

**NOVEL ZINC FINGER PROTEIN TRANSCRIPTION FACTORS
FOR REPRESSING ALPHA-SYNUCLEIN EXPRESSION**

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CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims priority from U.S. Provisional Application 63/087,164, filed October 2, 2020, the disclosure of which is incorporated herein by reference in its entirety.

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SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. The electronic copy of the Sequence Listing, created on September 30, 2021, is named
15 025297_WO035_SL.txt and is 167,361 bytes in size.

BACKGROUND OF THE INVENTION

[0003] Parkinson's disease (PD) is a neurodegenerative disorder characterized by motor deficits. Approximately 50% of PD patients eventually develop dementia. PD is the
20 second most prevalent neurodegenerative disease after Alzheimer's disease. In the United States, there are approximately one million PD patients and every year there are 50,000-60,000 new cases. Sporadic forms of PD have a typical onset age of 60-70 years. There is generally a span of 15-20 years from initial diagnosis to death from PD complications.

25 **[0004]** PD patients display a range of motoric symptoms such as bradykinesia, rigidity, stooped posture, masked facial expression, forward tilt of trunk, reduced arm swinging, flexed elbows, wrists, hips and knees, postural instability, tremor of extremities at rest, and shuffling, short-stepped gait. The patients often also have non-motoric symptoms, including anosmia, disordered sleep, reduced gut motility, neuropathic pain, and
30 dementia. See, e.g., Jeanjean and Aubert, *Lancet* (2011) 378(9805):1773-4; Kalia and Lang, *Lancet* (2015) 386(9996):896-912.

[0005] The brains of PD patients are characterized by the loss of dopamine-producing (dopaminergic) neurons in an area called the substantia nigra. PD patients' brains also are characterized by the presence of Lewy bodies, which are protein aggregates or clumps

formed inside neurons, and Lewy neurites, which are neurites (processes of neurons) containing protein aggregates similar to Lewy bodies. Lewy bodies were first discovered in the brains of PD patients by Friedrich Lewy in 1912 and were later found to contain fibrils of aggregated and insoluble forms of alpha-synuclein (Goedert and Spillantini, *Mol Psychiatry*. (1998) 3(6):462-5; Spillantini et al., *Neurosci Lett*. (1998) 251(3):205-8; Spillantini et al., *Nature* (1997) 388(6645):839-40). A mutation in the alpha-synuclein gene (*SNCA*) was identified in families with PD in 1997 (see, e.g., Polymeropoulos et al., *Science* (1997) 276(5321):2045-7). Later, duplication and triplication of the *SNCA* gene, as well as additional point mutations in alpha-synuclein, were shown to correlate with the genetic or familial forms of PD. Moreover, changes in parts of the genome that control the level of alpha-synuclein expression have been shown to be associated with an increased risk for PD in large unbiased population studies (genome-wide association studies, GWAS).

[0006] Alpha-synuclein is a membrane-bound protein involved in vesicle release at presynaptic terminals of neurons. It may also play a role in DNA repair. Mature alpha-synuclein is a small 14-kD protein with a central core region (residues 61-95) containing hydrophobic amino acids, known as the NAC (non-A-beta component of Alzheimer's disease amyloid) region. The NAC contributes to protein aggregation. Misfolded alpha-synuclein polypeptides aggregate into oligomers and protofibrils, which then come together to form the large, insoluble aggregates found in Lewy bodies. Accumulating evidence indicates that alpha-synuclein misfolding and aggregation play a central role in the cellular damage that occurs in PD and eventually lead to the death of neurons in the substantia nigra. Furthermore, smaller aggregates of alpha-synuclein have been shown to move from cell to cell and spread throughout the brain, similar to what is seen in prion diseases. Inhibition of alpha-synuclein aggregation may reduce damage to neurons and slow down or even halt the progression of PD.

[0007] Current approaches to reduce the levels of alpha-synuclein include the use of anti-sense oligonucleotides (ASOs), which target alpha-synuclein at the RNA level, and monoclonal antibodies (mAbs), which target specific 3D shapes or conformations of alpha-synuclein outside cells. However, there remains an urgent need for a clinically efficacious method of treating PD by targeting alpha-synuclein.

SUMMARY OF THE INVENTION

[0008] The present disclosure provides zinc finger protein (ZFP) domains that target sites in or near the human *SNCA* gene. The ZFP domains of the present disclosure may be fused to a transcription factor to specifically inhibit expression of the human *SNCA* gene at the DNA level. These fusion proteins contain (i) a ZFP domain that binds specifically to a target region in the *SNCA* gene and (ii) a transcription repressor domain that reduces the transcription of the gene.

[0009] In one aspect, the present disclosure provides a fusion protein comprising a zinc finger protein (ZFP) domain and a transcription repressor domain, wherein the ZFP domain binds to a target region of a human alpha-synuclein gene (*SNCA* gene). In some embodiments, the target region (i.e., target site) is within about 1 kb of a transcription start site (TSS, e.g., TSS 1, 2a, or 2b) in the *SNCA* gene. In further embodiments, the target region is within about 500 bps upstream of TSS 2a, within about 500 bps downstream of TSS 2b, and/or within about 500 bps upstream or downstream of TSS 1 of the *SNCA* gene as shown in **FIGs. 2B** and/or **4**. Nonlimiting examples of target regions are shown in **Table 1**.

[0010] In some embodiments, the fusion protein comprises one or more (e.g., two, three, four, five, or six) zinc fingers. It may repress expression of the *SNCA* gene by at least about 40%, 75%, 90%, 95%, or 99%, preferably with no or minimal detectable off-target binding or activity (e.g., binding to a gene that is not the *SNCA* gene). Nonlimiting examples of zinc finger domains are shown in **Table 1**. In some embodiments, the fusion protein comprises one or more recognition helix sequences shown in **Table 1**. In further embodiments, the fusion protein comprises some or all the recognition helix sequences from a single row of the table, with or without the indicated backbone mutation(s). In certain embodiments, the fusion protein comprises an amino acid sequence shown in **Table 2**.

[0011] In some embodiments, the transcription repressor domain of the fusion protein is from the KRAB domain of the KOX1 protein. The zinc finger domain may be linked to the transcription repressor domain through a peptide linker. In another aspect, the present disclosure provides a nucleic acid construct comprising a coding sequence for the present fusion protein, wherein the coding sequence is linked operably to a transcription regulatory element, such as a mammalian promoter that is constitutively active or inducible in a brain cell, and wherein the promoter is optionally a human synapsin I promoter. The present disclosure also provides a host cell comprising the nucleic acid

construct. The host cell may be a human cell, such as a brain cell or a pluripotent stem cell, wherein the stem cell is optionally an embryonic stem cell or an inducible pluripotent stem cell (iPSC).

[0012] In another aspect, the present disclosure provides a method of inhibiting expression of alpha-synuclein in a human brain cell, comprising introducing into the cell the present fusion protein (e.g., through introduction of a nucleic acid construct or a recombinant virus such as AAV (e.g., AAV2, AAV6, AAV9, or hybrids thereof), thereby inhibiting the expression of alpha-synuclein in the cell. The brain cell may be a neuron, a glial cell, an ependymal cell, or a neuroepithelial cell. The cell may be in the brain of a patient suffering from or at risk of developing Parkinson's disease, Lewy body dementia, Alzheimer's disease, multiple system atrophy, or other synucleinopathy.

[0013] The present disclosure also provides a method of treating (e.g., slowing the progression) of a synucleinopathy in a patient, comprising administering to the patient a recombinant AAV encoding a fusion protein of the present disclosure. In some embodiments, the AAV is introduced to the patient via intravenous, intrathecal, intracerebroventricular, intra-cisternal magna, intrastriatal, or intranigral injection, or injection into any brain region. The patient may have Parkinson's disease, Lewy body dementia, Alzheimer's disease, or multiple system atrophy.

[0014] The present disclosure also provides fusion proteins for use in the above methods and use of the present fusion proteins in the manufacture of a medicament for use in the above methods.

[0015] Other features, objectives, and advantages of the invention are apparent in the detailed description that follows. It should be understood, however, that the detailed description, while indicating embodiments and aspects of the invention, is given by way of illustration only, not limitation. Various changes and modification within the scope of the invention will become apparent to those skilled in the art from the detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] **FIG. 1** illustrates specific targeting of the *SNCA* gene by an engineered 6-finger zinc finger protein-transcription factor (ZFP-TF), which recognizes 18 base pairs in the gene. Binding of the ZFP-TF to the gene leads to reduced *SNCA* transcription, which in turn leads to reduced *SNCA* mRNA and alpha-synuclein protein levels. The figure

discloses the genomic target sequence (bound sequence; SEQ ID NO: 226) for an exemplary engineered ZFP (SBS-82110).

[0017] **FIG. 2A** is a diagram illustrating the genomic structure of the human *SNCA* gene. The gene has 7 exons (2 non-protein-coding and 5 protein-coding), with each transcript containing 5 introns. There are three transcription start sites (TSSs), named TSS 1, TSS 2a, and TSS 2b, which give rise to transcripts starting with non-coding exons 1, 2a, and 2b, respectively. The first protein-coding exon in all transcripts is exon 3.

[0018] **FIG. 2B** is a diagram illustrating an upstream genomic region of the human *SNCA* gene. The *SNCA* mRNA sequence is shown as a red bar. The small triangles in the clusters underneath the gene depict the regions in the *SNCA* gene targeted by the 416 representative ZFP-TFs exemplified herein. The figure also shows the effect of each of the ZFP-TFs on reducing human *SNCA* mRNA expression in SK-N-MC human neuroblastoma cells harvested 24 hours after transfection with ZFP-TF mRNA. Messenger RNA levels were measured by RT-qPCR. Normalized *SNCA* expression levels are indicated by the gradient bar “SNCA mRNA.” Deepest (red) color denotes 100% reduction. Lightest color (white) denotes 0% reduction. RT-qPCR data were normalized to the mean of mRNA levels of two housekeeping genes (*ATP5B* and *EIF4A2*). Triangles pointing to the right indicate that the ZFP-TFs bind the sense strand of the gene. Triangles pointing to the left indicate that the ZFP-TFs bind the antisense strand of the gene.

[0019] **FIGs. 3A-3E** are graphs showing the screening results from a library of 416 ZFP-TFs as described herein. The screening was done in the SK-N-MC human neuroepithelial cell line. The y-axis in each graph is alpha-synuclein mRNA expression normalized to the geometric mean of two housekeeping genes (*EIF4A2* and *ATP5B*) and assessed 24 hours after transfection with RNA coding for the different ZFP-TFs. The RNA dose increases from left to right (3, 10, 30, 100, 300, and 1,000 ng). The bars represent the mean of four technical replicates and the error bars represent standard deviation. The numbers below the graphs are the internal reference numbers for the ZFP-TFs.

[0020] **FIG. 4** is a diagram illustrating an upstream genomic region of the human *SNCA* gene. The *SNCA* mRNA sequence is shown as a red bar. The small triangles in the clusters underneath the gene depict the regions in the *SNCA* gene targeted by 27 representative ZFP-TFs with 1, 2, or 3 phosphate contact mutations. The figure also shows the effect of each of the ZFP-TFs on reducing human *SNCA* mRNA expression in

SK-N-MC human neuroblastoma cells harvested 24 hours after transfection with ZFP-TF mRNA. Messenger RNA levels were measured by RT-qPCR. Normalized *SNCA* expression levels are indicated by the gradient bar “SNCA mRNA.” Deepest (red) color denotes 100% reduction. Lightest color (white) denotes 0% reduction. RT-qPCR data were normalized to the mean of mRNA levels of two housekeeping genes (*ATP5B* and *EIF4A2*). Triangles pointing to the right indicate that the ZFP-TFs bind the sense strand of the gene. Triangles pointing to the left indicate that the ZFP-TFs bind the antisense strand of the gene.

[0021] FIG. 5 shows illustrative screening results from a library of 27 representative ZFP-TFs with 1, 2, or 3 phosphate contact mutations. The screening was done in the SK-N-MC human neuroepithelial cell line. The y-axis in each graph is alpha-synuclein mRNA expression normalized to the geometric mean of two housekeeping genes (*EIF4A2* and *ATP5B*) and assessed 24 hours after transfection with RNA coding for the different ZFP-TFs. The RNA dose increases from left to right (3, 10, 30, 100, 300, and 1,000 ng). The bars represent the mean of four technical replicates and the error bars represent standard deviation. The numbers below the graphs are the internal reference numbers for the ZFP-TFs.

[0022] FIGs. 6A and 6B are panels of graphs showing that 40 alpha-synuclein ZFP-TFs displayed a range of alpha-synuclein repression activity in SK-N-MC human neuroblastoma cells and human iPSC-derived neurons. Twenty illustrative ZFP-TFs described in Tables 1 and 2 are shown in panel A. Illustrative ZFP-TFs characterized in FIGs. 3A-3E but not described in Tables 1 and 2 are shown in panel B. The y-axis is alpha-synuclein mRNA expression normalized to the geometric mean of two housekeeping genes (*ATP5B* and *EIF4A2*) and assessed 24 hours after transfection of SK-N-MC cells with RNA coding for the different ZFP-TFs, or 28 days after transduction of iPSC-derived neurons with AAV6 encoding the different ZFP-TFs. The amount of RNA or AAV6 used is indicated at the x-axis, with the RNA (3, 10, 30, 100, 300, and 1,000 ng) or AAV6 (1E3, 3E3, 1E4, 3E4, 1E5, and 3E5) dose increasing from left to right. The blue and orange bars represent the mean of four technical replicates and the error bars represent standard deviation. Enlarged versions of the titration scales are shown at the bottom of the figure.

