or subsequent routes of administration. In an aspect, a disclosed nucleic acid molecule, a disclosed vector, and/or a disclosed pharmaceutical formulation can be concurrently and/or serially administered to a subject via multiple routes of administration. For example, in an aspect, administering a disclosed nucleic acid molecule, a disclosed vector, and/or a disclosed pharmaceutical formulation can comprise intravenous administration and intra-cistern magna (ICM) administration. In an aspect, administering a disclosed nucleic acid molecule, a disclosed vector, and/or a disclosed pharmaceutical formulation can comprise IV administration and intrathecal (ITH) administration.

[0549] In an aspect of a disclosed method of treating and/or preventing progression of a Alzheimer's or other APOE associated disease or disorder, a therapeutically effective amount of disclosed vector can be delivered via intravenous (IV) administration and can comprise a range of about 1×10^{10} vg/kg to about 2×10^{14} vg/kg. In an aspect, for example, a disclosed vector can be administered at a dose of about 1×10^{11} vg/kg to about 8×10^{13} vg/kg or about 1×10^{12} vg/kg to about 8×10^{13} vg/kg. In an aspect, a disclosed vector can be administered at a dose of about 1×10^{13} vg/kg to about 6×10^{13} vg/kg. In an aspect, a disclosed vector can be administered at a dose of at least about 1×10^{10} vg/kg, at least about 5×10^{10} vg/kg, at least about 1×10^{11} vg/kg, at least about 5×10^{11} vg/kg, at least about 1×10^{12} vg/kg, at least about 5×10^{12} vg/kg, at least about 1×10^{13} vg/kg, at least about 5×10^{13} vg/kg, or at least about 1×10^{14} vg/kg. In an aspect, a disclosed vector can be administered at a dose of no more than about 1×10^{10} vg/kg, no more than about 5×10^{10} vg/kg, no more than about 1×10^{11} vg/kg, no more than about 5×10^{11} vg/kg, no more than about 1×10^{12} vg/kg, no more than about 5×10^{12} vg/kg, no more than about 1×10^{13} vg/kg, no more than about 5×10^{13}, or no more than about 1×10^{14} vg/kg. In an aspect, a disclosed vector

can be administered at a dose of about 1 x 10^{12} vg/kg. In an aspect, a disclosed vector can be administered at a dose of about 1 x 10^{11} vg/kg. In an aspect, a disclosed vector can be administered in a single dose, or in multiple doses (such as 2, 3, 4, 5, 6, 7, 8, 9 or 10 doses) as needed for the desired therapeutic results.

[0550] In an aspect, a disclosed method of treating and/or preventing progression of a Alzheimer's or other APOE associated disease or disorder can further comprise monitoring the subject for adverse effects. In an aspect, in the absence of adverse effects, the method can further comprise continuing to treat the subject. In an aspect, in the presence of adverse effects, the method can further comprise modifying the treating step. Methods of monitoring a subject's well-being can include both subjective and objective criteria (and are discussed supra). Such methods are known to the skilled person.

[0551] In an aspect, a disclosed method of treating and/or preventing progression of a Alzheimer's or other APOE associated disease or disorder can further comprise administering to the subject a therapeutically effective amount of a therapeutic agent. A therapeutic agent can be any disclosed agent that effects a desired clinical outcome.

[0552] In an aspect, a disclosed method of treating and/or preventing progression of a Alzheimer's or other APOE associated disease or disorder can further comprise administering to the subject a therapeutically effective amount of an agent that can correct one or more aspects of a dysregulated metabolic or enzymatic pathway. In an aspect, such an agent can comprise an enzyme for enzyme replacement therapy. In an aspect, a disclosed enzyme can replace any enzyme in a dysregulated or dysfunctional metabolic or enzymatic pathway. In an aspect, a disclosed method can comprise replacing one or more enzymes in a dysregulated or dysfunctional metabolic pathway.

[0553] In an aspect, a disclosed method of treating and/or preventing progression of a Alzheimer's or other APOE associated disease or disorder can further comprise administering one or more immune modulators. In an aspect, a disclosed immune modulator can be methotrexate, rituximab, intravenous gamma globulin, or bortezomib, or a combination thereof. In an aspect, a disclosed immune modulator can be bortezomib or SVP-Rapamycin. In an aspect, a disclosed immune modulator can be Tacrolimus. In an aspect, a disclosed immune modulator such as methotrexate can be administered at a transient low to high dose. In an aspect, a disclosed immune modulator can be administered at a dose of about 0.1 mg/kg body weight to about 0.6 mg/kg body weight. In an aspect, a disclosed immune modulator can be administered at a dose of about 0.4 mg/kg body weight. In an aspect, a disclosed immune modulator can be administered at about a daily dose of 0.4 mg/kg body weight for 3 to 5 or greater cycles, with up to three days per cycle. In an aspect, a disclosed immune modulator can be administered at about a daily dose of 0.4 mg/kg

body weight for a minimum of 3 cycles, with three days per cycle. In an aspect, a person skilled in the art can determine the appropriate number of cycles. In an aspect, a disclosed immune modulator can be administered as many times as necessary to achieve a desired clinical effect.

[0554] In an aspect, a disclosed immune modulator can be administered orally about one hour before a disclosed therapeutic agent. In an aspect, a disclosed immune modulator can be administered subcutaneously about 15 minutes before a disclosed therapeutic agent. In an aspect, a disclosed immune modulator can be administered concurrently with a disclosed therapeutic agent. In an aspect, a disclosed immune modulator can be administered orally about one hour or a few days before a disclosed isolated nucleic acid molecule, a disclosed vector, a disclosed pharmaceutical formulation, or a combination thereof. In an aspect, a disclosed immune modulator can be administered subcutaneously about 15 minutes before or a few days before a disclosed isolated nucleic acid molecule, a disclosed vector, a disclosed pharmaceutical formulation, or a combination thereof. In an aspect, a disclosed immune modulator can be administered concurrently with a disclosed isolated nucleic acid molecule, a disclosed vector, a disclosed pharmaceutical formulation, or a combination thereof.

[0555] In an aspect, a disclosed method of treating and/or preventing progression of a Alzheimer's or other APOE associated disease or disorder can further comprise administering one or more proteasome inhibitors (*e.g.*, bortezomib, carfilzomib, marizomib, ixazomib, and oprozomib). In an aspect, a proteasome inhibitor can be an agent that acts on plasma cells (*e.g.*, daratumumab). In an aspect, an agent that acts on a plasma cell can be melphalan hydrochloride, melphalan, pamidronate disodium, carmustine, carfilzomib, carmustine, cyclophosphamide, daratumumab, doxorubicin hydrochloride liposome, doxorubicin hydrochloride liposome, elotuzumab, melphalan hydrochloride, panobinostat, ixazomib citrate, carfilzomib, lenalidomide, melphalan, melphalan hydrochloride, plerixafor, ixazomib citrate, pamidronate disodium, panobinostat, plerixafor, pomalidomide, pomalidomide, lenalidomide, selinexor, thalidomide, thalidomide, bortezomib, selinexor, zoledronic acid, or zoledronic acid.

[0556] In an aspect, a disclosed method of treating and/or preventing progression of a Alzheimer's or other APOE associated disease or disorder can further comprise administering one or more proteasome inhibitors or agents that act on plasma cells prior to administering a disclosed isolated nucleic acid molecule, a disclosed vector, or a disclosed pharmaceutical formulation. In an aspect, a disclosed method can comprise administering one or more proteasome inhibitors or one or more agents that act on plasma cells concurrently with administering a disclosed isolated nucleic acid molecule, a disclosed vector, or a disclosed pharmaceutical formulation. In an aspect, a disclosed method can comprise administering one or more proteasome inhibitors or one or more agents that

act on plasma cells after administering a disclosed isolated nucleic acid molecule, a disclosed vector, or a disclosed pharmaceutical formulation. In an aspect, a disclosed method can further comprise administering one or more proteasome inhibitors more than 1 time. In an aspect, a disclosed method can comprise administering one or more proteasome inhibitors repeatedly over time.

[0557] In an aspect, a disclosed method of treating and/or preventing progression of a Alzheimer's or other APOE associated disease or disorder can further comprise administering one or more immunosuppressive agents. In an aspect, an immunosuppressive agent can be, but is not limited to, azathioprine, methotrexate, sirolimus, anti-thymocyte globulin (ATG), cyclosporine (CSP), mycophenolate mofetil (MMF), steroids, or a combination thereof. In an aspect, a disclosed method can comprise administering one or more immunosuppressive agents more than 1 time. In an aspect, a disclosed method can comprise administering one or more one or more immunosuppressive agents repeatedly over time. In an aspect, a disclosed method can comprise administering a compound that targets or alters antigen presentation or humoral or cell mediated or innate immune responses.

[0558] In an aspect, a disclosed method of treating and/or preventing progression of a Alzheimer's or other APOE associated disease or disorder can further comprise administering a compound that exerts a therapeutic effect against B cells and/or a compound that targets or alters antigen presentation or humoral or cell mediated immune response. In an aspect, a disclosed compound can be rituximab, methotrexate, intravenous gamma globulin, anti CD4 antibody, anti CD2, an anti-FcRN antibody, a BTK inhibitor, an anti-IGF1R antibody, a CD19 antibody (*e.g.*, inebilizumab), an anti-IL6 antibody (*e.g.*, tocilizumab), an antibody to CD40, an IL2 mutein, or a combination thereof. Also disclosed herein are Treg infusions that can be administered as a way to help with immune tolerance (*e.g.*, antigen specific Treg cells to AAV).

[0559] In an aspect of a disclosed method, a subject can be a human. In an aspect, a subject can be suspected of having or can be diagnosed with having Alzheimer's or other APOE associated disease or disorder (such as, for example, PD or DLB). In an aspect, a disclosed subject can be symptomatic or asymptomatic.

[0560] In an aspect, a disclosed method can comprise repeating one or more steps of the method and/or modifying one or more steps of the method (such as, for example, an administering step).

[0561] In an aspect, a disclosed method of treating and/or preventing progression of a Alzheimer's or other APOE associated disease or disorder can comprise modifying one or more of the disclosed steps. For example, modifying one or more of steps of a disclosed method can comprise modifying or changing one or more features or aspects of one or more steps of a disclosed method. For

example, in an aspect, a method can be altered by changing the amount of one or more of the disclosed isolated nucleic acid molecules, disclosed vectors, disclosed pharmaceutical formulations, or a combination thereof administered to a subject, or by changing the frequency of administration of one or more of the disclosed isolated nucleic acid molecules, disclosed vectors, disclosed pharmaceutical formulations, or a combination thereof to a subject, or by changing the duration of time one or more of the disclosed isolated nucleic acid molecules, disclosed vectors, disclosed pharmaceutical formulations, or a combination are administered to a subject.

[0562] In an aspect, a disclosed method of treating and/or preventing progression of a Alzheimer's or other APOE associated disease or disorder can be altered by changing the amount of one or more disclosed therapeutic agents, disclosed immune modulators, disclosed proteasome inhibitors, disclosed immunosuppressive agents, disclosed compounds that exert therapeutic effect against B cells and/or disclosed compounds that targets or alters antigen presentation or humoral or cell mediated immune response administered to a subject, or by changing the frequency of administration of one or more of the disclosed therapeutic agents, disclosed immune modulators, disclosed proteasome inhibitors, disclosed immunosuppressive agents, disclosed compounds that exert therapeutic effect against B cells and/or disclosed compounds that targets or alters antigen presentation or humoral or cell mediated immune response administered to a subject.

[0563] In an aspect, a disclosed method of treating and/or preventing progression of a Alzheimer's or other APOE associated disease or disorder can further comprise generating and/or validating one or more of the disclosed isolated nucleic acid molecules, one or more of the disclosed vectors, one or more of the disclosed pharmaceutical formulations, or any combination thereof.

[0564] In an aspect, a disclosed method of treating and/or preventing progression of a synucleinopathy or other APOE associated disease or disorder can further comprise administering to the subject a second disclosed isolated nucleic acid molecule, a second disclosed vector, a second disclosed pharmaceutical formulations, or any combination thereof. In an aspect, a disclosed method of treating and/or preventing progression of a synucleinopathy or other APOE associated disease or disorder can further comprise administering to the subject additional disclosed isolated nucleic acid molecules, additional disclosed vectors, additional disclosed pharmaceutical formulations, or any combination thereof.

H. Methods of Treating and/or Preventing Parkinson's Disease Progression

[0565] Disclosed herein is a method of treating and/or preventing Parkinson's disease progression in a subject, the method comprising administering to a subject a therapeutically effective amount

of a disclosed isolated nucleic acid molecule, thereby reducing the pathological phenotype associated with Parkinson's disease.

[0566] Disclosed herein is a method of treating and/or preventing Parkinson's disease progression in a subject, the method comprising administering to a subject a therapeutically effective amount of a disclosed isolated nucleic acid molecule, comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, (ii) at least one polypeptide having an enzymatic activity, and (iii) at least one guide RNA targeting a *APOE* gene or portion thereof and (b) at least one microglia specific regulatory element operably linked to (a)(i), (a)(ii), (a)(iii) or any combination thereof, thereby reducing the pathological phenotype associated with Parkinson's disease. Disclosed herein is a method of treating and/or preventing Parkinson's disease progression in a subject, the method comprising administering to a subject a therapeutically effective amount of a disclosed isolated nucleic acid molecule, comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, (ii) at least one polypeptide having an enzymatic activity, and (iii) at least one guide RNA targeting a *APOE* gene or portion thereof and (b) at least one microglia specific promoter operably linked to (a)(i), (a)(ii), (a)(iii) or any combination thereof, thereby reducing the pathological phenotype associated with Parkinson's disease.

[0567] Disclosed herein is a method of treating and/or preventing Parkinson's disease progression in a subject, the method comprising administering to a subject a therapeutically effective amount of an isolated nucleic acid comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, (ii) a fusion product having one or more enzymatic activities, (iii) at least one guide RNA targeting the *APOE* gene or a portion of the *APOE* gene, and (b) at least one microglia specific promoter operably linked to (a)(i), (a)(ii), (a)(iii) or any combination thereof, thereby reducing the pathological phenotype associated with Parkinson's disease.

[0568] Disclosed herein is a method of treating and/or preventing Parkinson's disease progression in a subject, the method comprising administering to a subject a therapeutically effective amount of an isolated nucleic acid molecule, comprising: (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, wherein the dCas is a dSpCas9, dSaCas9, or dCjCas9, (ii) a fusion product having one or more enzymatic activities, wherein the fusion product comprises any combination of HP1a, HP1b, MBD1, MBD2, KRAB, NIPP1, MeCP2, or DNMT3A, and (iii) at least one guide RNA targeting an *APOE* gene or portion thereof and b) at least one microglia specific regulatory element operably linked to (a)(i), (a)(ii), (a)(iii) or any combination thereof, wherein the expression and/or activity the *APOE* gene in the one or more microglial cells is modulated, thereby reducing the pathological phenotype associated with Parkinson's disease. In some aspects, the microglia specific regulatory element of (b) can comprise a F4/80 promoter

(SEQ ID NO: 4), CD68 promoter (SEQ ID NO: 1-3), FCGR2A promoter (SEQ ID NO: 8), or IBA1 promoter (SEQ ID NO: 5-7), a derivative thereof, a fragment thereof, or a combination thereof, a derivative thereof, a fragment thereof, or any combination thereof.

[0569] Disclosed herein is a method of treating and/or preventing progression of a Parkinson's disease or disorder in a subject, the method comprising administering to a subject a therapeutically effective amount of an isolated nucleic acid molecule, comprising: (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, wherein the dCas is a dSpCas9, dSaCas9, or dCjCas9, (ii) a fusion product having one or more enzymatic activities, wherein the fusion product comprises any combination of HP1a, HP1b, MBD1, MBD2, KRAB, NIPP1, MeCP2, or DNMT3A, and (iii) at least one guide RNA targeting the *APOE* gene or a portion of the *APOE* gene and (b) at least one microglia specific promoter operably linked to (a)(i), (a)(ii), (a)(iii) or any combination thereof, thereby reducing the pathological phenotype associated with the Parkinson's disease or disorder. In some aspects, the microglia specific promoter of (b) can comprise a F4/80 promoter (SEQ ID NO: 4), CD68 promoter (SEQ ID NO: 1-3), FCGR2A promoter (SEQ ID NO: 8), or IBA1 promoter (SEQ ID NO: 5-7), a derivative thereof, a fragment thereof, or a combination thereof, a derivative thereof, a fragment thereof, or any combination thereof.

[0570] Disclosed herein is a method of treating and/or preventing progression of a Parkinson's disease or disorder in a subject, the method comprising administering to a subject a therapeutically effective amount of an isolated nucleic acid molecule, comprising: (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, wherein the dCas is a dSpCas9, dSaCas9, or dCjCas9, (ii) a fusion product having one or more enzymatic activities, wherein the fusion product comprises any combination of HP1a, HP1b, MBD1, MBD2, KRAB, NIPP1, MeCP2, or DNMT3A, and (iii) at least one guide RNA targeting the *APOE* gene or a portion of the *APOE* gene, (b) a microglia specific regulatory element operably linked to (a)(i) and/or (a)(ii), and (c) a promoter operably linked to (a)(iii), thereby reducing the pathological phenotype associated with the Parkinson's disease or disorder. In some aspects, the microglia specific regulatory element of (iv) may comprise a (b) can comprise a F4/80 promoter (SEQ ID NO: 4), CD68 promoter (SEQ ID NO: 1-3), FCGR2A promoter (SEQ ID NO: 8), or IBA1 promoter (SEQ ID NO: 5-7), a derivative thereof, a fragment thereof, or a combination thereof. In some aspects, the promoter of (c) comprises a U6 promoter, a derivative thereof or a fragment thereof.

[0571] Disclosed herein is a method of treating and/or preventing progression of a Parkinson's disease or disorder in a subject, the method comprising administering to a subject a therapeutically

effective amount of a disclosed viral vector, thereby reducing the pathological phenotype associated with the Parkinson's disease or disorder.

[0572] Disclosed herein is a method of treating and/or preventing progression of a Parkinson's disease or disorder in a subject, the method comprising administering to a subject a therapeutically effective amount of a viral vector comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, (ii) at least one polypeptide having an enzymatic activity, and (iii) at least one guide RNA targeting the *APOE* gene or a portion of the *APOE* gene and (b) at least one microglia specific regulatory element operably linked to (a)(i), (a)(ii), (a)(iii) or a combination thereof, thereby reducing the pathological phenotype associated with the Parkinson's disease or disorder. Disclosed herein is a method of treating and/or preventing progression of a Parkinson's disease or disorder in a subject, the method comprising administering to a subject a therapeutically effective amount of a disclosed viral vector, comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, (ii) at least one polypeptide having an enzymatic activity, and (iii) at least one guide RNA targeting a *APOE* gene or portion thereof and (b) at least one microglia specific promoter operably linked to (a)(i), (a)(ii), (a)(iii) or any combination thereof, thereby reducing the pathological phenotype associated with the Parkinson's disease or disorder.

[0573] Disclosed herein is a method of treating and/or preventing progression of a Parkinson's disease or disorder in a subject, the method comprising administering to a subject a therapeutically effective amount of a viral vector comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, (ii) a fusion product having one or more enzymatic activities, (iii) at least one guide RNA targeting the *APOE* gene or a portion of the *APOE* gene and (b) at least one microglia specific regulatory element operably linked to (a)(i), (a)(ii), (a)(iii), or a combination thereof, thereby reducing the pathological phenotype associated with the Parkinson's disease or disorder.

[0574] Disclosed herein is a method of treating and/or preventing progression of a Parkinson's disease or disorder in a subject, the method comprising administering to a subject a therapeutically effective amount of a viral vector comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, (ii) a fusion product having one or more enzymatic activities, (iii) at least one guide RNA targeting the *APOE* gene or a portion of the *APOE* gene and (b) at least one microglia specific promoter operably linked to (a)(i), (a)(ii), (a)(iii), or a combination thereof, thereby reducing the pathological phenotype associated with the Parkinson's disease or disorder.

[0575] Disclosed herein is a method of treating and/or preventing progression of a Parkinson's disease or disorder in a subject, the method comprising administering to a subject a therapeutically effective amount of a viral vector comprising (a) a nucleic acid sequence encoding (i) a deactivated

Cas (dCas) endonuclease, wherein the dCas is a dSpCas9, dSaCas9, or dCjCas9, (ii) a fusion product having one or more enzymatic activities, wherein the fusion product comprises any combination of HP1a, HP1b, MBD1, MBD2, KRAB, NIPP1, MeCP2, or DNMT3A, (iii) at least one guide RNA targeting the *APOE* gene or a portion of the *APOE* gene, and (b) at least one microglia specific regulatory element operably linked to (a)(i), (a)(ii), (a)(iii), or any combination thereof, thereby reducing the pathological phenotype associated with the Parkinson's disease or disorder. Disclosed herein is a method of treating and/or preventing progression of a Parkinson's disease or disorder in a subject, the method comprising administering to a subject a therapeutically effective amount of a viral vector comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, wherein the dCas is a dSpCas9, dSaCas9, or dCjCas9, (ii) a fusion product having one or more enzymatic activities, wherein the fusion product comprises any combination of HP1a, HP1b, MBD1, MBD2, KRAB, NIPP1, MeCP2, or DNMT3A, (iii) at least one guide RNA targeting the *APOE* gene or a portion of the *APOE* gene, and (b) at least one microglia specific promoter operably linked to (a)(i), (a)(ii), (a)(iii), or any combination thereof, thereby reducing the pathological phenotype associated with the Parkinson's disease or disorder.

[0576] Disclosed herein is a method of treating and/or preventing progression of a Parkinson's disease or disorder in a subject, the method comprising administering to a subject a therapeutically effective amount of a viral vector comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, wherein the dCas is a dSpCas9, dSaCas9, or dCjCas9, (ii) a fusion product having one or more enzymatic activities, wherein the fusion product comprises any combination of HP1a, HP1b, MBD1, MBD2, KRAB, NIPP1, MeCP2, or DNMT3A, and (iii) at least one guide RNA targeting the *APOE* gene or a portion of the APOE gene, (b) a microglia specific regulatory element operably linked to the (a)(i) and/or (a)(ii), and (c) a promoter operably linked to (a)(iii), thereby reducing the pathological phenotype associated with the Parkinson's disease or disorder. Disclosed herein is a method of treating and/or preventing progression of a Parkinson's disease or disorder in a subject, the method comprising administering to a subject a therapeutically effective amount of a viral vector comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, wherein the dCas is a dSpCas9, dSaCas9, or dCjCas9, (ii) a fusion product having one or more enzymatic activities, wherein the fusion product comprises any combination of HP1a, HP1b, MBD1, MBD2, KRAB, NIPP1, MeCP2, or DNMT3A, and (iii) at least one guide RNA targeting the *APOE* gene or a portion of the *APOE* gene, (b) a microglia specific promoter operably linked to the (a)(i) and/or (a)(ii), and (c) a promoter operably linked to (a)(iii), thereby reducing the pathological phenotype associated with the Parkinson's disease or disorder. In an aspect, the microglia specific regulatory element or

microglia specific promoter of (b) can comprise Alzheimer's. In an aspect, the fusion product of (a)(ii) can comprise KRAB-MeCP2.

[0577] Disclosed herein is a method of treating and/or preventing progression of Parkinson's disease or disorder in a subject, the method comprising administering to a subject a therapeutically effective amount of a viral vector comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, wherein the dCas is a dSpCas9, dSaCas9, or dCjCas9, (ii) a fusion product having one or more enzymatic activities, wherein the fusion product comprises any combination of HP1a, HP1b, MBD1, MBD2, KRAB, NIPP1, MeCP2, or DNMT3A, and (iii) at least one guide RNA targeting the *APOE* gene or a portion of the *APOE* gene, (b) a microglia specific regulatory element operably linked to (a)(i) and/or (a)(ii), and (c) a promoter operably linked to (a)(iii), thereby reducing the pathological phenotype associated with the Parkinson's disease or disorder. Disclosed herein is a method of treating and/or preventing progression of a Parkinson's disease or disorder in a subject, the method comprising administering to a subject a therapeutically effective amount of a viral vector comprising (a) a nucleic acid sequence encoding (i) a deactivated Cas (dCas) endonuclease, wherein the dCas is a dSpCas9, dSaCas9, or dCjCas9, (ii) a fusion product having one or more enzymatic activities, wherein the fusion product comprises any combination of HP1a, HP1b, MBD1, MBD2, KRAB, NIPP1, MeCP2, or DNMT3A, and (iii) at least one guide RNA targeting the *APOE* gene or a portion of the *APOE* gene, (b) a microglia specific promoter operably linked to (a)(i) and/or (a)(ii), and (c) a promoter operably linked to (a)(iii), thereby reducing the pathological phenotype associated with the Alzhiemer's or other APOE associated disease or disorder. In an aspect, the microglia specific regulatory element or microglia specific promoter of (b) can comprise a (b) can comprise a F4/80 promoter (SEQ ID NO: 4), CD68 promoter (SEQ ID NO: 1-3), FCGR2A promoter (SEQ ID NO: 8), or IBA1 promoter (SEQ ID NO: 5-7), a derivative thereof, a fragment thereof, or a combination thereof. In an aspect, the fusion product of (a)(ii) can comprise KRAB-MeCP2. In an aspect, the promoter of (c) comprises a U6 promoter, a derivative thereof, or a fragment thereof.

[0578] In an aspect, a disclosed method of treating and/or preventing progression of Parkinson's disease or disorder can reduce and/or decrease the expression and/or activity of APOE. In an aspect of a disclosed method of treating and/or preventing progression of Parkinson's disease or disorder, the expression and/or activity of APOE is reduced and/or decreased. In an aspect, a disclosed method can comprise diagnosing the subject with Parkinson's disease or disorder.

[0579] In an aspect, a disclosed method of treating and/or preventing Parkinson's disease or disorder comprises measuring the level of expression and/or the level of activity of the APOE

gene. In an aspect, a disclosed method can comprise repeating the measuring step one or more times.

[0580] In an aspect, prior to the administering step, the expression and/or activity level of APOE can be elevated and/or increased when compared to wild-type or control expression level. In an aspect, following the administering step, the expression and/or activity level of APOE can be reduced and/or decreased when compared to the pre-administering step level. In an aspect, a disclosed gRNA targeting a portion of the *APOE* gene can comprise at least two gRNAs. In an aspect, disclosed gRNAs can target the same portion of the *APOE* gene or different portions of the APOE gene. In an aspect, a disclosed gRNA can target the promoter of the APOE gene. In an aspect, a disclosed gRNA can target exon 1 of the APOE gene. In an aspect, a disclosed gRNA can target exon 1 of the APOE gene. In an aspect, a disclosed gRNA can target intron 1 of the APOE gene. In an aspect, a disclosed gRNA can target intron 4 of the APOE gene. In an aspect, a disclosed gRNA can target a CpG island region of intron 1 of the APOE gene. The CpG island region can include CpG1, CpG2, CpG3, CpG4, CpG5, CpG6, CpG7, CpG8, CpG9, CpG10, CpG11, CpG12, CpG13, CpG14, CpG15, CpG16, CpG17, CpG18, CpG19, CpG20, CpG21, CpG22, CpG23, or a combination thereof. For example, the CpG island region can include CpG1, CpG3, CpG6, CpG7, CpG8, CpG9, CpG18, CpG19, CpG20, CpG21, CpG22, or a combination thereof. In an aspect, the disclosed gRNA may comprise a nucleic acid sequence as set forth in SEQ ID NO: 15-33. In an aspect, the disclosed gRNA may comprise a nucleic acid sequence as set forth in SEQ ID NO: 15-33, or a complement thereof. In an aspect, the disclosed gRNA may comprise a nucleic acid sequence with at least about 90%, or about 91%, or about 92%, or about 93%, or about 94%, or about 95%, or about 96%, or about 97%, or about 98%, or about 99%, or more sequence identity to any one of the sequences set forth in SEQ ID NO: 15-33, or a complement thereof, or a fragment thereof.

[0581] In an aspect of a disclosed method, a disclosed viral vector or disclosed recombinant AAV vector can further comprise a gRNA scaffold. In an aspect, a disclosed gRNA scaffold can comprise the sequence set forth in SEQ ID NO: 129, SEQ ID NO: 130, or a fragment thereof.

[0582] In an aspect of a disclosed method, a disclosed dCas endonuclease can comprise a dCas9 endonuclease. In an aspect, a disclosed dCas9 endonuclease can comprise a deactivated *Staphylococcus aureus* Cas9 (dSaCas9), a deactivated *Streptococcus pyogenes* Cas9 (dSpCas9), a deactivated *Campylobacter jejuni* Cas9 (dCjCas9), or a variant dCas9 endonuclease. In an aspect, a disclosed variant dCas9 can comprise a variant dSaCas9, a variant dSpCas9, or a variant dCjCa9. In an aspect, a disclosed variant dSpCas9 can comprise dVQR, dEQR, or dVRER.