[0023] FIGs. 7A-7D are panels of volcano plots depicting the off-target activity of 40 alpha-synuclein ZFP-TFs in mouse primary neurons (A and B) and human iPSC-derived neurons (C and D). For easy reference, bar graphs from FIGs. 6A-6B illustrating the

repressing activity of the ZFP-TFs in human iPSC-derived neurons are shown next to the volcano plots of the corresponding ZFP-TFs. The volcano plots summarize microarray data showing changes in the transcriptome of mouse primary neurons 7 days after transduction (A and B) or human iPSC-derived neurons 19 days after transduction (C and D). Yellow circles indicate human alpha-synuclein. Green circles (right side of each volcano plot) represent genes significantly upregulated by >2-fold. The number of these genes is indicated in green text. Red circles represent genes significantly downregulated by >2-fold. The number of these genes is indicated in red text.

[0024] FIGs. 8A and 8B are panels of graphs showing (A) ZFP-TF and alpha-synuclein mRNA expression levels or (B) mRNA expression levels of glial fibrillary acidic protein (GFAP), ionized calcium binding adaptor molecule 1 (IBA1), and NeuN in different brain regions of female PAC synuclein mice (Kuo et al., *Hum Mol Genet.* (2010) 19(9):1633-50). The animals were bilaterally administered AAV9 coding for the indicated test article or vehicle at two sites in the striatum. All gene expression data are normalized to the geometric mean of 3 housekeeping genes (*ATP5B*, *EIF4A2*, and *GAPDH*). The y-axis is mRNA expression level of the indicated gene and the x-axis is brain region. The red squares indicate average expression data for n = 3 animals administered ZFP-TF 82195, and the green triangles indicate average expression data for n = 3 animals administered ZFP-TF 82264. In panel A, alpha-synuclein mRNA expression is normalized to the mean of 3 vehicle-treated animals, and ZFP-TF expression is provided on a log scale as copies per ng input RNA. In panel B, all expression data are normalized to the mean of the 3 vehicle-treated animals. Statistical analysis was performed using a one-way analysis of variance (ANOVA) followed by a Dunnett's multiple comparisons test. * p < 0.05, ** p < 0.01, ***, p < 0.001, ****, p < 0.0001.

DETAILED DESCRIPTION OF THE INVENTION

[0025] The present disclosure provides ZFP domains that target sites (i.e., sequences) in or near the human *SNCA* gene. A ZFP domain as described herein may be attached or fused to another functional molecule or domain. The ZFP domains of the present disclosure may be fused to a transcription factor to repress transcription of the human *SNCA* gene into RNA. The fusion proteins are called zinc finger protein transcription factors (ZFP-TFs). These ZFP-TFs comprise a zinc finger protein (ZFP) domain that binds specifically to a target region in or near the *SNCA* gene and a transcription repressor domain that reduces the transcription of the gene. Reducing the level of alpha-synuclein

in neurons by introducing the ZFP-TFs into the brain of a patient is expected to inhibit (e.g., reduce or stop) the assembly of alpha-synuclein into oligomeric (smaller soluble aggregates) or fibrillar (larger insoluble aggregates) forms. With a reduction in alpha-synuclein aggregation, the brain cells will have the capacity to timely remove misfolded and toxic forms of alpha-synuclein with their cellular quality control machinery. As a result, aggregation and cell-to-cell propagation of alpha-synuclein will be reduced or prevented.

[0026] Our ZFP-TF approach to alpha-synuclein inhibition has several advantages over the current approaches being tested by others. ZFP-TFs may need to be administered only once (by introducing to the patient a ZFP-TF expression construct), while ASOs require repeated dosing. In addition, the ZFP-TF approach only needs to engage the two alleles of the *SNCA* gene in the genome of each cell. By contrast, ASOs need to engage numerous copies of the *SNCA* mRNA in each cell.

[0027] Our ZFP-TF approach is advantageous over the antibody approach because antibodies can only bind a subset of alpha-synuclein shapes or conformations. This may not be sufficient for a robust therapeutic effect. In contrast, ZFP-TFs repress alpha-synuclein expression at the DNA level and lead to lower levels of all forms of alpha-synuclein. ZFP-TFs are therefore agnostic to the forms of the toxic species, unlike antibodies. In addition, antibodies are thought to largely act on alpha-synuclein on or outside the cells, whereas ZFP-TFs can reduce alpha-synuclein inside the cell directly and indirectly lower extracellular alpha-synuclein levels. Thus, the ZFP-TF approach is expected to be more effective because alpha-synuclein is largely an intracellular protein. Further, antibodies require repeated administration, while ZFP-TFs require only a one-time delivery of their expression constructs.

I. Targets of the ZFP Domains

[0028] The ZFP domains of the present fusion proteins bind specifically to a target region in or near the human *SNCA* gene. **FIG. 1** illustrates the binding of a ZFP domain to a target *SNCA* gene sequence. The ZFP domain in the figure has six zinc fingers; however, as further described below, a ZFP domain that has fewer or more zinc fingers can also be used.

[0029] The human *SNCA* gene spans about 117 kb and has been mapped to chr4:89,724,099-89,838,315(GRCh38/hg38). Its nucleotide sequence is available at GenBank accession number NC_000004 version 000004.12. The gene has 7 exons (2

non-protein-coding, and 5 protein-coding), with each transcript having 5 introns (**FIG. 2A**). There are three transcription start sites (TSSs) for the gene: one at the beginning of exon 1, and two in exon 2, resulting in the expression of exons 2a and 2b. The first protein-coding exon is exon 3. *See also* Touchman et al., *Genome Res.* (2001) 11:78-86.

5 Isoform 1 (full length) of human alpha-synuclein is shown below:

MDVFMKGLSK AKEGVVAAAE KTKQGVAAEA GKTKEGVLYV GSKTKEGVVH
GVATVAEKTQ EQVTNVGGAV VTGVTAVAQK TVEGAGSIAA ATGFVKKQDL
GKNEEGAPQE GILEDMPVDP DNEAYEMPSE EGYQDYEP EA (SEQ ID NO:1;
P37840-1)

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[0030] Isoform 2-4 differs from isoform 1 in that amino acid residues 103-130 are missing. Isoform 2-5 differs from isoform 1 in that amino acid residues 41-54 are missing. Genetic analysis of alpha-synuclein has pointed to gene copy amplification (*see, e.g.,* Brueggemann et al., *Neurology* (2008) 71:1294; Troiano et al., *Neurology* (2008) 71:1295; Uchiyama et al., *Neurology* (2008) 71:1289-90) and certain point mutations as potential causes for synucleinopathies such as PD and Lewy body dementia. For example, the following alpha-synuclein point mutations have been identified in some PD patients: A30P (Kruger et al., *Nature Genet.* (1998) 18:106-8); E46K (Zarranz et al., *Ann Neurol.* (2004) 55:164-73; Choi et al., *FEBS Lett.* (2004) 576:363-8); H50Q (Khalaf et al., *J Biol Chem.* (2014) 289:21856-76); G51D (Lesage et al., *Ann Neurol.* (2013) 73:459-71); and A53T (Polymeropoulos et al., *Science* (1997) 276:2045-7).

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[0031] The DNA-binding ZFP domain of the ZFP-TFs directs the fusion proteins to a target region of the *SNCA* gene and brings the transcription repressor domain of the fusion proteins to the target region. The repressor domain then represses the *SNCA* gene's transcription by RNA polymerase. The target region for the ZFP-TFs can be any suitable site in or near the *SNCA* gene that allows repression of gene expression. By way of example, the target region includes, or is adjacent to (either downstream or upstream of) an *SNCA* TSS or an *SNCA* transcription regulatory element (e.g., promoter, enhancer, RNA polymerase pause site, and the like).

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[0032] As described above, the human *SNCA* gene has three transcription start sites (TSSs). They are, from 5' to 3', TSS 1, TSS 2a, and TSS 2b, which are located at the 5' ends of exon 1 (TSS 1), exon 2a, and exon 2b (TSSs 2a and 2b) (**FIG. 2B**). Transcription at the three TSSs leads to RNA isoforms with different lengths. However, since translation of alpha-synuclein begins at exon 3, RNA produced from the different TSSs leads to the formation of the same protein. In some embodiments, the genomic target

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region of the present ZFP-TFs spans or is in the vicinity of TSS 1 (e.g., base pairs 529-1529) or TSSs 2a and 2b (e.g., base pairs 1613-2949). In certain embodiments, the target region is within about 500 bp upstream or downstream of TSS 1, and/or within about 500 bp upstream or downstream of TSS 2a, and/or within about 500 bp upstream or downstream of TSS 2b.

[0033] In some embodiments, the genomic target region is at least 8 bps in length. For example, the target region may be 8 bps to 40 bps in length, such as 12, 15, 18, 21, 24, 27, 30, 33, or 36 bps in length. The targeted sequence may be on the sense strand of the gene, or the antisense strand of the gene. To ensure targeting accuracy and to reduce off-target binding or activity by the ZFP-TFs, the sequence of the selected *SNCA* target region preferably has less than 75% homology (e.g., less than 70%, less than 65%, less than 60%, or less than 50%) to sequences in other genes. In certain embodiments, the target region of the present ZFP-TFs is 15 -18 bps in length and resides within 500 bps of TSSs 1, 2a, or 2b. Examples of target regions are shown in **FIG. 2B** and **Table 1**.

[0034] In some embodiments, the present engineered ZFPs bind to a target site (i.e., Binding Sequence) as shown in a single row of **Table 1**, preferably with no or little detectable off-target binding or activity.

[0035] Other criteria for further evaluating target segments include the prior availability of ZFPs binding to such segments or related segments, ease of designing new ZFPs to bind a given target segment, and off-target binding risk.

II. ZFP Domains

[0036] A “zinc finger protein” or “ZFP” refers to a protein having a DNA-binding domain that is stabilized by zinc. ZFPs bind to DNA in a sequence-specific manner. The individual DNA-binding unit of a ZFP is referred to as a zinc “finger.” Each finger contains a DNA-binding “recognition helix” that is typically comprised of seven amino acid residues and determines DNA binding specificity. A ZFP domain has at least one finger, each finger binds from two to four base pairs of DNA, typically three or four base pairs of DNA. Each zinc finger typically comprises approximately 30 amino acids and chelates zinc. An engineered ZFP can have a novel binding specificity, compared to a naturally occurring ZFP. Engineering methods include, but are not limited to, rational design and various types of selection. Rational design includes, for example, using databases comprising triplet (or quadruplet) nucleotide sequences and individual zinc finger amino acid sequences, in which each triplet or quadruplet nucleotide sequence is

- associated with one or more amino acid sequences of zinc fingers that bind the particular triplet or quadruplet sequence. *See, e.g.*, ZFP design methods described in detail in U.S. Pats. 5,789,538; 5,925,523; 6,007,988; 6,013,453; 6,140,081; 6,200,759; 6,453,242; 6,534,261; 6,979,539; and 8,586,526; and International Patent Publications
- 5 WO 95/19431; WO 96/06166; WO 98/53057; WO 98/53058; WO 98/53059; WO 98/53060; WO 98/54311; WO 00/27878; WO 01/60970; WO 01/88197; WO 02/016536; WO 02/099084; and WO 03/016496. A ZFP domain as described herein may be attached or fused to another molecule, for example, a protein. Such ZFP-fusions may comprise a domain that enables gene activation (e.g., activation domain), gene
- 10 repression (e.g., repression domain), ligand binding (e.g., ligand-binding domain), high-throughput screening (e.g., ligand-binding domain), localized hypermutation (e.g., activation-induced cytidine deaminase domain), chromatin modification (e.g., histone deacetylase domain), recombination (e.g., recombinase domain), targeted integration (e.g., integrase domain), DNA modification (e.g., DNA methyl-transferase domain), base
- 15 editing (e.g., base editor domain), or targeted DNA cleavage (e.g., nuclease domain). Examples of engineered ZFP domains are shown in **Table 1**.
- [0037] The ZFP domain of the present engineered ZFP fusion proteins may include at least one (e.g., one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, or more) zinc finger(s). A ZFP domain having one finger typically recognizes a
- 20 target site that includes 3 or 4 nucleotides. A ZFP domain having two fingers typically recognizes a target site that includes 6 or 8 nucleotides. A ZFP domain having three fingers typically recognizes a target site that includes 9 or 12 nucleotides. A ZFP domain having four fingers typically recognizes a target site that includes 12 to 15 nucleotides. A ZFP domain having five fingers typically recognizes a target site that includes 15 to 18
- 25 nucleotides. A ZFP domain having six fingers can recognize target sites that include 18 to 21 nucleotides.
- [0038] In some embodiments, the present engineered ZFPs comprise a DNA-binding recognition helix sequence shown in **Table 1**. For example, an engineered ZFP may comprise the sequence of F1, F2, F3, F4, F5, or F6 as shown in **Table 1**.
- 30 [0039] In some embodiments, the present engineered ZFPs comprise two adjacent DNA-binding recognition helix sequences shown in a single row of **Table 1**. For example, an engineered ZFP may comprise the sequences of F1-F2, F2-F3, F3-F4, F4-F5, or F5-F6 as shown in a single row of **Table 1**.

[0040] In some embodiments, the present engineered ZFPs comprise the DNA-binding recognition helix sequences shown in a single row of **Table 1**. For example, an engineered ZFP may comprise the sequences of F1, F2, F3, F4, F5, and F6 (e.g., F1-F6) as shown in a single row of **Table 1**.

- 5 [0041] The target specificity of the ZFP domain may be improved by mutations to the ZFP backbone sequence as described in, e.g., U.S. Pat. Pub. 2018/0087072. The mutations include those made to residues in the ZFP backbone that can interact non-specifically with phosphates on the DNA backbone but are not involved in nucleotide target specificity. In some embodiments, these mutations comprise mutating a cationic amino acid residue to a neutral or anionic amino acid residue. In some embodiments, these mutations comprise mutating a polar amino acid residue to a neutral or non-polar amino acid residue. In further embodiments, mutations are made at positions (-5), (-9) and/or (-14) relative to the DNA binding helix. In some embodiments, a zinc finger may comprise one or more mutations at positions (-5), (-9) and/or (-14). In further
- 10 15 20 25 30
- embodiments, one or more zinc fingers in a multi-finger ZFP domain may comprise mutations at positions (-5), (-9) and/or (-14). In some embodiments, the amino acids at positions (-5), (-9) and/or (-14) (e.g., an arginine (R) or lysine (K)) are mutated to an alanine (A), leucine (L), serine (S), aspartate (N), glutamate (E), tyrosine (Y), and/or glutamine (Q). Examples of engineered ZFPs with 1, 2, or 3 backbone mutations are shown in **FIGs. 4 and 5** and **Tables 1 and 2**. The symbol “^” in **Table 1** indicates that the arginine (R) residue at the 4th position upstream of the 1st amino acid in the indicated recognition helix is changed to glutamine (Q). In each recognition helix sequence, the positions of the seven DNA-binding amino acids are numbered -1, +1, +2, +3, +4, +5, and +6. Thus, the position for the R-to-Q substitution is numbered as (-5).
- [0042] In some embodiments, the present engineered ZFPs comprise a DNA-binding recognition helix sequence and associated backbone mutation as shown in **Table 1**. In some embodiments, the present engineered ZFPs comprise the DNA-binding recognition helix sequences and associated backbone mutations as shown in a single row of **Table 1**.
- [0043] In some embodiments, an engineered ZFP described herein comprises the recognition helix and backbone portions of a sequence shown in a single row of **Table 2**. In some embodiments, an engineered ZFP described herein comprises the recognition helix and backbone portions of a sequence shown in a single row of **Table 2** as the sequence would appear following post-translational modification. For example, post-

translational modification may remove the initiator methionine residue from a sequence as shown in **Table 2**.

[0044] In some embodiments, the present ZFP-TFs comprise one or more zinc finger domains. The domains may be linked together via an extendable flexible linker such that, for example, one domain comprises one or more (e.g., 4, 5, or 6) zinc fingers and another domain comprises additional one or more (e.g., 4, 5, or 6) zinc fingers. In some embodiments, the linker is a standard inter-finger linker such that the finger array comprises one DNA binding domain comprising 8, 9, 10, 11 or 12 or more fingers. In other embodiments, the linker is an atypical linker such as a flexible linker. For example, two ZFP domains may be linked to a transcription repressor TF in the configuration (from N terminus to C terminus) ZFP-ZFP-TF, TF-ZFP-ZFP, ZFP-TF-ZFP, or ZFP-TF-ZFP-TF (two ZFP-TF fusion proteins are fused together via a linker).

[0045] In some embodiments, the ZFP-TFs are “two-handed,” i.e., they contain two zinc finger clusters (two ZFP domains) separated by intervening amino acids so that the two ZFP domains bind to two discontinuous target sites. An example of a two-handed type of zinc finger binding protein is SIP1, where a cluster of four zinc fingers is located at the amino terminus of the protein and a cluster of three fingers is located at the carboxyl terminus (*see* Remacle et al., *EMBO J.* (1999) 18(18):5073-84). Each cluster of zinc fingers in these proteins is able to bind to a unique target sequence and the spacing between the two target sequences can comprise many nucleotides.

III. Zinc Finger Protein Transcription Factors

[0046] The ZFP domains described herein may be fused to a transcription factor. In some embodiments, the present fusion proteins contain a DNA-binding zinc finger protein (ZFP) domain and a transcription factor domain (i.e., ZFP-TF). In some embodiments, the transcription factor may be a transcription repressor domain, wherein the ZFP and repressor domains may be associated with each other by a direct peptidyl linkage or a peptide linker, or by dimerization (e.g., through a leucine zipper, a STAT protein N terminal domain, or an FK506 binding protein). As used herein, a “fusion protein” refers to a polypeptide with covalently linked domains as well as a complex of polypeptides associated with each other through non-covalent bonds. The transcription repressor domain can be associated with the ZFP domain at any suitable position, including the C- or N-terminus of the ZFP domain.

[0047] In some embodiments, the present ZFP-TFs bind to their target with a K_D of less than about 25 nM and repress transcription of a human *SNCA* gene by 20% or more (e.g., by 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% or more). In some embodiments, two or more of the present ZFP-TFs are used concurrently in a patient, where the ZFP-TFs
 5 bind to different target regions in the *SNCA* gene, so as to achieve optimal repression of *SNCA* expression.

A. Transcription Repressor Domains

[0048] The present ZFP-TFs comprise an engineered ZFP domain as described herein and one or more transcription repressor domains that dampen the transcription activity of
 10 the *SNCA* gene. One or more engineered ZFP domains and one or more transcription repressor domains may be joined by a flexible linker. Non-limiting examples of transcription repressor domains are KRAB domain of KOX1, KAP-1, MAD, FKHR, EGR-1, ERD, SID, TGF-beta-inducible early gene (TIEG), v-ERB-A, MBD2, MBD3, TRa, histone methyltransferase, histone deacetylase (HDAC), nuclear hormone receptor
 15 (e.g., estrogen receptor or thyroid hormone receptor), members of the DNMT family (e.g., DNMT1, DNMT3A, DNMT3B), Rb, and MeCP2. See, e.g., Bird et al. (1999) *Cell* 99:451-454; Tyler et al. (1999) *Cell* 99:443-446; Knoepfler et al. (1999) *Cell* 99:447-450; and Robertson et al. (2000) *Nature Genet.* 25:338-342. Additional exemplary repression domains include, but are not limited to, ROM2 and AtHD2A. See, for example, Chem et al. (1996) *Plant Cell* 8:305-321; and Wu et al. (2000) *Plant J.* 22:19-27.
 20

[0049] In some embodiments, the transcription repressor domain comprises a sequence from the Kruppel-associated box (KRAB) domain of the human zinc finger protein 10/KOX1 (ZNF10/KOX1) (e.g., GenBank No. NM_015394.4). An exemplary KRAB domain sequence is:
 25 DAKSLTAWSR TLVTFKDV FV DFTREEWKLL DTAQQIVYRN VMLENYKNLV
 SLGYQLTKPD VILRLEKGEE PWLVEREIHQ ETHPDSETAF EIKSSV
 (SEQ ID NO: 12) .

30 Variants of this KRAB sequence may also be used so long as they have the same or similar transcription repressor function.

[0050] In some embodiments, an engineered ZFP-TF described herein binds to a target site as shown in a single row of **Table 1**, preferably with no or little detectable off-target binding or activity. Off-target binding may be determined, for example, by measuring the activity of ZFP-TFs at off-target genes. In some embodiments, an engineered ZFP-TF
 35 described herein comprises a DNA-binding recognition helix sequence shown in **Table 1**.

In some embodiments, an engineered ZFP-TF described herein comprises two adjacent DNA-binding recognition helix sequences shown in a single row of **Table 1**. In some embodiments, an engineered ZFP-TF described herein comprises the DNA-binding recognition helix sequences shown in a single row of **Table 1**. In some embodiments, an engineered ZFP-TF described herein comprises the recognition helix and backbone portions of a sequence shown in a single row of **Table 2**. In some embodiments, an engineered ZFP-TF described herein comprises an amino acid sequence as shown in a single row of **Table 2**. In some embodiments, an engineered ZFP-TF described herein comprises the recognition helix and backbone portions of a sequence shown in a single row of **Table 2** as the sequence would appear following post-translational modification. In some embodiments, an engineered ZFP-TF described herein comprises an amino acid sequence as shown in a single row of **Table 2** as the sequence would appear following post-translational modification. For example, post-translational modification may remove the initiator methionine residue from a sequence as shown in **Table 2**.

15 **B. Peptide Linkers**

[0051] The ZFP domain and the transcription repressor domain of the present ZFP-TFs and/or the zinc fingers within the ZFP domains may be linked through a peptide linker, e.g., a noncleavable peptide linker of about 5 to 200 amino acids (e.g., 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more amino acids). Preferred linkers are typically flexible amino acid subsequences that are synthesized as a recombinant fusion protein. *See, e.g.,* description above; and U.S. Pats. 6,479,626; 6,903,185; 7,153,949; 8,772,453; and 9,163,245; and WO 2011/139349. The proteins described herein may include any combination of suitable linkers. Non-limiting examples of linkers are DGGGS (SEQ ID NO: 2), TGEKP (SEQ ID NO: 3), LRQKDGERP (SEQ ID NO: 4), GGRR (SEQ ID NO: 5), GGRRGGGS (SEQ ID NO: 6), LRQRDGERP (SEQ ID NO: 7), LRQKDGGGGERP (SEQ ID NO: 8), LRQKD(G₃S)₂ERP (SEQ ID NO: 9), and TGSQKP (SEQ ID NO: 10).

[0052] In some embodiments, TGEKPFA (SEQ ID NO: 15) and/or TGSQKPFQ (SEQ ID NO: 16) links the zinc fingers within the ZFP domain, and/or LRQKDAARGSGG (SEQ ID NO: 17) or LRGSGG (SEQ ID NO: 18) links the ZFP domain to the transcription repressor domain.

[0053] In some embodiments, the peptide linker is three to 20 amino acid residues in length and is rich in G and/or S. Non-limiting examples of such linkers are G₄S-type linkers (SEQ ID NO: 11), i.e., linkers containing one or more (e.g., 2, 3, or 4) GGGGS

(SEQ ID NO: 11) motifs, or variations of the motif (such as ones that have one, two, or three amino acid insertions, deletions, and substitutions from the motif).

IV. Expression of the ZFP-TFs

- 5 [0054] A ZFP-TF of the present disclosure may be introduced to a patient through a nucleic acid molecule encoding it. The nucleic acid molecule may be an RNA or cDNA molecule. The nucleic acid molecule may be introduced into the brain of the patient through injection of a composition comprising a lipid:nucleic acid complex (e.g., a liposome). Alternatively, the ZFP-TF may be introduced to the patient through a nucleic acid expression vector comprising a sequence encoding the ZFP-TF. The expression vectors may include expression control sequences such as promoters, enhancers, transcription signal sequences, and transcription termination sequences that allow expression of the coding sequence for the ZFP-TFs in the cells of the nervous system. In some embodiments, the expression vector remains present in the cell as a stable episome.
- 10 In other embodiments, the expression vector is integrated into the genome of the cell.
- [0055] In some embodiments, the promoter on the vector for directing the ZFP-TF expression in the brain is a constitutive active promoter or an inducible promoter. Suitable promoters include, without limitation, a retroviral RSV LTR promoter (optionally with an RSV enhancer), a CMV promoter (optionally with a CMV enhancer),
- 20 a CMV immediate early promoter, an SV40 promoter, a dihydrofolate reductase (DHFR) promoter, a β -actin promoter, a phosphoglycerate kinase (PGK) promoter, an EF1 α promoter, a MoMLV LTR promoter, a CK6 promoter, a transthyretin promoter (TTR), a TK promoter, a tetracycline responsive promoter (TRE), an HBV promoter, an hAAT promoter, chimeric liver-specific promoters (LSPs), an E2F promoter, the telomerase (hTERT) promoter, a CMV enhancer/chicken β -actin/rabbit β -globin promoter (CAG promoter; Niwa et al., *Gene* (1991) 108(2):193-9), and an RU-486-responsive promoter. Brain cell-specific promoters such as a synapsin I promoter, a MeCP2 promoter, a CAMKII promoter, a PrP promoter, a GFAP promoter, or an engineered or natural promoter that restricts expression to neurons and/or glial cells may also be used.
- 30 [0056] Any method of introducing the nucleotide sequence into a cell may be employed, including but not limited to, electroporation, calcium phosphate precipitation, microinjection, cationic or anionic liposomes, liposomes in combination with a nuclear localization signal, naturally occurring liposomes (e.g., exosomes), or viral transduction.

[0057] For *in vivo* delivery of an expression vector, viral transduction may be used. A variety of viral vectors known in the art may be adapted by one of skill in the art for use in the present disclosure, for example, vaccinia vectors, adenoviral vectors, lentiviral vectors, poxyviral vectors, adeno-associated viral (AAV) vectors, retroviral vectors, and hybrid viral vectors. In some embodiments, the viral vector used herein is a recombinant AAV (rAAV) vector. AAV vectors are especially suitable for CNS gene delivery because they infect both dividing and non-dividing cells, exist as stable episomal structures for long term expression, and have very low immunogenicity (Hadaczek et al., *Mol Ther.* (2010) 18:1458-61; Zaiss, et al., *Gene Ther.* (2008) 15:808-16). Any suitable AAV serotype may be used. For example, the AAV may be AAV1, AAV2, AAV3, AAV3B, AAV4, AAV5, AAV6, AAV7, AAV8, AAV8.2, AAV9, or AAVrh10, or of a pseudotype such as AAV2/8, AAV2/5, AAV2/6, or AAV2/9, or a serotype that is the variant or derivative of one of the AAV serotypes listed herein (i.e., AAV derived from multiple serotypes; for example, the rAAV comprises AAV2 inverted terminal repeats (ITR) in its genome and an AAV8, 5, 6, or 9 capsid). In some embodiments, the expression vector is an AAV viral vector and is introduced to the target human cell by a recombinant AAV virion whose genome comprises the construct, including having the AAV Inverted Terminal Repeat (ITR) sequences on both ends to allow the production of the AAV virion in a production system such as an insect cell/baculovirus production system or a mammalian cell production system. The AAV may be engineered such that its capsid proteins have reduced immunogenicity or enhanced transduction ability in humans or nonhuman primates. In some embodiments, AAV9 is used. Viral vectors described herein may be produced using methods known in the art. Any suitable permissive or packaging cells may be employed to produce the viral particles. For example, mammalian or insect cells may be used as the packaging cell line.