[0583] In an aspect of a disclosed method, a disclosed dCas endonuclease can comprise a dCas9 endonuclease. In an aspect, a disclosed dCas9 endonuclease can comprise a deactivated *Staphylococcus aureus* Cas9 (dSaCas9), a deactivated *Streptococcus pyogenes* Cas9 (dSpCas9), a deactivated *Campylobacter jejuni* Cas9 (dCjCas9), or a variant dCas9 endonuclease. In an aspect, a disclosed variant dCas9 can comprise a variant dSaCas9, a variant dSpCas9, or a variant dCjCa9. In an aspect, a disclosed variant dSpCas9 can comprise dVQR, dEQR, or dVRER.

[0584] In an aspect of a disclosed method, a nucleic acid sequence the disclosed dSpCas9 may comprise a sequence as set forth in any one of SEQ ID NO: 40 or SEQ ID NO: 41, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed dSpCas9 endonuclease may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 40 or SEQ ID NO: 41, or a fragment thereof. In an aspect, a disclosed dSpCas9 endonuclease can comprise the amino acid sequence as set forth in SEQ ID NO: 36 or SEQ ID NO: 37, or a fragment, or a derivative thereof. In an aspect, a disclosed dSpCas9 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 36 or SEQ ID NO: 37, or a fragment thereof.

[0585] In an aspect, the nucleic acid sequence encoding the disclosed dSaCas9 may comprise a sequence as set forth in SEQ ID NO: 42, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed dSaCas9 endonuclease may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequences set forth in SEQ ID NO: 42, or a fragment thereof. In an aspect, a disclosed dSaCas9 endonuclease can comprise the amino acid sequence as set forth in SEQ ID NO: 38 or a fragment, or a derivative thereof. In an aspect, a disclosed dSaCas9 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 38, or a fragment thereof.

[0586] In an aspect, the nucleic acid sequence encoding the disclosed dCjCas9 may comprise a sequence as set forth in SEQ ID NO: 43, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed dCjCas9 endonuclease may comprise a nucleic acid sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 43, or a fragment thereof. In an aspect, a disclosed dCjCas9 endonuclease can comprise the amino acid sequence as set forth in SEQ ID

NO: 39, or a fragment, or a derivative thereof. In an aspect, a disclosed dCjCas9 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 39 or a fragment thereof.

[0587] In an aspect, a disclosed variant of dCas9 dVQR can comprise D1135V, R1335Q, and T1337R with reference to SEQ ID NO: 36. In an aspect, a disclosed variant of dCas9 dEQR can comprise D1135E, R1335Q, and T1337R with reference to SEQ ID NO: 36. In an aspect, a disclosed variant of dCas9 dVRER can comprise D1135V, G1218R, R1335E, and T1337R with reference to SEQ ID NO: 36. In an aspect, a disclosed dVRER can comprise the sequence set forth in SEQ ID NO: 37 or a fragment thereof.

[0588] In an aspect, the nucleic acid sequence encoding the disclosed dCas9 endonuclease may comprise a sequence as set forth in any one of SEQ ID NO: 40-43. In an aspect, the sequence encoding the disclosed dCas9 endonuclease may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 40-43, or a fragment, or a derivative thereof.

[0589] In an aspect of a disclosed method, a disclosed encoded polypeptide can comprise transcription activation activity, transcription repression activity, transcription release factor activity, histone modulation activity, nucleic acid association activity, methyltransferase activity, demethylase activity, acetyltransferase activity, deacetylase activity, or any combination thereof.

[0590] In an aspect of a disclosed method, a disclosed encoded polypeptide can comprise HP1a, HP1b, MBD1, MBD2, Krüppel-Associated Box (KRAB), NIPP1, the Transcription Repression Domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof.

[0591] In an aspect of a disclosed method, a disclosed fusion or disclosed fusion product can comprise HP1a-HP1a, HP1a-HP1b, HP1a-MBD1, HP1a-MBD2, HP1a-KRAB, HP1a-NIPP1, HP1a-MeCP2, HP1a-DNMT3A, HP1b-HP1b, HP1b-HP1a, HP1b-MBD1, HP1b-MBD2, HP1b-KRAB, HP1b-NIPP1, HP1b-MeCP2, HP1b-DNMT3A, MBD1-MBD1, MBD1-HP1a, MBD1-HP1b, MBD1-MBD2, MBD1-KRAB, MBD1-NIPP1, MBD1-MeCP2, MBD1-DNMT3A, MBD2-MBD2, MBD2-HP1a, MBD2-HP1b, MBD2-MBD1, MBD2-KRAB, MBD2-NIPP1, MBD2-MeCP2, MBD2-DNMT3A, KRAB-KRAB, KRAB-HP1a, KRAB-HP1b, KRAB-MBD1, KRAB-MBD2, KRAB-NIPP1, KRAB-MeCP2, KRAB-DNMT3A, NIPP1-NIPP1, NIPP1-HP1a, NIPP1-HP1b, NIPP1-MBD1, NIPP1-MBD2, NIPP1-KRAB, NIPP1-MeCP2, NIPP1-DNMT3A, MeCP2-MeCP2, MeCP2-HP1a, MeCP2-HP1b, MeCP2-MBD1, MeCP2-MBD2, MeCP2-KRAB,

MeCP2-NIPP1, MeCP2-DNMT3A, DNMT3A-DNMT3A, DNMT3A-HP1a, DNMT3A-HP1b, DNMT3A-MBD1, DNMT3A-MBD2, DNMT3A-KRAB, DNMT3A-NIPP1, or DNMT3A-MeCP2. In an aspect of the disclosed method, a disclosed fusion or disclosed fusion product can comprise KRAB-MeCP2.

[0592] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide HP1a may comprise a sequence as set forth in any one of SEQ ID NO: 59 or SEQ ID NO: 60, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide HP1a may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 59 or SEQ ID NO: 60, or a fragment thereof. In an aspect, a disclosed polypeptide HP1a can comprise the amino acid sequence as set forth in SEQ ID NO: 44 or SEQ ID NO: 45, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide HP1a can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 44 or SEQ ID NO: 45, or a fragment thereof.

[0593] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide HP1b may comprise a sequence as set forth in any one of SEQ ID NO: 61 or SEQ ID NO: 62, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide HP1b may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 61 or SEQ ID NO: 62, or a fragment thereof. In an aspect, a disclosed polypeptide HP1b can comprise the amino acid sequence as set forth in SEQ ID NO: 46 or SEQ ID NO: 47, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide HP1b can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 46 or SEQ ID NO: 47, or a fragment thereof.

[0594] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide MBD1 may comprise a sequence as set forth in any one of SEQ ID NO: 63 or SEQ ID NO: 64, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide MBD1 may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 63 or

SEQ ID NO: 64, or a fragment thereof. In an aspect, a disclosed polypeptide MBD1 can comprise the amino acid sequence as set forth in any one of SEQ ID NO: 48-50, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide MDB1 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in any one of SEQ ID NO: 48-50, or a fragment thereof.

[0595] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide MBD2 may comprise a sequence as set forth in any one of SEQ ID NO: 65 or SEQ ID NO: 66, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide MBD2 may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 65 or SEQ ID NO: 66, or a fragment thereof. In an aspect, a disclosed polypeptide MBD2 can comprise the amino acid sequence as set forth in SEQ ID NO: 51 or SEQ ID NO: 52, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide MBD2 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 51 or SEQ ID NO: 52, or a fragment thereof.

[0596] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide NIPP1 may comprise a sequence as set forth in any one of SEQ ID NO: 67 or SEQ ID NO: 68, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide NIPP1 may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 67 or SEQ ID NO: 68, or a fragment thereof. In an aspect, a disclosed polypeptide NIPP1 can comprise the amino acid sequence as set forth in SEQ ID NO: 53 or SEQ ID NO: 54, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide NIPP1 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 53 or SEQ ID NO: 54, or a fragment thereof.

[0597] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide DNMT3A may comprise a sequence as set forth in SEQ ID NO: 69, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide DNMT3A may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%,

at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 69, or a fragment thereof. In an aspect, a disclosed polypeptide DNMT3A can comprise the amino acid sequence as set forth in SEQ ID NO: 55, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide DNMT3A can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 55, or a fragment thereof.

[0598] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide KRAB may comprise a sequence as set forth in any one of SEQ ID NO: 70 or SEQ ID NO: 71, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide KRAB may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 70 or SEQ ID NO: 71, or a fragment thereof. In an aspect, a disclosed polypeptide KRAB can comprise the amino acid sequence as set forth in SEQ ID NO: 56, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide KRAB can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 56, or a fragment thereof.

[0599] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide KRAB may comprise a sequence as set forth in any one of SEQ ID NO: 70 or SEQ ID NO: 71, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide KRAB may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one of the sequences set forth in SEQ ID NO: 70 or SEQ ID NO: 71, or a fragment thereof. In an aspect, a disclosed polypeptide KRAB can comprise the amino acid sequence as set forth in SEQ ID NO: 56, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide KRAB can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 56, or a fragment thereof.

[0600] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide MeCP2 may comprise a sequence as set forth in any one of SEQ ID NO: 72, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide MeCP2 may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or

100% sequence identity to the sequences set forth in SEQ ID NO: 72, or a fragment thereof. In an aspect, a disclosed polypeptide MeCP2 can comprise the amino acid sequence as set forth in SEQ ID NO: 57, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide MeCP2 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 57, or a fragment thereof.

[0601] In an aspect, the nucleic acid sequence encoding the disclosed fusion polypeptide KRAB-MeCP2 may comprise a sequence as set forth in any one of SEQ ID NO: 73 or SEQ ID NO: 74, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide KRAB-MeCP2 may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequences set forth in SEQ ID NO: 73 or SEQ ID NO: 74, or a fragment thereof. In an aspect, a disclosed polypeptide KRAB-MeCP2 can comprise the amino acid sequence as set forth in SEQ ID NO: 58, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide KRAB-MeCP2 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 58, or a fragment thereof.

[0602] In an aspect, a disclosed encoded polypeptide can comprise transcription activation activity. In an aspect, a disclosed encoded polypeptide comprising transcription activation activity can comprise a transactivation domain. In an aspect, a disclosed transactivation domain can comprise a VP16 protein, a series of linked VP16 proteins, a p65 domain of NFκB, or any combination thereof.

[0603] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide VP16 may comprise a sequence as set forth in any one of SEQ ID NO: 132 or SEQ ID NO: 133, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide VP16 may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequences set forth in SEQ ID NO: 132 or SEQ ID NO: 133, or a fragment thereof. In an aspect, a disclosed polypeptide VP16 can comprise the amino acid sequence as set forth in SEQ ID NO: 131, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide VP16 can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 131, or a fragment thereof.

[0604] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide p65 domain of NFκB or RELA may comprise a sequence as set forth in any one of SEQ ID NO: 135 or SEQ ID NO: 136, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide p65 domain of NFκB or RELA may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequences set forth in SEQ ID NO: 135 or SEQ ID NO: 136, or a fragment thereof. In an aspect, a disclosed polypeptide p65 domain of NFκB or RELA can comprise the amino acid sequence as set forth in SEQ ID NO: 134, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide p65 domain of NFκB or RELA can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 134, or a fragment thereof.

[0605] In an aspect, a disclosed encoded polypeptide can comprise transcription repression activity. In an aspect, a disclosed encoded polypeptide comprising transcription repression activity can comprise a Krüppel associated box domain, an ERF repressor domain, a MXI1 repressor domain, a SID4x repressor domain, a fused KRAB-MeCP2 domain, a MeCP2 TRD domain, a MAS-SID repressor domain, a TATA box binding protein activity, or any combination thereof. In an aspect, a disclosed encoded polypeptide comprising transcription repression activity can comprise HP1 repressor activity, MeCP2 repressor activity, MBD1 repressor activity, MBD2 repressor activity, MBD3 repressor activity, MBD4 repressor activity, KRAB repressor activity, SUV39H1 repressor activity, SUV39H2 repressor activity, CTCF insulator-repressor activity, LSD-1 histone-demethylase repressor activity, or any combination thereof.

[0606] In an aspect, the nucleic acid sequence encoding the disclosed polypeptide comprising Eukaryotic Release Factor 1 (ERF1) activity or Eukaryotic Release Factor 3 (ERF3) activity may comprise a sequence as set forth in any one of SEQ ID NO: 138, or a fragment, or a derivative thereof. In an aspect, the sequence encoding the disclosed polypeptide comprising ERF1 activity or ERF3 activity may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequences set forth in SEQ ID NO: 138, or a fragment thereof. In an aspect, a disclosed polypeptide comprising ERF1 activity or ERF3 activity can comprise the amino acid sequence as set forth in SEQ ID NO: 137, or a fragment, or a derivative thereof. In an aspect, a disclosed polypeptide comprising ERF1 activity or ERF3 activity can comprise a sequence having at least 60%, at least 65%, at least 70%, at least

75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the sequence set forth in SEQ ID NO: 137, or a fragment thereof.

[0607] In an aspect of a disclosed method, a disclosed encoded polypeptide can comprise histone modulation activity. A disclosed encoded polypeptide comprising histone modulation activity can comprise histone acetyltransferase, histone deacetylase, histone demethylase, histone methyltransferase activity, or any combination thereof.

[0608] In an aspect of a disclosed method, a disclosed encoded polypeptide can comprise nucleic acid association activity. A disclosed encoded polypeptide comprising nucleic acid association activity can comprise a helix-turn-helix region, a leucine zipper region, a winged helix region, a winged helix-turn-helix region, a helix-loop-helix region, an immunoglobulin fold, a B3 domain, a zinc finger, a HMG-box, a Wor3 domain, a TAL effector DNA-binding domain, or any combination thereof. In an aspect of a disclosed method, a disclosed encoded polypeptide can comprise methyltransferase activity. A disclosed encoded polypeptide comprising methyltransferase activity can comprise DNA (cytosine-5)-methyltransferase 3a (DNMT3A).

[0609] In an aspect of a disclosed method, a disclosed encoded polypeptide can comprise demethylase activity. A disclosed encoded polypeptide comprising demethylase activity can comprise ten-eleven translocation methylcytosine dioxygenase 1 (TET1) or lysine-specific histone demethylase 1 (LSD1).

[0610] In an aspect of a disclosed method, a disclosed encoded polypeptide can comprise acetyltransferase activity. A disclosed encoded polypeptide comprising acetyltransferase activity can comprise histone acetyltransferase. In In an aspect of a disclosed method, a disclosed encoded polypeptide can comprise deacetylase activity. A disclosed encoded polypeptide comprising deacetylase activity can comprise histone deacetylase.

[0611] In an aspect of a disclosed method, a disclosed dCas endonuclease can comprise dSpCas9 and a disclosed encoded polypeptide can comprise HP1a, HP1b, MBD1, MBD2, Krüppel-associated box (KRAB), NIPP1, the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof. In an aspect, a disclosed dCas endonuclease can comprise dSaCas9 and a disclosed encoded polypeptide can comprise HP1a, HP1b, MBD1, MBD2, Krüppel-associated box (KRAB), NIPP1, the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof. In an aspect, a disclosed dCas endonuclease can comprise dCjCas9 and a disclosed polypeptide can comprise HP1a, HP1b, MBD1, MBD2, Krüppel-associated box (KRAB), NIPP1, the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof.

[0612] In an aspect of a disclosed method, a disclosed dCas endonuclease can comprise dVQR, dEQR, or dVRER and a disclosed encoded polypeptide can comprise HP1a, HP1b, MBD1, MBD2, Krüppel-associated box (KRAB), NIPP1, the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof.

[0613] In an aspect of a disclosed method, a disclosed dCas endonuclease can comprise dSpCas9, dSaCas9, or dCjCas9 and a disclosed encoded polypeptide can comprise KRAB-MeCP2.

[0614] In an aspect of a disclosed method, a disclosed dCas endonuclease can comprise dSpCas9, dSaCas9, or dCjCas9, and a disclosed fusion can comprise HP1a-HP1a, HP1a-HP1b, HP1a-MBD1, HP1a-MBD2, HP1a-KRAB, HP1a-NIPP1, HP1a-MeCP2, HP1a-DNMT3A, HP1b-HP1b, HP1b-HP1a, HP1b-MBD1, HP1b-MBD2, HP1b-KRAB, HP1b-NIPP1, HP1b-MeCP2, HP1b-DNMT3A, MBD1-MBD1, MBD1-HP1a, MBD1-HP1b, MBD1-MBD2, MBD1-KRAB, MBD1-NIPP1, MBD1-MeCP2, MBD1-DNMT3A, MBD2-MBD2, MBD2-HP1a, MBD2-HP1b, MBD2-MBD1, MBD2-KRAB, MBD2-NIPP1, MBD2-MeCP2, MBD2-DNMT3A, KRAB-KRAB, KRAB-HP1a, KRAB-HP1b, KRAB-MBD1, KRAB-MBD2, KRAB-NIPP1, KRAB-MeCP2, KRAB-DNMT3A, NIPP1-NIPP1, NIPP1-HP1a, NIPP1-HP1b, NIPP1-MBD1, NIPP1-MBD2, NIPP1-KRAB, NIPP1-MeCP2, NIPP1-DNMT3A, MeCP2-MeCP2, MeCP2-HP1a, MeCP2-HP1b, MeCP2-MBD1, MeCP2-MBD2, MeCP2-KRAB, MeCP2-NIPP1, MeCP2-DNMT3A, DNMT3A-DNMT3A, DNMT3A-HP1a, DNMT3A-HP1b, DNMT3A-MBD1, DNMT3A-MBD2, DNMT3A-KRAB, DNMT3A-NIPP1, or DNMT3A-MeCP2.

[0615] In an aspect of a disclosed method, an isolated nucleic acid or viral vector can encode for a dCas endonuclease fused to the at least one encoded polypeptide having an enzymatic activity. In these or related aspects, the isolated nucleic acid can encode for a "fusion protein" wherein the fusion protein comprises a first component comprising a dCas endonuclease and a second component comprising at least one polypeptide having an enzymatic activity. In an aspect, the encoded fusion protein can comprise a dSpCas9 and a polypeptide having enzymatic activity comprising HP1a, HP1b, MBD1, MBD2, Krüppel-associated box (KRAB), NIPP1, the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof. In an aspect, the encoded fusion protein can comprise a dSaCas9 and a polypeptide having enzymatic activity comprising HP1a, HP1b, MBD1, MBD2, Krüppel-associated box (KRAB), NIPP1, the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof. In an aspect, the encoded fusion protein can comprise a dCjCas9 and a polypeptide having enzymatic activity comprising HP1a, HP1b, MBD1, MBD2, Krüppel-associated box

(KRAB), NIPP1, the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof. In an aspect, the encoded fusion protein can comprise dVQR, dEQR, or dVRER and a polypeptide having enzymatic activity comprising HP1a, HP1b, MBD1, MBD2, Krüppel-associated box (KRAB), NIPP1, the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof.

[0616] In an aspect, the encoded fusion protein can comprise dSpCas9, dSaCas9, dCjCas9, or a variant dCas9 (*e.g.*, dVQR, dEQR or dVRER) and one or more of HP1a-HP1a, HP1a-HP1b, HP1a-MBD1, HP1a-MBD2, HP1a-KRAB, HP1a-NIPP1, HP1a-MeCP2, HP1a-DNMT3A, HP1b-HP1b, HP1b-HP1a, HP1b-MBD1, HP1b-MBD2, HP1b-KRAB, HP1b-NIPP1, HP1b-MeCP2, HP1b-DNMT3A, MBD1-MBD1, MBD1-HP1a, MBD1-HP1b, MBD1-MBD2, MBD1-KRAB, MBD1-NIPP1, MBD1-MeCP2, MBD1-DNMT3A, MBD2-MBD2, MBD2-HP1a, MBD2-HP1b, MBD2-MBD1, MBD2-KRAB, MBD2-NIPP1, MBD2-MeCP2, MBD2-DNMT3A, KRAB-KRAB, KRAB-HP1a, KRAB-HP1b, KRAB-MBD1, KRAB-MBD2, KRAB-NIPP1, KRAB-MeCP2, KRAB-DNMT3A, NIPP1-NIPP1, NIPP1-HP1a, NIPP1-HP1b, NIPP1-MBD1, NIPP1-MBD2, NIPP1-KRAB, NIPP1-MeCP2, NIPP1-DNMT3A, MeCP2-MeCP2, MeCP2-HP1a, MeCP2-HP1b, MeCP2-MBD1, MeCP2-MBD2, MeCP2-KRAB, MeCP2-NIPP1, MeCP2-DNMT3A, DNMT3A-DNMT3A, DNMT3A-HP1a, DNMT3A-HP1b, DNMT3A-MBD1, DNMT3A-MBD2, DNMT3A-KRAB, DNMT3A-NIPP1, or DNMT3A-MeCP2. In an aspect, the encoded fusion protein can comprise a variant dCas9 selected from dVQR, dEQR, or dVRER and a fusion comprising KRAB-MeCP2. In an aspect, the encoded fusion protein can comprise a dCas9 selected from dSpCas9, dSaCas9, or dCjCas9 and a fusion comprising KRAB-MeCP2. In an aspect, the encoded fusion protein can comprise a dCas9 selected from dSpCas9, dSaCas9, or dCjCas9 and a fusion comprising KRAB-MeCP2. In an aspect, the encoded fusion protein can comprise a dSaCas9 and KRAB-MeCP2. In an aspect, the encoded fusion protein can comprise a dCas9 selected from dSpCas9, dSaCas9, or dCjCas9 and DNMTA. In an aspect, the encoded fusion protein can comprise a dSaCas9 and DNMTA.

[0617] In an aspect, the nucleic acid sequence encoding the fusion protein comprising dSpCas9 and DNMT3A or dVRER and DNMT3A may comprise a sequence as set forth in SEQ ID NO: 75 or SEQ ID NO: 76 respectively, or a derivative thereof. In an aspect, the nucleic acid sequence encoding the fusion protein comprising dSpCas9 and DNMT3A or dVRER and DNMT3A may comprise a sequence as set forth in SEQ ID NO: 77 or SEQ ID NO: 78 respectively, or a derivative thereof. In an aspect, the nucleic acid sequence encoding the fusion protein comprising dSpCas9 and DNMT3A may comprise a sequence having at least 60% at least 65%, at least 70%, at least

75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any of the sequences set forth in SEQ ID NO: 77 or SEQ ID NO: 78, or a fragment thereof. In an aspect, a disclosed polypeptide dSpCas9 can comprise the amino acid sequence as set forth in SEQ ID NO: 75 or SEQ ID NO: 76, or a fragment, or a derivative thereof.

[0618] In an aspect of a disclosed method, a disclosed gene of interest or portion thereof can demonstrate a reduced expression level when compared to wild-type or control expression level. In an aspect of a disclosed method, a disclosed gRNA can targes a gene of interest or portion thereof that demonstrates a reduced expression level when compared to wild-type or control expression level. In an aspect of a disclosed method, a disclosed gene of interest or portion thereof can demonstrate an increased expression level when compared to wild-type or control expression level. In an aspect of a disclosed method, a disclosed gRNA can target a gene of interest or portion thereof that demonstrates an increased expression level when compared to wild-type or control expression level.

[0619] In an aspect of a disclosed method, a disclosed gRNA can target the *APOE* gene. In an aspect, the at least one gRNA may target a region in the *APOE* gene comprising a nucleic acid sequence as set forth in any one of SEQ ID NO: 11-14, or a complement thereof. In an aspect, the at least one gRNA targets a nucleic acid sequence comprising at least 10 consecutive nucleotides as set forth in SEQ ID NO: 11-14, or a complement thereof. In an aspect, a disclosed gRNA can target the promoter of the APOE gene. In an aspect, a disclosed gRNA can target exon 1 of the APOE gene. In an aspect, a disclosed gRNA can target exon 1 of the *APOE gene*. In an aspect, a disclosed gRNA can target intron 1 of the *APOE gene*. In an aspect, a disclosed gRNA can target intron 4 of the APOE gene. In an aspect, a disclosed gRNA can target a CpG island region of intron 1 of the APOE gene. The CpG island region can include CpG1, CpG2, CpG3, CpG4, CpG5, CpG6, CpG7, CpG8, CpG9, CpG10, CpG11, CpG12, CpG13, CpG14, CpG15, CpG16, CpG17, CpG18, CpG19, CpG20, CpG21, CpG22, CpG23, or a combination thereof. For example, the CpG island region can include CpG1, CpG3, CpG6, CpG7, CpG8, CpG9, CpG18, CpG19, CpG20, CpG21, CpG22, or a combination thereof. In an aspect, the disclosed gRNA may comprise a nucleic acid sequence as set forth in SEQ ID NO: 15-33. In an aspect, the disclosed gRNA may comprise a nucleic acid sequence as set forth in SEQ ID NO: 15-33, or a complement thereof. In an aspect, the disclosed gRNA may comprise a nucleic acid sequence with at least about 90%, or about 91%, or about 92%, or about 93%, or about 94%, or about 95%, or about 96%, or about 97%, or about 98%, or about 99%, or more sequence identity to any one of the sequences set forth in SEQ ID NO: 15-33, or a complement thereof, or a fragment thereof.

[0620] In an aspect of a disclosed method, a disclosed viral vector or disclosed recombinant AAV vector can further comprise a gRNA scaffold. In an aspect, a disclosed gRNA scaffold can comprise the sequence set forth in SEQ ID NO: 129, SEQ ID NO: 130, or a fragment thereof.

[0621] In an aspect of a disclosed method, a disclosed isolated nucleic acid or disclosed viral vector comprises one or more microglia specific regulatory elements. In an aspect, the one or more microglia specific regulatory elements can comprise a microglia specific wild-type promoter, a microglia specific enhancer, a synthetic promoter, a synthetic enhancer, a transcriptional or translational regulatory sequence or any combination thereof. In an aspect, the one or more microglia specific regulatory elements comprise at least one microglia specific promoter (*e.g.*, a microglia specific wild-type promoter or a microglia specific synthetic promoter). In an aspect, the microglia specific regulatory element (*e.g.*, promoter) may be specific for a certain type of microglial cell (*e.g.*, a dopaminergic or a cholinergic microglial cell). In these instances, the microglia specific regulatory element may include a dopaminergic specific regulatory element (*i.e.*, a dopaminergic promoter) and/or a cholinergic microglia specific regulatory element (*i.e.*, a cholinergic promoter). In an aspect, the microglia specific regulatory element can comprise a microglia specific promoter. In an aspect, a disclosed microglia specific promoter can comprise a F4/80 promoter, CD68 promoter, FCGR2A promoter, HexB promoter or IBA1 promoter, a derivative thereof, a fragment thereof, or any combination thereof.

[0622] In an aspect, the promoter is a CD68 promoter, or a derivative thereof. In an aspect, the promoter may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to any one or more of the sequences set forth in SEQ ID NO: 1-3, or a fragment, or a derivative thereof. In an aspect, the promoter comprises a sequence as set forth in any one of SEQ ID NO: 1-3.

[0623] In an aspect, the promoter is a F4/80 promoter, or a derivative thereof. In an aspect, the promoter may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequence set forth in SEQ ID NO: 4, or a fragment, or a derivative thereof. In an aspect, the promoter comprises a sequence as set forth in SEQ ID NO: 4.

[0624] In an aspect, the promoter is a IBA1 promoter, or a derivative thereof. In an aspect, the promoter may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequence set forth in SEQ ID NO: 5-7,

or a fragment, or a derivative thereof. In an aspect, the promoter comprises a sequence as set forth in any one of SEQ ID NO: 5-7.

[0625] In an aspect, the promoter is a FCGR2A promoter, or a derivative thereof. In an aspect, the promoter may comprise a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequence set forth in SEQ ID NO: 8, or a fragment, or a derivative thereof. In an aspect, the promoter comprises a sequence as set forth in SEQ ID NO: 8.

[0626] In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) can be operably linked to the dCas endonuclease and the fusion protein having one or more enzymatic activities. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the dCas endonuclease and the fusion protein having one or more enzymatic activities can comprise a F4/80 promoter, or a derivative thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the dCas endonuclease and the fusion protein having one or more enzymatic activities can comprise a CD68 promoter, or a derivative thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the dCas endonuclease and the fusion protein having one or more enzymatic activities can comprise a IBA1 promoter, or a derivative thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the dCas endonuclease and the fusion protein having one or more enzymatic activities can comprise a FCGR2A promoter, or a derivative thereof.

[0627] In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the dCas endonuclease can be operably linked to the at least one polypeptide having enzymatic activity. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the at least one polypeptide having enzymatic activity can comprise a F4/80promoter, a derivative thereof, or a fragment thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the at least one polypeptide having enzymatic activity can comprise a CD68 promoter, a derivative thereof, or a fragment thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the at least one polypeptide having enzymatic activity can comprise a IBA1 promoter, a derivative thereof, or a fragment thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia

specific promoter) operably linked to at least one polypeptide having enzymatic activity can comprise a FCGR2A promoter, a derivative thereof, or a fragment thereof.

[0628] In an aspect of a disclosed method, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) can be operably linked to the dCas endonuclease and the at least one polypeptide having enzymatic activity. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the dCas endonuclease and the at least one polypeptide having enzymatic activity can comprise a FGCR2A promoter, a derivative thereof, or a fragment thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the at dCas endonuclease and the at least one polypeptide having enzymatic activity can comprise a F4/80 promoter, a derivative thereof, or a fragment thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the dCas endonuclease and the at least one polypeptide having enzymatic activity can comprise a CD68 promoter, a derivative thereof, or a fragment thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the dCas endonuclease and the at least one polypeptide having enzymatic activity can comprise a IBA1 promoter, a derivative thereof, or a fragment thereof.