V. Pharmaceutical Applications

[0058] The present ZFP-TFs can be used to treat patients in need of downregulation of alpha-synuclein expression. The patients suffer from, or are at risk of developing, neurodegenerative diseases such as Parkinson's disease, Lewy body dementia, Alzheimer's disease, multiple system atrophy, and any other synucleinopathies. Patients at risk include those who are genetically predisposed, those who have suffered repeated brain injuries such as concussions, and those who have been exposed to environmental neurotoxins. The present disclosure provides a method of treating a neurological disease

(e.g., a neurodegenerative disease) in a subject such as a human patient in need thereof, comprising introducing to the nervous system of the subject a therapeutically effective amount (e.g., an amount that allows sufficient repression of *SNCA* expression) of the ZFP-TF (e.g., an rAAV vector expressing it). The term “treating” encompasses
5 alleviation of symptoms, prevention of onset of symptoms, slowing of disease progression, improvement of quality of life, and increased survival.

[0059] The present disclosure provides a pharmaceutical composition comprising a viral vector such as a recombinant AAV (rAAV) whose recombinant genome comprises an expression cassette for the ZFP-TFs. The pharmaceutical composition may further
10 comprise a pharmaceutically acceptable carrier such as water, saline (e.g., phosphate-buffered saline), dextrose, glycerol, sucrose, lactose, gelatin, dextran, albumin, or pectin. In addition, the composition may contain auxiliary substances, such as, wetting or emulsifying agents, pH-buffering agents, stabilizing agents, or other reagents that enhance the effectiveness of the pharmaceutical composition. The pharmaceutical
15 composition may contain delivery vehicles such as liposomes, nanocapsules, microparticles, microspheres, lipid particles, and vesicles.

[0060] The cells targeted by the therapeutics of the present disclosure are cells in the brain, including, without limitation, a neuronal cell (e.g., a motor neuron, a sensory neuron, a dopaminergic neuron, a cholinergic neuron, a glutamatergic neuron, a
20 GABAergic neuron, or a serotonergic neuron); a glial cell (e.g., an oligodendrocyte, an astrocyte, a pericyte, a Schwann cell, or a microglial cell); an ependymal cell; or a neuroepithelial cell. The brain regions targeted by the therapeutics may be those most significantly affected in synucleinopathies, such as the striatum, caudate, putamen, substantia nigra, midbrain, olfactory bulb, cerebellum, locus coeruleus, pons, medulla,
25 brainstem, globus pallidus, hippocampus, and cerebral cortex, or other brain regions. These regions can be reached directly through intrastriatal injection, intranigral injection, intracerebral injection, intra-cisterna magna (ICM) injection, or more generally through intraparenchymal injection, intracerebroventricular (ICV) injection, intrathecal injection, or intravenous injection. Other routes of administration include, without limitation,
30 intraventricular, intranasal, or intraocular administration. In some embodiments, the viral vector spreads throughout the CNS tissue following direct administration into the cerebrospinal fluid (CSF), e.g., via intrathecal and/or intracerebroventricular injection, or intracisterna-magna injection. In other embodiments, the viral vectors cross the blood-brain barrier and achieve wide-spread distribution throughout the CNS tissue of a subject

following intravenous administration. In other embodiments, the viral vectors are delivered directly to the target regions via intraparenchymal injections. In some cases, the viral vectors may undergo retrograde or anterograde transport to other brain regions following intraparenchymal delivery. In some aspects, the viral vectors have distinct
5 CNS tissue targeting capabilities (e.g., CNS tissue tropisms), which achieve stable and nontoxic gene transfer at high efficiencies.

[0061] By way of example, the pharmaceutical composition may be provided to the patient through intraventricular administration, e.g., into a ventricular region of the forebrain of the patient such as the right lateral ventricle, the left lateral ventricle, the
10 third ventricle, or the fourth ventricle. The pharmaceutical composition may be provided to the patient through intracerebral administration, e.g., injection of the composition into or near the striatum, caudate, putamen, substantia nigra, midbrain, olfactory bulb, cerebrum, cerebellum, locus coeruleus, pons, medulla, brainstem, globus pallidus, hippocampus, cerebral cortex, intracranial cavity, meninges, dura mater, arachnoid mater,
15 or pia mater of the brain. Intracerebral administration may include, in some cases, administration of an agent into the cerebrospinal fluid (CSF) of the subarachnoid space surrounding the brain.

[0062] In some cases, intracerebral administration involves injection using stereotaxic procedures. Stereotaxic procedures are well known in the art and typically involve the use
20 of a computer and a 3-dimensional scanning device that are used together to guide injection to a particular intracerebral region, e.g., a ventricular region. Micro-injection pumps (e.g., from World Precision Instruments) may also be used. In some cases, a microinjection pump is used to deliver a composition comprising a viral vector. In some cases, the infusion rate of the composition is in a range of 0.1 $\mu\text{l}/\text{min}$ to 100 $\mu\text{l}/\text{min}$. As
25 will be appreciated by the skilled artisan, infusion rates will depend on a variety of factors, including, for example, species of the subject, age of the subject, weight/size of the subject, serotype of the AAV, dosage required, and intracerebral region targeted. Thus, other infusion rates may be deemed by a skilled artisan to be appropriate in certain circumstances.

[0063] Delivery of rAAVs to a subject may be accomplished, for example, by
30 intravenous administration. In certain instances, it may be desirable to deliver the rAAVs locally to the brain tissue, the spinal cord, cerebrospinal fluid (CSF), neuronal cells, glial cells, meninges, astrocytes, oligodendrocytes, microglia, interstitial spaces, and the like. In some cases, recombinant AAVs may be delivered directly to the CNS by injection into

the ventricular region, as well as to the striatum, caudate, putamen, substantia nigra, midbrain, olfactory bulb, cerebellum, locus coeruleus, pons, medulla, brainstem, globus pallidus, hippocampus, cerebral cortex, or other brain region. AAVs may be delivered with a needle, catheter or related device, using neurosurgical techniques known in the art, such as by stereotactic injection (*see, e.g.,* Stein et al., *J Vir.* (1999) 73:3424-9; Davidson et al., *PNAS.* (2000) 97:3428-32; Davidson et al., *Nat Genet.* (1993) 3:219-223; and Alisky and Davidson, *Hum. Gene Ther.* (2000) 11:2315-29).

[0064] Unless otherwise defined herein, scientific and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. Exemplary methods and materials are described below, although methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present disclosure. In case of conflict, the present specification, including definitions, will control. Generally, nomenclature used in connection with, and techniques of neurology, medicine, medicinal and pharmaceutical chemistry, and cell biology described herein are those well-known and commonly used in the art. Enzymatic reactions and purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Throughout this specification and embodiments, the words "have" and "comprise," or variations such as "has," "having," "comprises," or "comprising," will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers. All publications and other references mentioned herein are incorporated by reference in their entirety. Although a number of documents are cited herein, this citation does not constitute an admission that any of these documents forms part of the common general knowledge in the art. As used herein, the term "approximately" or "about" as applied to one or more values of interest refers to a value that is similar to a stated reference value. In certain embodiments, the term refers to a range of values that fall within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context.

[0065] In order that this invention may be better understood, the following examples are set forth. These examples are for purposes of illustration only and are not to be construed as limiting the scope of the invention in any manner.

EXAMPLES

Example 1: Screening of ZFP-TFs

[0066] In order to identify ZFP-TFs that repress the expression of alpha-synuclein, we designed and screened a library of 416 ZFP-TFs predicted to bind 15 or 18 bp sequences in the region of the human *SNCA* gene spanning from 500 bps upstream of TSS 1 to 500 bps downstream of TSS 1 or 500 bp upstream of TSS 2a to 500 bp downstream of TSS 2b (FIG. 2B). The target regions of the ZFP-TFs are denoted by arrowheads in FIG. 2B, with the direction of the arrowhead indicating the strand of DNA the ZFP-TF binds to (5' to 3'). FIG. 4 shows similar data to those displayed in FIG. 2B for ZFP-TFs with 1, 2, or 3 phosphate contact changes targeting TSSs 2a and 2b of the *SNCA* gene. In this study, a KRAB domain sequence (SEQ ID NO: 12) was used as the transcription repressor and fused to the C-terminus of the ZFP domain. The sequences of 47 representative ZFP-TFs are shown in Table 2 below. Templates for *in vitro* transcription were generated from pVAX-ZFP or pVAX-GFP plasmids using PCR (forward primer GCAGAGCTCTCTGGCTAACTAGAG (SEQ ID NO: 13); reverse primer T(180)CTGGCAACTAGAAGGCACAG (SEQ ID NO: 14). Messenger RNA was synthesized using a mMESSAGE mMACHINE T7 ULTRA Transcription Kit (Thermo Fisher Scientific) as per the manufacturer's instructions and purified using RNeasy96 columns (Qiagen). RNA encoding each ZFP-TF was then aliquoted into 96-well plates in a 6-dose dilution.

[0067] Recombinant rAAV vectors carrying the ZFP-TF coding sequences were generated in HEK293 cells according to well-known methods. Three days after the cells were transfected with plasmids encoding AAV helper genes and the rAAV genome, the cells were harvested. The cells were then lysed by three rounds of freeze/thaw and the cell debris was removed by centrifugation. The rAAV virions were precipitated using polyethylene glycol. After resuspension, the virions were purified by ultracentrifugation overnight on a cesium chloride gradient. The virions were formulated by dialysis and then filter-sterilized. The AAVs were aliquoted and stored at -80°C until use. The AAVs were not re-frozen after thawing.

[0068] The screening was performed in the SK-N-MC human neuroepithelial cell line. SK-N-MC cells express human alpha-synuclein at high levels and are thus appropriate for testing of ZFP-TFs that reduce alpha-synuclein expression. The SK-N-MC cells were cultured in tissue culture flasks until confluency. The cells were plated on 96-well plates

at 150,000 cells per well and were resuspended in Amaxa® SF solution. The cells were then mixed with ZFP-TF RNA (6 doses: 3, 10, 30, 100, 300, and 1000 ng) and transferred to Amaxa® shuttle plate wells. The cells were transfected using the Amaxa® Nucleofector® device (Lonza; program CM-137). Eagle's MEM cell media was added to each well of the plate. The cells were transferred to a 96-well tissue culture plate and incubated at 37°C for 24 hours.

[0069] The ZFP-TFs were also tested in human iPSC-derived GABAergic neurons (Cellular Dynamics International). The cells were plated onto poly-L-ornithine- and laminin-coated 96-well plates at a density of 40,000 cells per well and then maintained according to the manufacturer's instructions. The cells were transfected with AAV6 expressing the desired ZFP-TF at 6 different MOIs (1E3, 3E3, 1E4, 3E4, 1E5, and 3E5) 48 hours after plating. The transduced cells were maintained for up to 32 days (50-75% media changes performed every 3-5 days). The cells were harvested 28-30 days after AAV transfection.

[0070] Harvested cells were lysed and reverse transcription was performed using the C2CT kit following the manufacturer's instructions. TaqMan quantitative polymerase chain reaction (qPCR) was used to measure the expression levels of *SNCA*. *SNCA* expression levels were normalized to the geometric mean of the expression levels of the housekeeping genes *EIF4A2*, *ATP5B* and *GAPDH*. A mock transfection and transfection with a ZFP-TF known not to target *SNCA* were used as negative controls.

[0071] Dose-dependent repression of alpha-synuclein was demonstrated with many of the ZFP-TFs tested. The maximum repression achieved was more than 99%, but we also identified ZFP-TFs that repressed alpha-synuclein to a lesser degree (e.g., about 90%, about 75%, or about 40% at the highest dose). FIGs. 3A-E and 5 show the screening data.

Example 2: Off-Target Activity of Alpha-Synuclein ZFP-TFs

[0072] To evaluate the off-target impact of the alpha-synuclein ZFP-TFs on global gene expression, we performed microarray experiments on total RNA isolated from human iPSC-derived neurons and primary mouse cortical neurons treated with AAVs encoding the representative alpha-synuclein ZFP-TFs.

[0073] Human iPSC-derived neurons were treated as described in Example 1. For microarray analysis, the cells were plated onto poly-L-ornithine- and laminin-coated 24-well plates at a density of 260,000 cells per well, transfected with 1E5 VGs/cell 48 hours

after plating, and harvested 19 days after viral transfection. RNA isolated from the harvested cells was used for microarray analysis.

- [0074] Primary mouse cortical neurons were purchased from Gibco. Cells were plated onto poly-D-lysine-coated 24-well plates at 200,000 cells/well and maintained according to the manufacturer's specifications using Gibco Neurobasal Medium containing GlutaMAX™ I supplement, B27 supplement, and penicillin/streptomycin. Forty-eight hours after plating (at DIV2), the cells were infected with AAV6 at an MOI of 3E3 VGs/cell and harvested 7 days later (at DIV9; 50% media exchanges performed every 3-4 days). This was followed by RNA isolation and microarray analysis.
- [0075] Off-target analysis was performed using the GeneTitan™ platform (Clariom S kit) according to the manufacturer's instructions. The assay results were analyzed using TAC software. Differentially regulated genes with FDR-corrected p-values ≤ 0.05 that were regulated by >2-fold were called out in the analysis. A ZFP-TF known to have minimal off-targets and a mock transfection were used as negative controls.
- [0076] **FIGs. 7A-D** show the microarray results of 40 representative alpha-synuclein ZFP-TFs in human iPSC-derived neurons and primary mouse cortical neurons. There was a range of off-target activity, with some ZFP-TFs displaying very low to no detectable off-target activity.

20 **Example 3: *In Vivo* Repression of Alpha-Synuclein mRNA Expression**

- [0077] AAV9 constructs expressing two representative ZFP-TFs (82195 and 82264) with minimal to no detectable off-target activity in both human and mouse neurons and different maximal repression activity in human iPSC-derived neurons (82195, ~95%; 82264, ~80%) were used to demonstrate *in vivo* repression of human *SNCA* in the PAC synuclein mouse model (Kuo et al., *Hum Mol Genet.* (2010) 19(9):1633-50). This mouse model expresses the full human *SNCA* sequence along with its upstream regulatory sequence on a mouse alpha-synuclein-null background. AAV9 vectors expressing each ZFP-TF and vehicle were bilaterally administered to the striatum at two sites in 8-week-old female PAC synuclein mice (n = 3 per group) at a rate of 0.5 μ L/minute (2 sites per hemisphere: 5 μ L to anterior striatum and 4 μ L to the posterior striatum, a total of 9 μ L/hemisphere and 18 μ L/animal). Following infusion, the needle was left in place for 5 minutes to allow the test article to diffuse. The needle was then slowly retracted over 1-2 minutes. The stereotaxic coordinates for the injections were as follows (rostral striatum - AP: + 1.4 mm, ML: +/-1.7 mm, DV: -3.0 mm; caudal striatum: AP: +0.2 mm, ML: +/-

2.3 mm, DV: -2.7 mm). The mice were euthanized after 3 weeks and their brains were collected for molecular analyses. At the time of euthanasia, the animals were transcardially perfused with 0.9% saline, and the brain was removed and hemisected. The left hemisphere was further dissected into 12 different regions (olfactory bulb; rostral, medial, and caudal cortex; rostral, medial, and caudal striatum; hippocampus; thalamus; ventral midbrain; medulla; and cerebellum). The dissected tissues were placed in RNALater to preserve RNA integrity. After 24 h, RNALater was removed and the tissues were flash-frozen in liquid nitrogen and maintained on dry ice until storage at -80°C.

[0078] Brain tissues were transferred to 1.5 mL Eppendorf tubes containing 0.6 mL TRI reagent (Thermo Fisher) and two 3.2 mm steel beads (BioSpec Products) on ice. The samples were lysed using a Qiagen TissueLyser at 4°C using the following parameters: 5 cycles, 90 s duration, and 25.1 frequency. After a brief spin, 70 µL of 1-bromo-3-chloropropane was added to each sample at room temperature. The samples were vortexed for 10 s, centrifuged at 12,000 ×g for 10 min at 4°C, and 120 µL of the aqueous phase from each sample was transferred to wells of a 96-well plate.

[0079] Sixty microliters of isopropyl alcohol and 12 µL of MagMax magnetic beads (Thermo Scientific) were added to each well containing the aqueous phase of the tissue lysate. A Kingfisher 96 robot (Thermo Scientific) and the MagMax kit (Thermo Fisher) were used to isolate ribonucleic acid (RNA) from the tissue lysate following the manufacturer's protocol. One hundred microliters of the eluted RNA were separated from the magnetic beads using a magnetic stand. RNA yield and quality were evaluated using a Nanodrop 8000 instrument (Thermo Scientific).

[0080] Complementary deoxyribonucleic acid (cDNA) was prepared using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems), with 10 µL of RNA and 10 µL of RT Master Mix (10x RT buffer, 10x random primer, 25x dNTP mix, Multiscribe enzyme, and RNase-free water) by default. If needed, the RNA and RT Master Mix volumes were adjusted to ensure that the input was in the 100 to 1,000 ng range. Reverse transcription was performed on a C1000 Touch Biorad thermal cycler using the following program: 25°C for 10 min, 37°C for 120 min, 85°C for 5 min, and hold at 4°C.

[0081] cDNA was subjected to RT-qPCR using Biorad CFX384 thermal cyclers. cDNA was diluted 10 fold in nuclease-free water, and 4 µL of diluted cDNA were added to each 10 µL PCR reaction. Each sample was assayed in technical quadruplicate. 2x

Fast Multiplex PCR (Qiagen) master mix was used for triplex assays, and SsoAdvanced Universal Probes Supermix (Biorad) was used for other assays.

[0082] The following cycling conditions were used: Qiagen Fast Multiplex master mix → 95°C for 5 min, 95°C for 45 s, 60°C for 45 s, plate read, 40 cycles; Biorad

5 SsoAdvanced master mix → 95°C for 90 s, 95°C for 12 s, 60°C for 40 s, plate read, 42 cycles.

[0083] Spiking of GFP RNA before vs. after the RNA isolation step was used to assess % RNA recovery. A 4-sample 5-fold dilution series of pooled RNA from the animals in the study was used as a standard curve. Sample data were normalized to the geometric
10 mean of the three housekeeping genes *ATP5B*, *EIF4A2*, and *GAPDH*.

[0084] Alpha-synuclein, ZFP-TF, GFAP, IBA1, and NeuN mRNA expression data from the experiment are shown in **FIGs. 8A** and **8B**. The data show that there was repression of alpha-synuclein in brain regions with significant ZFP-TF expression. Furthermore, the GFAP, IBA1, and NeuN expression data indicate that the ZFP-TFs were
15 well-tolerated, as their administration did not lead to elevated expression of markers of neuroinflammation or reduced expression of the neuronal marker NeuN.

LIST OF SEQUENCES

[0085] **Table 1** below lists 47 exemplary engineered ZFPs of the present disclosure.
20 For each ZFP, the genomic target sequence (Binding Sequence) and the DNA-binding recognition helix sequences (i.e., F1-F6) of each zinc finger within the ZFP domain are shown in a single row. “^” in below table indicates that the arginine (R) residue at the 4th position upstream of the 1st amino acid in the indicated helix is changed to glutamine (Q). The SEQ ID NO for each sequence is shown in parenthesis underneath the sequence. In
25 the nucleotide sequences in column 2, nucleotides contacted by the ZFP are shown in capital letters.

Table 1 Exemplary ZFPs

ZFP name	Binding Sequence (capital letters)	F1	F2	F3	F4	F5	F6
SBS-82090	aTCCGAGATAG GGaCGAGGAg (19)	QSGHLAR (20)	QRKDLTQ (21)	RSDHLNR (22)	TRDGLRQ (23)	RSANLAR (24)	DSSDRKK (25)
SBS-82092	cCGAGATAGGG ACGAGGAGc (26)	RSDNLST (27)	RSDNLTR (28)	DRSDLSR (29)	RREHLRA (30)	TSGNLTR (31)	QRSHLSD (32)
SBS-82107	cAGGGAAaGCA GCGAGCGCCg (33)	ERGTLAR (34)	MACCRYA (35)	RSADLTR (36)	QSGDLTR (37)	QSGNLAR (38)	RQEHKVA (39)

ZFP name	Binding Sequence (capital letters)	F1	F2	F3	F4	F5	F6
SBS-82110	gAAAGCAGCGA GCGCCGGa (40)	RSAHLRS (41)	DRSDLSR (29)	MSCCRSA (42)	RSADLSR (43)	QSGDLTR (37)	QRSNLNI (44)
SBS-82136	gGACGGCGACG ACCAGAAg (45)	RSDNLSV (46)	ASWTLTQ (47)	DRSDLSR (29)	DRSNLTR (48)	DRSHLTR (49)	DRSNLTR (48)
SBS-82148	cGCGACGCGGA AGTGAGGTg (50)	LRHHLTR (51)	VAEYRYK (52)	RSDNLSV (46)	RHSHLTS (53)	RSDTLA (54)	RSADLSR (43)
SBS-82190	cGCTCAGGAGG GTGGAAAg (55)	RSDNLST (27)	QSGHLRS (56)	DAHHLHR (57)	RSANLTR (58)	DRSNRTT (59)	HRSSLRR (60)
SBS-82195	aTTCGACGACA GGTAGCGg (61)	RSDDLTR (62)	QKFPDRS (63)	RSDHLTQ (64)	DSANLSR (65)	DRSNLSR (66)	DRTSLKW (67)
SBS-82208	gGCCGCTGTGA GCCGGCGAGc (68)	DRSNLSR (66)	HLCCLGR (69)	RSCCLHL (70)	RNASRTR (71)	QSSDLRS (72)	HRKSLRS (73)
SBS-82215	aGGCGGCACCC GGgGGGACT (74)	DRSNLSR (66)	DRSHLAR (75)	RSDHLSE (76)	TSSDRTK (77)	DRSHLSR (78)	DRSHLAR (75)
SBS-82225	gAGCGCACAGG AAgGGCGAG (79)	DRSHLTR (49)	DRSHLAR (75)	QSGNLAR (38)	AKWNLDA (80)	QSGDLTR (37)	TQGYLRK (81)
SBS-82242	gGCGGGGAGGA GTCGGAGTTg (82)	TSGSLRS (83)	QAGHLAK (84)	DRSARTR (85)	QSGHLRS (56)	RSAHLRS (41)	QSSDLRR (86)
SBS-82294	gGTCGTGgGCA CCGGAGGGg (87)	RSDHLRS (22)	QHHRNT (88)	RPDTLRD (89)	QNATRTK (90)	RSDALRS (91)	DRSALAR (92)
SBS-82329	gGCTGCTTCTC CGGATCCGc (93)	NNRDLIN (94)	TSSNLSR (95)	RSDTLSE (96)	NNRDRTK (97)	QSSDLRS (72)	HRSTRNR (98)
SBS-82135	gGAGGACGGCG ACgACCAGAA (99)	QNAHRKT (100)	DNSTRIK (101)	DRSNLSR (66)	HLCCLGR (69)	DRSNLSR (66)	TSANLSR (102)
SBS-82144	aGAGGGGCGGA GCGACCGAG (103)	QRNHRRT (104)	DRSNLTR (48)	MSCCRSA (42)	RSADLSR (43)	RSAHLRS (41)	TSANLSR (102)
SBS-82150	cGTCGCGCGC TCgGTCGCTc (105)	QSSDLRS (72)	DRSALAR (92)	DRSYRNT (106)	RRSDLKR (107)	RSADLTR (36)	DRSALAR (92)
SBS-82158	cTGGCTGCAG CCcGCACGCa (108)	DSSTRKT (109)	QSATRTK (110)	ERGTLAR (34)	QSGDLTR (37)	QSSDLRS (72)	AASNRSK (111)
SBS-82197	cGTCGAAtGGC CACTCCAGt (112)	RSDNLSQ (113)	ASNDRKK (114)	YDYGRYT (115)	DRSHLAR (75)	QSGNLAR (38)	DRSALAR (92)
SBS-82264	gTACCTGTGGA TCTAAACGGg (116)	RSDHLSE (76)	QSNHRKT (117)	VYEGCLK (118)	QSGHLRS (56)	RSDVLSE (119)	DRSNRIK (120)
SBS-84948	aAAGTGGgGGC GCTTTTGCGc (121)	^RRADLSR (122)	YHWWLKK (123)	^QSSDLRS (72)	DRSHLAR (75)	^RSDHLSQ (124)	TSANRTT (125)
SBS-84953	gCAAGGCGGGA AAGTGGGg (126)	^RSDHLST (127)	RSDALAR (128)	^QSANRTT (129)	RSAHLRS (41)	DRSHLTR (49)	QSGNLHV (130)
SBS-84954	gCAAGGCGGGA AAGTGGGg (126)	^RSDHLST (127)	RSDALAR (128)	^QSANRTT (129)	RSAHLRS (41)	^DRSHLTR (49)	QSGNLHV (130)
SBS-84958	gCGCAAGgCGG GAAAGTGGGg (131)	^RSDHLSE (76)	AKHHRKT (132)	QSGNLAR (38)	RQEHVRA (39)	RSDNLSA (133)	QNRDRKN (134)