[0629] In an aspect of a disclosed method, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) can be operably linked to the fusion protein having one or more enzymatic activities. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the fusion protein having one or more enzymatic activities can comprise a F4/80 promoter, a derivative thereof, or a fragment thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the fusion protein having one or more enzymatic activities can comprise a CD68 promoter, a derivative thereof, or a fragment thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the fusion protein having one or more enzymatic activities can comprise a IBA1 promoter, a derivative thereof, or a fragment thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the fusion protein having one or more enzymatic activities can comprise a FCGR2A promoter, a derivative thereof, or a fragment thereof.

[0630] In an aspect of a disclosed method, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) can be operably linked to the dCas endonuclease and the fusion protein having one or more enzymatic activities. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the dCas endonuclease and the fusion protein having one or more enzymatic activities can comprise a F4/80 promoter, a

derivative thereof, or a fragment thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the dCas endonuclease and the fusion protein having one or more enzymatic activities can comprise a CD68 promoter, a derivative thereof, or a fragment thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the dCas endonuclease and the fusion protein having one or more enzymatic activities can comprise a IBA1 promoter, a derivative thereof, or a fragment thereof. In an aspect, a disclosed microglia specific regulatory element (*i.e.*, a microglia specific promoter) operably linked to the dCas endonuclease and the fusion protein having one or more enzymatic activities can comprise a FCGR2A promoter, a derivative thereof, or a fragment thereof.

[0631] In an aspect of a disclosed method, a disclosed isolated nucleic acid or disclosed viral vector can further comprise one or more regulatory elements (*i.e.*, promoters) in addition to the microglia specific regulatory element (*e.g.*, microglia specific promoter). In an aspect, a disclosed additional promoter can comprise a U6 promoter, a chicken β-actin promoter, an EF-1α, a CMV promoter, a CMV promoter/enhancer, a fragment thereof, a derivative thereof, or any combination thereof. In an aspect, a disclosed U6 promoter can comprise the sequence set forth in SEQ ID NO: 81, or a fragment thereof. In an aspect, a disclosed U6 promoter can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% identity to the sequence set forth in SEQ ID NO: 81 or a fragment thereof. In an aspect, a disclosed EF-1α promoter can comprise the sequence set forth in SEQ ID NO: 79, SEQ ID NO: 80, or a fragment thereof. In an aspect, a disclosed EF-1α promoter can comprise a sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% identity to the sequence set forth in SEQ ID NO: 79, SEQ ID NO:80, or a fragment thereof. In an aspect, a promoter can be a short EF1alpha (EFS-NF) promoter.

[0632] In an aspect of a disclosed method, a disclosed promoter operably can be linked to the at least one guide RNA targeting a gene of interest or portion thereof. In an aspect, a disclosed promoter operably linked to the at least one guide RNA can comprise a U6 promoter, a derivative thereof, or a fragment thereof. In an aspect, a disclosed promoter operably linked to the at least one guide RNA targeting an *APOE* gene can comprise a U6 promoter, a derivative thereof, or a fragment thereof.

[0633] In an aspect of the disclosed method, a disclosed isolated nucleic acid molecule or viral vector can further two or more promoters, wherein a first promoter can be operably linked to the dCas endonuclease, a polypeptide having enzymatic activity, a fusion of two or more polypeptides

having enzymatic activities or any combination thereof, and wherein a second promoter can be operably linked to the at least one guide RNA targeting a gene of interest or portion thereof. In an aspect, a disclosed isolated nucleic acid molecule can further comprise one or more promoters, wherein a first promoter comprises a microglia specific promoter that is operably linked to the dCas endonuclease, a polypeptide having enzymatic activity, a fusion of two or more polypeptides having enzymatic activities or any combination thereof, and wherein a second promoter can be operably linked to the at least one guide RNA targeting a gene of interest thereof. In an aspect, the second promoter operably linked to the gRNA may not be a microglia specific promoter. In an aspect, the isolated nucleic acid can comprise a first promoter selected from a F4/80 promoter, a CD68 promoter, a IBA1 promoter, or a FCGR2A promoter, a fragment thereof, a derivative thereof, or any combination thereof, and a second promoter comprising a U6 promoter.

[0634] In an aspect of the disclosed method, a disclosed isolated nucleic acid molecule or viral vector can further comprise one or more additional regulatory elements. In an aspect, a disclosed additional regulatory element can comprise a promoter (described supra), an enhancer, a promoter/enhancer, an internal ribosomal entry site, a transcription terminal signal, a polyadenylation signal, a Sp1 and/or NF-kB transcriptional factor binding site, a p2A signal, a woodchuck hepatitis virus post-transcriptional regulatory element, a Phi signal-packaging signal, a rev responsive element, a 5'-ITR, a 3'-ITR, an inverted terminal repeat, a nuclear localization signal (NLS), or any combination thereof.

[0635] In an aspect of the disclosed method, a disclosed 5'-ITR may comprise the sequence as set forth in any one of SEQ ID NO: 82 or SEQ ID NO: 83. In an aspect, a disclosed 3'-ITR may comprise the sequence as set forth in any one of SEQ ID NO: 84 or SEQ ID NO: 85. In an aspect, a disclosed NLS can comprise the sequence set forth in any one of SEQ ID NO: 88 – SEQ ID NO: 94, or a fragment thereof. In an aspect, a disclosed PolyA sequence can comprise the sequence set forth in SEQ ID NO: 86, SEQ ID NO: 87, or a fragment thereof.

[0636] In an aspect of a disclosed method, a disclosed viral vector or isolated nucleic acid can comprise a nucleic acid that is CpG depleted and codon-optimized for expression in a human cell. In an aspect, "CpG-free" can mean completely free of CpGs or partially free of CpGs. In an aspect, "CpG-free" can mean "CpG-depleted." In an aspect, "CpG-depleted" can mean "CpG-free." In an aspect, "CpG-depleted" can mean completely depleted of CpGs or partially depleted of CpGs. In an aspect, "CpG-free" can mean "CpG-optimized" for a desired and/or ideal expression level. CpG depletion and/or optimization is known to the skilled person in the art. In an aspect, any disclosed Cas9 endonuclease, a disclosed polypeptide having enzymatic activities, a disclosed fusion product or a disclosed fusion protein, or any combination thereof can be codon-optimized.

[0637] In an aspect of a disclosed method, a disclosed viral vector or disclosed recombinant AAV vector can comprise a sequence that is about 4.5 kilobases or less than about 4.5 kilobases.

[0638] In an aspect of a disclosed method of treating and/or preventing Parkinson's disease can comprise increasing expression and/or activity of one or more genes of interest. In an aspect of a disclosed method of effecting precision epigenetic modulation, modulating can comprise decreasing expression and/or activity of one or more genes of interest.

[0639] In an aspect, a disclosed method of treating and/or preventing Parkinson's disease can comprise reducing expression and/or activity of APOE in a cell.

[0640] In an aspect, the disclosed cells can be in a subject. In an aspect of a disclosed method, a subject can be a human. In an aspect, a subject can be suspected of having or can be diagnosed with having Parkinson's disease.

[0641] In an aspect, a disclosed method can comprise reducing the pathological phenotype associated with Parkinson's disease. In an aspect, reducing the pathological phenotype associated with Parkinson's disease can comprise reducing the number and/or amount of Tau aggregates.

[0642] In an aspect, restoring one or more aspects of cellular homeostasis can comprise improving, enhancing, restoring, and/or preserving one or more aspects of cellular structural and/or functional integrity. In an aspect, restoring the activity and/or functionality of a missing, deficient, and/or mutant protein or enzyme can comprise a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or any amount of restoration when compared to a pre-existing level such as, for example, a pre-treatment level. In an aspect, the amount of restoration can be 10-20%, 20-30%, 30-40%, 40-50%, 50-60%, 60-70%, 70-80%, 80-90%, or 90-100% more than a pre-existing level such as, for example, a pre-treatment level. In an aspect, restoration can be measured against a control level or a reference level (*e.g.*, determined, for example, using one or more subjects not having a missing, deficient, and/or mutant protein or enzyme). In an aspect, restoration can be a partial or incomplete restoration. In an aspect, restoration can be complete or near complete restoration such that the level of expression, activity, and/or functionality is like that of a wild-type or control level.

[0643] In an aspect, restoring the activity and/or functionality of the *APOE* gene or a portion of the *APOE* gene can comprise decreasing or reducing the expression and/or activity level of the *APOE* gene or a portion of the APOE gene. In an aspect, decreasing or reducing can comprise a decrease of at least about 5%, 10%, 15%, 20%, 25%, 35%, 50%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, 100%, 200%, 300%, 400%, 500%, or more as compared to a control (such as a pre-treatment level).

[0644] In an aspect of a disclosed method of treating and/or preventing Parkinson's disease progression, techniques to monitor, measure, and/or assess the restoring one or more aspects of cellular homeostasis and/or cellular functionality can comprise qualitative (or subjective) means as well as quantitative (or objective) means. These means are known to the skilled person. For example, representative regulated variables and sensors relating to systemic homeostasis are discussed supra.

[0645] In an aspect of a disclosed method of treating and/or preventing Parkinson's disease progression, administering can comprise intravenous administration, intracerebral administration, intra-CSF administration, intracerebroventricular (ICV) administration, intraventricular administration, intra-cisterna magna (ICM) administration, intraparenchymal administration, intrathecal (lumbar, cisternal, or both) administration, intrahepatic administration, hepatic intra-arterial administration, hepatic portal vein (HPV) administration, or any combination thereof. In an aspect, a disclosed vector can be administered via LNP administration.

[0646] In an aspect of a disclosed method of treating and/or preventing Parkinson's disease progression, administering can comprise use of non-viral vectors to deliver a disclosed isolated nucleic acid. These non-viral vectors can include, but are not limited to, nanoparticles or liposomes. Alternatively, the disclosed isolated nucleic acids may be delivered via viral vectors (such as the disclosed viral vectors provided herein). Exemplary viral vectors are described supra and include adenovirus, adeno-associated virus (AAV), and lentivirus (LV). Viral vectors are used more commonly due to their robust delivery, long-lasting expression, low immunogenicity and toxicity, and compatibility with the advanced manufacturing techniques. Most research in gene therapy has utilized AAV or LV due to their lowered immunogenicity compared to adenovirus. Derived from HIV, LV integrates its viral genome into the host gene to create permanent change in gene expression.

[0647] In an aspect, a disclosed method of treating and/or preventing Parkinson's disease progression can employ multiple routes of administration to the subject. In an aspect, a disclosed method can employ a first route of administration that can be the same or different as a second and/or subsequent routes of administration. In an aspect, a disclosed nucleic acid molecule, a disclosed vector, and/or a disclosed pharmaceutical formulation can be concurrently and/or serially administered to a subject via multiple routes of administration. For example, in an aspect, administering a disclosed nucleic acid molecule, a disclosed vector, and/or a disclosed pharmaceutical formulation can comprise intravenous administration and intra-cistern magna (ICM) administration. In an aspect, administering a disclosed nucleic acid molecule, a disclosed

vector, and/or a disclosed pharmaceutical formulation can comprise IV administration and intrathecal (ITH) administration.

[0648] In an aspect of a disclosed method of treating and/or preventing Parkinson's disease progression, a therapeutically effective amount of disclosed vector can be delivered via intravenous (IV) administration and can comprise a range of about 1 x 10^{10} vg/kg to about 2 x 10^{14} vg/kg. In an aspect, for example, a disclosed vector can be administered at a dose of about 1 x 10^{11} vg/kg to about 8 x 10^{13} vg/kg or about 1 x 10^{12} vg/kg to about 8 x 10^{13} vg/kg. In an aspect, a disclosed vector can be administered at a dose of about 1 x 10^{13} vg/kg to about 6 x 10^{13} vg/kg. In an aspect, a disclosed vector can be administered at a dose of at least about 1 x 10^{10} vg/kg, at least about 5 x 10^{10} vg/kg, at least about 1 x 10^{11} vg/kg, at least about 5 x 10^{11} vg/kg, at least about 1 x 10^{12} vg/kg, at least about 5 x 10^{12} vg/kg, at least about 1 x 10^{13} vg/kg, at least about 5 x 10^{13} vg/kg, or at least about 1 x 10^{14} vg/kg. In an aspect, a disclosed vector can be administered at a dose of no more than about 1 x 10^{10} vg/kg, no more than about 5 x 10^{10} vg/kg, no more than about 1 x 10^{11} vg/kg, no more than about 5 x 10^{11} vg/kg, no more than about 1 x 10^{12} vg/kg, no more than about 5 x 10^{12} vg/kg, no more than about 1 x 10^{13} vg/kg, no more than about 5 x 10^{13}, or no more than about 1 x 10^{14} vg/kg. In an aspect, a disclosed vector can be administered at a dose of about 1 x 10^{12} vg/kg. In an aspect, a disclosed vector can be administered at a dose of about 1 x 10^{11} vg/kg. In an aspect, a disclosed vector can be administered in a single dose, or in multiple doses (such as 2, 3, 4, 5, 6, 7, 8, 9 or 10 doses) as needed for the desired therapeutic results.

[0649] In an aspect, a disclosed method of treating and/or preventing Parkinson's disease progression can further comprise monitoring the subject for adverse effects. In an aspect, in the absence of adverse effects, the method can further comprise continuing to treat the subject. In an aspect, in the presence of adverse effects, the method can further comprise modifying the treating step. Methods of monitoring a subject's well-being can include both subjective and objective criteria (and are discussed supra). Such methods are known to the skilled person.

[0650] In an aspect, a disclosed method of treating and/or preventing Parkinson's disease progression can further comprise administering to the subject a therapeutically effective amount of a therapeutic agent. A therapeutic agent can be any disclosed agent that effects a desired clinical outcome.

[0651] In an aspect, a disclosed method of treating and/or preventing Parkinson's disease progression can further comprise administering to the subject a therapeutically effective amount of an agent that can correct one or more aspects of a dysregulated metabolic or enzymatic pathway. In an aspect, such an agent can comprise an enzyme for enzyme replacement therapy. In an aspect, a disclosed enzyme can replace any enzyme in a dysregulated or dysfunctional metabolic or

enzymatic pathway. In an aspect, a disclosed method can comprise replacing one or more enzymes in a dysregulated or dysfunctional metabolic pathway.

[0652] In an aspect, a disclosed method of treating and/or preventing Parkinson's disease progression can further comprise administering one or more immune modulators. In an aspect, a disclosed immune modulator can be methotrexate, rituximab, intravenous gamma globulin, or bortezomib, or a combination thereof. In an aspect, a disclosed immune modulator can be bortezomib or SVP-Rapamycin. In an aspect, a disclosed immune modulator can be Tacrolimus. In an aspect, a disclosed immune modulator such as methotrexate can be administered at a transient low to high dose. In an aspect, a disclosed immune modulator can be administered at a dose of about 0.1 mg/kg body weight to about 0.6 mg/kg body weight. In an aspect, a disclosed immune modulator can be administered at a dose of about 0.4 mg/kg body weight. In an aspect, a disclosed immune modulator can be administered at about a daily dose of 0.4 mg/kg body weight for 3 to 5 or greater cycles, with up to three days per cycle. In an aspect, a disclosed immune modulator can be administered at about a daily dose of 0.4 mg/kg body weight for a minimum of 3 cycles, with three days per cycle. In an aspect, a person skilled in the art can determine the appropriate number of cycles. In an aspect, a disclosed immune modulator can be administered as many times as necessary to achieve a desired clinical effect.

[0653] In an aspect, a disclosed immune modulator can be administered orally about one hour before a disclosed therapeutic agent. In an aspect, a disclosed immune modulator can be administered subcutaneously about 15 minutes before a disclosed therapeutic agent. In an aspect, a disclosed immune modulator can be administered concurrently with a disclosed therapeutic agent. In an aspect, a disclosed immune modulator can be administered orally about one hour or a few days before a disclosed isolated nucleic acid molecule, a disclosed vector, a disclosed pharmaceutical formulation, or a combination thereof. In an aspect, a disclosed immune modulator can be administered subcutaneously about 15 minutes before or a few days before a disclosed isolated nucleic acid molecule, a disclosed vector, a disclosed pharmaceutical formulation, or a combination thereof. In an aspect, a disclosed immune modulator can be administered concurrently with a disclosed isolated nucleic acid molecule, a disclosed vector, a disclosed pharmaceutical formulation, or a combination thereof.

[0654] In an aspect, a disclosed method of treating and/or preventing Parkinson's disease progression can further comprise administering one or more proteasome inhibitors (*e.g.*, bortezomib, carfilzomib, marizomib, ixazomib, and oprozomib). In an aspect, a proteasome inhibitor can be an agent that acts on plasma cells (*e.g.*, daratumumab). In an aspect, an agent that acts on a plasma cell can be melphalan hydrochloride, melphalan, pamidronate disodium,

carmustine, carfilzomib, carmustine, cyclophosphamide, daratumumab, doxorubicin hydrochloride liposome, doxorubicin hydrochloride liposome, elotuzumab, melphalan hydrochloride, panobinostat, ixazomib citrate, carfilzomib, lenalidomide, melphalan, melphalan hydrochloride, plerixafor, ixazomib citrate, pamidronate disodium, panobinostat, plerixafor, pomalidomide, pomalidomide, lenalidomide, selinexor, thalidomide, thalidomide, bortezomib, selinexor, zoledronic acid, or zoledronic acid.

[0655] In an aspect, a disclosed method of treating and/or preventing Parkinson's disease progression can further comprise administering one or more proteasome inhibitors or agents that act on plasma cells prior to administering a disclosed isolated nucleic acid molecule, a disclosed vector, or a disclosed pharmaceutical formulation. In an aspect, a disclosed method can comprise administering one or more proteasome inhibitors or one or more agents that act on plasma cells concurrently with administering a disclosed isolated nucleic acid molecule, a disclosed vector, or a disclosed pharmaceutical formulation. In an aspect, a disclosed method can comprise administering one or more proteasome inhibitors or one or more agents that act on plasma cells subsequent to administering a disclosed isolated nucleic acid molecule, a disclosed vector, or a disclosed pharmaceutical formulation. In an aspect, a disclosed method can further comprise administering one or more proteasome inhibitors more than 1 time. In an aspect, a disclosed method can comprise administering one or more proteasome inhibitors repeatedly over time.

[0656] In an aspect, a disclosed method of treating and/or preventing Parkinson's disease progression can further comprise administering one or more immunosuppressive agents. In an aspect, an immunosuppressive agent can be, but is not limited to, azathioprine, methotrexate, sirolimus, anti-thymocyte globulin (ATG), cyclosporine (CSP), mycophenolate mofetil (MMF), steroids, or a combination thereof. In an aspect, a disclosed method can comprise administering one or more immunosuppressive agents more than 1 time. In an aspect, a disclosed method can comprise administering one or more one or more immunosuppressive agents repeatedly over time. In an aspect, a disclosed method can comprise administering a compound that targets or alters antigen presentation or humoral or cell mediated or innate immune responses.

[0657] In an aspect, a disclosed method of treating and/or preventing Parkinson's disease progression can further comprise administering a compound that exerts a therapeutic effect against B cells and/or a compound that targets or alters antigen presentation or humoral or cell mediated immune response. In an aspect, a disclosed compound can be rituximab, methotrexate, intravenous gamma globulin, anti CD4 antibody, anti CD2, an anti-FcRN antibody, a BTK inhibitor, an anti-IGF1R antibody, a CD19 antibody (*e.g.*, inebilizumab), an anti-IL6 antibody (*e.g.*, tocilizumab), an antibody to CD40, an IL2 mutein, or a combination thereof. Also disclosed herein are Treg

infusions that can be administered as a way to help with immune tolerance (*e.g.*, antigen specific Treg cells to AAV).

[0658] In an aspect of a disclosed method, a subject can be a human. In an aspect, a subject can be suspected of having or can be diagnosed with having Parkinson's disease (such as, for example, PD). In an aspect, a disclosed subject can be symptomatic or asymptomatic.

[0659] In an aspect, a disclosed method can comprise repeating one or more steps of the method and/or modifying one or more steps of the method (such as, for example, an administering step).

[0660] In an aspect, a disclosed method of treating and/or preventing Parkinson's disease progression can comprise modifying one or more of the disclosed steps. For example, modifying one or more of steps of a disclosed method can comprise modifying or changing one or more features or aspects of one or more steps of a disclosed method. For example, in an aspect, a method can be altered by changing the amount of one or more of the disclosed isolated nucleic acid molecules, disclosed vectors, disclosed pharmaceutical formulations, or a combination thereof administered to a subject, or by changing the frequency of administration of one or more of the disclosed isolated nucleic acid molecules, disclosed vectors, disclosed pharmaceutical formulations, or a combination thereof to a subject, or by changing the duration of time one or more of the disclosed isolated nucleic acid molecules, disclosed vectors, disclosed pharmaceutical formulations, or a combination are administered to a subject.

[0661] In an aspect, a disclosed method of treating and/or preventing Parkinson's disease progression can be altered by changing the amount of one or more disclosed therapeutic agents, disclosed immune modulators, disclosed proteasome inhibitors, disclosed immunosuppressive agents, disclosed compounds that exert therapeutic effect against B cells and/or disclosed compounds that targets or alters antigen presentation or humoral or cell mediated immune response administered to a subject, or by changing the frequency of administration of one or more of the disclosed therapeutic agents, disclosed immune modulators, disclosed proteasome inhibitors, disclosed immunosuppressive agents, disclosed compounds that exert therapeutic effect against B cells and/or disclosed compounds that targets or alters antigen presentation or humoral or cell mediated immune response administered to a subject.

[0662] In an aspect, a disclosed method of treating and/or preventing Parkinson's disease progression can further comprise generating and/or validating one or more of the disclosed isolated nucleic acid molecules, one or more of the disclosed vectors, one or more of the disclosed pharmaceutical formulations, or any combination thereof.

[0663] In an aspect, a disclosed method of treating and/or preventing Parkinson's disease progression can further comprise administering to the subject a second disclosed isolated nucleic

acid molecule, a second disclosed vector, a second disclosed pharmaceutical formulations, or any combination thereof. In an aspect, a disclosed method of treating and/or preventing Parkinson's disease progression can further comprise administering to the subject additional disclosed isolated nucleic acid molecules, additional disclosed vectors, additional disclosed pharmaceutical formulations, or any combination thereof.

I. Kits

[0664] Disclosed herein is a kit comprising one or more disclosed isolated nucleic acid molecules, disclosed vectors, disclosed AAV vectors, disclosed pharmaceutical formulations, disclosed host cells, disclosed guide RNAs, disclosed plasmids, or any combination thereof with or without additional therapeutic agents to effect precision epigenetic modulation. Disclosed herein is a kit comprising one or more disclosed isolated nucleic acid molecules, disclosed vectors, disclosed AAV vectors, disclosed pharmaceutical formulations, disclosed host cells, disclosed guide RNAs, disclosed plasmids, or any combination thereof with or without additional therapeutic agents to treat and/or prevent Alzheimer's disease, Parkinson's disease or any APOE associated disease progression. Disclosed herein is a kit comprising one or more disclosed isolated nucleic acid molecules, disclosed vectors, disclosed AAV vectors, disclosed pharmaceutical formulations, disclosed host cells, disclosed guide RNAs, disclosed plasmids, or any combination thereof with or without additional therapeutic agents to treat and/or prevent Parkinson's disease progression.

[0665] In an aspect, a disclosed kit can be used in a disclosed method to reduce expression and/or activity of APOE regardless of the subject's genotype.

[0666] In an aspect, a disclosed kit can comprise at least two components constituting the kit. Together, the components constitute a functional unit for a given purpose (such as, for example, treating a subject diagnosed with or suspected of having Alzheimer's disease or Parkinson's disease). Individual member components may be physically packaged together or separately. For example, a kit comprising an instruction for using the kit may or may not physically include the instruction with other individual member components. Instead, the instruction can be supplied as a separate member component, either in a paper form or an electronic form which may be supplied on computer readable memory device or downloaded from an internet website, or as recorded presentation. In an aspect, a kit for use in a disclosed method can comprise one or more containers holding a disclosed pharmaceutical formulation, a disclosed therapeutic agent, a disclosed reagent, or a combination thereof, and a label or package insert with instructions for use. In an aspect, suitable containers include, for example, bottles, vials, syringes, blister pack, etc. The containers can be formed from a variety of materials such as glass or plastic. The container can hold, for example, a disclosed pharmaceutical formulation and/or a disclosed therapeutic agent and can

have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The label or package insert can indicate that a disclosed pharmaceutical formulation and/or a disclosed therapeutic agent can be used for treating, preventing, inhibiting, and/or ameliorating Alzheimer's disease or Parkinson's disease or complications and/or symptoms associated with Alzheimer's disease or Parkinson's disease. In an aspect, a disclosed kit can comprise additional components necessary for administration such as, for example, other buffers, diluents, filters, needles, and syringes. In an aspect, a disclosed kit can comprise those components (*e.g.*, primers) necessary to measure one or more times the level of expression and/or the level of activity of the disclosed gene of interest.

VII. EXAMPLES

[0667] Apolipoprotein E (ApoE) is encoded by the APOE gene (SEQ ID NO:01) positioned on chromosome 19q13.32 (GRCh 38: chr19:44,905,795-44,909,392). Two common coding SNPs in exon 4 of the gene give rise to three allelic variants, APOEe2 (SEQ ID NO:02), APOEe3 (SEQ ID NO:03), and APOEe4 (SEQ ID NO:04), encoding three corresponding protein isoforms that differ at two amino acid positions 112 and 158. The e4 allele of the apolipoprotein E gene (APOE e4) is the first, strongest, and most firmly established genetic risk factor for LOAD (Corder EH, et al. (1993) Science 261:921-923; Liu N, et al. (2008) Adv Genet. 60:335-405; Schmechel DE, et al. (1993) Proc Natl Acad Sci USA. 90:9649-9653; Saunders AM, et al. (1993) Neurology. 43:1467-1472). The initial discovery was made nearly 30 years ago by linkage analysis of pedigrees (Corder et al., 1993) and over the ensuing years it has become the most highly replicated genetic risk factor for LOAD (Corder et al., 1993; Liu et al. 2008; Schmechel et al., 1993; Saunders et al., 1993). Subsequent LOAD genome-wide association studies (GWAS) have confirmed strong associations with the APOE genomic region, and no other LOAD-association remotely approached the same level of significance (Harold D, et al. (2009) Nat Genet. 41:1088-1093; Lambert JC, et al. (2009) Nat Genet. 41:1094-1090; Heinzen EL, et al. (2009) J Alzheimers Dis. 19(1):69-77; Kamboh MI, et al. (2012) Mol Psychiatry. 17:1340-1346; Kamboh MI, et al. (2012) Transl Psychiatry. 2:e117; Seshadri, S. et al. (2010) JAMA. 303:1832-1840; Kunkle BW, et al. (2019) Nat Genet. 51:414-430; Lambert JC, et al. (2013) Nat Genet. 45:1452-1458; Coon KD, et al. (2007) J Clin Psychiatry. 68:613-618).

[0668] Carrying the APOE e4 variant significantly increases the lifetime risk for LOAD, whereas the number of e4 copies affects the level of risk and is associated with lower age of clinical disease onset (Corder et al., 1993; Farrer LA, et al. (1997) JAMA. 278:1349-1356), while APOE e3 is natural and APOE e2 conferred a protective effect (Saunders AM, et al. (1993) Neurology. 43:1467-1472; Reiman EM, et al. (2020) Nat Commun. 11:667). 40-65% of LOAD patients carry

the e4 allele compared to 10-15% in the general population. Although, the precise molecular mechanisms underlying ApoE e4-mediated risk effects have not been fully elucidated, it was suggested that ApoE e4 acquired hyperfunction (gain of toxic effects) (Gottschalk WK, et al. (2016) J Alzheimers Dis Parkinsonism. 6(1):209) and increasing data indicate several cellular pathways through which ApoE e4 may exert toxicity associated with LOAD pathologic phenotypes (Huang YA, et al. (2017) Cell. 168:427-441 e21; Sen A, et al. (2015) J Neurosci. 35:7538-7551; Theendakara V, et al. (2013) Proc Natl Acad Sci USA. 110:18303-18308; Theendakara V, et al. (2016) J Neurosci. 36:685-700; Min SW, et al. (2010) Neuron. 67:953-966; Tambini MD, et al. (2016) EMBO Rep. 17:27-36; Hatters DM, et al. (2006) J Mol Biol. 361:932-944). Collectively, these studies provide strong support to the concept that decreasing the levels of ApoE e4 specifically will have a therapeutic implication.

[0669] However, ApoE e4 as a target for LOAD remains significantly understudied, despite the few recent studies that have begun to pave the way. Another study utilized an antibody that specifically recognized the ApoE4 isoform and showed inhibition of Aβ accumulation in the hippocampus and reversed the cognitive impairments compared to the control APOE4 mice (Luz I, et al. (2016) Curr Alzheimer Res. 13:918-929). An additional study applied the anti-human ApoE4 antibody and also found a reduction in Aβ pathology characteristic of the APPS1-21/humanAPOE4 mice (Liao F, et al. (2018) J Clin Invest. 128:2144-2155). Collectively, these observations (Yang A, et al. (2021) Int J Mol Sci. 22(3):1244) demonstrated the beneficial effects of reducing the expression levels of ApoE, thus, supporting APOE as a promising therapeutics target for LOAD.