ZFP name	Binding Sequence (capital letters)	F1	F2	F3	F4	F5	F6
SBS-84961	cGCGCAAGGCG GGAAAGTGg (135)	^RSDSLSV (136)	QNANRKT (137)	RSAHLSR (41)	DRSHLAR (75)	QSANRTT (129)	RRSDLKR (107)
SBS-84962	cGCGCAAGGCG GGAAAGTGg (135)	^RSDSLSV (136)	QNANRKT (137)	^RSAHLSR (41)	DRSHLAR (75)	QSANRTT (129)	RRSDLKR (107)
SBS-84998	gGAGCACGCTG CAGGGAAAg (138)	^QSANRTT (129)	RSAHLSR (41)	^QSGDLTR (37)	QSSDLRR (86)	DQSNLRA (139)	RSANLTR (58)
SBS-84999	gGAGCACGCTG CAGGGAAAg (138)	^QSANRTT (129)	RSAHLSR (41)	^QSGDLTR (37)	QSSDLRR (86)	^DQSNLRA (139)	RSANLTR (58)
SBS-85000	aGCACGCTGCA GGGAAAGCAg (140)	^QSGDLTR (37)	QRSNLNI (44)	RSAHLSR (41)	QSGDLTR (37)	DSSTRKT (109)	QSATRTK (110)
SBS-85001	aGCACGCTGCA GGGAAAGCAg (140)	^QSGDLTR (37)	QRSNLNI (44)	^RSAHLSR (41)	QSGDLTR (37)	DSSTRKT (109)	QSATRTK (110)
SBS-85014	tGCAGGGAAAG CAGCGAGCg (141)	^MSCRSA (42)	RSADLSR (43)	^QSGDLTR (37)	QRSNLNI (44)	^RSAHLSR (41)	QSGDLTR (37)
SBS-85042	gTGGGGGCGGA GAGCCGAGg (142)	^RSDNLAR (143)	DRSVLHR (144)	QSAHRKN (145)	RHSHLTS (53)	RSDHLSE (76)	RNDHRTT (146)
SBS-85069	gAGAGGGgGCG AGCGACCGAg (147)	^QRNHRTT (104)	DRSNLTR (48)	MSCRSA (42)	RSADLSR (43)	RSDHLSE (22)	QGAHLGA (148)
SBS-85096	gcAGCGCAGAC CCCGGCCCGg (149)	^RSDALSV (150)	DSSHRTR (151)	DNRDLSL (152)	DRSNLTR (48)	QSGDLTR (37)	TQGYLRK (81)
SBS-85102	tCTCGGAGGGG CCgGGCCCGg (153)	^RSDHLSQ (124)	DNSHRTR (154)	DRSDLSR (29)	RSTHLVR (155)	DRSHLTR (49)	DRSYRNT (106)
SBS-85105	gGACGCTCTCG GAGGGGCCg (156)	^DRSDLSR (29)	RSTHLVR (155)	DRSHLTR (49)	DRSYRNT (106)	QESDLVR (157)	DRSNLTR (48)
SBS-85177	aAAAAGGAGCGC ACAGGAAGg (158)	^RSDNLVS (46)	RRAHLNQ (159)	DQSNLRA (139)	RRSDLKR (107)	QSGHLAR (20)	QRVALQA (160)
SBS-85180	gGAAAAGgAGC GCACAGGAAG (161)	^QSGNLAR (38)	AKWNLDA (80)	QSGDLTR (37)	TQGYLRK (81)	RSDNLVS (46)	QSGDLTR (37)
SBS-85216	tCAGGTAAgTA CCTGTGGATc (162)	^TSGNLTR (31)	RSELLQS (163)	ARSTRIT (164)	QSGSLTR (165)	QSGSLTR (165)	AKWNLDA (80)
SBS-85217	tCAGGTAAgTA CCTGTGGATc (162)	^TSGNLTR (31)	RSELLQS (163)	^ARSTRIT (164)	QSGSLTR (165)	QSGSLTR (165)	AKWNLDA (80)
SBS-85218	tCAGGTAAgTA CCTGTGGATc (162)	^TSGNLTR (31)	RSELLQS (163)	^ARSTRIT (164)	QSGSLTR (165)	^QSGSLTR (165)	AKWNLDA (80)
SBS-85256	tCTGGGAGGTG AGTACTTGt (166)	^RPYTLRL (167)	DNSNRIK (168)	^RSANLAR (24)	TSGHLSR (169)	QSGHLAR (20)	RKWTLQG (170)
SBS-85257	tCTGGGAGGTG AGTACTTGt (166)	^RPYTLRL (167)	DNSNRIK (168)	^RSANLAR (24)	TSGHLSR (169)	^QSGHLAR (20)	RKWTLQG (170)
SBS-85260	cCAAAGGGACA AGTACTCAc (171)	^QSADRTK (172)	DNSNRIK (168)	^RSDNLST (27)	DSANRIK (173)	^RSDHLSE (76)	QNANRIT (174)
SBS-85261	cCCAAAGgGAC AAGTACTCAc (175)	^QSADRTK (172)	DNSNRIK (168)	RSANLSV (176)	DRANLSR (177)	RSDNLVS (46)	QKATRTT (178)

ZFP name	Binding Sequence (capital letters)	F1	F2	F3	F4	F5	F6
SBS-85262	cCCAAAGgGAC AAGTACTCac (175)	[^] QSADRTK (172)	DNSNRIK (168)	[^] RSANLSV (176)	DRANLSR (177)	RSDNLSV (46)	QKATRIT (178)
SBS-85263	cCCAAAGgGAC AAGTACTCac (175)	[^] QSADRTK (172)	DNSNRIK (168)	[^] RSANLSV (176)	DRANLSR (177)	[^] RSDNLSV (46)	QKATRIT (178)

[0086] Table 2 below lists the full amino acid sequences of 47 exemplary ZFP-TFs of the present disclosure, wherein the DNA-binding recognition helix sequences are in boldface, intramodule and intermodule linkers are underlined. The R(-5)Q backbone mutations are indicated by boldface and underline. The linkers between the ZFP and KRAB domain and double-underlined.

Table 2 Exemplary ZFP-TF Sequences

ZFP-TF name	Amino Acid Sequences
SBS-82090	MAPKKKRRKVGVPAAAMAERPFCQCRICMRNFS QSGHLAR HIRTHTGEKPFAC DICGRKF AQRKDLTQ HTKIHTHPRAPIPKPFQCRICMRNFS RSDHLSR HI RTHHTGEKPFACDICGRKF TRDGLR QHTKIHTGSQKPFQCRICMRNFS RS ANLAR HIRTHTGEKPFACDICGRKF ADSSDRKK HTKIHL RQKDAARGSGG DAKSLTAWSRITLVTFKDVFDFTREEWKLLDTAQQIVYRNVMLNENYKNLV SLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS (SEQ ID NO: 179)
SBS-82092	MAPKKKRRKVGVPAAAMAERPFCQCRICMRNFS RSDNLS THIRTHTGEKPFAC DICGRKF ARSDNL TRHTKIHTGSQKPFQCRICMRNFS DRSDLSR HIRTHT GEKPFACDICGRKF ARREHLRA HTKIHTGSQKPFQCRICMRNFS TSGNLT RH IRTHTGEKPFACDICGRKF QRSHLSD HTKIHL RQKDAARGSGG DAKS LTAWSRITLVTFKDVFDFTREEWKLLDTAQQIVYRNVMLNENYKNLVSLGY QLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS (SEQ ID NO: 180)
SBS-82107	MAPKKKRRKVGVPAAAMAERPFCQCRICMRNFS ERGT LARHIRTHTGEKPFAC DICGRKF AMACCRYA HTKIHTGSQKPFQCRICMRNFS RSADL TRHIRTHT GEKPFACDICGRKF QSGDL TRHTKIHTHPRAPIPKPFQCRICMRNFS QS GNLAR HIRTHTGEKPFACDICGRKF ARQEHRVA HTKIHL RQKDAARGSGG DAKSLTAWSRITLVTFKDVFDFTREEWKLLDTAQQIVYRNVMLNENYKNLV SLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS (SEQ ID NO: 181)
SBS-82110	MAPKKKRRKVGVPAAAMAERPFCQCRICMRNFS RSAHLSR HIRTHTGEKPFAC DICGRKF ADRSDL SRHTKIHTGSQKPFQCRICMRNFS SMCCRS AHIRTHT GEKPFACDICGRKF ARSADLSR HTKIHTGSQKPFQCRICMRNFS QSGDLT RH IRTHTGEKPFACDICGRKF QRSNLNI HTKIHL RQKDAARGSGG DAKS LTAWSRITLVTFKDVFDFTREEWKLLDTAQQIVYRNVMLNENYKNLVSLGY QLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS (SEQ ID NO: 182)

SBS-82136	MAPKKRKRKVGVPAAAMAERPFOCRICMRNFS RSDNLSV HIRTHTGEKPFAC DICGRKF ASWTLTQ HHTKIHTGSQKPFQCRICMRNFS DRSDLSR HIRTHT GEKPFACDICGRKF ADRSNLTR HHTKIHTGSQKPFQCRICMRNFS DRSHLT R HIRTHTGEKPFACDICGRKF ADRSNLTR HHTKIHL RQKDAARGSGG DAKS LTAWSRTLVTFKDVFDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGY QLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS (SEQ ID NO: 183)
SBS-82148	MAPKKRKRKVGVPAAAMAERPFOCRICMRNFS LRHHLTR HIRTHTGEKPFAC DICGRKF VAEYRYK HHTKIHTGSQKPFQCRICMRNFS RSDNLSV HIRTHT GEKPFACDICGRKF ARHSHLTS HHTKIHTGSQKPFQCRICMRNFS RSDTLS A HIRTHTGEKPFACDICGRKF ARSADLSR HHTKIHL RQKDAARGSGG DAKS LTAWSRTLVTFKDVFDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGY QLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS (SEQ ID NO: 184)
SBS-82190	MAPKKRKRKVGVPAAAMAERPFOCRICMRNFS RSDNLS THIRTHTGEKPFAC DICGRKF QSGHLSR HHTKIHTGSQKPFQCRICMRNFS DAHHLHR HIRTHT GEKPFACDICGRKF ARSANL TRHHTKIHTGSQKPFQCRICMRNFS DRSNRT T HIRTHTGEKPFACDICGRKF AHRSSLR HHTKIHL RQKDAARGSGG DAKS LTAWSRTLVTFKDVFDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGY QLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS (SEQ ID NO: 185)
SBS-82195	MAPKKRKRKVGVPAAAMAERPFOCRICMRNFS RSDDLTR HIRTHTGEKPFAC DICGRKF QKFPRD SHTKIHTGSQKPFQCRICMRNFS RSDHLTQ HIRTHT GEKPFACDICGRKF ADSANLSR HHTKIHTGSQKPFQCRICMRNFS DRSNLS R HIRTHTGEKPFACDICGRKF ADRTSLK WHTKIHL RQKDAARGSGG DAKS LTAWSRTLVTFKDVFDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGY QLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS (SEQ ID NO: 186)
SBS-82208	MAPKKRKRKVGVPAAAMAERPFOCRICMRNFS DRSNLSR HIRTHTGEKPFAC DICGRKF AHLCC LGRHTKIHTHPRAPIPKPFQCRICMRNFS RSCCLHL HI RHTHTGEKPFACDICGRKF ARNASR TRHHTKIHTGSQKPFQCRICMRNFS QS SDL SRHIRTHTGEKPFACDICGRKF AHRKSL SRHHTKIHL RQKDAARGSGG DAKSLTAWSTRVLVTFKDVFDFTREEWKLLDTAQQIVYRNVMLENYKNLV SLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS (SEQ ID NO: 187)
SBS-82215	MAPKKRKRKVGVPAAAMAERPFOCRICMRNFS DRSNLSR HIRTHTGEKPFAC DICGRKF ADRS HLARHTKIHTHPRAPIPKPFQCRICMRNFS RSDDLSE HI RHTHTGEKPFACDICGRKF TSSDR TKHTKIHTGSQKPFQCRICMRNFS SDR SHLSR HIRTHTGEKPFACDICGRKF ADRS HLARHTKIHL RQKDAARGSGG DAKSLTAWSTRVLVTFKDVFDFTREEWKLLDTAQQIVYRNVMLENYKNLV SLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS (SEQ ID NO: 188)
SBS-82225	MAPKKRKRKVGVPAAAMAERPFOCRICMRNFS DRSHLTR HIRTHTGEKPFAC DICGRKF ADRS HLARHTKIHTHPRAPIPKPFQCRICMRNFS QSGNLAR HI RHTHTGEKPFACDICGRKF AKWN LDAHTKIHTGSQKPFQCRICMRNFS QS GDL TRHIRTHTGEKPFACDICGRKF ATQGYLR KHTKIHL RQKDAARGSGG DAKSLTAWSTRVLVTFKDVFDFTREEWKLLDTAQQIVYRNVMLENYKNLV SLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS (SEQ ID NO: 189)

SBS-82242	MAPKKRKRKVGVPAAAMAERPFQCRICMRNFS TSGSLSR HIRTHTGEKPFAC DICGRKFA QAGHLAK HTKIHTGSQKPFQCRICMRNFS DRSAR TRHIRTHT GEKPFACDICGRKFA QSGHLSR HHTKIHTHPRAPIPKPFQCRICMRNFS RS AHLSR HIRTHTGEKPFACDICGRKFA QSSDLRR HHTKIHLRQKDAARGSGG DAKSLTAWSR TLVTFKDVFDFTREEWKLLDTAQQIVYRNVMLNENYKNLV SLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS (SEQ ID NO: 190)
SBS-82294	MAPKKRKRKVGVPAAAMAERPFQCRICMRNFS RSDHLSR HIRTHTGEKPFAC DICGRKFA QHAHRN THTKIHTGSQKPFQCRICMRNFS RPD TLRDHIRTHT GEKPFACDICGRKFA QNATRT KHTKIHTHPRAPIPKPFQCRICMRNFS RS DALS RHIRTHTGEKPFACDICGRKF ADRSALAR HHTKIHLRQKDAARGSGG DAKSLTAWSR TLVTFKDVFDFTREEWKLLDTAQQIVYRNVMLNENYKNLV SLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS (SEQ ID NO: 191)
SBS-82329	MAPKKRKRKVGVPAAAMAERPFQCRICMRNFS NNRDLIN HIRTHTGEKPFAC DICGRKFAT SSNLSR HHTKIHTHPRAPIPKPFQCRICMRNFS RS DTLSEHI RHTHTGEKPFACDICGRKF ANNRDR THTKIHTGSQKPFQCRICMRNFS QS SDLSR HIRTHTGEKPFACDICGRKF AHRSTRNR HHTKIHLRQKDAARGSGG DAKSLTAWSR TLVTFKDVFDFTREEWKLLDTAQQIVYRNVMLNENYKNLV SLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS (SEQ ID NO: 192)
SBS-82135	MAPKKRKRKVGVPAAAMAERPFQCRICMRNFS QNAHRK THIRTHTGEKPFAC DICGRKF ADNSTR IKHTKIHTHPRAPIPKPFQCRICMRNFS DRSNLSR HI RHTHTGEKPFACDICGRKF AHLCCLGR HHTKIHTGSQKPFQCRICMRNFS SDR SNLSR HIRTHTGEKPFACDICGRKF ATSANLSR HHTKIHLRQKDAARGSGG DAKSLTAWSR TLVTFKDVFDFTREEWKLLDTAQQIVYRNVMLNENYKNLV SLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS (SEQ ID NO: 193)
SBS-82144	MAPKKRKRKVGVPAAAMAERPFQCRICMRNFS QRNHR TTHTIRTHTGEKPFAC DICGRKF ADRSNL TRHTKIHTGSQKPFQCRICMRNFS SMSCCR SAHIRTHT GEKPFACDICGRKF ARSADLSR HHTKIHTGSQKPFQCRICMRNFS RS SAHLS RH IRTHTGEKPFACDICGRKF ATSANLSR HHTKIHLRQKDAARGSGGDAKS LTAWSR TLVTFKDVFDFTREEWKLLDTAQQIVYRNVMLNENYKNLVSLGY QLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS (SEQ ID NO: 194)
SBS-82150	MAPKKRKRKVGVPAAAMAERPFQCRICMRNFS QSSDLSR HIRTHTGEKPFAC DICGRKF ADRSALAR HHTKIHTHPRAPIPKPFQCRICMRNFS DRSYR NTHI RHTHTGEKPFACDICGRKF ARRSDLKR HHTKIHTGSQKPFQCRICMRNFS RS ADL TRHIRTHTGEKPFACDICGRKF ADRSALAR HHTKIHLRQKDAARGSGG DAKSLTAWSR TLVTFKDVFDFTREEWKLLDTAQQIVYRNVMLNENYKNLV SLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS (SEQ ID NO: 195)
SBS-82158	MAPKKRKRKVGVPAAAMAERPFQCRICMRNFS DSSTRK THIRTHTGEKPFAC DICGRKFA QSATRT KHTKIHTHPRAPIPKPFQCRICMRNFS ERGT LARHI RHTHTGEKPFACDICGRKFA QSGDL TRHTKIHTGSQKPFQCRICMRNFS QS SDLSR HIRTHTGEKPFACDICGRKFA AASNRSK HHTKIHLRQKDAARGSGG DAKSLTAWSR TLVTFKDVFDFTREEWKLLDTAQQIVYRNVMLNENYKNLV SLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS (SEQ ID NO: 196)

SBS-82197	MAPKKRKRKVGVPAAAMAERPFQCRICMRNFS RS <u>DNLSQ</u> HIRTHTGEKPFAC DICGRKFA AS <u>NDRKK</u> HTKIHTGSQKPFQCRICMRNFS YDYG <u>RYTH</u> IRHTHT GEKPFACDICGRKF ADRS <u>HLAR</u> HTKIHTHPRAPIPKPFQCRICMRNFS QS GN <u>LAR</u> HIRTHTGEKPFACDICGRKF ADRS <u>SALAR</u> HTKIHL <u>RQKDA</u> ARGSGG DAKSLTAWSR TLVTFKDVFDFTREEWKLLDTAQQIVYRNVMLENYKNLV SLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS (SEQ ID NO: 197)
SBS-82264	MAPKKRKRKVGVPAAAMAERPFQCRICMRNFS RS <u>DHLS</u> EHIRTHTGEKPFAC DICGRKFA QSN <u>HRK</u> HTKIHTGSQKPFQCRICMRNFS VY <u>EGLK</u> HIRTHT GEKPFACDICGRKFA QSG <u>HL</u> SRHTKIHTHPRAPIPKPFQCRICMRNFS RS DVL <u>SE</u> HIRTHTGEKPFACDICGRKF ADRS <u>NR</u> IKHTKIHL <u>RQKDA</u> ARGSGG DAKSLTAWSR TLVTFKDVFDFTREEWKLLDTAQQIVYRNVMLENYKNLV SLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS (SEQ ID NO: 198)
SBS-84948	MAPKKRKRKVGVPAAAMAERPFQCRICM <u>Q</u> NFS RR <u>ADLS</u> RHIRTHTGEKPFAC DICGRKFA YH <u>WYLK</u> HTKIHTGSQKPFQCRICM <u>Q</u> NFS QSS <u>DL</u> SRHIRTHT GEKPFACDICGRKF ADRS <u>HLAR</u> HTKIHTHPRAPIPKPFQCRICM <u>Q</u> NFS RS DHLS <u>Q</u> HIRTHTGEKPFACDICGRKF AT <u>SAN</u> RTTHTKIHL <u>RQKDA</u> ARGSGG DAKSLTAWSR TLVTFKDVFDFTREEWKLLDTAQQIVYRNVMLENYKNLV SLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS (SEQ ID NO: 199)
SBS-84953	MAPKKRKRKVGVPAAAMAERPFQCRICM <u>Q</u> NFS RS <u>DHLS</u> THIRTHTGEKPFAC DICGRKF ARSD <u>ALAR</u> HTKIHTGSQKPFQCRICM <u>Q</u> NFS QS <u>AN</u> RTTHTHIRTHT GEKPFACDICGRKF ARSA <u>HL</u> SRHTKIHTGSQKPFQCRICMRNFS DR <u>SHLT</u> R HIRTHTGEKPFACDICGRKFA QSG <u>NL</u> HVHTKIHL <u>RQKDA</u> ARGSGGDAKS LTAWSR TLVTFKDVFDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGY QLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS (SEQ ID NO: 200)
SBS-84954	MAPKKRKRKVGVPAAAMAERPFQCRICM <u>Q</u> NFS RS <u>DHLS</u> THIRTHTGEKPFAC DICGRKF ARSD <u>ALAR</u> HTKIHTGSQKPFQCRICM <u>Q</u> NFS QS <u>AN</u> RTTHTHIRTHT GEKPFACDICGRKF ARSA <u>HL</u> SRHTKIHTGSQKPFQCRICM <u>Q</u> NFS DR <u>SHLT</u> R HIRTHTGEKPFACDICGRKFA QSG <u>NL</u> HVHTKIHL <u>RQKDA</u> ARGSGGDAKS LTAWSR TLVTFKDVFDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGY QLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS (SEQ ID NO: 201)
SBS-84958	MAPKKRKRKVGVPAAAMAERPFQCRICM <u>Q</u> NFS RS <u>DHLS</u> EHIRTHTGEKPFAC DICGRKFA AK <u>HHRK</u> HTKIHTGSQKPFQCRICMRNFS QSG <u>NLAR</u> HIRTHT GEKPFACDICGRKF ARQ <u>EH</u> VAHTKIHTHPRAPIPKPFQCRICMRNFS RS DNLS <u>A</u> HIRTHTGEKPFACDICGRKFA QNR <u>DR</u> KNHTKIHL <u>RQKDA</u> ARGSGG DAKSLTAWSR TLVTFKDVFDFTREEWKLLDTAQQIVYRNVMLENYKNLV SLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS (SEQ ID NO: 202)
SBS-84961	MAPKKRKRKVGVPAAAMAERPFQCRICM <u>Q</u> NFS RS <u>DSL</u> SVHIRTHTGEKPFAC DICGRKFA QNA <u>N</u> RKHTTKIHTGSQKPFQCRICMRNFS RS <u>AHL</u> SRHIRTHT GEKPFACDICGRKF ADRS <u>HLAR</u> HTKIHTGSQKPFQCRICMRNFS QS <u>AN</u> RT T HIRTHTGEKPFACDICGRKF ARR <u>SDL</u> KRHTKIHL <u>RQKDA</u> ARGSGGDAKS LTAWSR TLVTFKDVFDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGY QLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS (SEQ ID NO: 203)

SBS-84962	MAPKKRKRKVGVPAAAMAERPFQCRICM <u>Q</u> NF <u>S</u> RSDSL SVHIRTHTGEKPFAC DICGRKF <u>AQ</u> NANRKT HTKIHTGSQKPFQCRICM <u>Q</u> NF <u>S</u> SAHL SRHIRTHT GEKPFACDICGRKF <u>ADRSH</u> LARHT KIHTGSQKPFQCRICM <u>Q</u> NF <u>S</u> QSANRT TH IRTHTGEKPFACDICGRKF <u>ARRSD</u> LKRHT KIHL <u>RQKDA</u> ARGSGGDAKS LTAWSRTLVTFKDVFDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGY QLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS (SEQ ID NO: 204)
SBS-84998	MAPKKRKRKVGVPAAAMAERPFQCRICM <u>Q</u> NF <u>S</u> QSANRT THIRTHTGEKPFAC DICGRKF <u>ARS</u> AHL SRHTKIHTGSQKPFQCRICM <u>Q</u> NF <u>S</u> QSGDL TRHIRTHT GEKPFACDICGRKF <u>QSSDL</u> RRHT KIHTGSQKPFQCRICM <u>Q</u> NF <u>S</u> DQSNLR A HIRTHTGEKPFACDICGRKF <u>ARSANL</u> TRHT KIHL <u>RQKDA</u> ARGSGGDAKS LTAWSRTLVTFKDVFDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGY QLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS (SEQ ID NO: 205)
SBS-84999	MAPKKRKRKVGVPAAAMAERPFQCRICM <u>Q</u> NF <u>S</u> QSANRT THIRTHTGEKPFAC DICGRKF <u>ARS</u> AHL SRHTKIHTGSQKPFQCRICM <u>Q</u> NF <u>S</u> QSGDL TRHIRTHT GEKPFACDICGRKF <u>QSSDL</u> RRHT KIHTGSQKPFQCRICM <u>Q</u> NF <u>S</u> DQSNLR A HIRTHTGEKPFACDICGRKF <u>ARSANL</u> TRHT KIHL <u>RQKDA</u> ARGSGGDAKS LTAWSRTLVTFKDVFDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGY QLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS (SEQ ID NO: 206)
SBS-85000	MAPKKRKRKVGVPAAAMAERPFQCRICM <u>Q</u> NF <u>S</u> QSGDL TRHIRTHTGEKPFAC DICGRKF <u>QRSNLN</u> I HTKIHTGSQKPFQCRICM <u>Q</u> NF <u>S</u> SAHL SRHIRTHT GEKPFACDICGRKF <u>QSGDL</u> TRHT KIHTHPRAPIPKPFQCRICM <u>Q</u> NF <u>S</u> SDS STRK THIRTHTGEKPFACDICGRKF <u>Q</u> SATRT KHTKIHL <u>RQKDA</u> ARGSGG DAKSLTAWSTRVLVTFKDVFDFTREEWKLLDTAQQIVYRNVMLENYKNLV SLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS (SEQ ID NO: 207)
SBS-85001	MAPKKRKRKVGVPAAAMAERPFQCRICM <u>Q</u> NF <u>S</u> QSGDL TRHIRTHTGEKPFAC DICGRKF <u>QRSNLN</u> I HTKIHTGSQKPFQCRICM <u>Q</u> NF <u>S</u> SAHL SRHIRTHT GEKPFACDICGRKF <u>QSGDL</u> TRHT KIHTHPRAPIPKPFQCRICM <u>Q</u> NF <u>S</u> SDS STRK THIRTHTGEKPFACDICGRKF <u>Q</u> SATRT KHTKIHL <u>RQKDA</u> ARGSGG DAKSLTAWSTRVLVTFKDVFDFTREEWKLLDTAQQIVYRNVMLENYKNLV SLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS (SEQ ID NO: 208)
SBS-85014	MAPKKRKRKVGVPAAAMAERPFQCRICM <u>Q</u> NF <u>S</u> MSCCRS AHIRTHTGEKPFAC DICGRKF <u>ARSADL</u> SRHT KIHTGSQKPFQCRICM <u>Q</u> NF <u>S</u> QSGDL TRHIRTHT GEKPFACDICGRKF <u>QRSNLN</u> I HTKIHTGSQKPFQCRICM <u>Q</u> NF <u>S</u> SAHL R HIRTHTGEKPFACDICGRKF <u>QSGDL</u> TRHT KIHL <u>RQKDA</u> ARGSGGDAKS LTAWSRTLVTFKDVFDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGY QLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS (SEQ ID NO: 209)
SBS-85042	MAPKKRKRKVGVPAAAMAERPFQCRICM <u>Q</u> NF <u>S</u> RSDNL ARHIRTHTGEKPFAC DICGRKF <u>ADRSVL</u> HRHT KIHTGSQKPFQCRICM <u>Q</u> NF <u>S</u> QSAHRK NHIRTHT GEKPFACDICGRKF <u>ARHSHL</u> TSHT KIHTGSQKPFQCRICM <u>Q</u> NF <u>S</u> RSDHL E HIRTHTGEKPFACDICGRKF <u>ARNDHRT</u> TTHT KIHL <u>RQKDA</u> ARGSGGDAKS LTAWSRTLVTFKDVFDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGY QLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS (SEQ ID NO: 210)