[0670] Moreover, accumulating evidence indicates that the increased overall expression of APOE plays an important role in the etiology of LOAD. Significant higher levels of APOE-mRNA were found in brain tissues obtained from e3/3 LOAD patients compared to 3/3 healthy donors, consistent with other reports showing elevated levels of APOE-mRNA in LOAD brains (Linnertz C, et al. (2014) Alzheimers Dement. 10:541-551; Zarow C, et al. (1998) Exp. Neurol. 149:79-86; Matsui T, et al. (2007) Brain Res. 1161:116-123; Akram A, et al. (2012) Neurobiol. Aging. 33:628e1). Further, studies using the APP/PS1 transgenic mice showed that lowering the ApoE protein levels ameliorated cognitive dysfunctions and Aβ pathology (Huynh TV, et al. (2017) Neuron. 96:1013-1023.e1014: Zheng JY, et al. (2017) Neurobiol. Aging 2017, 54:112-132) independent of the APOE allele (Bien-Ly N, et al. (2012) J. Neurosci. 32:4803-4811; Kim J, et al. (2011) J. Neurosci. 31:18007-18012). While ApoE4 has received much attention for its LOAD-risk effect, there are clear changes in APOE expression associated with LOAD and independent of the e4 allele.

[0671] This means that the regulation of APOE expression can impact the risk to develop LOAD, making the modulation of the overall ApoE protein levels useful as a therapeutic target. To this end, reduction in APOE expression had beneficial effects, specifically a decrease in Aβ pathology. Antisense oligonucleotide (ASO) treatment lowered the APOE-mRNA and protein levels in the brains of APP/PS1-21 mouse model by at least 50%, leading to a significant decrease in Aβ pathology (Huynh TV, et al. (2017) Neuron 96:1013-1023 e4). Administration of anti-ApoE antibody had consistent effects on reducing Aβ pathology and improved brain function and cognitive abilities (Kim J, et al. (2012) Annu Rev Neurosci. 31:75-93; Liao F, et al. (2014) J Neurosci. 34:7281-7292)).

[0672] Thus, as described below, methods of modifying the e4 isoform and reducing APOE expression and/or activity levels provide a promising avenue towards precision medicine in AD and especially LOAD.

Example 1
Overview of Examples

[0673] The epigenome therapeutics strategy provided herein allows for the mitigation of the risk of LOAD conferred by the APOE gene and its pathogenic effect mediated by the microglia cells. The gene editing system described in the examples comprises all-in-one viral vectors comprising deactivated (d)Cas9, an optimized fusion of KRAB-MeCP2 repressors, or other transcriptional repressors/epigenome modifiers, and gRNA targeting the promoter/intron 1 region of APOE. The initial set of experiments described here found that the APOE promoter/intron 1 region in sorted glial nuclei from LOAD brains and in APOE hiPSCs-derived models, is characterized by open-chromatin organization, thus, defined the target site for epigenetic therapy. Microglial specific promoters for use in the described system were identified using the next set of experiments. To achieve microglia-specific expression, F4/80, CD68, FCGR2A, or 1BA1 promoters were utilized that showed high-level of specificity and efficiency in microglial cells. Finally, lentiviral and AAV platforms are described and used to establish proof-of-concept(POC) in *in vitro* hiPSC-derived microglia like cells, hiPSC-derived 3D AD models and *in vivo* in mouse AD models. In preliminary experiments, bilateral stereotaxic injection of the epigenome editing AAV all-in-one vector and a vector driving a reporter gene directly into the left hemisphere of the hippocampus and injection of a control vector with no repressor into the right hippocampus were performed. The results showed statistically significant robust and precise reduction in the expression of the reporter gene in the left side. Analysis of 11 mice demonstrated significant and efficient suppression in the hippocampus, the brain region affected in early stage of AD that contains relatively large subpopulation of microglia.

Example 2
snRNA-seq with 10X Genomics to determine differential expression of ApoE gene

[0674] Initially single nucleus multiomics analysis was conducted using microglia of normal and AD patients to determine the relevance of using APOE as a target and further to determine potential sites for epigenomic modification. For these experiments snRNA-seq with 10X Genomics experiments were conducted**.** Single nucleus (sn)RNA-seq of 12 LOAD and 12 control temporal cortex of aged and sex matched normal controls were analyzed. UMAP plot showing separation of cell type-specific clusters is provided in **FIG. 1A** with the micro1 cluster circled in red. ApoE expression in LOAD and in age-matched controls is shown in **FIG. 1B**. As clearly seen the UMAP and from APOE is overexpressed in microglia of LOAD patients, in comparison to normal individuals. Differential expression (DE) analysis identified APOE as significant Differential Expressed Gene (DEG) only in Micro1 cluster with $Log_2(FC)=0.45$ and adjusted $p=0.0086$.

[0675] Next, (sn)ATAC-seq data from the same 12 AD and 12 control temporal cortex were analyzed. A schematic representation of *APOE* gene, which is located at chromosome 19q13.2 is provided in **FIG. 1D**. Differential chromatin accessibility analysis of AD vs. control cells, identified differential accessible peak (DAP) in microglia cluster 1: DAP (red) and open chromatin peaks (orange). Load Micro1 APOE DAP and co-accessible peaks within the range of +/- 1k bp are further shown in **FIG. 1E**. **FIG. 1F** provides a schematic of positions for use in design of suitable gRNA for epigenetic modification based on chromatin accessibility analysis across APOE genomic locus shown in **FIG. 1G**. Coverage plots for all 6 cell-types and micro1 cluster are shown in the upper panels, and Micro1 differential accessible peak (DAP) in the promoter region of APOE is labeled in a red box.

[0676] Data from these studies is further represented in dot plots (**FIG. 1H**). Size of dot indicates % nuclei expressing APOE with bluer the dot, higher the expression value. Micro1 was identified as the only cluster with an obvious difference in expression. Dot plots on the left represents APOE expression in all brain cell clusters for both LOAD and control.

[0677] APOE as Differentially Expressed Gene (DEG) was tested in 5 clusters in which sufficient number of nuclei expressed APOE**.** DE results for all clusters in which APOE was expressed are illustrated in the bar plot of the logFCs, and Micro1 was found to be the most robust (**FIG. 1I**) . A scatter plot (see **FIG. 1J**) showing the adjusted p-value (-log10(fdr)) vs. the logFC (coef), with broken line defining the thresholds at p=0.05 and logFC=0.25. Micro1 was confirmed to be the only datapoint that passes both thresholds.

[0678] These data clearly show the differential expression of APOE in microglial cells of LOAD patients in comparison to normal individuals. DAP analysis further helped identify sites suitable

for epigenetic targeting. Suitable gRNA were identified based on these studies and are provided in FIG. 1K and Table 1 (SEQ ID NO: 15-33).

[0679] Table 1: List of gRNA identified from the multi-omics studies

SEQ ID NO:	STRAND	SEQUENCE
15	+ STRAND	GACAGGGGGAGCCCTATAAT
16	+ STRAND	TCAGGAGAGCTACTCGGGGT
17	+ STRAND	ACTGGGATGTAAGCCATAGC
18	+ STRAND	GTTGGAGCTTAGAATGTGAA
19	+ STRAND	GCCCTATCCCTGGGGGAGGG
20	+STRAND	TCGGGCTTGGGGAGAGGAGG
21	-STRAND	CTCTCCCCACCCCACCTTCT
22	+STRAND	TGTGAAGGGAGAATGAGGAA
23	-STRAND	GAGGAGGGGGCGGGACAGG
24	-STRAND	GCTTTTTAAAATGTGCTCTGT
25	-STRAND	GTAGCTCTTCCCTCCCAAGGT
26	-STRAND	GCGCTTGGCCCCCAGAATGGAG
27	-STRAND	GAATTGGACAAGTCTGGGATCC
28	-STRAND	GCCCACCCCACCTTCTAGCGGG
29	+STRAND	GGGGCGGCTTGGTAAATGTGC
30	+STRAND	GCTTGGCCCCCAGAATGGAG
31	+STRAND	ATTGGACAAGTCTGGGATCC
32	+STRAND	CCCGCTAGAAGGTGGGGTGG
33	+STRAND	GGGCGGCTTGGTAAATGTGC

[0680] Similar experiments were conducted to test microglial specificity of expression of the gene FCGR2A. LOAD and 12 aged and sex matched normal controls were tested. Chromatin accessibility analysis across the genomic region of FCGR2A is indicated in FIG. 1L and 1M. The ATAC-seq peak includes the promoter-like signature (ENCODE: cis-Regulatory Elements (cCREs)) for FCGR2A. These data indicate the possibility of using FCGR2A as a potential microglia specific promoter.

Example 3
Identification of suitable CRISPR/dCas9-repressor systems

[0681] Suitable CRISPR/dCas9-repressor systems were developed essentially as described in PCT/US2022/078260, the contents of which are incorporated herein by reference, in their entirety. Briefly, out of 150 Cas-Repressor pairs, 24 showed efficient packaging in AAV. Those AAV/dCas9-repressor vectors were screened *in vitro* using a luciferase reporter assay to characterize their repression effects on APOEe4 expression. A schematic of the experimental design and the results are provided in **FIG. 2**. Though multiple dCas9-repressor pairs worked well when incorporated into the system, dSaCas9- KRAB-MeCP2(TRD) or dCas9-DNMT3A (SEQ ID NO: 75) were used in subsequent experiments.

Example 4

Identification of Promoters with enhanced expression in Microglia

[0682] To find promoter sequences for genes that are overexpressed in microglial cells, human induced pluripotent stem cells harboring APOE3/3 (E3) and APOE4/4 (E4) were differentiated to microglia. Upon microglia maturation, RNA was isolated and qRT-PCR was performed to evaluate the expression of five microglia markers: IBA1(AIF1), CD68, CD11B, P2RY12, and TMEM119. **FIG. 3A** is a bar graph displaying the identified gene in the order of gene expression, with IBA1 having the highest expression. The results were normalized to GAPDH and PPIA1 internal controls. Based on these experiments, IBA and CD68 were identified as some of the suitable candidates and further characterized using the workflow described for IBA1 (AIF1) below.

[0683] First 'gencode.v32.primary_assembly.annotation.gtf" was used to find the chromosome range of AIF1. Peaks that are DAPs from ATAC differential analysis (with covariates) were identified. DAPs that overlap with AIF1 were then isolated and use to find peaks that are co-accessible with the DAPs from the results of cicero analysis (cluster Micro1). The DAPs and their co-accessible peaks were visualized on UCSC genome browser (with custom track) and sequences of the DAPS and peaks obtained. **Table 2**, and **FIG. 3B** provides a compilation of these data.

Table 2: DAPS and peaks in the IBA1 promoter sequence.

DAPS	PEAKS	COACCESS	DISTANCE TO AIF1
chr6-31614000-31616011	chr6-31619824- 31621400	0.338009843	2802
chr6-31614000-31616011	chr6-31598225-31599122	0.188310059	16094
chr6-31614000-31616011	chr6-31585879-31587380	0.400694803	27836
chr6-31614000-31616011	chr6-31580353-31581795	0.157331642	33421
chr6-31614000-31616011	chr6-31651504-31653391	0.267531204	34482

[0684] Based on these data, 3 synthetic promoters of separate were designed and tested. The sequences for these promoter sequences are provided below in **Table 3** below. Immortalized microglia HMC3 grown in 0%FBS EMEM or 0.5% FBS EMEM on matrigel plates were transduced with different GFP reporter AAV vectors (serotype 6) driven by synthetic human IBA1 promoters of 3 lengths (SEQ ID NO: 5-7). The media was changed the next day. Of the three sequences tested, the medium human IBA1 synthetic promoters (SEQ ID NO: 5) had the most robust expression in microglial cells (see **FIG. 3C** and **FIG. 3D**).

[0685] Based on the analysis of these, and other microglial specific promoters, and considering the promoter size for packaging in AAV, multiple suitable promoters were identified for use in to drive the expression of the dCas-repressor including CD68, IBA1 and F4/80 or combination of elements thereof. Examples presented herein use a derivative of the microglial specific promoter CD68 (**Table 3**, SEQ ID NO: 3).

Table 3: Promoter Sequences

SEQ ID NO:	SEQ TYPE	SEQUENCE
1	CD68 promoter	GACGGATCGGGAGATCCTAGCGTTTAAACTTAAGCTTGGTACCGAGCTCG GATCCACTAGTCCAGTGTGGTGGAATTCTGCAGATATCAAACTGCCTGTTT GGGCTTCTCATTTCTTACCTCCCCTTCCCTCTCCCACCTGCTACTGGGTGC ATCTCTGCTCCCCCCTTCCCCAGCAGATGGTTACCTTTGGGCTGTTGCTTT CTTGTCACCATCTGAGTTCTCAGACGCTGGAAAGCCATGTTCTCGGCTCTG TGAATGACAATGCTGACTGGAGTGCTGCCCCTCTGTAAAGGGCTGGGTGT GGATGGTCACAAGCCCCTCACATGCCTCAGCCAAGAGGAAGTAGTACAG GGGTCAGCCCAGAGGTCCAGGGGAAAGGAGTGGAAACCGATTTCCCCAC CAAGGGAGGGGCCTGTACCTCAGCTGTTCCCATAGCTTACTTGCCACAAC TGCCAAGCAAGTTTCGCTGAGTTTGACACATGGATCCCTGTGGATCAACT GCCCTAGGACTCCGTTTGCACCCATGTGACACTGTTGACTTTGCCCTGACG AAGCAGGGCCAACAGTCCCCTAACTTAATTACAAAAACTAATGACTAAGA GAGAGGTGGCTAGAGCTGAGGCCCCTGAGTCAGGCTGTGGGTGGGATCA TCTCCAGTACAGGAAGTGAGACTTTCATTTCCTCCTTTCCAAGAGAGGGCT GAGGGAGCAGGGTTGAGCAACTGGTGCAGACAGCCTAGCTGGACTTT
2	CD68 (microglia specific promoter)	GTGGCTAGAGCTGAGGCCCCTGAGTCAGGCTGTGGGTGGGATCATCTCCA GTACAGGAAGTGAGACTTTCATTTCCTCCTTTCCAAGAGAGGGCTGAGGG AGCAGGGTTGAGCAACTGGTGCAGACAGCCTAGCTGGACTTTGGGTGAG GCGGTTCAGCCATGAGGTAAGGAGGAAGGAGGCTGAGGGGAGGGGGCCC CTGGGAGGGAGCCTGCCCTGGGTTGCTAACCATCTCCTCTCTGCCAAAAG
3	CD68v2 promoter	GTGGCTAGAGCTGAGGCCCCTGAGTCAGGCTGTGGGTGGGATCATCTCCA GTACAGGAAGTGAGACTTTCATTTCCTCCTTTCCAAGAGAGGGCTGAGGG AGCAGGGTTGAGCAACTGGTGCAGACAGCCTAGCTGGACTTTGGGTGAG GCGGTTCAGCCATGAGGTAAGGAGGAAGGAGGCTGAGGGGAGGGGGCCC CTGGGAGGGAGCCTGCCCTGGGTTGCTAACCATCTCCTCTCTGCCAAAAG TATATAAGCAGAGCTGTAAGTATCAAGGTTACAAGACAGGTTTAAGGAGT CCAATAGAAACTGGGCTTGTCGAGACAGAGAAGACTCTTGCGTTTCTGAT AGGCACCTATTGGTCTTACTGACATCCACTTTGCCTTTCTCTCCACAG
4	F4/80 promoter	GGTTTTTTGTTATTCCAGATGAATTTGAGTTGGGATTTTGATAGGGAATAC ACTGAATTTGTAGATTGCTTTTGGCAAGATGGCCATCTTTCCATCTTCTGA GGTCTTTGATTTCTTTCTTCAGAGACTTGAAGTTCTTGTCATACAGATCTTT

		CAATTGCTTGGTTAGAGTCACACCAAGATATTTTATATTATTTGTGACTAT TGTGAAGGGTGTCATTTCTCTAATTTATTTCTCAGCCCGTTTATCCTTTGAG TAGAGGAAAACTACTGATTTGTTTGAATTAATTTTATATCTAGCCACTAAG CTGAAGTTGTTTATCAGCTGTAGGAGTTCTCTGGTGGATTATTTTTTAGGG TTATTTATGTATTCTACCACATCATCTGCAAATAGTGATATCTTGACTTCC TCCTTTCTAATTTGTATCCCTTTGACCTCCTTTTGTTGCCTAATTGTTCTGG GTAGAACTTTGAGTACTATATTAAATGATAGGGGAAAAGTGGACAGCCTT GGGTGTGGTGTGTGTGGTATGGTGTGGTGTGTGTATGGTGATGATGCATG TGGTGTGTATGGTGTGGTGTATGTGGTGTGGTGTGTGGTGGTGGTGATAA TGATGATGGTGGTCTCTCTGTCTCTGTCTGTCTCTTTCTATCTCTGCCTCTC TCTCTCTGTCTCTCTCTGCCCCCCTCTGTCTCCCTCTGTCTGTCTGTCTGTC TCTTTGGTGTGATGTATGTGGTGTGTGTGTATTCTACAAGGTTGACATGAT GACAGAATTTAATTTTCTTAGCAGCAAGCTCATGGATCCTGGTGATAAAT GCAGCATGACTTTACTGAAAAGGCTTTGTGATCTTGAAGAGTGGATTGAC TTCACTGTCGGCAGCACATGCAATCTCACTTGTTTGGTGTAATGAAAGAA GAGAATGAGAGGTGGAAGGGGGATGGTAATGTTGAAAAAAAGAATGGTA CAGAGGAAACTGAGGTTGGAGAGAGATGGGGTAGATGGTAAGAGATGGA GAAAGAGGGAAGGAAATGGAGAGAAAGACAGAGAGACAGAGAGAGACA CACAGAGAGACACACAGAGACAGAGAGGAAGGGAAAGGGAAAGAGAAA GGAAGAGGAAGAGGGGGAGGGGAAGGGGAAGGGGAAGGGGAAGGGAG AGGGAGAAATGTGGACACTAGCCAGATTTAAGGGAGAAATTAGGGGGTT GCCAGTCTGTCCACCTCTGATGGTGGCAACTCAGCAGAAAGCTGCTGGGC TCAGTCTGGCTTTGTTGAGCAACCCTGACTCCACCCCTTTTCTTCCCCACA AAGCAAGCTTTTAAAGGGAAGGCTTTCTTCATTGAATGACTGCCACAGTA CGATG
5	1985: Full-length	GCACTCTAGCCTGGGCGACAGAGTGAGACTCCGTTTAAAAAAAAAACAA AAAACAAAAAACAAAAAACTGGGGACCATTGGCAATAATACTCCTATGT CCCCTCTTCCCTACTTTGTTTTCCTCCATAGGCACCTGGCGCCTTTTTTTTT TTTTTTTTTTTTTTTTTTTGAGACGGAGTCTCACTCTGTTGCCCAGGCTGGA GTGCAATGGCGCGATCTCAGCTCACTGCAACCTCTGCCTCCCGGGTTTAA GCGATTCGCCTGCGTCAGCCTCCTGAGCAGCTGGGATTACAGGCACGCAC CACCAGGCCCTGCTAATTTTTGTATTTTTAGTAGAGATGGGGTTTCACCAT GTTGGTCAGGCTGGTCTCCAACTCCTGACCTTGTGATCCGCCTGCCCCAGC CTCCCAAAGTGCTGTGATTACAGGCGTGAGCCACTGCGCCTGGCCACCTA GCACCTTTAATATACTTATTTATTTGTATTGTCTGCCTTCCCCAATTAGATC AACCATGAAGACAAGAGTTTTCATTTGTTGGGTTCTCTGGGCCTAGAGGC ATGTCTGGCATATAGTAAGCATTCAGTAAATATCTGTTGAGTGAACGTAT GAATAAAGAAGTGAGTTCCTCCCAGCAGGCACTGAGAACATTGGGAGTA CAGGGTTGCAGCTCTCTCTGCAGCAGGAGAATGTAGCTGCAATAAAGGGA AGTCAAGAAGCCAGAGTCCAGCCAGGTGCAGTGGCTCATGCCTGTAATCC CAGCACTTTGGGAGGCTGAGGTGGGTGGATCACAAGGTCAAGAGATAGA GACCATCCTGGCCAACATGGCGAAACCCCATCTGTACTAAAAATACAAAA ATTAGCTGGGCGTGGTGGTGGGCGCCTGTAGTCCCAGCTACTCAGGAGGC TGAGGTAGGAGAATTGCTTGAACCCAGGAGGCAGTGGTTGCAGTGAGCC GAGATTGCACCATTGCACTCCCGCCTGGGCGACAGAGCAAGACTCCGACT CAAAAAAAAAAAAAAAGCAGCAGCAGCAGCCAGAGGCCACTCCAGCATCT CCCCTACCTGGCTTGGGTCAGGGAGAGGGCAGTGAGAAGTGAAAACTCC CAGCTACAGAAAAGGAAATATGTTGCGGGGAAGGGAGAAGGAAAGGTGT CTTCATCAATGCCGGGGCAGGGTAGATGGAGCCCTGGGCAGGGAGTTTGG ACCAGGAAATCTCAATGAGGGAAATGTGCTGTCCTCACCTCTCCAAGAAG CGACTGGCCAAACAGAGTGACAGAGGGGATAAAGGTTATGCCTAGGGAG GCATGTGTCAGAGGCTATCATCCACTCTGTTGAACCCACAGTGACCAGCA CCACCATCACACAAACATGCCTGCATGTGTGCACGCACGCGCAGTGTGCA AACCTGATGTCAGCCTCACTCCCTGGCTCTTCTGTCCACAAACGCTGTTTC TTTAAGTACCACTTTCAGTTCCTCCAAAGAATCTACTTAAACTCTTAAATT CCTGATCTCTATAGATTTTACTAAAGATTTCAAAGGAGATAAGATGAGAG GGTTACGTTGCACATTCTAAAGCAAACAAATTAAAATGTTTTGTTAGACA TTTCCATATTTTTAAGGGCCTCCTTGGAGCTGCCAGGCTGGGAGTGAGGTT TCTCTCCCTTTCTAAACCCTGTGCCCATCTTGTCACCCTCCTGGAGCTGCC AGCAGACTTCAGATTCTTCTCCGATCTACAGAGCAGAAAAATTCAGCCAG

		CCCTTCCTTGTCTTCCTATCCACAGCTGCCTGCCCAGACTCATGAAACCTG ACAAAATGCAAGGTCTTATCATTACCTGAACCTTGGACCTGTTCAAAAAT ACTAGTTCCTGAGAATAAATATCCCTGGTGTCTTCCTGCCCTTCCTGCACA CCTCCAGTGGCTTATCAAAATATTTGTTTCATGCGCACACTGGGCTCTCAT TTAAGAGGAATTTGGGAGAATGTTATTTTCTAATCTGCATTTCACACCAGG CTCCCCCTCCTTCCTGGGGTGCTAGTGTCAGCAGAACCTGATGGGGAAGT GAGGTCTGGGAGGCAGAGGAGGAAGGAATGAGGGGAAAGGGGAAGTTT GGGAGGAAGGCTTCTGAGAAGACTGGTGGGAGAGAAGGAGAGCCTGCAG ACAGAGGCCTCCAGCTTGGTCTGTCTCCCCACCTCTACCAGCATCTGCTGA GCTATGAGCCAAACCAGGGATTTACAGGGTAGGGAGGGTGGGATAGGCA GCGGCATTAGATCGGAGGAATGAGATGGACAGACCTGGGCTGTGGGCTA GGAGGGCAGTCAGCTGGCCTAGGGTAGCCCGGGCTGGTGTCAGGGTAAG GAGAGGAAGGGAGGGATGAGGGCTGATTAATTTTTTTCACCCCACAGGGT AAGTATCAAGGTTACAAGACAGGTTTAAGGAGTCCAATAGAAACTGGGC TTGTCGAGACAGAGAAGACTCTTGCGTTTCTGATAGGCACCTATTGGTCTT ACTGACATCCACTTTGCCTTTCTCTCCACAG
6	1986: Medium	CCTCACCTCTCCAAGAAGCGACTGGCCAAACAGAGTGACAGAGGGGATA AAGGTTATGCCTAGGGAGGCATGTGTCAGAGGCTATCATCCACTCTGTTG AACCCACAGTGACCAGCACCACCATCACACAAACATGCCTGCATGTGTGC ACGCACGCGCAGTGTGCAAACCTGATGTCAGCCTCACTCCCTGGCTCTTC TGTCCACAAACGCTGTTTCTTTAAGTACCACTTTCAGTTCCTCCAAAGAAT CTACTTAAACTCTTAAATTCCTGATCTCTATAGATTTTACTAAAGATTTCA AAGGAGATAAGATGAGAGGGTTACGTTGCACATTCTAAAGCAAACAAAT TAAAATGTTTTGTTAGACATTTCCATATTTTTAAGGGCCTCCTTGGAGCTG CCAGGCTGGGAGTGAGGTTTCTCTCCCTTTCTAAACCCTGTGCCCATCTTG TCACCCTCCTGGAGCTGCCAGCAGACTTCAGATTCTTCTCCGATCTACAGA GCAGAAAAATTCAGCCAGCCCTTCCTTGTCTTCCTATCCACAGCTGCCTGC CCAGACTCATGAAACCTGACAAAATGCAAGGTCTTATCATTACCTGAACC TTGGACCTGTTCAAAAATACTAGTTCCTGAGAATAAATATCCCTGGTGTCT TCCTGCCCTTCCTGCACACCTCCAGTGGCTTATCAAAATATTTGTTTCATG CGCACACTGGGCTCTCATTTAAGAGGAATTTGGGAGAATGTTATTTTCTA ATCTGCATTTCACACCAGGCTCCCCCTCCTTCCTGGGGTGCTAGTGTCAGC AGAACCTGATGGGGAAGTGAGGTCTGGGAGGCAGAGGAGGAAGGAATG AGGGGAAAGGGGAAGTTTGGGAGGAAGGCTTCTGAGAAGACTGGTGGGA GAGAAGGAGAGCCTGCAGACAGAGGCCTCCAGCTTGGTCTGTCTCCCCAC CTCTACCAGCATCTGCTGAGCTATGAGCCAAACCAGGGATTTACAGGTAA GTATCAAGGTTACAAGACAGGTTTAAGGAGTCCAATAGAAACTGGGCTTG TCGAGACAGAGAAGACTCTTGCGTTTCTGATAGGCACCTATTGGTCTTACT GACATCCACTTTGCCTTTCTCTCCACAG
7	1987: Short or core promoter	TGTCACCCTCCTGGAGCTGCCAGCAGACTTCAGATTCTTCTCCGATCTACA GAGCAGAAAAATTCAGCCAGCCCTTCCTTGTCTTCCTATCCACAGCTGCCT GCCCAGACTCATGAAACCTGACAAAATGCAAGGTCTTATCATTACCTGAA CCTTGGACCTGTTCAAAAATACTAGTTCCTGAGAATAAATATCCCTGGTGT CTTCCTGCCCTTCCTGCACACCTCCAGTGGCTTATCAAAATATTTGTTTCA TGCGCACACTGGGCTCTCATTTAAGAGGAATTTGGGAGAATGTTATTTTCT AATCTGCATTTCACACCAGGCTCCCCCTCCTTCCTGGGGTGCTAGTGTCAG CAGAACCTGATGGGGAAGTGAGGTCTGGGAGGCAGAGGAGGAAGGAATG AGGGGAAAGGGGAAGTTTGGGAGGAAGGCTTCTGAGAAGACTGGTGGGA GAGAAGGAGAGCCTGCAGACAGAGGCCTCCAGCTTGGTCTGTCTCCCCAC CTCTACCAGCATCTGCTGAGCTATGAGCCAAACCAGGGATTTACAGGTAA GTATCAAGGTTACAAGACAGGTTTAAGGAGTCCAATAGAAACTGGGCTTG TCGAGACAGAGAAGACTCTTGCGTTTCTGATAGGCACCTATTGGTCTTACT GACATCCACTTTGCCTTTCTCTCCACAG
8	FCGR2A	ACTATCTGCCACCGCGCCCGGCTAAATTTTTTTTGTATTATTAGTAGAGAC GGGGTTTCACCGTGTTAGCCAGGATGGTCTCGATCTCCTGACCTCGTGATC CACCCGCCTTGGCCTCCCAAAGTGCTGAGATTACAGGCGTGAGCCACCGC GCCCGGCCGACCAGGAATTATCTTAAACAGAAAAAGAATACTCTAAGGA

		GGGGTATACCGGCCAGCAGAACAGTAACCCCTCCCCGGGGTTTTCACAAG GACACAAGCTGGTGTGTAATAGACTTATCTATAAAGCAGACTCAACTCCT AACTGGAATTGTTCCTGATTGGTTAGCAGAAACAGAATTGAGGACTGACG ACAGCTGCACAAGAAGATGAAACCATTTCCTTCCTCTTTTCTAAGCTTGTC TCTTAAAACCCACTGGACGTTGGCACAGTGCTGGGATGACTATGGAGACC CAAATGTCTCAGAATGTATGTCCCAGAAACCTGTGGCTGCTTCAACCATT GACAGTTTTGCTGCTGCTGGGTGAGTGAGGGTCATTCTGAAATGGGGCAA TTTCAGACACTCCTACTGCCTGGACTCCAGGTACCAGTGTGGTAAGGAGC AGGCCTGGGCCCTGGAAGCAGGGGATAGATTGAAAGAGGAGAGAGGACC CTGAATTCTTAAGTGTTCCAATGGTTCCTAAGGTCAGAACTCAGAGGTAG TTTCATAGTCCCTGGAAAGTCCATTCTGCACCAAACATTCATGTGTACTTT GTAGTCATGAGGAGGAACAGGAAATGAGATAAAGGGGACATATGAAGTG AATACTTTTATAAAAGATCAACTGGAAACAGGATCTTGAGATGGGTCCTG GAGAAGGAAGAGCCCAAGCTCACCTCCCCAGCATTTCACATACGCAGCTA AATTTGAAGCCGTGTTCTCCTGCTCGACGTTGATCCACTCTCTTCTCTTTTA CAGCTTCTGCAGACAGTCAAGCTGGTGAGTATGCCCTTTGCTTCCTTGTAT TGACAGTGTTGTATCCTCATAATATGATGCTGTGTTTGCTGGGGCGGCGG GGGGGTATGTCTATTCCACTGAAAATCAAGCTTGGGTTCAGCATGGGCAG TTCCCCCATTTTAGTGGGGTCTGGGATTTGCTTCTTAGATCCACTGAAGAC CCAAGGGAATGAGGCCGCTGCAAGTACAGATCTTGAACTACATCTACAAT AGGACTCTTGGGTGCCTGACCTCCCTTGGGAGCTCCTTTGGCATCCACAGT CCCTTCAGGGTTATTTATTCCACACCCCTTTCCACTCTGCCCCTCAGCAGC TCCCCCAAAGGCTGTGCTGAAACTTGAGCCCCCGTGGATCAACGTGCTCC AGGAGGACTCTGTGACTCTGACATGCCAGGGGGCTCGCAGCCCTGAGAG CGACTCCATTCAGTGGTTCCACAATGGGAATCTCATTCCCACCCACACGC AGCCCAGCTACAGGTTCAAGGCCAACAACAATGACAGCGGGGAGTACAC GTGCCAGACTGGCCAGACCAGCCTCAGCGACCCTGTGCATCTGACTGTGC TTTCCGGTCAGTGGAGGAAGGCCCCAGGGTGGACCTGGGAGGGCCAGGA CGGATGAAATCTGTTTACAGACAGAGGTTTGCAGGAAAGAGTGGGCGTG GACTGCTTACTGGGAAGCACTGTTGTGAGTTGCTCATTCATTCCCCATTTC ACCCACCCTCTTTGCTTAGCATGCGTGGTGGGGGAAGGGGGAATTTCTAA TAATTTTCTCAGCATGTGTTAGGTTGTTTTTGCCTCAGTCTTGATTGAGCA AGGGGGTTACGAGGCCACAAACAGCTGAGTGTGGGAGAAGCAGAAGGAA ATCCCTACTTTAAAAAACTACCCTCCTCCTGTGGCAATGCGGTAAAGCAG

Example 5
Designing and validation of an All-In-One AAV vector for microglial specific targeting of APOE

[0686] **FIG. 4A** provides a schematic of the lentiviral vector design that was adapted into an all-in-one Adeno-Associated viral (AAV) vector for targeting APOE. AAV can transduce nondividing cells (*i.e.*, neurons) and can confer long-term stable gene expression without associated inflammation or toxicity, a feature that is particularly important for CNS gene therapy applications. AAV-based gene transfer vector genomes do not integrate into the chromosome in nondividing cells, instead generally persisting as nonintegrated episomal concatemers.

[0687] Comparison of transduction efficiency and stability of lentiviral vectors and AAV vectors was conducted using cerebral organoids derived from human induced pluripotent stem cell. The organoids were transduced with lentivirus or AAV serotype (phpeb) expressing GFP. Transduced organoids were spun down for 45 minutes at 300 g, and the media was changed the next day. GFP

fluorescence was monitored daily. Representative images (see **FIG. 4B** and **4C**) show a higher efficiency of transduction with the AAV serotype (phpeb).

[0688] Next, different AAV serotypes were tested for transduction efficiency *in vitro* in microglia. Immortalized microglia HMC3 in 0%FBS EMEM or 0.5% EMEM on Matrigel® plates were transduced with 2 uL of different serotypes. The media was changed the next day. Images were taken on days 3 and 6. On both days serotype 6 showed the strongest GFP expression (**FIG. 4D** and **FIG. 4E** respectively).

[0689] Initial *in vivo* validation of the all-in-one AAV vectors was done using GFP as the reporter gene with GFP specific gRNAs (SEQ ID NO: 34 and SEQ ID NO: 35) instead of APOE. A constitutive promoter was used in these experiments to derive the expression of the d-Cas9-repressor. The AAV/gRNA(GFP)p-dSaCas9- KRAB-MeCP2(TRD) repressor with the LV-dGFP-nLuc reporter (hereafter, LV-GFP) was stereotaxically co-injected into the left dorsal hippocampus (DH), and the control vector with no repressor and no gRNA (AAV/dSaCas9) with the LV-GFP reporter into the right DH, such that each mouse provided an internal control (**FIG. 4F**). Six weeks post injections brains were harvested and the GFP expression was compared between the left and the right hemisphere of the DH for each mouse. The experiment was performed using a total of 21 C57BL/6 mice, 11 (6 males, 5 females) represented middle aged (16 weeks) and 10 (5 males, 5 females) very-old (32 weeks old) mice. There was no significant difference in GFP expression between males and females, thus, the data was combined for subsequent analysis. The AAV/gRNA(GFP)p-dSaCas9-KRAB-MeCP2(TRD) vector significantly repressed the GFP expression in the left DH compared to the control right DH (AAV/dSaCas9) in both the middle aged and the very old mouse groups (Mann–Whitney $U = 18$, $n = 11$, $P = 0.004$, two-tailed, and Mann–Whitney $U = 21$, $n = 10$, $P = 0.028$, two-tailed, respectively, **FIG. 4G-4I**). The expression of GFP in the left DH with the AAV/gRNA(GFP)p-dSaCas9-MeCP2(TRD) vector was 51.94% ± 11 in the middle aged mice group and 40.98% ± 6 in the very old group relative to the right DH with the control vector. There was no significant difference in the efficacy of the repressor between the two age groups. Collectively, the AAV/gRNA(GFP)p-dSaCas9-MeCP2(TRD) repressor effectively reduced the GFP reporter expression in the mouse DH.

Example 7
In vivo validation of durable, targeted APOEe4 repression in mice models

[0690] Next, the *in vivo* efficacy of the all-in-one AAV epigenome-editing platform using APOE as an example for LOAD therapeutically relevant target was investigated. The expression of the mouse endogenous APOE was targeted in the DH of adult C57BL/6 mice and tested for efficacy in reducing its expression. Two all-in-one AAV repressor vectors carrying gRNA1 (SEQ ID NO:

23) and gRNA2 (SEQ ID NO: 25) targeted at APOE promoter sequences were used. These experiments used a constitutive promoter to express the dSaCas9-repressor protein. A total of sixteen mice (8 males, 8 females) received bilateral stereotaxic injection with the AAV/gRNA(*APOE*)p-dSaCas9-KRAB-MeCP2(TRD) repressor vector carrying either gRNA1 (n=8) or gRNA2 (n=8) into the right DH, and the AAV/dSaCas9 control vector (no gRNA, no repressor) into the left DH (see schematic in **FIG. 5A**). After 6 weeks, the expression of the mouse APOE was quantified and its level in the right hemisphere of the DH relative to the left DH was determined for each mouse. Since there was no significant difference in APOE expression between males and females the data was combined for subsequent analysis. The results showed that both AAV/gRNA(APOE)dSaCas9-KRAB-MeCP2(TRD) vectors with gRNA1 and gRNA2 significantly repressed the mouse endogenous APOE expression in the right DH compared to the left DH (gRNA1: Mann–Whitney U = 0, n = 8, P = 0.0002, two-tailed, and gRNA2: Mann–Whitney U = 3, n = 8, P = 0.0011, two-tailed, **FIG. 5C**). The expression of APOE in the right DH with gRNA1 and gRNA2 were 28.8% ± 7.07 and 47.16% ± 8.5 relative to the left DH with the control vector, respectively (**FIG. 5B** and **FIG. 5C**). There was no significant difference in the efficacy between the gRNA1 and the gRNA2 vectors (Unpaired t-test, p=0.14). In conclusion, the expression of the mouse APOE was significantly reduced by ~71% and 53% by the all-in-one AAV repressor platform carrying gRNA1 and gRNA2, respectively. These results suggested that the epigenome therapy platform was efficient for lowering Apoe expression and thus bears the translational potential towards the development of a therapeutics approach to treat, prevent and/or delay the progression of LOAD.

[0691] In addition, pilot safety evaluations including the mice daily weights and monitoring welfare criterions were conducted. In the GFP reporter experiment, while both age and sex had significant influence on mouse weights (middle aged; Male = 26.09 ± 0.06, Female = 22.37 ± 0.1, $F(1,10) = 28.97$, $p = 0.0003$. Very old; Male = 32.17 ± 0.09, Female = 25.43 ± 0.05, $F(1,10) = 37.48$, $p = 0.0001$; two-way ANOVAs), the mice weights were maintained for the 42 days following viral vector injection (**FIG. 5E**). Similarly, in the mouse endogenous APOE experiments, no changes were observed in the weights of the mice along the 42 days following the vectors injection (**FIG. 5D**). The mice also displayed normal grooming and eating/drinking behaviors. Overall, these analyses showed no safety issues upon the stereotaxic injections of our all-in-one AAV platform for gene repression mediated by epigenome editing.

[0692] Next, the approach was validated in an APOE humanized mice model in which the entire targeted mouse region is replaced with the human (h)APOE and the adjacent hTOMM40 loci including their upstream and downstream flanking regulatory sequences. AAV-

gRNA(h*APOE*)/dCas-KRAB/MeCP2 was injected into the left DH of 4 *APOE*-humanized mouse males and the right DH was injected with the control vector (**FIG. 5D**) and the impact on the expression of the human APOE gene was measured. As shown in **FIG. 5E** and further quantitated in **FIG. 5F**, the AAV was able to significantly repress the expression of ApoE in the left DH relative to the right DH amounting to ~75% reduction. In addition, no changes in weights or welfare criterions were observed, and the mice displayed normal grooming and eating/drinking behaviors indicating no safety issues.

Example 8
Controlled APOE4-targeted repression in 4 months old organoids models

[0693] Human induced pluripotent stem cells harboring APOE3/3 (E3) and APOE4/4 (E4) were differentiated to cerebral organoids. Next, 4 months old organoids were transduced with AAV virus harboring gRNA to target promoter region of APOE or no-gRNA (Control) virus. RNA was then isolated, and qRT-PCR was performed to evaluate the expression of APOE. Results show a significant downregulation of APOE expression in both E3 and E4 organoids (**FIG. 6A**). The results were normalized to GAPDH, PPIA1 and the controls.

[0694] Similarly, levels of p-Tau in the transduced organoids were tested using western blot analysis and showed that the mediated reduction in ApoE4 resulted in a significant reduction in pathological p-Tau (**FIG. 6B**).

Example 9
Controlled APOE4-targeted repression in microglial cells

[0695] Next modulation of endogenous APOE in microglial cells was studied. hiPSC-derived microglia were stained with microglia specific marker *IBA1*. *APOE*e4 microglia were transduced with gRNA/dCas-repressor or control (no-gRNA) viruses using a vector driven by a synthetic human CD68 regulatory sequence harboring gRNA to target the *APOE* locus. The results (**FIG. 7A-7B**) showed a significant decrease in *APOE*-mRNA expression levels. Repressing *APOE*e4 expression in the microglia led to an increase in Aβ uptake, comparable to that of *APOE*e3 microglia, (**FIG. 7C**) and an increase in TMEM119 level and decrease CD74, demonstrating rescue of neuroinflammation (**FIG. 7D**).

Summary of Examples

[0696] The Examples provided herein show that epigenome-based therapy targeting allele specific APOE, paired with AAVs is an advantageous strategy for the treatment of LOAD because it has versatility, low immunogenicity, and remarkable suitability for viral-mediated gene transfers. The Examples further provide evidence that epigenome therapy can be achieved in a cell-type specific manner.

[0697] The *APOE*-targeted epigenome therapy described herein combines emerging innovative genomic technologies and delivery techniques to overcome these limitations. Therapy provides several novel features and advantages: **a)** epigenetic editing at specifically targeted loci represents a novel appealing approach for gene therapy mediated by altering the expression of a specific gene. Epigenome editing represents new 'smart drugs' designed to target specific genes based on CRISPR/deactivated (d)Cas9 technology. While genome editing approaches change the genetic code via DNA cleavage, with the risk of introducing new mutations, epigenome editing uses deactivated nuclease. Thus, the DNA remains intact and is not exposed to potential mutations. Finally, this technology offers an unprecedented opportunity to modify epigenetic markers to control the expression of disease-associated genes and variants; **b)** The allelic discrimination approach is innovative as it allows a precise and fine-tuned downregulation of APOEe4 allele expression. APOEe4 is the most validated genetic risk factor for LOAD. While it was shown that the downregulation of APOEe4 expression leads to a beneficial effect, the combination of the epigenomic modification approach and the strategy to target allele-specific is novel; **c)** cell-type specific targeting ensures minimal therapeutic side effects; **d)** The all-in-one vector technology circumvents several challenges related to gene therapy: (i) optimized AAV backbone for high packaging and delivery efficiencies of the CRISPR/dCas9 components; (ii) low cytotoxicity, immunogenicity; (iii) inheritable, sustainable expression of the vector to ensure the durability of the epigenetic changes.

[0698] Pre-existing approaches including antisense oligonucleotides (ASO) and immunotherapy are plagued by significant disadvantages such as low efficiency and specificity, low stability and solubility, adverse immunoreactivity, and inability to penetrate blood-brain barrier (BBB). Epigenome editing also holds key advantages over direct gene knockout because epigenome editing triggers the natural cellular system that leads to gene silencing by a defined mechanism.

G. LIST OF SEQUENCES

SEQ ID NO:	SEQ TYPE
1	CD68 promoter
2	CD68 (microglia specific promoter)
3	CD68v2 promoter
4	F4/80 promoter
5	IBA Complete version
6	IBA1 Compact version
7	IBA1 Core promoter

8	THE PROMOTER REGION OF FCGR2A: (1) (1904BP) COORDINATES CHR1: 161505031-161506934 (HG38)
9	hSyn promoter (constitutive)
10	GfaABC1D (astrocyte specific promoter)
11	APOE-complete sequence
12	APOE e2
13	APOE e3
14	APOE e4
15	gRNA (APOE)
16	gRNA (APOE)
17	gRNA (APOE)
18	gRNA (APOE)
19	gRNA (APOE)
20	gRNA (APOE)
21	gRNA (APOE)
22	gRNA (APOE)
23	gRNA (APOE)
24	gRNA (APOE)
25	gRNA (APOE)
26	gRNA (APOE)
27	gRNA (APOE)
28	gRNA (APOE)
29	gRNA (APOE)
30	gRNA (APOE)
31	gRNA (APOE)
32	gRNA (APOE)
33	gRNA (APOE)
34	gRNA GFP
35	gRNA GFP
36	*Streptococcus pyogenes* dCas amino acid sequence
37	*Streptococcus pyogenes* dCas VRER
38	*Staphylococcus aureus (Sa)* dCas amino acid sequence
39	*Campylobacter jejuni (Cj)aureus* dCas amino acid sequence
40	*Streptococcus pyogenes* dCas nucleotide sequence
41	*Streptococcus pyogenes* dCas VRER nucleotide sequence
42	*Staphylococcus aureus (Sa)* dCas amino acid sequence
43	*Campylobacter jejuni (Cj)aureus* dCas amino acid sequence
44	HP1 alpha
45	HP1 alpha2

46	HP1 beta
47	HP1 beta 2
48	MBD1
49	MBD1 isoform 1
50	MBD1-Isoform 6
51	MBD2
52	MBD2-2
53	NIPP1
54	NIPP1-2
55	DNMT3A amino acid sequence
56	KRAB (aa)
57	MeCP2
58	KRAB-MeCP2 (aa)
59	HP1 alpha nuc
60	HP1a-hinge+CSD
61	HP1beta
62	HP1beta-hinge-csd
63	MBD1
64	MBD1-2
65	MBD2
66	MBD2-2
67	NIPP1-1
68	NIPP1-2
69	DNMT3A nucleotide sequence
70	KRAB (nucleotide)
71	KRAB (nucleotide 2)
72	MeCP2 (nucleotide)
73	KRAB-MeCP2 (nucleotide)
74	KRAB-MeCP2 -2(nucleotide)
75	dCas9-DNMT3A
76	SpCas9-dVRER-DNMT3A (aa)
77	dCas9-DNMT3A-nucleotide
78	SpCas9-dVRER-DNMT3A
79	EF-1a core-1
80	EF-1a-core2
81	U6 promoter
82	5’ ITR AAV2
83	5’ ITR AAV2
84	3’ ITR AAV2

85	3' ITR AAV2
86	PolyA
87	PolyA
88	NLS
89	NLS
90	NLS
91	NLS
92	NLS
93	NLS
94	NLS
95	pBK546 carrying dCas9-DNMT3A fused transgene linked to puromycin reporter via p2A cleavage signal
96	pBK539 carrying dCas9-DNMT3A fused transgene linked to GFP reporter via p2A cleavage signal
97	pBK1026 SEQ ID NO:47 dCas9- DNMT3A vector targeting promoter region of ApoE gene. gRNA1 cloned into this vector highlighted above. Active DNMT3A present in this construct. SpCas9 is present in this construct. Puromycin reporter is cloned in this version separated via p2a signal from the DNMT3A enzyme
98	pBK1027 SEQ ID NO:48 dCas9- DNMT3A vector targeting promoter region of ApoE gene. gRNA2 cloned into this vector highlighted above. Active DNMT3A present in this construct. SpCas9 is present in this construct. Puromycin reporter is cloned in this version separated via p2a signal from the DNMT3A enzyme
99	pBK1028 SEQ ID NO:49 dCas9- DNMT3A vector targeting promoter region of ApoE gene. gRNA3 cloned into this vector highlighted above. Active DNMT3A present in this construct. SpCas9 is present in this construct. Puromycin reporter is cloned in this version separated via p2a signal from the DNMT3A enzyme
100	pBK1029 SEQ ID NO:50 dCas9- DNMT3A vector targeting promoter region of ApoE gene. gRNA4 cloned into this vector highlighted above. Active DNMT3A present in this construct. SpCas9 is present in this construct. Puromycin reporter is cloned in this version separated via p2a signal from the DNMT3A enzyme
101	pBK1030 SEQ ID NO:51 dCas9- DNMT3A vector targeting promoter region of ApoE gene. gRNA1 cloned into this vector highlighted above. Active DNMT3A present in this construct. SpCas9 is present in this construct. Puromycin reporter is cloned in this version separated via p2a signal from the DNMT3A enzyme
102	pBK1031 dCas9- DNMT3A vector targeting promoter region of ApoE gene. gRNA2 cloned into this vector highlighted above. Active DNMT3A present in this construct. SpCas9 is present in this construct. Puromycin reporter is cloned in this version separated via p2a signal from the DNMT3A enzyme
103	pBK1032 dCas9- DNMT3A vector targeting promoter region of ApoE gene. gRNA3 cloned into this vector highlighted above. Active DNMT3A present in this construct. SpCas9 is present in this construct. Puromycin reporter is cloned in this version separated via p2a signal from the DNMT3A enzyme
104	pBK1033 dCas9- DNMT3A vector targeting promoter region of ApoE gene. gRNA4 cloned into this vector highlighted above. Active DNMT3A present in this construct. SpCas9 is present in this construct. Puromycin reporter is cloned in this version separated via p2a signal from the DNMT3A enzyme
105	Lentiviral vector carried dCas9-VRER-MeCP2 Transcription Repression Domain (TRD) with gRNA1
106	Lentiviral vector carried dCas9-VRER-MeCP2 Transcription Repression Domain (TRD) with gRNA2

107	pBK1531 carrying a Lentiviral vector with dCas9-VRER-MeCP2 Transcription Repression Domain (TRD) and gRNA1
108	pBK1532 carrying a Lentiviral vector with dCas9-VRER-MeCP2 Transcription Repression Domain (TRD) and gRNA2
109	pBK1536 Lentiviral vector carried dCas9-VRER-KRAB-MeCP2 Transcription Repression Domain (TRD)
110	pBK1340 Lentiviral vector carried reporter system comprised of CMVp-dGFP-Nano-Luc-WPRE-CV40-Puro
111	pCL35 Lentiviral vector carried reporter system comprised of CAGp-SNCA-INTRON1- SNCA-ORF-dGFP-Nano-Luc- WPRE-CV40-Puro
112	pBK1314 AAV vector carried dCas9-VRER-KRAB-MeCP2 Transcription Repression Domain (TRD) without gRNA full plasmid sequence
113	AAV vector carried dCas9-VRER-KRAB-MeCP2 Transcription Repression Domain (TRD) with gRNA1 targeting CMV promoter full plasmid sequence
114	AAV vector carried dCas9-VRER-KRAB-MeCP2 Transcription Repression Domain (TRD) with gRNA2 targeting CMV promoter full plasmid sequence
115	AAV vector carried dCas9-VRER-KRAB-MeCP2 Transcription Repression Domain (TRD) without gRNA expression driven from CD68 glial-specific promoter full plasmid sequence
116	AAV vector carried dCas9-VRER-KRAB-MeCP2 Transcription Repression Domain (TRD) without gRNA expression driven from F4/80 glial-specific promoter full plasmid sequence
117	AAV vector carried dCas9-VRER-KRAB-MeCP2 Transcription Repression Domain (TRD) with gRNA targeting CMV promoter; expression driven from CD68 glial-specific promoter full plasmid sequence
119	AAV vector carried dCas9-VRER-KRAB-MeCP2 Transcription Repression Domain (TRD) without gRNA expression driven from F4/80 glial-specific promoter full plasmid sequence
120	AAV vector carried dCas9-VRER-KRAB-MeCP2 Transcription Repression Domain (TRD) with gRNA1 targeting promoter region of ApoE gene; expression driven from CD68 glial-specific promoter full plasmid sequence
121	AAV vector carried dCas9-VRER-KRAB-MeCP2 Transcription Repression Domain (TRD) with gRNA1 targeting promoter region of ApoE gene; expression driven from F4/80 glial-specific promoter full plasmid sequence
122	AAV vector carried dCas9-VRER-KRAB-MeCP2 Transcription Repression Domain (TRD) with Grna2 targeting promoter region of ApoE gene; expression driven from CD68 glial-specific promoter full plasmid sequence
123	AAV vector carried dCas9-VRER-KRAB-MeCP2 Transcription Repression Domain (TRD) with gRNA2 targeting promoter region of ApoE gene; expression driven from F4/80 glial-specific promoter full plasmid sequence
124	AAV-CD68v2-dSaCas9-KRAB-MeCp2
125	AAV-CD68V2-DSACAS9-LINKER/MINIMCS [NO GRNA]
126	1. AAV-IBA1FULL-CHINT-EGFP-WPRE-HGHPA PBK1985
127	2. AAV-IBA1COMPACT-CHINT-EGFP-WPRE-HGHPA PBK1986
128	3. AAV-IBA1CORE-CHINT-EGFP-WPRE-HGHPA PBK1987
129	Cj gRNA Scaffold
130	Sa gRNA Scaffold
131	VP16
132	VP16

133	VP16
134	NF-Kβ subunit / RELA Proto-Oncogene
135	NF-Kβ subunit / RELA Proto-Oncogene-full gene
136	NF-Kβ subunit / RELA Proto-Oncogene (mRNA)
137	Eukaryotic Release Factor 1
138	Eukaryotic Release Factor 1

VIII. CLAIMS

What is claimed is:

1. An isolated nucleic acid molecule, comprising:

(a) a nucleic acid sequence encoding:

(i) a deactivated Clustered Regularly Interspaced Short Palindromic Repeats associated (dCas) endonuclease,

(ii) at least one polypeptide having an enzymatic activity, and

(iii) at least one guide RNA targeting an APOE gene sequence or a regulatory element thereof; and

(b) one or more microglia specific regulatory elements operably linked to (a)(i), (a)(ii), (a)(iii), or any combination thereof.

2. The isolated nucleic acid molecule of claim 1, wherein the one or more microglia specific regulatory element comprises a promoter, wherein the promoter comprises a wild-type promoter, a synthetic promoter, or a minimal promoter.

3. The isolated nucleic acid molecule of either claim 1 or claim 2, wherein the one or more microglia specific regulatory elements are operably linked to the nucleic acid sequence encoding the dCas endonuclease.

4. The isolated nucleic acid molecule of either claim 1 or claim 2, wherein the one or more microglia specific regulatory element are operably linked to the nucleic acid encoding the at least one polypeptide having enzymatic activity.

5. The isolated nucleic acid molecule of either claim 1 or claim 2, wherein the one or more microglia specific regulatory elements are operably linked to the nucleic acid encoding the dCas endonuclease and the nucleic acid encoding the at least one polypeptide having enzymatic activity.

6. The isolated nucleic acid molecule of either claim 1 or claim 2, wherein the one or more microglia specific regulatory elements is operably linked to the at least one guide RNA.

7. The polypeptide sequence of claim 2, wherein the promoter is a F4/80, CD68, FCGR2A or IBA1 promoter, or a derivative thereof, or any combination thereof.

8. The isolated nucleic acid molecule of claim 2, wherein the promoter drives the expression of the at least one gRNA, the dCas endonuclease, the at least one polypeptide or any combination thereof.

9. The polypeptide sequence of claim 7 wherein the promoter is a CD68 promoter, or a derivative thereof.

10. The isolated nucleic acid molecule of claim 9, wherein the promoter comprises a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequence set forth in SEQ ID NO: 1-3, or a derivative thereof.
11. The polypeptide sequence of claim 7 wherein the promoter is a IBA1 promoter, or a derivative thereof.
12. The isolated nucleic acid molecule of claim 11, wherein the promoter comprises a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequence set forth in SEQ ID NO: 5-7, or a derivative thereof.
13. The polypeptide sequence of claim 7 wherein the promoter is a F4/80 promoter, or a derivative thereof.
14. The isolated nucleic acid molecule of claim 13, wherein the promoter comprises a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequence set forth in SEQ ID NO: 4 or a derivative thereof.
15. The isolated nucleic acid molecule of claim 1, wherein the at least one gRNA targets the APOE gene, or promoter region thereof, or an untranslated region (UTR) thereof, or any combination thereof.
16. The isolated nucleic acid molecule of claim 15, wherein the at least one gRNA targets the promoter region, or intron 1, or a flanking sequence thereof, of APOE.
17. The isolated nucleic acid molecule of claim 1, wherein the at least one gRNA targets the nucleic acid sequence set forth in SEQ ID NO: 15-33, or a sequence at least about 90% identical thereto.
18. The isolated nucleic acid molecule of claim 1, wherein the Cas endonuclease comprises a deactivated Cas9 (dCas9) endonuclease.
19. The isolated nucleic acid molecule of claim 18, wherein the dCas endonuclease comprises a deactivated *Staphylococcus aureus* Cas9 (dSaCas9), a deactivated *Streptococcus pyogenes* Cas9 (dSpCas9), a deactivated *Campylobacter jejuni* Cas9 (dCjCas9), or a variant dCas9 endonuclease.

20. The isolated nucleic acid molecule of claim 19, wherein the dCas endonuclease comprises a variant dCas9 endonuclease selected from a VRER, EQR or VQR variant of dCas9.

21. The isolated nucleic acid molecule of either claim 19 or claim 20, wherein the dCas9 endonuclease comprises an amino acid sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequence set forth in SEQ ID NO: 44-47, or a fragment thereof.

22. The isolated nucleic acid molecule of claim 1, wherein the enzymatic activity comprises transcription activation activity, transcription repression activity, transcription release factor activity, histone modification activity, nucleic acid association activity, methyltransferase activity, demethylase activity, acetyltransferase activity, deacetylase activity, or any combination thereof.

23. The isolated nucleic acid molecule of claim 22, wherein the at least one polypeptide having enzymatic activity comprises HP1a, HP1b, MBD1, MBD2, Krüppel-Associated Box (KRAB), NIPP1, Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A, any combination thereof, or any fusion thereof.

24. The isolated nucleic acid molecule of claim 22, wherein the at least one polypeptide having an enzymatic activity comprises Krüppel-associated box (KRAB), the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), DNMT3A or any fusion polypeptide thereof.

25. The isolated nucleic acid molecule of claim 24, wherein the fusion polypeptide comprises KRAB-MeCP2 (KRAB-MeCP2).

26. The isolated nucleic acid molecule of claim 23, wherein the at least one polypeptide having enzymatic activity comprises an amino acid sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequence set forth in SEQ ID NO: 36-39, or a derivative thereof.