SBS-85069	MAPKKRKRKVGVPAAAMAERPFOCRICM <u>Q</u> NFS <u>Q</u> RNHRTTHIRHTHTGEKPFAC DICGRKFADRSNLTRHTKIHTGSQKPFQCRICMRNFSMSCCRS <u>A</u> HIRHTHT GEKPFACDICGRKFARSADLSRHTKIHTHPRAPIPKPFQCRICMRNFSRS <u>DHLS</u> SRHIRTHTGEKPFACDICGRKFAQGAHLGAHTKIHLRQKDAARGSGG DAKSLTAWSR TLVTFKDVFDFTREEWKLLDTAQQIVYRNVMLENYKNLV SLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS (SEQ ID NO: 211)
SBS-85096	MAPKKRKRKVGVPAAAMAERPFOCRICM <u>Q</u> NFSRSDALSVHIRTHTGEKPFAC DICGRKFADSSHRTRHTKIHTGSQKPFQCRICMRNFSDNRDLS <u>T</u> HIRTHT GEKPFACDICGRKFADRSNLTRHTKIHTGSQKPFQCRICMRNFSQSGDLT RHIRTHTGEKPFACDICGRKFATQGYLRKHTKIHLRQKDAARGSGGDAKS LTAWSR TLVTFKDVFDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGY QLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS (SEQ ID NO: 212)
SBS-85102	MAPKKRKRKVGVPAAAMAERPFOCRICM <u>Q</u> NFSRSDHLSQHIRTHTGEKPFAC DICGRKFADNSHRTRHTKIHTHPRAPIPKPFQCRICMRNFSDRSDLSRH RHTHTGEKPFACDICGRKFARSTHLVRHTKIHTGSQKPFQCRICMRNFSDR SHLTRHIRTHTGEKPFACDICGRKFADRSYRNTHTKIHLRQKDAARGSGG DAKSLTAWSR TLVTFKDVFDFTREEWKLLDTAQQIVYRNVMLENYKNLV SLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS (SEQ ID NO: 213)
SBS-85105	MAPKKRKRKVGVPAAAMAERPFOCRICM <u>Q</u> NFSDRSDLSRHIRTHTGEKPFAC DICGRKFARSTHLVRHTKIHTGSQKPFQCRICMRNFSDRSHLTRHIRTHT GEKPFACDICGRKFADRSYRNTHTKIHTGSQKPFQCRICMRNFSQESDLV RHIRTHTGEKPFACDICGRKFADRSNLTRHTKIHLRQKDAARGSGGDAKS LTAWSR TLVTFKDVFDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGY QLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS (SEQ ID NO: 214)
SBS-85177	MAPKKRKRKVGVPAAAMAERPFOCRICM <u>Q</u> NFSRSDNLSVHIRTHTGEKPFAC DICGRKFARRAHLNQHHTKIHTGSQKPFQCRICMRNFSDQSNLRAHIRTHT GEKPFACDICGRKFARRSDLKRRHTKIHTGSQKPFQCRICMRNFSQSGHLA RHIRTHTGEKPFACDICGRKFAQRVALQAHTKIHLRQKDAARGSGGDAKS LTAWSR TLVTFKDVFDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGY QLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS (SEQ ID NO: 215)
SBS-85180	MAPKKRKRKVGVPAAAMAERPFOCRICM <u>Q</u> NFSQSGNLARHIRTHTGEKPFAC DICGRKFAAKWNLDAHTKIHTGSQKPFQCRICMRNFSQSGDLTRHIRTHT GEKPFACDICGRKFATQGYLRKHTKIHTHPRAPIPKPFQCRICMRNFSRS DNLSVHIRTHTGEKPFACDICGRKFAQSGDLTRHTKIHLRQKDAARGSGG DAKSLTAWSR TLVTFKDVFDFTREEWKLLDTAQQIVYRNVMLENYKNLV SLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS (SEQ ID NO: 216)
SBS-85216	MAPKKRKRKVGVPAAAMAERPFOCRICM <u>Q</u> NFSTSGNLTRHIRTHTGEKPFAC DICGRKFARSELLQSHHTKIHTGSQKPFQCRICMRNFSARSTRITHIRTHT GEKPFACDICGRKFAQSGSLTRHTKIHTHPRAPIPKPFQCRICMRNFSQS GSLTRHIRTHTGEKPFACDICGRKFAAKWNLDAHTKIHLRQKDAARGSGG DAKSLTAWSR TLVTFKDVFDFTREEWKLLDTAQQIVYRNVMLENYKNLV SLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS (SEQ ID NO: 217)

SBS-85217	MAPKKRKRKVGVPAAAMAERPFQCRICM <u>Q</u> NFS TSGNLTR HIRTHTGEKPFAC DICGRKF ARSELLQ SHTKIHTGSQKPFQCRICM <u>Q</u> NFS ARSTRIT HIRTHT GEKPFACDICGRKFA QSGSLTR HHTKIHTHPRAPIPKPFQCRICMRNFS QS GSLTR HIRTHTGEKPFACDICGRKFA AKWNLDA HHTKIHLRQKDAARGSGG DAKSLTAWSR TLVT FKDVFDFTREEWKLLDTAQQIVYRNVMLENYKNLV SLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS (SEQ ID NO: 218)
SBS-85218	MAPKKRKRKVGVPAAAMAERPFQCRICM <u>Q</u> NFS TSGNLTR HIRTHTGEKPFAC DICGRKF ARSELLQ SHTKIHTGSQKPFQCRICM <u>Q</u> NFS ARSTRIT HIRTHT GEKPFACDICGRKFA QSGSLTR HHTKIHTHPRAPIPKPFQCRICM <u>Q</u> NFS QS GSLTR HIRTHTGEKPFACDICGRKFA AKWNLDA HHTKIHLRQKDAARGSGG DAKSLTAWSR TLVT FKDVFDFTREEWKLLDTAQQIVYRNVMLENYKNLV SLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS (SEQ ID NO: 219)
SBS-85256	MAPKKRKRKVGVPAAAMAERPFQCRICM <u>Q</u> NFS RPYTLRL HIRTHTGEKPFAC DICGRKF ADNSNRIK HHTKIHTGSQKPFQCRICM <u>Q</u> NFS RSANLAR HIRTHT GEKPFACDICGRKFA TSGHLSR HHTKIHTGSQKPFQCRICMRNFS QSGHLA R HIRTHTGEKPFACDICGRKFA ARKWTLQ GHTKIHLRQKDAARGSGGDAKS LTAWSR TLVT FKDVFDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGY QLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS (SEQ ID NO: 220)
SBS-85257	MAPKKRKRKVGVPAAAMAERPFQCRICM <u>Q</u> NFS RPYTLRL HIRTHTGEKPFAC DICGRKF ADNSNRIK HHTKIHTGSQKPFQCRICM <u>Q</u> NFS RSANLAR HIRTHT GEKPFACDICGRKFA TSGHLSR HHTKIHTGSQKPFQCRICM <u>Q</u> NFS QSGHLA R HIRTHTGEKPFACDICGRKFA ARKWTLQ GHTKIHLRQKDAARGSGGDAKS LTAWSR TLVT FKDVFDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGY QLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS (SEQ ID NO: 221)
SBS-85260	MAPKKRKRKVGVPAAAMAERPFQCRICM <u>Q</u> NFS Q SADRTKHIRTHTGEKPFAC DICGRKF ADNSNRIK HHTKIHTGSQKPFQCRICM <u>Q</u> NFS RS DNLS TH HIRTHT GEKPFACDICGRKF AD SANRIKHTKIHTGSQKPFQCRICM <u>Q</u> NFS RS DNLS E HIRTHTGEKPFACDICGRKFA Q NANRITHTKIHLRQKDAARGSGGDAKS LTAWSR TLVT FKDVFDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGY QLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS (SEQ ID NO: 222)
SBS-85261	MAPKKRKRKVGVPAAAMAERPFQCRICM <u>Q</u> NFS Q SADRTKHIRTHTGEKPFAC DICGRKF ADNSNRIK HHTKIHTGSQKPFQCRICMRNFS RS ANLSVHIRTHT GEKPFACDICGRKF ADRANLSR HHTKIHTHPRAPIPKPFQCRICMRNFS RS DNLSV HIRTHTGEKPFACDICGRKFA Q KATRITHTKIHLRQKDAARGSGG DAKSLTAWSR TLVT FKDVFDFTREEWKLLDTAQQIVYRNVMLENYKNLV SLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS (SEQ ID NO: 223)
SBS-85262	MAPKKRKRKVGVPAAAMAERPFQCRICM <u>Q</u> NFS Q SADRTKHIRTHTGEKPFAC DICGRKF ADNSNRIK HHTKIHTGSQKPFQCRICM <u>Q</u> NFS RS ANLSVHIRTHT GEKPFACDICGRKF ADRANLSR HHTKIHTHPRAPIPKPFQCRICMRNFS RS DNLSV HIRTHTGEKPFACDICGRKFA Q KATRITHTKIHLRQKDAARGSGG DAKSLTAWSR TLVT FKDVFDFTREEWKLLDTAQQIVYRNVMLENYKNLV SLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS (SEQ ID NO: 224)

SBS- 85263	MAPKKRKRKVGVPAAEAERPFQCRICM <u>Q</u> NFS <u>Q</u> SADRTKHIRTHTGEKPFAC DICGRKFAD <u>NSNR</u> IKHTKIHTGS <u>Q</u> KPFQCRICM <u>Q</u> NFS <u>RSANLSV</u> HIRTHT GEKPFACDICGRKFAD <u>RANLSR</u> HTKIH TH PRAPIPKPFQCRICM <u>Q</u> NFS <u>RS</u> <u>DNLSV</u> HIRTHTGEKPFACDICGRKFA <u>Q</u> KATRITHTKIHL <u>RQ</u> KDAARGSGG DAKSLTAWSR ^{TL} VTFKDVFDFTREEWKLLDTAQQIVYRNVML ^{ENY} KNLV SLGYQLTKPDVILRLEKGEEPW ^L VEREIHQETHPDSETAFEIKSSVRS (SEQ ID NO: 225)
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What is claimed is:

1. A fusion protein comprising a zinc finger protein (ZFP) domain and a transcription repressor domain, wherein the ZFP domain binds to a target region of a human alpha-synuclein gene (*SNCA* gene).
2. The fusion protein of claim 1, wherein the target region is within 1 kb of a transcription start site (TSS) in the *SNCA* gene.
3. The fusion protein of claim 2, wherein the TSS is TSS1, 2a, or 2b.
4. The fusion protein of claim 3, wherein the target region is within 500 bps upstream of TSS 1, within 500 bps downstream of TSS 1, within 500 bps upstream of TSS 2a, and/or within 500 bps downstream of TSS 2b, of the *SNCA* gene.
5. The fusion protein of any one of the preceding claims, wherein the ZFP domain comprises six zinc fingers and the fusion protein optionally represses expression of the *SNCA* gene by at least about 40%, 75%, 90%, 95%, or 99% with no or minimal detectable off-target binding or activity.
6. The fusion protein of any one of the preceding claims, wherein the transcription repressor domain comprises a KRAB domain amino acid sequence from human KOX1.
7. The fusion protein of any one of the preceding claims, wherein the ZFP domain is linked to the transcription repressor through a peptide linker.
8. The fusion protein of any one of the preceding claims, wherein the ZFP domain comprises a DNA-binding recognition helix sequence shown in Table 1.
9. The fusion protein of any one of the preceding claims, wherein the ZFP domain comprises the DNA-binding recognition helix sequences as shown in a single row of Table 1.

10. A nucleic acid construct comprising a coding sequence for the fusion protein of any one of claims 1-9, wherein the coding sequence is linked operably to a transcription regulatory element.
11. The nucleic acid construct of claim 10, wherein the transcription regulatory element is a mammalian promoter that is constitutively active or inducible in a brain cell, and wherein the promoter is optionally a human synapsin I promoter.
12. A recombinant virus comprising the nucleic acid construct of claim 10 or 11.
13. The recombinant virus of claim 12, wherein the recombinant virus is an adeno-associated viral vector, an adenoviral vector, or a lentiviral vector.
14. A pharmaceutical composition comprising the nucleic acid construct of claim 10 or 11 or the recombinant virus of claim 12 or 13, and a pharmaceutically acceptable carrier.
15. A host cell comprising the nucleic acid construct of claim 10 or 11, or the recombinant virus of claim 12 or 13.
16. The host cell of claim 15, wherein the host cell is a human cell.
17. The host cell of claim 15, wherein the host cell is a brain cell or a pluripotent stem cell, wherein the stem cell is optionally an embryonic stem cell or an inducible pluripotent stem cell (iPSC).
18. A method of inhibiting expression of alpha-synuclein in a human brain cell, comprising introducing into the cell a fusion protein of any one of claims 1-9, optionally through introduction of a nucleic acid construct of claim 10 or 11 or a recombinant virus of claim 12 or 13, thereby inhibiting the expression of alpha-synuclein in the cell.
19. The method of claim 18, wherein the human brain cell is a neuron, a glial cell, an ependymal cell, or a neuroepithelial cell.

20. The method of claim 18 or 19, wherein the cell is in the brain of a patient suffering from or at risk of developing Parkinson's disease, Lewy body dementia, Alzheimer's disease, multiple system atrophy, or another synucleinopathy.
21. The method of any one of claims 18-20, comprising introducing into the cell a recombinant virus that expresses the fusion protein.
22. The method of claim 21, wherein the recombinant virus is an adeno-associated virus (AAV), optionally of serotype 9.
23. A method of treating a synucleinopathy in a patient, comprising administering to the patient a recombinant AAV encoding a fusion protein of any one of claims 1-9.
24. The method of claim 23, wherein the AAV is introduced to the patient via intravenous, intrathecal, intracerebroventricular, intra-cisternal magna, intrastriatal, or intranigral injection, or injection into any brain region.
25. The method of claim 23 or 24, wherein the synucleinopathy is Parkinson's disease, Lewy body dementia, Alzheimer's disease, or multiple system atrophy.
26. A fusion protein of any one of claims 1-9, a nucleic acid construct of claim 10 or 11, a recombinant virus of claim 12 or 13, a pharmaceutical composition of claim 14, for use in the method of any one of claims 18-25.
27. Use of a fusion protein of any one of claims 1-9, a nucleic acid construct of claim 10 or 11, a recombinant virus of claim 12 or 13, for the manufacture of a medicament in the method of any one of claims 18-25.