27. The isolated nucleic acid molecule of claim 1, where the dCas endonuclease comprises dCas9 VRER and the polypeptide having enzymatic activity comprises Krüppel-associated box (KRAB), the transcription repression domain (TRD) of Methyl-CpG Binding Protein 2 (MeCP2), or a fusion of KRAB-MeCP2 (KRAB-MeCP2).

28. The isolated nucleic acid molecule of either claim 1 or claim 26, wherein the nucleic acid sequence encodes a fusion protein comprising the dCas endonuclease and the at least one polypeptide having an enzymatic activity.

29. The isolated nucleic acid molecule of either claim 1 or claim 26, wherein the isolated nucleic acid molecule comprises one of more of a P2A, T2A, E2A, F2A and IRES.
30. The isolated nucleic acid molecule of claim 23, wherein the fusion protein comprises an amino acid sequence set forth in SEQ ID NO: 36-39, or a sequence at least 80% identical thereto.
31. The isolated nucleic acid molecule of claim 1, further comprising one or more additional regulatory elements.
32. The isolated nucleic acid molecule of claim 31, wherein the one or more additional regulatory elements are selected from one or more primer binding sites (PBS), one or more splice donor (SD) sites, one or more splice acceptor (SA) sites, one or more central polypurine tracts (cPPT), one or more polypurine tracts (PPT), one or more Rev Response elements (RRE), one or more Woodchuck Hepatitis Virus Posttranscriptional Regulatory Elements (WPRE), Sp1 responsive element, a p2A signal, a woodchuck hepatitis virus post-transcriptional regulatory element, a Phi signal-packaging signal, a rev responsive element, a 5'-LTR, and a 3'-LTR one or more retroviral vector packaging elements, or any combination thereof.
33. A viral vector comprising the isolated nucleic acid molecule of any one of claims 1-32.
34. The viral vector of claim 33, wherein the vector is a recombinant adenovirus-associated viral vector (rAAV).
35. The viral vector of claim 34, comprising a nucleic acid sequence having at least 60% at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the sequence set forth in SEQ ID NO: 112-128.
36. A pharmaceutical formulation comprising the isolated nucleic acid molecule of any one of claims 1 - 30 or the rAAV vector of any one of claims 33 - 35, and a pharmaceutically acceptable carrier.
37. The pharmaceutical formulation of claim 36, for the treatment of neurological disease associated with APOE overexpression.
38. The pharmaceutical formulation of claim 36, wherein the neurodegenerative disease is Alzheimer's disease (AD) or Parkinson's disease (PD).
39. A method of effecting an epigenetic modulation in a cell, comprising contacting the cell with an isolated nucleic acid molecule of any one of claims 1-30 or a viral vector of any one of claims 33-35.
40. The method of claim 39, wherein the cell is a microglial cell.

41. The method of claim 39, wherein the epigenetic modulation comprises modification of methylation of at least one nucleotide in the promoter region of APOE.
42. A method of effecting a precision gene therapy, comprising administering to a subject in need thereof a therapeutically effective amount of the pharmaceutical composition of claim 36.
43. The method of claim 42, wherein administering of the pharmaceutical composition enhances DNA-methylation of the APOE promoter region in at least one microglial cells.
44. A method of treating and/or preventing a neurological disease in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the pharmaceutical composition of claim 36.
45. The method of any claim 44, wherein the subject is a human.
46. The method of claim 44, wherein the neurological disease is Alzheimer's disease, Parkinson's disease, or a neuroinflammatory disease.
47. The method of claim 46, wherein the subject is suspected of having or has been diagnosed with having Alzheimer's disease.
48. The method of claim 47, further comprising reducing the pathological phenotype associated with Alzheimer's disease.
49. The method of claim 44, wherein administering the pharmaceutical composition comprises intravenous administration, intracerebral administration, intra-CSF administration, intracerebroventricular (ICV) administration, intraventricular administration, intra-cisterna magna (ICM) administration, intraparenchymal administration, intrathecal (lumbar, cisternal, or both) administration, or any combination thereof.
50. The method of claim 44, further comprising administering to the subject a therapeutically effective amount of a therapeutic agent.
51. The method of claim 44, further comprising administering to the subject a therapeutically effective amount of an immune modulator.
52. A method of treating and/or preventing Alzheimer's disease progression in a subject, the method comprising administering to the subject a therapeutically effective amount of the pharmaceutical composition of claim 36, wherein the administering of the pharmaceutical composition reduces the expression of APOE at least one microglial cell of the subject, thereby reducing the pathological phenotype associated with Alzheimer's disease.
53. The method of claim 52, wherein the subject is a human subject.
54. The method of claim 52, wherein administering the vector comprises intravenous administration, intracerebral administration, intra-CSF administration,

intracerebroventricular (ICV) administration, intraventricular administration, intra-cisterna magna (ICM) administration, intraparenchymal administration, intrathecal (lumbar, cisternal, or both) administration, or any combination thereof.

55. The method of claim 52, further comprising administering to the subject at least one additional therapeutic effective agent.

56. The method of claim 53, wherein therapeutically effective agent is an immune modulator.

57. A method of controlling expression of APOE in a target cell comprising, contacting the target cell with the isolated nucleic acid molecule of any one of claims 1-30 or a viral vector of any one of claims 33-35 or the pharmaceutical composition of claim 36, wherein the APOE expression in the target cell is reduced after contacting the cell.

58. The method of claim 57, wherein the target cell comprises a microglial cell.

59. The method of either claim 57 or claim 58, wherein the target cell is located *in vivo*, *ex vivo*, or *in vitro*.

60. The method of claim 57 or claim 58, wherein the target cell is located in a brain tissue.

61. The method of any one of claims 42-60, wherein the target cell is located in a human brain.

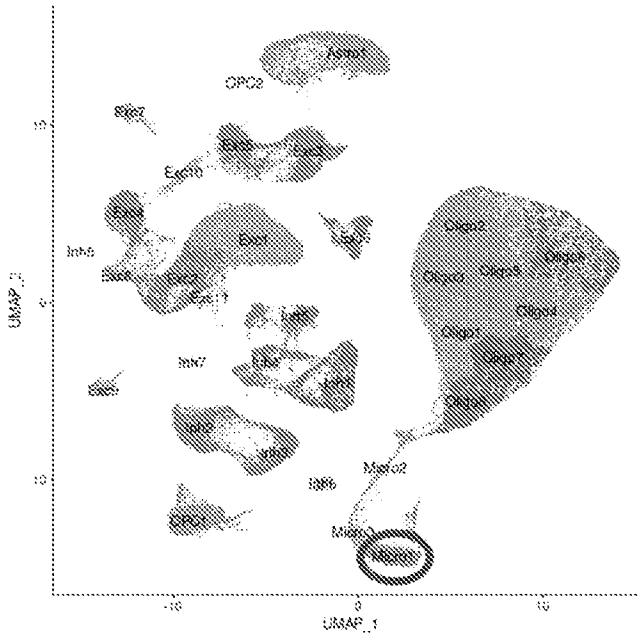

Astro1
OPC2
Epen1
Oligo2
Oligo3
Oligo4
Oligo1
Mac/Mo2
Mac/Mo1
Mic
UMAP_1
UMAP_2

FIG. 1A

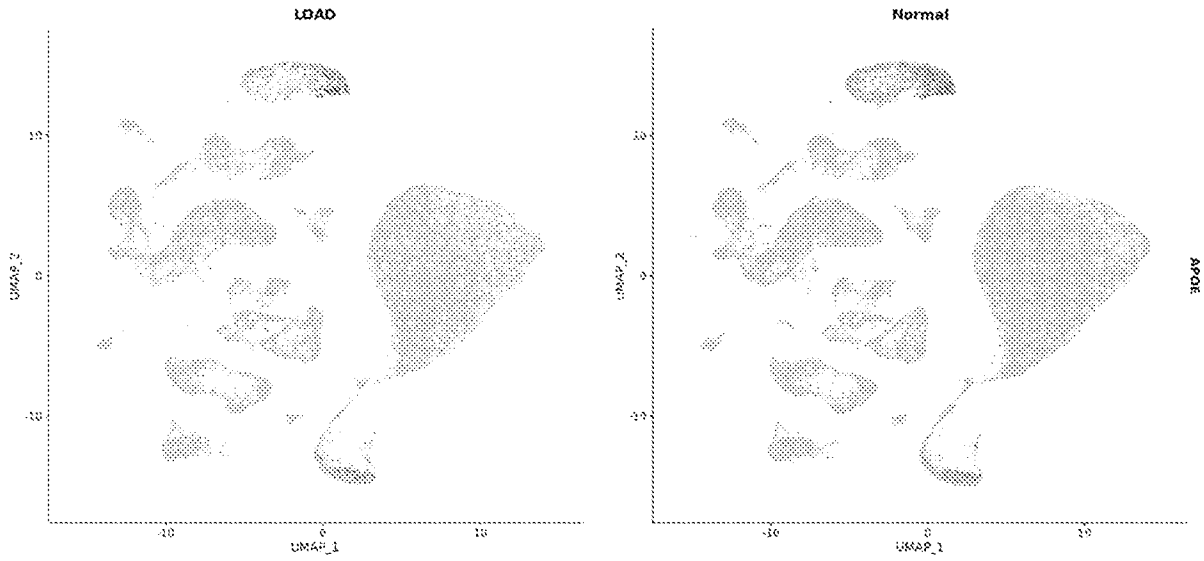

LOAD
Normal
APOE
UMAP_1
UMAP_2

FIG. 1B

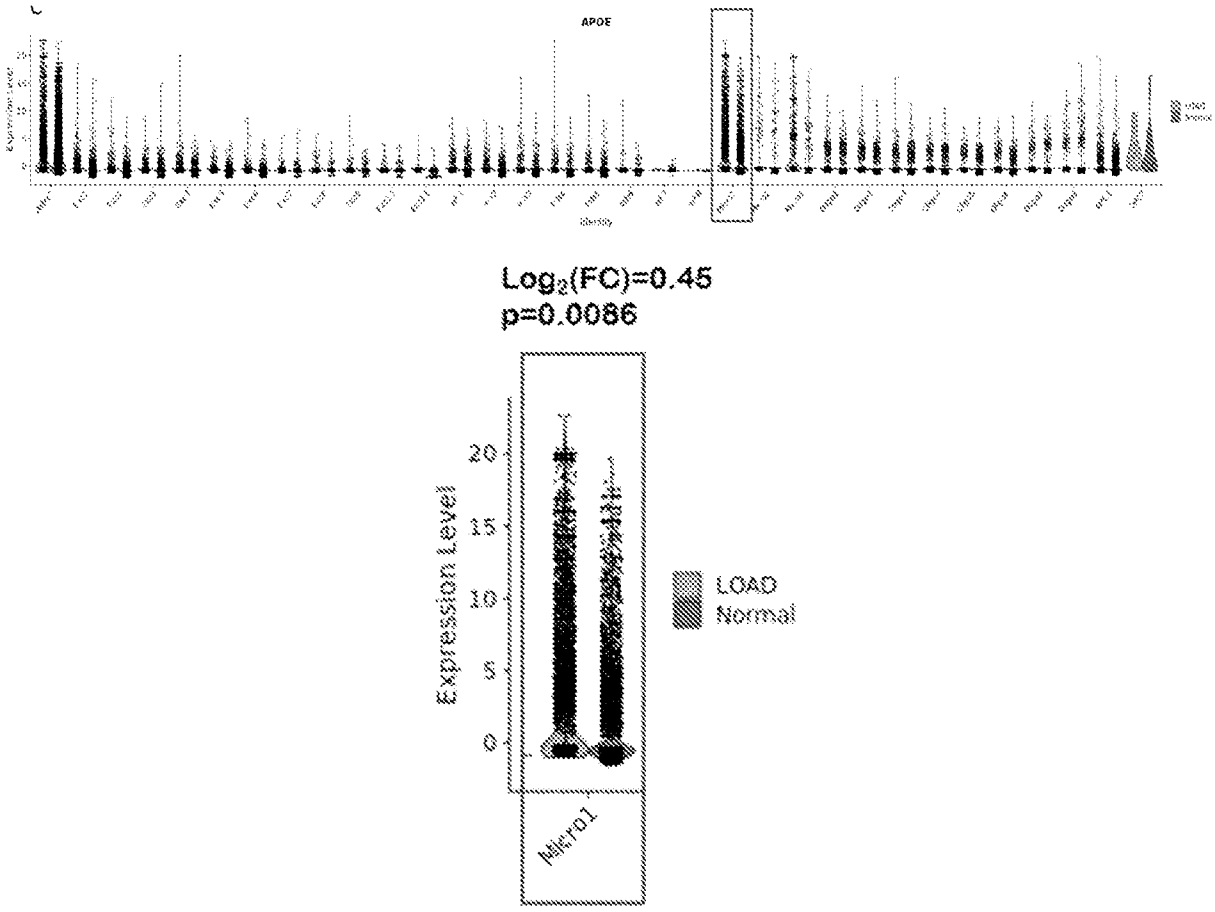

APOE
Log2(FC)=0.45
p=0.0086
Expression Level
20
15
10
5
0
LOAD
Normal
Mertk

FIG. 1C

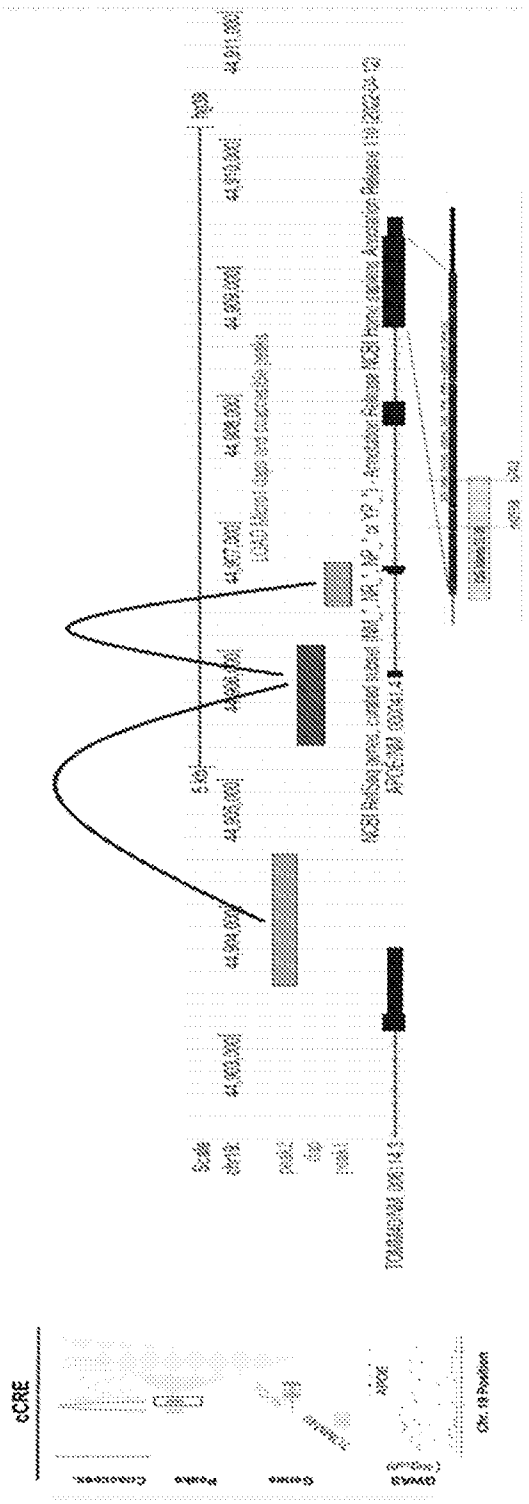


FIG. 1D

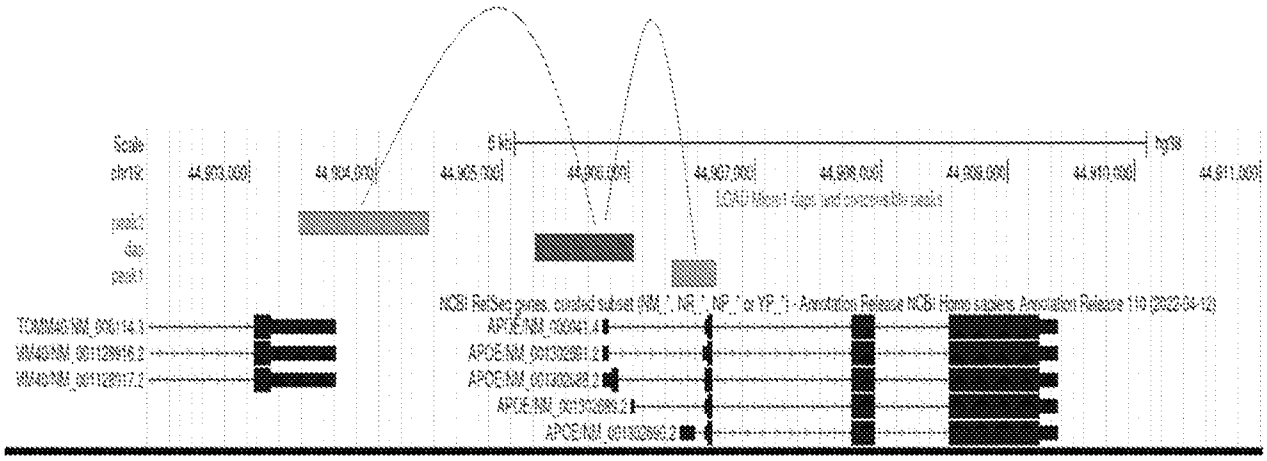


FIG. 1E

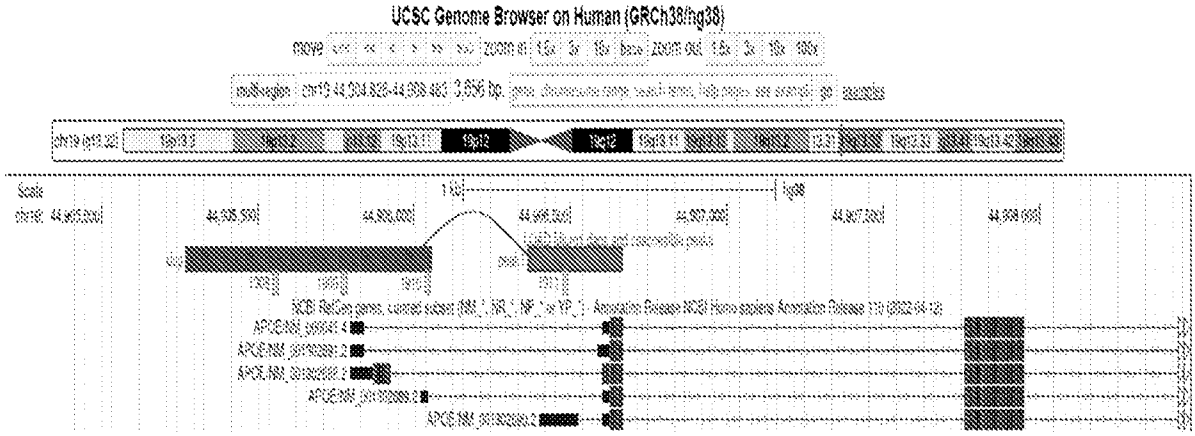


FIG. 1F

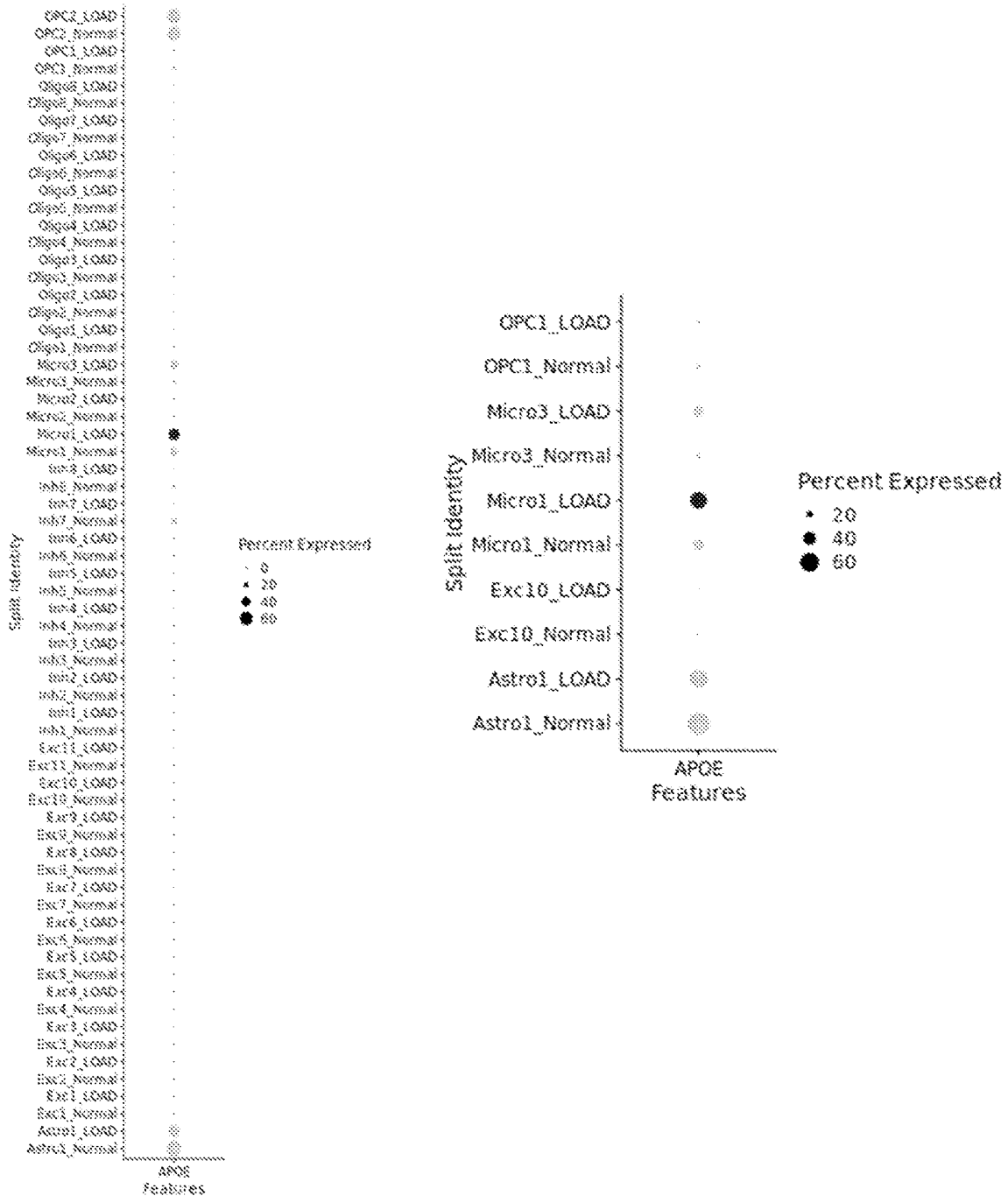

Split Identity
OPC1_LOAD
OPC1_Normal
Micro3_LOAD
Micro3_Normal
Micro1_LOAD
Micro1_Normal
Exc10_LOAD
Exc10_Normal
Astro1_LOAD
Astro1_Normal
APOE
Features
Percent Expressed
20
40
60

FIG. 1G

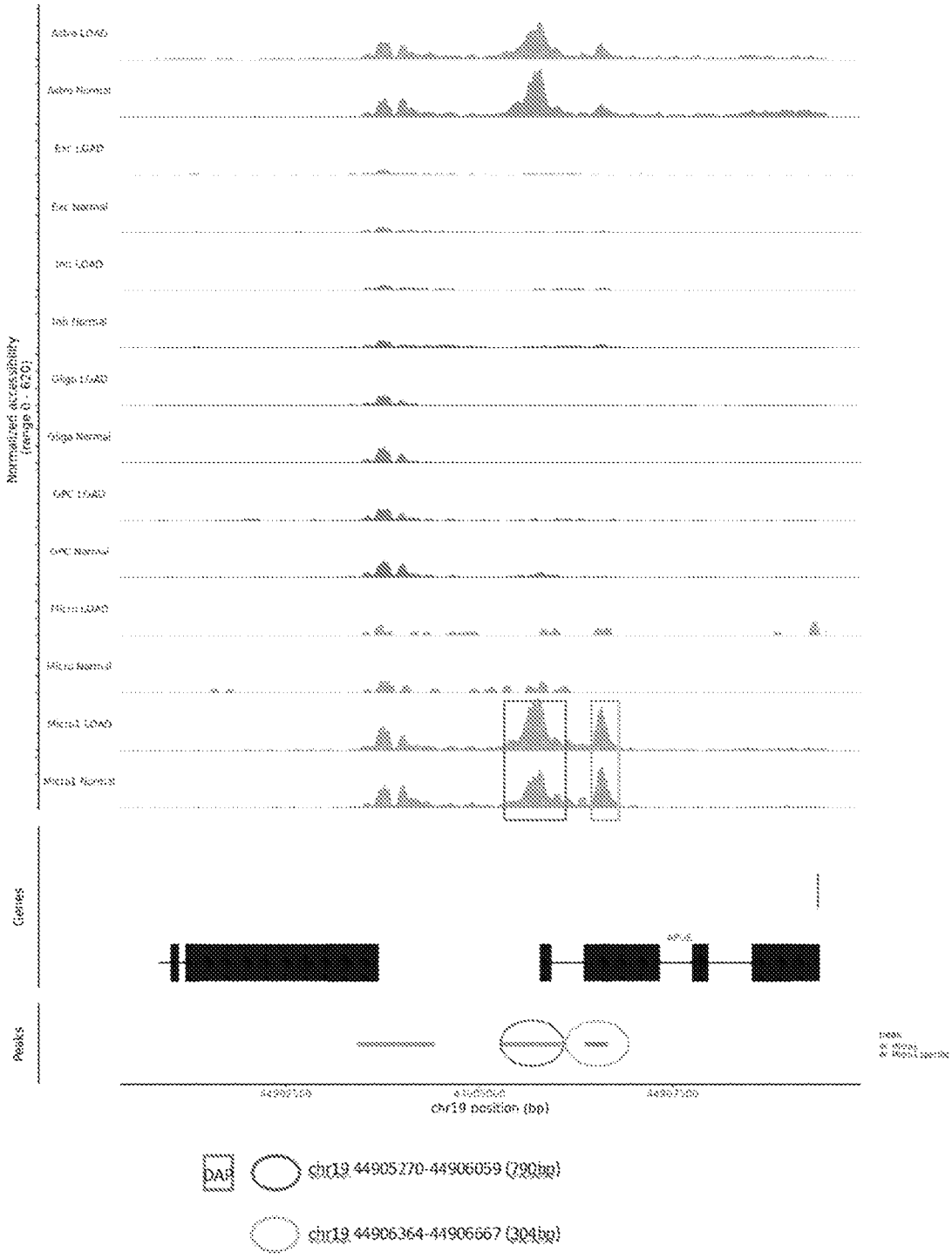

Astro LOAD
Astro Normal
Exc LOAD
Exc Normal
Inh LOAD
Inh Normal
Oligo LOAD
Oligo Normal
OPC LOAD
OPC Normal
Micro LOAD
Micro Normal
Normalized accessibility
(range 0 - 6.20)
Genes
Peaks
chr19 position (bp)
DAPI
chr19 44905270-44906059 (790bp)
chr19 44906364-44906667 (304bp)

FIG. 1H

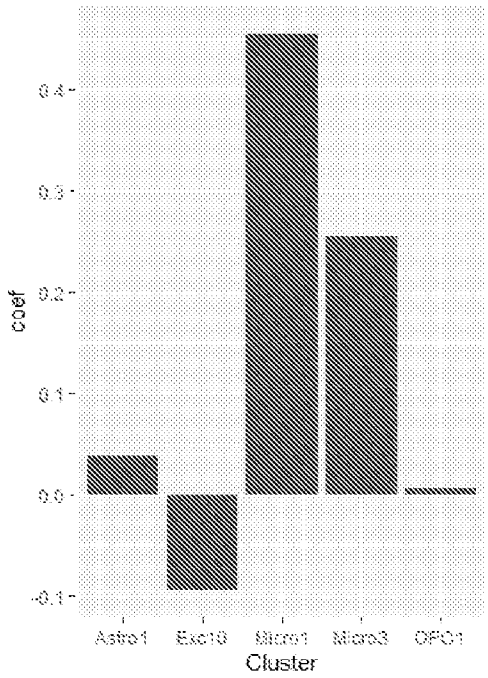

0.4
0.3
0.2
0.1
0.0
-0.1
coef
Astro1
Exc10
Micro1
Micro3
OPC1
Cluster

FIG. 1I

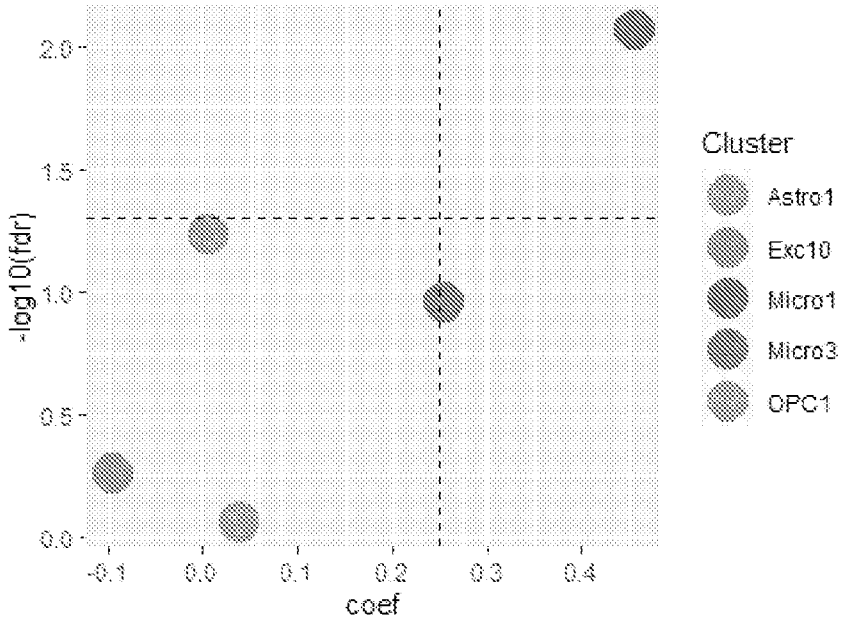
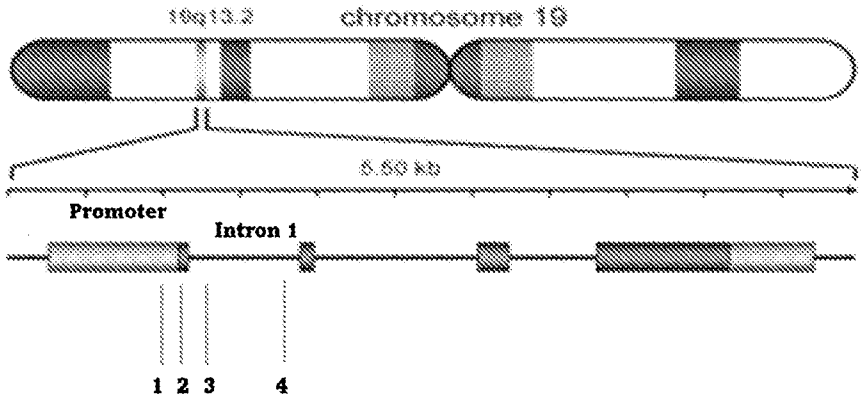
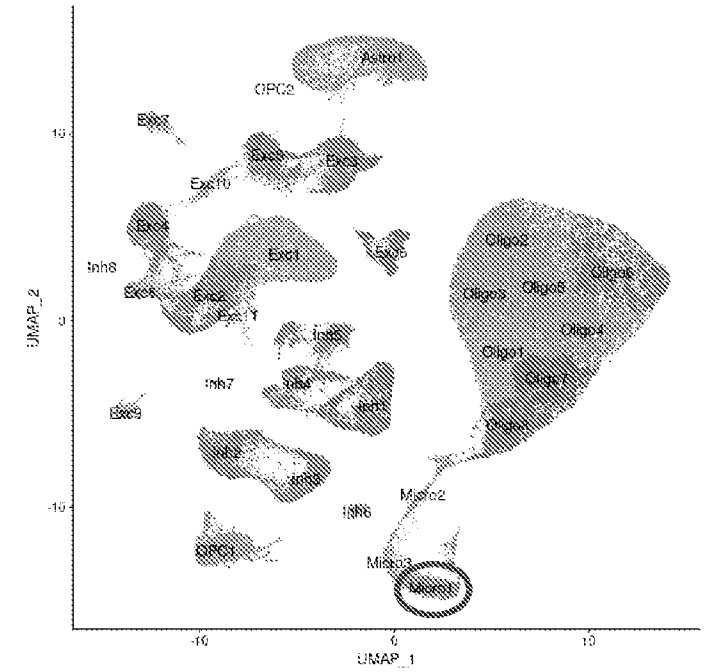

2.0
1.5
1.0
0.5
0.0
-log10(fdr)
-0.1
0.0
0.1
0.2
0.3
0.4
coef
Cluster
Astro1
Exc10
Micro1
Micro3
OPC1

FIG. 1J



1908 gRNA1 -235: -215 GCTTGGCCCCCAGAATGGAG + STRAND
1909 gRNA2 -13: +7 ATTGGACAAGTCTGGGATCC +STRAND
1910 gRNA3 Intron 1 CCACCCCACCTTCTAGCGGG -STRAND
1911 gRNA4 Intron 1 GGGCGGCTTGGTAAATGTGC +STRAND

FIG. 1K



FCGR2A

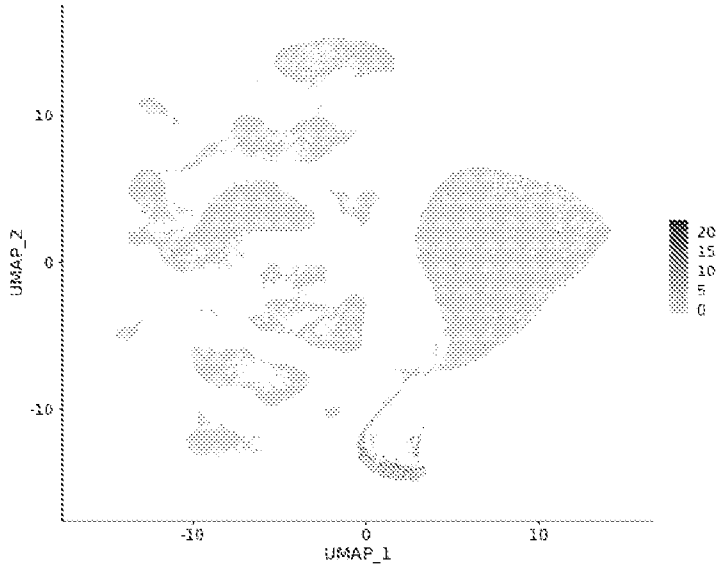


FIG. 1L

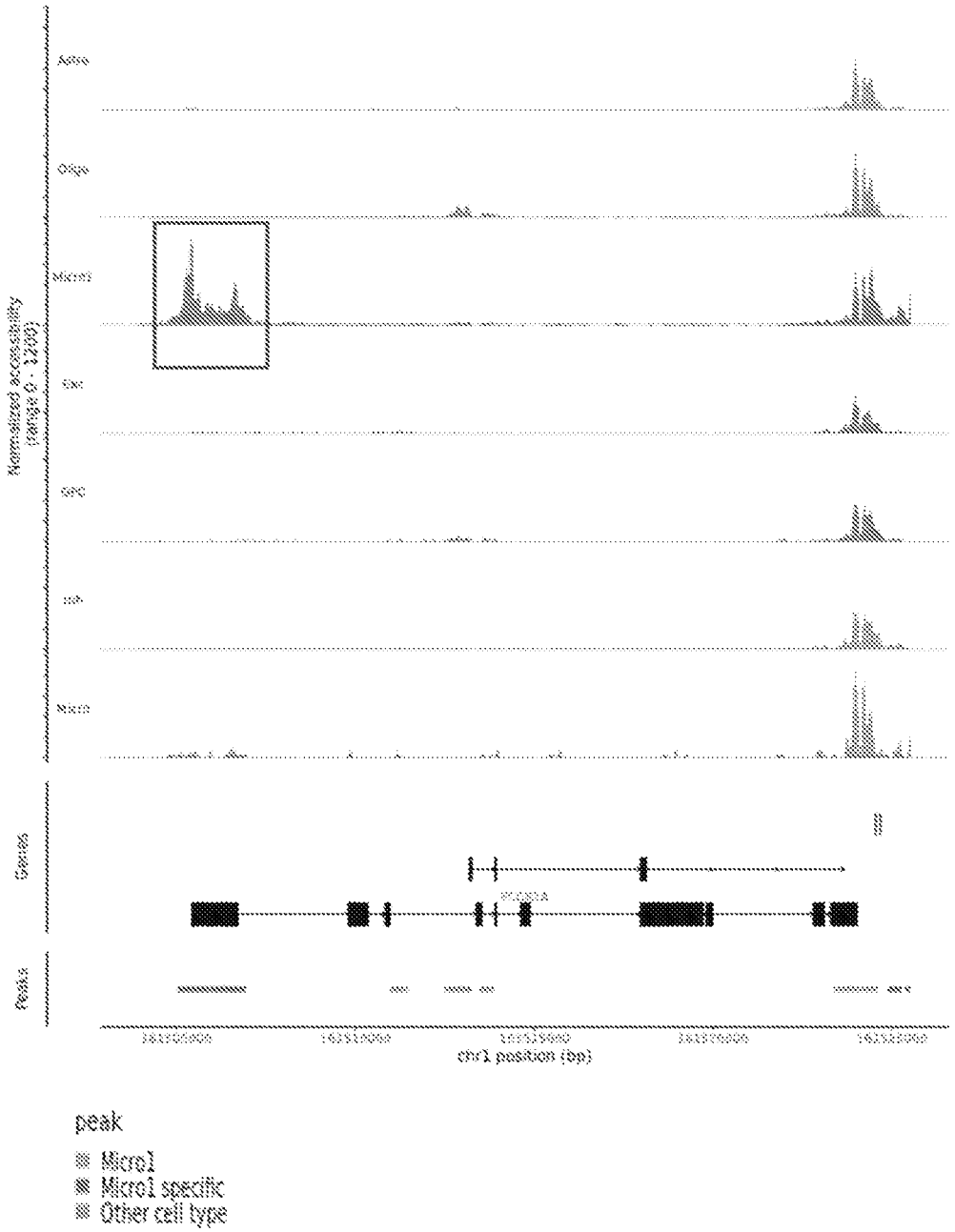

Astro
Oligo
Micro1
Exc
Inh
Micro
Normalized accessibility
(range: 0 - 12000)
Genes
Peaks
chr1 position (bp)
peak
Micro1
Micro1 specific
Other cell type

FIG. 1M

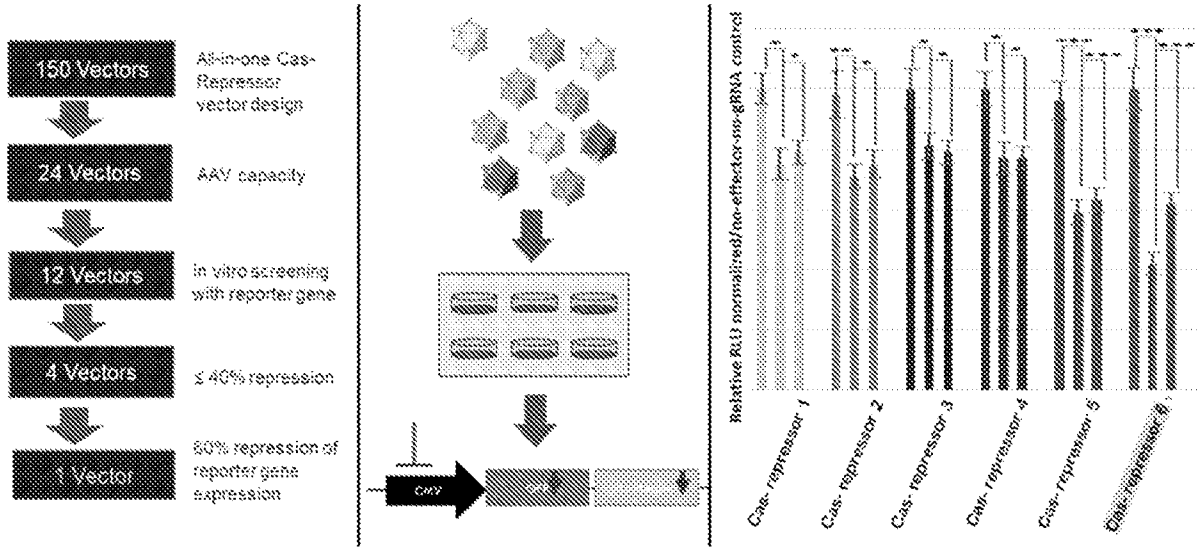

150 Vectors
All-in-one Cas-Repressor vector design
24 Vectors
AAV capacity
12 Vectors
In vitro screening with reporter gene
4 Vectors
≤ 40% repression
1 Vector
60% repression of reporter gene expression
AAV
Relative RLU normalized to no-effector-no-gRNA control
Cas- repressor 1
Cas- repressor 2
Cas- repressor 3
Cas- repressor 4
Cas- repressor 5
Cas- repressor 6

FIG. 2

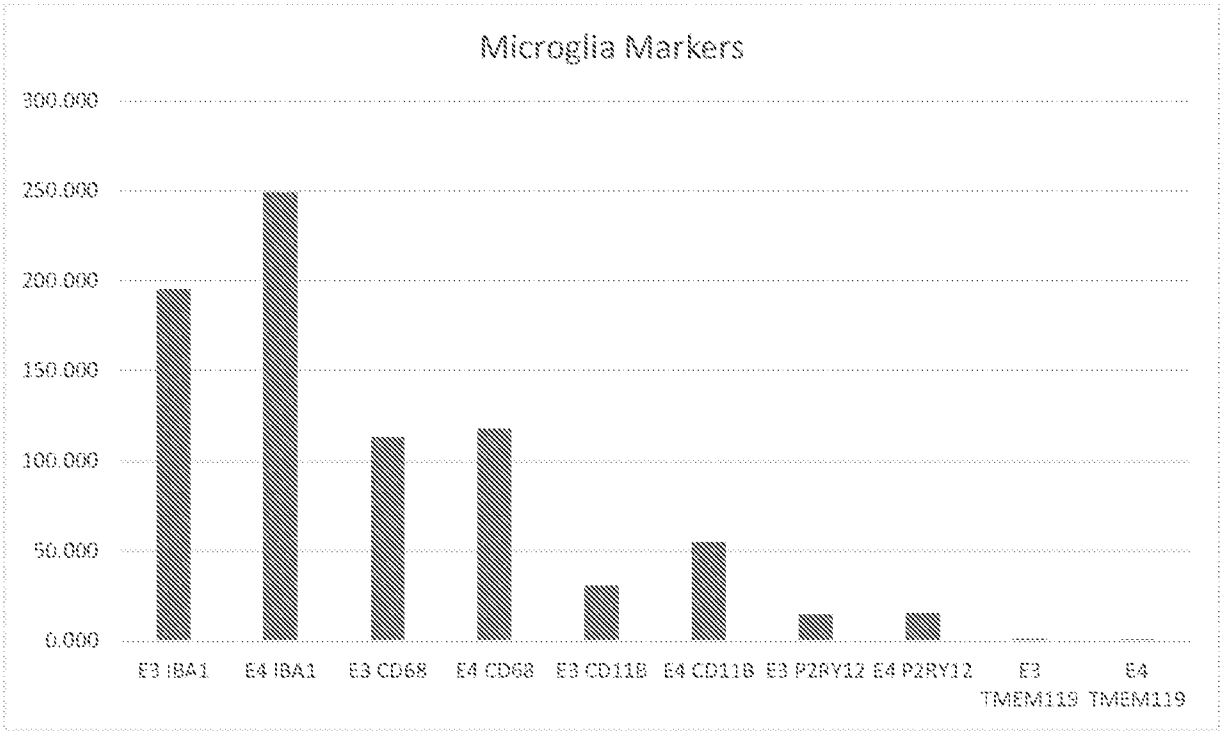

Microglia Markers
300.000
250.000
200.000
150.000
100.000
50.000
0.000
E3 IBA1
E4 IBA1
E3 CD68
E4 CD68
E3 CD11B
E4 CD11B
E3 P2RY12
E4 P2RY12
E3 TMEM119
E4 TMEM119

FIG. 3A

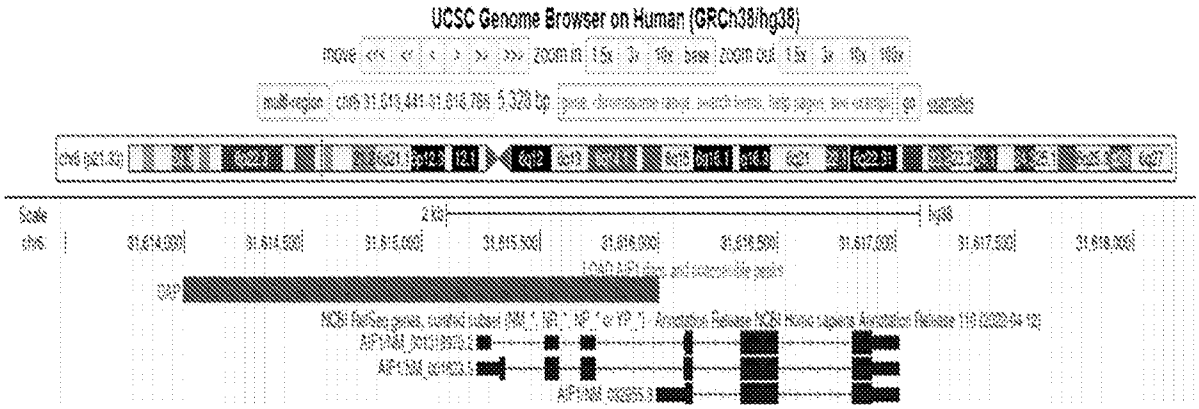
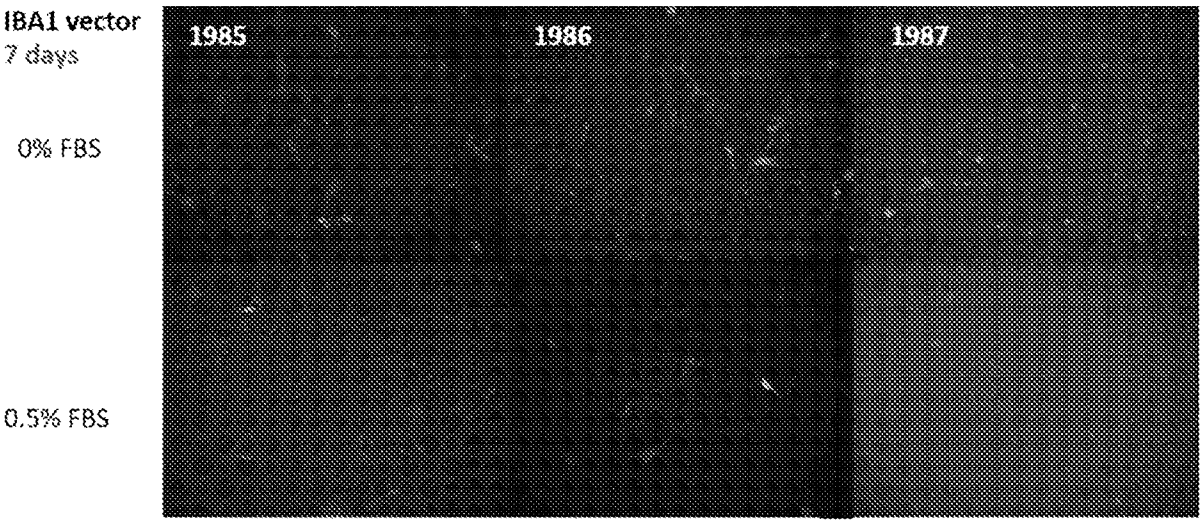


FIG. 3B



Human IBA1 synthetic promoters:
1985 full
1986 medium
1987 short

FIG. 3C

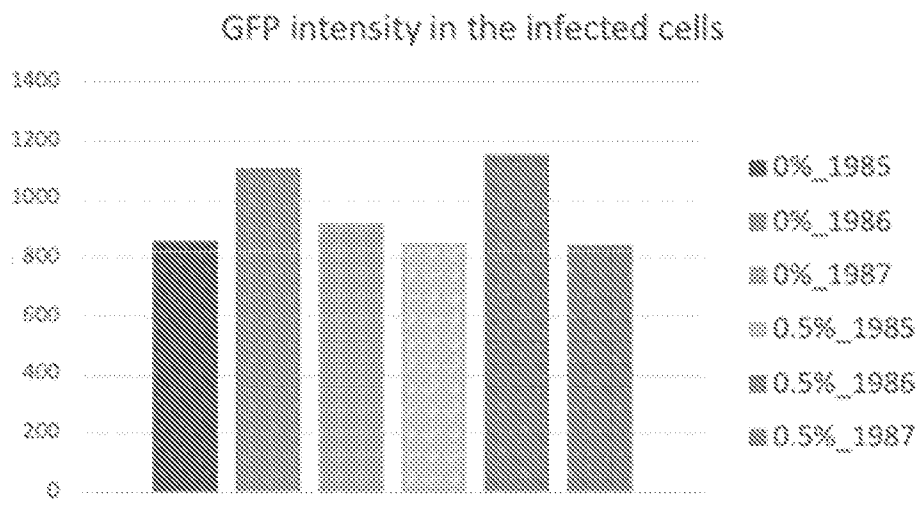

GFP intensity in the infected cells
1400
1200
1000
800
600
400
200
0
0%_1985
0%_1986
0%_1987
0.5%_1985
0.5%_1986
0.5%_1987

FIG. 3D

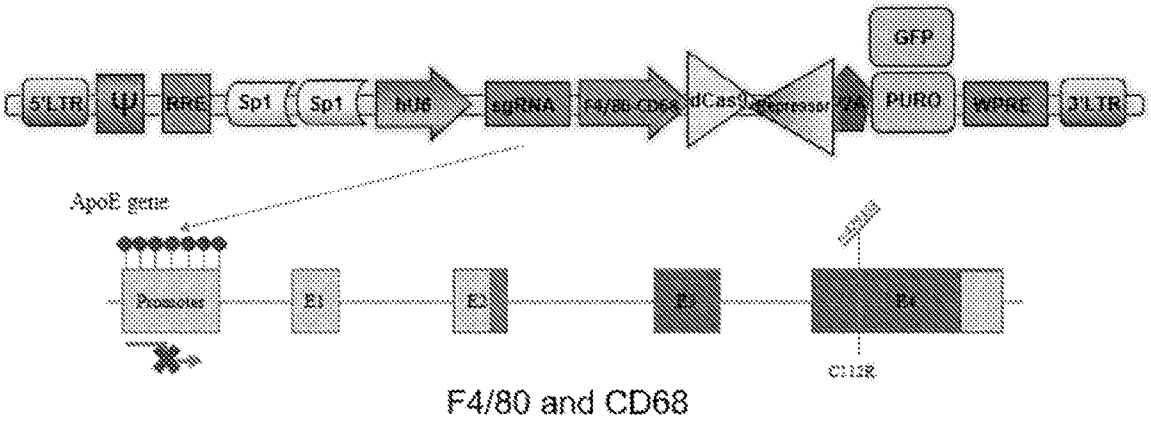

5'LTR
Sp1
Sp1
sgRNA
GFP
PURO
WPRE
3'LTR
ApoE gene
E1
E2
E3
E4
C112R
F4/80 and CD68

FIG. 4A

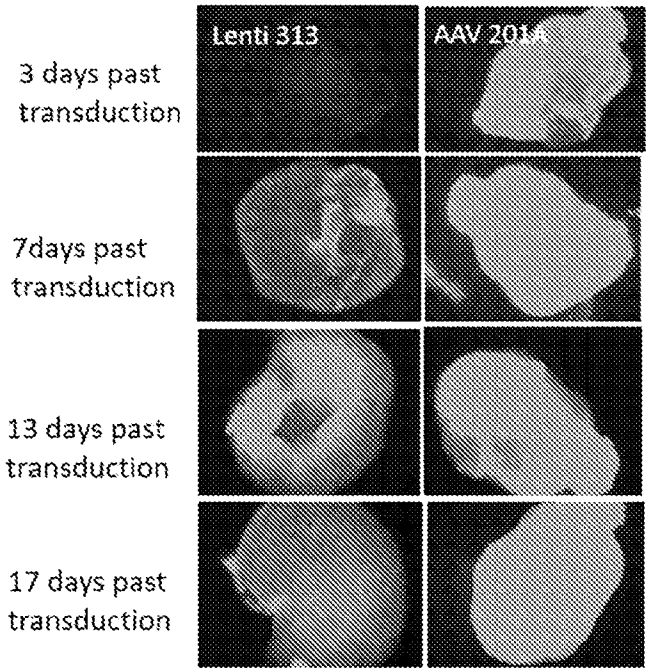

Lenti 313
AAV 201A
3 days past transduction
7days past transduction
13 days past transduction
17 days past transduction

FIG. 4B

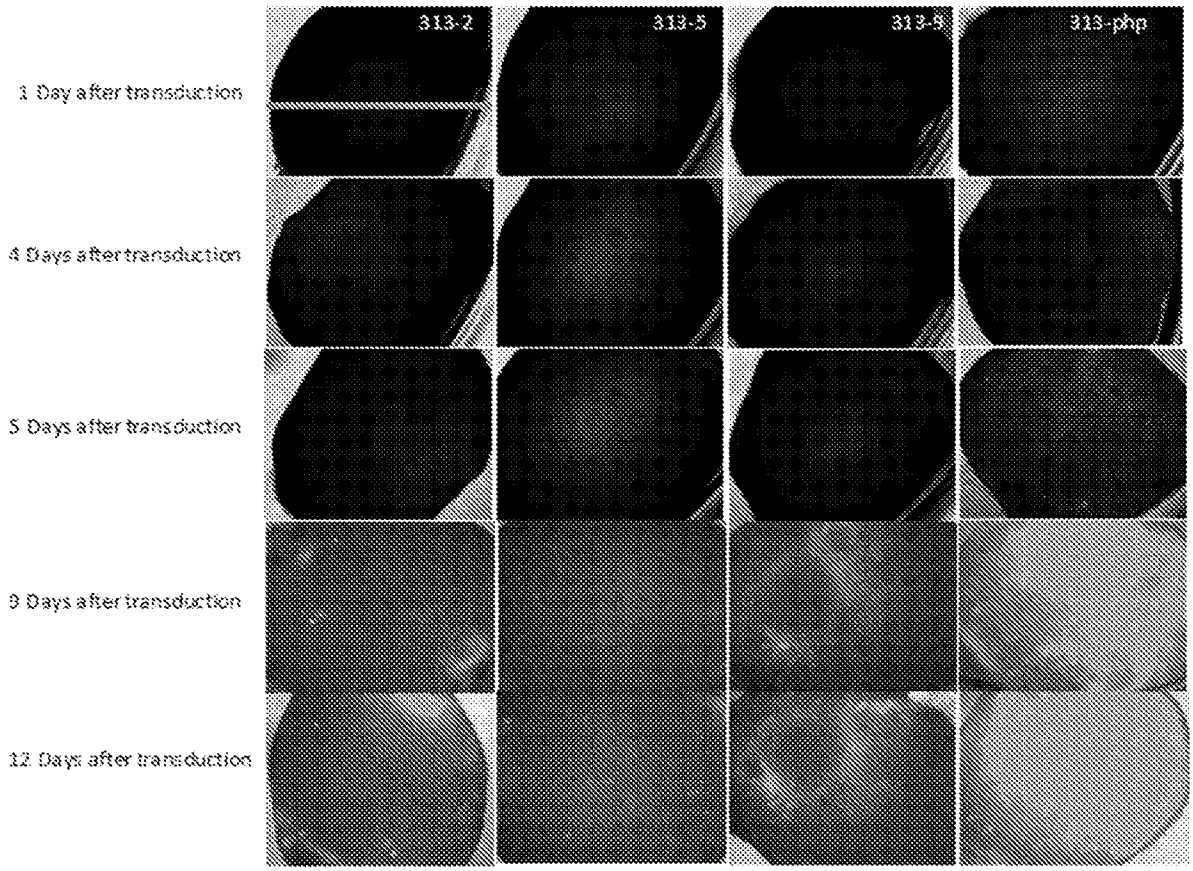

313-2
313-5
313-9
313-php
1 Day after transduction
4 Days after transduction
5 Days after transduction
9 Days after transduction
12 Days after transduction

FIG. 4C

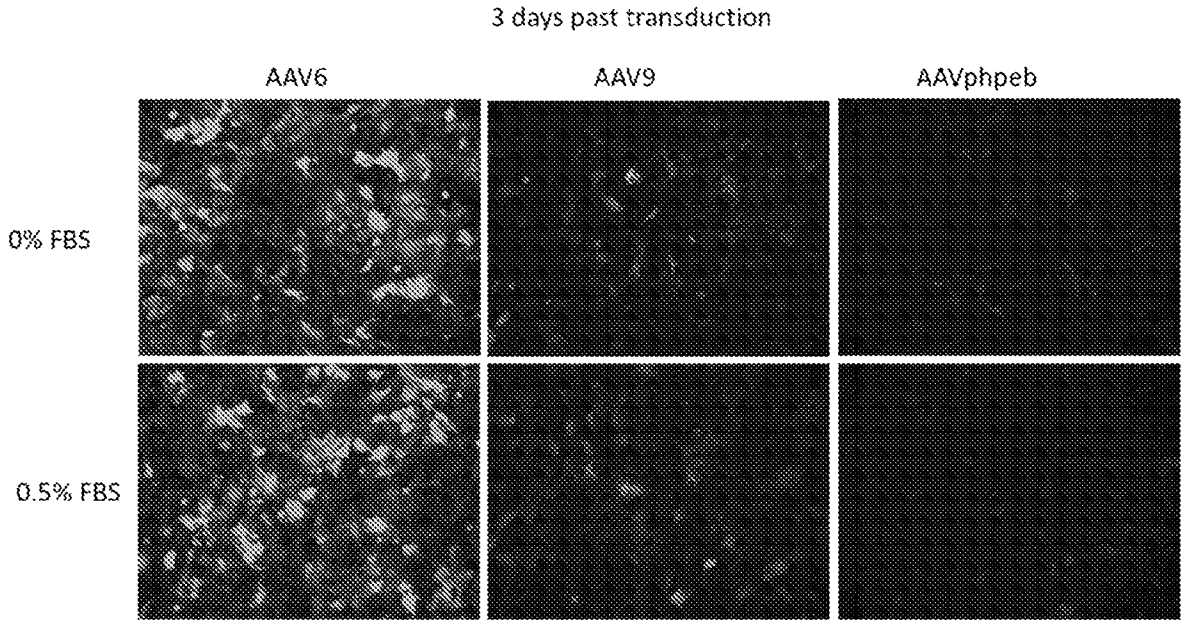

3 days past transduction
AAV6
AAV9
AAVphpeb
0% FBS
0.5% FBS

FIG. 4D

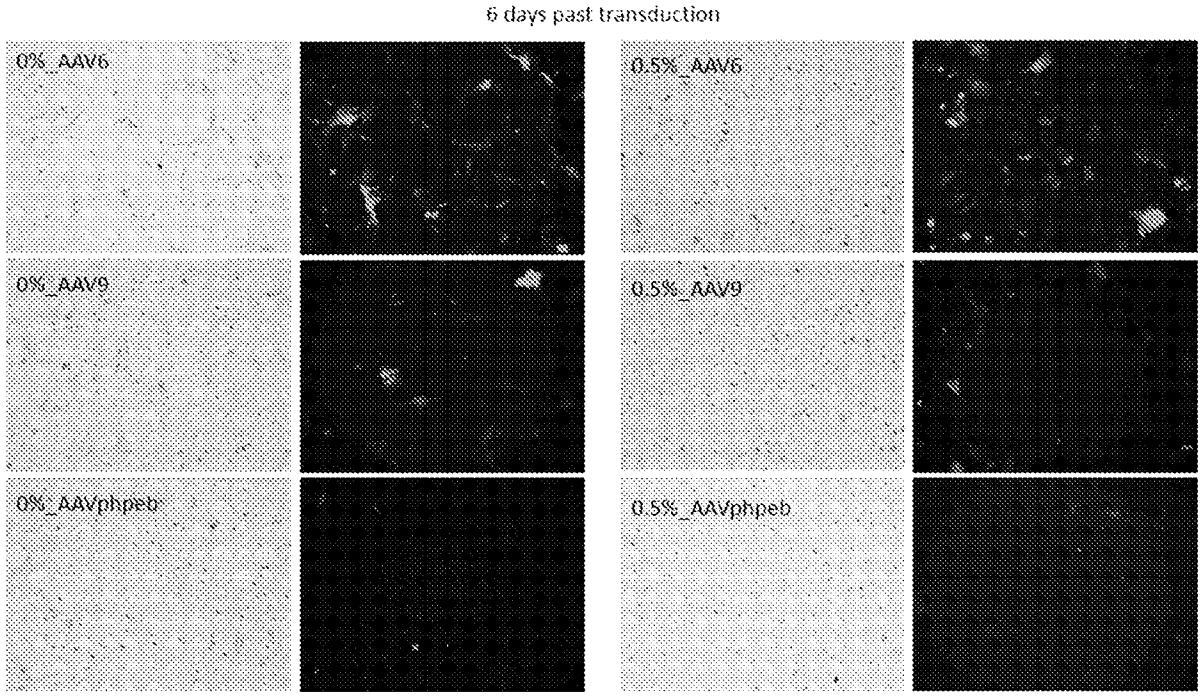

6 days past transduction
0%_AAV6
0.5%_AAV6
0%_AAV9
0.5%_AAV9
0%_AAVphpeb
0.5%_AAVphpeb

FIG. 4E

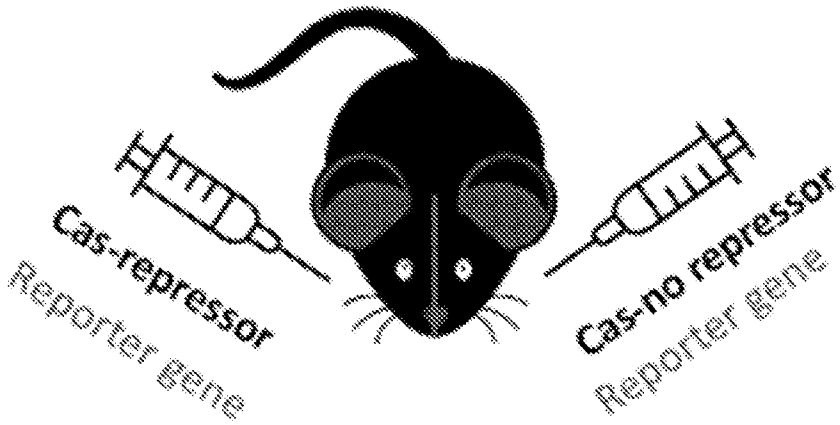

Cas-repressor
Reporter gene
Cas-no repressor
Reporter gene

FIG. 4F

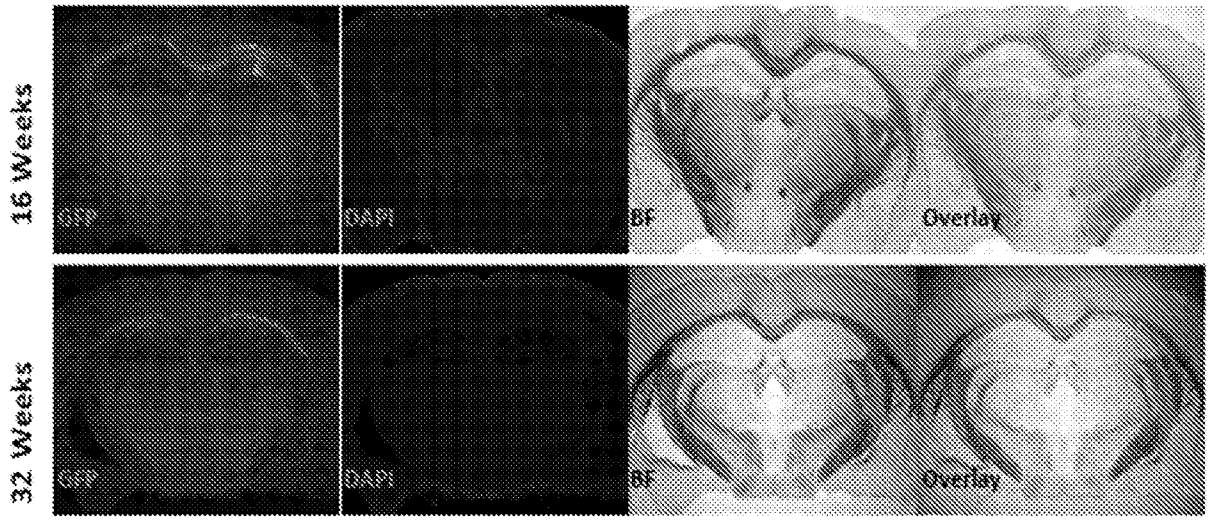

16 Weeks
32 Weeks
GFP
DAPI
BF
Overlay

FIG. 4G

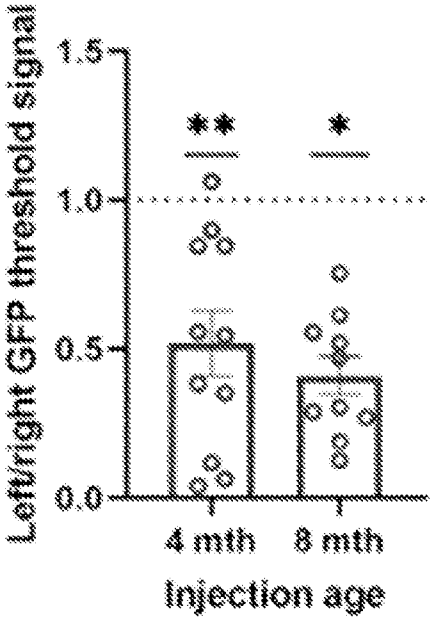

Left/right GFP threshold signal
1.5
1.0
0.5
0.0
**
*
4 mth
8 mth
Injection age

FIG. 4H

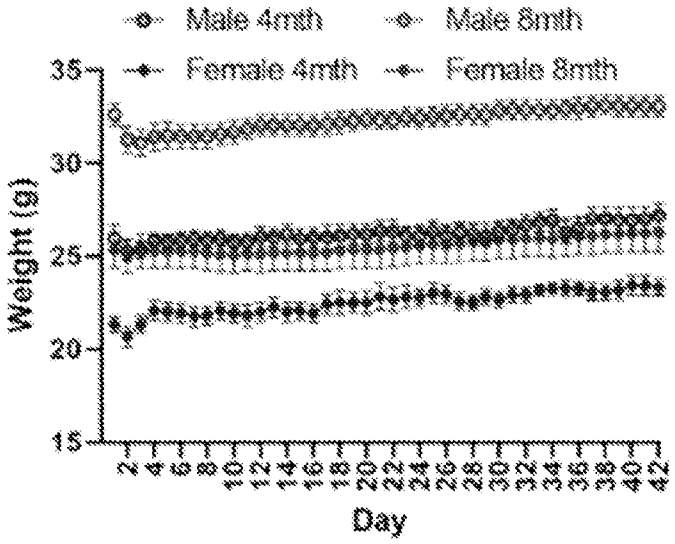

Male 4mth
Male 8mth
Female 4mth
Female 8mth
Weight (g)
35
30
25
20
15
Day

FIG. 4I

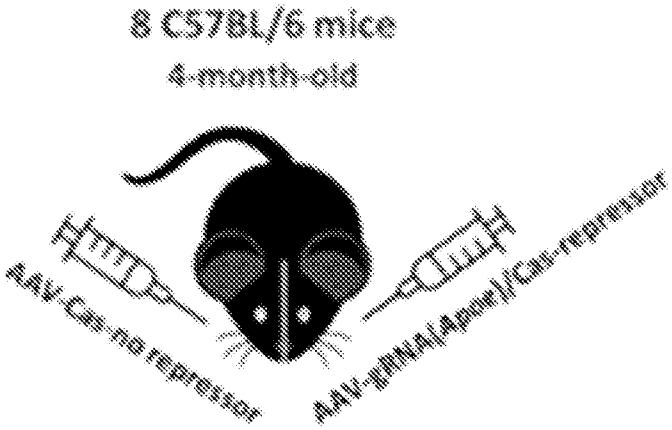

8 C57BL/6 mice
4-month-old
AAV-Cas-no repressor
AAV-gRNA(Apoe)/Cas-repressor

FIG. 5A

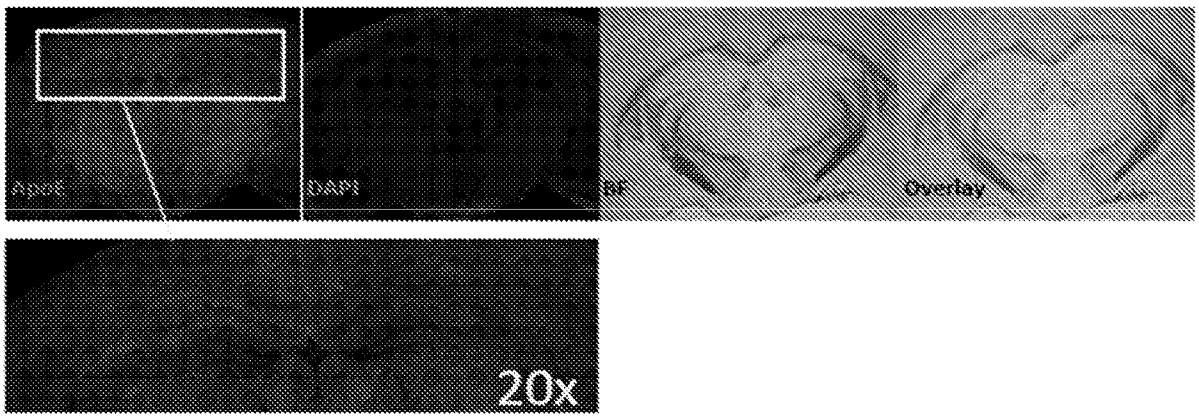

ApoE
DAPI
Overlay
20x

FIG. 5B

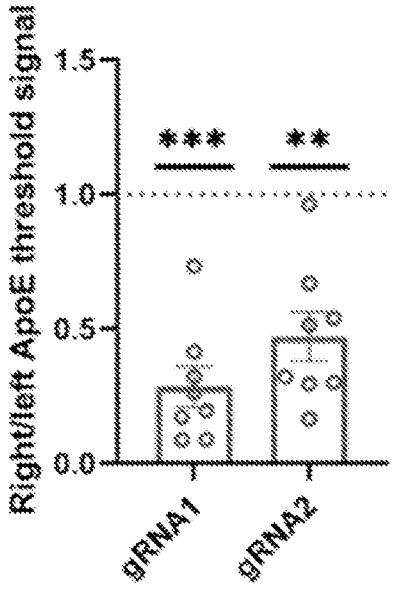

Right/left ApoE threshold signal
1.5
1.0
0.5
0.0
***
**
gRNA1
gRNA2

FIG. 5C

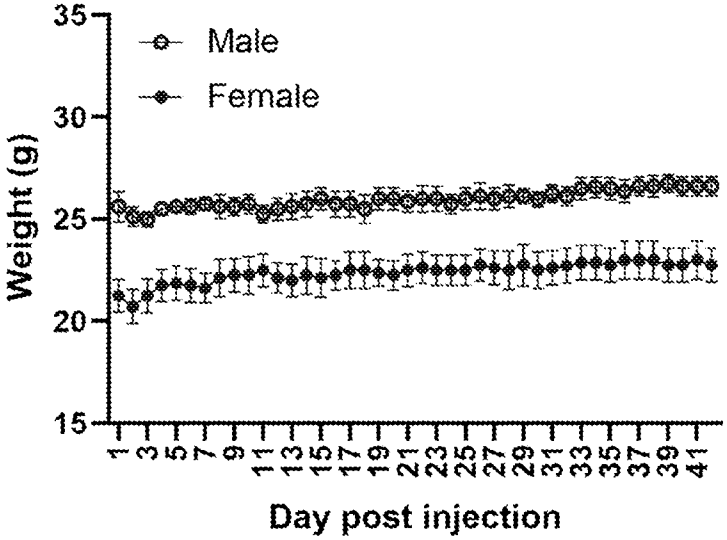

Male
Female
Weight (g)
35
30
25
20
15
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41
Day post injection

FIG. 5D

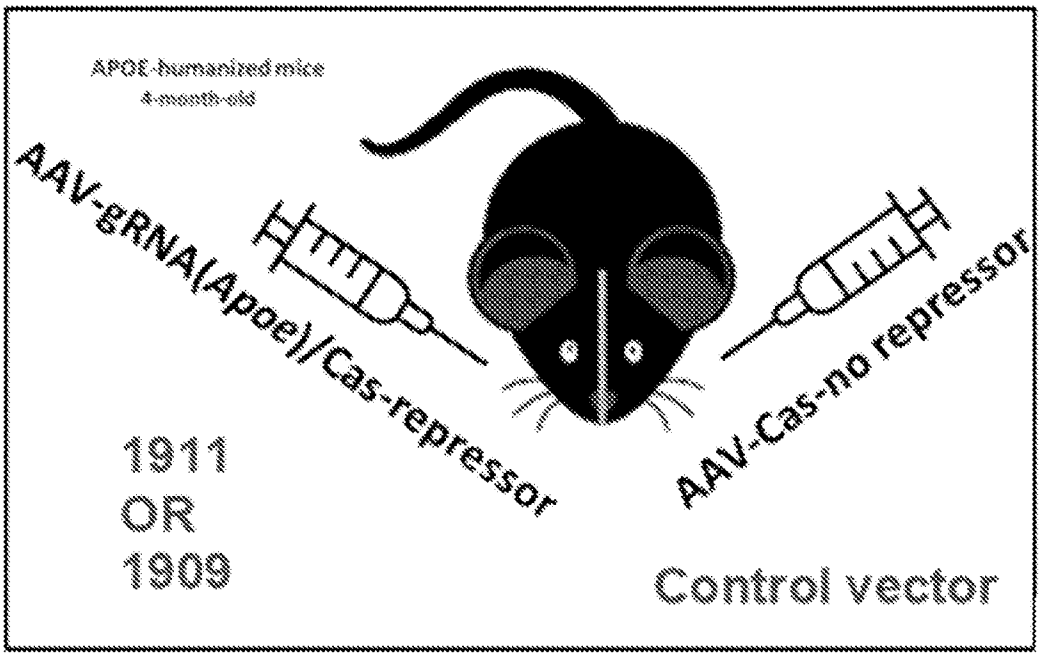

APOE-humanized mice
4-month-old
AAV-gRNA(Apoe)/Cas-repressor
AAV-Cas-no repressor
1911
OR
1909
Control vector

FIG. 5E

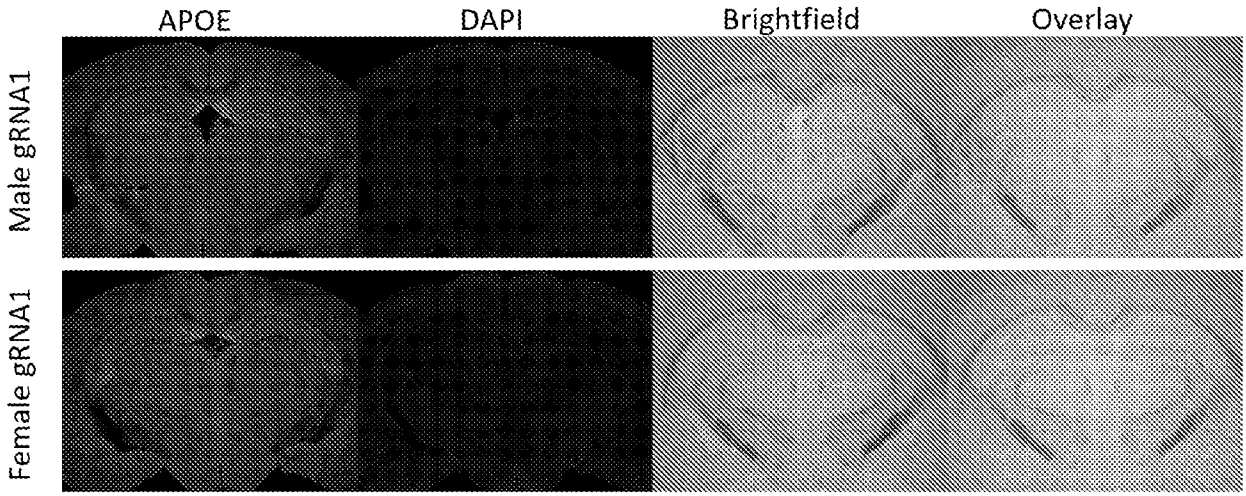

APOE
DAPI
Brightfield
Overlay
Male gRNA1
Female gRNA1

FIG. 5F

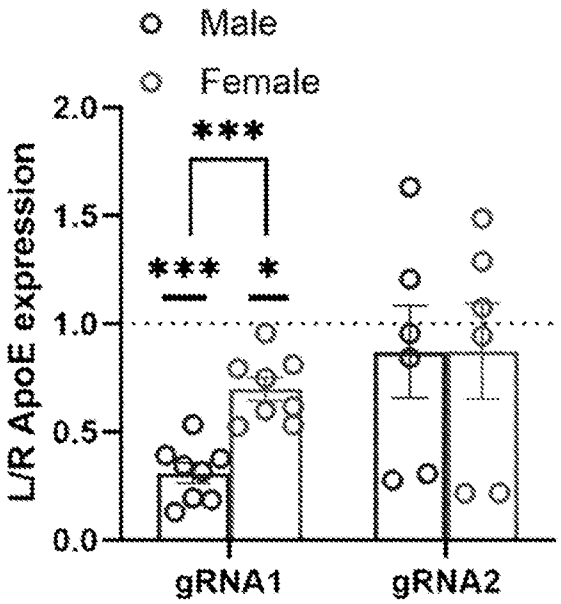

Male
Female
L/R ApoE expression
2.0
1.5
1.0
0.5
0.0
***
***
*
gRNA1
gRNA2

FIG. 5G

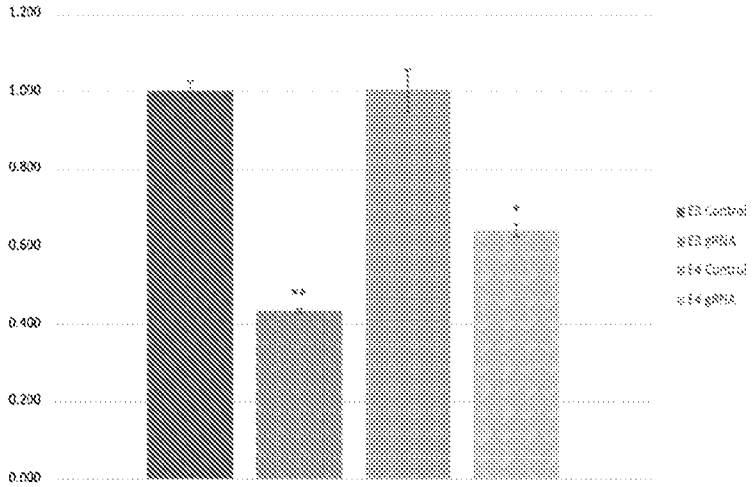

1.200
1.000
0.800
0.600
0.400
0.200
0.000

FIG. 6A

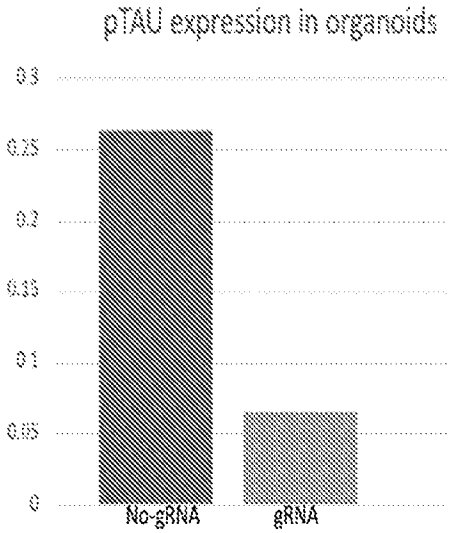

pTAU expression in organoids
0.3
0.25
0.2
0.15
0.1
0.05
0
No-gRNA
gRNA

FIG. 6B

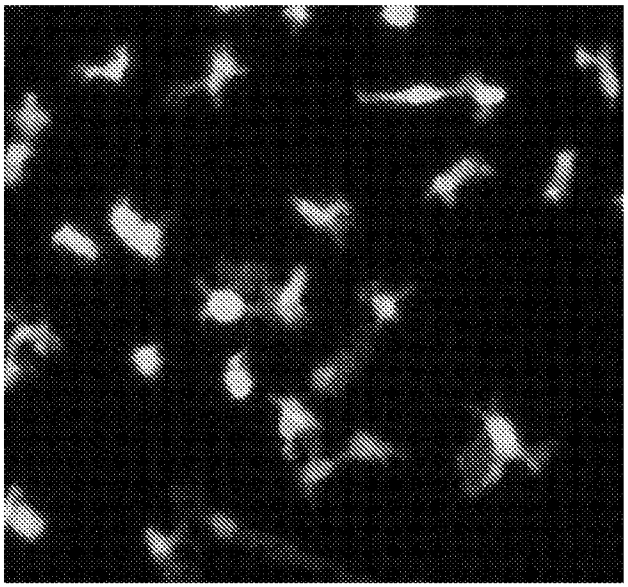


FIG. 7A

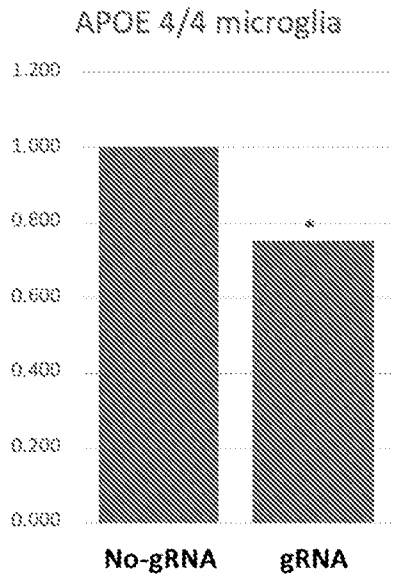

APOE 4/4 microglia
1.200
1.000
0.800
0.600
0.400
0.200
0.000
*
No-gRNA
gRNA

FIG. 7B

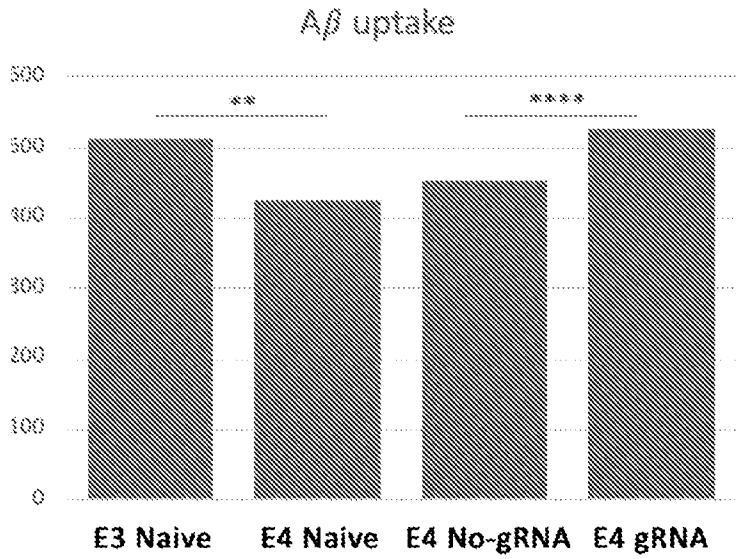

Aβ uptake
500
400
300
200
100
0
**
****
E3 Naive
E4 Naive
E4 No-gRNA
E4 gRNA

FIG. 7C

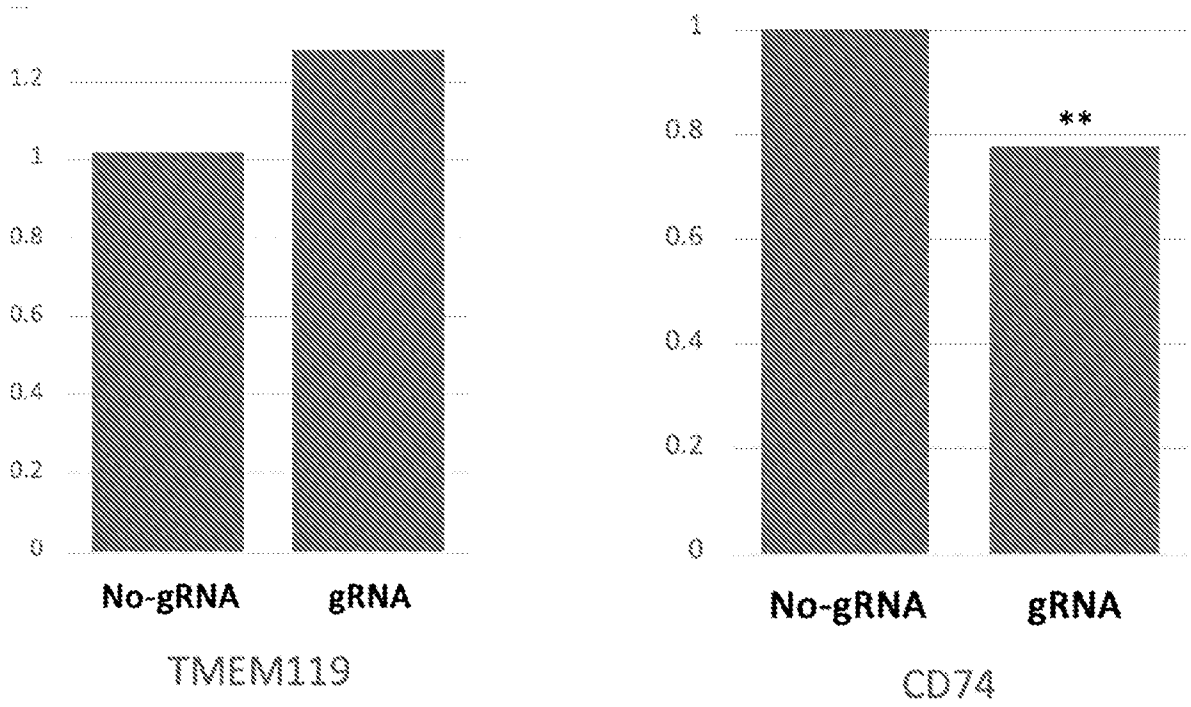

1.2
1
0.8
0.6
0.4
0.2
0
No-gRNA
gRNA
TMEM119
1
0.8
0.6
0.4
0.2
0
**
No-gRNA
gRNA
CD74

FIG. 7D